VYTAUTAS MAGNUS UNIVERSITY
LITHUANIAN RESEARCH CENTRE FOR AGRICULTURE AND FORESTRY

Rūta SUTULIENĖ

ENVIRONMENTALLY INDUCED OXIDATIVE STRESS IN GREEN PEA AND ITS MANAGEMENT MEASURES

Doctoral Dissertation
Agricultural Sciences, Agronomy (A 001)

Kaunas, 2022

Dissertation was prepared during 2018–2022 in at Lithuanian Research Centre for Agriculture and Forestry, under the doctoral program right conferred to Vytautas Magnus University, Lithuanian Research Centre for Agriculture and Forestry on 16 October 2022 by the Order No. CV-1-452E of the Minister of Education and Science of the Republic of Lithuania.

Scientific Supervisors:

Dr. **Jurga Miliauskienė** (Lithuanian Research Centre for Agriculture and Forestry, Agricultural Sciences, Agronomy A 001).

Scientific Consultant:

Assoc. Prof. Dr. **Lina Ragelienė** (Vytautas Magnus University, Natural Sciences, Biochemistry N 004).

The defence of doctoral thesis will be held at the Council of Defence for Agricultural Sciences, Agronomy.

Chairman:

Prof. Dr. **Pranas Viškelis** (Lithuanian Research Centre for Agriculture and Forestry, Agricultural Sciences, Agronomy A 001).

Members:

Dr. **Giedrė Kacienė** (Vytautas Magnus University, Natural Sciences, Ecology and Environmental Science N 012);

Dr. **Sigita Jurkonienė** (Nature Research Centre, Natural Sciences, Ecology and Environmental Science N 012);

Assoc. Dr. **Irina Vaseva** (Bulgarian Academy of Sciences, Agricultural Sciences, Agronomy A 001);

Dr. **Julė Jankauskienė** (Lithuanian Research Centre for Agriculture and Forestry, Agricultural Sciences, Agronomy A 001).

Defence of dissertation will take at the public meeting of the Council of Agronomy on 20th of December 2022, at 10 a.m. in room No 1-12, Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture.

Address: Instituto al. 1, Akademija, 58344 Kėdainiai distr., Lithuania

The doctoral dissertation is available in the libraries of the Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry, Vytautas Magnus University Agriculture Academy and Martynas Mažvydas National Library of Lithuania.

VYTAUTO DIDŽIOJO UNIVERSITETAS

LIETUVOS AGRARINIŲ IR MIŠKŲ MOKSLŲ CENTRAS

Rūta SUTULIENĖ

APLINKOS VEIKSNIŲ INDUKUOJAMAS OKSIDACINIS STRESAS ŽIRNIUOSE IR JO VALDYMO PRIEMONĖS

Mokslo daktaro disertacija

Žemės ūkio mokslai, Agronomija (A 001)

Kaunas, 2022

Daktaro disertacija rengta 2018–2022 metais Lietuvos agrarinių ir miškų mokslo centro Sodininkystės ir daržininkystės institute pagal Lietuvos Respublikos švietimo ir mokslo ministro 2022 lapkričio 16 d. įsakymu Nr.CV-1-452E suteiktą doktorantūros teisę Lietuvos agrarinių ir miškų mokslų centrui su Vytauto Didžiojo universiteto Žemės ūkio akademija.

Mokslinis vadovas

Dr. **Jurga Miliauskienė** (Lietuvos agrarinių ir miškų mokslų centras, Žemės ūkio mokslai, Agronomija A 001).

Mokslinis konsultantas

Doc. dr. **Lina Ragelienė** (Vytauto Didžiojo universitetas, Gamtos mokslai, Biochemija, N 004).

Disertacija ginama Agronomijos mokslo krypties taryboje:

Pirmininkas

Prof. Dr. **Pranas Viškelis** (Lietuvos agrarinių ir miškų mokslų centras, Žemės ūkio mokslai, Agronomija A 001).

Nariai:

Dr. **Giedrė Kacienė** (Vytauto Didžiojo universitetas, Gamtos mokslai, Ekologija ir aplinkotyra N 012);

Dr. **Sigita Jurkonienė** (Gamtos tyrimų centras, Gamtos mokslai, Ekologija ir aplinkotyra N 012);

Doc. Dr. **Irina Vaseva** (Bulgarijos mokslų akademija, Žemės ūkio mokslai, Agronomija A 001);

Dr. **Julė Jankauskienė** (Lietuvos agrarinių ir miškų mokslų centras, Žemės ūkio mokslai, Agronomija A 001).

Disertacija ginama viešame Agronomijos mokslo krypties tarybos posėdyje 2022 m. gruodžio mėn. 20 d. 10 val. LAMMC Žemdirbystes instituto posėdžių salėje 1-12.

Adresas: Instituto al. 1, Akademija, 58344 Kėdainių r., Lietuva

Disertaciją galima peržiūrėti Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės instituto, Vytauto Didžiojo universiteto Žemės ūkio akademijos ir Lietuvos nacionalinėje Martyno Mažvydo bibliotekose.

TABLE OF CONTENTS

ABBREVIATIONS

$^1O_2$ – singlet oxygen

3Chl – triplet state of Chl

ABTS – 2,2 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

AsA – ascorbate

APX – ascorbate peroxidase

BCF – bioconcentration factor

CAT – catalase

DHAR – dehydroascorbate reductase

DPPH – 2-diphenyl-1-picrylhydrazyl

ETC – electron transport chain

FAD – flavin adenine dinucleotide

FRAP – Fe^{2+} reducing antioxidant power assay

GPOX – glutathione peroxidase

GPX – guaiacol peroxidase

GR – glutathione reductase

GSSG – glutathione disulfide

GST – glutathione S-transferases

H_2O_2 – hydrogen peroxide

LHCs – light-harvesting complexes

MDA – malondialdehyde

MDHAR – monodehydroascorbate reductase

NADH – nicotinamide adenine dinucleotide

NPs – nanoparticles

$O_2^{\bullet-}$ – superoxide radicals

OH• – hydroxyl radicals

PS II – photosystem II

ROS – reactive oxygen species

SM – substrate moisture

SOD – superoxide dismutase

TBA – thiobarbituric acid

TF – translocation factor

Ti – tolerance index

TPC – total phenolic compound

INTRODUCTION

Relevance of the topic

Agricultural crops are increasingly adversely affected by heavy rains or droughts, temperature extremes, strong winds, and the spread of new pathogens due to a changing climate (Shahzad et al., 2021; Rivero et al., 2022). Drought stress is the most common environmental factor limiting crop productivity (Basu et al., 2016; Liliane at al., 2020). Soil drought characteristics can vary from water deficit and depletion to long periods of water deficit or when the soil water content is below total capacity (Seleiman et al., 2021). Drought stress reduces photosynthesis by reducing leaf area and photosynthetic rate. In addition, during drought, the plant closes its stomata to reduce water loss through transpiration, reducing CO_2 and nutrient uptake (Khan et al., 2018; Gambetta et al., 2020; Kapoor et al., 2020; Ozturk et al., 2021). These induced damages are due to the plant's redox balance disruption, resulting in the formation of reactive oxygen species (ROS) (Yang et al., 2021). ROS causes oxidative stress in plant cells. Plants have adapted to survive under stressful conditions by developing an antioxidant system (Basu et al., 2016; Kapoor et al., 2020). Balancing between oxidative and reductive reactions is essential in maintaining redox homeostasis in plants, leading to plants forming an enzymatic and non-enzymatic antioxidant system. External measures, such as the application of various nanoparticles (NPs), can be used to reduce the harmful effects of adverse environmental factors on plants. (Kandhol at el., 2022; Ghani et al., 2022; Ahmad et al., 2022). The application of NPs has been discussed in recent decades to improve the resistance of various crops to abiotic stress. However, to date, there are still considerable gaps in knowledge on the benefits of NPs for plant growth and yields. Besides, there is still no consensus on the effects of NPs on the environment, exclusively on plant oxidative stress biomarkers and antioxidant systems.

Nanoparticles are materials of up to 100 nm, characterized by unique shapes, surface charge, and a large surface area (Modena et al., 2019; Raval et al., 2019). Therefore, in studying the effects of NPs on plants, it is essential to study the properties of NPs suspensions, as well as different methods of application pathways (Tarafdar et al., 2012; Mittal et al., 2020). Furthermore, it is crucial to choose the optimal concentration, which can be different for each type of plant and cause the opposite effect as expected.

The green pea (*Pisum sativum* L.) is one of the most popular plants of the legume family, which is exceptionally sensitive to drought (Nadeem et al., 2019). Molybdenum and boron are essential for pea-specific nodule bacteria and their differentiation into a nitrogen-fixing form. However, molybdenum and boron have not yet been studied on the nanoscale. In addition, the

use of silicon and copper NPs, due to their exceptional properties in regulating oxidative stress, makes this work not only scientific but also of practical importance.

Research hypothesis. Likely, the enzymatic and non-enzymatic antioxidant system induced by the application of nanoparticles neutralizes the oxidative burst in drought-affected pea plants.

The subject of research. The green pea (*Pisum sativum* L.) plant oxidative and antioxidant systems.

The aim. To select the most appropriate concentration and method of application of SiO_2, MoO_3, B_2O_3, CuO nanoparticles for green peas (*Pisum sativum* L.) by enhancing their resistance to drought and the combined effects of heavy metal copper and drought.

Research tasks:

1. To evaluate the effects of SiO_2, CuO, MoO_3, and B_2O_3 nanoparticles on morphological parameters, oxidative stress, and antioxidant system in green peas.
2. To investigate the effects of agrometeorological drought and SiO_2, CuO, MoO_3, and B_2O_3 nanoparticles on oxidative stress and antioxidant system in green peas.
3. To investigate the combined effect of environmental factors (agrometeorological drought, heavy metals, and nanoparticles) on oxidative stress and antioxidant system in green peas.
4. To evaluate the application pathway of various NPs in strengthening the activity of the green pea antioxidant system and resistance to adverse environmental factors.

Defended statements:

1. Using optimal concentrations of SiO_2, MoO_3, B_2O_3, and CuO nanoparticle suspensions for green peas grown in a substrate of normal moisture stimulate the activity of the antioxidant and oxidative system, which positively affects morphological parameters and increases plant productivity.
2. The resistance of peas to agrometeorological drought is increased by suspensions of SiO_2, MoO_3, B_2O_3, and CuO nanoparticles. It enriches the mineral nutrition of plants and activates enzymatic and non-enzymatic antioxidants, which reduce the content of oxidative biomarkers and positively affect the morphological parameters and yield of green peas.
3. Suspensions of SiO_2, MoO_3, and B_2O_3 nanoparticles increase the resistance of peas to the combined effects of agrometeorological drought and heavy metal copper by strengthening the antioxidant system, weakening the impact of oxidative stress, and

increasing the tolerance index of plants to copper. Conversely, the effects of CuO nanoparticles act synergistically with excess copper by increasing oxidative biomarkers and reducing productivity in green peas.

4. The effect of nanoparticle SiO_2, MoO_3, B_2O_3, and CuO suspensions on the antioxidant system, morphological parameters, and accumulation of macro- and microelements in peas depends on their stability, surface charge, and mode of exposure.

Novelty. The study provided new knowledge about the effect of SiO_2, CuO, MoO_3, and B_2O_3 nanoparticles on oxidative stress biomarkers, antioxidant system, and macro- and microelement changes in pea plants when the plants were grown in normal moisture substrate, moisture deficit conditions and was exposed to combined drought and heavy metal copper stress. It has been found that nanoparticle suspensions have positive and negative effects, which depend on the properties of the applied particles, their concentration, and the application method. The investigated zeta potential of SiO_2, CuO, MoO_3, and B_2O_3 nanoparticles showed that all suspensions were stable and anionic, and nano-sized particles were found in all aqueous suspensions. The most optimal concentrations of nanoparticles that had a positive effect on oxidative stress biomarkers, antioxidant system, macro- and microelement changes, and productivity of peas were selected: 50 ppm - SiO_2, CuO, MoO_3, 12.5 ppm - B_2O_3. It was also found that spraying with CuO and B_2O_3 nanoparticles affects pea productivity more effectively than watering, and watering plants with MoO_3 nanoparticle suspension was more effective than spraying.

Practical importance. Research on the effects of metal-based NPs on pea enriches the knowledge about the benefits or risks of NPs to plants, helps to include a wider variety of NPs, and expand the use of nanotechnology in crop production while contributing to the improvement of general agricultural practices. All this has great value for the development of agronomic science. In addition, the thesis has a social benefit, as the development of such research would help meet the needs for plant-based food in society and improve the quality of plant-based products.

Approval of dissertation. The results of the research were published in 2 scientific articles published in international journals with the Clarivate Analytics Web of Science citation index of the Scientific Information Institute database (Horticulturae (Q1, IF – 2.331), Journal of Soil Science and Plant Nutrition (Q1, IF – 3.872)) journals indexed in Clarivate Analytics Web of Science database and one scientific article in a peer-reviewed journal (Sodininkystė ir daržininkystė). The main research results were presented at five international conferences: International Conference on Agriculture and Horticulture (ICAH2020, oral presentation, online,

2020); 11th Scandinavian Plant Physiology Society Ph.D. Student conference (SPPS, poster presentation, online, 2020); 17th International Conference of young scientists on energy and natural sciences issues (CYSENI, oral presentations, online, 2021, 2022); 1st International Electronic Conference on Horticulturae (IECHo, poster presentation, online, 2022); 31st International Horticultural Congress (IHC, poster presentation, France, 2022)

Volume and structure of the dissertation. The dissertation consists of an introduction, literature analysis, description of research methods, analysis of results, conclusions, list of used literature, and list of publications published together with co-authors and appendices. Fifteen figures and 22 tables illustrate the literature analysis, description of the research methodology, and results. There are 295 sources in the bibliography, and the volume of the work is 155 pages.

1. LITERATURE REVIEW

1.1 The response of plants to environmental change

Plants are highly complex multicellular organisms with many organs and tissues above or below ground. To meet environmental challenges (abiotic and/or biotic stress), plants must adapt their development and metabolism to ensure survival. The concept of stress consists of four stages: alarm, resistance, exhaustion, and regeneration (Selye et al.,1936; Lichtenthaler, 1998). Plants are at a particular optimal physiological stage, depending on the environmental conditions of growth, light, water, and availability of minerals before stress. Stressors or combined stresses lead to the first three phases of the stress response and then to the regeneration phase if the damage is not too severe after removing the stressor. At the beginning of stress, plants react with a decrease in photosynthesis, metabolite transport, and ion uptake and transfer. Due to this decrease in metabolic activity, plants deviate from the usual physiological standard. Acute damage and senescence occur quickly in plants with only weak stress resistance mechanisms or none - in such plants, the resistance minimum is low (Selye et al.,1936; Lichtenthaler, 1998). However, most plants with stress-coping mechanisms, such as acclimatization of metabolic flows, repair processes, and long-term metabolic and morphological adaptations, activate theirs in the alarm phase. This general adaptation of the plant sets a new physiological standard, which is the optimal physiology stage under the influence of a stressor and corresponds to the maximum plant resistance. In the case of long-term stress or a dose of stress that overloads the plant's stress-coping mechanism, the exhaustion stage (termination phase) occurs. This causes severe damage to the plant and, ultimately, cell death. However, when stressors are removed right before senescence processes become dominant, plants regenerate and move to new physiological standards (regeneration phase). The time of exhaustion and the stage at which the stressors are removed from the plant determine to which new physiological standard the plants will grow within the minimum and maximum resistance limits (Lichtenthaler, 1998).

However, the approach to stress has become broader, and it is not limited to general adaptation syndrome and only adverse effects on live organisms nowadays. Scientists have noticed that a weak stimulus, causing a lower impact of stressors, can increase plant productivity and positively affect metabolic processes. Hormesis is a phenomenon where low doses of environmental factors can stimulate plant mechanisms to cope with stress, increasing the growth and production of secondary metabolites, and wound healing capacity to ultimately maintain homeostasis (Poschenrieder et al., 2013; Duarte-Sierra et al., 2020). Modern scientists distinguish two possible effects of stressors on plants: oxidative distress as harmful and

oxidative eustress as positive (Sies, 2019). Excessive oxidative stress is detrimental to plant biomolecules, but maintenance of physiological oxidative levels, oxidative eustress, is essential for plants growing under optimal conditions due to redox signaling (Seis et al., 2017).

Acclimating plants to stress conditions successfully requires an efficient, timely, and coordinated response that spans most, if not all, plant parts and tissues (Zandalinas, 2020). Plants have developed several systemic signaling pathways that allow the transmission of different stress signals from a particular part of a plant. It should be noted that different plant cells and tissues can communicate with each other through simplastic, vascular, and apoplastic connections. That allows short, long-distance intercellular communication (systemic signaling) (Sevilem et al., 2013). The systemic response to the biotic stimulus is called systemic acquired resistance and is usually caused by the interaction of certain cells with pathogens (bacteria, fungi, viruses). A systemic response to abiotic stimulus (heat, cold, high light, UV, salinity, water deficiency, osmotic stress) is called systemic acquired acclimatization. A systemic response to mechanical stress that can be induced by biotic (e.g., insect feeding) or abiotic stress (wind, hail, heavy rain) is called a systemic wound reaction (Conrath, 2006, Perez and Brown, 2014). These systemic reactions warn distant and non-stressed plant tissues of a biotic or abiotic threat and activate resistance or acclimatization pathways in these tissues.

Among the main systemic signals in plants are electrical, calcium, reactive oxygen species (ROS) and hydraulic waves, and plant hormones. Systematic signals spread from the affected tissue to the whole plant in a few minutes (Gilroy et al., 2016; Vega-Muñoz et al., 2020; Fichman and Mittler, 2020; Zandalinas, 2020). Due to the rapid and systematic transmission of calcium, electricity, and ROS waves, the plant adapts to light, heat, and other stressors (Gilroy et al., 2016). Most research has focused on how these systemic signals respond to a single stress stimulus affecting a specific plant leaf or root. Still, plants are often exposed to more than one environmental stress simultaneously, known as a "stress combination" (Lamers et al., 2020). Acclimation of plants to a combination of stress (such as a combination of drought and heat) includes responses to each stress that affects the plant simultaneously (Zandalinas, 2020).

1.2 Oxidative stress in plants

Oxygen (O_2), which has appeared in the Earth's atmosphere mainly as a product of photosynthesis, is a double-edged sword for aerobic organisms. It allows efficient energy production by enzymatic combustion of organic compounds but also damages the formation of reactive oxygen species (ROS) that react with all biomolecules (Bartosz, 1997). Over millions of years of evolution, aerobic organisms have adapted to the threat posed by O_2 by developing antioxidant systems that reduce the harmful effects of ROS and even by using ROS as a defense

and possibly as secondary signaling (Mansoor et al., 2022). As a result, each aerobic cell has a dynamic balance between ROS-generating reactions, thus promoting non-specific oxidations and antioxidants (Figure 1). The shift in this balance in favor of oxidative reactions is called oxidative stress (OS) (Inzé and Montagu, 1995; Das and Roychoudhury, 2014; Noctor and Foyer, 2016).

OS results in redox imbalance due to the formation of ROS (Demidchik, 2015). The use of O_2 in many essential metabolic processes of living systems has had an evolutionary cost because its metabolism leads to the production of ROS. Cells generate both non-radical and free-radical ROS. Free radicals include hydroxyl radicals (OH•), superoxide radicals ($O_2^{\bullet-}$), organic hydroperoxides (ROO•), organic hydroperoxyl radicals (RO_2•), and alkoxyl radicals (RO•). Non-radicals include singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). The most prevalent cellular ROS in plants is H_2O_2, OH•, $O_2^{\bullet-}$, and $^1O_2$ (Zhou et al., 2014; Inzé and Montagu, 1995; Das and Roychoudhury, 2014; Noctor and Foyer, 2016).

The superoxide anion radical ($O_2^{\bullet-}$) is the primary ROS generated in cells, leading to a cascade of reactions and the generation of secondary ROS, either directly or via enzymatic and metal-catalyzed processes, depending on the cellular compartment (Huang et al., 2019; Gill and Tuteja, 2010). The $O_2^{\bullet-}$ is generated during PSI's acyclic electron transport chain (ETC) (Foyer, 2018). Typically, cytochrome c oxidase interacts with O_2 to form H_2O, but when O_2 reacts with different ETC components, $O_2^{\bullet-}$ can be created. $O_2^{\bullet-}$ is moderately reactive, does not cause much damage by itself, and has a short half-life of 2–4 μs (Gill and Tuteja, 2010). However, it is rapidly converted to H_2O_2 by superoxide dismutase (SOD). Thus, preventing its accumulation in cells and protecting proteins from damage, especially those containing Fe-S clusters (Imlay, 2006; Gill and Tuteja, 2010). The most important reaction of $O_2^{\bullet-}$ is dismutation. Dismutation can be non-enzymatic or catalyzed by SOD when it reacts with another $O_2^{\bullet-}$ molecule. It results in the reduction of one of its molecules to H_2O_2 and the oxidation of the other to oxygen (Das and Roychoudhury, 2014; Sharma et al., 2012; Dumanović et al., 2021).

Hydrogen peroxide (H_2O_2) is a moderately reactive ROS usually generated by the neutralization of $O_2^{\bullet-}$ via univalent reduction or protonation. However, it can be formed non-enzymatically due to low pH or a reaction catalyzed by the enzyme SOD (Das and Roychoudhury, 2014).

$2\ O_2^{\bullet-}+2H^+ \rightarrow H_2O_2+O_2$

H_2O_2 plays a dual role: at low concentrations, it is involved in signal transduction; at high concentrations, it is toxic to the cell. It acts as a signal for regulating essential physiological processes such as senescence, photorespiration and photosynthesis, stomatal movement, cell cycle, and growth and development at low intracellular concentrations. Under normal

conditions, the amount of H_2O_2 in plant leaves ranges from about 1 μmol per gram of fresh tissue weight (10 μmol/L H_2O_2 in peroxisomes) (Raja et al., 2022). The elimination half-life of H_2O_2 is 1 ms, which allows it to cross membranes through aquaporins (Bienert et al., 2006) and cover the cell over long distances (Foyer et al., 1997) and cause oxidative damage (Vilchis-Landeros et al., 2020). High intracellular concentrations (10–100 μmol L^{-1} in peroxisomes) of H_2O_2 oxidize the residues of cysteine (-SH) and methionine (-SCH_3). Further, oxidize the thiol groups of the Cu/Zn-SOD and Fe-SOD enzymes. Excess production of H_2O_2 in plant cells during oxidative stress is caused by factors such as chilling, drought, UV radiation, wounding, intense light, and pathogens (Sharma et al., 2012).

Limited internal carbon dioxide (CO_2) concentration and fixation due to stomatal closure, oxygenation via ribulose 1,5-bisphosphate is favored, resulting in enhanced photorespiration. As a result, 70% more H_2O_2 is formed in the case of water shortage than when plants grow in favorable conditions (Das and Roychoudhury, 2014). The main sources of H_2O_2 production in plant cells are the ETC in the chloroplast, endoplasmic reticulum (ER), cell membrane, mitochondria, photorespiration, and β-oxidation of fatty acids. Additional hydrogen peroxide is produced by photooxidation by NADPH oxidase and xanthine oxidase. (Anjum et al., 2016).

$O_2^{\bullet-}$ and H_2O_2 are moderately reactive, but the full damage caused by them is realized only after transformation into more reactive species. An indirect effect of H_2O_2 is expressed by crossing the cellular membranes via peroxiporins and by Fenton (Fenton, 1984) and Haber-Weiss reaction (Koppenol, 2001) with the iron (Fe^{2+}) or copper (Cu^{+}) ions, resulting in the formation of more potent toxic species such as OH• (Dumanović et al., 2021).

The Haber-Weiss reaction:

$Fe^{3+}+O_2^{\bullet-}\longrightarrow Fe^{2+}+O_2$

The Fenton reactions:

$Fe^{2+}+H_2O_2\longrightarrow Fe^{3+}+OH^{-}+\bullet OH$

$O_2^{\bullet-}+H_2O_2\longrightarrow \bullet OH+OH^{-}+O_2$

The hydroxyl radical (OH•) is the most reactive and toxic ROS due to its short half-life, positive redox potential (close to +2 V), and high affinity for biomolecules, non-selectively oxidize sugars, amino acids, DNA, lipids, proteins, and metals, leading to damage or genetic instability (Bano et al., 2021; Dumanović et al., 2021; Sharma et al., 2012).

Lipid peroxidation, which leads to the destruction of proteins and membrane damage, is caused by OH• (Ayal et al., 2014; Repetto et al., 2012). Plants have no enzymatic system to eliminate this radical, but mechanisms to maintain iron homeostasis have evolved in cells. Plants' propensity to oxidative stress is also determined by the absorption of metals from the soil (Rai et al., 2021). Special efforts are made to prevent the reaction of transition metals and H_2O_2

by sequestering them. In this way, plants are provided with ferritin and metallothionein, which help to accumulate iron, zinc, and copper (Ravet and Pilon, 2013.

Singlet oxygen ($^1O_2$) reacts with the antenna system's triplet state of chlorophyll (Chl) (Krieger-Liszkay, 2005; Ma et al., 2009; Dogra and Kim, 2019). The lack of intracellular CO_2 stimulates the formation of $^1O_2$ during stressors. $^1O_2$ is particularly dangerous for photosystems I and II (PSI, PSII) (Krieger-Liszkay, 2005). Its half-life is shorter, about a few ns, but it can diffuse 100 nanometers in the cell and damage pigments, nucleic acids, proteins, and lipids (Dogra and Kim, 2019). Plants have been able to adapt and efficiently remove $^1O_2$ using tocopherol, plastoquinone, and β-carotene. Genes responsible for protection against photooxidative stress can be regulated by $^1O_2$ (Foyer, 2018; Dmitrieva et al., 2020).

Plants are particularly affected by $^1O_2$ because they are rich in Chl, which acts as a photosensitizer, resulting in the continuous formation of $^1O_2$ in the leaves. Chl absorbs light in light-harvesting complexes (LHCs), photosystem II (PS II) reaction centers, and internal antennas (Krieger-Liszkay, 2005; Triantaphylidès and Havaux, 2009; Dogra and Kim, 2019). Its excited state is long-lived enough that the excitation energy can be converted into an electrochemical potential using the charge separation method during photosynthesis (Dmitrieva et al., 2020). However, when energy is not efficiently used, the excited triplet state of Chl (3Chl) near molecular oxygen can provide sufficient energy to cause the formation of $^1O_2$ (Triantaphylidès and Havaux, 2009). $^1O_2$ is one of the most critical ROS because it can reduce the activity of PS II by degrading the D1 protein, which causes pigment "bleaching" (Tripathy and Oelmüller, 2012). Plants use two strategies to protect themselves from photoinhibition. For prevention, it is the dissipation of excessive 3Chl excitation energy in PS II antennas in the form of heat (non-photochemical quenching). Another method is based on the ability of PS II to transfer electrons to nearby acceptors such as carotenoids and α-tocopherol. Due to this, excess energy is released in the form of heat and returns to its ground state (Derks et al., 2015; Roach and Krieger-Liszkay, 2014). This transfer of Chl and carotenoids is efficient, 5% 3Chl remains, and this incomplete quenching is evidence that antenna pigments are considered a potential source of $^1O_2$ in chloroplasts, which tend to damage the D1 protein (Roach and Krieger-Liszkay, 2014).

1.2.1 Sources of reactive oxygen species

Redox balance is a crucial factor that ensures the smooth functioning of proteins and enzymes involved in essential metabolic pathways. Plant cell organelles chloroplasts, mitochondria, peroxisomes, plasma membranes, endoplasmic reticulum (ER), and the cell wall

produce ROS during normal and stress conditions (Sharma et al., 2012; Zhou and Shao, 2014; Noctor and Foyer, 2016; Bano et al., 2021).

The chloroplast consists of an orderly arranged system of thylakoid membranes, where photosynthetic mechanisms responsible for light capture and efficient light collection are located. PSI and PSII photosystems are the core of the light collection system in thylakoids and the main ROS formation sources. The formation of $O_2^{\bullet-}$ in PS via the Mehler reaction ($2O_2 + 2Fd_{red} \rightarrow 2O_2^{\bullet-} + 2Fd_{ox}$) is determined by abiotic stressors such as water stress, CO_2 limitation, and excess light (Cruz de Carvalho, 2008; Foyer, 2018; Das and Roychoudhury, 2014). Cu/Zn SOD enzymes in PSI convert $O_2^{\bullet-}$ to H_2O_2. Fe-S clusters can also be accomplices of electron leakage from the PSI ETC (Imlay, 2006; Gill and Tuteja, 2010). Through the QA and QB electron acceptors, electron penetration into PS II occurs and is responsible for the formation of $O_2^{\bullet-}$ (Pospíšil, 2016). The superoxide radical converts to a more toxic ROS such as OH• via the H_2O_2 intermediate via a Fenton reaction at the Fe-S centers (Imlay, 2006; Gill and Tuteja, 2010). PSII also produces $^1O_2$, and this occurs in two ways: by the formation of triplet Chl (Chl→ ^{3}Chl), which reacts with dioxide to release singlet oxygen ($^3Chl + {}^3O_2 \rightarrow Chl + {}^1O_2$), when environmental stress disturbs the balance between energy utilization and LHC) PSII generates $^1O_2$ when the ETC is too reduced (Dogra and Kim, 2019). Accumulation of $^1O_2$ in chloroplasts leads to peroxidation of membrane lipids. Polyunsaturated fatty acids (PUFA) and membrane proteins are particularly affected due to damage to the PSII P680 reaction center (Foyar, 2018; Li and Kim, 2021). The primary source of ROS production in plants is the chloroplast. Controlling and scavenging ROS in the chloroplast is necessary to ensure the continued survival of stressed plants.

To a lesser extent, harmful ROS such as H_2O_2 and $O_2^{\bullet-}$ are also produced in mitochondria (Huang et al., 2016; Cruz de Carvalho, 2008). Mitochondria of plants participate in photorespiration and have O_2 and carbohydrate circulation. The mitochondrial ETC (mtETC) is the site of ROS production, as it generates electrons with sufficient energy. Complex I, III, and IV are the main components of mtETC responsible for ROS production (Møller, 2001). Complex I directly reduce O_2 to $O_2^{\bullet-}$ in its flavoprotein domain using nicotinamide adenine dinucleotide dehydrogenase (NADH). When the bound of NAD^+ substrates is lacking, reverse electron flow from complex III to complex I occur, and ROS production is enhanced. By the principle of ATP hydrolysis, this reverse flow of electrons is controlled (Vanlerberghe, 2013). An electron is transferred to cytochrome c1 when ubiquinone is completely reduced. This results in an unstable ubisemiquinone half-radical that promotes electron leakage to O_2, thereby generating $O_2^{\bullet-}$ in complex III. (Rhoads et al., 2006). Although $O_2^{\bullet-}$ is the predominant ROS in mitochondria, it is converted to H_2O_2 by ascorbate peroxidase and Mn-SOD (Sharma et al.,

2012). About 1-5% of the total O_2 consumed by mitochondria is directed to the production of H_2O_2 (Møller, 2001). Mitochondrial ROS production is activated under abiotic stress conditions (Vanlerberghe, 2013). Stress affects the close connection between ETC and ATP synthesis, resulting in a significant reduction of electron carriers (ubiquinone pool), which influences the formation of ROS (Huang et al., 2016; Nadarajah, 2020). During drought, to compensate for the lower rate of chloroplast ATP synthesis, mitochondrial ATP synthesis increases due to increased respiration and, thus, the production of ROS (Pastore et al., 2007; Cruz de Carvalho, 2008; Miller et al., 2010). Two enzymes neutralize ROS in mitochondria, alternative oxidase and mitochondrial Mn-SOD (Huang et al., 2016; Møller, 2001; Vanlerberghe, 2013).

Peroxisomes are metabolically plastic because the amount of enzyme in them can change depending on environmental conditions, plant species, and cell and tissue type (Corpas et al., 2019). Peroxisomes are 0.1–1 μm spherical microbodies bound by a single membrane and are the main sites of intracellular H_2O_2 production due to integrated oxidative metabolism (Corpas et al., 2019; Smirnoff and Arnaud). Xanthine is converted to uric acid by a reaction catalyzed by xanthine oxidoreductase. This hydroxylase enzyme, which contains flavin adenine dinucleotide (FAD), molybdenum, iron, and sulfur, exists in two interconvertible forms. Oxygen-dependent xanthine oxidase and NAD-dependent xanthine dehydrogenase may be present. As a result, either superoxide radicals or NADH can be formed during this reaction. Allantoin and H_2O_2 are then produced by urate oxidase (Corpas et al., 2008; Corpas et al., 2019; Kostić et al., 2015). Another pathway for the formation of H_2O_2 occurs when the stomata close during stress. As a result, increased photorespiration initiates glycolate production. Glycolate oxidase in peroxisomes oxidizes glycolate, releasing H_2O_2 (Del Rio et al., 1996; Rojas et al., 2012). In addition, fatty acid β-oxidation and flavin oxidase pathways influence unbalanced ROS production in peroxisomes.

The apoplast is the intercellular space filled with water and gas, formed between the cell membranes, the intermycelial and interfibrillar space of the cell walls, and the xylem, which extends to the rhizoplane and cuticle (Farvardin et al., 2020). In the apoplast, CO_2 is converted into a soluble form, which then enters the cytosol and participates in photosynthesis (Tränkner et al., 2018). It becomes an important site for H_2O_2 production when a stress signal with abscisic acid occurs. Enzymes such as NADPH oxidases, peroxidases, oxalate oxidases, and polyamine oxidases (Xue et al., 2009; Kärkönen and Kuchitsu, 2015) produce H_2O_2 and $O_2^{\bullet-}$ in the apoplast.

The endoplasmic reticulum can be a source of $O_2^{\bullet-}$ production. The reaction depends on NAD(P)H electron transfer involving $CytP_{450}$. After the reaction of the substrate RH with Cyt P450, the compound is reduced with the flavoprotein, resulting in the formation of a radical

intermediate Cyt P_{450} R−. Cyt P_{450} R− reacts with triplet oxygen ($^3O_2$) to form the oxygenated complex Cyt P_{450}-ROO−. This oxygenated complex can be reduced with cytochrome b or complexes to release $O_2^{\bullet-}$ (Sharma et al., 2012).

The plasma membrane surrounds the entire plant cell. It is responsible for transmitting information about the changing environment necessary for the cell's survival (Schapire et al., 2008). At the plasma membrane, the transfer of electrons from cytosolic NADPH to O_2 is dependent on NADPH oxidase, resulting in the formation of $O_2^{\bullet-}$ (Das and Roychoudhury, 2014). Then, either spontaneously or catalyzed by SOD, $O_2^{\bullet-}$ is converted to H_2O_2 (Schopfer and Liszkay, 2006). NADPH oxidase is essential in defense of plants against abiotic stresses. Hydroperoxidation of PUFA with the participation of lipoxygenase is induced in the cell wall under stress conditions, and polyamines or diamines are used by diamine oxidases, resulting in the formation of ROS in the cell wall (Hasanuzzaman et al., 2020).

1.3 Plant antioxidant system

Balancing between oxidative and reductive reactions is vital in maintaining redox homeostasis in plants. This led to the formation of an antioxidant system in plants. Antioxidants are molecules that inhibit or slow down the reactions of free radicals, thereby preventing cell damage. Antioxidants in plants are divided into two groups: enzymatic and non-enzymatic. Enzymatic antioxidants (Table 1.3.1) include superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPOX), glutathione S-transferases (GST), dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR).

SOD is essential in oxidative stress, as it catalyzes the dismutation of $O_2^{\bullet-}$, thereby reducing the risk of OH• formation through metal-catalyzed reactions. SOD is found in the plant in three forms: Fe SOD, Mn-SOD, and Cu/Zn-SOD (Suman et al., 2021). APX is one of the most critical antioxidants in plants, with an essential role in scavenging H_2O_2 (Amir et al., 2019). Its principal function is to neutralize ROS generated during stress. It removes H_2O_2 by using ascorbate (AsA) as an electron donor in the ascorbate-glutathione (AsA/GSH) cycle. Four isoforms of the APX family are distinguished according to localization: tAPX, gmAPX, sAPX, and cAPX (Habi, 2014). The highest amounts of CAT are found in peroxisomes, with smaller quantities of CAT found in mitochondria (Mhamdi et al., 2010). Three CAT genes exist: class I CAT in photosynthetic tissues, class II CAT in vascular tissue, and class III in seeds and young seedlings (Willekens et al., 1995). CAT binds H_2O_2 produced in peroxisomes during photorespiration and β-oxidation of fatty acids. Four heme subunits with Fe^{2+} ions are included in the composition of CAT. They catalyze the dissociation of two H_2O_2 molecules into water

and oxygen. The GPX enzyme contains a heme group; its main function is to remove excess H_2O_2 during normal metabolism and stress. It is critical for lignin biosynthesis and is also important for degrading indole acetic acid (IAA) using H_2O_2, thus protecting the plant from the effects of stress. GPX electron donors are guaiacol and pyragol. GPX is active in the cytosol, vacuoles, and cell wall. GPX activity depends on plant species and stress conditions (Mika and Lüthje. 2003; Das and Roychoudhury, 2014). GR is known as flavoprotein oxidoreductase. The highest amounts of this enzyme are found in chloroplasts, less in mitochondria and cytosol (Das and Roychoudhury, 2014). GR belongs to the AsA-GSH cycle and, by maintaining a reduced GSH state, plays an essential role in the defense system against ROS. GR catalyzes the reduction of GSH, a molecule involved in much metabolic regulation and antioxidant processes in plants, where GR catalyzes the NADPH-dependent disulfide bond reaction of GSSG and is, therefore important in supporting GSH formation. GR and GSH are crucial in determining plant tolerance to various stresses.

GPOX are thiol-based enzymes that protect plant cells from oxidative stress damage by scavenging ROS by catalyzing the reduction of H_2O_2 and other organic hydroperoxides (Eshdat et al., 1997). According to their localization and composition, eight different genes were found encoding GPOX activity in plants (Bela et al., 2015). Glutathione-dependent peroxidase activity can be associated with glutathione transferase isozymes. They are essential in detoxifying lipid hydroperoxides and other reactive molecules under stress conditions (Hasanuzzaman et al., 2019). GST is one of the most versatile enzymes that catalyze reactions involving the conjugation of glutathione (GSH; γ-Glu-Cys-Gly) with electrophilic compounds (Moons, 2005). One of the functions of GST is to scavenge ROS. GSTs can also function as anthocyanin transporters into the vacuole, phenylpropanoid and auxin carriers, and enzymes in tyrosine catabolism. Plant GSTs have been divided into seven classes: GSTU (Tau), GSTF (Phi), GSTL (Lambda), GSTT (Theta), GSTZ (Zeta), DHAR (dehydroascorbate reductase) (Estévez et al., 2020). MDAR reduces MDHA to AsA using electron donors such as NADH/NADPH. Considering that MDHA is an unstable radical, MDAR must quickly convert it to AsA because if it is not there, it is spontaneously converted to AsA and dehydroascorbate (DHA) (Eltayeb et al., 2007). In the DHAR-catalyzed reaction, DHA is reduced to AsA using GSH as the reducing agent (Eltayeb et al., 2006). Therefore, DHAR and MDAR are critical components in maintaining reduced AsA levels and are extremely important for oxidative stress resistance (Yin et al., 2010).

Table 1.3.1. Enzymatic antioxidants functions and sites in plants

Enzymatic antioxidant	Function (as catalysator or directly involved)	Location in the cell
Superoxide dismutase, SOD (EC 1.15.1.1)	$O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$	Fe-SOD in chloroplast; Mn-SOD in mitochondria and peroxisomes. Cu/Zn-SOD in chloroplast and cytosol.
Ascorbate peroxidase, APX (EC 1.11.1.11)	$H_2O_2 + AsA \rightarrow 2H_2O + DHA$	tAPX – thylakoid, gmAPX - glyoxisome membrane, sAPX – chloroplast, cAPX – cytosol.
Catalase, CAT (EC 1.11.1.6)	$2H_2O_2 \rightarrow 2H_2O + O_2$	Peroxisomes and mitochondria.
Guaiacol peroxidase, GPX (EC 1.11.1.7)	$H_2O_2 + GSH \rightarrow H_2O + GSSG$	Cytosol, vacuoles, and cell wall
Glutathione reductase, GR (EC 1.6.4.2)	$GSSG + NADP(H) \rightarrow GSH + NAD(P)^+$	Chloroplast, mitochondria, and cytosol
Glutathione peroxidase, GPOX (EC 1.11.1.9)	$2\ GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$	GPX1, GPX7 - chloroplast GPX2 - chloroplast, mitochondria, and cytosol GPX3 - mitochondria, Golgi apparatus GPX4 - mitochondria and cytosol GPX5 - plasma membrane and endoplasmic reticulum GPX6 – chloroplast, plasma membrane, apoplast GPX8 – nucleus and cytosol
Glutathione S-transferases, GST (EC 2.5.1.18)	RX + glutathione = HX + R-S-glutathione	Tau - cytosol and nucleus Phi - cytosol and chloroplast Theta - peroxisome and nucleus Zeta - cytosol DHAR - cytosol, chloroplast, and peroxisome Lambda - cytosol, chloroplast, and peroxisome
Monodehydroascorbate reductase, MDHAR (EC 1.6.5.4)	$H^+ + 2MDHA + NADH \rightarrow 2AsA + NAD^+$	Chloroplast, peroxisomemitochondria, and cytosol

The main non-enzymatic antioxidants in plants are ascorbic acid, phenols, tocopherols, flavonoids, carotenoids, and glutathione (Gill and Tuteja, 2010; Hasanuzzaman et al., 2019). Ascorbic Acid (AsA) is one of the most powerful and widely studied antioxidants. It can donate

electrons for various enzymatic and non-enzymatic reactions (Akram et al., 2017). The catalysis of L-galactan-γ-lactone dehydrogenase produces the majority of AsA in plant cells in plant mitochondria via the Smirnoff-Wheeler pathway (Linster et al., 2007). The majority of AsA is concentrated in the apoplast. AsA can be oxidized in two steps: oxidation to MDHA, which can be converted to AsA and DHA. AsA reacts with H_2O_2, OH•, $O_2^{\bullet-}$ and regenerates α-tocopherol from the tocopheroxyl radical, thereby protecting membranes from oxidative damage (Das and Roychoudhury, 2014). AsA in the chloroplast is also a cofactor for violaxanthin deep oxidase, where it is involved in the production of xanthophylls, which are directly engaged in quenching the excessive excitation energy of PS II (Davey et al., 2000). It also protects the activity of metal-binding enzymes.

There are four tocopherol isomers: α-, β-, γ-, δ-. α-tocopherol has the highest antioxidant capacity (Sadiq et al., 2019). It belongs to lipophilic antioxidants, which are effective scavengers of ROS and lipid radicals, making them essential defenders and essential components of biological membranes. α-tocopherol is synthesized from γ-tocopherol by γ-tocopherol-methyltransferase. Tocopherols protect lipids and other chloroplast membrane components by reacting with $^1O_2$ and quenching its excess energy, thereby protecting PSII structurally and functionally. Tocopherol is also an effective free radical scavenger. It reacts with lipid radicals RO•, ROO•, and RO• at the membrane-water interface, where α-tocopherol reduces them and is itself converted to TOH•. TOH•, which is then reduced by interaction with GSH and AsA (Munné-Bosch and Alegre, 2002). Tocopherols are synthesized only in photosynthetic organisms.

Carotenoids also belong to a family of lipophilic antioxidants, such as lycopene, β-carotene, xanthophyll, lutein, and zeaxanthin, that can be found in most plant tissues. In addition, they belong to a group of antenna molecules that absorb light between 450 and 570 nm and transfer the energy to the ChL molecule. Carotenoids demonstrate their antioxidant activity by protecting photosynthetic mechanisms in four ways (Ramel et al., 2012; Das and Roychoudhury, 2014): 1) reacting with LPO products; 2) remove $^1O_2$ and generate heat as a byproduct, 3) react with 3Chl* and excited Chl (Chl*) to prevent $^1O_2$ formation, and 4) dissipates excess excitation energy through the xanthophyll cycle.

Phenols are a group of secondary metabolites such as flavonoids, tannins, hydroxycinnamate esters, and lignin (Roaa, 2020; Grace et al., 2000). The antioxidant properties of phenols are due to their constituent aromatic ring with –OH or $–OCH_3$ substituents, which are adapted to scavenge free radicals (Pietta, 2000). They can donate an electron or a hydrogen atom to neutralize ROS. Phenols can modify the kinetics of peroxidation by changing the lipid

package, which determines the reduced fluidity of the membrane (Arora et al., 2000). These changes can limit the diffusion of free radicals and inhibit the peroxidation reaction.

Glutathione (GSH) is a thiol tripeptide (γ-glutamyl-cysteinyl-glycine) abundantly found in chloroplasts, ER, cytosol, vacuoles, peroxisomes, mitochondria, and even the apoplast (Das and Roychoudhury, 2014). It is important for cell differentiation, cell growth and division, cell senescence, and expression of stress-responsive genes. This versatility of GSH is due to its high reducing potential. GSH scavenges ROS by reducing them when present and generating GSSG as a byproduct (Garcia-Caparros et al., 2021). GSH also aids in the formation of phytochelatins via phytochelatin synthase, which helps to chelate heavy metal ions, thereby eliminating another potential source of ROS generation in plants (Roychoudhury et al., 2012). Therefore, a delicate balance between GSH and GSSG is necessary to maintain the cell's redox state.

GSH and most enzymatic antioxidants are involved in the ascorbate-glutathione cycle (AsA-GSH) (Figure 1.3.1), which is essential in maintaining the redox state in plants (Roychoudhury et al., 2012; Garcia-Caparros et al., 2021). The cycle begins when H_2O_2 is detoxified in the AsA-GSH cycle by APX, which in turn oxidizes two AsA molecules (Dumanović et al., 2021). This results in the formation of the short-lived radical monodehydroascorbate, which can spontaneously convert to AsA and dehydroascorbate (DHA) and/or be reduced to AsA by NAD(P)H catalysis (Gallie, 2013). Unlike AsA, DHA has no antioxidant properties and is converted back to AsA. This occurs using DHA reductases and the addition of two electrons from GSH. GSH-dependent DHAR activity is expressed in chloroplasts, mitochondria, and peroxisomes (Nakano and Asada, 1981). GSH is regenerated from the oxidized state by catalyzing the reaction of glutathione disulfide (GSSG) with GR and using electrons from NAD(P)H, thus closing the regeneration cycle of AsA and GSH (Roychoudhury et al., 2012; Dumanović et al., 2021).

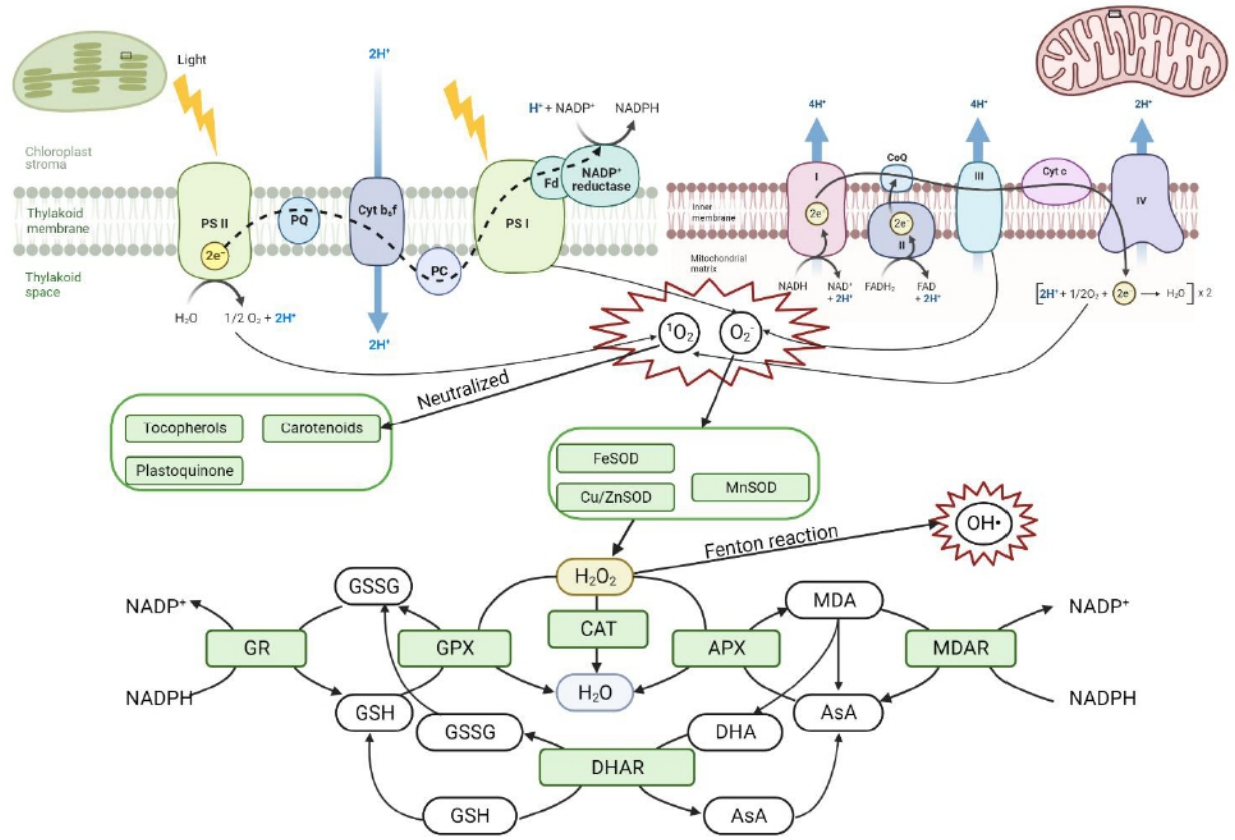


Figure 1.3.1. Main sources of ROS production and antioxidant defense mechanisms in the plant cell

1.4 Specific effects of drought and heavy metals stresses on plants

The impact of abiotic stress on plants in both natural and agricultural environments is a crucial topic due to the potential effects of climate change on precipitation patterns and temperature extremes, salinization of agricultural lands due to irrigation and fertilization, and maintaining increased agrarian productivity in marginal lands (Mitter, 2006; Pinheiro and Chaves, 2011; Corwin, 2021). In field conditions, plants can experience several different abiotic stresses at the same time, for example: decreased water availability during drought or increased excessively during flood, extreme temperatures, including heat waves and freezing, reduced availability of nutrients from the soil (or, conversely, the accumulation of toxic ions during salt stress), heavy metals, excess light (especially when photosynthesis is limited) or increased soil hardness limiting root growth (Seleiman et al., 2021). Different stresses cause different responses in plants, which requires plants to use other acclimation processes. In addition, when several strains affect plants simultaneously, their adverse effects overlap, and the plant becomes more stressed (Rivero et al., 2022).

1.4.1 Effects of drought (water deficit) on plants

Drought is one of the significant constraints limiting crop production worldwide. Crop growth models predict that this issue will be more severe in the future. The conceptual definition

of drought is related to physical processes such as meteorological drought resulting from a lack of precipitation, agricultural drought resulting from a lack of soil moisture, hydrological drought resulting from water shortages in lakes and streams, and water shortages resulting from water management (Mukherjee et al., 2010; Zargar et al., 2011). Drought impairs normal growth, disturbs water relations, and reduces plant water use efficiency (Farooq et al., 2012). The diversity of plant species growing in climate regions with arid conditions suggests that some plants are drought-tolerant. Drought resistance is a broader term applied to plant species with adaptive traits that allow them to escape, avoid, or tolerate drought stress (Basu et al., 2016; Bowles et al., 2021; Santos et al., 2022). "Drought escape" is the ability of a plant species to complete its life cycle before the onset of drought (Carraro et al., 2022, Abid et al., 2018). Plants survive drought stress through two distinct mechanisms: rapid phenological development and developmental plasticity, which can alter their vegetative and reproductive growth according to water availability (Basu et al., 2016; Shavrukov et al., 2017). Plants using rapid phenological development grow faster, form a minimal number of seeds against water deficits, and are thought to have no particular physiological, morphological, or biochemical adaptations. Plants with developmental plasticity mechanisms inhibit growth during drought stress, forming few flowers and seeds, but when more moisture appears, they grow indefinitely, forming many seeds (Carraro et al., 2022, Abid et al., 2018; Osakabe et al., 2014). "Drought avoidance" is when plants retain a relatively higher amount of water in their tissues despite reduced substrate moisture. This is achieved through various adaptations: reducing water loss (reducing transpiration, transpiration area, radiation absorption) and optimizing water uptake (increasing hydraulic conductivity and rooting) (Chaves and Oliveira, 2004; Chaves et al., 2002; Santos et al., 2022). "Drought tolerance" is when plants can withstand a decrease in the amount of water in the tissues by maintaining cell turgor, osmotic regulation, cell elasticity, and increasing protoplasmic resistance (Ashraf et al., 2011; Zivcak et al., 2016).

A sudden plant reaction is a stomatal closure during drought stress. However, stomatal closure reduces water loss through reduced transpiration and CO_2 and nutrient uptake, thus altering metabolic pathways such as photosynthesis (Basu et al., 2016; Chaves and Oliveira, 2004). Drought stress reduces photosynthesis by reducing leaf area and photosynthetic rate. During drought, a lack of intracellular CO_2 leads to the accumulation of photosynthetic electron transport components, leading to the generation of ROS (Gupta et al., 2020). However, plants have developed adaptive responses to mitigate drought-induced damage to photosynthesis, such as thermal dissipation of light energy, dissociation of light-harvesting complexes from photosynthetic reaction centers, xanthophyll, and water-water cycling. Disturbances in plant metabolism are caused by changes in photosynthetic carbon metabolism during drought stress.

Biochemical photosynthetic efficiency depends on ribulose-1,5-bisphosphate (RuBP) regeneration and ribose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity (Portis, 1992) in drought-stressed plants.

Plants growing in dry soils may have xeromorphic properties that reduce transpiration (Chaves et al., 2002). Transpiration reduction under drought conditions could be achieved by shedding leaves and reducing leaf number, size, and branching (Basu et al., 2016). Sclerophylly is another adaptation to combat drought stress, where plants develop stiff leaves that do not suffer permanent damage from wilting and can fully function when conditions return to normal (Basu et al., 2016; Chaves et al., 2002). During drought stress, reduced stomatal conductance is associated with reduced expression of aquaporin genes and reduced chloroplast surface area. The interaction of the leaf developmental stage and light availability with drought is also known to alter the differentiation of mesophyll and chloroplasts, influencing photosynthetic capacity (Chaves et al., 2002; Basu et al., 2016; Gupta et al., 2020). Another important factor is stomatal density and size. Scientists have found that under mild drought stress, the density of stomata increases and decreases under severe drought. All these plant adaptations positively affect water use efficiency (WUE) (Bertolino et al., 2019).

Crops important for agriculture first sense the lack of water through the root system (Ghosh and Xu, 2014, Kim et al., 2020). Drought stress does not affect primary root growth, but lateral root growth is significantly reduced, inhibiting the activation of lateral root meristems (Ghosh and Xu, 2014). Lateral root meristem activity is regulated through the ABA signaling cascade. Auxin signaling is also attenuated. As a result, the plant may begin to form small roots that increase water uptake by providing a more absorbent surface area (Kim et al., 2020).

Osmotic regulation (OA) is the process by which the water potential decreases and solutes accumulate in dividing cells, thereby helping to maintain turgor (Zivcaket al., 2016; Blum, 2017). Cell division and growth in plants depend on water availability and turgor. During drought stress, cell growth is inhibited by OA, although turgor in plant leaves and stems shows little or no reduction. OA is associated with stomatal conductance, photosynthesis, leaf water volume, and growth maintenance under drought conditions. In plants, the content of water decreases, and the salt concentration and mechanical resistance increase when growing in drought conditions (Bertolino et al., 2019). In addition, inorganic cations, organic acids, free amino acids, and carbohydrates accumulate. The rate at which cell volume will decrease depends on the elasticity of the cells or tissues due to reduced water uptake. Water moves out of the plant cells, and the water potential of the cells decreases. The osmotic potential decreases because the solutes in the cells are concentrated. If the cells accumulate solutes during dehydration and the osmotic potential decreases, it is like the decrease in water potential, and

turgor is maintained (Chen and Jiang, 2010). In practice, turgor maintenance is more often mentioned when the accumulation of soluble substances does not correspond to the decrease in water potential and turgor decreases. The maintenance of turgor depends on the elasticity of tissues and the speed of stress development, which leads to OA. It is noteworthy that when plant water deficit develops slowly, the degree of OA is higher (Blum, 2017).

1.4.2 Effects of heavy metals on plants

The group of metals and metalloids with an atomic density greater than 4 g/cm3 or at least five times that of water is collectively called "heavy metals". However, the chemical properties of heavy metals have a more significant influence in terms of toxicity (Raskin et al., 1994). Heavy metals are considered nickel (Ni), cadmium (Cd), lead (Pb), cobalt (Co), silver (Ag), chromium (Cr), copper (Cu), zinc (Zn), iron (Fe), and arsenic (As) (Nagajyoti et al., 2010; Ravet et al., 2013; Viehweger, 2014; Morkunas, 2018). The excess of these elements in nature can be formed naturally (rock outcroppings or geological parent material) or due to anthropogenic factors (agricultural, industrial, atmospheric, and domestic waste) (Nagajyoti et al., 2010; Viehweger, 2014; Morkunas, 2018). Metals can interact with macromolecules and affect the structure and activity of proteins, so their balance is particularly important in plants (Ravet et al., 2013). Biological activity is essential for these transition metals Mo, Mn, Fe, Cu, and Ni. These metal ions bind to the active centers of certain proteins and participate in many oxidation-reduction reactions. Plants have evolved different mechanisms to maintain the balance of metal ions. As the first defense stage, plants exposed to heavy metals try to reduce or prevent their entry into the root cells. They work to confine metal ions in the apoplast by binding them to the cell wall or cell exudates or by inhibiting long-distance transport (Rsdkin et al., 1994; Morkunas, 2018). If the cell fails to protect itself in this way, it tries to transport, chelation, trafficking, and sequester the metal into the vacuole. When this occurs, plants activate oxidative stress defense mechanisms and synthesize stress-related proteins and signaling molecules such as heat shock proteins, reactive oxygen species, and hormones (Emamverdian et al., 2015).

Increased concentration of metals in the soil causes toxic effects on the environment. In Europe, the average concentration of Cu in the soil is about 11.4 - 17 mg Cu kg^{-1}. Using Cu preparations in agriculture as a fungicide can increase its amount in the soil hundreds of times (Printz et al., 2016). In small amounts, Cu is beneficial to both plants and humans, but an increase in Cu concentration in the soil can cause harmful changes in plants (Yruela, 2005). The bioavailability of Cu depends on total soil Cu content, soil pH, and cation exchange capacity. Meanwhile, the activity of free Cu^{2+} in the soil solution increases as the soil pH decreases, ultimately reducing Cu adsorption (Adrees et al., 2015). Plant roots also play an important role

in Cu bioavailability, but this depends on the plant species and the availability of Cu in the soil (Yruela, 2009). Similarly, Cu bioavailability is also affected by physical, chemical and biological processes occurring at the soil-root interface in the rhizosphere, such as changes in pH or dissolved organic matter.

Excess Cu inhibits plant growth and disrupts photosynthetic electron transport. The most common effect of higher Cu concentration in the growing medium is a reduction in the amount of photosynthetic pigments. A decrease in the amount of chlorophyll and damage to the structural photosynthesis apparatus leads to a decrease in photosynthesis. Cu has an inhibitory effect on both photosystems, but photosystem II is the most sensitive to excess Cu (Adrees et al., 2015). At high concentrations of Cu, oxidative stress is induced in the plant, and ROS production is generated, which leads to reduced plant biomass and yield (Fernandes and Henriques, 1991). Scientists have published a study on the effects of the heavy metal Cu on peas (Hattab et al., 2009). They found that the excess amount of Cu negatively affected the morphological parameters of peas (length of roots and shoots, leaf area), the content of chlorophylls and carotenoids, and significantly reduced photosynthetic rate (Hattab et al., 2009).

The effect of trace metals depends not only on the chemical properties, concentration, and density of the metal but also on the type of plant (Viehweger, 2014.). The toxic effect of Cu on biomass depends on plant species, for example, wheat (An, 2006) and sorghum (*Sorghum bicolor* L.) (Kasim, 2006) were more sensitive to Cu stress compared to maize plants and showed a decreasing trend with increasing Cu excess. Oilseed rape growth was more inhibited than mustard under Cu stress (Feigl et al., 2013). For soybeans, a significant decrease in biomass was caused by a concentration of >100 ppm Cu, while for chickpeas, a concentration of >60 ppm Cu was sufficient (Adhikari et al., 2012). Plants that accumulate metals or metalloids in their tissues hundreds or thousands of times higher than usual for most plants are hyperaccumulators. Plants can be metal scavengers, metal indicators, and metal hyperaccumulators according to their sensitivity to heavy metals (Pajuelo et al., 2011). Plants of the *Brassicaceae* family are characterized by hyperaccumulation of almost all heavy metals (Pandey and Bajpai, 2019). Due to Rhizobium bacteria, the Legumes family is characterized by hyperaccumulation of As, Cd, Cu, Zn, and Pb heavy metals in roots (Pajuelo et al., 2011). Such plants are adapted to survive in heavy metal environments. They have well-developed metal detoxification mechanisms involving mycorrhiza, cell exudates, plasma membrane, heat shock proteins, phytochelatins (PCs), metallothioneins (MTs), organic acids and amino acids (Singh et al., 2016). Extracellular plants play the role of mycorrhizae and extracellular exudates in the plasma membrane, falling by absorbing heavy metals or causing metal ion resorption. On the

other hand, intracellular heat shock proteins, MTs, organic acids, amino acids, and PCs also play essential roles in the tolerance of different heavy metals (Kumar et al., 2016).

1.5 Agronomic measures for stress mitigation in crops

Agronomic measures used to mitigate the effects of drought and heavy metals on crops range from cultivar selection and sowing time to tillage, cropping systems, fallowing, mulching, use of soil inoculants, irrigation, and nutrient management (Nguyen et al., 2018).

Selecting suitable crops and cultivars is perhaps the most important factor in soil suitability (Nguyen et al., 2018). Not all plants are suitable for growing in sand or sandy clay because water and elements do not stagnate. Besides, not all plants can grow in clay, where in wet conditions, water stagnates, and in drought conditions, it dries out, hardens, reduces the mobility of trace elements, and makes it difficult for roots to grow. The selection of crops and varieties for drought and heavy metal resistance should be based on the tolerance level (Reinhardt et al., 2021). It is crucial to consider the vegetation period - when the crop or variety matures under favorable conditions for survival. For example, under drought conditions, it is better to choose early-maturing crop varieties to grow before the drought stress peak (Rosenow et al., 1983; Shavrukov et al., 2017). It is also recommended to choose varieties with short stems and a small leaf surface area to help reduce transpiration in the event of stress. It would be helpful to select varieties with deep and wide root systems to increase water availability in soil (Wasson et al., 2012). If the soil is contaminated with heavy metals, it would be better to choose varieties that hyper-accumulate those metals (Varsha et al., 2010). As a new tool, geovisualization application was developed to help farmers choose the suitable variety for cultivation (Peter et al., 2020). Maps can show the most convenient places to grow certain varieties or, according to your location and parameters such as rainfall, seasonality, and geographical limit, suggest which variety would be the most suitable to grow in such conditions.

Another critical factor is the choice of sowing time. If the climate tends to be dry, the plants should be sown as early as possible so that they have time to develop deeper roots that will increase the availability of water, as well as the plants, have time to flower and produce crops before the drought sets in (Nguyen et al., 2018). In addition, it is essential to consider the temperature of the soil for sowing. For example, when sowing wheat, gram, peas, and turnips, the soil temperature may be lower (Singh and Dhaliwal, 1972) so they can be sown in early spring, while maize is better suited to warmer soil, so it is suitable for later sowing (Bollero et al., 1996). When planted at the right time, plants will grow healthier and stronger, making them more resistant to, or even avoiding, stressful conditions.

Tillage practices also affect soil hydraulic properties, such as hydraulic conductivity, which determines soil moisture retention (Azooz et al., 1996). Reducing tillage reduces the soil's total porosity and macropore volume but increases water accumulation in delicate pores. Smaller diameter pores (<7.5 μm) are classified as efficient pores that retain water in the soil and make it available to plants. However, pores with a larger diameter (>150 μm) are efficient pores where gravity allows water to drain freely and not stagnate in water (Bhattacharyya et al., 2006). According to this, a tillage method should be chosen that leads to the formation of smaller pores.

Covering the soil surface with an organic or inorganic protective layer (mulching) reduces water evaporation by up to 28% and retains 8-22% more moisture in the soil, thus supporting crop growth in unfavorable conditions associated with water scarcity (Rana et al., 2016; Nguyen et al., 2018; Sekara et al., 2019). However, a large amount of mulch is required to cover cultivated areas, which complicates the use of this tool.

Watering plants is inefficient because about 50% of the water used during irrigation is wasted (Nguyen et al., 2018). Optimizing water use is especially important when growing plants in dry environments (Hsiao et al., 2007). The most effective way is to irrigate plants at night, thereby reducing the amount of water that evaporates. Another way to save water is to modernize irrigation systems to a precise type and irrigate crops only during critical stages of growth.

Crop rotation is recognized to reduce the impact of climate change on soils and yields (Bowles et al., 2020). Crop rotation improves yields, restores soil health, and breaks weed and pathogen cycles. In addition, crop rotation increases crop resistance to drought and other stresses through improved soil properties, increased soil water capture and storage, and an abundance of beneficial soil microbes. It is important to note that leguminous plants are useful in crop rotation (Fischer et al., 2002). This is determined by the Rhizobium bacteria in their roots, which fix atmospheric nitrogen and convert it into a form available to plants, thus enriching the soil.

Crop treatment with arbuscular mycorrhizal fungi and rhizobacteria helps plants to resist various stresses. Arbuscular mycorrhizal fungi integrate diverse mechanisms of cumulative stress response tolerance in plants by regulating the activity of antioxidant enzymes, osmolytes, and phytohormone synthesis (Hashem et al., 2018). Plant growth-promoting rhizobacteria contribute to plant stress tolerance mechanisms include phosphorus solubilization, siderophore production, nitrogen fixation, organic acid, and 1-aminocyclopropane-1-carboxylic acid deaminase, chitinase, and glucanase production (Al-Garni, 2006; Egamberdievaet al., 2017; Saghafi et al., 2018).

Plant growth regulators or phytohormones such as auxin, gibberellin, abscisic acid, jasmonic acid, and salicylic acid are said to be strongly associated with plant resistance to drought, heavy metals, heat waves, flooding stresses. Plants treated with phytohormones

increase water potential and chlorophyll content, stimulate antioxidant activity, regulate oxidative stress, and reduce transpiration, thereby increasing stress tolerance. (Egamberdieva et al., 2017; Nguyen et al., 2018).

Targeted nutrient management at macro and micro levels can improve plant resistance to stress and maintain or even increase crop yields. The most critical macronutrients are phosphorus (P), calcium (Ca), and potassium (K). At the same time, the essential micronutrients are selenium (Se), silicon (Si), copper (Cu), zinc (Zn), iron (Fe), molybdenum (Mo), and boron (B). Application of P and K to drought-stressed plants increased root growth, stomatal conductance, photosynthesis, membrane stability, and leaf water potential. Additional K and Ca use in plants improves water uptake, which helps regulate stomatal permeability, osmosis, and high tissue water potential and increases resistance to temperature stress (Waraich et al., 2012). Micronutrients such as B, Mn, and Se activate plants' physiological, biochemical, and metabolic processes, thereby increasing plant stress resistance. Using Se and salicylic acid increases the activity of enzymatic antioxidants and reduces the effect of ROS on membranes. Se increases the water uptake capacity of roots under drought conditions, and the addition of Si to drought-stressed plants increased relative water content by increasing proline and glycine betaine content. Applying Si alone or in combination with K increased dry matter yield in chickpea plants exposed to drought (Ahlawat et al., 2007). The exogenous application of Si has been reported to reduce drought stress in wheat, rice, pea, and chickpea. To reduce the effects of drought, the external use of N, K, Mg, B, Zn, and Si elements can reduce the production of ROS and increase the activity of enzymatic antioxidants SOD, CAT, and PX in plant cells (Waraich et al., 2011). This results in reduced photooxidation, maintenance of chloroplast membrane integrity, and increased or maintained photosynthetic rates in crop plants, improving water use efficiency. Nutrients P, K, Mg, and Zn can improve root growth, it increases water availability, which helps regulate stomata, increases the rate of photosynthesis, and increases water use efficiency (Fageria, 2001). Applications of K, Ca, and chloride (Cl) help regulate osmosis in plant cells during stress. Using Zn and Fe increases the relative amount of water and positively affects the accumulation of proteins and trace elements in plants. B improves nodule number and mass in soybean and pea grown under drought conditions when applied through the leaves. Besides, it is important to mention another form of nutrient management related to using nano-sized elements in agronomy. The application of nanoparticles in agronomy is a relatively new field, so the following chapter is devoted to an overview of their effects and applications.

1.6 Characterization and application of nanoparticles in agriculture

Nanotechnology is an advanced field of science, the use of which prevails from standard household chemicals and cosmetics to precision agriculture, the development of medicines and medical devices, and their use in space technology for effective shielding and energy storage. According to the International Organization for Standardization, nanotechnology is the knowledge and control of 1-100 nm nanoscale objects and organisms (ISO/TC 229). NPs stand out from other bulk materials because they are composed of three layers (Khan et al., 2019; Ijaz et al., 2020). A surface layer is functionalized with metal ions, polymers, and surfactants. The shell layer is usually chemically different from the core material (Kang et al., 2018). The core is essentially the central part of the NP and usually refers to the NP itself. Nanoparticles can be divided into six groups: carbon-based NPs, metal and metal oxide NPs, ceramics NPs, semiconductor NPs, polymeric NPs, and lipid-based NPs.

The properties of nanomaterials differ not only from their bulk counterparts but also from different nanoforms of the same chemical material (Schwirn et al., 2014). Therefore, different synthesis methods can create other morphological properties, sizes, shapes, and surface areas of NPs from the same chemical substance. These parameters can affect the reactivity and photo-catalytic activity of the particles, which can lead to different cell penetration abilities, effects, and toxicity/benefits levels (Ridolfo et al., 2020).

Nanotechnology has been specifically applied in agricultural applications as nano -pesticides, -herbicides, -insecticides, -fungicides, -fertilizers. Specific nanoparticles (NPs) or their derivatives can be applied to increase plant productivity and protect plants from weeds, insects, and pathogens without harming soil and water (Prasad et al. 2017). The properties of NPs differ from other micro-macro elements due to size manipulation (Guo et al., 2013). NPs can be synthesized in two ways. Bottom-Up method, during which NPs are formed from relatively more straightforward materials using template support synthesis, plasma/flame laser pyrolysis, spraying synthesis, atomic or molecular condensation, and spinning (Khan et al., 2019; Wang and Xia, 2004). This method includes the biological synthesis of NPs by bacteria, yeast, fungi, plants, and algae, otherwise known as green synthesis (Ijaz et al., 2020). Top-down synthesis methods are characterized by destructiveness, starting from a larger molecule, which is decomposed into smaller units and converted into NPs. This method synthesizes NPs using mechanical melting, sputtering, laser ablation, chemical etching, and electro-explosion (Khan et al., 2019; Ijaz et al., 2020). Studying the effects of various nano-derivatives on nature is essential to avoid pollution and their possible toxic effects (Prasad et al., 2017; Pestovsky and Martínez-Antonio, 2017; Singh et al., 2021).

1.6.1 Routes of entry and movement of nanoparticles in plants

The chemical and morphological properties of NPs affect their absorption and accumulation in plant tissues. NPs can enter the plant through the roots (irrigation application) and the leaves (foliar application). In the soil, NPs can interact by directly diffusing through the root surface or be transported to the plant with the help of specific protein carriers or through microorganisms and specific surface compounds that can facilitate or hinder their absorption. Notably, the effect of NPs depends on soil type, granulometric composition, and pH (Simonin et al., 2015; Ben-Moshe et al., 2013). For NPs to reach the xylem, they must overcome the ectoderm, cortex, endoderm, and Casparian strip (Schwab et al., 2016). NPs in plants often accumulate near the root tips, especially the root cap and root hairs (Geisler-Lee et al., 2012; Sun et al., 2020). Accumulation of NPs on the root surface (mucilage) may coincide with reduced root–shoot translocation, which is often associated with the positive surface charge of NPs (Sun et al., 2020). Positively charged NPs were more likely to accumulate on the negatively charged mucilage than negatively charged NPs. Negatively charged NPs exhibit higher translocation rates due to less association with the negatively charged mucilage (Spielman-Sun et al., 2019).

The interaction of pathogenic organisms and symbiotic bacteria with roots begins in the region of cell wall elongation, where they strongly interact with plant mucus and exudates. Microorganisms living with plants create a symbiotic microenvironment, biomineralized products, and soil aggregates. The symbiotic microenvironment can affect the mobility and uptake of metal and their oxide NPs in plants (Schwab et al., 2016; Tian et al., 2019). Rhizobia, symbionts of legumes (Fabaceae) are N-fixing bacteria (*Rhizobium leguminosarum*). They create holes in the cell walls of the root hair to induce the formation of bacteria-containing nodules. These nodules help legumes survive the effects of heavy metal accumulation. For soybean and chickpea symbiotic bacteria *Bradyrhizobium japonicum* iron (Fe_3O_4) and molybdenum (Mo) NPs suspensions have a positive impact associated with stimulation of plant nodule formation, increase in their size and weight (Ghalamboran, 2011; Taran et al., 2014).

Widespread mycorrhizal fungi can form symbiotic relationships with higher plants and increase the effective surface area of plant roots up to 10 times. It also improves plant water and nutrient uptake, which may affect NPs uptake (Tian et al., 2019). In addition, mycorrhizae can protect roots from heavy metal stress due to increased glutathione levels in the hyphae. Fungi form symbiotic spores through mantles around the root tip and elongation zone, penetrating the apoplast or through arbuscular punctate penetration into cell walls (Ban et al., 2021). Overall, scientific studies with a negative effect on symbiotic bacteria have been found, but it should be

noted that the impact mostly depends on the concentration and particle sizes, so more extensive studies on this topic are needed.

After NPs enter the plant through the roots in the ways mentioned above, they can move further through the ectoderm, cortex, and endoderm to the Caspariant strip. The Casparian strip is a layer of interstitial cell walls between endodermal cells sealed by lipophilic hydrocarbons such as lignin and suberin. It forms a mostly water-impermeable barrier between the cortex and the blood vessels in the apoplast. The Casparian strip is the last barrier for apoplastically traveling NPs before entering the vasculature (Lv et al., 2019; Schwab et al., 2015). Such travel around cells can take place through intracellular spaces and longitudinal channels in cell walls. NPs with properties unsuitable for translocation through the Casparian strip accumulate near it (Schwab et al., 2015). However, most NPs in plants travel symplastically directly through cells. In symplastic transport, NPs must cross the cell membrane via fluid-phase endocytic or non-endocytic pathways and move from cell to cell via plasmodesmata, sieve plates or primary pit fields (Su et al., 2019).

The rate of transpiration in plants correlates with the absorption rate of NPs. This means that if plants evaporate less water or slowly, NPs accumulation is small or occurs more slowly (Dietz and Herth, 2011). Transpiration is proportional to the amount of water entering the plant, which is often determined by the plant's leaf area. Thus, some NPs accumulate in the roots, while others move through the plant's xylem along with water and mineral substances to the plant's leaves (Su et al., 2019). NPs in leaves can both positively and negatively affect stomatal rotation, photosynthetic rate, and chlorophyll formation, induce oxidative stress and hormesis or activate antioxidant activity (Dietz and Herth, 2011).

Using the foliar spray method, NPs can form a protective surface on the leaf or enter the plant through the waxy layer and cuticle (Schwab et al., 2015; Su et al., 2019; Lv et al., 2019; Spielman-Sun et al., 2019). Wax crystals of various sizes and shapes are formed on the surface of the cuticle of plants, which increases the roughness and resistance of the leaf surface. The molecular permeability of waxes and cuticles increases as the lipophilicity and diffusion coefficient of the substance increase. The permeability can be increased by using surfactants that dissolve epicuticular waxes. Lipophilic molecules accumulate in cuticles above anticlinal cell walls and then move along pectin through the apoplast (Schwab et al., 2015).

Considering the resistance of the cuticle, it is likely that NPs do not cross it directly but enter the plant through the thinned and more permeable areas of the cuticle. Such areas on leaves can include trichomes and hydathodes. Hydathodes are small openings at the top of the leaf that allow the cuticle to absorb water and other substances (Banerjeeet al., 2019; Jauneau et al., 2020). Trichomes, unicellular and multicellular formations in the epidermis, are found on most plant leaf surfaces (Levin, 1973; Hülskamp, 2004). Their main function is protective, but they also have secretory properties that make them permeable to polar substances. Stomata are most abundant on the abaxial side of the leaf. The permeability of the stomata to water and carbon dioxide is regulated by guard cells. Guard cells use osmotic pressure to open the stomata during the day and close them at night. Their size can vary from 18 to more than 300 μm^2 depending on the plant type and growing conditions, and they are more permeable to polar substances (Eckerson, 1908). For these reasons, these are potentially permeable sites for NPs deposition and uptake after foliar application. Also, wounds found in plants, a pathway for many plant pathogens (Savatin et al., 2014), can facilitate the penetration of NPs directly into the plant cytoplasm. It has been described that NPs ranging in size from 4-10 nm can cross the epidermis by disrupting the wax layer, and fluorescently labeled 50 nm NPs can accumulate in the epidermis. Also, the charge of the particles is significant for the effect, as the negatively charged plant cell walls act as an ion exchange surface, which favors the penetration of cationic NPs rather than anionic NPs (Nadmint et al., 2013).

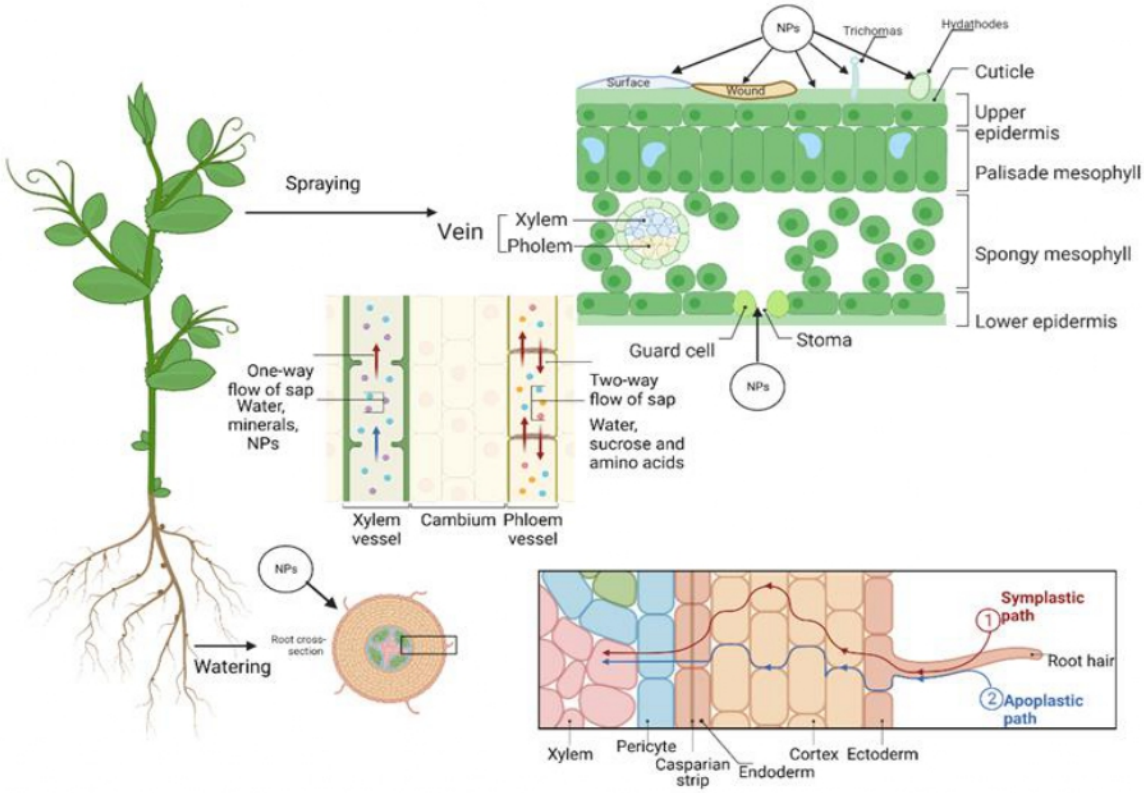


Figure 1.6.1. Routes of entry and movement of nanoparticles in plants

1.6.2 Application of nanoparticles in stress mitigation

The application of nanoparticles in agronomy to reduce the effects of stresses needs to be carefully studied. NPs can have both positive and negative impacts, unlike bulk elements. As mentioned earlier, the elements P, Ca, K, Se, Si, Cu, Zn, Fe, Mo, and B are essential for plant resistance to stressors. However, NPs synthesized from these elements may have different properties.

K NPs on pea plants have not yet been studied, especially the combination of their effects with stresses. However, a positive impact of K_2O NPs on plants growing in sandy soil was observed, as MDA and ion leakage decreased, Chl content increased significantly in leaves, and the use of these NPs can promote K^+ uptake, modulate Na^+ levels and reduce cell wall damage (Lo'ay et al., 2021). It was also reported that applying K NPs-based mulch increased the average grain yield and yield quality of maize grown under normal substrate moisture (Kandil et al., 2020).

One of the NPs with a strong effect is calcium phosphate (CaP) NPs. They were tested using plants exposed to drought, salinity, and heavy metals (Nasrallah et al., 2022). CaP NPs enhance cellular defense mechanisms, especially enzymatic antioxidants CAT, POX, and SOD, increase the total amount of phenolic compounds and increase the accumulation of soluble sugars, resulting in a reduction of oxidative stress biomarkers. Another critical compound containing the crucial elements Fe and P is $FePO_4$ NPs. After treating cucumbers and corn grown in hydroponics with $FePO_4$ NPs, an increase in the amount of P and Fe, as well as an effect on the absorption of manganese and zinc, as well as lengthening of the roots, which led to a lower shoot-to-root ratio, were found (Sega et al., 2020). However, these NPs have not yet been studied concerning stress.

Exogenous treatment of plants with CaO NPs significantly reduces Cd and As-induced growth inhibition in barley. CaO NPs stimulate the absorption of nutrients and increase the antioxidant defense capacity by stimulating the activity of enzymatic antioxidants by stimulating the expression of the genes encoding antioxidant enzymes (Nazir et al., 2022[a]; Nazir et al., 2022[b]).

The positive effects of silica (SiO_2) NPs have been found against the impact of heavy metals such as mercury (Li et al., 2020), cadmium (Ali et al., 2019), and chromium (Tripathi et al., 2015) on plants. It reduced the accumulation of these metals in plants, increased antioxidant activity, and reduced oxidative stress. SiO_2 NPs showed a positive effect against strains related to water deficit (Behboudi et al., 2018; Zahedi et al., 2020; Sutulienė et al., 2021) and salinity (Kalteh et al., 2018). NPs maintained or increased plant biomass, productivity, Chls, carotenoids, and polyphenols and reduced H_2O_2 and MDA levels in plants. It also had a positive effect on the activity of plant enzymatic antioxidants such as CAT, SOD, and APX and positively affected the

water content of plants. It was also found that SiO_2 effectively protects plants from cold stress by maintaining photosynthetic activity and increasing the chlorophylls and carotenoids content (Elsheery et al.,2020). Depending on the discussed publications, the sizes of the SiO_2 particles usually vary from 5-30 nm and effective concentrations from 25-1000 ppm.

Zinc (ZnO) NPs can increase proline content and activate SOD, CAT, and APX enzyme activities, promote photosynthetic pigment synthesis, correct osmoregulation, and reduce MDA and Na levels in plants that have been affected by water deficit stress (Abdel Latef et al., 2017; Faizan et al., 2021; Semida et al., 2021; Sun et al., 2021). ZnO NPs can also protect the plant from the effects of Pb, Cd, and Hg heavy metals (Venkatachalam et al., 2017; Hussain et al.,2018; Raghib et al., 2020). Using ZnO NPs in plants exposed to heavy metals can significantly increase plant growth rate, biomass, photosynthetic pigment, and protein content, inducing the overexpression of antioxidant enzymes and initiating desirable genetic changes. In addition, ZnO also has positive effects against cold stress (Elsheery et al., 2020; Song et al., 2021), as it can restore Chl accumulation and significantly reduce chilling-induced oxidative stress by reducing H_2O_2, MDA, proline and increasing the activities of key antioxidant enzymes SOD, CAT, and POD. It should be noted that ZnO NPs can be toxic to plants. This can depend on the concentration and particle size used, the type of plant, and even the route of exposure (García-Gómez et al., 2017).

Iron (Fe) NPs are commonly used as Fe_2O_3 and FeO, and they positively affect plant resistance to drought, salinity, and heavy metals such as cadmium (Rizwan et al., 2019; Adreeset al., 2020; Manzoor et al., 2021). The use of Fe NPs in plants can increase the content of Chl and affect the accumulation of important macroelements such as N, P, K. In addition, the increasing concentration of Fe NPs decreases the concentration of electrolyte leakage, MDA and H_2O_2 in plant leaves, but increases the content of phenolic compounds and activates SOD and POD. Also, the accumulation of Fe is significantly increased by using NPs instead of the bulk structure so that it can be effectively applied in biofortification.

Copper (CuO) NPs are often toxic to plants growing under normal conditions (Tang et al., 2016; Dai et al., 2018). CuO NPs can significantly reduce the activity of complexes I and III in the mitochondrial electron transport chain, block electron transfer from NADH to ubiquinone and from ubisemiquinone to ubiquinol, and cause oxidative stress (Dai et al., 2018). However, when CuO NPs of appropriate size and concentration are applied to plants stressed by salinity and drought, can eliminate adverse effects. For example, in wheat affected by salinity stress, using CuO-NPs in different ways can compensate for the harmful effects of salinity on DNA damage and cytosine methylation (Hosseinpour et al., 2021). Using Cu NPs maintained leaf water status as well as Chl and carotenoid content under drought conditions and increased the

activity of ROS scavenging enzymes and antioxidants in maize (Van Nguyen et al., 2022). To the lack of publications, Cu NPs on plants under stress have not yet been widely studied. There is a lack of data on their effects on heavy metal stress and on plants with symbiotic relationships with bacteria, which may lead to different results.

The use of selenium (Se) NPs on drought-affected plants positively affects photosynthesis, increases metabolite accumulation, activates antioxidants, and maintains the osmotic state of cells, thus increasing growth and plant biomass (Zahedi et al., 2021). Treatment with Se NPs reduced the adverse effects of cold on sugarcane, maintained the photochemical efficiency of PSII, and increased the content of Chl and carotenoids. In addition, foliar application with Se/SiO_2 increases drought resistance in strawberries by increasing the activity of antioxidant enzymes CAT, APX, GPX, and SOD and reducing MDA and H_2O_2 levels (Zahedi et al., 2020). Treatment with Se NPs reduced the harmful effects of cold on sugarcane, maintained the photochemical efficiency of PSII, and increased the content of Chl and carotenoids (Elsheery et al., 2020). Applying chitosan-coated (Cs) Se NPs by foliar spray increased plant growth and yield by increasing the expression of photosynthetic pigments in leaves and increasing overall photosynthetic capacity in salinity-stressed plants (Sheikhalipour et al., 2021). Application of Cs–Se NPs also increased enzymatic antioxidant activity and decreased H_2O_2 content in leaves, thereby reducing oxidative damage under salinity stress conditions. Cs-Se NPs increased the expression of phenolics and flavonoids, ascorbate, and anthocyanins.

Plants need molybdenum (Mo) in small quantities, but it is crucial for symbiotic bacteria, which can improve plant's properties by acting synergistically with the plant. For example, green synthesized Mo NPs and *Bacillus* sp. were used to reduce As's harmful effects on wheat (Ahmed et al., 2022). Mo NPs induced the phyto-beneficial traits of strain ZH16, such as IAA synthesis, phosphate solubilization, and ACC deaminase activity under As stress. The combined use of bacterial strain ZH16 and Mo NPs reduced As accumulation in wheat, maintained their growth parameters, and improved nutrient accumulation. In addition, Mo NPs positively affected chickpeas and their symbiotic bacteria by promoting nodulation and increasing their number (Taran et al., 2014). Other studies have shown that Mo NPs can regulate nitrate accumulation and nitrate reductase activity in spinach (Abbasifar et al., 2020), and affect the antioxidant system in rice (Sharma et al., 2021). Still, there are no studies on their effects on water deficit stress.

Boron (B) NPs have only been studied in plants grown under normal conditions. Plants treated with B NPs showed higher Chl content, higher MDA and H_2O_2 concentrations, and more active SOD and CAT enzymes (Daglioglu et al., 20.) Besides, B NPs can also influence shoot height, flower number, and B, N, and K accumulation in soybeans (Dimkpa et al. 2017).

2. RESEARCH OBJECT, CONDITIONS, AND METHODS

Object of research

The research object is the green pea (*Pisum sativum* L.) plants. Peas were chosen as model plants because of their susceptibility to drought and their significant role in crop rotation. In addition, due to their ability to fix atmospheric nitrogen in a symbiotic association with *Rhizobium* bacteria and fulfill the nitrogen demand of the subsequent crops, they can be used for both human food and animal feed. Green pea (*Pisum sativum* L.) cultivar 'Respect' (Maribo Seed International ApS, Denmark) was used in all experiments. It is a medium-early semi-leafless pea variety.

Preparation of nanoparticles suspension

Silica (SiO_2) NPs (particle size: 20-30nm; purity: 99%), copper oxide (CuO) NPs (particle size: 25-55 nm; purity: 99.95%), molybdenum trioxide (MoO_3) NPs (particle size: 35-45 nm; purity: 95%), boron trioxide (B_2O_3) NPs (particle size: 100nm; purity: 99.9%) were used in the experiments (US Research Nanomaterials, Inc, Houston, TX USA). The NPs with concentrations of 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm were suspended in deionized water and ultrasonically dispersed for 60 min. The NPs size and suspension stability were measured using Delsa™ Nano Submicron Particle Size (Beckman Coulter Instruments. Corporation, Fullerton, California) and Zeta Potential device (Dispersion Technology Inc., Bedford Hills, New York).

The obtained data showed that all prepared NPs suspensions were strongly anionic and stable, also monodispersed according to the polydispersity index (PDI) (Table 1). The pH value of all suspensions was equalized to 7.

Table 2.2.1. Properties of SiO_2, B_2O_3, CuO, MoO_3 NPs suspension in DI water: zeta potential, results represent the mean ± standard error, polydispersity index, and percentage of nanoparticles between 1 – 100 nm

	SiO_2 NPs	**B_2O_3 NPs**	**CuO NPs**	**MoO_3 NPs**
Zeta potential (ζ; mV)	-20.64 ± 0,333	-28.54±0.223	-26.68±0.631	-24.92±0.314
The polydispersity index (PI)	0.34	0.237	0.245	0.218
NPs size 1-100 nm in suspension, %	70%	43%	54%	68%
pH value	6.6	8.4	8.6	7.8

Experimental design

A visually summarized methodology is presented in Figure 2.3. Before sowing, green pea seeds were sterilized in 5% sodium hypochlorite solution for 15 min to ensure surface sterility (Lehotai et al., 2011) and rinsed gently with deionized water several times. Then, the seeds were soaked in water for 24 h.

The following experiments were performed:

The initial (primary) experiment was performed to determine the appropriate concentrations of SiO_2, B_2O_3, CuO, and MoO_3 NPs for pea plants. The research was carried out in a walk-in controlled environment growth chamber (4 × 6 m, h = 3.2 m) at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. The three seeds were sown in each 500 mL plastic pot which was treated as a single biological replication, and 16 pots were used for each treatment. Plants were seeded and grown in peat substrate, pH 6 (Profi 1, JSC Durpeta, Lithuania) for 30 days. The substrate's average amounts of nutrients (mg L^{-1}) were as follows: N, 110; P_2O_5, 50; K_2O, 160. The average amounts of microelements (mg L^{-1}) Fe (4,5), Mn (0,5), Cu (0,1), B (0,02), Mo (0,03), and Zn (0,04), were also present. Electrical conductivity (EC) varied between 2.0 and 2.5 mS cm^{-1} (± 0.03 mS cm^{-1}). The growth conditions were as follows: 16 h photoperiod, 20 ± 2/16 ± 2 °C day/night ambient air temperature, 60 % relative air humidity, ~220 µmol m^{-2} s^{-1} photon flux density of photosynthetically active radiation (PAR) provided by high-pressure sodium (HPS) lamp (SON-T Agro, 400 W; Philips, Somerset, NJ, USA). At the 14 BBCH growth stage, when plants unfolded their 3-4 true leaf or had 3-4 tendrils developed (14 days after sowing) (Meier, 2018), peas were watered with 100 mL of SiO_2, CuO, Mo, or B NPs suspension each containing 0.0125; 0.025; 0.05; 0.1 ppm concentrations. After that, all plants were watered daily with deionized water, and no additional fertilizer or nutrient solution was added. Randomization and regular spatial rearrangement of 10 pots per treatment were applied to minimize the potential effect of the growth chamber on plant response. Others were left unrotated to avoid border effects. At the end of the experiment, when plants reached the 31 BBCH growth stage – the beginning of stem elongation (Meier, 2018) the peas were harvested for biometric, non-destructive measurements of leaf chlorophyll and nitrogen balance (NBI) indexes and biochemical analyses. After a thorough examination and analysis of the affected plants, three concentrations of each NPs were selected for use in further experiments. These experiments aimed to select the most effective concentrations of different NPs against the effects of drought on pea plants.

The following experiments were performed over a two-year spring-summer period (2019-2020) in two greenhouses (3 × 6 m, h = 2 m) at the Lithuanian Research Centre for Agriculture

and Forestry, Institute of Horticulture, Babtai, Lithuania (55°05'08.4"N 23°48'03.5"E, at an altitude of 51 m; moderate climate zone of the northern hemisphere). For experiments, pea seeds were prepared for sowing as described above. Ten seeds were sown in 10-L volume plastic pots (7 pots per treatment, arranged randomized), and filled with ~ 8 kg of soil mixture (volume of 7:1 soil to perlite ratio, respectively). The soil was heavy loam particle size distribution, pH 7.4 ± 0.1; concentration of humus – 3.6±0.1%; P_2O_5 - 243±8 mg kg^{-1}; K_2O - 348±37 mg kg^{-1}; NH_4 – 4 ±0.6 mg kg^{-1}; NO_3 – 22±0.9 mg kg^{-1}; SiO_2 – 39±0.8 mg kg^{-1}. Pea seedlings were thinned to 7 plants per pot five days after sowing. After 16 days of cultivation, the peas were fertilized with 7 g pot^{-1} ammonium nitrate. The peas were sprayed with fungicides because the green pea cultivar 'Respect' is more susceptible to powdery mildew, even when grown in a greenhouse. Pots were irrigated with water by graduated cylinder daily to 80% of substrate moisture (SM) using a substrate moisture sensor (Delta-T devices, HH2 moisture meter, Cambridge, United Kingdom) for 35 days. Plants were grown under a natural day-length photoperiod. The average day/night temperature was 22.2/14.4 °C; relative air humidity - 58/77 ± 5% before exposure; during the ten days of drought treatment, the average day/night temperature was 25.4/16.6 °C, and the relative air humidity was 53/75 ± 5%, data were measured throughout the experiment (Termio+ data logger, Poland) at the first year.

When the peas reached the 40 BBCH growth stage (Meier, 2018), they were watered (100±1 mL per pot) or foliar sprayed until full wetting (ca. 14±0.5 mL $plant^{-1}$) with solutions containing different concentrations of SiO_2, MoO_3, CuO and B_2O_3 NPs: 0 (watered or sprayed with water, NPs-untreated), 12.5 ppm, 25 ppm, and 50 ppm. After the application of NPs, the watering of one part of the pea plants was stopped, and drought stress was initiated (30% SM). In contrast, another part of the pea and control plants was irrigated with water to maintain regular soil moisture (80% SM) throughout the experiment. These regimes were applied for ten days until harvest. After each treatment, plants were harvested after reaching the BBCH 50 growth stage (Meier, 2018) to assess their morphophysiological responses.

In subsequent experiments, we sought to determine whether NPs suspensions could protect plants from the complex effects of agrometeorological drought and copper (Cu) as heavy metal.

The conditions of the following experiment: the average day/night temperature was 24.2/14.4 °C; relative air humidity - 54/75 ± 5% before exposure; during the ten days of drought treatment, the average day/night temperature was 26.2/17.0 °C, and the relative air humidity was 50/73 ± 5%. Soil preparation, pea sowing, and treatment with NPs were kept the same as described above. In addition, aqueous solution of 160 mg kg^{-1} $CuSO_4$ was added to half of the pots to cause Cu as heavy metal stress. When the peas reached the 40 BBCH growth stage

(Meier, 2018), they were watered (100±1 mL per pot) or foliar sprayed until full wetting (ca. 14±0.5 mL per plant) with solutions containing one concentration of each SiO_2 (50 ppm), CuO (50 ppm), MoO_3 (50 ppm), B_2O_3 (12.5 ppm) NPs selected from previous experiments. After the application of NPs, the watering of one part of the pea plants was stopped, and drought stress was initiated (30% SM). In contrast, another part of the pea and control plants was irrigated with water to maintain regular soil moisture (80% SM) throughout the experiment. These regimes were applied for ten days until harvest. After each treatment, plants were harvested after reaching the BBCH 50 growth stage (Meier, 2018) to assess their morphophysiological responses.

Morphological and non-destructive measurements

Ten plants per treatment were randomly selected (n = 10) for biometric measurements. First, the shoots were separated from the roots, and then shoot height, root length, fresh weight (FW), and dry weight (DW) were determined. FW and DW were measured with an electronic scale (Mettler Toledo AG64, Columbus, OH, USA). DW was determined following forced-air convection drying at 105ºC to a constant dry weight (VENTICELL 222, MBT, Czech Republic). After shoot FW determination, ten matured plants per treatment were floated on deionized water for 24h, and turgid weights (TW) were measured. Relative water content (RWC) was calculated using the following equation (Baris and Weatherley, 1962):

$$\text{RWC}, \% = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \text{x} 100 \quad (1)$$

The leaf area was measured using an automatic leaf area meter (AT Delta-T Devices, Wallingford, UK) expressed as $cm^2\ g^{-1}$. For the calculation of specific leaf area (SLA), the total plant leaf area (n=10) was divided by the shoot DW. The root/shoot ratio was determined as the ratio of root DW to aboveground DW.

Pods were collected from each pea plant, and the average number of heads/pods m^{-2} was counted (A, average number of pods m^{-2}). Then, the number of grains in the pods in each variant was calculated, and the average (B, average number of grains per pod) was derived. The weight of 100 grains (C, weight of 100 grains of peas) was calculated. The pea yield was calculated according to the following formula (Koiter and Bill Ashton, 2021):

$$\text{The pea yield (t ha}^{-1}) = (A \times B \times C) / 10{,}000 \quad (2)$$

Antioxidant properties and total phenolic compounds

Non-enzymatic antioxidant activity

Antioxidant properties of pea leaves were evaluated as the DPPH (2-diphenyl-1-picrylhydrazyl), ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) diammonium salt, radical scavenging activities, and Fe^{2+} reducing antioxidant power assay (FRAP). Also, the total contents of phenolic compounds were determined. Extracts were prepared by grinding 0,3 g of plant leaves with liquid nitrogen and diluting them with 5 mL of 80% methanol. After 24 h, the samples were centrifuged for 10 min at 3000 rpm (Hermle Z300K, Germany), extracts were filtered through cellulose filters, and the supernatant was used for further analyses. All biochemical analysis was performed in 3 biological replications. Each of the three biological replicates consisted of at least three conjugated plants and was repeated in three analytical replicates.

The total content of phenolic compounds was determined as gallic acid equivalents. 250 µL aliquot of the sample extract was mixed with 250 µL of 10% (w/v) Folin-Ciocalteu reagent, 500 µL of 1 M Na_2CO_3 solution, and 2 mL of distilled water (Ainsworth and Gillespie, 2007). After 20 min in the dark incubation, the absorbance was measured at 765 nm (M501, Spectronic Camspec Ltd., UK). The total phenolic compounds quantity mg g^{-1} was calculated from the calibration curve of the gallic acid (0.01 – 0.1 mg mL^{-1}, R^2=0,99).

The ABTS (2,2 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation was obtained by incubating the 7 mM ABTS stock solution (100 mL) with 2.45 mM potassium persulfate ($K_2S_2O_8$; final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use (Re et al., 1999). Thereafter, 50 µL of the prepared sample was mixed with 2 mL of ABTS solution (ABTS stock solution was diluted 1:7), and the absorbance was measured after 11 min (plateau phase) at 734 nm (M501, Spectronic Camspec Ltd., UK). The ABTS scavenging activity of pea leaves extracts was calculated as the difference between the initial absorbance and after reacting for 10 min. A calibration curve was determined using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; 97% purity; Sigma-Aldrich, USA) as an external standard with a range of concentrations from 0.1 to 0.8 mM (R^2 = 0.99). It was expressed as ABTS µmol scavenged per 1 g of fresh weight (µmol g^{-1} FW).

For DPPH (2-diphenyl-1-picrylhydrazyl) assay, a stable 126,8 µM DPPH (100% purity; Sigma-Aldrich, USA) solution was prepared in methanol (Sharma and Bhat, 2009). Subsequently, 1 mL of the DPPH solution was transferred to a test tube and mixed with 100 µL of the diluted pea extract with 400 µL methanol. The absorbance was scanned at 515 nm (M501, Spectronic Camspec Ltd., UK) while reacting for 16 min. The free radical scavenging capacity was expressed as µmol of DPPH radicals scavenged per 1 g of fresh weight (µmol g^{-1} FW). A calibration curve was determined using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; 97% purity; Sigma-Aldrich, USA) as an external standard with a range of concentrations from 0.1 to 0.6 mM (R^2 = 0.99).

The FRAP method is based on reducing ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The fresh working solution was prepared by mixing 300 mM, pH 3.6 acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \times 6H_2O$ at 10:1:1 (*v*/*v*/*v*) (Benzie and Strain, 1996). 20 µL of the sample was mixed with 3 mL of working solution and incubated in the dark for 30 min. Readings of the colored product (ferrous tripyridyl-triazine complex) were then taken at 593 nm. A calibration curve was determined using $Fe_2(SO_4)_3$ (Iron (III) sulfate; 97% purity; Sigma-Aldrich, USA) as an external standard with a range of concentrations from 0.005 to 0.5 mM (R^2 = 0.99). The antioxidant power is expressed as Fe^{2+} antioxidant capacity (Fe^{2+} µmol g^{-1} FW).

Enzymatic antioxidant activity

The extracts used to determine the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX) in pea leaves were prepared by grinding 0.5 g of fresh sample with liquid nitrogen and diluting within 5 mL extraction buffer (100 mM potassium-phosphate buffer, pH 7.8, containing 0.1 mM EDTA). After centrifugation for 10 min at 3000 rpm (Hermle Z300K, Germany), the supernatant was collected and used for the assays of enzymatic activities. All steps in the preparation of the enzyme extract were carried out at 4 °C.

For soluble protein determination, the dye-binding method and bovine serum albumin as standard were used to determine soluble proteins. 30µL of enzyme extract was mixed with 1,5 mL of Bradford reagent diluted by 1:5 with DI water. Absorbance was read after 2 min through a spectrophotometer (M501, Spectronic Camspec Ltd., UK) at 595 nm (Bradford, 1976).

Total SOD activity was estimated by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by the enzyme (Dhindsa et al., 1981). 3 mL of reaction mixture consisted of 13 mM methionine, 75 µM NBT, 100 mM potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 50 µL enzyme extract, and 13 µM riboflavin. The tubes were under 150 µmol m^{-2} s^{-1} for 1 min to initiate the reaction and then covered. The absorbance was recorded after 30 min by spectrophotometer (M501, Spectronic Camspec Ltd., UK) at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme expressed as unit mg^{-1} protein min^{-1}.

CAT activity was measured as the disappearance of H_2O_2 (Aebi, 1984). 100 µL enzyme extract was added in 1.275 mL of 0.1 M phosphate buffer (pH 7.8, containing 0.1 mM EDTA). The reaction started by adding 125 µL of 30 mM H_2O_2. The decrease in absorbance measured by spectrophotometer (M501, Spectronic Camspec Ltd., UK) at 240 nm was observed for 1 min,

and enzyme activity was computed by calculating the amount of H_2O_2 decomposed (μmol H_2O_2 mg^{-1} protein min^{-1}).

APX activity was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm (Nakano and Asada, 1981). The 1 mL assay mixture contained 0.1 M potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 0.5 mM ascorbic acid, 0.1 mL enzyme extract, and 0.1 mL of 30 mM H_2O_2 was added to initiate the reaction. The decrease in absorbance was measured spectrophotometrically (M501, Spectronic Camspec Ltd., UK) for 1 min. The extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate was used to calculate the enzyme activity that was expressed as μmol AsA mg^{-1} protein min^{-1}.

Measuring GR activity based on the decrease in the absorbance of oxidized glutathione (GSSG), at 340 nm (Sofo et al., 2005). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 1 mM GSSG, 100 μL enzyme extract and 75 μL 0.1 mM NADPH added last to initiate the reaction. The decrease in absorbance measured by spectrophotometer (M501, Spectronic Camspec Ltd., UK) was recorded every 5 min until 20 min. An absorption coefficient of 6.22 mM^{-1} cm^{-1} was used for calculations. GR activity was defined as μmol NADPH mg^{-1} protein min^{-1}.

GPX activity measurements are based on the increase in the absorbance of oxidized guaiacol at 470 nm (Kvaratskhelia et al., 1997). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 31 mM guaiacol, 100 μL enzyme extract, and 75 μL 3.6 mM H_2O_2 added last to initiate the reaction. The increase in absorbance measured by spectrophotometer (M501, Spectronic Camspec Ltd., UK) was recorded for 2 min. Therefore, GPX activity was expressed as μmol H_2O_2 mg^{-1} protein min^{-1}.

Oxidative stress biomarkers

The extracts used to determine the concentration of lipid peroxidation and hydrogen peroxide (H_2O_2) in pea leaves were prepared by grinding 0.1 g of fresh sample with liquid nitrogen and diluting with 4 mL of 0.1% TCA (trichloroacetic acid). After centrifugation for 10 min at 3000 rpm (Hermle Z300K, Germany), the supernatant was used for further analyses.

For H_2O_2 measurements in plant leaves, 500 μL of the supernatant was added to 1 mL of 1 M potassium iodide (KI). The absorbance of the mixture was scanned at 390 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., UK). A calibration curve was determined using H_2O_2 (30% hydrogen peroxide) as an external standard with a range of concentrations from 0,6 – 24,3 mM (R^2 = 0.99). The content of H_2O_2 is expressed in fresh weight (μmol g^{-1} FW) (Velikova et al., 2000).

The thiobarbituric acid (TBARS) test determines malondialdehyde (MDA) content in pea leaves samples as the end product of lipid peroxidation. 500 µL of the supernatant was added to 1 mL 0.5% (w/v) thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The mixture was incubated in boiling water for 30 min. The reaction stopped after the samples had cooled. The samples were centrifuged at 10 000×*g* for 5 min, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., UK). The value for non-specific absorbance at 600 nm was subtracted (Heath and Packer, 1968). The amount of MDA–TBA complex (red pigment) in leaves was calculated and expressed as nmol g^{-1} FW:

$$C_{MDA} = (A532 - A600) / E_{MDA} \quad (3)$$

C_{MDA}– concentration of MDA, µM

A532, A600 – Absorbance at wavelengths

E_{MDA} – MDA extinction coefficient 155 mM^{-1} cm^{-1}.

Determination of macro- and microelements

The macro-and microelements quantity in pea leaves, stems, and roots were determined using the microwave digestion technique combined with inductively coupled plasma optical emission spectrometry. Complete digestion of dry plant material (0.3 g) was achieved with 8 mL 65% HNO_3 using a microwave digestion system Multiwave GO (Anton Paar GmbH, Graz, Austria). The digestion program was as follows: (1) 170 °C reached within 3 min, digested for 10 min; (2) 180 °C reached within 10 min, digested for 10 min. Full-digested samples were diluted to 50 mL with deionized water. The elemental profile was analyzed by an ICP–OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments, Kleve, Germany). The operating conditions employed for ICP-OES determination were 1300 W RF power, 12 L min^{-1} plasma flow, 1 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, and 1 mL min^{-1} sample uptake rate. The analytical wavelengths chosen were P I 213.618 nm, K I 766.491 nm, S I 182.034 nm, Ca II 445.478 nm, Mg II 279.079 nm, Fe II 259.941 nm, Zn I 213.856 nm, Mn II 259.373 nm, Cu I 324.754 nm. The calibration standards were prepared by diluting a stock multi-elemental standard solution (1000 mg L^{-1}) in 6.5% (v/v) nitric acid and by diluting stock phosphorus and standard sulfur solutions (1000 mg L^{-1}) in deionized water. The calibration curves for all the studied elements were in the range of 0.01–400 mg L^{-1}. The contents of macro and microelements in the dry weight of pea are presented (Viršilė et al., 2020).

Bio-concentration factor, translocation factor, and tolerance index calculation

Bioaccumulation is the ability of plants to absorb elements and retain them. The efficiency of this process depends on environmental conditions, and the plant type most influences the

elements' retention. Due to the complexity of the methods, the bioconcentration factor (BCF) of specific elements was calculated to assess the environmental risk that may arise from the substances under investigation. Depending on the component, a high BCF value means a low element solubility in water and a high relative octanol-water partition coefficient besides a high soil adsorption coefficient.

Therefore, BCF was calculated as an index of the pea plants' ability to accumulate the heavy metal copper Cu. It was calculated as the ratio of the Cu concentration (mg kg^{-1}) in the soil and the Cu concentration (mg kg^{-1}) in the pea plant (Zacchini et al., 2009):

$$\mathrm{BCF} = \frac{C_{\mathrm{shoot}}}{C_{\mathrm{soil}}}. \quad (4)$$

The translocation factor (TF) was estimated to determine the relative translocation of specific elements from roots to shoots of pea plants (Zacchini et al., 2009). TF was calculated using the following formula:

$$\mathrm{Tf} = \frac{C_{\mathrm{shoot}}}{C_{\mathrm{root}}} \mathrm{x}100. \quad (5)$$

C_{shoot} and C_{root} correspond to element concentrations (mg kg^{-1}) in the pea shoot and roots for which TF was calculated.

The tolerance index (Ti) was calculated to evaluate the plant's ability to grow under the influence of the heavy metal Cu (Zacchini et al., 2009):

$$\mathrm{Ti} = \frac{\text{Dry weight of pea plants grown under copper stress}}{\text{Dry weight of pea plants grown under control conditions}} x100 . \quad (6)$$

Statistical Analysis

All the values were expressed as mean ± standard deviation. Data were analyzed using the Analysis of Variance (ANOVA) test followed by Tukey HSD at $p \leq 0.05$ to identify significant differences. Differences were analyzed between variants when peas were grown under normal conditions and separately between peas grown under drought conditions. The micro- and macro-elemental analysis results were also compared between control - peas grown in a drought not treated with NPs (SM 30%) and treated with NPs and between control peas grown under normal conditions (SM 80%) not treated with NPs and treated with NPs.

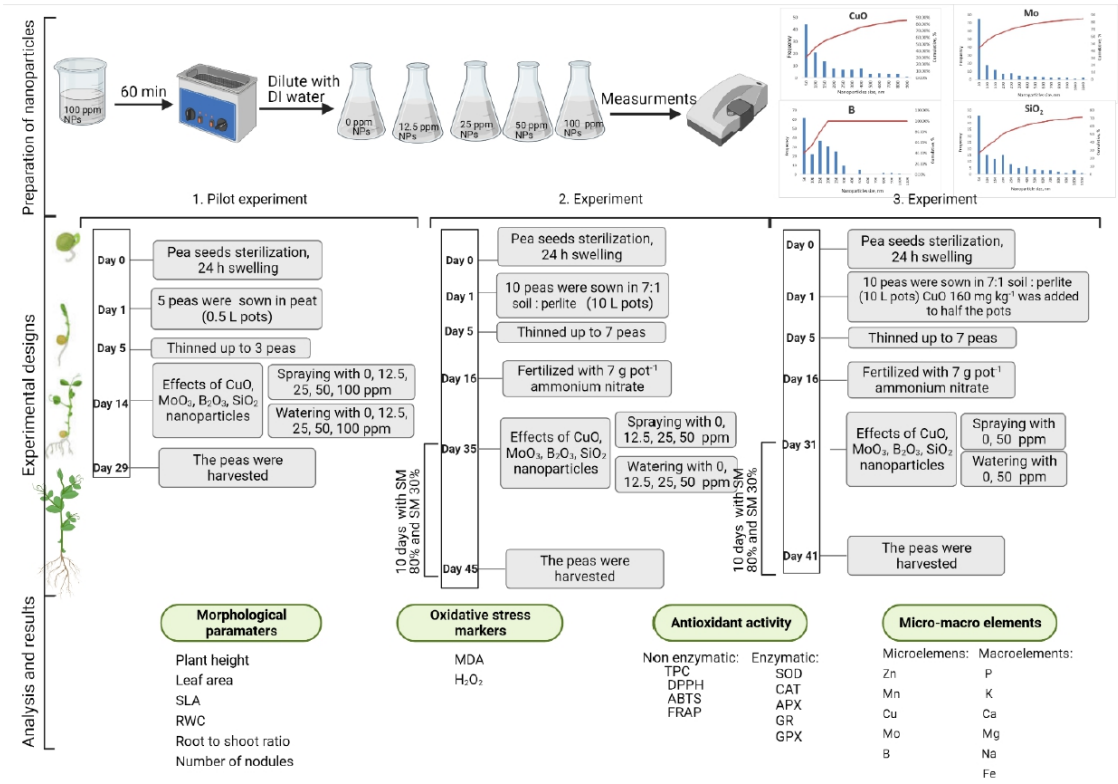


Figure 2.3.1. Methodology: preparation of nanoparticle suspensions, experiments design, analysis, and results

3. RESULTS

This section analyzes the effect of SiO_2, MoO_3, B_2O_3 and CuO nanoparticles (ND) on pea seedlings grown in optimal conditions (3.1), fully developed pea plants grown under agrometeorological drought (3.2) and complex copper and agrometeorological drought (3.3).

3.1 Effect of nanoparticles on the morphological parameters and antioxidant system in pea seedlings

The first experiment was conducted to determine how pea seedlings react to the effect of nanoparticles (NPs) of different concentrations when growing them under optimal conditions. This section analyses the effects of silica (SiO_2), copper oxide (CuO), molybdenum trioxide (MoO_3), and boron trioxide(B_2O_3) NPs on pea morphological parameters and antioxidant activity when the plants were grown in a closed climate-controlled chamber.

The results showed that using SiO_2 NPs increased peas' height by 28-45%, depending on the increasing concentration (Table 3.1.1). When peas were watered with the suspension of concentrations of 12.5, 25, 50, and 100 ppm, the average fresh weight increased by 81, 104, 116, and 109%, respectively. The dry mass increased from 62 to 135% when the concentration of SiO_2 NPs in the solution increased. Root elongation was increased by 76% after watering plants with the suspension of 100 ppm concentration, and with other solutions, it got longer, up to 106%. Root fresh and dry weight increased with higher concentrations of SiO_2 NPs. The study confirmed the positive effect of SiO_2 NPs on pea height, fresh and dry biomass, and root length at all concentrations in the solution (Table 3.1.1).

MoO_3 NPs and SiO_2 NPs had a more positive effect on peas with increasing concentration in the solution (Table 3.1.1). The higher concentration of MoO_3 NPs in the solution promoted an increase in the height of peas from 23 to 48%. In addition, an increase in shoot fresh up to 61% and dry up to 84% biomass were determined. As a result, root length was longer by 56 - 85%, and their biomass accumulated more intensively, both fresh 58 - 121% and dry 30 - 71% according to used concentrations of 12.5 – 100 ppm.

The lowest CuO NPs concentration in the solution had the most positive effect on the morphological parameters of the pea (Table 3.1.1). Pea height increased by 41, 32, and 16%, shoot fresh biomass increased by 87, 78, and 41%, and shoot dry biomass increased by 47, 50, and 35% using 12.5, 25, and 50 ppm suspensions, compared to NPs untreated plants. Root length was affected by all concentrations of CuO NPs, lengthening them by 85 - 108%. Increasing concentrations of CuO NPs in the solution caused a positive effect in fresh root biomass from 108% to 28%, but it should be emphasized that the increase in biomass slowed down with increasing concentration.

The effect of B_2O_3 NPs was closer to the development of CuO NPs - the decreasing concentration of B_2O_3 NPs had a more positive impact on the morphological parameters of peas (Table 3.1.1). Watering B_2O_3 NPs suspension at 12.5, 25, and 50 ppm of concentrations increased pea's height by 52, 21, and 2%, fresh biomass by 95, 60, and 14%, and dry biomass by 116, 35, and 52%, respectively. Root length was increased from 92 to 11%, and fresh biomass was accumulated more by 91 – 21% using the B_2O_3 NPs solutions.

Table 3.1.1. Impact of SiO_2, CuO, MoO_3, B_2O_3 NPs (12,5; 25; 50; 100 ppm) on P. sativum L. morphological parameters: plants shoot height, fresh and dry weight (FW, DW), root length, FW, and DW. 0 – control plants watered with deionized water. Mean values in columns with different letters are significantly different from the control at $p < 0.05$ (n=10) by Tukey (HSD) test

Nanoparticle concentration, ppm		Shoot			Root		
		Height, cm	FW, g	DW, g	Length, cm	FW, g	DW, g
H_2O	0	20.0 b	1.3 b	0.240 b	4.5 b	0.365 b	0.044 b
SiO_2	12.5	25.7 a	2.4 a	0.388 a	9.1 a	0.624 a	0.073 a
	25	28.2 a	2.7 a	0.465 a	9.2 a	0.782 a	0.082 a
	50	28.7 a	2.9 a	0.501 a	9.2 a	1.049 a	0.089 a
	100	28.8 a	2.8 a	0.564 a	7.8 a	1.166 a	0.105 a
CuO	12.5	28.1 a	2.5 a	0.353 a	9.1 a	0.763 a	0.069 a
	25	26.3 a	2.4 a	0.361 a	8.3 a	0.668 a	0.058 b
	50	23.3 a	1.9 a	0.323 a	8.4 a	0.501 a	0.055 b
	100	20.3 b	1.2 b	0.217 c	9.3 a	0.469 a	0.037 c
MoO_3	12.5	24.6 a	1.7 b	0.259 b	6.9 a	0.627 a	0.063 b
	25	24.7 a	1.5 b	0.304 a	7.4 a	0.577 a	0.058 b
	50	27.7 a	2.2 a	0.380 a	7.3 a	0.667 a	0.065 a
	100	29.6 a	2.5 a	0.388 a	8.3 a	0.809 a	0.076 a
B_2O_3	12.5	30.3 a	2.6 a	0.518 a	8.6 a	0.696 a	0.061 a
	25	24.3 a	2.1 a	0.323 a	7.3 a	0.598 a	0.053 b
	50	20.4 b	1.5 b	0.366 a	4.9 b	0.444 a	0.044 b
	100	19.3 c	1.4 b	0.270 b	5.5 a	0.341 b	0.043 b

FW – fresh weight; DW – dry weight

All applied NPs suspensions influenced the antioxidant response in pea leaves (Figure 1). Increasing concentration of SiO_2 NPs increased total phenolic compounds (TPC) content by 10 (12.5 ppm), 22 (25 ppm), 30 (50 ppm), and 32% (100 ppm) in pea leaves (Figure 1A). The same effect was observed in the 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging activities (Figure 1B, C). The activity of DPPH radical scavenging increased up to 35%, corresponding to the increasing concentration of SiO_2 NPs in the solution. ABTS increased by 12%, 24%, and 25% after applying the solution containing 25 ppm, 50 ppm, and 100 ppm SiO_2 NPs, respectively.

MoO_3 NPs effectively induce higher TPC content by 29, 31, 33, and 24% after exposure to suspensions containing 12.5, 25, 50, and 100 ppm NPs (Figure 3.1.1A). The DPPH and ABTS free radicals scavenging activities showed the same tendency. The DPPH free radical scavenging activity increased intensively at 12.5 ppm - 28%, 25 ppm - 29%, and 50 ppm - 34%, but after watering with 100 ppm, the activity increased less significantly - 18% (Figure 3.1.1B). The ABTS free radical scavenging activity was also affected by increasing MoO_3 NPs concentration by 24, 31, 36, and 23%, respectively (Figure 3.1.1C). In general, a MoO_3 concentration of 100 ppm was observed to have a weaker effect on TPC accumulation and antioxidant capacity in pea leaves than lower concentrations.

TPC content in pea leaves increased by 25, 19, 18, and 15% after exposure to 12.5, 25, 50, and 100 ppm CuO NPs (Figure 3.1.1A). Also, stimulation of DPPH free radical scavenging activity by 31, 28, 23, and 21%, and ABTS free radical scavenging activity by 21, 18, 17, and 15% after exposure to CuO NPs at 12.5, 25, 50, 100 ppm were determined (Figure 3.1.1B, C). The results showed that the effect on antioxidant capacity decreased with increasing concentration of CuO NPs in the suspension.

B_2O_3 NPs concentrations of 12.5, 25, 50, and 100 ppm strongly stimulated TPC accumulation in peas, increasing it by 23, 24, 28, and 22%, respectively (Figure 3.1.1A). On the other hand, the DPPH free radical scavenging activity was weakly affected (Figure 3.1.1B). At the same time, B_2O_3 NPs concentrations of 12.5, 25, 50, and 100 ppm increased the activity of ABTS free radical scavenging by 12, 20, 30, and 22%, respectively (Figure 3.1.1C).

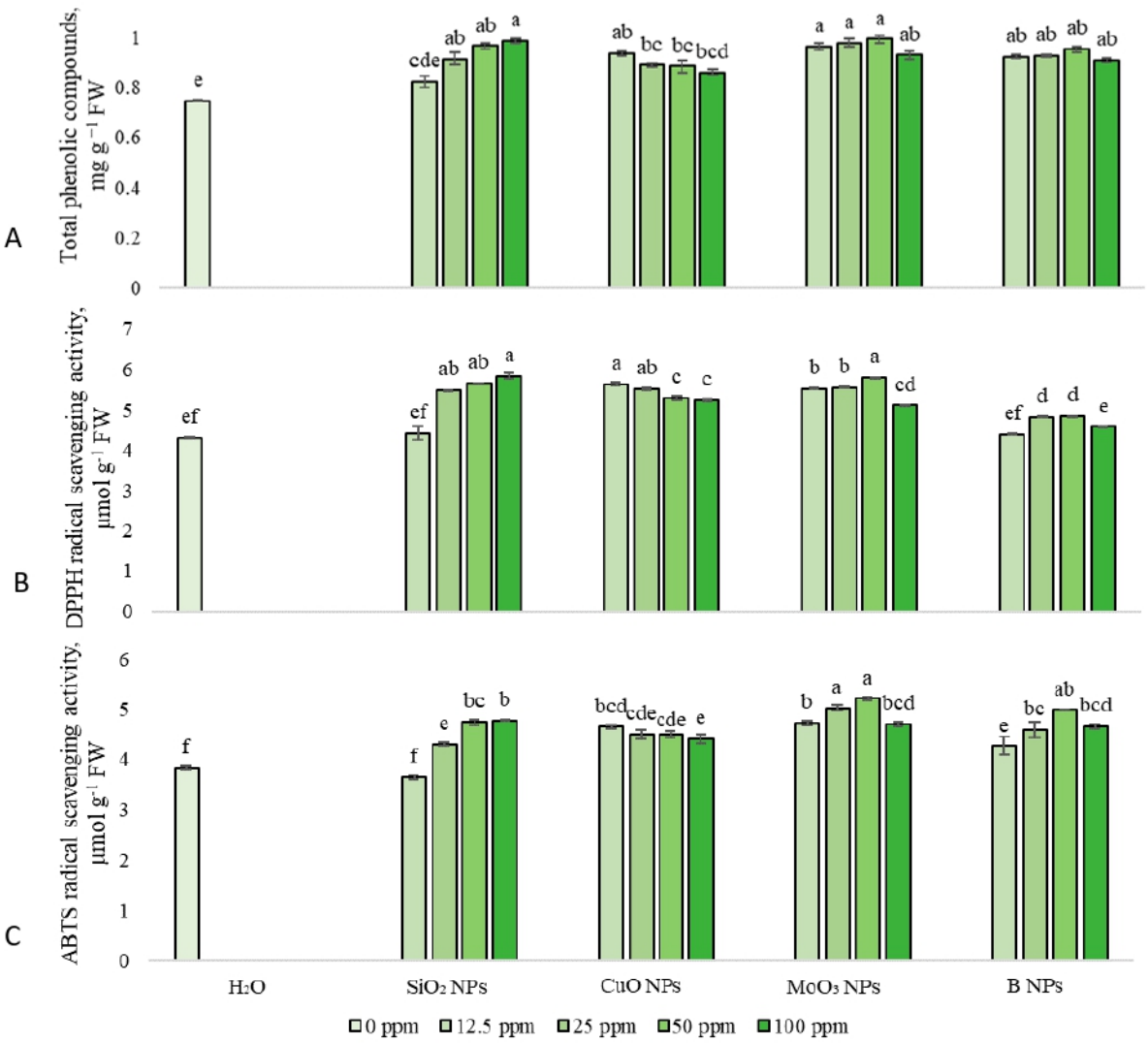


Figure 3.1.1. Influence of SiO_2, CuO, MoO_3, and B_2O_3 (B in the figure) NPs (0; 12,5; 25; 50, 100 ppm) on total phenolic compounds (A), DPPH (B) free radicals scavenging activity, and ABTS (C) free radicals scavenging activity in P. sativum L. H_2O – control plants watered with deionized water. Values are mean ± SE of nine replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

SiO_2 NPs were effective in reducing hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) concentrations in pea leaves (Figure 3.1.2A, B). The concentration of H_2O_2 decreased up to 10% in pea leaves after treatment with 25, 50, and 100 ppm SiO_2 solutions. A slight increase in H_2O_2 (6%) was determined after irrigation with SiO_2 NPs at 12.5 ppm. MDA, which indicates lipid peroxidation, was effectively attenuated by 17, 30, 43, and 35% after irrigating peas with SiO_2 NPs solutions containing 12.5, 25, 50, and 100 ppm concentrations.

MoO_3 exposure with concentrations of 50 and 100 ppm decreased H_2O_2 content in pea leaves by 6 and 14%. Furthermore, lipid peroxidation weakened with increasing concentrations of MoO_3 NPs. This was shown by the decrease in MDA concentration by 11, and 14% depending on higher concentration.

12.5 ppm CuO NPs suspension substantially reduced H_2O_2 (17%) and MDA (30%) content in pea leaves. A slightly weaker effect was detected after the application of 25 ppm suspension. However, a significant increase in H_2O_2 content (26%) was found after irrigation with CuO NPs solution at 100 ppm.

B_2O_3 NPs at concentrations of 12.5 and 25 effectively reduced the amount of H_2O_2 by 14 and 15%. However, H_2O_2 content did not differ from the control plants after exposure to a concentration of 100 ppm B_2O_3 NPs. The amount of MDA in pea leaves significantly decreased with concentrations of 12.5 and 25 ppm B_2O_3 NPs. A significant increase in MDA content was found after applying suspension with 100 ppm B_2O_3 NPs concentration.

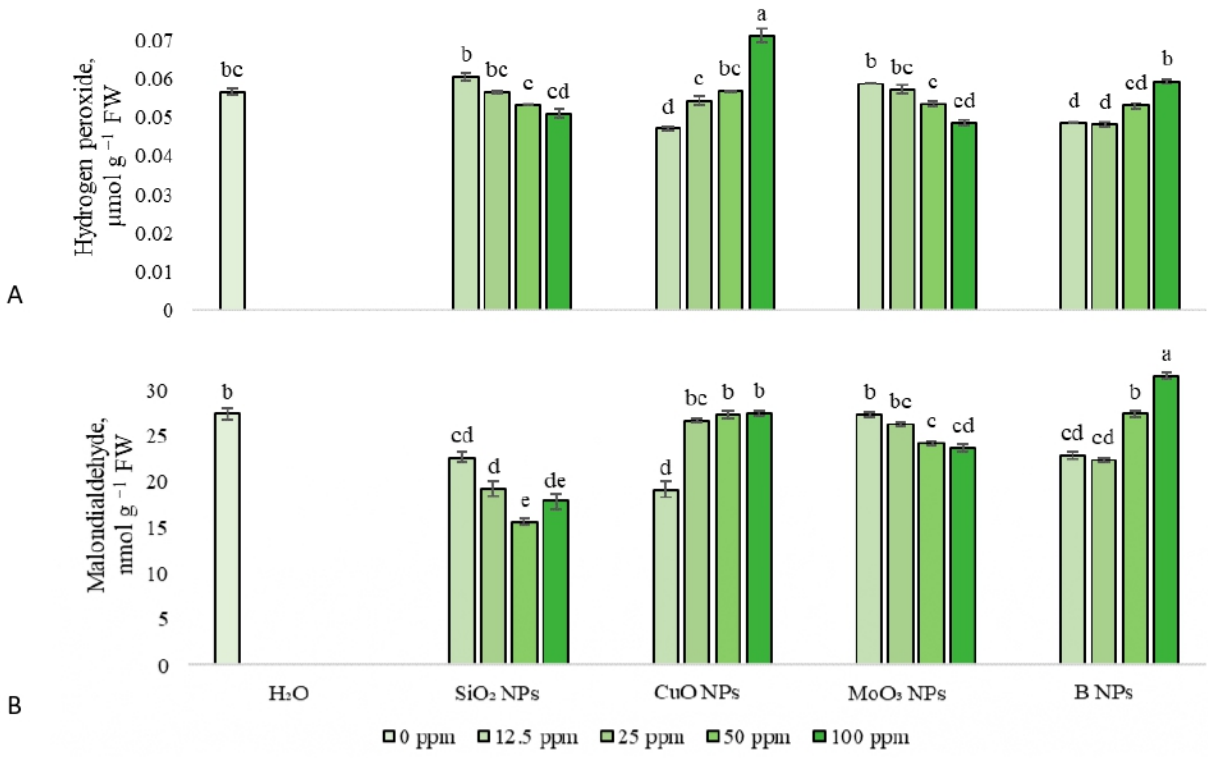


Figure 3.1.2. Influence of SiO_2, CuO, MoO_3, and B_2O_3 (B in the figure) NPs (0; 12,5; 25; 50, 100 ppm) on hydrogen peroxide (A) and malondialdehyde (B) content in P. sativum L. H_2O – control plants watered with deionized water. Values are mean ± SE of nine replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Summary

- *As the concentration of SiO_2 NPs increased, the morphological parameters of peas, the activity of antioxidant radicals, and TPC accumulation increased, and the concentration of H_2O_2 and MDA in peas decreased. However, no significant difference was found between 50 and 100 ppm concentrations, and it was chosen to use 12.5, 25, and 50 ppm concentrations for further studies.*
- *MoO_3 NPs showed a strong positive effect on morphological parameters with increasing concentration. However, the antioxidant response was considered: a concentration of 100 ppm was rejected due to a reduced antioxidant response.*
- *CuO NPs showed that using a concentration of 100 ppm is useless or even harmful to peas, so it was chosen to use concentrations of 12.5, 25, and 50 ppm for further experiments.*
- *B_2O_3 NPs concentration of 100 ppm showed an increase in H_2O_2 and MDA content in pea leaves and a decrease in pea morphological parameters, so it was rejected and not used in further studies.*

3.2 Effects of nanoparticles on drought-stressed pea plants

This section discusses the effects of NPs on drought-stressed peas grown at 30% substrate moisture (SM 30%) compared to plants grown at regular substrate moisture (SM 80%). The influence of NPs on morphological parameters, antioxidant system activity, and macro- and microelement accumulation in peas is discussed in detail. The different effects of NPs on peas were highlighted by applying two methods of exposure: foliar spraying and watering through the roots. This section discusses the results obtained between plants affected by drought and plants affected by drought and NPs. The subsections are divided according to the NPs, at the first paragraph were discussed the watered NPs effects on plants, and the second one is the foliar NPs application through the leaves.

3.2.1 Effects of silica nanoparticles on peas under different substrate moisture

Impact on morphological parameters

Irrigation of peas with 12.5, 25, and 50 ppm SiO_2 NPs suspensions significantly affected pea height (Table 3.2.1.1, 80% SM) when substrate moisture was sufficient. Also, plants irrigation with 50 ppm suspension stimulated leaf area development effectively. In addition, spraying with 12.5, 25, and 50 ppm SiO_2 NPs suspension increased leaf area by up to 15%. Relative water content (RWC) increased by 6% when plants were irrigated with a concentration of 50 ppm suspension. The ratio of root to shoot increased by 7, 34, and 68% when watering with suspensions of 12.5, 25, and 50 ppm, respectively. However, the influence of SiO_2 NPs decreased by 70, 23, and 4% after spraying with solutions containing 12.5, 25, and 50 ppm NPs.

No differences were found in specific leaf area (SLA) after watering peas with SiO_2 NPs, while spraying with 12.5 ppm reduced SLA by 13%. A positive influence was found on nodulation. The number of nodules increased to 5 times when peas were watered with SiO_2 NPs solutions. After spraying with SiO_2 NPs solutions, the number of nodules in pea roots increased slightly to 1.4 times. A 41% increase in pea yield was found after 50 ppm SiO_2 NPs suspension was applied to plants.

SiO_2 NPs showed a significant positive effect on pea plants as they grow in the substrate with insufficient moisture content (Table 3.2.1.1, 30% SM). Pea height increased by 23, 20, and 18% after irrigation and by 8, 11, and 24% after spraying with solutions containing 12.5, 25, and 50 ppm SiO_2 NPs. Leaf area increased by 11 and 13% after peas irrigation with 25 and 50 ppm and by 12, 18, and 10% after foliar application with 12.5, 25, and 50 ppm SiO_2 NPs solutions. RWC increased by 7% and 10% after irrigation of peas with 12.5 and 50 ppm SiO_2 NPs suspension when plants were grown under 30% substrate moisture. There was a substantial increase in root-to-shoot ratio (up to 30%) after irrigating peas with SiO_2 NPs solutions or spraying with 50 ppm concentration. However, the root-to-shoot ratio decreased after peas were sprayed with 12.5 and 25 ppm SiO_2 NPs solutions. The SiO_2 NPs did not affect SLA of peas grown under drought conditions, except for plants sprayed with 25 ppm solution. The application of SiO_2 NPs induced the formation of nodules in pea roots. This is shown by the 30% increase in nodules number after watering with 50 ppm suspension and 33%, 50%, and 180 % increase after spraying with 12.5, 25, and 50 ppm solutions, respectively. A significant increase in the pea yield (up to 43%) of drought-affected peas was found after the plants were irrigated with 12.5, 25, and 50 ppm SiO_2 NPs suspensions. A positive effect of 40, 35, and 45% on yield was found after peas were sprayed with the suspensions containing mentioned concentrations.

SiO_2 NPs slightly increased H_2O_2 concentration in pea leaves, 12.5 ppm - 25%, 25 ppm - 20%, 50 ppm - 12% when watered and 12.5 ppm - 28% when peas were sprayed and grown under 80% substrate moisture (Figure 3.2.1.1A). Knowing that plants need peroxide in small amounts, it can be noted that such an increase is optimal for plants and does not have a harmful effect. The concentration of malondialdehyde (MDA) in the plant indicates lipid peroxidation. MDA concentration in pea leaves was affected by SiO_2 NPs (Figure 3.2.1.1B, 80% SM). After watering with suspensions containing 12.5 and 25 ppm of SiO_2 NPs, the MDA content in the plant increased significantly. Pea exposure by watering or spraying with 50 ppm of SiO_2 NPs suspension significantly reduced the MDA content in pea leaves.

Table 3.2.1.1. Impact of drought stress and SiO_2 NPs (12.5; 25; 50 ppm) on P. sativum L. height, leaf area, specific leaf area (SLA), relative water content (RWC), root-to-shoot ratio, and the number of nodules.

0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Mean values within columns followed by different letters differ significantly at $p < 0.05$ (n=10) according to Tukey (HSD) test

		SiO_2 NPs, ppm	Plants height, cm	Leaf area, cm^2	SLA, $m^2\ kg^{-1}$	RWC, %	Root/shoot ratio	Nr. of nodules	Yield, $t\ ha^{-1}$
SM 80%	Watered	0	28.4 d	36.1 c	5.3 ab	82.5 b	7.8 c	1.7 c	3.9 b
		12.5	30.1 bc	36.3 c	4.7 ab	83.6 ab	8.3 b	5.0 ab	3.8 b
		25	32.5 b	37.6 b	5.1 ab	85.7 ab	10.4 ab	6.7 ab	3.7 b
		50	34.4 a	47.2 a	4.8 ab	87.6 a	13.0 a	9.3 a	5.5 a
	Sprayed	12.5	29.4 cd	38.8 b	4.6 c	84.6 ab	13.2 a	3.3 bc	3.4 bc
		25	29.3 cd	39.3 ab	5.6 a	83.8 ab	9.5 ab	4.0 b	4.0 b
		50	29.2 c	41.5 ab	5.1 ab	84.8 ab	8.1 b	3.3 bc	5.4 a
SM 30%	Watered	0	26.0 e	33.1 d	5.0 b	53.0 d	9.2 b	2.0 b	2.5 c
		12.5	32.0 d	33.2 d	3.8 bc	56.9 ab	11.7 a	2.0 b	3.3 ab
		25	31.2 a	36.6 bc	4.0 bc	56.6 ab	10.5 a	2.0 b	3.2 ab
		50	30.7 bc	37.3 b	4.8 b	53.9 d	11.9 a	2.7 b	4.5 a
	Sprayed	12.5	28.1 cd	37.0 b	5.1 ab	54.7 c	8.9 bc	2.7 b	3.4 ab
		25	28.9 ab	39.0 ab	6.2 a	55.4 bc	8.5 c	3.0 b	3.2 ab
		50	30.7 cd	43.1 a	4.9 b	58.9 a	9.2 b	2.0 b	4.6 a

Effects on oxidative stress biomarkers

Focusing on the effects of drought on the concentration of H_2O_2 in the pea, it has increased to a concentration that is dangerous to the plant, causing an imbalance in the cells. In pea leaves affected by drought, the concentration of H_2O_2 increased 2.5 times compared to plants grown in normal substrate moisture (Figure 3.2.1.1A, 30% SM). Watering with 12.5, 25, and 50 ppm SiO_2 NPs solutions positively affected plants by reducing H_2O_2 concentration by 15, 25, and 27%, while spraying with 25 and 50 ppm SiO_2 NPs solutions reduced H_2O_2 by 13 and 21%, respectively. An increase in MDA concentration of over 26% was found when plants were exposed to drought compared with peas grown under 80% SM (Figure 3.2.1.1B). In the results, when peas were watered or sprayed with SiO_2 NPs solutions, the amount of MDA in plants decreased significantly compared to untreated NPs.

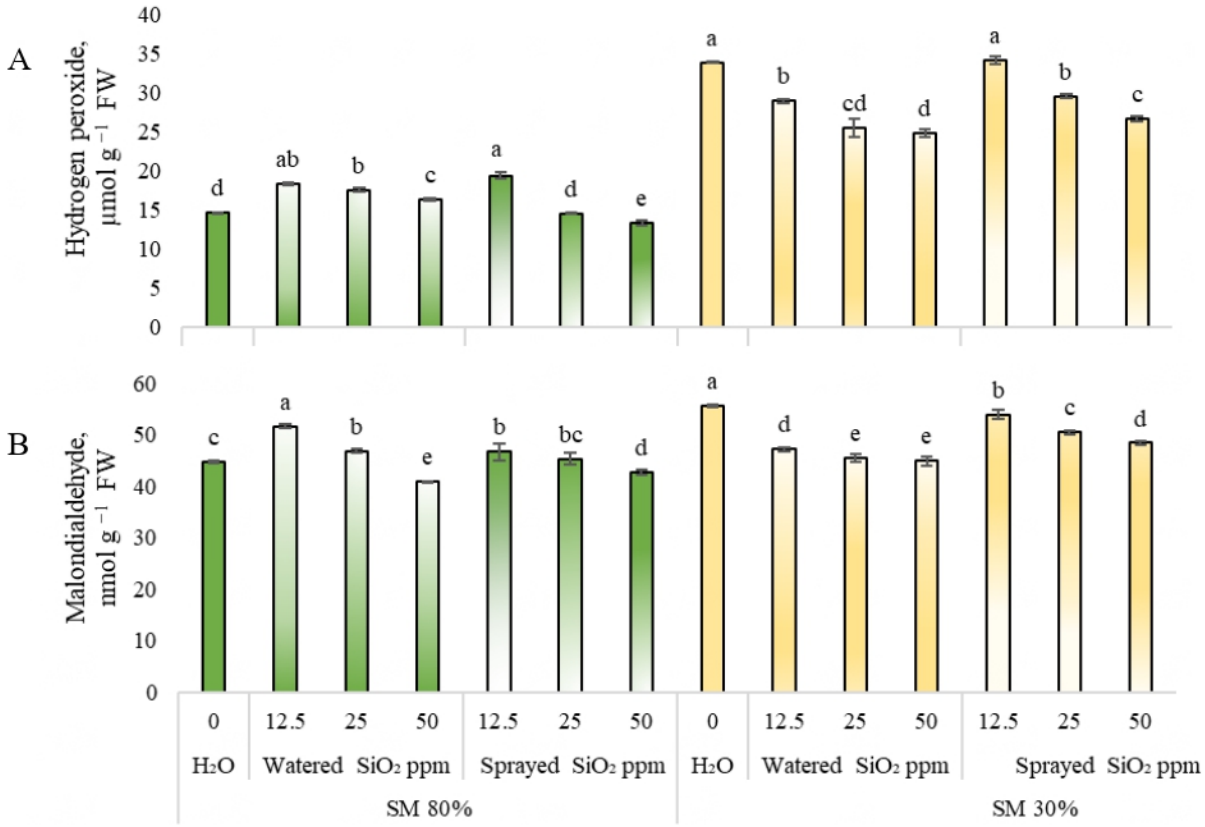


Figure 3.2.1.1. Influence of drought stress and SiO_2 NPs (0; 12,5; 25; 50 ppm) on hydrogen peroxide (A) and malondialdehyde (B) content in P. sativum L. H_2O – control plants sprayed or watered, with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of nine replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Effects on non-enzymatic antioxidants

The inhibitory effect of SiO_2 NPs on the antioxidant system of peas grown in 80% substrate moisture was determined (Figure 3.2.1.2, 80% SM). The TPC content decreased by 17% when the plants were watered with the suspension at 12.5 ppm concentration, and after spraying with suspensions containing 12.5, 25, and 50 ppm of SiO_2 NPs decreased it by 21, 23, and 12%, respectively. (Figure 3.2.1.2A). A statistically reliable effect in FRAP antioxidant power was caused by irrigation and foliar application of SiO_2 NPs suspensions (Figure 3.2.1.2D). The impact on the DPPH free radical scavenging activity was multifaceted. The activity of the DPPH radical scavenging decreased by 13% after treatment through roots with 25 ppm suspension, but after foliar application of 12.5 ppm suspension, it increased by 25% (Figure 3.2.1.2B). ABTS free radical scavenging activity increased significantly after spraying peas with the suspension of 50 ppm (Figure 3.2.2C).

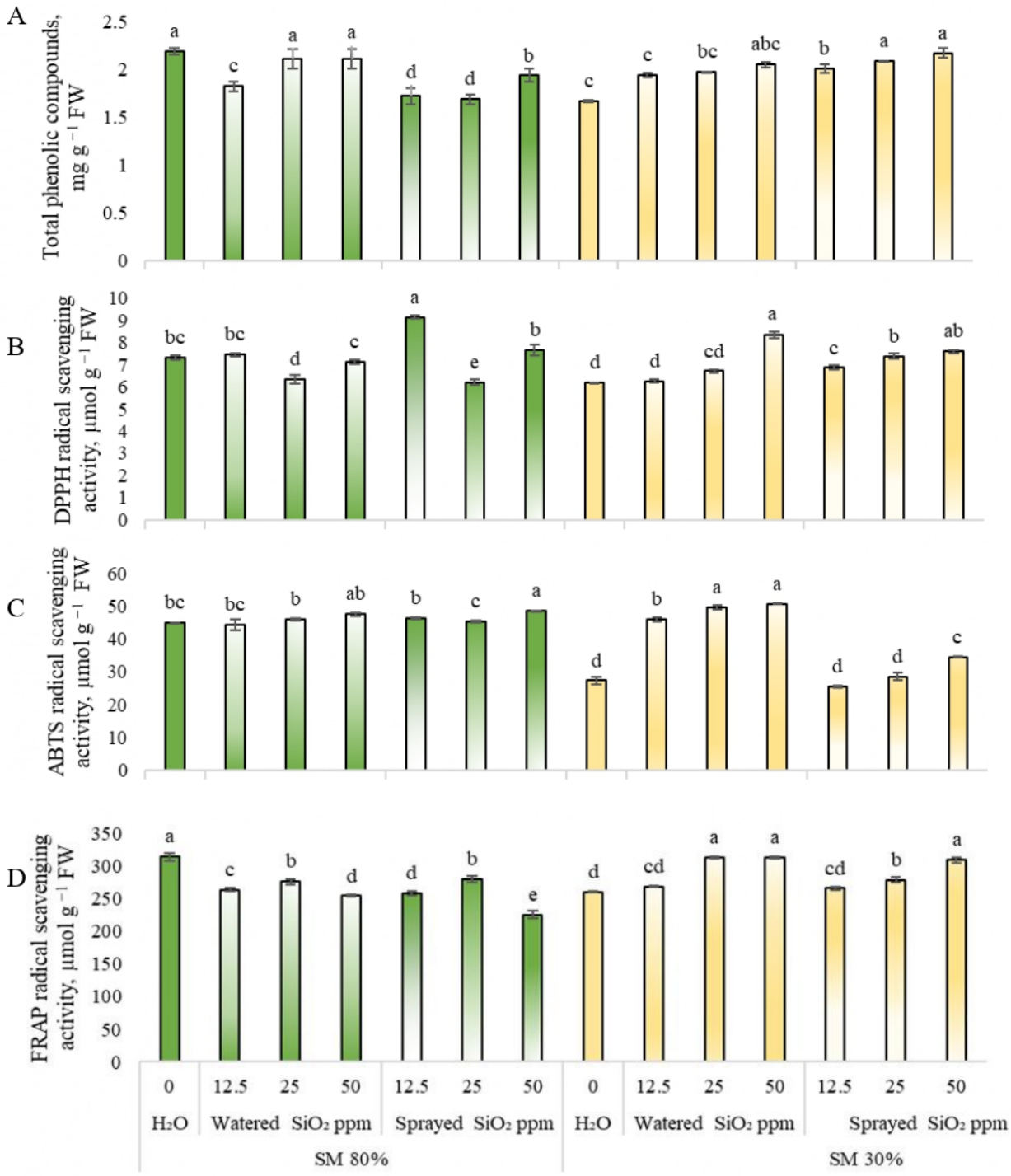


Figure 3.2.1.2. Influence of drought stress and SiO_2 NPs (0; 12,5; 25; 50 ppm) on A – total phenolic compounds (TPC), B – DPPH free radical scavenging activity, C – ABTS free radical scavenging activity D – FRAP antioxidant power in P. sativum L. H_2O – control plants, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Opposite results were found when studying the effects of SiO_2 NPs on pea plants grown in the substrate with reduced moisture content (Figure 3.2.1.2, 30% SM). Significant increases of 16, 18, and 23% in TPC content were found in pea leaves after irrigation with increasing

concentrations of SiO_2 NPs, and 20, 25, and 30% increases in TPC after spraying with 12.5, 25, and 50 ppm suspensions (Figure 3.2.1.2A). The results showed that irrigation with 50 ppm suspension of SiO_2 NPs DPPH free radical scavenging activity increased by 35%; after spraying with 12.5, 25, and 50 ppm suspensions - by 11, 19, and 23%, respectively (Figure 3.2.1.2B). FRAP antioxidant power increased to 20% after irrigation with 25 and 50 ppm SiO_2 NPs suspensions and up to 19% after spraying drought-affected peas (Figure 3.2.1.2D). SiO_2 NPs and drought strongly affected ABTS free radical scavenging activity (Figure 3.2.1.2C). The ABTS free radical scavenging activity increased by 69, 82, and 86% after using SiO_2 NPs through roots at 12.5, 25, and 50 ppm concentrations. However, the effect was different during spraying because only 50 ppm concentration of suspension increased it up to 26%.

Effects on enzymatic antioxidants

SiO_2 NPs stimulated the CAT activity after spraying peas grown under sufficient substrate moisture with 12.5 ppm by 93% and 25 ppm by 89% (Figure 3.2.1.3B, 80% SM). An inhibitory effect was found for the APX and GR enzymes (Figure 3.2.1.3A, D 80% SM). APX activity was inhibited after watering or spraying 50 ppm suspension by 32% and 38%. GR activity inhibition by 13% at a concentration of 50 ppm SiO_2 NPs applied through the roots and after spraying with 50 ppm by 19% were found. The activities of GPX and SOD were strongly affected by SiO_2 NPs (Figure 3.2.1.3E, C 80% SM). GPX activity increased 9, 8, and 5 times after irrigating peas with suspensions of 12.5, 25, and 50 ppm SiO_2 NPs, and after spraying, all used concentrations increased GPX activity by 9 times. SOD activity increased after 12.5, 25, and 50 ppm SiO_2 NPS application: by watering 49, 44, and 47%, and by spraying 42, 47, and 48%.

CAT activity in pea leaves after exposure to drought and 12.5, 25, and 50 ppm SiO_2 NPs increased by 36, 84, and 153% after watering and by 13, 41, and 119% after spraying (Figure 3.2.1.3B 30% SM). Increasing SiO_2 NPs concentration increased SOD activity after watering up to 37% and after spraying up to 23% (Figure 3.2.1.3C 30% SM) in drought-affected pea plants. GR activity was affected by 25 and 50 ppm SiO_2 NPs increasing the activity by 21 and 100% after watering. Furthermore, an even more substantial effect of up to 128% on GR activity was found after spraying with SiO_2 NPs drought-affected peas (Figure 3.2.1.3D 30% SM). Notably, decreases in APX and GPX activities were found (Figure 3.2.1.3A, E 30% SM). APX activity decreased by 37 and 16% after watering with 12.5 and 25 ppm SiO_2 NPs, with an even more substantial negative effect of up to 37% after spraying. GPX activity was inhibited after watering drought-affected peas with 12.5 ppm SiO_2 NPs by 19% while spraying inhibited the activity by up to 30%. A slight GPX activity increase was found only after irrigating peas with a concentration of 50 ppm SiO_2 NPs.

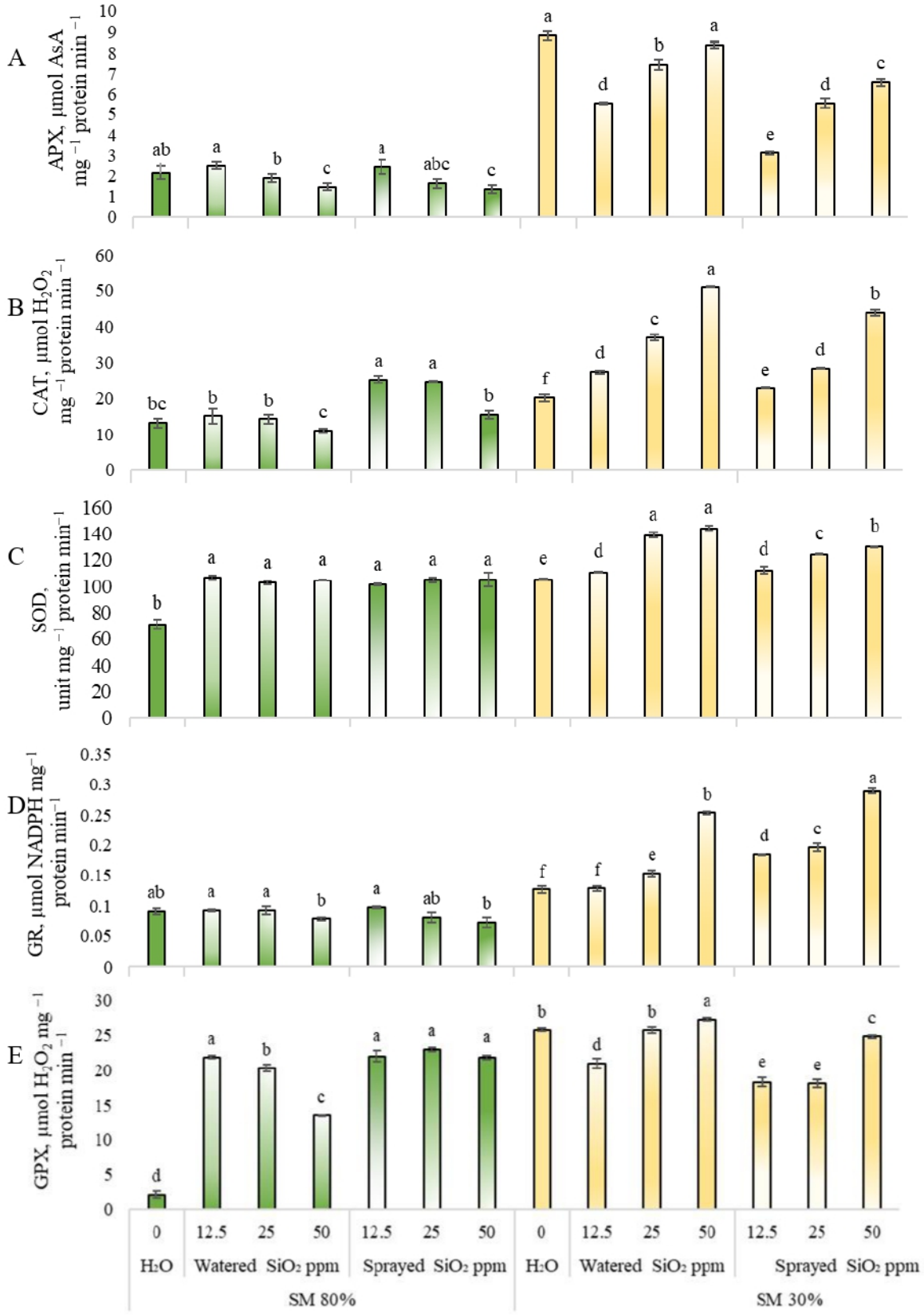


Figure 3.2.1.3. Response of A – ascorbate peroxidase (APX), B –catalase (CAT), C –superoxide dismutase (SOD), D –glutathione reductase (GR), and E –guaiacol peroxidase (GPX) activity to drought stress and SiO_2 NPs (0; 12,5; 25; 50 ppm) in P. sativum L. H_2O – control plants sprayed or watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Changes in macro- and microelement composition

The effects of SiO_2 NPs suspension on macro- and microelements in pea's leave, stems, and roots are presented in Table 3.2.1.2. The results showed that drought reduces P, Mg, Na, Mn, Mo, and B but increases the accumulation of K, Ca, Fe, and Zn in pea plant leaves. Spraying at a concentration of 50 ppm when plants were grown with 30% SM increased the accumulation of K, Ca, Mg, Fe, Mn, and Mo in the pea leaves. SiO_2 NPs solution of 50 ppm had an effect against drought by increasing the amount of all micro- and macroelements in the plant stems except for Na, Fe, and B elements. In addition, the SiO_2 suspension strongly affected the accumulation of all elements in the root, significantly increasing the amount of P, K, Mg, Fe, Zn, Mn, Cu, and Mo.

Table 3.2.1.2. Effects of SiO_2 NPs suspension on macro- and microelements of peas leaves, stem, and root. Mean values in bold indicate a statistically significant difference at $p < 0.05$ according to Tukey (HSD) test (n=9)

Treatment SiO_2 NPs, ppm			Macroelements, mg g^{-1} DW						Microelements, µg g^{-1} DW				
Leaves			P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Mo	B
SM 80%	Watered	0	3.79	18.76	45.73	9.20	2.64	19.86	25.71	42.54	10.32	2.99	33.20
		12.5	3.60	21.27	**48.76**	9.99	**3.41**	**23.50**	**33.10**	**48.30**	11.03	2.40	**16.51**
		25	3.00	**14.53**	**35.74**	**6.95**	**1.44**	19.78	23.29	**30.89**	**7.78**	**1.58**	**15.48**
		50	**4.91**	**23.63**	46.18	9.32	**1.98**	20.76	**34.70**	**40.59**	9.65	**1.60**	**15.82**
	Sprayed	12.5	3.26	19.07	40.76	8.99	**3.30**	**13.55**	**30.12**	**43.44**	10.23	2.51	**17.51**
		25	3.41	**17.64**	43.27	8.55	**1.71**	**15.94**	**29.23**	**44.82**	9.78	2.46	**16.70**
		50	**4.41**	**23.63**	**57.59**	**11.98**	**4.15**	**25.15**	**42.63**	**53.93**	**11.50**	2.19	**16.31**
SM 30%	Watered	0	3.21	20.56	46.19	8.81	2.07	20.03	37.24	37.65	10.02	0.70	11.66
		12.5	3.75	**23.65**	**48.93**	**9.57**	**1.65**	21.86	37.12	**44.00**	**8.82**	**1.97**	**4.20**
		25	3.53	**22.58**	44.91	8.71	**1.59**	**15.38**	**35.05**	38.94	**7.59**	**3.11**	**9.83**
		50	3.01	21.22	42.15	**8.16**	**1.27**	**18.99**	**39.01**	**41.42**	**7.51**	**2.82**	11.32
	Sprayed	12.5	**2.13**	**24.26**	**40.72**	8.90	**5.65**	**14.47**	**29.72**	**27.48**	**12.08**	**3.06**	**3.70**
		25	3.08	19.10	**43.75**	8.26	**1.76**	**14.71**	**31.78**	38.78	**7.31**	0.38	**9.77**
		50	**4.11**	**24.69**	**47.76**	**9.82**	**1.82**	**22.45**	**35.19**	**47.62**	**8.28**	**1.52**	11.75
Stem			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	2.00	17.77	37.81	8.71	7.73	15.49	19.81	26.09	10.08	3.66	1.05
		12.5	**1.62**	**19.78**	**41.52**	8.34	7.82	14.24	**26.24**	25.42	**12.89**	**2.74**	**9.28**
		25	**1.52**	**15.76**	**29.50**	**6.26**	**5.02**	14.60	18.09	**18.80**	**8.46**	3.08	**4.26**
		50	2.26	**20.86**	**42.90**	8.31	**6.84**	**17.10**	**24.35**	**27.08**	**12.05**	**2.63**	**4.61**
	Sprayed	12.5	**1.65**	17.83	**42.06**	8.39	7.48	**16.60**	**25.91**	**29.11**	**13.03**	**4.56**	**8.08**
		25	**1.49**	16.23	36.17	7.36	**5.53**	**12.82**	20.52	**24.56**	9.72	3.38	**7.50**
		50	2.11	**20.71**	**47.87**	**9.89**	**8.73**	**16.39**	**25.88**	**30.40**	**16.20**	**5.85**	**9.87**
SM 30%	Watered	0	1.43	20.13	38.10	7.98	6.83	15.46	22.31	24.19	8.92	1.17	10.57
		12.5	1.89	**24.25**	**43.95**	**8.53**	6.22	**19.28**	**27.43**	**30.08**	**12.17**	**2.55**	11.11
		25	1.87	**24.75**	37.25	7.39	**5.33**	**12.55**	20.98	24.52	**10.88**	1.38	**7.94**
		50	1.74	21.73	37.61	**7.06**	**4.02**	**13.37**	**30.57**	**26.51**	**9.69**	1.33	**6.88**

	Sprayed	12.5	**2.13**	**24.26**	**40.72**	**8.90**	**5.65**	**14.47**	**29.72**	**27.48**	**12.08**	**3.06**	**3.70**
		25	1.54	20.16	36.91	7.49	**5.58**	**13.67**	**24.81**	23.53	**10.96**	**2.39**	9.83
		50	**2.18**	**22.81**	**42.93**	**8.52**	**5.43**	**13.88**	**27.44**	**30.93**	**13.21**	**3.86**	**7.59**
Root			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%		0	0.81	16.16	10.25	6.91	3.18	258.88	15.88	46.44	18.46	9.40	57.91
	Watered	12.5	**2.96**	**37.36**	**22.85**	**18.47**	**8.42**	**797.28**	**45.21**	**149.63**	**36.13**	**25.06**	**27.27**
		25	**2.72**	**42.93**	**23.14**	**18.55**	**7.94**	**758.35**	**43.47**	**144.16**	**37.75**	**22.71**	**16.89**
		50	**3.44**	**48.48**	**31.12**	**21.51**	**11.42**	**895.74**	**56.39**	**164.75**	**42.82**	**27.62**	**26.67**
	Sprayed	12.5	**2.78**	**34.62**	**24.21**	**15.82**	**11.30**	**667.76**	**51.21**	**129.32**	**38.21**	**21.71**	**21.91**
		25	**5.07**	**65.36**	**44.07**	**31.90**	**14.62**	**1591.93**	**120.9**	**320.39**	**65.65**	**48.16**	**27.04**
		50	**3.23**	**37.08**	**24.69**	**19.56**	**7.49**	**1138.92**	**53.11**	**217.61**	**42.07**	**31.86**	**24.55**
SM 30%		0	1.58	28.33	19.57	15.45	7.33	657.85	34.15	118.04	22.49	16.58	9.84
	Watered	12.5	**2.22**	**31.42**	20.43	15.60	7.73	**774.18**	**48.50**	146.08	**31.38**	**22.69**	**7.04**
		25	**2.80**	**47.33**	**24.74**	**18.75**	**9.86**	**906.39**	**46.80**	**169.12**	**40.77**	**24.70**	10.07
		50	**2.92**	**42.44**	**31.10**	**22.50**	**12.41**	**1353.64**	**68.41**	**249.53**	**45.04**	**36.94**	**8.78**
	Sprayed	12.5	**2.48**	**37.92**	**23.92**	**17.01**	**11.60**	**960.38**	**64.23**	**166.31**	**38.08**	**25.69**	9.00
		25	**3.94**	**59.53**	**42.14**	**31.60**	**15.60**	**1321.75**	**70.93**	**244.31**	**61.44**	**41.37**	**4.17**
		50	**3.16**	**46.26**	**32.11**	**22.40**	**12.75**	**1398.81**	**57.86**	**248.60**	**47.33**	**38.15**	**2.13**

Macroelements: P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, Na – sodium, Fe – iron; Microelements: Zn – zinc, Mn – manganese, Cu – copper, Mo – molybdenum, B – boron. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%.

Table 3.2.1.3. The impact of SiO_2 NPs (12,5; 25; 50 ppm) on P. sativum L. grown in the substrate with sufficient (SM 80%) and insufficient (SM 30%) moisture is expressed as a percentage change (%) compared to the control (for SM 80% control means plants grown under SM 80% and NPs untreated; SM 30% control means drought affected but NPs untreated plants) in the heat map. Statistically, significant differences are marked in bold

	SM 80%						SM 30%					
	Watered			Sprayed			Watered			Sprayed		
Treatment SiO_2 NPs, ppm	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Plant height	6	**15**	**21**	4	3	3	**23**	**20**	**18**	**8**	**11**	**24**
Leaf area	1	**4**	**31**	**8**	**9**	**15**	0	**11**	**13**	**12**	**18**	**10**
Nodules	**200**	**300**	**460**	100	**140**	100	0	0	**33**	**33**	**50**	**183**
RWC	1	4	**6**	3	2	3	**7**	**7**	2	**3**	**4**	**10**
Root/shoot	**7**	**34**	**68**	**70**	**23**	**4**	**27**	**14**	**30**	-3	**-8**	16
SLA	-11	-3	-9	**-13**	6	-3	-24	-20	-4	2	**23**	-25
Yield	-2	-5	**40**	-14	4	**38**	**37**	**37**	**44**	**40**	**35**	**45**
ABTS	-1	2	6	3	1	**8**	**69**	**82**	**86**	-7	5	**26**
DPPH	2	**-13**	-3	**25**	**-15**	5	1	9	**35**	**11**	**19**	**23**
TPC	**-17**	-4	-4	**-21**	**-23**	**-12**	**16**	**18**	**23**	**20**	**25**	**30**
FRAP	**-16**	**-12**	**-19**	**-18**	**-11**	**-28**	3	**20**	**20**	2	**7**	**19**
HP	**26**	**20**	**12**	**78**	0	**-9**	**-15**	**-25**	**-27**	1	**-13**	**-21**
MDA	**15**	**5**	**-9**	**4**	1	**-5**	**-15**	**-18**	**-19**	**-3**	**-9**	**-13**
GR	2	2	**-13**	7	-11	-19	2	**21**	**100**	**46**	**55**	**128**
GPX	**914**	**841**	**525**	**919**	**965**	**912**	-19	0	6	**-29**	**-30**	**-4**
APX	16	-12	**-32**	13	-24	**-38**	**-37**	**-16**	-5	**-65**	**-37**	**-25**
SOD	6	**15**	**21**	4	3	3	**23**	**20**	**18**	**8**	**11**	**24**
CAT	1	**4**	**31**	**8**	**9**	**15**	0	**11**	**13**	**12**	**18**	**10**

RWC – relative water content, SLA – specific leaf area, TPC – total phenolic compounds, HP – hydrogen peroxide, MDA – malondialdehyde, GR – glutathione reductase, GPX – guaiacol peroxidase, APX – ascorbate peroxidase, SOD – superoxide dismutase, CAT – catalase. 0 – control plants watered with deionized water, drought stress – 30% substrate moisture (SM 30%).

Summary (Table 3.2.1.3)

- *SiO_2 NPs protect peas from drought by strengthening the antioxidant system and activating enzyme-catalyzed reactions.*
- *SiO_2 suspension reduced the concentration of H_2O_2 and MDA in the pea plant, thus reducing the oxidative stress caused by drought.*
- *The selected SiO_2 concentration of 50 ppm is the most effective for the pea plant against drought-induced stress.*
- *No significant difference was found between applying SiO_2 NPs on pea plants by foliar spray or root irrigation.*

3.2.2 Effects of copper oxide nanoparticle on peas under different substrate moisture

Impact on pea morphological parameters

CuO NPs had different effects on pea plants grown at 80% SM. The results in Table 3.2.2.1 show that plant height, SLA, and root-to-shoot ratio were not affected by CuO NPs (80% SM). Leaf area decreased by 13% after watering with suspensions of 25 or 50 ppm CuO NPs. Spraying peas with 12.5 and 25 ppm suspensions of CuO NPs slightly decreased the leaf area. RWC was reduced when peas were treated with 50 ppm CuO NPs by watering or spraying. The effect of CuO NPs on pea yield was not significant.

Table 3.2.2.1. Impact of drought stress and CuO NPs (12.5; 25; 50 ppm) on P. sativum L. height, leaf area, specific leaf area (SLA), relative water content (RWC), root to shoot ratio, and the number of nodules. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Mean values within columns followed by different letters differ significantly at $p < 0.05$ (n=10) according to Tukey (HSD) test

CuO NPs, ppm			Plants height, cm	Leaf area, cm^2	SLA, $m^2\ kg^{-1}$	RWC, %	Root/shoot ratio	Number of nodules	Yield, $t\ ha^{-1}$
SM 80%	Watered	0	28.4 a	36.1 ab	5.3 a	82.5 b	7.8 a	1.7 c	3.9 a
		12.5	27.8 a	33.4 bc	3.9 ab	83.9 ab	9.7 a	2.0 bc	3.7 a
		25	28.5 a	31.8 c	4.0 ab	84.1 ab	5.5 a	1.0 c	3.8 a
		50	27.3 a	31.5 c	4.4 ab	78.6 cd	7.7 a	5.3 a	3.8 a
	Sprayed	12.5	26.9 a	28.5 c	4.0 ab	80.1 bc	12.6 a	2.7 bc	3.5 ab
		25	27.4 a	29.4 c	5.9 a	80.1 bc	6.2 a	3.7 b	3.5 ab
		50	26.1 ab	38.9 a	5.8 a	79.5 cd	4.9 a	4.7 ab	3.6 a
SM 30%	Watered	0	26.0 ab	33.1 ab	5.0 a	53.0 a	9.2 a	2.0 b	2.5 b
		12.5	27.4 a	33.5 ab	4.4 b	52.6 a	11.2 a	2.3 b	2.6 ab
		25	26.0 ab	28.3 c	4.4 b	49.4 ab	6.9 a	1.7 b	2.5 b
		50	23.7 c	27.2 cd	5.2 ab	45.3 ab	6.9 a	3.7 a	3.3 a
	Sprayed	12.5	26.6 ab	36.9 a	5.7 ab	51.6 a	7.5 a	2.3 b	2.7 ab
		25	27.3 a	26.5 d	4.7 ab	50.7 ab	8.3 a	3.0 b	2.9 ab
		50	21.6 c	32.7 ab	4.9 ab	48.2 ab	6.0 a	3.7 a	3.7 a

The highest concentration (50 ppm) of CuO NPs reduced pea height after watering or spraying pea plants grown in insufficient substrate moisture (Table 3.2.2.1, 30% SM). Leaf area was reduced by 14 and 18% after the application of suspensions of 25 and 50 ppm and after spraying with 25 ppm suspensions by 20%. SLA decreased by 12% after watering plants with

12.5 and 25 ppm CuO NPs solutions. RWC and root-to-shoot ratio were not affected significantly by CuO NPs treatment. CuO NPs positively affected nodule formation in pea roots when 50 ppm CuO NPs concentration was used. A significant positive effect of 27% on pea yield was found after exposure to drought and irrigation with 50 ppm CuO NPs. The yield of sprayed peas increased up to 47% depending on the concentration of CuO NPs.

Effects on oxidative stress biomarkers

CuO NPs strongly affected both H_2O_2 and MDA accumulation in pea plants grown in the substrate with sufficient moisture (Figure 3.2.2.1A, B). After irrigation with CuO NPs concentrations of 12.5, 25, and 50 ppm, H_2O_2 content increased by 70, 41, and 16%, while after spraying, an increase of 45, 74, and 72% were found in pea leaves (Figure 3.2.2.1A 80% SM). MDA amount in pea leaf cells also significantly increased up to 54% after irrigating or spraying peas with CuO NPs solutions. (Figure 3.2.2.1B 80% SM). From these results, we can conclude that CuO NPs strongly activate lipid peroxidation and can cause oxidative stress in plants.

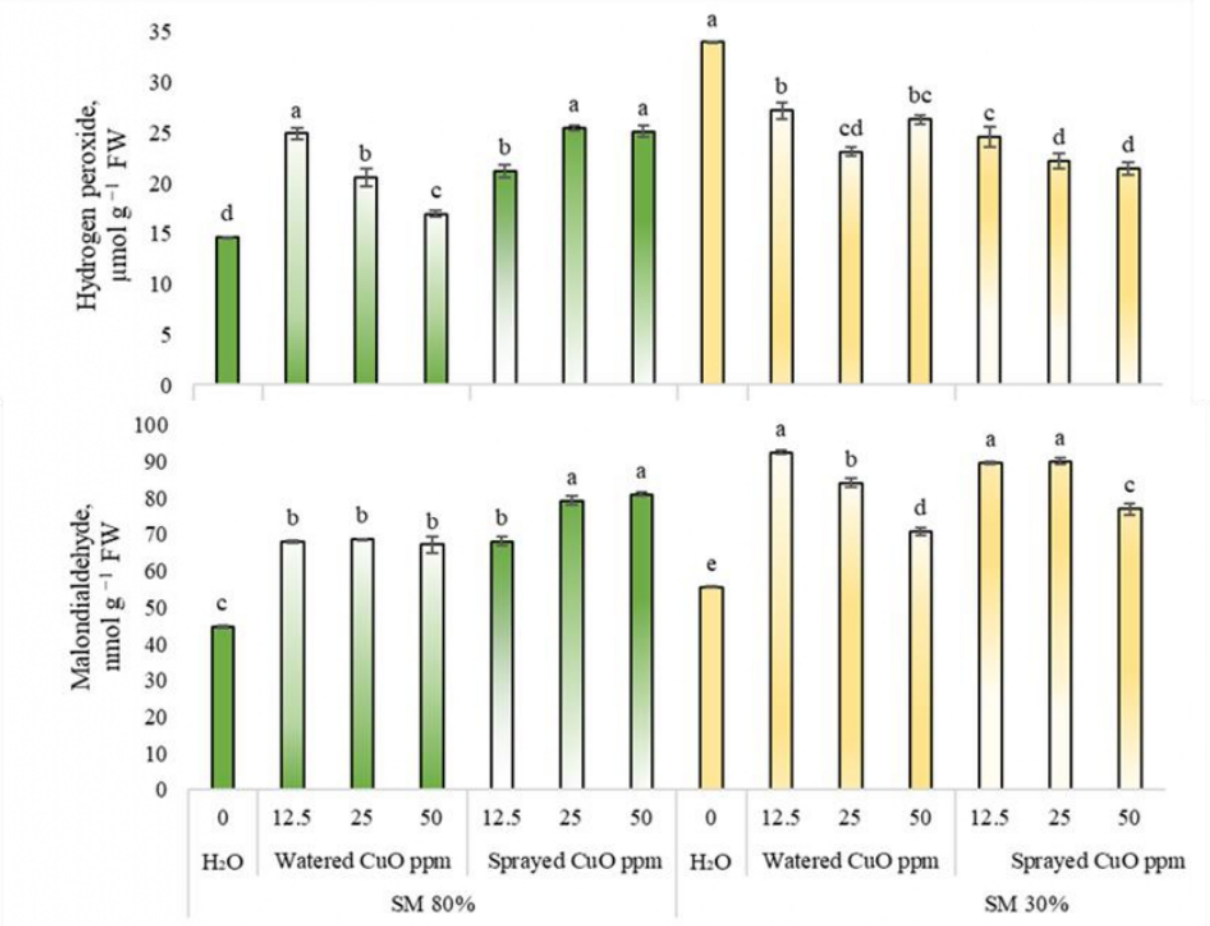


Figure 3.2.2.1. Influence of drought stress and CuO NPs (0; 12,5; 25; 50 ppm) on hydrogen peroxide and malondialdehyde content in P. sativum L. H_2O – control plants watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

However, the effect of CuO NPs on the amount of H_2O_2 in the plant changed along with drought exposure (Figure 3.2.2.1A 30% SM). The content of H_2O_2 was reduced remarkably after irrigating or spraying peas with CuO NPs solutions containing different concentrations. The MDA-increasing effect in peas persisted under drought conditions (Figure 3.2.2.1B 30% SM). The MDA content increased to 66 % depending on the CuO NPs concentration and application method.

Effects on non-enzymatic antioxidants

Pea irrigation with 12.5 ppm CuO NPs solution significantly decreased the TPC in pea leaves. In contrast, 25 and 50 ppm exposure significantly increased when plants were grown in the substrate with sufficient moisture (Figure 3.2.2.2A 80% SM). Pea spraying with solutions containing 12.5 and 25 ppm CuO NPs reduced TPC content by 17 and 10%, respectively. A significant increase in ABTS free radical scavenging activity was found after peas were watered with 12.5 ppm and sprayed with 50 ppm CuO NPs solutions (Figure 3.2.2.2C 80% SM). However, inhibition of ABTS free radical scavenging activity was found after watering peas with 25 and 50 ppm or after spraying with 12.5 and 25 ppm CuO NPs compared with control plants.

DPPH free radical scavenging activity decreased by 36 and 13% as peas were irrigated with 12.5 and 50 ppm CuO NPs suspensions, respectively (Figure 3.2.2.2B 80% SM). However, significant stimulation of DPPH free radical scavenging activity was determined after peas were sprayed with concentrations of 25 and 50 ppm CuO NPs. FRAP antioxidant power in pea leaves was strongly affected (Figure 3.2.2.2D 80% SM). It increased 1.7 and 3 times after watering peas with 12.5 and 25 ppm CuO NPs and increased up to 2 times when peas were sprayed with CuO NPs. A decreased value of FRAP was found in peas watered with 50 ppm CuO NPs suspension.

Although more positive indicators were found in pea plants exposed to drought stress and CuO NPs application. For instance, TPC content in pea leaves increased when peas were watered or sprayed with any CuO NPs concentration (Figure 3.2.2.2A 30% SM). In addition, the activity of DPPH free radical scavenging in pea leaves increased with increasing CuO NPs concentration after watering or spraying (Figure 3.2.2.2B 30% SM). ABTS free radical scavenging activity was stimulated up to 70% depending on applied concentration after irrigation and by 50 and 48% after spraying with 25 and 50 ppm CuO NPs solution (Figure 3.2.2.2C 30% SM). A significant reduce in ABTS free radical scavenging activity was found by spraying plants with 12.5 ppm CuO NPs. A positive effect on the FRAP antioxidant power in

pea leaves was observed under all applied CuO NPs concentrations within any application manner (Figure 3.2.2.2D 30% SM).

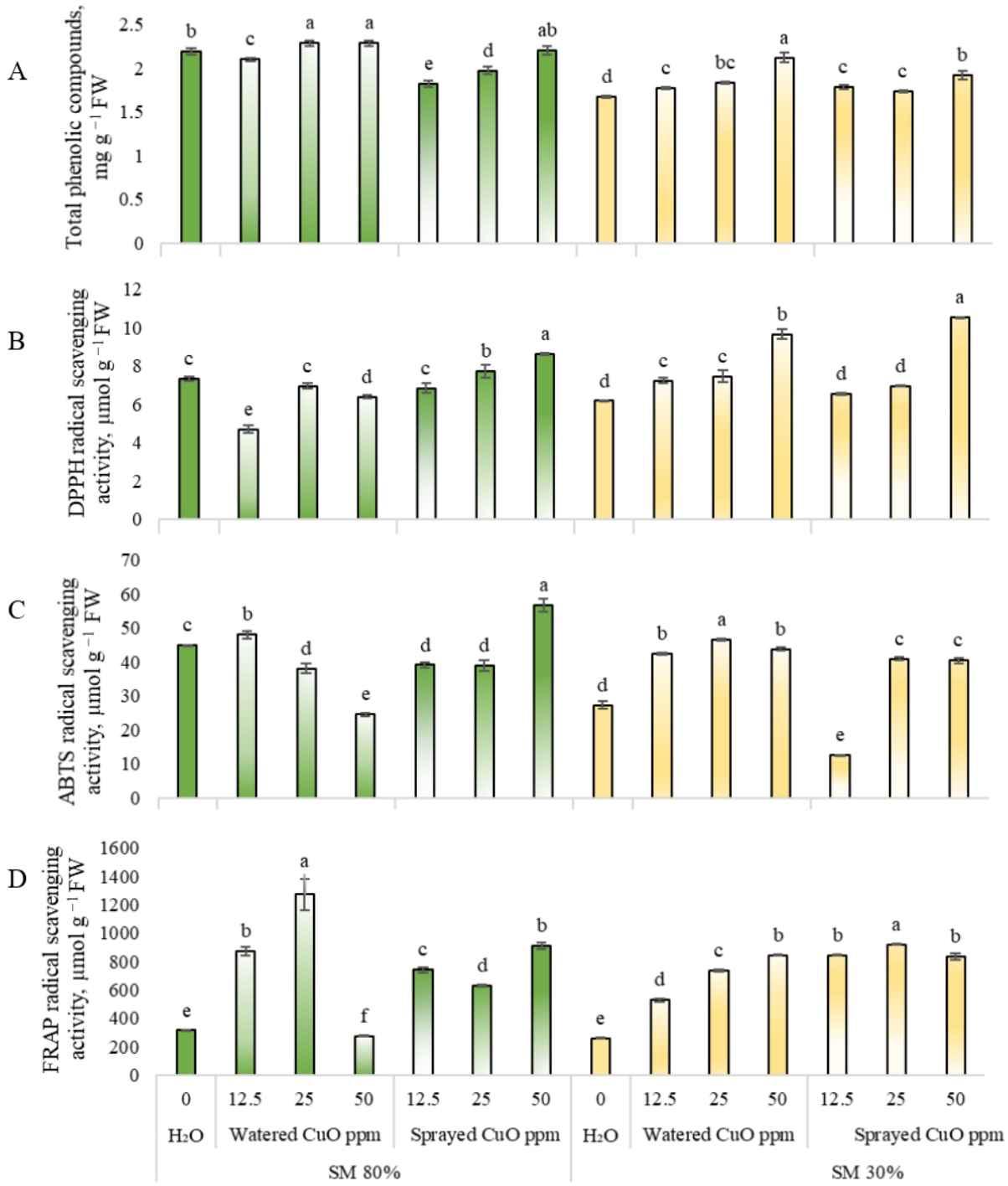


Figure 3.2.2.2. Influence of drought stress and CuO NPs (0; 12,5; 25; 50 ppm) on A – total phenolic compounds, B – DPPH free radical scavenging activity, C – ABTS free radical scavenging activity D – FRAP antioxidant power in P. sativum L. H_2O – control plants, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Effects on enzymatic antioxidants

A robust inhibitory effect on CAT and GR activity in pea leaves was found when the plant grew in the substrate with sufficient moisture content (Figure 3.2.2.3A, C 80% SM). CAT activity was reduced by 57, 37, and 63% when peas irrigation with 12.5, 25, and 50 ppm CuO NPs suspensions and by 48, 68, and 65% when sprayed with the same concentrations. Also, GR activity decreased by 37, 27, and 17% when peas were irrigated with 12.5, 25, and 50 ppm CuO NPs, respectively. An inhibitory effect on GR activity was found after spraying plants with concentrations of 25 and 50 ppm CuO NPs. The activity promotion was found for the APX enzyme (Figure 3.2.2.3B 80% SM), as it increased by 3.6, 5.6, and 1.7 times after watering and 4.6, 3.2, and 3 times after spraying with concentrations of 12.5, 25, and 50 ppm CuO NPs respectively. GPX activity (Figure 3.2.2.3E 80% SM) was significantly increased up to 94% after watering plants with any concentration solution, while spraying with CuO NPs had a significant effect only using the solution at 25 ppm concentration. SOD activity (Figure 3.2.2.3D 80% SM) was stimulated by watering peas with concentrations of 25 and 50 ppm and spraying with 12.5 ppm CuO NPs.

The negative effect remained on GR activity in pea leaves (Figure 3.2.2.3C 30% SM) during drought and CuO NPs exposure, reducing it up to 93% depending on applied solution concentration. An inhibitory effect on GPX activity (Figure 3.2.2.3E 30% SM) was also found when peas were sprayed with 12.5, 25, and 50 ppm and watered with 12.5 ppm CuO NPs. A stimulation of GPX activity (Figure 3.2.2.3E 30% SM) was determined only with watering exposure to 25 ppm CuO NPs. CAT activity (Figure 3.2.2.3A 30% SM) was positively influenced up to 1.8 times within any applied CuO NPs concentration. In addition, APX activity (Figure 3.2.2.3B 30% SM) was also significantly stimulated after peas were exposed to CuO NPs. Irrigation of peas with CuO NPs significantly increased SOD activity (Figure 3.2.2.3D 30% SM) up to 33%, depending on the used suspension concentration, and in addition, spraying with 50 ppm induced SOD activity by 53%. However, spraying with 12.5 ppm of CuO NPs had no significant effect but spraying with 25 ppm solution significantly reduced SOD activity.

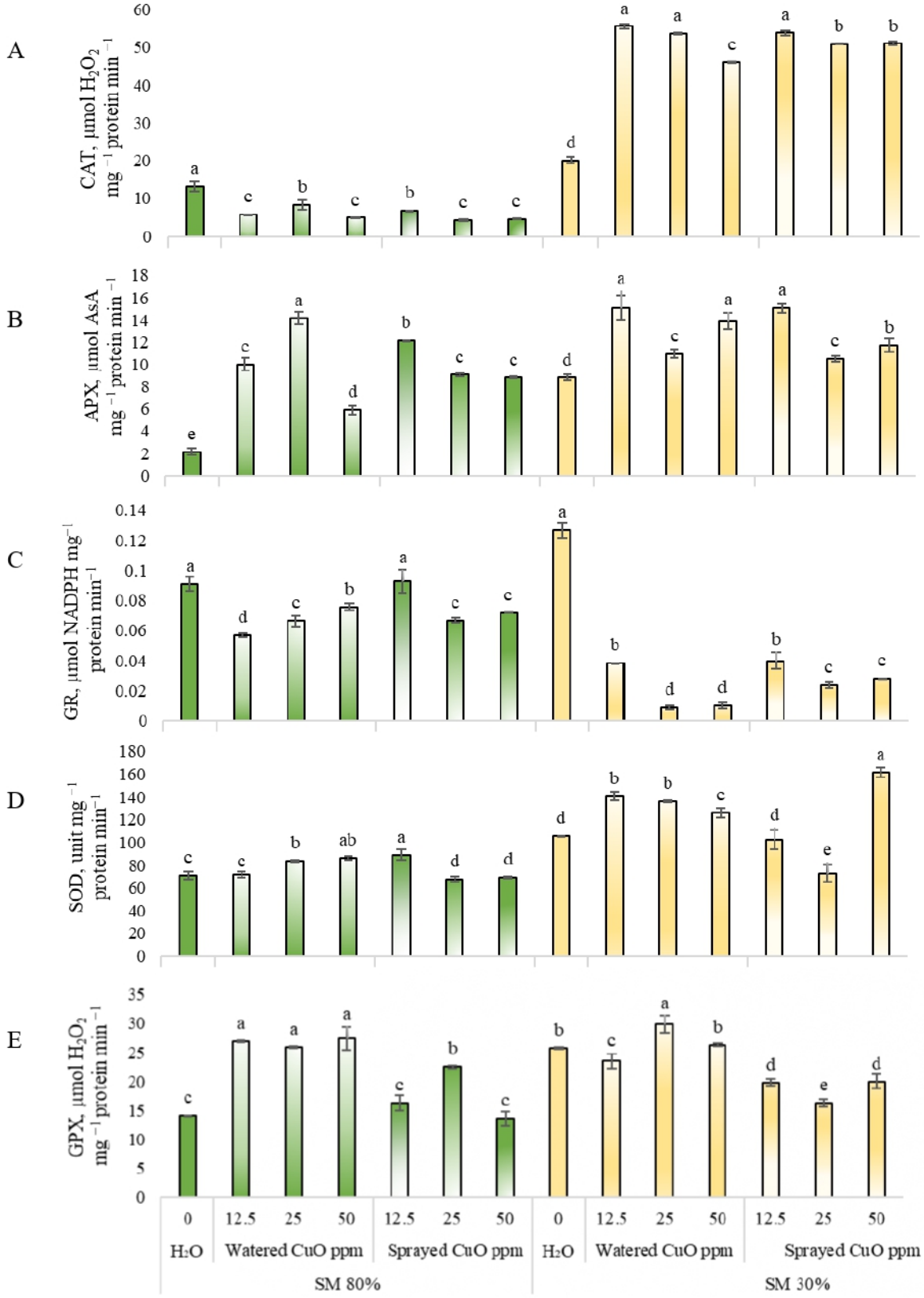


Figure 3.2.2.3. Response of A – ascorbate peroxidase (APX), B –catalase (CAT), C –superoxide dismutase (SOD), D –glutathione reductase (GR), and E –guaiacol peroxidase (GPX) activity to drought stress and CuO NPs (0; 12,5; 25; 50 ppm) in P. sativum L. H_2O – control plants sprayed or watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Changes in macro- and microelement composition

Cu content in pea leaves grown at 80% SM increased by 24% after irrigating peas with a concentration of 50 ppm (Table 3.2.2.2). Cu content in pea leaves increased with increasing CuO NPs concentration: 12.5 ppm by 49%, 25 ppm – 112%, and 50 ppm – 233%, the same trend was observed for Cu accumulation in pea stems. An increase in the amount of Cu by 59, 101, and 159% in the roots was found after pea irrigation with 12.5, 25, and 50 ppm CuO NPs suspensions, but an even higher accumulation of 77, 113, and 230% of Cu were found after spraying.

Irrigation with CuO NPs did not cause significant Cu content differences in drought-affected pea plant leaves. However, the increase in Cu content by 14, 35, and 55% in the leaves was found after spraying the drought-affected peas with suspensions at 12.5, 25, and 50 ppm concentrations. In addition, Cu accumulated more in the roots after irrigation compared to the Cu amount in roots not affected by NPs. Spraying with CuO NPs had a positive effect on the accumulation of Cu in all parts of the pea, and it is also worth noting that the amount of Cu increased with the increasing concentration of CuO NPs. Cu content increased in leaves up to 4 times, stems up to 3.5, and roots up to 1.7 times.

The Ca concentration in pea leaves increases during their growth with 80% SM and after spraying or watering at a concentration of 50 ppm. After drought exposure, the Ca content increased when peas were irrigated with 25 ppm and sprayed with 25 and 50 ppm suspensions. The amount of Fe increased when the plants were exposed to CuO NPs suspension with a concentration of 50 ppm. CuO NPs significantly increased the Fe, Zn, and Mn content in roots.

Table 3.2.2.2. Effects of CuO NPs suspension on macro- and microelements of peas leaves, stem, and root. Mean values in bold indicate a statistically significant difference at $p < 0.05$ according to Tukey (HSD) test (n=9)

Treatment CuO NPs			Macroelements, mg g^{-1} DW						Microelements, µg g^{-1} DW				
Leaves			P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Mo	B
SM 80%	Watered	0	3.79	18.76	45.73	9.20	2.64	19.86	25.71	42.54	10.32	2.99	3.58
		12.5	3.41	18.29	46.85	9.49	2.21	22.10	**33.14**	**54.10**	10.38	2.14	3.35
		25	**2.95**	16.35	**37.41**	8.17	2.06	20.53	**27.83**	**49.61**	9.14	**1.25**	4.28
		50	3.98	19.39	**48.84**	10.20	2.86	**31.06**	**30.50**	**56.97**	**12.82**	**1.54**	4.65
	Sprayed	12.5	3.41	18.29	46.85	9.49	2.21	22.10	**33.14**	**54.10**	**15.38**	2.14	4.19
		25	3.50	18.01	44.24	8.26	**2.02**	25.91	**30.49**	**49.19**	**21.88**	2.33	2.43
		50	**4.04**	20.45	**53.80**	10.15	**2.11**	**29.73**	**37.69**	**63.38**	**34.39**	2.27	3.87
SM 30%	Watered	0	3.21	20.56	46.19	8.81	2.07	20.03	37.24	37.65	10.02	0.70	1.30
		12.5	3.31	20.93	48.73	8.51	**1.68**	**27.86**	**31.14**	**50.62**	9.17	**1.50**	**2.60**
		25	3.39	**17.85**	**49.51**	9.81	2.55	**29.61**	**33.09**	**54.21**	9.60	**1.30**	1.96
		50	3.60	19.53	47.44	9.31	**1.66**	**60.76**	36.38	**64.81**	8.36	**1.16**	**2.72**
	Sprayed	12.5	**1.86**	18.31	44.51	8.02	**5.94**	18.17	**22.23**	34.62	**15.05**	**1.61**	**4.52**
		25	3.34	**16.45**	**42.35**	8.11	**1.59**	**23.62**	**30.74**	**50.56**	**25.40**	**1.26**	**3.98**
		50	3.14	21.33	**52.29**	**10.12**	2.15	**24.33**	34.36	**64.64**	**51.99**	**1.54**	**2.81**
Stem			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	2.00	17.77	37.81	8.71	7.73	15.49	19.81	26.09	10.08	3.66	1.05
		12.5	2.18	**20.12**	**47.97**	9.24	5.98	**19.44**	**22.37**	**34.92**	11.40	2.54	1.19
		25	1.67	17.29	37.26	7.57	6.41	**18.62**	**21.19**	**30.99**	10.07	3.03	1.11
		50	2.06	17.15	**46.37**	9.21	8.30	**20.21**	**27.08**	**33.66**	12.05	3.59	0.86
	Sprayed	12.5	1.77	**15.72**	**46.11**	8.55	6.01	**17.33**	**22.28**	**34.78**	**15.13**	3.86	1.53
		25	2.34	16.58	**41.67**	7.48	**4.93**	**20.95**	**26.60**	**34.47**	**18.92**	3.65	1.33
		50	2.10	**20.09**	**51.22**	9.58	7.98	**25.06**	**25.33**	**39.38**	**30.14**	**4.53**	1.72
SM 30%	Watered	0	1.43	20.13	38.10	7.98	6.83	15.46	22.31	24.19	8.92	1.17	10.57
		12.5	1.97	21.78	**46.83**	8.84	5.92	**21.48**	**29.72**	**33.69**	10.19	1.35	**5.90**
		25	2.19	**17.45**	**48.84**	**9.50**	7.05	**29.12**	**33.34**	**41.45**	**12.08**	1.38	**3.29**
		50	1.73	20.40	**42.92**	7.92	**7.56**	**34.94**	**39.52**	**36.46**	**13.81**	0.75	**4.39**

	Sprayed	12.5	1.86	18.31	**44.51**	8.02	5.94	**18.17**	22.23	**34.62**	**15.05**	1.61	**4.52**
		25	1.77	19.38	36.29	7.21	6.45	15.74	**27.02**	**27.93**	**16.91**	1.41	**6.40**
		50	1.91	**22.82**	**49.94**	**9.45**	**8.38**	**19.89**	**35.58**	**38.88**	**40.97**	1.55	**5.83**
Root			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%		0	0.81	16.16	10.25	6.91	3.18	258.88	15.88	46.44	18.46	9.40	2.68
	Watered	12.5	**2.13**	**36.49**	**25.45**	**15.18**	**7.28**	**781.52**	**40.59**	**135.20**	**29.29**	**12.33**	1.89
		25	**2.51**	**34.83**	**27.74**	**13.80**	**10.74**	**1002.32**	**51.54**	**173.10**	**37.18**	10.13	2.51
		50	**3.01**	**42.45**	**31.05**	**21.64**	**9.69**	**1571.45**	**84.47**	**278.69**	**47.73**	**12.22**	2.41
	Sprayed	12.5	**2.24**	**39.14**	**28.93**	**17.65**	**7.50**	**1057.82**	**48.79**	**180.70**	**32.67**	7.56	1.05
		25	**4.49**	**69.29**	**51.35**	**30.96**	**12.89**	**2134.70**	**104.78**	**382.71**	**39.25**	8.90	2.01
		50	**2.63**	**46.95**	**33.46**	**21.66**	**9.05**	**1201.86**	**61.77**	**205.61**	**60.94**	9.38	1.13
SM 30%		0	1.58	28.33	19.57	15.45	7.33	657.85	34.15	118.04	22.49	8.29	6.70
	Watered	12.5	**2.03**	**33.83**	**23.36**	14.06	7.53	**906.12**	**47.73**	**163.88**	**29.10**	6.72	5.19
		25	**2.85**	**37.31**	**29.91**	**18.00**	**9.11**	**1153.86**	**60.92**	**200.61**	**41.80**	8.41	**1.34**
		50	**3.21**	**39.42**	**31.21**	**18.90**	**10.67**	**1260.26**	**110.09**	**211.62**	**43.69**	8.73	**3.01**
	Sprayed	12.5	**2.72**	**40.68**	**29.84**	15.08	**9.72**	**1047.25**	**65.12**	**182.99**	**36.94**	**6.35**	**9.59**
		25	**3.93**	**67.11**	**44.51**	**22.27**	**15.83**	**1539.12**	**79.43**	**262.40**	**45.13**	**9.33**	**2.40**
		50	**3.09**	**43.97**	**39.53**	**19.78**	**9.67**	**1536.57**	**75.82**	**278.21**	**59.78**	**9.51**	**8.71**

Macroelements: P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, Na – sodium, Fe – iron; Microelements: Zn – zinc, Mn – manganese, Cu – copper, Mo – molybdenum, B – boron. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%.

Summary of the chapter (Table 3.2.2.3)

- *CuO NPs positively affected the antioxidant capacity and activated APX, SOD, and CAT enzymes but suppressed the activity of GPX and GR enzymes.*
- *CuO NPs significantly reduced H_2O_2 content but induced an MDA in pea leaves.*
- *The highest Cu amount was accumulated in the leaves of plants sprayed with 50 ppm NPs suspension as well as in the stem and roots*
- *CuO NPs suspensions were more effective when sprayed on plants than when watered.*

Table 3.2.2.3. The impact of drought stress and CuO NPs (12,5; 25; 50 ppm) on P. sativum L. grown in the substrate with sufficient (SM 80%) and insufficient (SM 30%) moisture is expressed as a percentage change (%) compared to the control (for SM 80% control means plants grown under SM 80% and NPs untreated; SM 30% control means drought affected but NPs untreated plants) in the heat map. Statistically, significant differences are marked in bold

Treatment CuO	SM 80%						SM 30%					
	Watered			Sprayed			Watered			Sprayed		
NPs, ppm	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Plant height	-2	0	-4	-6	-4	-8	5	0	**-9**	2	5	**-17**
Leaf area	-7	**-12**	**-13**	**-21**	**-19**	8	1	**-14**	**-18**	12	**-20**	-1
Nodules	20	-40	**220**	-60	**120**	**180**	-83	-17	**83**	17	-100	**283**
RWC	2	2	**-5**	-3	-3	**-4**	-1	-7	-15	-3	-4	-9
Root/shoot	25	-29	-1	62	-20	-36	22	-24	-24	-18	-9	-34
SLA	-26	-24	-17	-24	12	10	**-12**	**-13**	4	13	-7	-3
Yield	-4	-2	-1	-9	-11	-7	2	1	**27**	7	14	**47**
ABTS	**7**	**-15**	**-45**	**-13**	**-13**	**26**	**55**	**70**	**60**	**-54**	**50**	**48**
DPPH	**-36**	-5	**-13**	-7	**6**	**18**	**17**	**21**	**56**	6	13	**70**
TPC	**-4**	**4**	**4**	**-17**	**-10**	1	**6**	**10**	**27**	**7**	**4**	**15**
FRAP	**177**	**304**	**-13**	**136**	**100**	**190**	**103**	**182**	**224**	**224**	**253**	**220**
HP	**70**	**41**	**16**	**45**	**74**	**72**	**-20**	**-32**	**-23**	**-28**	**-35**	**-37**
MDA	**52**	**54**	**50**	**52**	**77**	**81**	**66**	**51**	**27**	**61**	**62**	**38**
GR	**-37**	**-27**	**-17**	2	**-27**	**-21**	**-70**	**-93**	**-92**	**-68**	**-81**	**-78**
GPX	**90**	**83**	**94**	15	**59**	-4	**-9**	**16**	2	**-23**	**-37**	**-22**
APX	**364**	**556**	**173**	**463**	**320**	**308**	**72**	**25**	**58**	**71**	**19**	**33**
SOD	1	**18**	**21**	**25**	**-5**	**-3**	**33**	**29**	**20**	-3	**-31**	**53**
CAT	**-57**	**-37**	**-63**	**-48**	**-68**	**-65**	**176**	**166**	**128**	**167**	**153**	**153**
Cu (leaves)	1	-11	**24**	**49**	**112**	**233**	-8	-4	-17	**50**	**153**	**419**
Cu (stem)	**13**	0	**19**	**50**	**88**	**199**	**14**	**35**	**55**	**69**	**89**	**359**
Cu (roots)	**59**	**101**	**159**	**77**	**113**	**230**	**29**	**86**	**94**	**64**	**101**	**166**

RWC – relative water content, SLA – specific leaf area, TPC – total phenolic compounds, HP – hydrogen peroxide, MDA – malondialdehyde, GR – glutathione reductase, GPX – guaiacol peroxidase, APX – ascorbate peroxidase, SOD – superoxide dismutase, CAT – catalase, Cu content in leaves, stem, and root. 0 – control plants watered with deionized water, drought stress – 30% substrate moisture.

3.2.3 Effects of molybdenum trioxide nanoparticle on peas under different substrate moisture

Impact on morphological parameters

Molybdenum trioxide nanoparticle (MoO_3 NPs) suspensions significantly affected the morphological parameters of peas grown under 80% substrate moisture (Table 3.2.3.1). Pea height increased after watering with MoO_3 NPs suspensions of any concentration. The suspension of 50 ppm concentration induced leaf area formation in both watering (25%) and spraying (15%). The RWC in plants was positively affected by MoO_3 NPs, and the most significant increase was found after watering and spraying peas at 50 ppm. The ratio of root to shoot increased by 22% when peas were watered with a 50 ppm concentration of MoO_3 NPs suspension. In addition, 33 and 11% increase was found after spraying with 12.5 and 50 ppm concentration. The SLA was reduced by 28% after peas grown in the substrate with sufficient moisture were watered with 12.5 ppm. SLA reduction of up to 30% was found after spraying with 12.5 and 50 ppm suspensions. MoO_3 NPs concentrations of 25 and 50 ppm strongly influenced the formation of nodules on the roots, as their number was 3 and 5.6 times higher after watering and 1.4 times higher after spraying with 50 ppm compared to the control plants. Yield increased significantly after irrigating peas with 12.5, 25, and 50 ppm MoO_3 solution. A significant increase in yield was observed in plants treated through leaves with 50 ppm MoO_3 and grown under 80% substrate moisture.

MoO_3 NPs effectively reduced the effects of drought on pea morphological parameters (Table 3.2.3.1, 30 % SM). The height of peas increased up to 40% after watering and up to 24% after spraying with MoO_3 NPs solutions. The formation of leaf area increased when drought-affected peas were watered with a suspension of 50 ppm MoO_3 NPs by 30%. Spraying the suspensions of MoO_3 NPs increased leaf area up to 12%. RWC in peas increased by 10% when 50 ppm MoO_3 NPs were applied through roots. The reducing effects of MoO_3 NPs were found on SLA at all concentrations. A decrease of 30% was determined after watering and up to 25% after spraying. Even during drought conditions, a positive effect of MoO_3 NPs on pea root nodule number was recorded. An increase of 1.2 to 5 times was found when peas were watered with 25 and 50 ppm and 1.2 – 2 times when spraying with MoO_3 NPs. The pea yield showed a strong dependence on the increasing concentration of MoO_3 NPs during drought. It increased by 11, 26, and 80% after irrigation with 12.5, 25, and 50 ppm suspensions of MoO_3 NPs, and after spraying increased by 3, 15, and 64%, respectively.

Table 3.2.3.1. Impact of drought stress and MoO_3 NPs (12,5; 25; 50 ppm) on P. sativum L. height, leaf area, specific leaf area (SLA), relative water content (RWC), root-to-shoot ratio, and the number of nodules. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Mean values within columns followed by different letters differ significantly at $p < 0.05$ (n=10) according to Tukey (HSD) test

		MoO_3 NPs, ppm	Plants height, cm	Leaf area, cm^2	SLA, $m^2\ kg^{-1}$	RWC, %	Root/shoot ratio	Number of nodules	Yield, $t\ ha^{-1}$
SM 80%	Watered	0	28.4 d	36.1 bcd	5.3 a	82.5 e	7.8 b	1.7 c	3.9 b
		12.5	30.1 c	35.0 cd	3.8 bc	86.4 cd	7.6 bc	2.3 c	4.1 a
		25	34.4 b	41.3 abc	4.2 abc	87.2 bc	7.9 b	7.0 b	4.1 a
		50	35.5 a	45.2 a	4.0 abc	88.9 a	9.4 ab	11.0 a	4.1 a
	Sprayed	12.5	30.5 c	32.9 d	3.5 c	85.1 d	10.3 a	2.3 c	3.9 b
		25	29.8 c	36.7 bcd	5.0 ab	86.8 c	6.6 c	2.0 c	4.0 ab
		50	33.4 b	41.6 ab	3.7 bc	88.5 ab	8.6 ab	4.0 b	4.2 a
SM 30%	Watered	0	26.0 f	33.1 cd	5.0 a	53.0 b	9.2 ab	2.0 b	2.5 c
		12.5	28.5 d	30.8 d	4.4 ab	60.1 ab	5.1 b	1.0 b	2.8 ab
		25	30.6 c	30.4 d	3.5 b	58.0 ab	9.2 ab	4.3 a	3.2 a
		50	36.3 a	43.1 a	3.6 b	64.4 a	11.3 ab	12.7 a	4.5 a
	Sprayed	12.5	27.0 e	37.1 b	4.4 ab	56.7 b	12.5 a	1.7 b	2.6 b
		25	28.6 d	36.3 b	4.1 ab	56.7 b	8.4 ab	4.3 a	2.9 ab
		50	32.2 b	36.3 b	3.8 b	58.5 ab	10.7 ab	5.7 a	4.2 a

Effects on oxidative stress biomarkers

H_2O_2 content in normal substrate moisture (SM 80%) grown pea leaves was promoted from 1.2 to 2 times after pea application with 12.5, 25, and 50 ppm MoO_3 NPs suspensions. MoO_3 NPs promoted lipid peroxidation in pea plants grown under SM 80% (Figure 3.2.3.1B 80% SM). This is shown by the MDA concentration increase in peas when they were exposed to a concentration of 12.5 and 50 ppm by watering and by spraying with 12.5 and 25 ppm MoO_3 NPs.

In drought-affected peas, H_2O_2 content decreased by 8, 12, and 20% when plants were irrigated with 12.5, 25, and 50 ppm MoO_3 NPs suspensions, but MDA concentration increased by 9, 11, and 25% (Figure 3.2.3.1A, B 30% SM). Spraying with 12.5, 25, and 50 ppm MoO_3 NPs suspensions similarly reduced H_2O_2 content in pea leaves. The reduced MDA content was determined in peas sprayed with 50 ppm MoO_3 NPs suspension compared to plants grown in drought conditions and untreated with NPs.

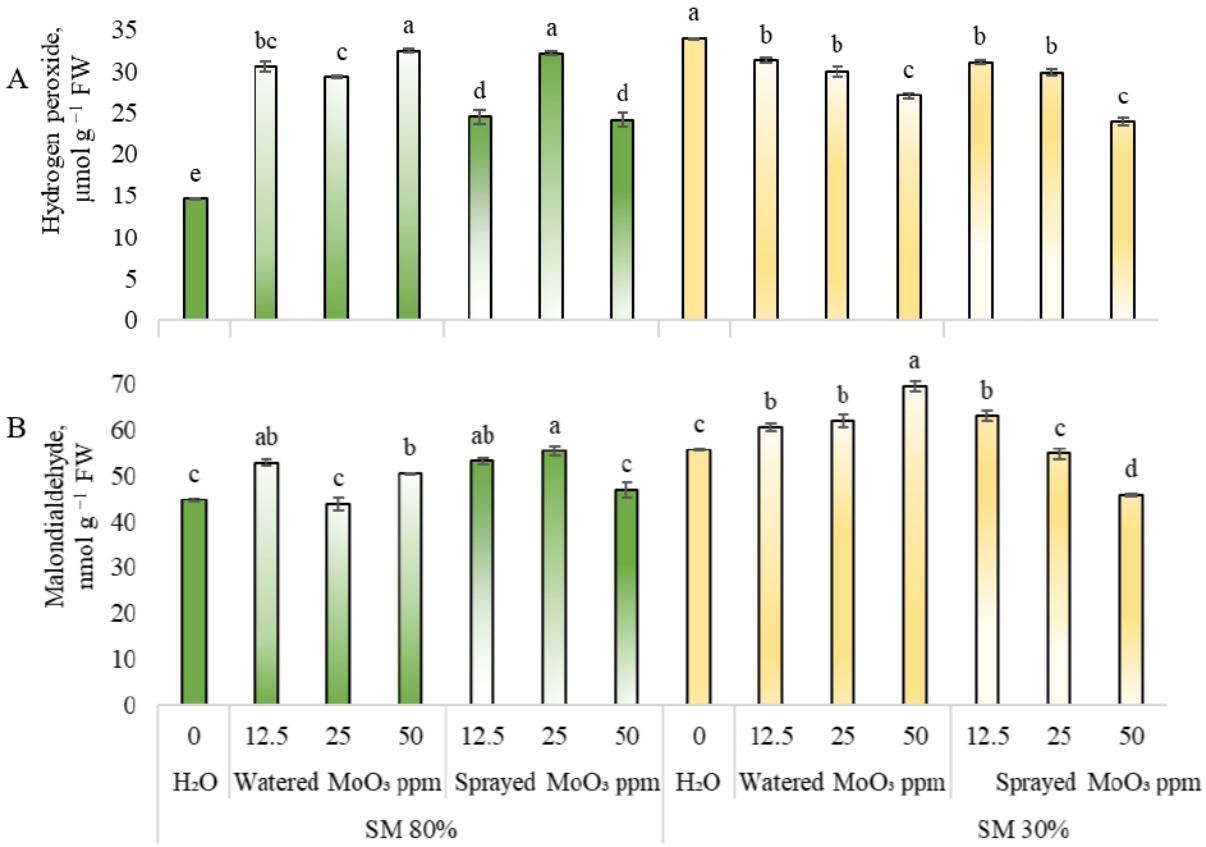


Figure 3.2.3.1. Influence of drought stress and MoO_3 NPs (0; 12,5; 25; 50 ppm) on hydrogen peroxide and malondialdehyde content in P. sativum L. H_2O – control plants watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Effects on non-enzymatic antioxidants

MoO_3 NPs have an ambiguous effect on plant non-enzymatic antioxidants in peas growing at 80% SM (Figure 3.2.3.2). The amount of TPC (Figure 3.2.3.2A 80% SM) decreased by 20% after 12.5 ppm MoO_3 NPs exposure through the roots, and after foliar application with 12.5 and 25 ppm, the amount of TPC decreased by 23 and 15%. In pea leaves, FRAP antioxidant power (Figure 3.2.3.2D 80% SM) increased significantly after watering or spraying with MoO_3 NPs solution of any concentration. Irrigation of peas with 25 and 50 ppm MoO_3 NPs solutions influenced the stimulation of DPPH free radical scavenging activity by 27 and 36%, respectively. Spraying with 25 ppm suspension of MoO_3 NPs caused a 7% decrease in DPPH free radical scavenging activity (Figure 3.2.3.2B 80% SM). ABTS free radical scavenging activity (Figure 3.2.3.2C 80% SM) was inhibited by watering peas with 12.5 ppm by 11% and by spraying with 25 ppm - 8%.

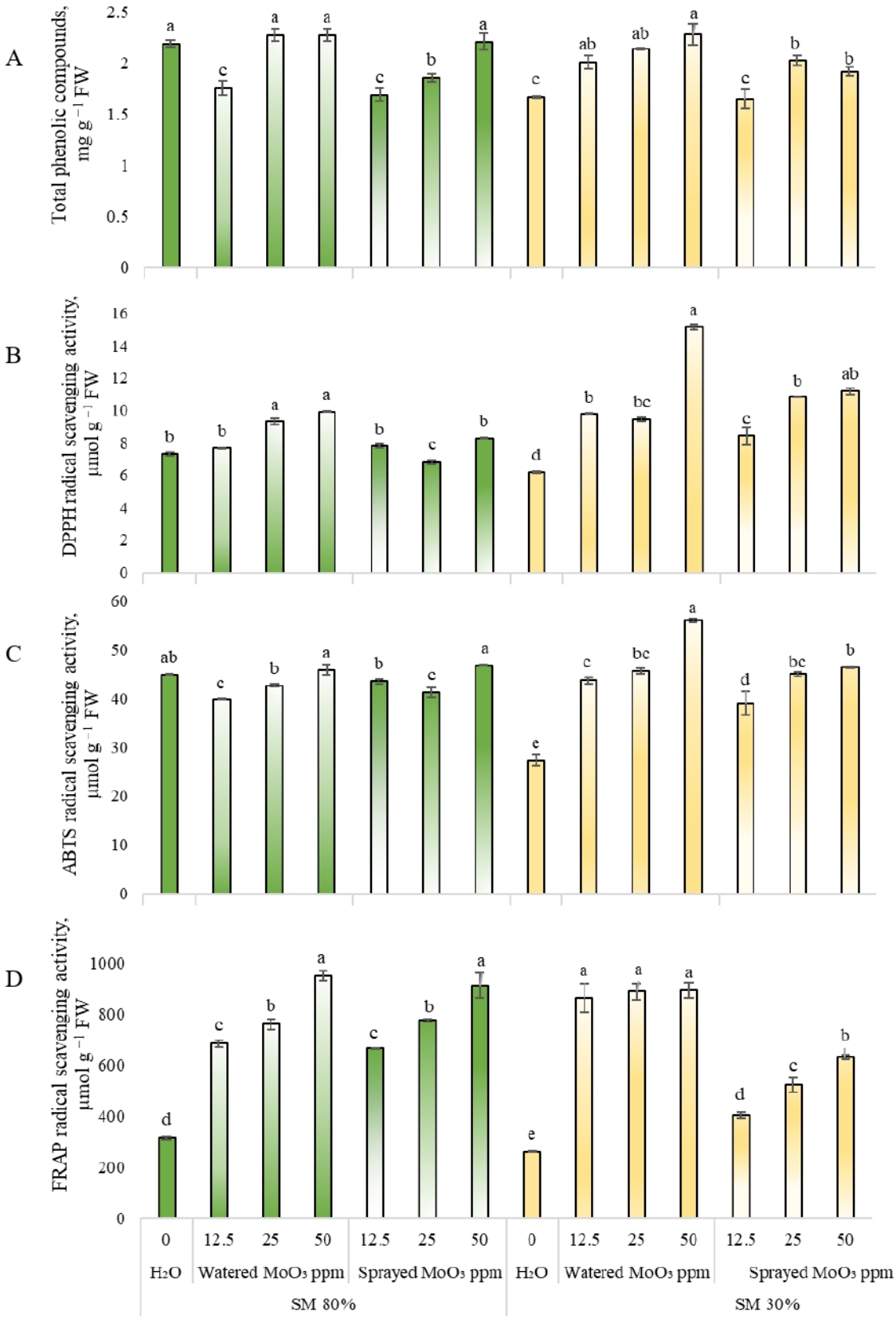


Figure 3.2.3.2. Influence of drought stress and MoO_3 NPs (0; 12,5; 25; 50 ppm) on A – total phenolic compounds, B – DPPH free radical scavenging activity, C – ABTS free radical scavenging activity, D – FRAP antioxidant power in P. sativum L. H_2O – control plants, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

The effects of drought (Figure 3.2.3.2, 30% SM) and MoO_3 NPs on the non-enzymatic antioxidants of peas were significant. The amount of TPC (Figure 3.2.3.2A 30%SM) in drought-affected peas was increased up to 37% after exposure with MoO_3 NPs through roots and up to 15% after spraying. The activity of the ABTS free radical scavenging (Figure 3.2.3.2C 30% SM) was particularly enhanced. It increased by 60, 67, and 105% in plants exposed to 12.5, 25, and 50 ppm MoO_3 NPs through roots. Also, foliar exposure to MoO_3 NPs increased ABTS free radical scavenging activity by 43, 65, and 70% at the above concentrations. The results show that exposure through roots to MoO_3 NPs induced DPPH free radical scavenging activity (Figure 3.2.3.2B 30%SM) up to 145% and through leaves up to 81%. The positive influence of MoO_3 NPs of any concentration was significant for FRAP antioxidant power in pea leaves.

Effects on enzymatic antioxidants

A strong effect of MoO_3 NPs on the activity of enzymatic antioxidants was found in plants grown at 80% substrate moisture (Figure 3.2.3.3). CAT activity (Figure 3.2.3.3A 80%SM) increased 41% after spraying with 12.5 ppm MoO_3 NPs suspension. Concentrations of 12.5, 25, and 50 ppm MoO_3 NPs caused significantly higher APX activity (Figure 3.2.3.3B 80% SM) after watering or spraying pea plants. In contrast, the GR activity decreased after watering or spraying with MoO_3 NPs solution of any concentration. MoO_3 NPs strongly induced GPX activity in pea leaves depending on applied concentration and application manner (Figure 3.2.3.3E 80% SM).

Drought exposure and MoO_3 NPs stimulated APX activity in pea leaves (Figure 3.2.3.3B 30% SM). After peas' exposure to 12.5, 25, and 50 ppm MoO_3 NPs, from 2 to 6 times increase in APX activity was found, and after exposure through leaves – 2 – 4 times. Similar results were found for CAT activity in pea leaves during drought stress (Figure 3.2.3.3A 30% SM). An increase in CAT activity of 2 times was found after watering peas with 12.5, 25, and 50 ppm MoO_3 NPs while spraying promoted CAT activity from 1.6 to 1.9 times. Pea plant treatment with 12.5, 25, and 50 ppm MoO_3 NPs trough roots inhibited GR activity in leaves by 56, 57, and 31% and foliar exposure by 88, 79, and 71%, respectively. GPX activity (Figure 3.2.3.3E 30% SM) in drought-affected peas stimulated the irrigation with suspensions of 25 and 50 ppm by 43 and 55%, respectively. GPX activity increased by 17 and 56% after plants were sprayed with 12.5 and 50 ppm suspensions of MoO_3 NPs. SOD activity (Figure 3.2.3.3D 30% SM) was inhibited when peas were watered with 25 ppm and increased with the application of 50 ppm MoO_3 NPs by watering or spraying.

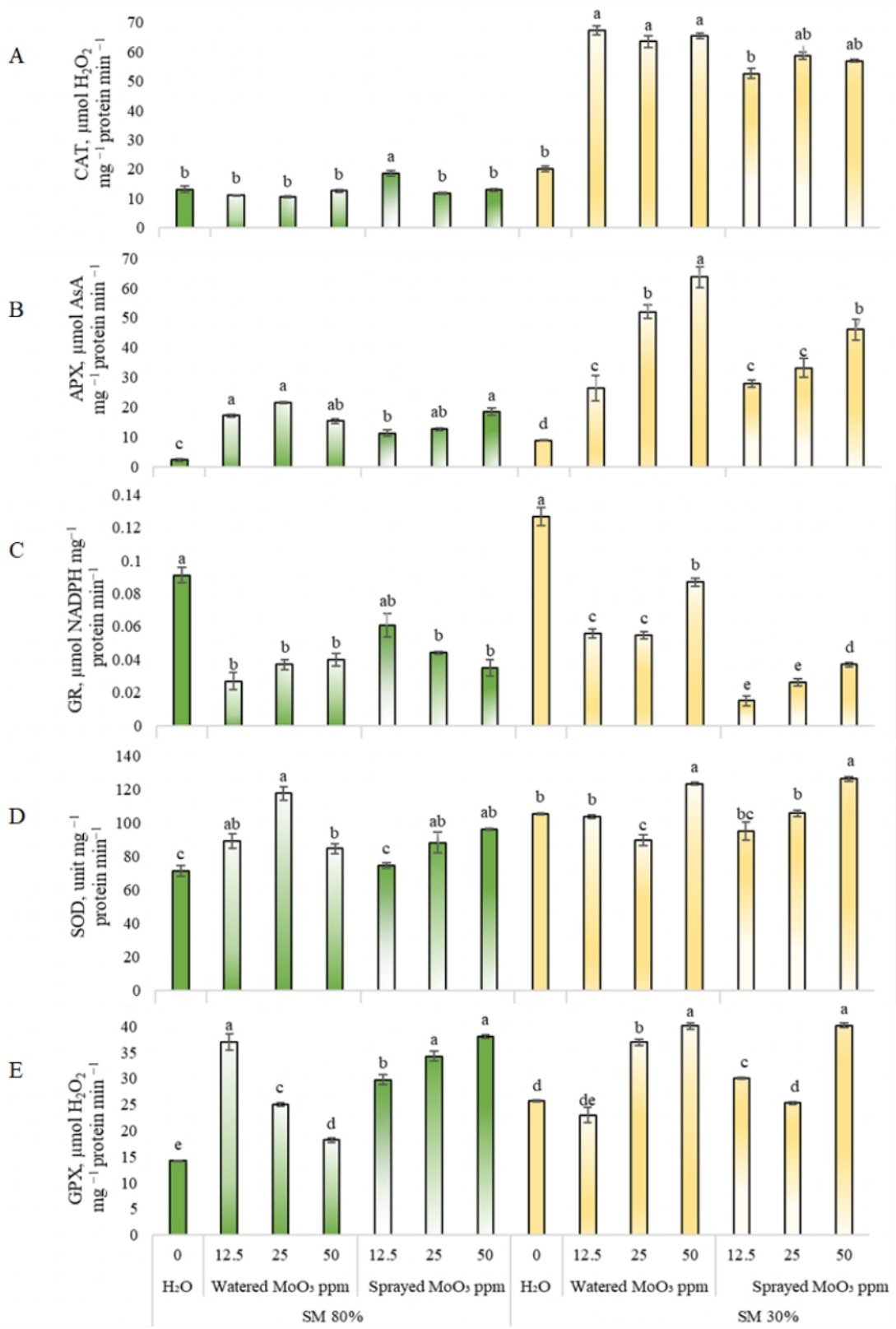


Figure 3.2.3.3. Response of A – ascorbate peroxidase (APX), B –catalase (CAT), C –superoxide dismutase (SOD), D –glutathione reductase (GR), and E –guaiacol peroxidase (GPX) activity to drought stress and MoO_3 NPs (0; 12,5; 25; 50 ppm) in P. sativum L. H_2O – control plants sprayed or watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Changes in macro- and micro-elemental composition

The content of Mo in pea leaves increased with increasing concentration of MoO_3 NPs in suspension (Table 3.2.3.2). For instants, in pea leaves grown under normal conditions (SM 80%), up to 43% increase in Mo amount was found after MoO_3 NPs exposure through roots, and up to 47% after foliar treatment. A similar tendency was observed for the accumulation of Mo content in the stem. Mo content increased up to 34% in pea stems when plants were treated through roots, and an even more substantial increase in Mo content up to 46% was found when plants were sprayed. Mo accumulated 2-3.6 times more in the roots when peas were irrigated with MoO_3 NPs and when they were sprayed 1.3-1.8 times.

Exposure to drought and MoO_3 NPs significantly increased the Mo content in pea leaves and stems. Watering with MoO_3 NPs suspensions was influenced to accumulate 1.6 - 3 times more Mo in leaves, while spraying increased Mo content up to 4.5 times. In addition, a higher Mo accumulation was observed in the stem after spraying the plants with MoO3 NPs.

Furthermore, MoO_3 NPs had a strong effect in reducing Ca accumulation in pea leaves and stems when they were grown under 80% SM. Accumulation of Mg, Na, and Fe also decreased in stems. However, in the roots, after watering and spraying with MoO_3 NPs, a positive effect on all elements was found.

Table 3.2.3.2. Effects of MoO_3 NPs suspension on macro- and microelements of peas leaves, stem, and root. Mean values in bold indicate a statistically significant difference at $p < 0.05$ according to Tukey (HSD) test (n=9)

Treatment MoO_3 NPs, ppm			Macroelements, mg g^{-1} DW						Microelements, µg g^{-1} DW				
Leaves			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	3.79	18.76	45.73	9.20	2.64	19.86	25.71	42.54	10.32	2.99	3.58
		12.5	3.93	19.33	**42.80**	8.58	2.26	18.35	24.90	41.32	**7.50**	**3.40**	3.36
		25	3.63	17.55	**40.75**	8.13	2.26	20.82	**26.45**	**46.29**	**12.77**	**3.79**	**4.96**
		50	**5.48**	19.16	**37.28**	**7.67**	**1.43**	**17.34**	**26.56**	41.23	9.68	**4.27**	**4.53**
	Sprayed	12.5	3.93	19.33	**42.80**	8.58	2.26	18.35	24.90	41.32	**7.50**	**3.40**	3.36
		25	2.66	**13.74**	**32.55**	**7.62**	2.60	**15.16**	**24.01**	41.47	9.71	**3.76**	**4.77**
		50	3.95	18.47	**40.40**	8.25	2.10	18.11	**24.00**	45.51	**11.62**	**4.40**	**4.65**
SM 30%	Watered	0	3.21	20.56	46.19	8.81	2.07	20.03	37.24	37.65	10.02	0.70	2.92
		12.5	3.31	**15.47**	**35.02**	**6.58**	**1.23**	**14.95**	**23.90**	38.54	8.58	**1.83**	**4.80**
		25	3.78	**18.30**	45.14	8.98	2.16	20.75	**30.10**	**49.45**	11.27	**2.36**	**4.42**
		50	**5.52**	21.83	**44.86**	8.87	**1.55**	20.00	**33.79**	**48.59**	**12.45**	**2.89**	**4.84**
	Sprayed	12.5	2.27	21.20	**41.08**	8.54	**6.33**	**12.18**	**15.62**	**29.62**	8.96	0.51	**3.20**
		25	3.72	**18.43**	46.85	8.66	1.82	21.55	**33.70**	**53.09**	**12.75**	**3.67**	**4.17**
		50	3.92	**18.81**	47.51	8.70	**1.49**	22.01	**30.96**	**53.51**	**14.02**	**3.90**	**3.63**
Stem			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	2.00	17.77	37.81	8.71	7.73	15.49	19.81	26.09	10.08	3.66	1.05
		12.5	**1.62**	**15.40**	**34.36**	**6.78**	**4.22**	**13.28**	**13.40**	**24.80**	9.26	4.02	2.03
		25	**1.53**	**15.50**	36.53	**7.30**	**6.24**	**11.57**	**17.93**	25.31	**8.37**	**4.91**	**2.25**
		50	2.14	**15.46**	**26.60**	**5.77**	**4.74**	**9.31**	**12.47**	**20.17**	**7.19**	**4.89**	**2.79**
	Sprayed	12.5	**2.61**	**19.49**	**43.13**	8.73	**5.37**	**13.45**	**21.52**	**33.23**	10.06	**4.73**	0.80
		25	**1.14**	**13.24**	**31.40**	**6.70**	**6.22**	**9.74**	**13.13**	**23.71**	**6.78**	**5.28**	**1.61**
		50	1.70	**14.31**	**33.33**	**7.01**	**5.78**	**11.22**	**12.00**	**21.96**	**8.83**	**5.36**	**2.78**
SM 30%	Watered	0	1.43	20.13	38.10	7.98	6.83	15.46	22.31	24.19	8.92	1.17	2.11
		12.5	1.56	**15.47**	**28.56**	**5.88**	**3.94**	**8.34**	**11.79**	**19.51**	**6.15**	**3.53**	2.51
		25	**1.83**	**18.69**	38.74	8.32	6.92	**13.09**	**18.92**	**28.34**	8.33	**3.58**	**1.61**
		50	**2.43**	20.78	37.50	7.84	**5.49**	**11.96**	**16.20**	**26.29**	8.27	**3.70**	2.34

	Sprayed	12.5	**2.27**	**21.20**	**41.08**	8.54	6.33	**12.18**	**15.62**	**29.62**	8.96	**2.51**	2.14
		25	1.53	**18.87**	**41.54**	7.87	**6.29**	**14.26**	**17.46**	**29.51**	**10.12**	**2.61**	**1.70**
		50	**1.95**	**18.78**	**42.06**	7.55	**4.27**	**18.25**	**17.84**	**30.52**	**10.02**	**2.77**	**1.61**
Root			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%		0	0.81	16.16	10.25	6.91	3.18	258.88	15.88	46.44	18.46	9.40	2.68
	Watered	12.5	**2.65**	**30.54**	**26.31**	**19.02**	**6.09**	**1542.53**	**53.70**	**269.59**	**16.85**	**28.18**	**6.05**
		25	**1.74**	**24.06**	**18.78**	**13.48**	**4.74**	**1085.63**	**38.42**	**185.89**	**16.27**	**29.43**	2.92
		50	**2.60**	**26.79**	**20.12**	**13.39**	**5.66**	**1098.77**	**44.99**	**192.17**	19.96	**43.19**	2.30
	Sprayed	12.5	**2.65**	**35.00**	**26.38**	**17.11**	**6.88**	**777.16**	**39.92**	**134.38**	**21.16**	**21.63**	**5.37**
		25	**1.71**	**24.34**	**21.29**	**12.18**	**6.44**	**756.67**	**32.37**	**131.22**	**16.09**	**26.04**	**0.74**
		50	**2.26**	**27.44**	**22.20**	**13.67**	**5.80**	**947.33**	**38.04**	**162.72**	18.57	**26.69**	**0.30**
SM 30%		0	1.58	28.33	19.57	15.45	7.33	657.85	34.15	118.04	14.99	16.58	1.97
	Watered	12.5	1.76	**25.67**	**21.46**	**13.76**	**5.91**	**1123.38**	**48.53**	**206.49**	**18.28**	**20.12**	**3.02**
		25	1.52	**22.28**	18.62	**12.25**	7.10	**851.83**	**39.13**	**143.28**	**16.89**	**21.21**	**0.91**
		50	**2.89**	**38.30**	**28.11**	16.08	**10.07**	**847.52**	**48.35**	**152.10**	**20.37**	**28.07**	**4.28**
	Sprayed	12.5	**2.15**	28.40	**22.71**	**11.62**	**6.85**	**819.77**	35.10	**154.88**	**18.16**	**22.91**	1.54
		25	**2.27**	**35.22**	**27.72**	16.93	**7.92**	**1077.09**	**53.24**	**202.91**	**21.94**	**25.80**	**0.61**
		50	1.67	**25.03**	20.39	**12.45**	**5.46**	**1034.68**	**36.24**	**183.94**	**16.29**	**28.65**	1.37

Macroelements: P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, Na – natrium, Fe – iron; Microelements: Zn – zinc, Mn – manganese, Cu – copper, Mo – molybdenum, B – boron. 0 – control plants watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%.

Summary of the chapter (Table 3.2.3.3)

- *MoO_3 NPs suspension positively affected plant morphological parameters determined by the effective reduction of oxidative biomarkers, increased total phenolics, and non-enzymatic antioxidant activity under drought conditions.*
- *MoO_3 NPs had an effect in enhancing the activity of CAT, APX, SOD, and GPX but reducing the activity of GR under both drought and normal conditions.*
- *The highest accumulation of Mo was found in pea plants when they were watered with 50 ppm MoO_3 NPs suspension.*
- *Comparing the application methods, MoO_3 NPs through the roots have a more substantial effect on peas.*

Table 3.2.3.3. The impact of drought stress and MoO_3 NPs (12,5; 25; 50 ppm) on P. sativum L. grown in the substrate with sufficient (SM 80%) and insufficient (SM 30%) moisture is expressed as a percentage change (%) compared to the control (for SM 80% control means plants grown under SM 80% and NPs untreated; SM 30% control means drought affected but NPs untreated plants) in the heat map. Statistically, significant differences are marked in bold

Treatment MoO_3 NPs, ppm	SM 80%						SM 30%					
	Watered			Sprayed			Watered			Sprayed		
	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Plant height	**6**	**21**	**25**	**7**	**5**	**17**	**10**	**18**	**40**	**4**	**10**	**24**
Leaf area	-3	15	**25**	**-9**	2	**15**	-7	-8	**30**	**12**	**10**	**10**
Nodules	40	**320**	**560**	40	20	**140**	-50	**117**	**533**	-17	**117**	**183**
RWC	**5**	**6**	**8**	**3**	**5**	**7**	13	9	**21**	7	7	10
Root/shoot	-1	2	22	**33**	**-15**	11	-45	1	23	36	-8	16
SLA	**-28**	-22	-24	**-34**	-6	**-30**	-12	**-30**	**-29**	-12	-18	**-25**
Yield	**6**	**5**	**6**	1	-12	**7**	**11**	**26**	**80**	**3**	**15**	**64**
ABTS	**-11**	-5	2	-3	**-8**	4	**60**	**67**	**105**	**43**	**65**	**70**
DPPH	5	**27**	**36**	6	**-7**	13	**59**	**53**	**145**	**36**	**76**	**81**
TPC	**-20**	4	4	**-23**	**-15**	1	**20**	**28**	**37**	-1	**22**	**15**
FRAP	**117**	**141**	**202**	**111**	**146**	**190**	**231**	**241**	**242**	**55**	**100**	**142**
HP	**109**	**101**	**122**	**68**	**120**	**65**	**-8**	**-12**	**-20**	**-9**	**-12**	**-30**
MDA	**18**	-2	**13**	**19**	**24**	**5**	**9**	**11**	**25**	**13**	-1	**-17**
GR	**-70**	**-59**	**-56**	-33	**-52**	**-62**	**-56**	**-57**	**-31**	**-88**	**-79**	**-71**
GPX	**161**	**76**	**28**	**110**	**142**	**168**	-11	**43**	**55**	**17**	-2	**56**
APX	**692**	**899**	**607**	**423**	**481**	**748**	**198**	**490**	**622**	**216**	**276**	**422**
SOD	**25**	**65**	**19**	5	**24**	**35**	-2	**-15**	**17**	-10	0	**20**
CAT	-16	-19	-4	**41**	-10	-2	**234**	**215**	**224**	**161**	**191**	**183**
Mo (leaves)	**14**	**27**	**43**	**14**	**26**	**47**	**160**	**234**	**310**	**27**	**421**	**454**
Mo (stem)	**10**	**34**	**34**	**29**	**44**	**46**	**201**	**205**	**215**	**114**	**122**	**136**
Mo (roots)	**200**	**213**	**360**	**130**	**177**	**184**	**21**	**28**	**69**	**38**	**56**	**73**

RWC – relative water content, SLA – specific leaf area, TPC – total phenolic compounds, HP – hydrogen peroxide, MDA – malondialdehyde, GR – glutathione reductase, GPX – guaiacol peroxidase, APX – ascorbate peroxidase, SOD – superoxide dismutase, CAT – catalase, B content in leaves, stem, and root. 0 – control plants watered with deionized water, drought stress – 30% substrate moisture.

3.2.4 Effects of boron nanoparticle on peas under different substrate moisture

Impact on morphological parameters

Pea height increased by 14 and 27% when watered and by 28 and 19% when sprayed with 12.5 and 50 ppm B_2O_3 NPs suspension under sufficient substrate moisture (Table 3.2.4.1, 80% SM). Leaf area also significantly increased when plants were watered with 12.5 and 50 ppm B_2O_3 NPs suspensions while spraying increased it within any applied concentration. Furthermore, a positive effect of B_2O_3 NPs was found in RWC. At the same time, a decrease of 15% in SLA was observed after spraying with 12.5 ppm solution. Root to shoot ratio statistically reliably increased after watering plants at 12.5 ppm by 21%, 25 ppm - by 36%, and 50 ppm - by 68%. This shows that in plants grown at 80% substrate moisture, increasing the concentration of B_2O_3 nanoparticle suspension promotes root growth. In addition, an increase in the root-to-shoot ratio by 68% (12.5 ppm), 18% (25 ppm), and 34% (50 ppm) was observed when plants were sprayed. The results also show that B_2O_3 NPs positively affect the number of nodules on plant roots by increasing their amount up to 5.6 times when plants were watered and up to 3.4 times when plants were sprayed. The results showed that pea irrigation with 50 ppm B_2O_3 NPs had a significant positive effect on yield, while foliar treatment increased pea yield at suspensions containing 12.5 and 25 ppm B_2O_3 NPs.

B_2O_3 NPs strongly affected pea plants grown in drought conditions (Table 3.2.4.1 30% SM). Applied B_2O_3 NPs suspension with different concentrations increased plant height as they were watered or sprayed. Furthermore, watering the plants with 25 and 50 ppm B_2O_3 NPs suspensions increased the leaf area by 30 and 40%, respectively. There was a statistically significant increase in RWC at higher B_2O_3 NPs concentrations. The root-to-shoot ratio increased to 30% after watering drought-affected peas with B_2O_3 NPs solutions, while foliar application increased the ratio to 14%. 50 ppm B NPs concentration influenced the number of root nodules increasing it by 3 times during watering and up to 6 times during spraying. Irrigation with the suspension of 12.5 and 25 ppm B_2O_3 NPs positively affected pea yield. Additionally, spraying drought-stressed peas with 12.5, 25, and 50 ppm B_2O_3 NPs increased yield by 16%.

Table 3.2.4.1. Impact of drought stress and B_2O_3 NPs (12,5; 25; 50 ppm) on P. sativum L. height, leaf area, specific leaf area (SLA), relative water content (RWC), root-to-shoot ratio, and the number of nodules. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Mean values within columns followed by different letters differ significantly at $p < 0.05$ (n=10) according to Tukey (HSD) test

		B_2O_3 NPs, ppm	Plants height, cm	Leaf area, cm^2	SLA, $m^2\ kg^{-1}$	RWC, %	Root/shoot ratio	Number of nodules	Yield, $t\ ha^{-1}$
SM 80%	Watered	0	28.4 d	36.1 c	5.3 ab	82.5 d	7.8 c	1.7 d	3.9 b
		12.5	32.4 bc	46.1 a	6.1 a	84.3 c	9.4 b	11.0 a	3.1 c
		25	30.3 cd	39.2 bc	5.1 ab	86.1 ab	10.5 b	5.0 c	4.0 ab
		50	36.1 a	46.8 a	4.7 ab	86.8 ab	13.1 a	9.7 ab	4.7 a
	Sprayed	12.5	36.3 a	49.9 a	4.5 b	87.3 a	13.1 a	7.3 bc	4.4 a
		25	30.9 bcd	44.9 ab	5.0 ab	85.5 bc	9.2 b	5.7 c	4.3 a
		50	33.8 ab	44.6 ab	5.1 ab	85.6 bc	10.4 b	5.0 c	3.9 b
SM 30%	Watered	0	26.0 e	33.1 b	5.0 a	53.0 c	9.2 c	2.0 c	2.5 c
		12.5	28.4 d	27.5 b	4.2 ab	52.6 c	9.6 b	2.3 c	2.9 a
		25	32.7 a	42.9 a	4.8 a	58.3 ab	10.5 a	6.0 b	2.7 ab
		50	30.4 bc	46.4 a	4.2 ab	59.1 a	11.9 a	8.3 b	2.6 bc
	Sprayed	12.5	29.3 cd	31.6 b	4.4 ab	51.9 c	10.2 a	2.0 c	3.0 a
		25	31.2 ab	33.4 b	3.9 ab	55.2 bc	10.5 a	3.3 c	2.9 a
		50	29.6 cd	31.2 b	3.5 b	57.8 ab	9.6 b	13.7 a	2.9 a

Effects on oxidative stress biomarkers

The results show that exposure to B_2O_3 NPs through the roots increased the amount of H_2O_2 in plants, regardless of its concentration, when peas were grown under sufficient substrate moisture (Figure 3.2.4.1A 80% SM). When plants were sprayed, a statistically reliable 65% increase in H_2O_2 content was found at 12.5 ppm B_2O_3 NPs. A significant decrease in MDA concentration (Figure 3.2.4.1B 80% SM) was also found in pea leaves as plants were watered or sprayed with a solution containing any concentration of B_2O_3 NPs

Significant inhibition of H_2O_2 and MDA was found as their concentration decreased after plants' exposure to drought and B_2O_3 NPs (Figure 3.2.4.1A, B 30% SM). The amount of H_2O_2 decreased by 18, 24, and 45% after spraying the plants with 12.5, 25, and 50 ppm, and by 22, 37, and 9% after watering. The reduction in the MDA content by 22, 13, and 17% was found after pea irrigation with 12.5, 25, and 50 ppm suspensions of B_2O_3 NPs and after foliar application by 20, 25, and 22%.

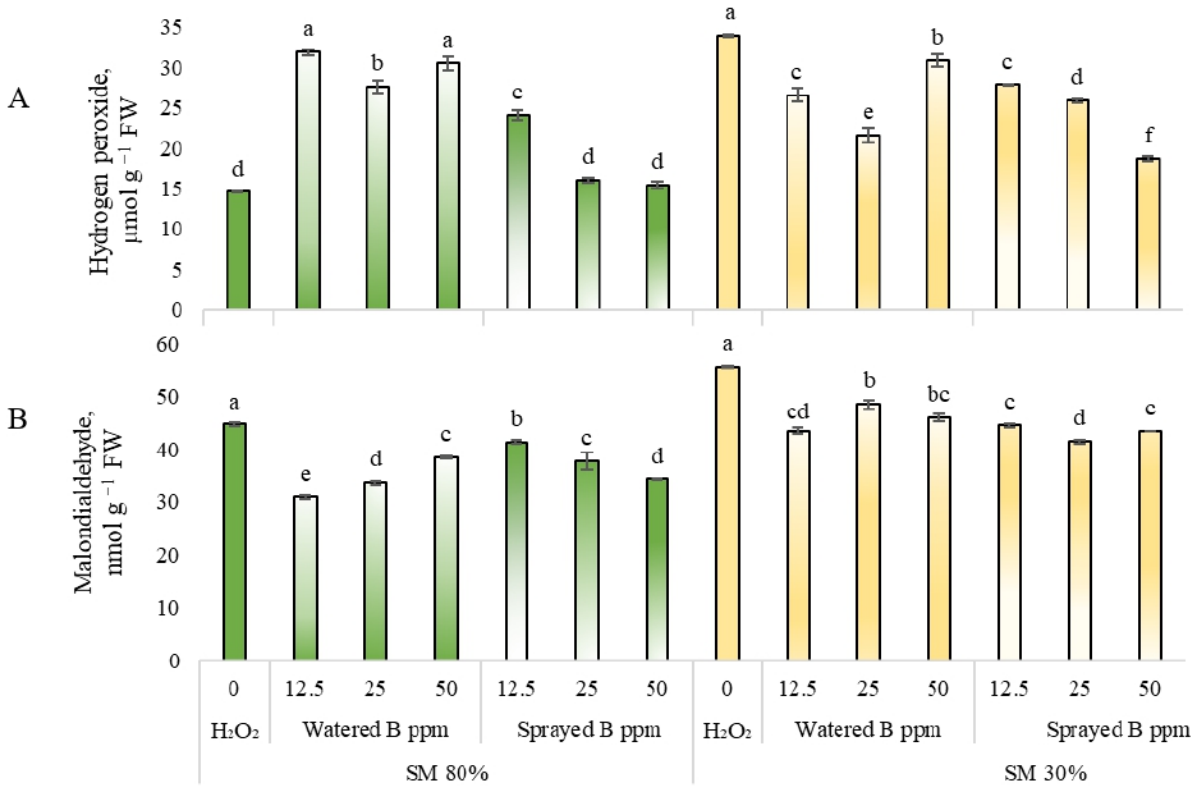


Figure 3.2.4.1. Influence of drought stress and B_2O_3 (B in the figure) NPs (0; 12,5; 25; 50 ppm) on hydrogen peroxide and malondialdehyde content in P. sativum L. H_2O – control plants watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Effects on non-enzymatic antioxidants

It was found that at 80% substrate moisture, both watering and spraying with B_2O_3 NPs reduced the TPC in pea leaves up to 30% (Figure 3.2.4.2A 80% SM). B_2O_3 NPs treatment did not affect ABTS free radical scavenging activity (Figure 3.2.4.2C 80% SM). However, it was determined that after spraying peas with 25 and 50 ppm suspensions, the DPPH free radical scavenging activity increased by 25 and 24% (Figure 3.2.4.2B 80% SM). Furthermore, concentrations of B_2O_3 NPs suspensions of 12.5, 25, and 50 ppm increased the FRAP antioxidant power (Figure 3.2.4.2D 80% SM): as plants were watered or sprayed.

The results showed that spraying drought-affected peas with 12.5, 25, and 50 ppm B_2O_3 NPs suspensions increased TPC content to 18% while watering with 12.5 significantly reduced it (Figure 3.2.4.2A 30% SM). ABTS free radical scavenging activity showed its sensitivity to the impact of B_2O_3 NPs (Figure 3.2.4.2C 30% SM); it increased to 73% after watering and 96% after spraying compared to drought-affected plants without NPs exposure. Similar results were found for FRAP antioxidant power in peas (Figure 3.2.4.2D 30% SM). The exposure of drought and B_2O_3 NPs 12.5 and 25 ppm suspension through the roots caused a slight impact (20%) on DPPH free radical scavenging activity (Figure 3.2.4.2B 30% SM). In addition, spraying with

12.5, 25, and 50 ppm B_2O_3 NPs suspensions induced DPPH free radical scavenging activity by 35, 24, and 25%, respectively.

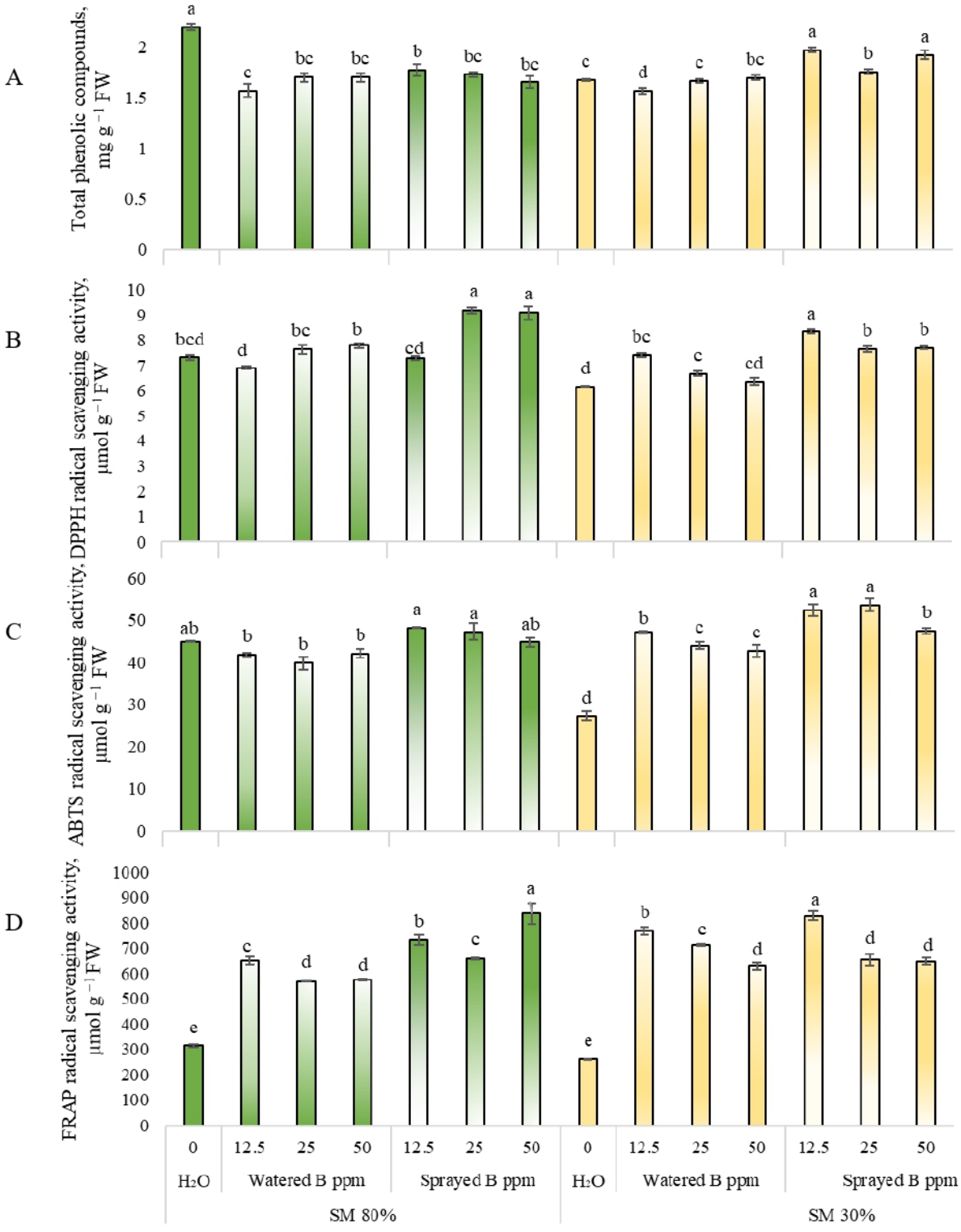


Figure 3.2.4.2. Influence of drought stress and B_2O_3 NPs (B in the figure) (0; 12,5; 25; 50 ppm) on A – total phenolic compounds, B – DPPH free radical scavenging activity, C – ABTS free radical scavenging activity D – FRAP antioxidant power in P. sativum L. H_2O – control plants, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Effects on enzymatic antioxidants

B_2O_3 NPs induced the activities of CAT, APX, SOD, and GPX in pea leaves when they were grown in 80% SM (Figure 3.2.4.3A, B, D, E). APX activity increased particularly strongly after watering plants with B_2O_3 NPs suspensions, while a slightly weaker effect was caused by spraying. CAT activity increased up to 2 times when plants were watered with suspensions of B_2O_3 NPs. When peas were sprayed, the CAT activity increased by 1.3, 1.8, and 2 times when the concentration was 12.5, 25, and 50 ppm. SOD activity was induced up to 41% by exposure to B_2O_3 NPs through roots, and foliar treatment activated the enzyme up to 46%. GPX activity was distinguished because lower concentrations of 12.5 and 25 ppm had a more substantial positive effect during watering, while higher concentrations of 25 and 50 ppm increased activity more strongly during spraying. B_2O_3 NPs suspension reduced GR activity (Figure 3.2.4.3C 80% SM) when suspensions of concentrations 12.5 and 50 ppm were used for plant watering or spraying.

A substantial decrease in GR activity was caused by drought and B_2O_3 NPs exposure, with a 55% reduction after irrigation and a 45% reduction after spraying (Figure 3.2.4.3C 30% SM). Also, an adverse effect was found on SOD activity (Figure 3.2.4.3D 30% SM) after peas irrigation with 25 and 50 ppm solutions of B_2O_3 NPs, a 36% increase in SOD activity was determined after using 12.5 ppm B_2O_3 NPs suspension. Furthermore, SOD activity was induced up to 51% when drought-affected peas were sprayed with B_2O_3 NPs solution. The strong effect of B_2O_3 NPs on the APX activity (Figure 3.2.4.3B 30% SM) remained in pea leaves as plants were grown in drought conditions. After watering peas with B_2O_3 NPs suspensions, APX activation occurred up to 1.4 times after spraying up to 8 times. Additionally, GPX activity in drought-affected peas (Figure 3.2.4.3E 30% SM) was increased up to 91% after foliar exposure with all B_2O_3 NPs concentrations. CAT activity (Figure 13A 30% SM) was strongly activated by watering or spraying plants with B_2O_3 NPs solutions of any concentration.

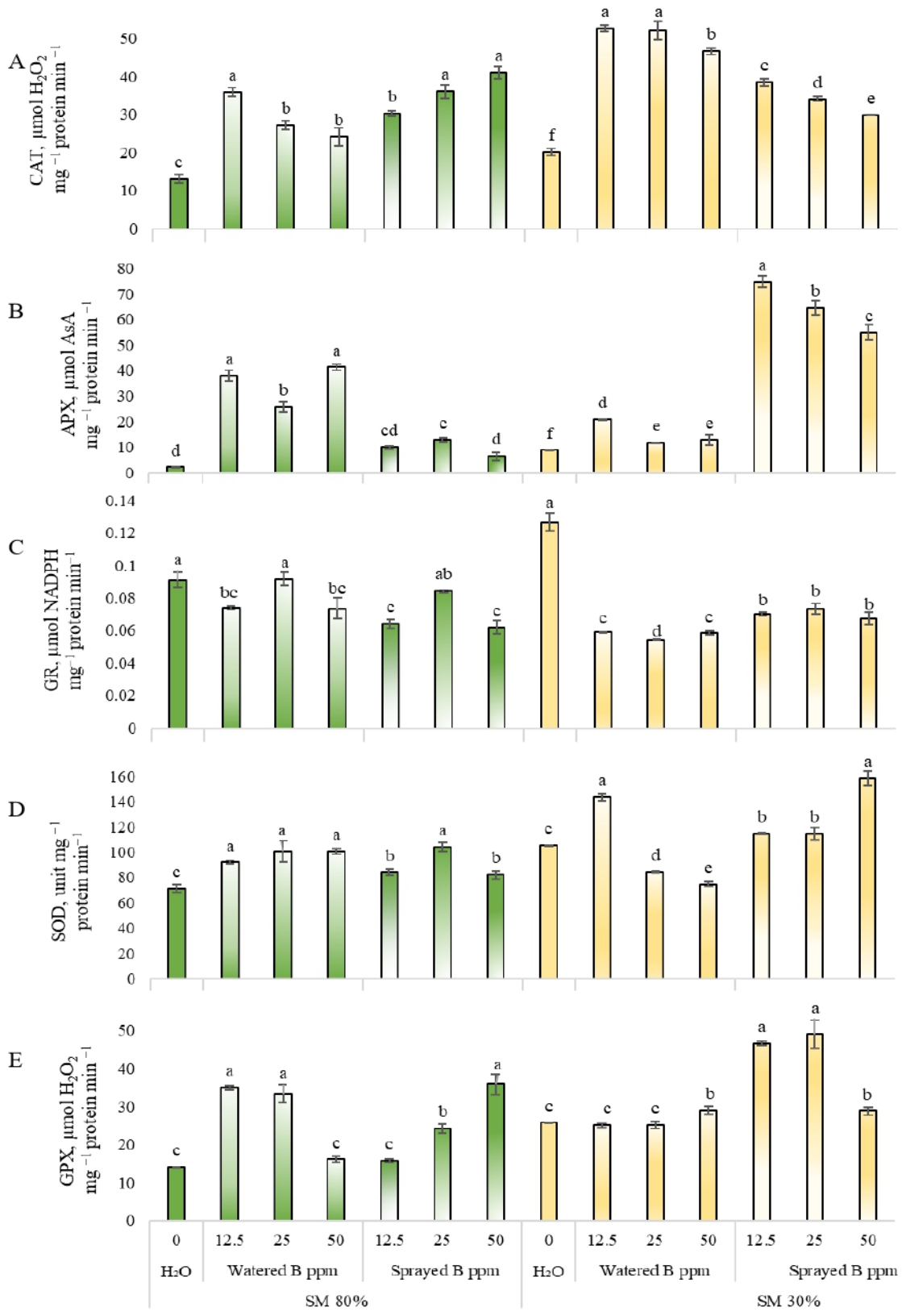


Figure 3.2.4.3. Response of A – ascorbate peroxidase (APX), B –catalase (CAT), C –superoxide dismutase (SOD), D –glutathione reductase (GR), and E –guaiacol peroxidase (GPX) activity to drought stress and B_2O_3 (B in the figure) NPs (0; 12,5; 25; 50 ppm) in P. sativum L. H_2O – control plants sprayed or watered with deionized water, substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean ± SE of three replicates, and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Changes in macro- and micro-elemental composition

B content in peas grown in sufficient substrate moisture (Table 3.2.4.2, 80% SM) leaves increased by 2, 3, and 11 times after watering with B_2O_3 NPs suspensions of 12.5, 25, and 50 ppm after spraying with the same concentrations by 3, 5 and 12 times. B content increased 10 times in the stem after watering with B_2O_3 NPs solutions and up to 17 times after foliar application. Furthermore, a 3, 7, and 20 times increase in B content was found in the roots after watering plants grown during normal substrate moisture conditions with B_2O_3 NPs. Foliar application increased the amount of B in the roots from 1 to 3 times.

When drought-affected peas were sprayed with 25 and 50 ppm B_2O_3 NPs solutions, leaf B content increased 3 and 5 times, respectively. In addition, 3 times increase in B content in the leaves was found when peas were irrigated with suspensions of B_2O_3 NPs. Moreover, substantial increases (up to 18 times) in B content were found in stems after irrigating peas with suspensions containing B_2O_3 NPs. B amount increased 1.5, 3, and 4 times after watering or spraying with the suspensions containing 12.5, 25, and 50 ppm B_2O_3 NPs.

B_2O_3 NPs affected the accumulation of Ca, Mg, Fe, Zn, and Mn in pea leaves. Spraying with B_2O_3 NPs resulted in higher K concentration in the leaves regardless of the exposure. It was found that the application of B_2O_3 NPs decreased the content of Ca in plant stems during both normal substrate conditions and drought. Furthermore, the reduction of Mg content in plant leaves and stems was found after exposure to B_2O_3 NPs solutions. However, pea leaves exposed to water deficit Mo content increased to 7 times. K, Ca, Mg, and Fe increased in roots when peas were grown at 80% SM. Drought and 50 ppm B_2O_3 NPs suspension increased the P, Ca, Mg, Na, and Fe content in roots.

Table 3.2.4.2. Effects of B_2O_3 NPs suspension on macro- and microelements of peas leaves, stem, and root. Mean values in bold indicate a statistically significant difference at $p < 0.05$ according to Tukey (HSD) test (n=9)

Treatment B_2O_3 NPs, ppm			Macroelements, mg g^{-1} DW						Microelements, µg g^{-1} DW				
Leaves			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	3.79	18.76	45.73	9.20	2.64	19.86	25.71	42.54	10.32	2.99	3.58
		12.5	**4.63**	**22.01**	**39.80**	**6.34**	2.34	22.78	25.03	40.54	9.43	2.64	**6.28**
		25	2.87	17.22	**32.84**	**5.69**	2.08	**16.00**	**18.50**	**33.35**	**5.84**	**0.27**	**9.52**
		50	4.05	**19.65**	**36.95**	**6.43**	**1.51**	**16.41**	**22.54**	**35.32**	**6.27**	**1.63**	**33.20**
	Sprayed	12.5	4.63	**22.01**	**39.80**	**6.34**	2.34	22.78	25.03	40.54	9.43	2.64	**9.52**
		25	3.36	**19.22**	**42.14**	**6.17**	2.33	**23.64**	**23.93**	**44.82**	**8.78**	**1.01**	**14.07**
		50	4.16	**19.05**	**47.94**	**8.04**	**3.92**	**32.44**	24.72	**50.31**	**8.36**	**1.21**	**35.14**
SM 30%	Watered	0	3.21	20.56	46.19	8.81	2.07	20.03	37.24	37.65	10.02	0.70	11.66
		12.5	4.14	21.69	**44.88**	8.74	2.02	**22.28**	**35.03**	**48.08**	11.52	**3.34**	**30.52**
		25	4.07	19.84	**47.46**	8.23	**1.30**	**22.62**	**35.06**	**44.97**	**13.53**	**5.30**	**36.38**
		50	**5.37**	**24.67**	**47.94**	8.87	**1.61**	**25.63**	**43.90**	**49.17**	**16.07**	**4.49**	**38.23**
	Sprayed	12.5	2.22	**23.66**	**36.52**	**6.09**	2.62	18.84	**17.67**	**27.98**	**13.08**	**3.47**	10.40
		25	**4.49**	**21.91**	**49.75**	8.55	**1.51**	**21.30**	**39.24**	**42.55**	**15.95**	**5.82**	**31.71**
		50	**4.29**	**21.83**	**47.76**	9.00	**1.56**	**20.81**	**42.85**	**49.19**	**16.90**	**4.48**	**53.25**
Stem			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	2.00	17.77	37.81	8.71	7.73	15.49	19.81	26.09	10.08	3.66	1.05
		12.5	1.71	**15.97**	**34.96**	**5.10**	**4.04**	**17.45**	**17.66**	26.81	**13.08**	**5.02**	**3.25**
		25	1.44	**13.77**	**28.02**	**4.32**	**4.73**	**10.66**	**11.81**	**20.91**	9.39	3.55	**9.37**
		50	2.21	17.38	**33.61**	**5.44**	**5.96**	**17.34**	**16.73**	**24.64**	10.76	3.25	**11.71**
	Sprayed	12.5	1.87	16.49	**29.69**	**6.02**	**4.20**	**10.62**	**12.38**	**19.37**	**7.71**	**1.23**	**3.37**
		25	1.24	**15.42**	**30.53**	**6.52**	**5.61**	**12.25**	**12.04**	**19.65**	8.23	**1.25**	**16.86**
		50	1.81	**14.98**	**33.27**	**6.99**	**5.65**	**13.58**	**17.09**	**23.33**	**7.07**	**1.75**	**18.56**
SM 30%	Watered	0	1.43	20.13	38.10	7.98	6.83	15.46	22.31	24.19	8.92	1.17	10.57
		12.5	1.71	20.38	**33.69**	**5.72**	5.91	**17.62**	**19.07**	**29.16**	**12.00**	**3.26**	**12.40**
		25	1.50	20.41	**30.73**	**5.14**	6.06	**11.02**	**15.44**	**22.76**	**11.17**	**2.98**	**17.27**
		50	2.05	22.79	**34.61**	**5.96**	5.48	**20.04**	**16.95**	**27.47**	**12.16**	**2.97**	**19.39**

	Sprayed	12.5	2.22	**23.66**	**36.52**	**6.09**	**7.62**	**18.84**	**17.67**	**27.98**	**13.08**	**3.47**	9.65
		25	2.17	21.02	38.79	**5.88**	**5.72**	**17.45**	**24.28**	**29.92**	**13.71**	**4.21**	10.40
		50	1.74	**23.02**	**33.99**	**5.73**	**7.13**	14.66	**16.47**	24.82	**11.23**	**2.62**	**11.11**
Root			**P**	**K**	**Ca**	**Mg**	**Na**	**Fe**	**Zn**	**Mn**	**Cu**	**Mo**	**B**
SM 80%	Watered	0	0.81	16.16	10.25	6.91	3.18	258.88	15.88	46.44	18.46	9.40	2.68
		12.5	1.35	**21.65**	**13.90**	**10.16**	3.66	**752.51**	**27.30**	**133.15**	19.50	**16.38**	**11.90**
		25	1.40	**21.30**	**13.11**	**9.61**	**4.91**	**532.65**	**23.54**	**100.79**	17.05	10.79	**22.36**
		50	**1.95**	**24.23**	**13.64**	**9.21**	**5.57**	**400.15**	**33.52**	**78.58**	17.35	**6.56**	**57.91**
	Sprayed	12.5	1.73	**19.96**	**12.67**	**9.44**	**4.50**	**548.87**	**23.91**	**95.76**	**16.32**	**12.93**	**5.00**
		25	**1.99**	**28.53**	**19.54**	**14.55**	**6.28**	**656.22**	**29.92**	**115.93**	**23.04**	**15.34**	**8.09**
		50	**1.94**	**25.32**	**14.36**	**10.01**	**5.39**	**547.88**	**35.78**	**97.03**	**21.05**	**10.78**	**11.11**
SM 30%	Watered	0	1.58	28.33	19.57	15.45	7.33	657.85	34.15	118.04	22.49	16.58	6.70
		12.5	1.58	29.73	18.37	**12.77**	7.41	570.32	**26.72**	102.34	**19.85**	**8.54**	**9.84**
		25	**1.88**	28.24	19.24	**12.89**	**5.76**	693.32	**29.81**	123.37	22.36	**10.63**	**17.91**
		50	**2.21**	**33.13**	**23.25**	14.22	7.96	**852.68**	**98.65**	**150.67**	23.30	**12.23**	**23.68**
	Sprayed	12.5	**1.91**	**35.33**	19.62	**12.79**	7.05	642.51	**29.47**	113.23	23.58	**10.32**	**10.78**
		25	**2.52**	**41.54**	**25.40**	**16.58**	7.84	**1035.42**	**44.91**	**187.73**	**29.58**	**18.86**	**17.32**
		50	**2.39**	**39.01**	**25.40**	**17.32**	**8.59**	**1044.14**	**46.56**	**196.38**	**29.07**	**19.95**	**21.22**

Macroelements: P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, Na – sodium, Fe – iron; Microelements: Zn – zinc, Mn – manganese, Cu – copper, Mo – molybdenum, B – boron. 0 – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%.

Summary of the chapter (Table 3.2.4.3)

- *Spraying with B_2O_3 NPs at a 12.5 ppm most effectively stimulated TPC accumulation, antioxidant capacity, and APX, SOD, and GPX enzyme activity in pea leaves exposed to drought.*
- B_2O_3 *NPs reduced the amount of* H_2O_2 *and MDA in pea plants grown on a substrate with insufficient moisture.*
- B_2O_3 *accumulation was most significant in drought-affected peas when irrigated with 25 and 50 ppm* B_2O_3 *NPs suspensions.*
- *The most substantial positive effect was found on peas affected by drought after spraying them with 12.5 ppm of* B_2O_3 *NPs.*

Table 3.2.4.3. The impact of drought stress and B_2O_3 NPs (12.5; 25; 50 ppm) on P. sativum L. grown in the substrate with sufficient (SM 80%) and insufficient (SM 30%) moisture is expressed as a percentage change (%) compared to the control (for SM 80% control means plants grown under SM 80% and NPs untreated; SM 30% control means drought affected but NPs untreated plants) in the heat map. Statistically, significant differences are marked in bold

Treatment	SM 80%						SM 30%					
B_2O_3 NPs,	Watered			Sprayed			Watered			Sprayed		
ppm	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Plant height	**14**	7	**27**	**28**	9	**19**	**9**	**26**	**17**	**13**	**20**	**14**
Leaf area	**28**	9	**30**	**38**	**25**	**24**	-17	**30**	**40**	-4	1	-6
Nodules	**560**	**200**	**480**	**340**	**240**	**200**	17	**200**	**317**	0	67	**583**
RWC	**2**	**4**	**5**	**6**	**4**	**4**	-1	**10**	**11**	-2	4	**9**
Root/shoot	**21**	**36**	**68**	**69**	**18**	**34**	**4**	**14**	**30**	**11**	**14**	**5**
SLA	14	-5	-11	-15	-6	-3	-17	-4	-16	-13	-23	**-29**
Yield	**-21**	2	**20**	**12**	**11**	-1	**16**	**10**	6	**19**	**16**	**14**
ABTS	-7	-11	-6	7	5	0	**73**	**61**	**56**	**92**	**96**	**74**
DPPH	-5	4	7	0	**25**	**24**	**20**	**8**	3	**35**	**24**	**25**
TPC	**-29**	**-22**	**-22**	**-19**	**-21**	**-25**	**-6**	-1	1	**18**	**5**	**15**
FRAP	**106**	**81**	**83**	**132**	**109**	**166**	**194**	**174**	**141**	**217**	**151**	**148**
HP	**119**	**89**	**109**	**65**	10	6	**-22**	**-37**	**-9**	**-18**	**-24**	**-45**
MDA	**-31**	**-25**	**-14**	**-7**	**-15**	**-23**	**-22**	**-13**	**-17**	**-20**	**-25**	**-22**
GR	**-19**	0	**-19**	**-30**	-8	**-32**	**-53**	**-57**	**-54**	**-45**	**-42**	**-47**
GPX	**147**	**136**	14	11	**71**	**153**	-3	-2	**13**	**81**	**91**	**12**
APX	**1657**	**1100**	**1817**	363	**498**	200	**136**	**33**	**46**	**750**	**634**	**522**
SOD	**29**	**41**	**41**	**18**	**46**	**15**	**36**	**-20**	**-29**	**9**	**9**	**51**
CAT	**173**	**107**	**84**	**131**	**175**	**214**	**161**	**159**	**132**	**91**	**69**	**48**
B (leaves)	**18**	**16**	7	8	4	**20**	-3	**36**	**52**	**-18**	**-20**	**-24**
B (stem)	**210**	**794**	**1017**	**221**	**1508**	**1670**	**17**	**63**	**83**	-9	-2	5
B (roots)	**344**	**735**	**2062**	**87**	**202**	**315**	**47**	**167**	**253**	**61**	**158**	**217**

RWC – relative water content, SLA – specific leaf area, TPC – total phenolic compounds, HP – hydrogen peroxide, MDA – malondialdehyde, GR – glutathione reductase, GPX – guaiacol peroxidase, APX – ascorbate peroxidase, SOD – superoxide dismutase, CAT – catalase, B content in leaves, stem, and root. 0 – control plants watered with deionized water, drought stress – 30% substrate moisture.

3.3 Effects of nanoparticles on peas exposed to the combined stress of drought and heavy metal copper

In field conditions, plants can experience several different abiotic stresses simultaneously, for example: decreased water availability during drought or increased excessively during a flood, extreme temperatures, including heavy metals, or increased soil hardness limiting root growth. Different stresses cause different responses in plants, which requires plants to use other acclimation processes. When several stresses affect plants simultaneously, their adverse effects overlap, and the plant suffers stronger strains. This chapter will analyze in detail the impact of NPs on combined drought, and heavy metal copper (Cu) stresses on pea plants.

This section discusses the results obtained between plants affected by drought and heavy metal cooper and plants affected by drought, heavy metal cooper, and NPs. The subsections are divided according to the NPs. The first paragraph discussed the watered NPs effects on drought, and heavy metal cooper exposed plants. The second one is the foliar NPs application on drought, and heavy metal cooper exposed plants.

3.3.1 Effects of silica nanoparticles on peas exposed to the combined stress of drought and heavy metal copper

SiO_2 NPs strongly affected peas to protect them from the combined effects of drought and Cu (Table 3.3.1.1). Pea height, root-to-shoot ratio, RWC, and yield increased by 24, 86, 24, and 50%, respectively, when plants were watered with SiO_2 NPs under drought and Cu treatment (WxDxCu). The activity of DPPH free radical scavenging and FRAP were significantly increased by 21 and 10% and the amount of TPC by 21% when plants were affected by WxDxCu. Besides, SiO_2 NPs stimulated GR and CAT activity by 50 and 67%, respectively, when plants were grown under WxDxCu. Irrigation with SiO_2 NPs reduced H_2O_2 and MDA content in leaves by 12 and 14% when plants were treated by WxDxCu. An increase in BCF was found in leaves by 75% and in roots by 94%, as well as in Tf by 1.5 times and Ti by 85%.

Spraying with SiO_2 NPs positively affected peas as they were affected by drought and Cu (SxDx Cu). In these plants, an increase in height, RWC, root-to-shoot ratio, and yield was determined by 34, 23, 99, and 44%, respectively. Also, SiO_2 NPs positively affected antioxidant capacity (ABTS, DPPH, FRAP) and increased TPC content in pea leaves during these conditions SxDxCu. However, the spraying of SiO_2 NPs strongly reduced the activities of enzymes such as GPX, APX, and SOD in peas exposed to drought and Cu. In addition, spraying with SiO_2 NPs significantly reduced the content of H_2O_2 and MDA by 57 and 55%. An increase in BCF was found in leaves by 99% and in roots by 81%, as well as in Tf by 200% and Ti by 90% after exposure to SxDxCu.

Table 3.3.1.1. The impact of drought stress, Cu, and SiO_2 NPs (50 ppm) on P. sativum L. is presented. H_2O – control plants sprayed or watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean and different letters differed significantly by Tukey HSD Test ($P < 0.05$).

Treatment with SiO_2 NPs, 50 ppm	80% SM	30 % SM					
	H_2O	H_2O	Cu	Watered NPs	Sprayed NPs	Cu+Watered NPs	Cu+Sprayed NPs
Morphological parameters							
Plants height	32.9 a	22.2 cd	20.0 d	28.3 ab	30.4 a	24.9 c	26.8 bc
Leaf area	76.9 a	49.9 c	43.9 cd	55.9 b	62.7 ab	44.8 d	50.4 c
No. of nodules	8.3 a	3.7 d	2.0 e	6.0 bc	6.3 b	3.6 d	2.6 de
RWC	80.6 a	41.7 c	29.3 e	47.3 b	45.8 bc	36.3 cd	35.9 cd
Root/shoot	11.3 a	5.4 bc	4.1 c	6.0 bc	8.9 a	6.8 bc	8.3 ab
SLA	9.5 bc	11.0 ab	13.5 a	8.5 b	9.4 bc	8.4 b	9.6 bc
Yield	3.4 a	2.4 b	1.8 c	3.3 a	2.6 b	2.7 b	2.6 b
Non-enzymatic antioxidant activity							
ABTS	113.2 a	103.1 b	96.4 bc	111.4 a	109.1 ab	93.9 c	106.6 a
DPPH	45.1 ab	38.5 c	36.8 cd	45.9 ab	49.2 a	44.5 ab	44.8 ab
TPC	2.7 ab	2.3 bc	1.9 d	2.7 ab	2.9 a	2.3 bc	2.4 b
FRAP	1475.8 a	1158.6 b	939.5 d	1380.3 a	1405.9 a	1027.6 bc	1117.3 bc
Enzymatic antioxidant activity							
Gr	0.053 c	0.074 bc	0.084 b	0.041 c	0.027 c	0.126 a	0.094 b
GPX	11.1 c	15.5 b	44.9 a	8.4 d	8.1d	19.3 b	10.6 cd
APX	25.1 e	51.3 b	74.3 a	61.5a	55.6 ab	48.2 cd	34.5 d
SOD	46.8 c	67.2 b	74.5 a	73.9 a	79.7 a	14.7 de	11.1 e
CAT	16.7 d	31.9 c	33.1 c	37.7 ab	15.2 d	55.8 a	43.2 ab
Oxidative stress biomarkers							
HP	12.3 e	25.5 ab	26.2 a	21.6 bc	19.6 d	23.1 b	20.3 bc
MDA	54.7 f	62.3 de	97.3 a	89.9 b	76.8 d	83.4 bc	74.7 d
Copper accumulation							
BCF (leaves)			0.04 b			0.07 a	0.08 a
BCF (roots)			1.6 b			3.1 a	2.9 a
Tf			2.1 b			5.2 a	6.1 a
Ti			62.7 c			116.2 b	119.3 a

BCF – bioconcentration factor, Tf – translocation factor, Ti tolerance index

Summary of the chapter

- *SiO_2 NPs increased the morphological parameters of peas, stimulated DPPH free radicals scavenging activity and antioxidant power of FRAP, and stimulated the activity of GR and CAT enzymes when plants were exposed to combined stress. Reduced oxidative stress biomarkers (H_2O_2, MDA) in peas exposed to drought, Cu, and watered SiO_2 NPs. Irrigation with SiO_2 NPs increased the TF and Ti.*
- *SiO_2 NPs increased the morphological parameters of peas and induced positive effects on antioxidant capacity and TPC content. Reduced oxidative stress biomarkers in peas exposed to drought, Cu, and sprayed with SiO_2 NPs. Foliar application with SiO_2 NPs increased the TF and Ti.*
- *Pea irrigation with SiO_2 NPs has more statistically reliable effects when peas are affected by drought (Table 3.3.1.2).*
- *More statistically reliable effects were found for peas exposed to drought, Cu, and spraying with SiO_2 NPs (Table 3.3.1.2).*

*Table 3.3.1.2. The impact of drought stress, Cu, and SiO_2 NPs (50 ppm) on P. sativum L. is presented. * Significant difference $p<0.05$*

Treatment with SiO_2 NPs, 50 ppm			Watered		Sprayed	
	D	D x Cu	D x NPs	D x Cu x NPs	D x NPs	D x Cu x NPs
Plants height	*	*	*	*	*	*
Leaf area	*	*	*		*	
Nodules	*	*	*	*	*	
RWC	*	*	*		*	*
Root/shoot	*	*				*
SLA		*		*		*
Yield	*	*	*			
ABTS	*	*	*		*	*
DPPH	*	*	*		*	
TPC		*			*	*
FRAP	*	*	*		*	*
Gr		*		*		
GPX	*	*	*	*	*	*
APX	*	*	*	*		*
SOD	*	*	*	*	*	*
CAT	*	*	*	*	*	*
HP	*	*	*	*	*	*
MDA	*	*	*	*		*

3.3.2 Effects of copper oxide nanoparticle on peas exposed to the combined stress of drought and heavy metal copper

A significant increase in the root-to-shoot ratio was found after peas were exposed to drought, Cu, and watered with CuO NPs (Table 3.3.2.1). Besides, a significant decrease was found in leaf area and yield. Furthermore, a reduction in FRAP antioxidant power and TPC content and no statistical significance for DPPH and ABTS free radicals scavenging activity in pea leaves were determined. In these plants, CuO NPs increased the activity of SOD, and no statistical reliability was found for GR, GPX, APX, and CAT enzymes. CuO NPs significantly reduced H_2O_2 content, but no effect on MDA content was detected in peas. Finally, CuO NPs increased BCF by 13% in roots, decreased Tf by 33%, and did not affect Ti.

When pea plants were exposed to drought and Cu, spraying with CuO NPs produced more positive results. An increase in RWC of up to 44% was found, and no differences were determined in all other morphological parameters. In these plants, stimulation of ABTS free radical scavenging activity and increase in TPC content were determined, while no differences were found in enzymatic antioxidants. However, there was a significant decrease in H_2O_2 and MDA in pea leaves. Foliar CuO NPs application did not affect both leaf and root BCF and Ti but significantly reduced Tf.

Table 3.3.2.1. *The impact of drought stress, Cu, and CuO NPs (50 ppm) on P. sativum L. is presented. H_2O – control plants sprayed or watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean (n=9) and different letters differed significantly by Tukey HSD Test ($P < 0.05$)*

Treatment with CuO NPs, 50 ppm	80% SM	30 % SM					
	H_2O	H_2O	Cu	Watered NPs	Sprayed NPs	Cu+Watered NPs	Cu+Sprayed NPs
Morphological parameters							
Plants height	32.9 a	22.2 cd	20.0 d	25.0 b	24.3 b	19.9 d	21.8 cd
Leaf area	76.9 a	49.9 b	43.9 c	40.6 cd	46.8 bc	24.3 e	40.2 cd
Nr. Of nodules	8.3 a	3.7 b	2.0 cd	2.1 cd	3.1 c	1.7 d	1.9 cd
RWC	80.6 a	41.7 bc	29.3 d	48.1 b	47.2 b	38.2 c	42.2 bc
Root/shoot	11.3 a	5.4 bc	4.1 c	10.2 a	6.8 b	8.1 b	6.1 bc
SLA	9.5 cd	11.0 c	13.5 ab	10.0 c	9.2 cd	13.2 b	14.7 a
Yield	3.4 a	2.4 b	1.8 c	2.4 b	2.7 ab	1.4 de	1.6 cd
Non-enzymatic antioxidant activity							
ABTS	113.2 a	103.1 ab	96.4 bc	108.7 b	111.5 a	90.7 bc	110.2 a
DPPH	45.1 a	38.5 b	36.8 c	36.6 c	35.9 c	35.2 c	35.3 c
TPC	2.7 a	2.3 bc	1.9 c	2.6 ab	2.6 ab	1.2 d	2.4 b
FRAP	1475.8 a	1158.6 b	939.5 de	1082.9 d	1201.8 c	683.4 f	890.0 e

Enzymatic antioxidant activity							
Gr	0.053 c	0.074 b	0.084 ab	0.073 b	0.066 bc	0.094 a	0.083 ab
GPX	11.1 f	15.5 e	44.9 ab	21.4 cd	19.6 cd	47.0 a	37.5 b
APX	25.1 d	51.3 c	74.3 a	63.0 b	63.7 b	70.3 ab	72.3 ab
SOD	46.8 e	67.2 d	74.5 cd	133.3 a	82.6 c	105.8 b	73.8 cd
CAT	16.7 d	31.9 bc	33.1 b	28.0 c	38.5 a	34.0 ab	32.2 bc
Oxidative stress biomarkers							
HP	12.3 d	25.5 ab	26.2 ab	24.8 ab	22.4 bc	19.7 c	20.1 c
MDA	54.7 e	62.3 d	97.3 a	84.5 b	81.9 bc	97.1 a	73.3 c
Copper accumulation							
BCF (leaves)			0.04 ab			0.05 ab	0.06 a
BCF (roots)			1.6 b			1.8 a	1.7 ab
Tf			2.1 a			1.4 c	1.7 b
Ti			62.7 ab			60.2 b	63.0 a

BCF – bioconcentration factor, Tf – translocation factor, Ti – tolerance index

Summary of the chapter

- *CuO NPs decreased FRAP antioxidant power and TPC content in peas but increased SOD activity when peas were exposed to drought, Cu, and watered with CuO NPs.*
- *An increase in ABTS radical scavenging activity and TPC content in peas was determined, and a decrease in H_2O_2 and MDA content when peas were exposed to drought, Cu, and sprayed with CuO NPs.*
- *CuO NPs had a more significant effect on peas exposed only to drought than drought and Cu affected but NPs untreated plants. More statistically reliable effects on antioxidants were found after foliar CuO NPs application (spraying) (Table 3.3.2.2).*
- *During the combined stress of drought, Cu, and CuO NPs, CuO NPs did not positively affect peas.*

*Table 3.3.2.2. The impact of drought stress, Cu, and CuO NPs (50 ppm) on P. sativum L. is presented. * Significant difference p<0,05*

Treatment with CuO NPs, 50 ppm			Watered		Sprayed	
	D	D x Cu	D x NPs	D x Cu x NPs	D x NPs	D x Cu x NPs
Plants height	*	*	*		*	
Leaf area	*	*	*	*		
Nodules	*	*	*		*	
RWC	*	*	*	*		*
Root/shoot	*			*		
SLA	*	*				
Yield	*	*			*	*
ABTS		*				*
DPPH	*	*	*		*	
TPC	*	*		*		*
FRAP	*	*	*	*	*	
Gr	*					
GPX	*	*	*		*	
APX	*	*	*		*	
SOD	*		*	*	*	
CAT	*				*	
HP	*	*				
MDA	*	*	*		*	*

3.3.3. Effects of molybdenum trioxide nanoparticles on peas exposed to the combined stress of drought and heavy metal copper

The results presented in Table 3.3.3.1 show the complex effects of drought, Cu, and MoO_3 NPs on peas. Pea height increased by 29%, leaf area by 28%, the number of nodules by 500%, RWC by 44%, yield by 88%, root to shoot ratio by 120%, and no effect was found on SLA when peas were exposed to drought, Cu and watered with 50 ppm MoO_3 NPs. A significant increase in the scavenging capacity of ABTS, DPPH, and FRAP radicals was determined. Besides, TPC content in peas increased by over 35%. Activation was found in GPX and CAT enzymes, but inhibition in GR and SOD activity was determined in peas exposed to drought, Cu, and watered with MoO_3 NPs. In addition, a 22% reduction in H_2O_2 and MDA content was found in pea leaves. MoO_3 NPs increased the Tf by 44% and the Ti by 76%.

Statistically reliable positive changes in the number of nodules, RWC, and yield were determined when peas were exposed to drought, Cu, and sprayed with MoO_3 NPs. The antioxidant capacity (DPPH, ABTS, and FRAP) increased to 13%, and TPC content in pea leaves increased by 32% after foliar application of MoO_3 NPs on plants exposed to drought and Cu. There, a 17% inhibitory effect was found only on GR activity, while others, GPX, APX, SOD, and CAT activity increased by 10, 35, 36, and 20%, respectively. A 23% reduction in

MDA content was found. Besides, the BCF in the roots increased by 25% and Ti - by 13%, while differences were not determined for Tf.

Table 3.3.3.1. The impact of drought stress, Cu, and MoO_3 NPs (50 ppm) on P. sativum L. is presented. H_2O – control plants watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean and different letters differed significantly by multiple comparisons of Tukey HSD Test ($P < 0.05$).

Treatment with MoO_3 NPs, 50 ppm	80% SM	30 % SM					
	H_2O	H_2O	Cu	Watered NPs	Sprayed NPs	Cu+Watered NPs	Cu+Sprayed NPs
Morphological parameters							
Plants height	32.9 a	22.2 c	20.0 d	27.8 b	20.0 d	25.8 bc	23.7 bcd
Leaf area	76.9 a	49.9 c	43.9 cd	52.1 bc	43.9 cd	56.5 b	43.5 c
Nr. of nodules	8.3 b	3.7 d	2.0 e	7.0 bc	2.0 d	11.7 a	7.3 bc
RWC	80.6 a	41.7 c	29.3 e	49.4 b	43.0 bc	43.8 bc	33.6 d
Root/shoot	11.3 a	5.4 bc	4.1 c	6.1 bcd	9.3 ab	8.9 ab	6.1 bc
SLA	9.5 abc	11.0 ab	13.5 a	9.3 bc	14.0 a	9.4 ab	8.5 d
Yield	3.4 ab	2.4 c	1.8 d	4.2 a	3.8 a	3.4 ab	3.3 ab
Non-enzymatic antioxidant activity							
ABTS	113.2 a	103.1 bc	96.4 e	110.3 a	96.4 e	105.0 bc	101.8 d
DPPH	45.1 a	38.5 c	36.8 d	42.2 b	36.9 d	42.5 b	41.8 b
TPC	2.7 a	2.3 cd	1.9 d	2.5 b	1.8 e	2.5 b	2.4 bc
FRAP	1475.8 a	1158.6 c	939.5 de	1216.2 b	924.6 de	1184.2 b	1058.2 c
Enzymatic antioxidant activity							
Gr	0.053 c	0.074 b	0.084 a	0.089 a	0.070 b	0.072 b	0.070 b
GPX	11.1 c	15.5 bc	44.9 b	15.1 bc	19.6 bc	48.1 a	49.3 a
APX	25.1 d	51.3 c	54.3 c	58.2 b	74.5 a	71.8 ab	72.9 ab
SOD	46.8 e	67.2 c	74.5 b	73.3 b	74.2 b	64.4 cd	101.2 a
CAT	16.7 f	31.9 e	33.1 e	44.4 b	48.9 a	35.3 d	39.6 c
Oxidative stress biomarkers							
HP	12.3 e	25.5 bc	26.2 b	21.9 d	26.2 b	30.2 a	25.4 bc
MDA	54.7 e	62.3 c	97.3 a	58.6 d	63.3 c	75.7 b	75.3 b
Copper accumulation							
BCF_{Cu} (leaves)			0.04 b			0.06 a	0.05 ab
BCF_{Cu} (roots)			1.6 c			2.3 a	1.8 b
Tf_{Cu}			2.1 b			3.7 a	1.9 b
Ti_{Cu}			62.7 c			131.8 a	112.5 b

BCF – bioconcentration factor, Tf – translocation factor, Ti – tolerance index

Summary of the chapter

- *An increase in morphological parameters, antioxidant capacity, as well as GPX and CAT enzymes, an increase in the amount of TPC, and a decrease in the amount of H_2O_2 and MDA in peas irrigated with MoO_3 NPs suspension and exposed to drought with Cu were determined.*
- *Stimulation of the activity of ABTS, DPPH, and FRAP radicals scavenging, as well as GPX and APX, SOD, and CAT enzymes, increased TPC content, and decreased MDA content were found in peas sprayed with MoO_3 NPs suspension and exposed to drought with Cu. (Table 3.3.3.2).*
- *Irrigation with MoO_3 NPs had more statistically reliable effects on the results of peas exposed to drought as well as on the results of peas exposed to the combined stresses (drought, MH_{Cu}, and NPs).*

*Table 3.3.3.2. The impact of drought stress, Cu, and MoO_3 NPs (50 ppm) on P. sativum L. is presented. * Significant difference $p<0,05$*

Treatment with MoO_3 NPs, 50 ppm			Watered		Sprayed	
	D	D x Cu	D x NPs	D x Cu x NPs	D x NPs	D x Cu x NPs
Plants height	*	*	*	*		
Leaf area	*	*		*		
Nodules	*	*		*		*
RWC	*	*	*	*		*
Root/shoot	*	*				
SLA		*		*		*
Yield	*	*	*	*	*	*
ABTS	*	*	*	*	*	*
DPPH	*	*	*	*	*	*
TPC	*	*	*	*	*	*
FRAP	*	*	*	*	*	*
Gr	*	*	*	*		*
GPX		*				*
APX	*	*	*		*	
SOD	*	*	*	*	*	*
CAT	*	*	*	*	*	*
HP	*	*	*		*	
MDA		*	*	*	*	*

3.3.4. Effects of boron nanoparticle on peas exposed to the combined stress of drought and heavy metal copper

Based on the results presented in Table 3.3.4.1, B_2O_3 NPs positively affect the morphological parameters of plants exposed to complex stress. After watering peas (affected by drought and Cu) with B_2O_3 NPs, it was found that the number of nodules increased by 1.5 times, RWC by 39%, root and shoot ratio by 75%, and yield by 56% compared to the data of peas grown in drought and Cu. Irrigation with a concentration of 12.5 ppm B_2O_3 NPs induced the activity of DPPH and FRAP radicals by 18 and 36% in complex-treated peas, but no statistically significant results were found on ABTS radical activity. The amount of TPC in pea leaves increased by 34%. SOD and CAT activities were positively influenced by 97 and 66% after watering complex-treated peas with 12.5 ppm B_2O_3 NPs while an inhibitory effect occurred on APX and GR enzymes. MDA concentration was suppressed by 22%, and no statistically significant difference was found for H_2O_2 content. No statistically reliable effect was found on the BCF. Still, an impact on the translocation factor was found after irrigation with B NPs it decreased by 14%, but the copper tolerance index increased by 42%.

Spraying 12.5 ppm B_2O_3 NPs suspension increased the resistance of peas to drought and heavy metal Cu by increasing pea height by 28%, RWC pre by 20%, root/shoot by 64%, and yield by 44%. The yield increase could be determined by a 20 and 10% increase in DPPH and FRAP radical activities and a 38% decrease in MDA content after spraying peas with B NPs compared to plants affected by drought and Cu. No statistically reliable results were found when analyzing ABTS radical activity, TPC content, Gr activity, and H_2O_2 content in pea leaves. Besides, a substantial increase of SOD over 57% and CAT 1.6 times activity was determined. Spraying peas with 12.5 ppm B_2O_3 NPs suspension affected the translocation factor and tolerance index by increasing them by 33 and 64%.

Table 3.3.4.1. The impact of drought stress, Cu, and B_2O_3 NPs (12.5 ppm) on P. sativum L. is presented. H_2O – control plants sprayed or watered with deionized water; substrate moisture (SM) 80%; drought stress – SM 30%. Values are mean and different letters differed significantly by Tukey HSD Test ($p < 0.05$)

Treatment with B_2O_3 NPs, 12.5 ppm	80% SM	30 % SM					
	H_2O	H_2O	Cu	Watered NPs	Sprayed NPs	Cu+Watered NPs	Cu+Sprayed NPs
Morphological parameters							
Plants height	32.9 a	22.2 c	20.0 d	27.2 b	31.2 a	21.0 d	25.6 bc
Leaf area	76.9 a	49.9 c	43.9 cd	40.9 c	57.1 b	49.0 c	36.9 d
Nr. of nodules	8.3 a	3.7 b	2.0 c	5.0 b	7.0 a	5.0 a	3.7 bc
RWC	80.6 a	41.7 b	29.3 d	44.2 bc	38.1 bc	40.7 bc	35.3 bc
Root/shoot	11.3 a	5.4 bc	4.1 c	7.2 b	5.7 bc	7.2 b	6.7 b
SLA	9.5 bc	11.0 ab	13.5 a	7.4 d	11.1 ab	9.4 bc	8.5 cd
Yield	3.4 a	2.4 c	1.8 d	2.8 ab	2.7 ab	2.8 ab	2.6 ab
Non-enzymatic antioxidant activity							
ABTS	113.2 a	103.1 b	96.4 bc	112.3 a	112.1 a	91.9 c	94.9 c
DPPH	45.1 a	38.5 c	36.8 c	43.5 b	47.6 a	43.4 b	43.9 b
TPC	2.7 ab	2.3 cd	1.9 e	2.8 ab	2.9 a	2.6 bc	2.2 de
FRAP	1475.8 a	1158.6 b	939.5 de	979.6 d	1029.4 c	1280.3 a	1033.8 c
Enzymatic antioxidant activity							
Gr	0.053 b	0.074 a	0.084 a	0.031 c	0.078 a	0.050 b	0.073 a
GPX	11.1 d	15.5 c	44.9 b	65.2 a	49.4 b	15.1 c	13.6 cd
APX	25.1 f	51.3 d	74.3 a	70.0 ab	57.5 bc	66.5 b	34.2 e
SOD	46.8 e	67.2 d	74.5 cd	100.1 b	117.0 b	146.4 a	116.9 b
CAT	16.7 e	31.9 c	33.1 c	25.2 d	25.8 d	54.8 b	87.4 a
Oxidative stress biomarkers							
HP	12.3 c	25.5 ab	26.2 ab	26.4 ab	25.3 ab	23.8 b	26.7 ab
MDA	54.7 f	62.3 e	97.3 a	91.6 b	64.6 d	75.7 c	60.4 e
Copper accumulation							
BCF_{Cu} (leaves)			0.04 a			0.03 a	0.04 a
BCF_{Cu} (roots)			1.6 b			1.4 bc	1.9 ab
Tf_{Cu}			2.1 b			1.8 c	2.8a
Ti_{Cu}			62.7 c			89.2 b	103.0 a

BCF – bioconcentration factor, Tf – translocation factor, Ti – tolerance index

Summary of the chapter

- *Irrigation with B_2O_3 NPs positively affected morphological parameters, DPPH and FRAP values, TPC content, SOD, and CAT enzymes when peas were exposed to the stress complex.*
- *Foliar application with B_2O_3 NPs positively affected morphological parameters, DPPH and FRAP values, SOD and CAT enzymes, and reduced MDA content when peas were exposed to the combined stress.*
- *After watering with 12.5 ppm B_2O_3 NPs (D x Cu x NPs), the most statistically reliable changes in peas were determined compared to spraying (Table 3.3.4.2).*

Table 3.3.4.2. The impact of drought stress, Cu, and B_2O_3 NPs (12.5 ppm) on P. sativum L. is presented.
** Significant difference p<0,05*

Treatment with B_2O_3 NPs, 12.5 ppm			Watered		Sprayed	
	D	D x Cu	D x NPs	D x Cu x NPs	D x NPs	D x Cu x NPs
Plants height	*	*	*	*	*	*
Leaf area	*	*	*	*	*	
Nodules	*	*	*	*	*	
RWC	*	*	*			
Root/shoot	*	*	*	*		
SLA		*	*	*		
Yield	*	*	*	*	*	*
ABTS	*	*		*	*	
DPPH	*	*		*	*	*
TPC	*	*		*	*	
FRAP	*	*	*	*	*	*
Gr	*	*	*	*		
GPX	*	*	*	*	*	
APX	*	*	*	*	*	*
SOD	*	*	*	*	*	*
CAT	*	*	*	*	*	*
HP	*	*		*		
MDA	*	*	*	*	*	*

4. DISCUSSION

This study elucidated the effects of SiO_2, MO_3, B_2O_3, and CuO NPs on pea plants grown under optimal agrometeorological drought and the combined stress of heavy metal Cu and agrometeorological drought conditions. The study considered NPs concentrations and various applications, such as foliar spray and root irrigation, to elucidate the underlying physiological, non-enzymatic, and enzymatic antioxidant mechanisms. The study showed that SiO_2, MO_3, B_2O_3, and CuO NPs positively affect plants grown in optimal conditions and reduce the harmful effects caused by drought and combined stresses. NPs physical and chemical properties, size, surface charge, concentration in suspensions, interaction with plants, and plant physiology determine the physical and chemical interaction of NPs with plants and can lead to the modification of specific membrane surface proteins, receptors, and transporters (Juárez-Maldonado et al., 2019). Biological membranes with hydrophobic and hydrophilic components and uneven fibers of lignin and cellulose create an uneven negative surface charge of -45 to -15 mV (Mittal et al., 2020). Negatively charged plant cell walls are observed to act as an ion exchange surface that potentially favors cationic permeation rather than anionic NPs. Considering the effect of irrigation when NPs reach plants through the soil (roots), it should be noted that soil particles are usually negatively charged, and NPs with a higher negative charge are more mobile in such soil. In contrast, positively charged NPs readily attract negatively charged soil surface particles. As the average soil grain size decreases, NP mobility decreases. The clay content in the soil can act as an anionic adjuvant, preventing the accumulation of NPs and increasing their mobility. Therefore, it is crucial to study the properties of NPs suspensions to understand the mechanisms of their entry and effect in the plant. NPs with a zeta potential of −10 to +10 mV are neutral and less stable than NPs with a zeta potential greater than +30 mV or less than −30 mV (strong cations and anions, respectively) (Clogston and Patri 2011).

NPs can cause various reactions in plants, increasing the activity of enzymes, promoting the conversion of nitrates into ammonia, intensifying the processes of respiration and photosynthesis, synthesizing enzymes and amino acids, enhancing carbon and nitrogen nutrition and/or directly affecting plant mineral nutrition. However, despite the potential advantages mentioned above, the use of nanotechnology in the agricultural sector is relatively limited and, compared to other industries, has not yet reached the market. Therefore, considering the possible practical application of NPs in the agricultural sector, a preliminary cost-benefit analysis should be considered, and risks to the environment and

human health should be adequately assessed before placing NPs-related products on the market.

Effects of different applications and concentrations of SiO_2 nanoparticles on pea plants

In our studies, the zeta potential of the aqueous suspension of SiO_2 NPs was -20.64 mV (Table 2.2.1.). Another scientific article reported that applying 10 g kg^{-1} SiO_2 NPs resulted in a zeta potential of −40 mV. This indicates that the suspensions are stable and anionic.

Our study indicates that pea plant treatments with SiO_2 at higher concentrations (50 and 100 ppm) increased antioxidant activity and reduced H_2O_2 content and MDA in their leaves when grown under optimal conditions (Figure 3.1.2 A, B; Figure 3.2.1.1 A, B). In addition, the height of pea shoots, root length, and biomass accumulation was also increased when they were grown under normal conditions (Table 3.1.1; Table 3.2.1.1, 80% SM). A particular effect was observed on the accumulation of macroelements in pea plants; the content of P, K, Ca, and Mg in pea leaves and stems increased significantly (Table 3.2.1.2). SiO_2 NPs positively affected different plant species' growth, increased their biomass and physiological properties, modified tissue differentiation, activated antioxidant systems, and helped to adapt to stressful conditions (Luyckx et al. 2017). In general, the effects of SiO_2 NPs are widely studied in different plants (Lu et al., 2007; Janmohammadi et al., 2015). However, the SiO_2 NPs effects of the legume family have only been studied in lentils and soybeans. It has been reported that the nano-sized mixture of SiO_2 increased the nitrate reductase activity, stimulated the antioxidant system, enhanced the absorption and utilization of water and fertilizers, and accelerated the germination and growth of soybean (*Glycine max*) (Lu et al. 2007). Previous studies suggest that the higher SiO_2 NPs concentrations should be used with caution as an inhibitory effect of 120.16 ppm concentration of NPs on lentils germination was found (Janmohammadi et al. 2015).

Our established results indicate that deficiency of water causes a reduction in the pea plant's height, leaf area, SLA, RWC, and an increase in the root-to-shoot ratio (Table 3.2.1.1), and it is in agreement with other researchers (Arafa et al., 2021; Khatun et al., 2021; Bangar et al., 2019). It should be noted that after irrigating with NPs suspensions, SiO_2 NPs had a more significant effect on peas grown with optimal substrate moisture than sprayed peas. Peas have a group of aquaporins MIP - Si influx transporters (SiT1 and SiT2) and efflux transporters (SiT6) located in the central and lateral roots, facilitating the entry of Si into the xylem and enabling it to move freely in the plant (Maurel et al., 2015; Raoi and

Susmitha, 2017). However, in the case of drought, the movement of materials in the xylem is disrupted, and, therefore, Si irrigation becomes less effective. However, SiO_2 NPs foliar exposure may form an additional layer on the leaves, protecting plants from transpiration and turgor pressure changes, and allow SiO_2 NPs to penetrate the wax layer and diffuse directly into the plant and therefore have a more substantial positive effect against drought.

Based on the obtained data, we claim that SiO_2 NPs successfully activate antioxidant activity (Figure 3.2.1.2; Figure 3.2.1.3), thereby reducing the effects of oxidative stress on drought-affected peas and preserving their yield. SiO_2 NPs have a strong relationship with water content in plants, but there are just a few published scientific articles specifically on the effects of drought. For instance, spraying with Si NPs during drought stress increased the activity of CAT, APX, SOD, and GR enzymes and decreased the MDA and H_2O_2 in strawberries (Zahedi et al., 2020). In addition, an increase in leaf greenness, relative water content, and yield was found in wheat grown with SiO_2 NPs under drought conditions (Behboudi et al., 2018).

The relationship between Si NPs and abiotic factors such as salinity, exposure to heavy metals, and UV radiation in plants was also explored. Spraying salinity-treated potato seedlings with 50 ppm SiO_2 NPs suspension, the researchers found (Gowayed et al., 2017) that enzymatic antioxidants such as GPX and SOD were more active compared to potatoes untreated with SiO_2 NPs solution. SiO_2 (60.08 ppm) NPs significantly mitigated the adverse effects of salt stress on lentil seedling weight, germination, shoot and root length, and seedling vigor indices (Janmohammadi et al. 2015). Furthermore, SiO_2 NPs promoted the antioxidant system of soybeans, including the activity of SOD, POD, and CAT enzymes (Shen et al. 2010). A recent publication (Ismail et al., 2022) analyzed the effects of SiO_2 NPs on peas grown under salinity stress. Researchers found that SiO_2 NPs improved peas' morphological parameters and yielded under normal and salinity conditions. Additionally, they observed that SOD and POD genes were down-regulated at 200 mM salinity, but at higher salinity levels, these genes were induced by SiO_2 NPs. The CAT gene was strongly up-regulated by SiO_2 NPs regardless of salinity level. This was consistent with the activity of the enzymes themselves. The increase in H_2O_2 and MDA content was found in peas after exposure to heavy metal Cr (VI), but spraying them with Si NPs resulted in enzymatic antioxidants such as SOD, CAT, GR, APX stimulation and a decrease in oxidative stress biomarkers content (Tripathi et al., 2015). Moreover, when heavy metal Cd-treated common beans were sprayed with 30 ppm SiO_2 NPs, the activation of antioxidant enzymes and a decrease in the concentration of MDA and H_2O_2 were observed (Rizwan et al., 2019).

Results were similar when researchers studied (Hussain et al., 2020) the Cd-, Pb heavy metals effects and foliar application of SiO_2 NPs on rice. The sprayed plants with 20 ppm SiO_2 NPs accumulated smaller amounts of heavy metals and produced higher yields. Compared to the effects of heavy metals on peas studied by other researchers, our results also confirm that SiO_2 NPs increased the tolerance index to the heavy metal copper in peas. The SiO_2 NPs (Tripathi et al., 2017) protected wheat seedlings from UV-B stress by stimulating the antioxidant defense system, increasing fresh weight and chlorophyll content, and reducing tissue damage. The researchers noted that nitric oxide levels peaked after exposure to UV-B and Si NPs, suggesting that stress protection may be due to the modulation of NO levels.

Effects of different applications and concentrations of CuO nanoparticles on pea plants

In our studies, the zeta potential of the aqueous suspension of CuO NPs was -26.68 mV (Table 2.2.1.). Researchers reported that the zeta potential of CuO NPs is -34.4 ± 0.5 mV in suspension in deionized water at pH 7 when NPs were purchased at 10-100 nm in primary size (Keller et al. 2018; Adeleye et al. 2014; Hong et al. 2015). This indicates that the suspensions were stable and anionic.

Based on our research, significant changes were detected in the antioxidant activity of drought-affected plants. Exceptionally high activation was found in CAT, APX, and SOD (Figure 3.2.2.3). Also, FRAP antioxidant power was strongly influenced by CuO NPs. Using CuO NPs in small amounts can improve the quality of plants and protect them from environmental stresses. Our results established that the suspensions of CuO NPs significantly reduced the amount of H_2O_2 in pea plants but increased the concentration of MDA (Figure 3.2.2.1), indicating that CuO NPs are actively involved in lipid peroxidation processes. When mung bean seedlings were exposed to solutions of CuO NPs concentrations of 20, 50, 100, 200, and 500 ppm, researchers found that H_2O_2 and MDA content increased significantly in plant roots, but no effect was detected in plant leaves (Gopalakrishnan et al., 2014). They highlighted that the up-regulation of the SOD gene or Cu excess resulted in increased H_2O_2 formation in the roots of mung bean plants exposed to CuO NPs. CAT gene expression was also found to be up-regulated at lower concentrations of CuO NPs. In addition, the expression level of the APX gene was down compared to the CAT gene, which may have resulted in the attenuation of the activity of the H_2O_2 neutralization system. Therefore, the researchers hypothesized that plant antioxidant defense mechanisms were activated during CuO NPs stress; incomplete removal of H_2O_2 may cause

increased ROS levels in the roots of mung bean plants exposed to CuO NPs (Gopalakrishnan et al., 2014). The effect on Arabidopsis of 0, 0.5, 1, 2, 5, 10, 20, 50, and 100 mg L^{-1} CuO NPs suspensions in the nutrient medium were investigated (Nair and Chung, 2014). This study demonstrated that up-regulation of genes encoding enzymatic and non-enzymatic antioxidant defense mechanisms, sulfur assimilation pathways, and proline biosynthesis showed increased ROS production and rapid activation of plant defense mechanisms to neutralize oxidative stress damage during exposure to CuO NPs. One year later, the same scientist investigated the effects of CuO NPs on pea plants. Our findings agree that the higher the concentration of CuO NPs, the higher the toxicity determined in peas, starting at 100 ppm (Nair and Chung 2015). Toxic effects were confirmed in soybean by elevated lipid peroxidation and H_2O_2 content in plants at concentrations of 100 ppm CuO NPs and higher (Yusefi-Tanha et al. 2020). A reduction in soybean yield was also observed using CuO NPs at concentrations of 50-100 ppm (Ochoa et al. 2017).

Our research showed that CuO NPs had a more positive effect on the antioxidant system and Cu accumulation when plants grown in water deficit were sprayed through the leaves. Root uptake and biotransformation of CuO NPs have been extensively investigated in rice plants (Peng et al., 2015). During the biotransformation of CuO NPs, dissolved Cu bound to cysteine, citrate, and phosphate ligands, and part of Cu (II) was transformed into Cu (I). It was determined that CuO NPs could move to the root epidermis, exoderm, and cortex and reach the endodermis, but CuO NPs have difficulty penetrating the Casparian strip (Peng et al., 2015). This hindered entry of CuO NPs into the plant through the roots could have resulted in a lower distribution through the plant and a localized effect on peas when they were exposed to the CuO NPs through the roots. This explains our results, in which peas watered with CuO NPs had a higher copper accumulation in the roots, and when the plants were sprayed, the copper content increased in all parts of the plant.

But there are also reports about the positive effects of CuO NPs on plants. For example, spraying lettuce with 0, 0.5, 1.0, 2.0, 4, and 6 ppm CuO NPs suspensions positively impacted total phenols, flavonoids, antioxidant capacity, and chlorophyll content (Gaucin-Delgado et al., 2022). In addition, CuO NPs initiated the activity of APX, SOD, and CAT and significantly increased Cu accumulation in lettuce leaves. In addition, when tomato seedlings were exposed to a concentration of 10 ppm CuO NPs suspension, significant increases in fresh biomass and total chlorophyll content, as well as an increase in sugar content, NR and CAT activity, and a decrease in MDA and electrolyte leakage were found (Singh et al., 2017). As we can see from the discussed publications, the effect of CuO

NPs on plants is usually determined by the concentration. In connection with the fact that NPs are many times smaller particles than bulk materials and have a much larger surface area, the concentrations used for plants should be reduced to achieve a positive response and avoid toxic effects.

Effects of different applications and concentrations of MoO_3 nanoparticles on pea plants

The zeta potential of the aqueous suspension of MoO_3 NPs was -24.92 mV (Table 2.2.1.). According to scientific publications, the zeta potential of MoO_3 NPs suspension can be -32 mV, confirming that the solution is stable and strongly anionic.

MoO_3 NPs significantly promoted the formation of nodules on pea roots (Table 3.2.3.1) and strongly affected non-enzymatic (Figure 3.2.3.2) and such enzymatic antioxidants as APX, and GPX (Figure 3.2.3.3), thereby increasing yield during drought. A comprehensive study was investigating MoO_3 NPs on hydroponically grown rice seedlings (Sharma et al., 2021). The researchers used high concentrations of 100, 500, and 1000 ppm. The most effective concentration for rice was found to be 100 ppm, the application of which induced hormesis in rice (Sharma et al., 2021). In rice, chlorophylls and carotenoids decreased with increasing concentration of MoO_3 NPs. Also, MoO_3 NPs in rice increased MDA concentration in shoots but dropped it in roots. Such adverse effects may be associated with the use of excessive concentration.

In comparison with our results, there is a remarkable agreement that SOD activity decreases in pea leaves treated with MoO_3 NPs, while APX, GPX, and CAT increase, which leads to faster neutralization of reactive oxygen species. Another study used suspensions of MoO_3 NPs at 200 and 1000 ppm concentrations (Huang et al., 2021). Researchers observed that corn leaves could accumulate higher amounts of Mo than wheat leaves. This highlights the differences in plant species' responses to MoO_3 NPs. They concluded that wheat is more sensitive than corn regarding the number of destabilized metabolites.

Mo is not biologically active, but a specific prosthetic group complexes it in small amounts. This Mo cofactor (Moco) is distributed after biosynthesis to both Mo-enzyme families of Moco-binding proteins (sulfite oxidase, nitrate reductase (NR), mitochondrial amidoxime reductant component) and Mo-enzymes (aldehyde oxidase (AO), xanthine dehydrogenase). However, the most crucial Mo-enzyme for plant survival is cytosolic NR, which catalyzes the first step in nitrate uptake (Kaiser et al. 2005; Mendel and Schwarz 2011). The conversion of nitrates to nitrites is essential for plant growth and development. A

scheme of the mechanism of Mo-induced oxidative tolerance under drought stress was proposed by Wu (2018). It is noted that when the moisture of the substrate decreases, the plant defense system is activated through ABA and NO signals. As a component of Mo-enzymes in the form of Moco, Mo stimulates ABA synthesis and NO production via AO and NR, respectively, and thus regulates oxidative tolerance. Furthermore, NO acts downstream of ABA in Mo-mediated oxidative tolerance in plants under drought stress (Wu et al., 2018). However, it is not clear whether the exact mechanism can be applied with nano-sized Mo. For instance, it is reported that MoO_3 NPs 0.1 and 1 ppm suspensions activate nitrate reductase activity in spinach and increase chlorophyll content (Abbasifar et al., 2020). Also, using a solution with a lower concentration of Mo NPs, such as 8 ppm, increased the antioxidant activity in chickpeas (Taran et al. 2014).

The research used MoO_3 NPs with a zeta potential of -6 mV, but they did not specify the solvent in which the tested suspensions were prepared (Yang et al., 2020). However, a lower zeta potential indicates an unstable suspension and its neutral charge. They found that adding 1, 10 mg kg^{-1} MoO_3 NPs improved the morphological parameters of soybeans, but higher concentrations (100 and 1000 mg kg^{-1} MoO_3 NPs) were toxic. Furthermore, the accumulation of Mo in soybean leaves increased with increasing concentration, and MoO_3 NPs increased the activity of enzymatic antioxidants such as POD, SOD, and CAT.

Spraying MoO_3 NPs concentrations of 10, 20, 30, 40, and 50 ppm on leaves of common bean plants significantly increased morphological parameters such as fresh and dry biomass, plant height, and root length compared to untreated plants (Osman et al., 2020). An essential parameter in that study was the percentage of genomic template stability (GTS%) directly related to the degree of DNA alteration and the competence of DNA repair and replication. Genomic instability includes changes in structure, such as an increased frequency of base pair mutations. The researchers found that GTS% values decreased with increasing MoO_3 NPs concentrations up to 10, 20, and 30 ppm, then raised up to 40 ppm, 84.61%, 80.77%, 73.08%, and 78.21%, respectively. In addition, the mutagenic effect of MoO_3 NPs on the common bean genomic DNA affected the expression of several genes encoding specific proteins, turning certain genes on or off during the seedling and flowering stages (Osman et al., 2020). This may have determined that all concentrations of MoO_3 NPs significantly affected the physiological characteristics of common bean plants, resulting in increased yield and yield quality.

Since tiny amounts of MoO_3 NPs are more effective than bulk molybdenum salts, they can be used in agriculture to increase plant productivity during optimal and stressful plant

growth conditions. As found in our results, pea yield can be increased by 6% with MoO_3 NPs under regular soil moisture, and the drought-stressed pea yield can be maintained up to 80% by exposure to 50 ppm suspension. Although producing MoO_3 NPs on an industrial scale is costly, the expanded plant yield per hectare covers the resulting difference and even increases profits.

Effects of different applications and concentrations of B_2O_3 nanoparticles on pea plants

In our studies, the zeta potential of the aqueous suspension of B_2O_3 NPs was -28.54 mV (Table 2.2.1.). However, other researchers found that the aqueous suspension of B_2O_3 NPs with 0.2% triton x-100 was -30.3 mV (Barreto et al., 2021). Such zeta potential values indicate that the solutions are stable and anionic. In addition, the PDI of this suspension was 0.23, while other scientists have found 0.4 (Barreto et al., 2021), indicating the suspensions are monodisperse.

The study shows that the number of nodules formed on pea roots (Table 3.2.4.1) increases strongly after exposure to B_2O_3 NPs in both regular and deficient substrate moisture. B is transported in the xylem, and about 90% is incorporated into plant cell walls (Goldbach and Wimmer 2007). B is the main element in forming esters with rhamnogalacturonan II (RG-II). This borate ester is required to maintain normal cell wall functions and structure (Ryden et al., 2003). Under normal conditions, when there is sufficient B in the soil, RGII-glycoproteins are also formed in the plasma membranes of pea root nodules and root cells. However, RGII-glycoproteins are not synthesized, and their absence destabilizes the plasma membrane and nodule formation in B deficiency (Bolaños et al. 2001). Moreover, B, as a component of glycoproteins, is essential for differentiating nodule bacteria into a nitrogen-fixing form (Bolaños et al., 2004).

B is also known to stimulate both enzymatic and non-enzymatic antioxidant activity. Many scientific publications are highlighting the benefits of bulk B on the plant antioxidant system during different stress conditions (Alpaslan and Gunes, 2001; Bonilla et al., 2004; Bastías et al., 2004), but only a few are investigating the effect of B_2O_3 NPs on plants (Dimkpa et al., 2019; Zewail et al., 2021; Mahmoud et al., 2020).

Recent research has shown that the application of 150 mg l^{-1} arbuscular mycorrhiza (AM) with 100 mg l^{-1} B_2O_3 NPs can significantly increase the height, the number of leaves, fresh and dry biomass, and herb g $plant^{-1}$ of stevia (Zewail et al., 2021). It also positively affected the content of chlorophyll, carotenoids, and the amount of nutrients N, P, K, Zn,

and B in stevia leaves. Another critical study highlighted that B_2O_3 NPs in soybean increased B content, grain yield, and nitrogen accumulation compared to untreated plants (Dimkpa et al., 2019). In addition, B_2O_3 NPs have a positive effect in protecting tomatoes against salinity stress. It increased shoot fresh and dry weight, chlorophyll content, photosynthesis rate, stomatal conductance, intercellular CO_2 concentration, water use efficiency, and decreased transpiration rate in saline soil-grown tomatoes (Mahmoud et al., 2020). It is worth mentioning that our study expands the knowledge about the effect of B NPs on the antioxidant system of plants. It should be emphasized that B_2O_3 NPs effectively protect plants from drought stress by stimulating ABTS free radical scavenging activity and FRAP antioxidant power non-enzymatic and APX, GPX, and CAT enzymatic antioxidants.

Effects of combined stress and applications of nanoparticles on pea plants

Scientists note that plants are more often affected by combined than single stresses. There are still many unexplored possible stress combinations between drought, salinity, heat waves, chilling, freezing, ozone, pathogens, UV, nutrient deficiency, excessive CO_2, excessive lighting, and heavy metal stress (Suzuki et al., 2014). One of the objectives of this scientific work was to investigate the potential interactions of the combined effects of drought, heavy metal stresses, and NPs on peas. In studies of combined strains in different plant species, transcriptomic and proteomic analyses have implicated antioxidant defense mechanisms as a significant pathway. The observed higher antioxidant capacity and/or lower accumulation of reactive oxygen species (ROS) appears to be a mechanism acting in plants to increase resistance to combined stress. When studying the combined effect of drought and heavy metal Cu, the results show reduced morphological parameters and the activity of non-enzymatic antioxidants but significantly increased activity of enzymatic antioxidants and the content of biomarkers of oxidative stress in plants compared to peas grown only under drought or normal conditions. A study was conducted on the combined effects of drought and heavy metal Cd on maize plants (Naz et al., 2021). In that study, the researchers found that such a combination did not affect plant morphological parameters but significantly reduced the total amount of chlorophylls, proteins, carotenoids, and relative water content in the plants. Combined stress significantly increased the levels of oxidative stress biomarkers and activated SOD, CAT, APX, and POD enzymatic antioxidants. Both drought and its combination with Cu heavy metal stress reduced pea growth and resulted in nearly identical plant changes. The effects were additive, with both strains acting simultaneously, resulting in smaller plants and more robust stress responses than each stress alone. The exception was only after using NPs.

Foliar treatment of SiO_2 and B_2O_3 NPs was more effective in peas grown under combined stress conditions, although different treatments did not differ under drought conditions. This can be linked to the negative effect of the heavy metal Cu on root growth. Because under drought conditions, plants focus most of their energy on root growth to find more water. The excessive presence of Cu in the soil inhibits this, hindering the uptake of both essential elements and inhibiting root growth (Kumar et al., 2021). The researchers showed that SiO_2 NPs improved the growth of wheat subjected to combined drought and heavy metal Cd stress and reduced the Cd concentration in different wheat tissues (Khan et al., 2020). The treatment reduced oxidative stress in plants and increased chlorophyll content, and the effect was significant at the highest ppm concentration of the treatment under both limited water and normal conditions. The researchers also note that the application of SiO_2 NPs can be an effective way to reduce Cd concentration in cereal grains. Significant changes in the accumulation of Cu in plants after the use of NPs were determined in our study. Both spraying and watering of SiO_2 NPs significantly increased Cu bioconcentration in pea leaves and roots, translocation factor, and tolerance index. This could be related to higher relative water content and increased antioxidant activity in plants exposed to combined stress and SiO_2 NPs.

Interesting results were found in peas exposed to combined stress and B_2O_3 NPs, as there was a substantial decrease in MDA concentration in pea leaves, while MDA was at its highest concentration in all cases with Cu excess. This is related to the fact that, as mentioned above, most of the B that enters the plant is bound to the plant cell walls and thus protects the cells from lipid peroxidation (Blevins and Lukaszewski, 1998). Also, B_2O_3 NPs increased the Cu tolerance index in peas and significantly increased the activity of the essential enzymatic antioxidants SOD and CAT. SOD breaks down the superoxide radical into H_2O_2; after that, CAT converts H_2O_2 into water. Significant activation of these enzymes helped peas to survive the combined stress.

Root treatment with MoO_3 NPs of peas under combined stress significantly affected nodulation, which was strongly reduced only by drought and Cu heavy metal exposure. A significant increase in the relative water content of the plants was also found. As a result, we see increased Cu bioaccumulation in roots, translocation factor, and Cu tolerance index. There was also a significant increase in the activity of enzymatic antioxidants GPX, APX, SOD, and CAT, which corresponded to the decreased levels of oxidative stress indicators. Another critical effect found is that in the case of the combined effects of drought and heavy

metal Cu, CuO NPs have strengthened their negative influence. This shows that CuO NPs in peas act synergistically with heavy metal Cu.

This work expands the knowledge about the potential effects of combined stresses on peas. Additionally, this study investigates and discusses in detail the effects of combined stresses and different NPs on pea morphological parameters, yield, antioxidant and oxidative systems, and elemental composition.

CONCLUSIONS

1. The most effective concentrations of SiO_2, CuO, and MoO_3 nanoparticle suspensions were 50 ppm and B_2O_3 – 12.5 ppm for pea seedlings and fully developed peas grown in a substrate of normal moisture or under drought conditions.

2. The used suspension of SiO_2 nanoparticles increased the drought resistance of peas. Under conditions of moisture deficiency, SiO_2 nanoparticles reduced the concentration of oxidative biomarkers by 27%. They raised the total amount of phenolic compounds and the activity of non-enzymatic antioxidants in peas by 30%. A significant activating effect was found on the catalase (159%), superoxide dismutase (37%), and glutathione reductase (128%) after exposure of plants to drought and a solution of 50 ppm SiO_2 nanoparticles. The yield of peas affected by drought was maintained by 45% after using a suspension of SiO_2 nanoparticles.

3. CuO nanoparticles reduced hydrogen peroxide by 37% but increased malondialdehyde by 66% in pea plants affected by drought. The highest concentration of 50 ppm CuO nanoparticles increased the activity of non-enzymatic antioxidants up to 2.5 times. It stimulated the activity of catalase (167%) and ascorbate peroxidase (72%) in peas grown under drought conditions. The highest accumulation of Cu in peas leaves, stems, and roots were determined after spraying the plants with a suspension of 50 ppm CuO nanoparticles.

4. After using the suspension of MoO_3 nanoparticles in drought conditions, a positive effect on the height of peas (40%) and leaf area (30%) was determined, and the indicators of oxidative stress decreased by up to 30%. A 37% increase in the total amount of phenolic compounds and a 145% increase in the activity of non-enzymatic antioxidants were determined in plants exposed to drought and MoO_3 nanoparticles. MoO_3 nanoparticles increased the activity of catalase and ascorbate peroxidase by 2 times and the activity of superoxide dismutase by 20%, and guaiacol peroxidase by 56%. The highest Mo accumulation was found in pea roots when the plants were watered with nanoparticle suspensions.

5. B_2O_3 nanoparticles positively affected pea height (26%) and leaf area (40%) and increased antioxidant activity by 2.5 times. Ascorbate peroxidase was stimulated 6 times, guaiacol peroxidase by 91%, and superoxide dismutase by 51%. The highest accumulation of B was found in the roots, regardless of the route of exposure. B accumulation occurred most intensively in stems and leaves of peas affected by drought and watered with B_2O_3 nanoparticles.

6. More substantial oxidative stress caused by the combined effects of Cu as heavy metal and drought on pea plants was inhibited by the used suspensions of SiO_2, MoO_3, and B_2O_3

nanoparticles compared to plants that were affected by drought and excess Cu. It effectively reduced the concentrations of hydrogen peroxide and malondialdehyde in the pea plant and increased the activity of the antioxidant system. Conversely, irrigation with CuO nanoparticles enhanced the adverse effects of drought and excess Cu on plants. In peas, the Cu tolerance index was statistically reliably increased by: irrigation and spraying of SiO_2, spraying B_2O_3, and irrigation with MoO_3 nanoparticle suspensions.

7. Watering with MoO_3 nanoparticle suspension more effectively reduced the adverse effects caused by stress in peas than spraying and saved 80% of the yield. On the other hand, the effectiveness of B_2O_3 and CuO nanoparticle suspensions was more substantial when sprayed on peas and preserved 92% and 47% of the yield, respectively. The effect of SiO_2 nanoparticles according to the application method did not differ substantially, and the yield was about 40% higher compared to plants affected by drought but not by nanoparticles.

LIST OF PUBLICATIONS

Articles in journals indexed in Clarivate Analytics Web of Science database:

1. **Sutulienė, R.**, Ragelienė, L., Samuolienė, G., Brazaitytė, A., Urbutis, M. and Miliauskienė, J., 2021. The Response of Antioxidant System of Drought-Stressed Green Pea (*Pisum sativum* L.) Affected by Watering and Foliar Spray with Silica Nanoparticles. Horticulturae, 8(1), 35.
2. **Sutulienė, R.**, Ragelienė, L., Duchovskis, P. and Miliauskienė, J., 2022. The Effects of Nano-copper, -molybdenum, -boron, and -silica on Pea (*Pisum sativum* L.) Growth, Antioxidant Properties, and Mineral Uptake. Journal of Soil Science and Plant Nutrition, 22(1), pp.801-814.

Articles in journals in Clarivate Analytics Web of Science database:

1. **(Sutulienė, R.) Paulauskaitė, R.**, Miliauskienė, J. and Ragelienė, L., 2019. Uptake and effect of nanoparticles in plants. Sodininkyste ir Daržininkyste, 38(1/2), pp.31-46.

Conferences:

1. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2022, poster: Influence of Silica Nanoparticles on the Enzymatic and Non-Enzymatic Antioxidant Activity of Green Pea (*Pisum sativum* L.) Affected by Drought. 31st International Horticultural Congress (IHC).
2. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2022, poster: The response of drought-stressed green pea (*Pisum sativum* L.) to boron nanoparticle application. 1st International Electronic Conference on Horticulturae (IECHo).
3. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2022, oral presentation: Impact of copper oxide nanoparticles on the antioxidant system of drought affected pea. 17 th International Conference of young scientists on energy and natural sciences issues (CYSENI).
4. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2021, oral presentation: Growth properties of pea plants in response to the complex effects of different nanoparticles and drought stress.17 th International Conference of young scientists on energy and natural sciences issues (CYSENI).
5. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2020, poster: The effect of CuO, Mo, B and SiO_2 nanoparticles on pea (*Pisum sativum* L.) plants during the agrometeorological drought. Scandinavian Plant Physiology Society PhD Student (SPPS).

6. **Sutulienė, R.**, Ragelienė, L., Miliauskienė, J., 2020, oral presentation: The Effect of CuO, Mo, B, SiO_2 Nanoparticles and Agrometeorological Drought Stress on Growth Parameters of Pea (*Pisum sativum* L.). International Conference on Agriculture and Horticulture (ICAH).

ABOUT THE AUTHOR

Rūta Sutulienė was born on March 1, 1992, in Marijampole, Lithuania. She successfully graduated from Marijampole "Sūduva" high school in 2011. She obtained a bachelor's degree in biology in 2015 at Vytautas the Great University, Faculty of Natural Sciences. She received a master's degree in applied biochemistry in 2018 at Vytautas Magnus University, Faculty of Natural Sciences. From 2019 until now, she has been working as a junior researcher at the Lithuanian Centre for Agricultural and Forestry Sciences, Institute of Horticulture and Horticulture. In addition, she participated in a short-term research internship at the University of Antwerp, Belgium, in 2021 and at the University of Agronomy in Krakow, Poland, in 2019.

ACKNOWLEDGEMENTS

I sincerely thank the scientific supervisor, Dr. Jurga Miliauskienei, and Assoc. Dr. Lina Rageliene, for your patience, time, support, and advice during the research and preparation of the dissertation. I am grateful to all the colleagues of the Plant Physiology Laboratory, who managed to turn the workplace into a second home where you always feel welcome. Thank you, Prof. Dr. Pavelas Duchovskis, an academician who trained outstanding female scientists: Dr. Giedrė Samuolienė, Dr. Akvilė Viršile, Dr. Aušra Brazaitytė, Dr. Viktorija Vaštakaitė-Kairienė, Dr. Kristina Laužikė, to whom you can always turn for help and advice, to be sad and happy together. I am also grateful to LAMMC SDI for the opportunity to study in the Ph.D. program and for encouraging me to expand my horizons through internships and participation in international conferences. Finally, I sincerely thank my parents and husband for their support and encouragement to study.

REFERENCES

1. Abbasifar, A., ValizadehKaji, B. and Iravani, M.A., 2020. Effect of green synthesized molybdenum nanoparticles on nitrate accumulation and nitrate reductase activity in spinach. Journal of Plant Nutrition, 43 (1), pp.13-27.
2. Abdel Latef, A.A.H., Abu Alhmad, M.F. and Abdelfattah, K.E., 2017. The possible roles of priming with ZnO nanoparticles in mitigation of salinity stress in lupine (*Lupinus termis*) plants. Journal of plant growth regulation, 36 (1), pp.60-70.
3. Abid, M., Ali, S., Qi, L.K., Zahoor, R., Tian, Z., Jiang, D., Snider, J.L. and Dai, T., 2018. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). Scientific reports, 8(1), pp.1-15.
4. Adhikari, T., Kundu, S., Biswas, A.K., Tarafdar, J.C. and Rao, A.S., 2012. Effect of copper oxide nano particle on seed germination of selected crops. Journal of Agricultural Science and Technology, 2(6A), 815.
5. Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M.K. and Bharwana, S.A., 2015. The effect of excess copper on growth and physiology of important food crops: a review. Environmental Science and Pollution Research, 22(11), pp.8148-8162.
6. Adrees, M., Khan, Z.S., Ali, S., Hafeez, M., Khalid, S., Zia Ur Rehman, M., Hussain, A., Hussain, K., Chatha, S.A.S. and Rizwan, M., 2020. Simultaneous mitigation of cadmium and drought stress in wheat by soil application of iron nanoparticles. Chemosphere, 238, 124681.
7. Ahlawat, I.P.S., Gangaiah, B. and Zahid, M.A., 2007. Nutrient management in chickpea. Chickpea breeding and management, pp.213-232.
8. Ahmad, J., Qamar, S., Kausar, N., and Qureshi, M.I., 2020. Nanoparticles: the magic bullets in mitigating drought stress in plants. Nanobiotechnology in agriculture, pp.145-161.
9. Ahmed, T., Noman, M., Rizwan, M., Ali, S., Ijaz, U., Nazir, M.M., AL Haithloul, H.A.S., Alghanem, S.M., Abdulmajeed, A.M. and Li, B., 2022. Green molybdenum nanoparticles-mediated bio-stimulation of Bacillus sp. strain ZH16 improved the wheat growth by managing in planta nutrients supply, ionic homeostasis and arsenic accumulation. Journal of Hazardous Materials, 423, 127024.
10. Ayala, A., Muñoz, M.F., Argüelles, S., Ayala, A., Munoz, M.F. and Arguelles, S., 1987. Lipid peroxidation: production, metabolism, and signaling mechanisms of

malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 360438. Science, 235(4792), pp.1043-1049.

11. Akram, N.A., Shafiq, F. and Ashraf, M., 2017. Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Frontiers in plant science, 8, pp.613-623.
12. Al-Garni, S.M.S., 2006. Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer Rhizobium bacterium. African Journal of Biotechnology, 5(2), pp.133-142.
13. Ali, S., Rizwan, M., Hussain, A., Zia Ur Rehman, M., Ali, B., Yousaf, B., Wijaya, L., Alyemeni, M.N. and Ahmad, P., 2019. Silicon nanoparticles enhanced the growth and reduced the cadmium accumulation in grains of wheat (*Triticum aestivum* L.). Plant Physiology and Biochemistry, 140, pp.1-8.
14. Alpaslan, M. and Gunes, A., 2001. Interactive effects of boron and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. Plant and Soil, 236(1), pp.123-128.
15. Amir, R., Munir, F., Kubra, G., Iqbal, T. and Khan, M., 2019. Plant signaling molecules and cadmium stress tolerance. In Cadmium Tolerance in Plants (pp. 367-399). Academic Press.
16. a Nazir, M.M., Li, Q., Noman, M., Ulhassan, Z., Ali, S., Ahmed, T., Zeng, F. and Zhang, G., 2022. Calcium Oxide Nanoparticles Have the Role of Alleviating Arsenic Toxicity of Barley. Frontiers in plant science, 13, 843795.
17. An, Y.J., 2006. Assessment of comparative toxicities of lead and copper using plant assay. Chemosphere, 62(8), pp.1359-1365.
18. Anjum, N.A., Khan, N.A., Sofo, A., Baier, M. and Kizek, R., 2016. Redox homeostasis managers in plants under environmental stresses. Frontiers in environmental science, pp.35-46.
19. Arafa, S.A., Attia, K.A., Niedbała, G., Piekutowska, M., Alamery, S., Abdelaal, K., Alateeq, T.K., AM Ali, M., Elkelish, A. and Attallah, S.Y., 2021. Seed Priming boost adaptation in pea plants under drought stress. Plants, 10(10), 2201.
20. Arora, A., Byrem, T.M., Nair, M.G. and Strasburg, G.M., 2000. Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Archives of Biochemistry and Biophysics, 373(1), pp.102-109.
21. Ashraf, M., Akram, N.A., Al-Qurainy, F. and Foolad, M.R., 2011. Drought tolerance: roles of organic osmolytes, growth regulators, and mineral nutrients. Advances in agronomy, 111, pp.249-296.

22. Azooz, R.H., Arshad, M.A. and Franzluebbers, A.J., 1996. Pore size distribution and hydraulic conductivity affected by tillage in Northwestern Canada. Soil Science Society of America Journal, 60(4), pp.1197-1201.
23. Ban, Y., Xiao, Z., Wu, C., Lv, Y., Meng, F., Wang, J. and Xu, Z., 2021. The positive effects of inoculation using arbuscular mycorrhizal fungi and/or dark septate endophytes on the purification efficiency of CuO-nanoparticles-polluted wastewater in constructed wetland. Journal of Hazardous Materials, 416, p.126095.
24. Banerjee, K., Pramanik, P., Maity, A., Joshi, D.C., Wani, S.H. and Krishnan, P., 2019. Methods of using nanomaterials to plant systems and their delivery to plants (Mode of entry, uptake, translocation, accumulation, biotransformation and barriers). In Advances in Phytonanotechnology (pp. 123-152). Academic Press.
25. Bangar, P., Chaudhury, A., Tiwari, B., Kumar, S., Kumari, R. and Bhat, K.V., 2019. Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turkish Journal of Biology, 43(1), pp.58-69.
26. Bano, A., Gupta, A., Rai, S., Fatima, T., Sharma, S. and Pathak, N., 2021. Mechanistic role of reactive oxygen species and its regulation via the antioxidant system under environmental stress. Plant Stress Physiology—Perspectives in Agriculture, pp.1-18.
27. Barreto, A., Santos, J., Amorim, M.J. and Maria, V.L., 2021. Environmental Hazards of Boron and Vanadium Nanoparticles in the Terrestrial Ecosystem—A Case Study with Enchytraeus crypticus. Nanomaterials, 11(8), 1937.
28. Bartosz, G., 1997. Oxidative stress in plants. Acta Physiologiae Plantarum, 19(1), pp.47–64.
29. Bastías, E.I., González-Moro, M.B. and González-Murua, C., 2004. *Zea mays* L. amylacea from the Lluta Valley (Arica-Chile) tolerates salinity stress when high levels of boron are available. Plant and Soil, 267(1), pp.73-84.
30. Basu, S., Ramegowda, V., Kumar, A. and Pereira, A., 2016. Plant adaptation to drought stress. F1000Research, 5.
31. Behboudi, F., Tahmasebi Sarvestani, Z., Kassaee, M.Z., Modares Sanavi, S.A.M. and Sorooshzadeh, A., 2018. Improving growth and yield of wheat under drought stress via application of SiO_2 nanoparticles. Journal of Agricultural Science and Technology, 20(7), pp.1479-1492.
32. Bela, K., Horváth, E., Gallé, Á., Szabados, L., Tari, I. and Csiszár, J., 2015. Plant glutathione peroxidases: emerging role of the antioxidant enzymes in plant development and stress responses. Journal of Plant Physiology, 176, pp.192-201.

33. Ben-Moshe, T., Frenk, S., Dror, I., Minz, D. and Berkowitz, B., 2013. Effects of metal oxide nanoparticles on soil properties. Chemosphere, 90(2), pp. 640-646.
34. Bertolino, L.T., Caine, R.S. and Gray, J.E., 2019. Impact of stomatal density and morphology on water-use efficiency in a changing world. Frontiers in plant science, 10, 225.
35. Bhattacharyya, R., Prakash, V., Kundu, S. and Gupta, H.S., 2006. Effect of tillage and crop rotations on pore size distribution and soil hydraulic conductivity in sandy clay loam soil of the Indian Himalayas. Soil and Tillage Research, 86(2), pp.129-140.
36. Bienert, G.P., Schjoerring, J.K. and Jahn, T.P., 2006. Membrane transport of hydrogen peroxide. Biochimica et Biophysica Acta (BBA)-Biomembranes, 1758(8), pp. 994-1003.
37. Panda Blevins, D.G. and Lukaszewski, K.M., 1998. Boron in plant structure and function. Annual review of plant biology, 49(1), pp. 481-500.
38. Blum, A., 2017. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant, cell & environment, 40(1), pp. 4-10.
39. b Nazira, M.M., Noman, M., Ahmed, T., Ali, S., Ulhassan, Z., Zeng, F. and Zhang, G., 2022. Exogenous calcium oxide nanoparticles alleviate cadmium toxicity by reducing Cd uptake and enhancing antioxidative capacity in barley seedlings. Journal of Hazardous Materials, 438, 129498.
40. Bollero, G.A., Bullock, D.G. and Hollinger, S.E., 1996. Soil temperature and planting date effects on corn yield, leaf area, and plant development. Agronomy Journal, 88(3), pp. 385-390.
41. Bonilla, I., El-Hamdaoui, A. and Bolaños, L., 2004. Boron and calcium increasePisum sativum seed germination and seedling development under salt stress. Plant and soil, 267(1), pp. 97-107.
42. Bowles, A.M., Paps, J. and Bechtold, U., 2021. Evolutionary origins of drought tolerance in spermatophytes. Frontiers in plant science, 12, 655924.
43. Bowles, T.M., Mooshammer, M., Socolar, Y., Calderón, F., Cavigelli, M.A., Culman, S.W., Deen, W., Drury, C.F., y Garcia, A.G., Gaudin, A.C. and Harkcom, W.S., 2020. Long-term evidence shows that crop-rotation diversification increases agricultural resilience to adverse growing conditions in North America. One Earth, 2(3), pp. 284-293.
44. Cabane, M., Afif, D. and Hawkins, S., 2012. Lignins and abiotic stresses. In Advances in botanical research (Vol. 61, pp. 219-262). Academic Press.
45. Carraro, E., and Di Iorio, A., 2022. Eligible strategies of drought response to improve drought resistance in woody crops: a mini-review. Plant Biotechnol Rep 16, pp. 265–282.

46. Chaves, M.M. and Oliveira, M.M., 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. Journal of experimental botany, 55(407), pp. 2365-2384.
47. Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P., Osório, M.L., Carvalho, I., Faria, T. and Pinheiro, C., 2002. How plants cope with water stress in the field? Photosynthesis and growth. Annals of botany, 89(7), pp. 907-916.
48. Chen, H. and Jiang, J.G., 2010. Osmotic adjustment and plant adaptation to environmental changes related to drought and salinity. Environmental Reviews, 18(NA), pp. 309-319.
49. Conrath, U., 2006. Systemic acquired resistance. Plant signaling & behavior, 1(4), pp. 179-184.
50. Corpas, F.J., Del Río, L.A. and Palma, J.M., 2019. Plant peroxisomes at the crossroad of NO and H2O2 metabolism. Journal of integrative plant biology, 61(7), pp. 803-816.
51. Corpas, F.J., Palma, J.M., Sandalio, L.M., Valderrama, R., Barroso, J.B. and Luis, A., 2008. Peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (Pisum sativum L.) leaves. Journal of plant physiology, 165(13), pp.1319-1330.
52. Corwin, D.L., 2021. Climate change impacts on soil salinity in agricultural areas. European Journal of Soil Science, 72(2), pp. 842-862.
53. Cruz de Carvalho, M.H., 2008. Drought stress and reactive oxygen species: production, scavenging and signaling. Plant signaling & behavior, 3(3), pp. 156-165.
54. Barrs, H.D. and Weatherley, P.E., 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian journal of biological sciences, 15(3), pp. 413-428.
55. Daglioglu, Y. and Türkiş, S., A Comparative Study on the Effect of Acute Toxicity of Nano and Micro Boron Particles in Lemna minor (Linneaus 1753). Journal of Boron, 6(2), pp. 263-273.
56. Dai, Y., Wang, Z., Zhao, J., Xu, L., Xu, L., Yu, X., Wei, Y. and Xing, B., 2018. Interaction of CuO nanoparticles with plant cells: internalization, oxidative stress, electron transport chain disruption, and toxicogenomic responses. Environmental Science: Nano, 5(10), pp. 2269-2281.
57. Das, K. and Roychoudhury, A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in environmental science, 2, 53.
58. Davarpanah, S., Tehranifar, A., Davarynejad, G., Abadía, J. and Khorasani, R., 2016. Effects of foliar applications of zinc and boron nano-fertilizers on pomegranate (Punica granatum cv. Ardestani) fruit yield and quality. Scientia horticulturae, 210, pp. 57-64.

59. Davey, M.W., Montagu, M.V., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D. and Fletcher, J., 2000. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. Journal of the Science of Food and Agriculture, 80(7), pp. 825-860.
60. Del Rio, L.A., Palma, J.M., Sandalio, L.M., Corpas, F.J., Pastori, G.M., Bueno, P. and López-Huertas, E., 1996. Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. Biochemical Society Transactions, 24(2), pp. 434-438.
61. Demidchik, V., 2015. Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. Environmental and Experimental Botany, 109, pp. 212–228.
62. Derks, A., Schaven, K. and Bruce, D., 2015. Diverse mechanisms for photoprotection in photosynthesis. Dynamic regulation of photosystem II excitation in response to rapid environmental change. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 1847(4-5), pp. 468-485.
63. Dietz, K.J. and Herth, S., 2011. Plant nanotoxicology. Trends in plant science, 16(11), pp. 582-589.
64. Dmitrieva, V.A., Tyutereva, E.V. and Voitsekhovskaja, O.V., 2020. Singlet oxygen in plants: Generation, detection, and signaling roles. International journal of molecular sciences, 21(9), 3237.
65. Dogra, V. and Kim, C., 2020. Singlet oxygen metabolism: from genesis to signaling. Frontiers in plant science, p.1640.
66. dos Santos, T.B., Ribas, A.F., de Souza, S.G.H., Budzinski, I.G.F. and Domingues, D.S., 2022. Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. Stresses, 2(1), pp. 113-135.
67. Duarte-Sierra, A., Tiznado-Hernández, M.E., Jha, D.K., Janmeja, N. and Arul, J., 2020. Abiotic stress hormesis: An approach to maintain quality, extend storability, and enhance phytochemicals on fresh produce during postharvest. Comprehensive Reviews in Food Science and Food Safety, 19(6), pp. 3659-3682.
68. Dumanović, J., Nepovimova, E., Natić, M., Kuča, K. and Jaćević, V., 2021. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. Frontiers in plant science, 11, 552969.
69. Eckerson, S.H., 1908. The number and size of the stomata. Botanical Gazette, 46(3), pp. 221-224.
70. Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F. and Hashem, A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. Frontiers in microbiology, 8, 2104.

71. Elsheery, N.I., Sunoj, V.S.J., Wen, Y., Zhu, J.J., Muralidharan, G. and Cao, K.F., 2020. Foliar application of nanoparticles mitigates the chilling effect on photosynthesis and photoprotection in sugarcane. Plant Physiology and Biochemistry, 149, pp. 50-60.
72. Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Morishima I, Shibahara T, Inanaga S, Tanaka K (2006) Enhanced tolerance to ozone and drought stresses in transgenic tobacco overexpressing dehydroascorbate reductase in cytosol. Physiol Plant 127, pp. 57–65.
73. Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T, Inanaga S, Tanaka K (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225, pp. 1255-1264.
74. Emamverdian, A., Ding, Y., Mokhberdoran, F. and Xie, Y., 2015. Heavy metal stress and some mechanisms of plant defense response. The scientific world journal, 756120.
75. Eshdat, Y., Holland, D., Faltin, Z. and Ben-Hayyim, G., 1997. Plant glutathione peroxidases. Physiologia Plantarum, 100(2), pp. 234-240.
76. Estévez, I.H. and Hernández, M.R., 2020. Plant glutathione S-transferases: an overview. Plant Gene, 23, 100233.
77. Fageria, N.K., 2001. Nutrient management for improving upland rice productivity and sustainability. Communications in Soil Science and Plant Analysis, 32(15-16), pp. 2603-2629.
78. Faizan, M., Bhat, J.A., Chen, C., Alyemeni, M.N., Wijaya, L., Ahmad, P. and Yu, F., 2021. Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. Plant Physiology and Biochemistry, 161, pp. 122-130.
79. Farooq, M., Hussain, M., Wahid, A. and Siddique, K.H.M., 2012. Drought stress in plants: an overview. Plant responses to drought stress, pp. 1-33.
80. Farvardin, A., González-Hernández, A.I., Llorens, E., García-Agustín, P., Scalschi, L. and Vicedo, B., 2020. The apoplast: a key player in plant survival. Antioxidants, 9(7), 604.
81. Feigl, G., Kumar, D., Lehotai, N., Pető, A., Molnár, Á., Rácz, É., Ördög, A., Erdei, L., Kolbert, Z. and Laskay, G., 2015. Comparing the effects of excess copper in the leaves of *Brassica juncea* (L. Czern) and *Brassica napus* (L.) seedlings: Growth inhibition, oxidative stress and photosynthetic damage. Acta Biologica Hungarica, 66(2), pp. 205-221.
82. Fenton, H. J. H., 1984. Oxidation of tartaric acid in the presence of iron. J. Chem. Soc. Trans. 65, pp. 899–910.
83. Fernandes, J.C. and Henriques, F.S., 1991. Biochemical, physiological, and structural effects of excess copper in plants. The botanical review, 57(3), pp. 246-273.

84. Fichman, Y. and Mittler, R., 2020. Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades? The Plant Journal, 102(5), pp. 887-896.
85. Fischer, R.A., Santiveri, F. and Vidal, I.R., 2002. Crop rotation, tillage and crop residue management for wheat and maize in the sub-humid tropical highlands: I. Wheat and legume performance. Field Crops Research, 79(2-3), pp. 107-122.
86. Foyer, C.H., 2018. Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environmental and experimental botany, 154, pp. 134-142.
87. Foyer, C.H., Lopez-Delgado, H., Dat, J.F. and Scott, I.M., 1997. Hydrogen peroxide-and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiologia plantarum, 100(2), pp. 241-254.
88. Gallie, D.R., 2013. The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. Journal of experimental botany, 64(2), pp. 433-443.
89. Gambetta, G.A., Herrera, J.C., Dayer, S., Feng, Q., Hochberg, U. and Castellarin, S.D., 2020. The physiology of drought stress in grapevine: towards an integrative definition of drought tolerance. Journal of experimental botany, 71(16), pp. 4658-4676.
90. Garcia-Caparros, P., De Filippis, L., Gul, A., Hasanuzzaman, M., Ozturk, M., Altay, V. and Lao, M.T., 2021. Oxidative stress and antioxidant metabolism under adverse environmental conditions: a review. The Botanical Review, 87(4), pp. 421-466.
91. García-Gómez, C., Obrador, A., González, D., Babín, M. and Fernández, M.D., 2017. Comparative effect of ZnO NPs, ZnO bulk and ZnSO4 in the antioxidant defences of two plant species growing in two agricultural soils under greenhouse conditions. Science of the Total Environment, 589, pp. 11-24.
92. Gaucin-Delgado, J.M., Ortiz-Campos, A., Hernandez-Montiel, L.G., Fortis-Hernandez, M., Reyes-Pérez, J.J., Gonzáles-Fuentes, J.A. and Preciado-Rangel, P., 2022. CuO-NPs Improve Biosynthesis of Bioactive Compounds in Lettuce. Plants, 11(7), 912.
93. Geisler-Lee, J., Wang, Q., Yao, Y., Zhang, W., Geisler, M., Li, K., Huang, Y., Chen, Y., Kolmakov, A. and Ma, X., 2012. Phytotoxicity, accumulation and transport of silver nanoparticles by Arabidopsis thaliana. Nanotoxicology, 7(3), pp.323-337.
94. Ghalamboran, M.R., 2011. Symbiotic nitrogen fixation enhancement due to magnetite nanoparticles (Doctoral dissertation, Cranfield University).
95. Ghani, M.I., Saleem, S., Rather, S.A., Rehmani, M.S., Alamri, S., Rajput, V.D., Kalaji, H.M., Saleem, N., Sial, T.A. and Liu, M., 2022. Foliar application of zinc oxide nanoparticles: An effective strategy to mitigate drought stress in cucumber seedling by modulating antioxidant defense system and osmolytes accumulation. Chemosphere, 289, 133202.

96. Ghosh, D. and Xu, J., 2014. Abiotic stress responses in plant roots: a proteomics perspective. Frontiers in plant science, 5, 6.
97. Gill, S.S. and Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry, 48(12), pp. 909-930.
98. Gilroy, S., Białasek, M., Suzuki, N., Górecka, M., Devireddy, A.R., Karpiński, S. and Mittler, R., 2016. ROS, calcium, and electric signals: key mediators of rapid systemic signaling in plants. Plant physiology, 171(3), pp. 1606-1615.
99. Gopalakrishnan Nair, P.M., Kim, S.H. and Chung, I.M., 2014. Copper oxide nanoparticle toxicity in mung bean (*Vigna radiata* L.) seedlings: physiological and molecular level responses of in vitro grown plants. Acta Physiologiae Plantarum, 36(11), pp. 2947-2958.
100. Gowayed, M.H., Al-Zahrani, H.S. and Metwali, E.M., 2017. Improving the salinity tolerance in potato (Solanum tuberosum) by exogenous application of silicon dioxide nanoparticles. International Journal of Agriculture and Biology, 19(1).
101. Grace, S.C. and Logan, B.A., 2000. Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 355(1402), pp. 1499-1510.
102. Guo, D., Xie, G. and Luo, J., 2013. Mechanical properties of nanoparticles: basics and applications. Journal of physics D: applied physics, 47(1), 013001.
103. Gupta, A., Rico-Medina, A. and Caño-Delgado, A.I., 2020. The physiology of plant responses to drought. Science, 368(6488), pp. 266-269.
104. Habibi, G., 2014. Hydrogen peroxide (H_2O_2) generation, scavenging and signaling in plants. In Oxidative Damage to Plants (pp. 557-584). Academic Press.
105. Hasanuzzaman, M., Bhuyan, M.B., Anee, T.I., Parvin, K., Nahar, K., Mahmud, J.A. and Fujita, M., 2019. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants, 8(9), 384.
106. Hasanuzzaman, M., Bhuyan, M.B., Parvin, K., Bhuiyan, T.F., Anee, T.I., Nahar, K., Hossen, M.S., Zulfiqar, F., Alam, M.M. and Fujita, M., 2020. Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. International Journal of Molecular Sciences, 21(22), 8695.
107. Hashem, A., Alqarawi, A.A., Radhakrishnan, R., Al-Arjani, A.B.F., Aldehaish, H.A., Egamberdieva, D. and Abd_Allah, E.F., 2018. Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. Saudi journal of biological sciences, 25(6), pp. 1102-1114.

108. Hattab, S., Dridi, B., Chouba, L., Kheder, M.B. and Bousetta, H., 2009. Photosynthesis and growth responses of pea *Pisum sativum* L. under heavy metals stress. Journal of Environmental Sciences, 21(11), pp. 1552-1556.
109. Hosseinpour, A., Ilhan, E., Özkan, G., Öztürk, H.İ., Haliloglu, K. and Cinisli, K.T., 2021. Plant growth-promoting bacteria (PGPBs) and copper (II) oxide (CuO) nanoparticle ameliorates DNA damage and DNA Methylation in wheat (*Triticum aestivum* L.) exposed to NaCl stress. Journal of Plant Biochemistry and Biotechnology, pp. 1-14.
110. Hsiao, T.C., Steduto, P. and Fereres, E., 2007. A systematic and quantitative approach to improve water use efficiency in agriculture. Irrigation science, 25(3), pp. 209-231.
111. Huang, S., Van Aken, O., Schwarzländer, M., Belt, K. and Millar, A.H., 2016. The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. Plant physiology, 171(3), pp. 1551-1559.
112. Huang, H., Ullah, F., Zhou, D.X., Yi, M. and Zhao, Y., 2019. Mechanisms of ROS regulation of plant development and stress responses. Frontiers in Plant Science, 10, 800.
113. Huang, X., Cervantes-Avilés, P., Li, W. and Keller, A.A., 2021. Drilling into the metabolomics to enhance insight on corn and wheat responses to molybdenum trioxide nanoparticles. Environmental Science & Technology, 55(20), pp. 13452-13464.
114. Hülskamp, M., 2004. Plant trichomes: a model for cell differentiation. Nature Reviews Molecular Cell Biology, 5(6), pp. 471-480.
115. Hussain, A., Ali, S., Rizwan, M., ur Rehman, M.Z., Javed, M.R., Imran, M., Chatha, S.A.S. and Nazir, R., 2018. Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. Environmental Pollution, 242, pp. 1518-1526.
116. Hussain, B., Lin, Q., Hamid, Y., Sanaullah, M., Di, L., Khan, M.B., He, Z. and Yang, X., 2020. Foliage application of selenium and silicon nanoparticles alleviates Cd and Pb toxicity in rice (Oryza sativa L.). Science of the Total Environment, 712, 136497.
117. Yang, J., Duan, G., Li, C., Liu, L., Han, G., Zhang, Y. and Wang, C., 2019. The crosstalks between jasmonic acid and other plant hormone signaling highlight the involvement of jasmonic acid as a core component in plant response to biotic and abiotic stresses. Frontiers in plant science, 10, 1349.
118. Yang, J., Song, Z., Ma, J. and Han, H., 2020. Toxicity of molybdenum-based nanomaterials on the soybean–rhizobia symbiotic system: implications for nutrition. ACS Applied Nano Materials, 3(6), pp.5773-5782.
119. Yang, X., Lu, M., Wang, Y., Wang, Y., Liu, Z. and Chen, S., 2021. Response mechanism of plants to drought stress. Horticulturae, 7(3), 50.

120. Yin, L., Wang, S., Eltayeb, A.E., Uddin, M., Yamamoto, Y., Tsuji, W., Takeuchi, Y. and Tanaka, K., 2010. Overexpression of dehydroascorbate reductase, but not monodehydroascorbate reductase, confers tolerance to aluminum stress in transgenic tobacco. Planta, 231(3), pp. 609-621.
121. Yruela, I., 2009. Copper in plants: acquisition, transport and interactions. Functional Plant Biology, 36(5), pp. 409-430.
122. Ijaz, I., Gilani, E., Nazir, A. and Bukhari, A., 2020. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles. Green Chemistry Letters and Reviews, 13(3), pp. 223-245.
123. Imlay, J.A., 2006. Iron-sulphur clusters and the problem with oxygen. Molecular microbiology, 59(4), pp. 1073-1082.
124. Inze, D. and Van Montagu, M., 1995. Oxidative stress in plants. Current opinion in Biotechnology, 6(2), pp. 153-158.
125. Yruela, I., 2005. Copper in plants. Brazilian Journal of Plant Physiology, 17, pp. 145-156.
126. Ismail, L.M., Soliman, M.I., Abd El-Aziz, M.H. and Abdel-Aziz, H.M., 2022. Impact of silica ions and nano silica on growth and productivity of pea plants under salinity stress. Plants, 11(4), 494.
127. Janmohammadi, M., Sabaghnia, N. and Ahadnezhad, A., 2015. Impact of silicon dioxide nanoparticles on seedling early growth of lentil (*Lens culinaris*) genotypes with various origins. Agriculture & Forestry/Poljoprivreda i Sumarstvo, 61(3).
128. Jauneau, A., Cerutti, A., Auriac, M.C. and Noël, L.D., 2020. Anatomy of leaf apical hydathodes in four monocotyledon plants of economic and academic relevance. Plos one, 15(9), 0232566.
129. Kaiser, B.N., Gridley, K.L., Ngaire Brady, J., Phillips, T. and Tyerman, S.D., 2005. The role of molybdenum in agricultural plant production. Annals of botany, 96(5), pp. 745-754.
130. Kalteh, M., Alipour, Z.T., Ashraf, S., Marashi Aliabadi, M. and Falah Nosratabadi, A., 2018. Effect of silica nanoparticles on basil (*Ocimum basilicum*) under salinity stress. Journal of Chemical Health Risks, 4(3).
131. Kandhol, N., Jain, M. and Tripathi, D.K., 2022. Nanoparticles as potential hallmarks of drought stress tolerance in plants. Physiologia Plantarum, 174(2), 13665.
132. Kandil, E.E., Abdelsalam, N.R., Mansour, M.A., Ali, H.M. and Siddiqui, M.H., 2020. Potentials of organic manure and potassium forms on maize (*Zea mays* L.) growth and production. Scientific Reports, 10(1), pp. 1-11.

133. Kang, E., Kim, H., Gray, L.A., Christie, D., Jonas, U., Graczykowski, B., Furst, E.M., Priestley, R.D. and Fytas, G., 2018. Ultrathin shell layers dramatically influence polymer nanoparticle surface mobility. Macromolecules, 51(21), pp. 8522-8529.
134. Kapoor, D., Bhardwaj, S., Landi, M., Sharma, A., Ramakrishnan, M. and Sharma, A., 2020. The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Applied Sciences, 10(16), 5692.
135. Kärkönen, A. and Kuchitsu, K., 2015. Reactive oxygen species in cell wall metabolism and development in plants. Phytochemistry, 112, pp. 22-32.
136. Kasim, W.A., 2006. Changes induced by copper and cadmium stress in the anatomy and grain yield of *Sorghum bicolor* (L.) Moench. Int J Agric Biol, 8(1), pp. 123-128.
137. Kaur, H. and Garg, N., 2021. Zinc toxicity in plants: a review. Planta, 253(6), pp. 1-28.
138. Khan, A., Pan, X., Najeeb, U., Tan, D.K.Y., Fahad, S., Zahoor, R. and Luo, H., 2018. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. Biological research, 51.
139. Khan, A.U., Wilson, T. 1995. Reactive oxygen species as cellular messengers. Chem. Biol. 2, pp. 437-445.
140. Khan, I., Saeed, K. and Khan, I., 2019. Nanoparticles: Properties, applications and toxicities. Arabian journal of chemistry, 12(7), pp. 908-931.
141. Khan, Z.S., Rizwan, M., Hafeez, M., Ali, S., Adrees, M., Qayyum, M.F., Khalid, S. and Sarwar, M.A., 2020. Effects of silicon nanoparticles on growth and physiology of wheat in cadmium contaminated soil under different soil moisture levels. Environmental Science and Pollution Research, 27(5), pp. 4958-4968.
142. Kim, Y., Chung, Y.S., Lee, E., Tripathi, P., Heo, S. and Kim, K.H., 2020. Root response to drought stress in rice (*Oryza sativa* L.). International journal of molecular sciences, 21(4), 1513.
143. Koppenol, W.H., 2001. The Haber-Weiss cycle–70 years later. Redox Report, 6(4), pp. 229-234.
144. Kostić, D.A., Dimitrijević, D.S., Stojanović, G.S., Palić, I.R., Đorđević, A.S. and Ickovski, J.D., 2015. Xanthine oxidase: isolation, assays of activity, and inhibition. Journal of Chemistry, 2015.
145. Krieger-Liszkay, A., 2005. Singlet oxygen production in photosynthesis. Journal of experimental botany, 56(411), pp. 337-346.

146. Ku, Y.S., Sintaha, M., Cheung, M.Y. and Lam, H.M., 2018. Plant hormone signaling crosstalks between biotic and abiotic stress responses. International Journal of Molecular Sciences, 19(10), 3206.
147. Kumar, R., Mishra, R.K., Mishra, V., Qidwai, A., Pandey, A., Shukla, S.K., Pandey, M., Pathak, A. and Dikshit, A., 2016. Detoxification and tolerance of heavy metals in plants. In Plant metal interaction (pp. 335-359). Elsevier.
148. Kumar, V., Pandita, S., Sidhu, G.P.S., Sharma, A., Khanna, K., Kaur, P., Bali, A.S. and Setia, R., 2021. Copper bioavailability, uptake, toxicity and tolerance in plants: a comprehensive review. Chemosphere, 262, 127810.
149. Kvaratskhelia, M., Winkel, C. and Thorneley, R.N., 1997. Purification and characterization of a novel class III peroxidase isoenzyme from tea leaves. Plant Physiology, 114(4), pp. 1237-1245.
150. Lamers, J., Van Der Meer, T. and Testerink, C., 2020. How plants sense and respond to stressful environments. Plant Physiology, 182(4), pp. 1624-1635.
151. Lehotai, N., Pető, A., Bajkán, S., Erdei, L., Tari, I. and Kolbert, Z., 2011. In vivo and in situ visualization of early physiological events induced by heavy metals in pea root meristem. Acta Physiologiae Plantarum, 33(6), pp. 2199-2207.
152. Levin, D.A., 1973. The role of trichomes in plant defense. The quarterly review of biology, 48(1, Part 1), pp. 3-15.
153. Li, M. and Kim, C., 2021. Chloroplast ROS and stress signaling. Plant Communications, 100264.
154. Li, Y., Zhu, N., Liang, X., Bai, X., Zheng, L., Zhao, J., Li, Y.F., Zhang, Z. and Gao, Y., 2020. Silica nanoparticles alleviate mercury toxicity via immobilization and inactivation of Hg (ii) in soybean (*Glycine max*). Environmental Science: Nano, 7(6), pp. 1807-1817.
155. Lichtenthaler, H.K., 1998. The stress concept in plants: an introduction. Annals of the New York Academy of sciences, 851, pp. 187-198.
156. Liliane, T.N. and Charles, M.S., 2020. Factors affecting yield of crops. Agronomy-climate change and food security, 9.
157. Linster, C.L., Gomez, T.A., Christensen, K.C., Adler, L.N., Young, B.D., Brenner, C. and Clarke, S.G., 2007. Arabidopsis VTC2 Encodes a GDP-l-Galactose Phosphorylase, the Last Unknown Enzyme in the Smirnoff-Wheeler Pathway to Ascorbic Acid in Plants*. Journal of Biological Chemistry, 282(26), pp. 18879-18885.
158. Liu, C., Li, F., Luo, C., Liu, X., Wang, S., Liu, T. and Li, X., 2009. Foliar application of two silica sols reduced cadmium accumulation in rice grains. Journal of Hazardous Materials, 161(2-3), pp. 1466-1472.

159. Lo'ay, A.A., EL-Ezz, S.F.A. and Awadeen, A.A., 2021. Effect of different foliar potassium fertilization forms on vegetative growth, yield, and fruit quality of kaki trees grown in sandy soil. Scientia Horticulturae, 288, 110420.
160. Luyckx, M., Hausman, J.F., Lutts, S. and Guerriero, G., 2017. Silicon and plants: current knowledge and technological perspectives. Frontiers in plant science, 8, 411.
161. Lv, J., Christie, P. and Zhang, S., 2019. Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges. Environmental Science: Nano, 6(1), pp. 41-59.
162. Ma, F., Chen, X.B., Sang, M., Wang, P., Zhang, J.P., Li, L.B. and Kuang, T.Y., 2009. Singlet oxygen formation and chlorophyll a triplet excited state deactivation in the cytochrome b 6 f complex from *Bryopsis corticulans*. Photosynthesis research, 100(1), pp. 19-28.
163. Mahmoud, A.W.M., Abdeldaym, E.A., Abdelaziz, S.M., El-Sawy, M.B. and Mottaleb, S.A., 2019. Synergetic effects of zinc, boron, silicon, and zeolite nanoparticles on confer tolerance in potato plants subjected to salinity. Agronomy, 10(1), 19.
164. Mansoor, S., Ali Wani, O., Lone, J.K., Manhas, S., Kour, N., Alam, P., Ahmad, A. and Ahmad, P., 2022. Reactive Oxygen Species in plants: From source to sink. Antioxidants, 11(2), 225.
165. Manzoor, N., Ahmed, T., Noman, M., Shahid, M., Nazir, M.M., Ali, L., Alnusaire, T.S., Li, B., Schulin, R. and Wang, G., 2021. Iron oxide nanoparticles ameliorated the cadmium and salinity stresses in wheat plants, facilitating photosynthetic pigments and restricting cadmium uptake. Science of the Total Environment, 769, 145221.
166. Maurel, C., Boursiac, Y., Luu, D.T., Santoni, V., Shahzad, Z. and Verdoucq, L., 2015. Aquaporins in plants. Physiological reviews, 95(4), pp. 1321-1358.
167. Meier, U. Growth Stages of Mono- and Dicotyledonous Plants: BBCH Monograph; Meier, U., Ed.; Julius Kühn-Institut: Quedlinburg, Germany, 2018.
168. Mika, A. and Luthje, S., 2003. Properties of guaiacol peroxidase activities isolated from corn root plasma membranes. Plant Physiology, 132(3), pp. 1489-1498.
169. Miller, G.A.D., Suzuki, N., Ciftci-Yilmaz, S.U.L.T.A.N. and Mittler, R.O.N., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, cell & environment, 33(4), pp. 453-467.
170. Mittal, D., Kaur, G., Singh, P., Yadav, K. and Ali, S.A., 2020. Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. Frontiers in Nanotechnology, 2, 10.

171. Mittler, R., 2006. Abiotic stress, the field environment and stress combination. Trends in plant science, 11(1), pp. 15-19.
172. Modena, M.M., Rühle, B., Burg, T.P. and Wuttke, S., 2019. Nanoparticle characterization: what to measure?. Advanced Materials, 31(32), 1901556.
173. Møller, I.M., 2001. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annual review of plant biology, 52(1), pp. 561-591.
174. Moons, A., 2005. Regulatory and functional interactions of plant growth regulators and plant glutathione S-transferases (GSTs). Vitamins and hormones, 72, pp. 155-202.
175. Morkunas, I., Woźniak, A., Mai, V.C., Rucińska-Sobkowiak, R. and Jeandet, P., 2018. The role of heavy metals in plant response to biotic stress. Molecules, 23(9), 2320.
176. Mukherjee, S., Mishra, A. and Trenberth, K.E., 2018. Climate change and drought: a perspective on drought indices. Current Climate Change Reports, 4(2), pp. 145-163.
177. Munné-Bosch, S. and Alegre, L., 2002. The function of tocopherols and tocotrienols in plants. Critical Reviews in Plant Sciences, 21(1), pp. 31-57.
178. Nadarajah, K.K., 2020. ROS homeostasis in abiotic stress tolerance in plants. International journal of molecular sciences, 21(15), 5208.
179. Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X. and Qiu, L., 2019. Research progress and perspective on drought stress in legumes: A review. International journal of molecular sciences, 20(10), 2541.
180. Nadiminti, P.P., Dong, Y.D., Sayer, C., Hay, P., Rookes, J.E., Boyd, B.J. and Cahill, D.M., 2013. Nanostructured liquid crystalline particles as an alternative delivery vehicle for plant agrochemicals. ACS Applied Materials & Interfaces, 5(5), pp. 1818-1826.
181. Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M., 2010. Heavy metals, occurrence and toxicity for plants: a review. Environmental chemistry letters, 8(3), pp. 199-216.
182. Nair, P.M.G. and Chung, I.M., 2014. Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignificaion, and molecular level changes. Environmental Science and Pollution Research, 21(22), pp. 12709-12722.
183. Nakano, Y. and Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and cell physiology, 22(5), pp. 867-880.
184. Nasrallah, A.K., Kheder, A.A., Kord, M.A., Fouad, A.S., El-Mogy, M.M. and Atia, M.A., 2022. Mitigation of Salinity Stress Effects on Broad Bean Productivity Using Calcium Phosphate Nanoparticles Application. Horticulturae, 8(1), 75.
185. Naz, R., Sarfraz, A., Anwar, Z., Yasmin, H., Nosheen, A., Keyani, R. and Roberts, T.H., 2021. Combined ability of salicylic acid and spermidine to mitigate the individual and

interactive effects of drought and chromium stress in maize (*Zea mays* L.). Plant Physiology and Biochemistry, 159, pp. 285-300.

186. Nguyen, H.C., Lin, K.H., Ho, S.L., Chiang, C.M. and Yang, C.M., 2018. Enhancing the abiotic stress tolerance of plants: from chemical treatment to biotechnological approaches. Physiologia Plantarum, 164(4), pp. 452-466.
187. Noctor, G. and Foyer, C.H., 2016. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiology, 171(3), pp. 1581-1592.
188. Osakabe, Y., Osakabe, K., Shinozaki, K. and Tran, L.S.P., 2014. Response of plants to water stress. Frontiers in plant science, 5, 86.
189. Osman, S.A., Salama, D.M., Abd El-Aziz, M.E., Shaaban, E.A. and Abd Elwahed, M.S., 2020. The influence of MoO3-NPs on agro-morphological criteria, genomic stability of DNA, biochemical assay, and production of common dry bean (*Phaseolus vulgaris* L.). Plant Physiology and Biochemistry, 151, pp. 77-87.
190. Ozgur, R., Turkan, I., Uzilday, B. and Sekmen, A.H., 2014. Endoplasmic reticulum stress triggers ROS signalling changes the redox state and regulates the antioxidant defence of *Arabidopsis thaliana*. Journal of experimental botany, 65(5), pp. 1377-1390.
191. Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A. and Hasanuzzaman, M., 2021. Osmoregulation and its actions during the drought stress in plants. Physiologia Plantarum, 172(2), pp. 1321-1335.
192. Pajuelo, E., Rodríguez-Llorente, I.D., Lafuente, A. and Caviedes, M.Á., 2011. Legume–rhizobium symbioses as a tool for bioremediation of heavy metal polluted soils. In Biomanagement of metal-contaminated soils (pp. 95-123). Springer, Dordrecht.
193. Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany, 2012.
194. Pandey, V.C. and Bajpai, O., 2019. Phytoremediation: from theory toward practice. In Phytomanagement of polluted sites (pp. 1-49). Elsevier.
195. Pastore, D., Trono, D., Laus, M.N., Di Fonzo, N. and Flagella, Z., 2007. Possible plant mitochondria involvement in cell adaptation to drought stress: a case study: durum wheat mitochondria. Journal of experimental botany, 58(2), pp. 195-210.
196. Peleg, Z. and Blumwald, E., 2011. Hormone balance and abiotic stress tolerance in crop plants. Current opinion in plant biology, 14(3), pp. 290-295.
197. Peng, C., Duan, D., Xu, C., Chen, Y., Sun, L., Zhang, H., Yuan, X., Zheng, L., Yang, Y., Yang, J. and Zhen, X., 2015. Translocation and biotransformation of CuO nanoparticles in rice (*Oryza sativa* L.) plants. Environmental Pollution, 197, pp. 99-107.

198. Perez, I.B. and Brown, P.J., 2014. The role of ROS signaling in cross-tolerance: from model to crop. Frontiers in plant science, 5, 754.
199. Pestovsky, Y.S. and Martínez-Antonio, A., 2017. The use of nanoparticles and nanoformulations in agriculture. Journal of nanoscience and nanotechnology, 17(12), pp. 8699-8730.
200. Peter, B.G., Messina, J.P., Lin, Z. and Snapp, S.S., 2020. Crop climate suitability mapping on the cloud: A geovisualization application for sustainable agriculture. Scientific Reports, 10(1), pp. 1-17.
201. Pietta, P.G., 2000. Flavonoids as antioxidants. Journal of natural products, 63(7), pp. 1035-1042.
202. Pinheiro, C. and Chaves, M.M., 2011. Photosynthesis and drought: can we make metabolic connections from available data?. Journal of experimental botany, 62(3), pp. 869-882.
203. Portis Jr, A.R., 1992. Regulation of ribulose 1, 5-bisphosphate carboxylase/oxygenase activity. Annual review of plant biology, 43(1), pp. 415-437.
204. Poschenrieder, C., Cabot, C., Martos, S., Gallego, B. and Barceló, J., 2013. Do toxic ions induce hormesis in plants? Plant science, 212, pp. 15-25.
205. Pospíšil, P., 2016. Production of reactive oxygen species by photosystem II as a response to light and temperature stress. Frontiers in plant science, 7, 1950.
206. Prasad, R., Bhattacharyya, A. and Nguyen, Q.D., 2017. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Frontiers in microbiology, 8, 1014.
207. Printz, B., Lutts, S., Hausman, J.F. and Sergeant, K., 2016. Copper trafficking in plants and its implication on cell wall dynamics. Frontiers in plant science, 7, 601.
208. Raghib, F., Naikoo, M.I., Khan, F.A., Alyemeni, M.N. and Ahmad, P., 2020. Interaction of ZnO nanoparticle and AM fungi mitigates Pb toxicity in wheat by upregulating antioxidants and restricted uptake of Pb. Journal of Biotechnology, 323, pp. 254-263.
209. Rai, S., Singh, P.K., Mankotia, S., Swain, J. and Satbhai, S.B., 2021. Iron homeostasis in plants and its crosstalk with copper, zinc, and manganese. Plant Stress, 1, 100008.
210. Raja, V., ullah Qadir, S., Majeed, U. and Dar, T.A., 2022. Redox Homeostasis Managers in Plants under Environmental Stresses. In Plant Abiotic Stress Physiology (pp. 31-70). Apple Academic Press.
211. Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J.L. and Havaux, M., 2012. Chemical quenching of singlet oxygen by carotenoids in plants. Plant physiology, 158(3), pp. 1267-1278.

212. Rana, D.S., Dass, A., Rajanna, G.A. and Kaur, R., 2016. Biotic and abiotic stress management in pulses. Indian J Agron, 61, pp. S238-S248.
213. Rao, G.B. and Susmitha, P., 2017. Silicon uptake, transportation and accumulation in Rice. J Pharmacogn Phytochem, 6(6), pp.290-293.
214. Raskin, I., Kumar, P.N., Dushenkov, S. and Salt, D.E., 1994. Bioconcentration of heavy metals by plants. Current Opinion in biotechnology, 5(3), pp. 285-290.
215. Raval, N., Maheshwari, R., Kalyane, D., Youngren-Ortiz, S.R., Chougule, M.B. and Tekade, R.K., 2019. Importance of physicochemical characterization of nanoparticles in pharmaceutical product development. In Basic fundamentals of drug delivery (pp. 369-400). Academic Press.
216. Ravet, K. and Pilon, M., 2013. Copper and iron homeostasis in plants: the challenges of oxidative stress. Antioxidants & redox signaling, 19(9), pp. 919-932.
217. Reinhardt, J., Hilgert, P. and Von Cossel, M., 2021. A review of industrial crop yield performances on unfavorable soil types. Agronomy, 11(12), 2382.
218. Repetto, M., Semprine, J. and Boveris, A., 2012. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. Lipid peroxidation, 1, pp. 3-30.
219. Rhoads, D.M., Umbach, A.L., Subbaiah, C.C. and Siedow, J.N., 2006. Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant physiology, 141(2), pp. 357-366.
220. Ridolfo, R., Tavakoli, S., Junnuthula, V., Williams, D.S., Urtti, A. and van Hest, J.C., 2020. Exploring the impact of morphology on the properties of biodegradable nanoparticles and their diffusion in complex biological medium. Biomacromolecules, 22(1), pp. 126-133.
221. Rivero, R.M., Mittler, R., Blumwald, E. and Zandalinas, S.I., 2022. Developing climate-resilient crops: improving plant tolerance to stress combination. The Plant Journal, 109(2), pp. 373-389.
222. Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A., ur Rehman, M.Z. and Waris, A.A., 2019. Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. Chemosphere, 214, pp. 269-277.
223. Rizwan, M., Ali, S., Malik, S., Adrees, M., Qayyum, M.F., Alamri, S.A., Alyemeni, M.N. and Ahmad, P., 2019. Effect of foliar applications of silicon and titanium dioxide nanoparticles on growth, oxidative stress, and cadmium accumulation by rice (*Oryza sativa*). Acta physiologiae plantarum, 41(3), pp. 1-12.

224. Roaa, M.H., 2020. A review article: The importance of the major groups of plants secondary metabolism phenols, alkaloids, and terpenes. International Journal for Research in Applied Sciences and Biotechnology, 7(5), pp. 354-358.
225. Roach, T. and Krieger-Liszkay, A., 2014. Regulation of photosynthetic electron transport and photoinhibition. Current Protein and Peptide Science, 15(4), pp. 351-362.
226. Roychoudhury, A.R.Y.A.D.E.E.P. and Basu, S.U.P.R.A.T.I.M., 2012. Ascorbate-glutathione and plant tolerance to various abiotic stresses. Oxidative stress in plants: causes, consequences and tolerance. IK International Publishers, New Delhi, pp. 177-258.
227. Rojas, C.M., Senthil-Kumar, M., Wang, K., Ryu, C.M., Kaundal, A. and Mysore, K.S., 2012. Glycolate oxidase modulates reactive oxygen species–mediated signal transduction during nonhost resistance in Nicotiana benthamiana and Arabidopsis. The Plant Cell, 24(1), pp. 336-352.
228. Rosenow, D.T., Quisenberry, J.E., Wendt, C.W. and Clark, L.E., 1983. Drought tolerant sorghum and cotton germplasm. Agricultural Water Management, 7(1-3), pp. 207-222.
229. Sadiq, M., Akram, N.A., Ashraf, M., Al-Qurainy, F. and Ahmad, P., 2019. Alpha-tocopherol-induced regulation of growth and metabolism in plants under non-stress and stress conditions. Journal of Plant Growth Regulation, 38(4), pp. 1325-1340.
230. Saghafi, D., Ghorbanpour, M. and Lajayer, B.A., 2018. Efficiency of Rhizobium strains as plant growth promoting rhizobacteria on morpho-physiological properties of Brassica napus L. under salinity stress. Journal of soil science and plant nutrition, 18(1), pp. 253-268.
231. Savatin, D.V., Gramegna, G., Modesti, V. and Cervone, F., 2014. Wounding in the plant tissue: the defense of a dangerous passage. Frontiers in plant science, 5, 470.
232. Schapire, A.L., Voigt, B., Jasik, J., Rosado, A., Lopez-Cobollo, R., Menzel, D., Salinas, J., Mancuso, S., Valpuesta, V., Baluska, F. and Botella, M.A., 2008. Arabidopsis synaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. The Plant Cell, 20(12), pp. 3374-3388.
233. Schopfer, P. and Liszkay, A., 2006. Plasma membrane-generated reactive oxygen intermediates and their role in cell growth of plants. Biofactors, 28(2), pp. 73-81.
234. Schwab, F., Zhai, G., Kern, M., Turner, A., Schnoor, J.L. and Wiesner, M.R., 2016. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants–Critical review. Nanotoxicology, 10(3), pp. 257-278.
235. Schwirn, K., Tietjen, L. and Beer, I., 2014. Why are nanomaterials different and how can they be appropriately regulated under REACH? Environmental Sciences Europe, 26(1), pp. 1-9.

236. Sega, D., Baldan, B., Zamboni, A. and Varanini, Z., 2020. $FePO_4$ NPs are an efficient nutritional source for plants: combination of nano-material properties and metabolic responses to nutritional deficiencies. Frontiers in plant science, 11, 586470.
237. Sekara, A., Pokluda, R., Cozzolino, E., del Piano, L., Cuciniello, A. and Caruso, G., 2019. Plant growth, yield, and fruit quality of tomato affected by biodegradable and non-degradable mulches. Horticultural Science, 46(3), pp. 138-145.
238. Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H. and Battaglia, M.L., 2021. Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants, 10(2), 259.
239. Selye, H., 1936. A syndrome produced by diverse nocuous agents. Nature, 138(3479), pp. 32-32.
240. Semida, W.M., Abdelkhalik, A., Mohamed, G.F., Abd El-Mageed, T.A., Abd El-Mageed, S.A., Rady, M.M. and Ali, E.F., 2021. Foliar application of zinc oxide nanoparticles promotes drought stress tolerance in eggplant (*Solanum melongena* L.). Plants, 10(2), 421.
241. Sevilem, I., Miyashima, S. and Helariutta, Y., 2013. Cell-to-cell communication via plasmodesmata in vascular plants. Cell adhesion & migration, 7(1), pp. 27-32.
242. Shahzad, A., Ullah, S., Dar, A.A., Sardar, M.F., Mehmood, T., Tufail, M.A., Shakoor, A. and Haris, M., 2021. Nexus on climate change: Agriculture and possible solution to cope future climate change stresses. Environmental Science and Pollution Research, 28(12), pp. 14211-14232.
243. Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M., 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany, 2012.
244. Sharma, P.K., Raghubanshi, A.S. and Shah, K., 2021. Examining the uptake and bioaccumulation of molybdenum nanoparticles and their effect on antioxidant activities in growing rice seedlings. Environmental Science and Pollution Research, 28(11), pp. 13439-13453.
245. Shavrukov, Y., Kurishbayev, A., Jatayev, S., Shvidchenko, V., Zotova, L., Koekemoer, F., De Groot, S., Soole, K. and Langridge, P., 2017. Early flowering as a drought escape mechanism in plants: how can it aid wheat production? Frontiers in plant science, 8, 1950.
246. Sheikhalipour, M., Esmaielpour, B., Behnamian, M., Gohari, G., Giglou, M.T., Vachova, P., Rastogi, A., Brestic, M. and Skalicky, M., 2021. Chitosan–selenium nanoparticle (Cs–Se NP) foliar spray alleviates salt stress in bitter melon. Nanomaterials, 11(3), 684.
247. Sies, H., 2019. Oxidative stress: eustress and distress in redox homeostasis. In stress: physiology, biochemistry, and pathology (pp. 153-163). Academic Press.

248. Sies, H., Berndt, C. and Jones, D.P., 2017. Oxidative stress. Annual review of biochemistry, 86, pp. 715-748.
249. Simonin, M., Guyonnet, J.P., Martins, J.M., Ginot, M. and Richaume, A., 2015. Influence of soil properties on the toxicity of TiO2 nanoparticles on carbon mineralization and bacterial abundance. Journal of Hazardous Materials, 283, pp. 529-535.
250. Singh, A., Singh, N.B., Hussain, I. and Singh, H., 2017. Effect of biologically synthesized copper oxide nanoparticles on metabolism and antioxidant activity to the crop plants Solanum lycopersicum and Brassica oleracea var. botrytis. Journal of biotechnology, 262, pp. 11-27.
251. Singh, N.T. and Dhaliwal, G.S., 1972. Effect of soil temperature on seedling emergence in different crops. Plant and soil, 37(2), pp. 441-444.
252. Singh, R.P., Handa, R. and Manchanda, G., 2021. Nanoparticles in sustainable agriculture: An emerging opportunity. Journal of Controlled Release, 329, pp. 1234-1248.
253. Singh, S., Parihar, P., Singh, R., Singh, V.P. and Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Frontiers in plant science, 6, 1143.
254. Smirnoff, N. and Arnaud, D., 2019. Hydrogen peroxide metabolism and functions in plants. New Phytologist, 221(3), pp. 1197-1214.
255. Song, Y., Jiang, M., Zhang, H. and Li, R., 2021. Zinc oxide nanoparticles alleviate chilling stress in rice (*Oryza Sativa* L.) by regulating antioxidative system and chilling response transcription factors. Molecules, 26(8), 2196.
256. Spielman-Sun, E., Avellan, A., Bland, G.D., Tappero, R.V., Acerbo, A.S., Unrine, J.M., Giraldo, J.P. and Lowry, G.V., 2019. Nanoparticle surface charge influences translocation and leaf distribution in vascular plants with contrasting anatomy. Environmental Science: Nano, 6(8), pp. 2508-2519.
257. Su, Y., Ashworth, V., Kim, C., Adeleye, A.S., Rolshausen, P., Roper, C., White, J. and Jassby, D., 2019. Delivery, uptake, fate, and transport of engineered nanoparticles in plants: a critical review and data analysis. Environmental Science: Nano, 6(8), pp. 2311-2331.
258. Suman, S., Bagal, D., Jain, D., Singh, R., Singh, I.K. and Singh, A., 2021. Biotic stresses on plants: reactive oxygen species generation and antioxidant mechanism. In Frontiers in Plant-Soil Interaction (pp. 381-411). Academic Press.
259. Sun, L., Song, F., Zhu, X., Liu, S., Liu, F., Wang, Y. and Li, X., 2021. Nano-ZnO alleviates drought stress via modulating the plant water use and carbohydrate metabolism in maize. Archives of Agronomy and Soil Science, 67(2), pp. 245-259.

260. Sun, X.D., Yuan, X.Z., Jia, Y., Feng, L.J., Zhu, F.P., Dong, S.S., Liu, J., Kong, X., Tian, H., Duan, J.L. and Ding, Z., 2020. Differentially charged nanoplastics demonstrate distinct accumulation in Arabidopsis thaliana. Nature nanotechnology, 15(9), pp. 755-760.
261. Sutulienė, R., Ragelienė, L., Samuolienė, G., Brazaitytė, A., Urbutis, M. and Miliauskienė, J., 2021. The Response of Antioxidant System of Drought-Stressed Green Pea (*Pisum sativum* L.) Affected by Watering and Foliar Spray with Silica Nanoparticles. Horticulturae, 8(1), 35.
262. Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E. and Mittler, R., 2014. Abiotic and biotic stress combinations. New Phytologist, 203(1), pp.32-43.
263. Tang, Y., He, R., Zhao, J., Nie, G., Xu, L. and Xing, B., 2016. Oxidative stress-induced toxicity of CuO nanoparticles and related toxicogenomic responses in Arabidopsis thaliana. Environmental pollution, 212, pp. 605-614.
264. Tarafdar, J.C., Xiong, Y., Wang, W.N., Quinl, D. and Biswas, P., 2012. Standardization of size, shape and concentration of nanoparticle for plant application. Applied Biological Research, 14(2), pp. 138-144.
265. Taran, N.Y., Gonchar, O.M., Lopatko, K.G., Batsmanova, L.M., Patyka, M.V. and Volkogon, M.V., 2014. The effect of colloidal solution of molybdenum nanoparticles on the microbial composition in rhizosphere of *Cicer arietinum* L. Nanoscale research letters, 9(1), pp. 1-8.
266. Tian, H., Kah, M. and Kariman, K., 2019. Are nanoparticles a threat to mycorrhizal and rhizobial symbioses? A critical review. Frontiers in Microbiology, 10, 1660.
267. Tränkner, M., Tavakol, E. and Jákli, B., 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiologia plantarum, 163(3), pp. 414-431.
268. Triantaphylidès, C. and Havaux, M., 2009. Singlet oxygen in plants: production, detoxification and signaling. Trends in plant science, 14(4), pp.219-228.
269. Tripathy, B.C. and Oelmüller, R., 2012. Reactive oxygen species generation and signaling in plants. Plant signaling & behavior, 7(12), pp.1621-1633.
270. Tripathi, D.K., Singh, V.P., Prasad, S.M., Chauhan, D.K. and Dubey, N.K., 2015. Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in *Pisum sativum* (L.) seedlings. Plant Physiology and Biochemistry, 96, pp. 189-198.
271. Tripathi, D.K., Singh, S., Singh, V.P., Prasad, S.M., Dubey, N.K. and Chauhan, D.K., 2017. Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. Plant Physiology and Biochemistry, 110, pp. 70-81.

272. Van Nguyen, D., Nguyen, H.M., Le, N.T., Nguyen, K.H., Nguyen, H.T., Le, H.M., Nguyen, A.T., Dinh, N.T.T., Hoang, S.A. and Van Ha, C., 2022. Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. Journal of Plant Growth Regulation, 41(1), pp. 364-375.
273. Vanlerberghe, G.C., 2013. Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. International journal of molecular sciences, 14(4), pp. 6805-6847.
274. Varsha, M., Nidhi, M. and Anurag, M., 2010. Heavy metals in plants: phytoremediation: plants used to remediate heavy metal pollution. Agriculture and Biology Journal of North America, 1(1), pp. 40-46.
275. Vega-Muñoz, I., Duran-Flores, D., Fernández-Fernández, Á.D., Heyman, J., Ritter, A. and Stael, S., 2020. Breaking bad news: dynamic molecular mechanisms of wound response in plants. Frontiers in Plant Science, 11, 610445.
276. Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E.R., Sharma, N.C. and Sahi, S.V., 2017. Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in Leucaena leucocephala seedlings: a physiochemical analysis. Plant Physiology and Biochemistry, 110, pp. 59-69.
277. Verma, V., Ravindran, P. and Kumar, P.P., 2016. Plant hormone-mediated regulation of stress responses. BMC plant biology, 16(1), pp. 1-10.
278. Verslues, P.E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J. and Zhu, J.K., 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal, 45(4), pp. 523-539.
279. Viehweger, K., 2014. How plants cope with heavy metals. Botanical Studies, 55(1), pp.1-12.
280. Vilchis-Landeros, M.M., Matuz-Mares, D. and Vázquez-Meza, H., 2020. Regulation of metabolic processes by hydrogen peroxide generated by NADPH oxidases. Processes, 8(11), 1424.
281. Wang, Y. and Xia, Y., 2004. Bottom-up and top-down approaches to the synthesis of monodispersed spherical colloids of low melting-point metals. Nano letters, 4(10), pp. 2047-2050.
282. Waraich, E.A., Ahmad, R., Ashraf, M.Y., Saifullah and Ahmad, M., 2011. Improving agricultural water use efficiency by nutrient management in crop plants. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science, 61(4), pp. 291-304.
283. Waraich, E.A., Ahmad, R., Halim, A. and Aziz, T., 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. Journal of soil science and plant nutrition, 12(2), pp. 221-244.

284. Wasson, A.P., Richards, R.A., Chatrath, R., Misra, S.C., Prasad, S.S., Rebetzke, G.J., Kirkegaard, J.A., Christopher, J. and Watt, M., 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. Journal of experimental botany, 63(9), pp. 3485-3498.
285. Willekens, H., Inzé, D., Van Montagu, M. and Van Camp, W., 1995. Catalases in plants. Molecular Breeding, 1(3), pp.207-228.
286. Wu, S., Hu, C., Tan, Q., Zhao, X., Xu, S., Xia, Y. and Sun, X., 2018. Nitric oxide acts downstream of abscisic acid in molybdenum-induced oxidative tolerance in wheat. Plant cell reports, 37(4), pp. 599-610.
287. Xue, B., Zhang, A. and Jiang, M., 2009. Involvement of polyamine oxidase in abscisic acid-induced cytosolic antioxidant defense in leaves of maize. Journal of Integrative Plant Biology, 51(3), pp. 225-234.
288. Zahedi, S.M., Moharrami, F., Sarikhani, S. and Padervand, M., 2020. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. Scientific reports, 10(1), pp. 1-18.
289. Zandalinas, S.I., Fichman, Y. and Mittler, R., 2020. Vascular bundles mediate systemic reactive oxygen signaling during light stress. Plant Cell, 32(11), pp. 3425-3435.
290. Zandalinas, S.I., Fichman, Y., Devireddy, A.R., Sengupta, S., Azad, R.K. and Mittler, R., 2020. Systemic signaling during abiotic stress combination in plants. Proceedings of the National Academy of Sciences, 117(24), pp. 13810-13820.
291. Zargar, A., Sadiq, R., Naser, B. and Khan, F.I., 2011. A review of drought indices. Environmental Reviews, 19(NA), pp. 333-349.
292. Zeeshan, H.M.A., Lee, G.H., Kim, H.R. and Chae, H.J., 2016. Endoplasmic reticulum stress and associated ROS. International journal of molecular sciences, 17(3), 327.
293. Zewail, R.M., Ali, M., El-Gamal, I.S., Al-Maracy, S.H., Islam, K.R., Elsadek, M., Azab, E., Gobouri, A.A., ElNahhas, N., Mohamed, M.H. and El-Desouky, H.S., 2021. Interactive effects of arbuscular mycorrhizal inoculation with nano boron, zinc, and molybdenum fertilization on stevioside contents of stevia (*Stevia rebaudiana*, L.) plants. Horticulturae, 7(8), 260.
294. Zhou, D., Shao, L. and Spitz, D.R., 2014. Reactive oxygen species in normal and tumor stem cells. In Advances in cancer research (Vol. 122, pp. 1-67). Academic Press.
295. Zivcak, M., Brestic, M. and Sytar, O., 2016. Osmotic adjustment and plant adaptation to drought stress. In Drought Stress Tolerance in Plants, Vol 1 (pp. 105-143). Springer, Cham.

horticulturae

MDPI

Article

The Response of Antioxidant System of Drought-Stressed Green Pea (*Pisum sativum* L.) Affected by Watering and Foliar Spray with Silica Nanoparticles

Rūta Sutulienė [1,*], Lina Ragelienė [2], Giedrė Samuolienė [1,3], Aušra Brazaitytė [1], Martynas Urbutis [4] and Jurga Miliauskienė [1,*]

[1] Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Kaunas Str. 30, LT-54333 Babtai, Lithuania; giedre.samuoliene@lammc.lt (G.S.); ausra.brazaityte@lammc.lt (A.B.)
[2] Faculty of Natural Sciences, Vytautas Magnus University, Vileikos Str. 8, LT-44404 Kaunas, Lithuania; lina.rageliene@vdu.lt
[3] Faculty of Agronomy, Agriculture Academy, Vytautas Magnus University, Studentų Str. 11, Akademija, LT-52261 Kaunas, Lithuania
[4] UAB Ikarai, Vakarų Str. 6, LT-57238 Kėdainiai, Lithuania; Fitolab@ikarfactory.eu
* Correspondence: ruta.sutuliene@lammc.lt (R.S.); jurga.miliauskiene@lammc.lt (J.M.)

Abstract: Abiotic stress caused by drought impairs plant growth and reduces yields. This study aimed to investigate the impact of silica nanoparticles (SiO_2 NPs) through the adverse effects of drought on the growth, oxidative stress, and antioxidative response of pea 'Respect'. Pea plants were grown in a greenhouse before being watered (100 ± 1 mL per pot) or foliar sprayed (ca. 14 ± 0.5 mL plant^{-1}) with suspensions containing SiO_2 NPs (0, 12.5 ppm, 25 ppm, and 50 ppm) and were exposed to drought stress for 10 days. Drought stress was created by maintaining 30% of the soil moisture while the control was 80%. The growth parameters of pea grown under drought stress conditions were improved by spraying or watering plants with SiO_2 NPs (12.5, 25, and 50 ppm). At drought stress, peas treated with SiO_2 NPs (50 ppm) increased their relative water content by 29%, specific leaf area by 17%, and decreased root/shoot ratio by 4% as compared to plant non-treated with SiO_2 NPs. In addition, spraying or watering of SiO_2 NPs increased peas tolerance to drought by increasing the activity of antioxidant enzymes at least three times including catalase, ascorbate peroxidase, glutathione reductase, and superoxide dismutase, as well as reducing hydrogen peroxide and lipid peroxidation in plant tissue. It was observed the increase in total phenolic compounds and non-enzymatic antioxidant activity (DPPH, ABTS, FRAP) in peas treated with SiO_2 NPs under drought stress. The physiological response of peas to drought and the effects of SiO_2 NPs studied in this experiment based on the use of the concentration of 50 ppm nanoparticles can protect peas from the damaging effects of drought and could help reduce global food shortages.

Keywords: antioxidant activity; drought; green pea; oxidative stress biomarkers; SiO_2 nanoparticles

Citation: Sutulienė, R.; Ragelienė, L.; Samuolienė, G.; Brazaitytė, A.; Urbutis, M.; Miliauskienė, J. The Response of Antioxidant System of Drought-Stressed Green Pea (*Pisum sativum* L.) Affected by Watering and Foliar Spray with Silica Nanoparticles. *Horticulturae* **2022**, *8*, 35. https://doi.org/10.3390/horticulturae8010035

Academic Editors: Othmane Merah, Purushothaman Chirakkuzhyil Abhilash, Magdi T. Abdelhamid, Hailin Zhang and Bachar Zebib

Received: 4 November 2021
Accepted: 28 December 2021
Published: 30 December 2021

1. Introduction

Field peas play an important role in crop rotation due to their ability to fix atmospheric nitrogen in a symbiotic association with *Rhizobium bacteria* and fulfill the nitrogen demand of the succeeding crops, as well as can be used for both human food and animal feed. However, climate change has led to increased heterogeneity of precipitation, ranging from heavy to drought [1], leading to unstable crop yields and seed protein content, thus contributing to the reduction in field pea cultivation in the world according to Food and Agriculture Organization Statistics (FAOSTAT). To restore pea cultivation in the world, new ways such as nanotechnology need to be discovered to maintain a constant pea harvest regardless of environmental factors, as well as to maintain soil quality [2–5].

Nanoparticle (NPs), according to their unique properties: size, surface charge, shape, and potential interaction with plants could help to reduce the impact of drought [6]. It has

been described that NPs ranging in size from 4 to 100 nm can cross the cuticle by disrupting the wax layer [7], and fluorescently tagged >50 nm NPs can accumulate in the epidermis under the cuticle where stomata are absent [7,8]. Negatively charged plant cell walls act as an ion exchange surface that potentially promotes the penetration of cationic NPs rather than anionic NPs [9], but show much higher adsorption on the root surface of positively charged NPs [10]. With increasing interest in silica NPs (SiO_2 NPs), it has been found that SiO_2 NPs through symplastic and apoplastic pathways (through cell wall microchannels) penetrate the roots and then reach other parts of the plant through conductive tissues [5,11].

Silicon (Si) is not essential for plants, but in drought stress, it can affect the water ratio in drought-affected plants, reduce stomatal conduction associated with protective cell turgor loss, improve the ability to extract water from the soil due to the promotion of root elongation and the regulation of aquaporin genes [12]. Si NPs can reduce oxidative stress by increasing antioxidant enzyme activities and decreasing reactive oxygen species (ROS) in plant leaf [7,13–15]. In a salinity experiment with sweet peppers and different forms of Si, it was found that using SiO_2 NPs resulted in 15% higher plants, 34% higher fresh, and 36% dry biomasses and 11% higher total chlorophyll content and 30% yield compared to bulk Si [16]. In wheat seedlings, Si NPs (size 20–95 nm) were found to be more effective than bulk SiO_2 in response to UV-B stress as well [17]; Si NPs successfully reduced lipid peroxidation by 82% and electrolyte leakage, as well as increased catalase (CAT) and superoxide dismutase (SOD) activity, resulting in increased antioxidant resistance of wheat to UV-B. More studies have demonstrated the effectiveness of SiO_2 NPs against abiotic stress in different plants. In strawberries, peroxide concentrations and total phenols, vitamin C, CAT, SOD, ascorbate peroxidase (APX), and glutathione peroxidase (GPX) were increased by spraying with 125 ppm SiO_2 NPs under severe to moderate drought stress [14]. SiO_2 NPs had a positive effect on photosynthesis and gas exchange in rice seedlings [18]. Besides 30 ppm of Si NPs foliar application reduced oxidative stress as evidenced by low levels of malondialdehyde (MDA) and electrolyte leakage and increased production of antioxidant enzymes such as SOD by 5%, APX by 41%, and CAT by 40% in rice tissues. In potatoes, increased GPX and SOD activity was observed after spraying with 50 ppm SiO_2 NPs under salinity stress [19]. Si NPs protected from oxidative stress in pea seedlings treated with Cr (VI) [20]; the application of SiO_2 NPs on Cr (VI) treated peas significantly increased the activity of enzymes such as SOD, APX, CAT, glutathione, and dehydroascorbate reductases. Another study [21] investigated the effects of SiO_2 NPs on marigold plants after spraying and watering them, observed that the CAT activity in plant leaves was higher when irrigated with the same concentration of SiO_2 NPs as compared to spray, but the opposite effect of the suspension was found for peroxidase—higher activity was found in sprayed plants.

In recent decades, the application of NPs to improve the resistance of various crops to abiotic stress was discussed, but there is still no consensus on the benefits of NPs for plant growth and yields. An array of plant species, different NPs, their particle size and concentrations, and application pathways have been used in the literature; however, knowing the beneficial role of Si to drought-affected plants [12], little data is available on the effects of SiO_2 NPs against drought stress in the Legumes family which are popular crops in the world. It was hypothesized that watering or foliar application of SiO_2 NPs alleviates oxidative stress in drought-affected peas by inducing non-enzymatic and enzymatic antioxidant activity. Therefore, the present study aimed to investigate the effect of watering and foliar application of different SiO_2 NPs concentrations on the growth traits of drought-affected green peas (*Pisum sativum* L.), to elucidate the influence of SiO_2 NPs on oxidative stress and antioxidative response in peas exposed to drought.

2. Materials and Methods

2.1. Study Site and Treatments

The research was carried out in a greenhouse (3 × 6 m, h = 2 m) at the Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Babtai, Lithuania

(55°05′08.4″ N 23°48′03.5″ E, at an altitude of 51 m; moderate climate zone of the northern hemisphere), from 6 May to 22 June. Before sowing, green pea (*Pisum sativum* L. cv. Respect, Maribo Seed International ApS, Holeby, Denmark) seeds were sterilized in 5% sodium hypochlorite solution for 15 min to assure surface sterility and rinsed gently with deionized water several times. Then, seeds were soaked in water for 24 h. Ten seeds were sown in 10-L volume plastic pots (7 pots per treatment, arranged randomized), filled with ~8 kg of soil mixture (7:1 soil to perlite ratio, respectively). The granulometric composition of the soil was heavy loam, pH 7.4 ± 0.1; concentration of humus—3.6 ± 0.1%; P_2O_5—243 ± 8 mg kg^{-1}; K_2O—348 ± 37 mg kg^{-1}; NH_4—4 ± 0.6 mg kg^{-1}; NO_3—22 ± 0.9 mg kg^{-1}; SiO_2—39 ± 0.8 mg kg^{-1}. Pea seedlings were thinned to 7 plants per pot 5 days after sowing. After 16 days of cultivation, the peas were fertilized with 7 g pot^{-1} ammonium nitrate. Pots were irrigated with water by graduated cylinder daily to 80% of substrate moisture (SM) using substrate moisture sensor (Delta-T devices, HH2 moisture meter, Cambridge, UK) for 23 days (6 May till 12 June). In the greenhouse with a natural day length photoperiod, the average day/night temperature was 24.2/14.4 °C; relative air humidity—54/75 ± 5% before exposure; during the drought treatment the average day/night temperature was 26.2/17.0 °C and the relative air humidity was 50/73 ± 5%, data were measured throughout the experiment (Termio + data logger, Poland). When the peas reached the 40 BBCH growth stage [22], they were watered (100 ± 1 mL per pot) or foliar sprayed until full wetting (ca. 14 ± 0.5 mL $plant^{-1}$) with solutions containing different concentrations of SiO_2 NPs: 0 (watered or sprayed with water, NPs-untreated), 12.5 ppm, 25 ppm, and 50 ppm. After the application of SiO_2 NPs, the watering of pea plants was stopped and drought stress was initiated (30% SM), while control plants were irrigated with water to maintain normal soil moisture (80% SM) throughout the experiment. These regimes were applied for 10 days (12 June till 22 June) until harvest. Plants were harvested after reaching the BBCH 50 growth stage from each treatment to assess their morphophysiological responses.

SiO_2 NPs solutions were prepared with silica (SiO_2) NPs (particle size: 20–30 nm; purity: 99%; US Research Nanomaterials, Inc, Houston, TX, USA). The NPs with concentrations of 12.5 ppm, 25 ppm, 50 ppm, were suspended in deionized (DI) water and ultrasonically dispersed for 60 min. The NPs size and suspension stability were measured using Delsa™ Nano Submicron Particle Size (Beckman Coulter Instruments Corporation, Fullerton, CA, USA) and Zeta Potential device (Dispersion Technology Inc., Bedford Hills, NY, USA). The data in Table 1 show the negative charge of NPs in the suspensions and the stability of the systems, furthermore the NPs suspensions tend to be more monodisperse according to the polydispersity index (PDI).

Table 1. Properties of SiO_2 NPs suspension in DI water (pH = 7.05): zeta potential, results represent the mean ± standard error, polydispersity index, and percentage of nanoparticles between 1–100 nm.

Suspension of SiO_2 NPs	
Zeta potential (ζ; mV)	−20.64 ± 0.333
Polydispersity index (PI)	0.34
NPs size 1–100 nm in suspension (%)	70

2.2. Relative Water Content, Specific Leaf Area, and Root/Shoot Ratio

Ten pea plants were randomly selected from each treatment for biometric measurements. The shoots were separated from the roots and then fresh weight (FW) and dry weight (DW) were determined (n = 10). The FW and DW were measured with an electronic scale (Mettler Toledo AG64, Columbus, OH, USA) and DW was determined following forced-air convection drying at 105 °C to a constant dry weight (Venticell 222, MBT, Brno, Czech Republic). After shoot FW determination, 10 matured plants per each treatment

were floated on deionized water for 24 h and then turgid weights (TW) were measured. Relative water content (RWC) was calculated, using the following equation [23]:

$$\text{RWC, \%} = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100 \quad (1)$$

The leaf area was measured using an automatic leaf area meter (AT Delta-T Devices, Wallingford, UK) expressed as $cm^2\ g^{-1}$. For calculation of specific leaf area (SLA), the total plant leaf area ($n = 10$) was divided by the shoot DW. Root/shoot ratio was determined as the ratio of root DW to aboveground DW.

2.3. Antioxidant Properties and Total Phenolic Compounds

Antioxidant properties of pea leaves were evaluated as the DPPH (2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) diammonium salt, radical scavenging activities, and Fe^{2+} reducing antioxidant power assay (FRAP); the total contents of phenolic compounds were also determined. Extracts were prepared by grinding 0.3 g of plant leaves with liquid nitrogen and diluting with 5 mL of 80% methanol. After 24 h, the samples were centrifuged for 10 min at 3000 rpm (Hermle Z300K, Baden-Württemberg, Germany), extracts were filtered through cellulose filters and the supernatant was used for further analyses. All biochemical analysis was performed in 3 biological replications. Each of three biological replicates consisted of at least three conjugated plants and was repeated in three analytical replicates.

The total content of phenolic compounds was determined as gallic acid equivalents. A 250 µL aliquot of the sample extract was mixed with 250 µL of 10% (*w/v*) Folin–Ciocalteu reagent, 500 µL of 1 M Na_2CO_3 solution, and 2 mL of distilled water [24]. After incubation for 20 min in the dark, the absorbance was measured at 765 nm (M501, Spectronic Camspec Ltd., Leeds, UK). The total phenolic compounds quantity mg g^{-1} was calculated from the calibration curve of the gallic acid (0.01–0.1 mg mL^{-1}, $R^2 = 0.99$).

The ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation was obtained by incubating the 7 mM ABTS stock solution (100 mL) with 2.45 mM potassium persulfate ($K_2S_2O_8$; final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use [25]. Thereafter, 50 µL of the prepared sample was mixed with 2 mL of ABTS solution (ABTS stock solution was diluted 1:7) and the absorbance was measured after 11 min (plateau phase) at 734 nm (M501, Spectronic Camspec Ltd., Leeds, UK). The ABTS scavenging activity of pea leaves extracts was calculated as the difference between the initial absorbance and after reacting for 10 min. A calibration curve was determined using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; 97% purity; Sigma-Aldrich, Burlington, MA, USA) as an external standard with a range of concentrations from 0.1 to 0.8 mM ($R^2 = 0.99$). It was expressed as ABTS µmol scavenged per 1 g of fresh weight (µmol g^{-1} FW).

For DPPH (2-diphenyl-1-picrylhydrazyl) assay, a stable 126.8 µM DPPH (100% purity; Sigma-Aldrich, Burlington, MA, USA) solution was prepared in methanol [26]. Subsequently, 1 mL of the DPPH solution was transferred to a test tube and mixed with 100 µL of the diluted pea extract with 400 µL methanol. The absorbance was scanned at 515 nm (M501, Spectronic Camspec Ltd., Leeds, UK) while reacting for 16 min. The free radical scavenging capacity was expressed as µmol of DPPH radicals scavenged per 1 g of fresh weight (µmol g^{-1} FW). A calibration curve was determined using Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid; 97% purity; Sigma-Aldrich, Burlington, MA, USA) as an external standard with a range of concentrations from 0.1 to 0.6 mM ($R^2 = 0.99$).

The FRAP method is based on reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The fresh working solution was prepared by mixing 300 mM, pH 3.6 acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \times 6H_2O$ at 10:1:1 (*v/v/v*) [27]. 20 µL of the sample was mixed with 3 mL of working solution and incubated in the dark for 30 min. Readings of the colored product (ferrous tripyridyl-triazine complex) were then taken at 593 nm. A calibration curve was determined using $Fe_2(SO_4)_3$ (Iron

(III) sulfate; 97% purity; Sigma-Aldrich, Burlington, MA, USA) as an external standard with a range of concentrations from 0.005 to 0.5 mM ($R^2 = 0.99$). The antioxidant power is expressed as Fe^{2+} antioxidant capacity (Fe^{2+} µmol g^{-1} FW).

2.4. Malondialdehyde and Hydrogen Peroxide

The extracts used to determine the concentration of lipid peroxidation and hydrogen peroxide (H_2O_2) in pea leaves were prepared by grinding 0.1 g of fresh sample with liquid nitrogen and diluting with 4 mL of 0.1% TCA (trichloroacetic acid). After centrifugation for 10 min at 3000 rpm (Hermle Z300K, Baden-Württemberg, Germany), the supernatant was used for further analyses.

For H_2O_2 measurements in plant leaves, 500 µL of the supernatant was added to 1 mL of 1 M potassium iodide (KI). The absorbance of the mixture was scanned at 390 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK). A calibration curve was determined using H_2O_2 (30% hydrogen peroxide) as an external standard with a range of concentrations from 0.6–24.3 mM ($R^2 = 0.99$). The content of H_2O_2 is expressed in fresh weight (µmol g^{-1} FW) [28].

The TBARS test determines malondialdehyde (MDA) content in pea leaves samples as the end product of lipid peroxidation. 500 µL of the supernatant was added to 1 mL 0.5% (*w/v*) thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The mixture was incubated in boiling water for 30 min. The reaction stopped after the samples have cooled. The samples were centrifuged at 10,000× *g* for 5 min, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK). The value for non-specific absorbance at 600 nm was subtracted [29]. The amount of MDA–TBA complex (red pigment) in leaves was calculated and expressed as nmol g^{-1} FW:

$$C_{MDA} = (A532 - A600)/E_{MDA} \quad (2)$$

C_{MDA}—concentration of MDA, µM
A532, A600—Absorbance at wavelengths
E_{MDA}—MDA extinction coefficient 155 mM^{-1} cm^{-1}

2.5. Antioxidant Enzymes Activities

The extracts used to determine the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) in pea leaves were prepared by grinding 0.5 g of fresh sample with liquid nitrogen and diluting within 5 mL extraction buffer (100 mM potassium-phosphate buffer, pH 7.8, containing 0.1 mM EDTA). After centrifugation for 10 min at 3000 rpm (Hermle Z300K, Baden-Württemberg, Germany), the supernatant was collected and used for the assays of enzymatic activities. All steps in the preparation of the enzyme extract were carried out at 4 °C.

For soluble protein determination, the dye-binding method and bovine serum albumin as standard were used. A volume of 30 µL of enzyme extract was mixed with 1.5 mL of Bradford reagent diluted by 1:5 with DI water. Absorbance was read after 2 min. through a spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK) at 595 nm [30].

Total SOD activity was estimated by the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) by the enzyme [31]. Three mL of reaction mixture consisted of 13 mM methionine, 75 µM NBT, 100 mM potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 50 µL enzyme extract, and 13 µM riboflavin. The tubes were under 150 µmol m^{-2} s^{-1} for 1 min to initiate the reaction and then covered. The absorbance was recorded after 30 min by spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK) at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme expressed as unit mg^{-1} protein min^{-1}.

CAT activity was measured as the disappearance of H_2O_2 [32]. A volume of 100 µL enzyme extract was added in 1.275 mL of 0.1 M phosphate buffer (pH 7.8, containing 0.1 mM EDTA); the reaction started by adding 125 µL of 30 mM H_2O_2. The decrease in

absorbance measured by spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK) at 240 nm was observed for 1 min and enzyme activity was computed by calculating the amount of H_2O_2 decomposed (µmol H_2O_2 mg^{-1} protein min^{-1}).

APX activity was assayed by recording the decrease in optical density due to ascorbic acid at 290 nm [33]. The 1 mL assay mixture contained 0.1 M potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 0.5 mM ascorbic acid, 0.1 mL enzyme extract, and 0.1 mL of 30 mM H_2O_2 was added to initiate the reaction. The decrease in absorbance was measured spectrophotometrically (M501, Spectronic Camspec Ltd., Leeds, UK) for 1 min and the extinction coefficient of 2.8 mM^{-1} cm^{-1} for reduced ascorbate was used in calculating the enzyme activity that was expressed as µmol AsA mg^{-1} protein min^{-1}.

Measuring GR activity based on the rate of decrease in the absorbance of oxidized glutathione (GSSG), at 340 nm [34]. The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.8, containing 0.1 mM EDTA), 1 mM GSSG, 100 µL enzyme extract and 75 µL 0.1 mM NADPH added last to initiate the reaction. The decrease in absorbance measured by spectrophotometer (M501, Spectronic Camspec Ltd., Leeds, UK) was recorded every 5 min until 20 min. An absorption coefficient of 6.22 mM^{-1} cm^{-1} was used for calculations, and GR activity was defined as µmol NADPH mg^{-1} protein min^{-1}.

2.6. Statistical Analysis

All the values were expressed as mean ± standard error. Data were analyzed using the Analysis of Variance (ANOVA) test followed by Tukey HSD at $p \leq 0.001$ to identify significant differences. All statistical analyses were performed using XLSTAT (Xlstat, Addinsoft, Paris, France, 2021).

3. Results

3.1. Effects of Drought Stress and SiO_2 NPs on Plants Specific Leaf Area, Relative Water Content, and Root/Shoot Ratio

The relative water content (RWC) and specific leaf area (SLA) decreased during drought stress (Figure 1a,b).

Drought stress significantly decreased SLA and RWC (38 and 27%, respectively) compared with control plants. Watering or spraying peas with SiO_2 NPs (50 ppm) increased the values of these two parameters compared to plants watered or sprayed with water. Moreover, watering SiO_2 NPs had a better effect on SLA than spraying at all concentration levels.

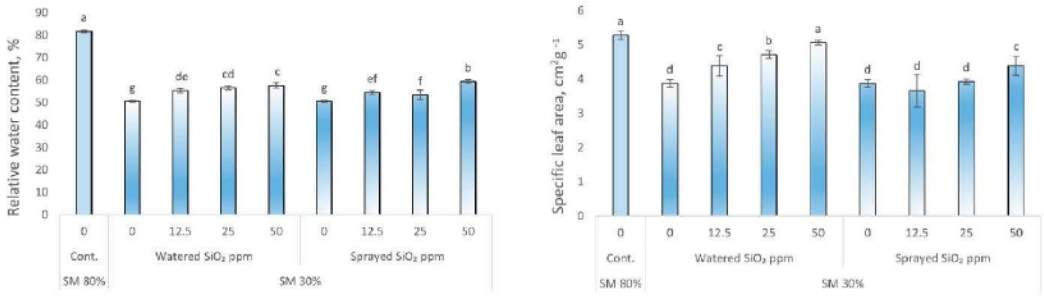
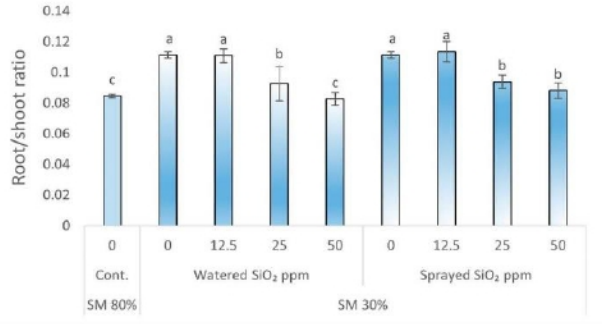


Figure 1. *Cont.*



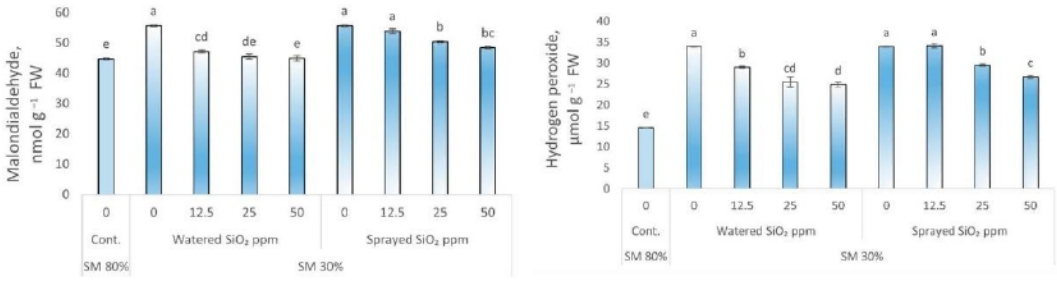
(c)

Figure 1. Effect of drought stress and SiO_2 NPs (0; 12.5; 25; and 50 ppm) on relative water content (%), specific leaf area ($cm^2\ g^{-1}$), and root/shoot ratio in *P. sativum* L. Cont.—control plants, substrate moisture (SM) 80%; drought stress—SM 30%, RWC (**a**); specific leaf area (**b**); root/shoot ratio (**c**). Values are mean ± SE of 10 replicates and different letters are differed significantly by Tukey HSD Test ($p < 0.001$).

There was a statistically significant increase in the root/shoot ratio (Figure 1c) when comparing drought-affected (0 ppm SiO_2 NPs, SM 30%) and control plants (SM 80%). Comparing the effects of 0 ppm and 12.5 ppm SiO_2 on plant root/shoot ratio when watered or sprayed in drought treatment, no statistically significant difference was found. However, a statistically significant effect on root/shoot ratio was found by watering or spraying peas at a concentration of 25 ppm SiO_2 compared to SiO_2 NPs untreated plants (0 ppm) in drought-affected peas. Meanwhile, the root/shoot ratio of peas grown under drought did not differ significantly from control plants when were watered or sprayed with 50 ppm SiO_2 NPs.

3.2. Influence of Drought Stress and SiO_2 NPs on Lipid Peroxidation and Hydrogen Peroxide

The malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content in peas increased significantly under drought stress (0 ppm SiO_2 NPs, SM 30%) compared to control plants, i.e., 24 and 132%, respectively (Figure 2a,b).



(a) (b)

Figure 2. Influence of drought stress and SiO_2 NPs (0; 12.5; 25; and 50 ppm) on malondialdehyde content (MDA), (**a**); hydrogen peroxide (H_2O_2), (**b**) in *P. sativum* L. Cont.—control plants, substrate moisture (SM) 80%; drought stress—SM 30%. Values are mean ± SE of three replicates and different letters are differed significantly by Tukey HSD Test ($p < 0.001$).

All applied SiO_2 NPs concentrations significantly reduced the content of MDA and H_2O_2 in peas compared to NPs-untreated (0 ppm SiO_2 NP, SM 30%) plants as they were

watered with SiO_2 NPs. However, no statistical reliability was found when comparing MDA levels in plants watered with 25 and 50 ppm SiO_2 NPs concentrations with control plants. Spraying peas with SiO_2 NPs resulted in a statistically significant reduction in MDA and H_2O_2 content at 25 ppm (9 and 13%, respectively) and 50 ppm (13 and 21%, respectively) SiO_2 NPs concentrations compared to NP-untreated plants under drought stress (0 ppm SiO_2 NPs, SM 30%), but still exceeded the level of control plants.

3.3. Effect of Drought Stress and SiO_2 NPs on Antioxidant Activity

The total phenolic compounds (TPC), DPPH and ABTS free radical scavenging activity, and FRAP antioxidant power in pea leaves were significantly reduced by drought stress (0 ppm SiO_2 NPs, SM 30%) (24, 16, 39, and 17%, respectively) compared to control plants (Figure 3). There were no statistically significant changes in TPC and DPPH value between control plants and peas sprayed with 50 ppm SiO_2 NPs. Moreover, there was no significant effect on FRAP value when peas were watered with 25, 50 ppm and sprayed with 50 ppm SiO_2 NPs as compared to control plants. ABTS free radical scavenging activity in peas sprayed with 12.5 and 25 ppm 50 ppm SiO_2 NPs under drought stress was significantly reduced compared to control plants. However, a statistically significant increase in ABTS value was found in plants watered with 12.5, 25, and 50 ppm SiO_2 NPs under drought exposure.

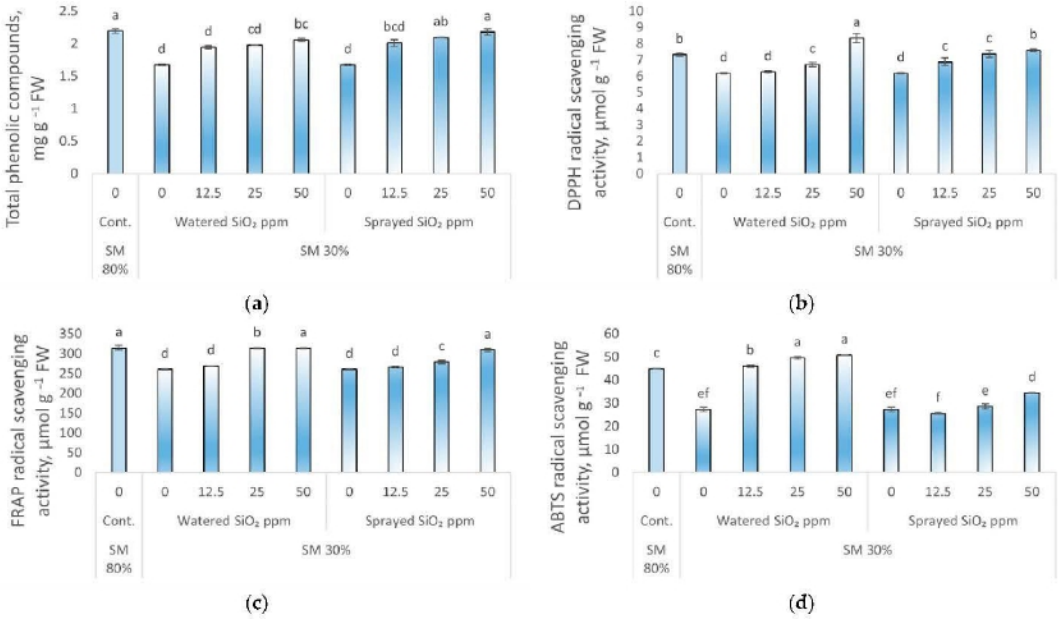


Figure 3. Influence of drought stress and SiO_2 NPs (0; 12.5; 25; and 50 ppm) on total phenolic compounds (**a**); DPPH radical scavenging activity (**b**); FRAP radical scavenging activity (**c**); ABTS radical scavenging activity (**d**) in *P. sativum* L. Cont.—control plants, substrate moisture (SM) 80%; drought stress—SM 30%. Values are mean ± SE of three replicates and different letters are differed significantly by Tukey HSD Test ($p < 0.001$).

3.4. Effect of Drought Stress and SiO_2 NPs on Antioxidant Enzymes Activities

Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) activity increased in peas affected by drought compared with control plants (Figure 4). The most active antioxidants were found when plants were wa-

tered with SiO_2 NPs suspensions. SOD activity was most increased by watering plants with 25 ppm (95%) and 50 ppm (103%) or by spraying with 50 ppm (82%) SiO_2 NPs compared with control plants (Figure 4a). Increased CAT activity was observed in both watered and sprayed plants at all applied concentrations but was most pronounced when peas were exposed to 50 ppm SiO_2 NPs suspension (Figure 4b). The activity of the APX enzyme in peas was also increased in drought conditions (Figure 4c) when plants were watered at 0, 12.5, 25, and 50 ppm NPs concentrations, APX activity increased by 158, 244, 288, and 309% respectively, compared to control plants. As with all enzymes tested, GR activity in peas also increased under drought conditions (Figure 4d) when plants were watered at 0, 12.5, 25, and 50 ppm SiO_2 NPs suspension, GR activity increased by 39, 41, 69, and 178%, respectively, compared to control plants.

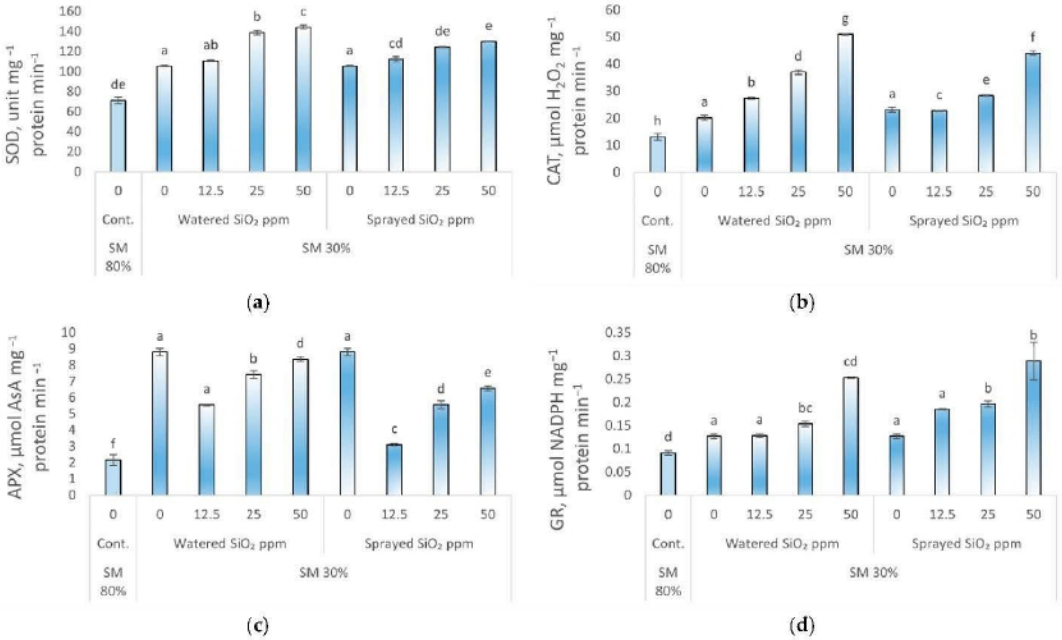


Figure 4. Response of (**a**), superoxide dismutase (SOD); (**b**), catalase (CAT); (**c**), ascorbate peroxidase (APX); (**d**), glutathione reductase (GR) activity to drought stress and SiO_2 NPs (0; 12.5; 25; and 50 ppm) in *P. sativum* L. Cont.—control plants, substrate moisture (SM) 80%; drought stress—SM 30%. Values are mean ± SE of three replicates and different letters are differed significantly by Tukey HSD Test ($p < 0.001$).

4. Discussion

This study elucidated the effects of SiO_2 NPs on drought stress in pea plants based on NP concentration and different applications (leaf application and root irrigation) to explain the main physiological, non-enzymatic, and enzymatic antioxidant defense mechanisms. The results of this study showed that SiO_2 NPs can alleviate drought-induced stress in plants. The above-presented research data indicate that the studied defense mechanisms significantly varied in SiO_2 NPs non-treated and treated pea plants under drought stress.

4.1. Effects of Different Applications and Concentration of NPs

Several factors are responsible for the transformation and uptake of NPs in plants: physicochemical properties of NP itself, size, surface charge, concentration in the suspen-

sion, potential interaction with plants, and plant physiology. Dissolved bulk silicon (Si) is known to be absorbed by plants in the form of mono-silicic acid ($Si(OH)_4$) and ultimately polymerizes with the loss of water molecules to form hydrated silica ($SiO_2 \cdot nH_2O$) [35]. In plants with a high capacity for metalloid accumulation, it is assisted by one of aquaporin group MIP [36]—Si influx transporters (SiT1 and SiT2) and efflux transporters (SiT6) located in the main and lateral roots, responsible for the transport of Si from the cortical cells to the xylem [35–38]. The researchers found that pea plants tend to the silicification process using Si transporters [39]. However, NPs can have different properties than their bulk materials [40]. Plants can be affected by NPs in two ways, sprayed through shoots and watered through roots. Spraying—exposure through the leaves can form an additional layer on the leaves [17] to protect plants from increased transpiration and disease [41], but can also allow some NPs to penetrate through the wax layer and diffuse directly into the plant (limited to pore size 5–20 nm) [42]. Considering the composition of the cell wall, which has hydrophobic and hydrophilic components and unequal distribution of fixed negative charges (cellulose fibers and lignin surface potential are −15 and −45 mV, respectively) [9,43], it is noticeable that negatively charged plant cell walls act as an ion exchange surface that potentially promotes the penetration of cationic NPs rather than anionic ones. Considering the effects of irrigation when NPs are reached through the soil, it should be noted that the soil particles are usually negatively charged, and NPs with a higher negative charge, are more agile in such soil [44]. On the contrary, positively charged NPs easily attract negatively charged soil surface particles. In general, NPs mobility decreases as the average soil grain size decreases. The clay content of the soil can act as an anionic adjuvant to prevent NPs accumulation and increase their mobility [45]. Zeta potential measurement is a technique for determining the surface charge in a colloidal solution [46]. NPs with a zeta potential of −10 to +10 mV are approximately neutral, and NPs with a zeta potential greater than +30 mV or less than −30 mV are considered to be strongly cationic and anionic, respectively [47]. The zeta potential of the plasma membrane of various plants can vary from 20 to −39 mV, but the zeta potential of the plasma membrane of pea has not been studied, making it difficult to estimate [48]. In future studies, it would be valuable to evaluate the zeta potential of the pea leaf plasma membrane when grown under normal and drought conditions. However, in our results, the zeta potential of the SiO_2 suspension in distilled water was −20 meaning anion (Table 1). There is another scientific article describing that the application of 10 g kg^{-1} SiO_2 NPs with a zeta potential of −40 mV through the soil had a positive effect on photosynthesis, yield quality, and increased productivity of maize plants [49]. Moreover, the researchers [50] found that negatively charged Au NPs accumulate less on the root surface but move most efficiently through the root epidermis to plant shoots, especially in rice and ryegrass.

The effect of NPs on plants is highly dependent on their concentration. In this study, the most effective concentration against drought-induced effects in peas was spraying and watering with 50 ppm SiO_2 NPs. The researchers found that spraying strawberries with 125 ppm SiO_2 NPs increased their resistance to drought [14]. Using concentrations of 100, 200, 300, and 400 ppm SiO_2 NPs, a statistically significant effect was found for common bean germination [51]. Other researchers used Si NPs as leaf sprays at concentrations of 0, 5, 10, 20, 30 ppm and found that the Cd-induced negative growth rates of rice were significantly reduced [18]. Previous comparative studies showed that approximately 140 ppm Si NPs were effective in reducing Cr (VI) toxicity in pea (*Pisum sativum*) [20], arsenate toxicity in maize (*Zea mays*) [13], and UV-B stress in wheat (*Triticum aestivum*) [17]. In another experiment with cucumbers [52], SiO_2 NPs concentrations at 100, 200, 300, 400 ppm were used, of which the concentration at 200 ppm caused the greatest positive effect in plants. In addition, researchers [19] sprayed potatoes with SiO_2 NPs concluded that a concentration of 50 ppm had a positive, but a concentration of 100 ppm had a statistically significant negative effect on plants. In contrast, the opposite, negative effect was found for SiO_2 NPs at concentrations 10, 100, 500, and 2000 ppm on Bt-transgenic cotton height and weight [5].

These results confirm that the effects of NPs depend not only on the NPs and applied concentration, but also on their interaction with the plant species.

4.2. Influence of Drought Stress and SiO_2 NPs on Oxidative Stress Markers and Antioxidant Enzymes Activities in Peas

The effects of appropriately selected and studied doses of NPs on plants and the soil ecosystem could address the food insecurity caused by climate change, which is challenging agriculture. In our research, the deficiency of water in soil caused oxidative stress resulting in increased malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels in pea plants (Figure 2a,b), but watering or spraying plants with SiO_2 NPs reduced the harmful effects of drought stress. Drought stress increased the activity of antioxidant enzymes, including CAT, APX, SOD, and GR (Figure 4), in the leaves of pea plants compared to control plants grown with insufficient moisture supply, while exposure to SiO_2 NPs further strengthened the activity of these enzymes. Previous studies [53,54] have shown that severe drought causes oxidative stress due to the accumulation of reactive oxidative species (ROS) in plant cells, including superoxide radicals ($O_2^{\bullet-}$), alkoxy radicals (RO•) and hydroxyl radicals (OH•), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). However, plants have adapted to eliminate excessive oxidative stress agents using antioxidant defense systems consisting of enzymatic (SOD, CAT, GSH, etc.) and non-enzymatic (ascorbic acid, phenolic compounds, carotenoids, etc.) antioxidants [53,55,56]. As the amount of oxidative stress products in plants increases, the antioxidant system activates, for example, superoxide dismutase converts the superoxide radical to hydrogen peroxide (which is less harmful to the plant) then the peroxide decomposes into the water using antioxidants such as CAT, APX, GPX (using different reaction catalysts) [54]. As can be seen in Figure 3, the application of SiO_2 NPs tends to stimulate a non-enzymatic antioxidant response by increasing TPC, DPPH, FRAP, and most strongly ABTS radical scavenging activity in pea plants exposed to drought stress. The ABTS [57] and FRAP methods show a greater correlation with non-enzymatic hydrophilic antioxidants such as α-tocopherols, flavonoids, and ascorbic acid, and the DPPH method showed a higher correlation with lipophilic antioxidants such as carotenoids [25]. According to the results of ABTS, FRAP, and TPC, it can be stated that peas contain more hydrophilic antioxidants than lipophilic in terms of DPPH results.

In general, increased levels of MDA, H_2O_2, and other antioxidant enzymes have been reported in various plant species under drought stress [14,56,58,59]. In this study, a significant increase in MDA and H_2O_2 in pea leaves (Figure 2) in response to water deficiency caused strong oxidative stress resulting in retarded plant growth, decreased RWC, SLA, and increased root/shoot ratio (Figure 1), indicating that pea plants in the lack of soil moisture encourage root growth. MDA can generally be described as a process in which oxidants, such as free radicals, attack lipids having a carbon–carbon double bond(s), especially polyunsaturated fatty acids (PUFAs) [60]. According to the results obtained by other researchers [61], PUFAs in peas are less than in other plants of the Legumes family. Besides, the amount of PUFAs had a positive correlation with DPPH antioxidants, which in pea plants also had lower activity compared to ABTS (Figure 3b,d). In this study, a very strong increase was found in the activity of enzymatic antioxidants such as SOD, CAT, APX, and GR (Figure 4) when pea plants were watered or sprayed with SiO_2 NPs especially at 50 ppm (enzyme activity increased with increasing SiO_2 NPs concentration). In response to water deficit, due to stomatal closure, low CO_2 availability, and limited fixation, oxygen saturation of ribulose 1,5-bisphosphate (RuBP) is preferred that enhances photorespiration [59] leading to more than 70% of the H_2O_2 generation in plants. Besides, H_2O_2 is formed in the plant when the enzyme SOD performs the monovalent reduction and protonation of the superoxide radical [53]. In this study, significant activation of SOD along with other enzymatic antioxidants activity was observed in pea plants under water deficit. We suppose that, due to this, a significant decrease in H_2O_2 (Figure 2b) was observed in peas as plants were watered with SiO_2 NPs in the presence of a water deficit. As mentioned above, SiO_2 NPs may enter the pea plant through special channels in the roots, then move

in a symplastic pathway in the plant and enter the cell cytosol containing most of the enzymatic antioxidants and thus affect plant with abnormal particle surface area, size, and charge. This explains the more efficient watering of SiO_2 NPs than foliar application to plants.

SiO_2 NPs have a very strong relationship with water content in plants; there are just a few published scientific articles specifically on the effects of drought. For instance, in strawberries, spraying with Si NPs during drought stress increased the activity of CAT, APX, SOD, and GR enzymes and decreased the content of MDA and H_2O_2 [14]. The relationship of Si NPs and other abiotic factors such as salinity, exposure to heavy metals, and UV radiation to plants were also explored by researchers. For example, when spraying with 50 ppm SiO_2 NPs suspension in salinity-treated potato seedlings, the researchers found [19] that enzymatic antioxidants such as GPX and SOD were more active compared to potatoes not sprayed with SiO_2 suspension. The negative effects of Cr (VI) were found in peas with increased H_2O_2 and MDA concentrations but spraying them with Si NPs resulted in a clear decrease in oxidative stress biomarkers and activated enzymatic antioxidants such as SOD, CAT, GR, APX [20]. Moreover, when common beans were sprayed with 30 ppm SiO_2 NPs, a statistically positive increase in the activity of antioxidant enzymes and a decrease in the concentration of MDA and H_2O_2 were observed under Cd stress [18]. Similar results were obtained when researchers studied [62] the effects of Cd-, Pb-affected rice and foliar application of SiO_2 NPs, the sprayed plants with 20 ppm SiO_2 NPs accumulated less heavy metals and produced the highest yields. The SiO_2 NPs have been shown [17] to protect wheat seedlings from UV-B stress by stimulating the antioxidant defense system and reducing the negative effects of UV-B stress, such as low fresh weight, chlorophyll content, and tissue damage. Researchers noted that as nitric oxide levels reached a peak after exposure to UV-B and Si NPs, the protection arises from modulating NO levels.

4.3. Effects of Drought Stress and SiO_2 NPs on Peas Specific Leaf Area, Relative Water Content, and Root/Shoot Ratio

In plants, decreased growth parameters may be due to decreased relative water content (RWC) and corresponding cell contraction, decreased meristematic cell division, decreased leaf growth, accelerated aging, blocked leaf production, and leaf fall. Water stress can also directly affect the biochemical processes involved in photosynthesis and indirectly reduce the uptake of carbon dioxide into the stoma, which closes during drought. In this study, relative water content (RWC) (Figure 1a) and specific leaf area (SLA) (Figure 1b) of pea plants significantly decreased during drought stress, but watering or spraying with a suspension containing SiO_2 NPs increased their contents. Plant membranes are the first place in the cell which are influenced under stress conditions and the ability of plants to protect the integrity of membranes under drought stress determines the tolerance of the plants to drought stress [63]. Under drought stress, the water potential of soil decreases, and plants prevent transpiration phenomenon using different mechanisms such as closing stomata, increasing stomatal resistance, and decreasing stomatal conductivity. Scientists showed that the improving effect of Si on the hydration status of plants may help to reduce leaf and stem transpiration or the deposition of phytolith under epidermal cells, resulting in a decreased waste of water from cuticle layers [64]. According to that concentration of 50 ppm, SiO_2 NPs play an important role in increasing RWC in pea plants (Figure 1a) under stressful conditions. Similar RWC results were found in drought-affected strawberries [14], wheat [58], and cucumber [52]. The researchers also found a statistically positive increase in rice height, shoot, and root weight under Cd stress and spraying of SiO_2 NPs [18]. A study by other scientists has shown that drought stress has negatively affected wheat growth rates and yields [58], but the use of SiO_2 NPs, especially through soil application at 30 and 60 ppm, reduced the negative effects of drought stress by reducing transpiration, improving the rate of photosynthesis, increasing chlorophyll content and RWC. Moreover, similar results were found in peas [20], maize [13], and wheat [17] affected by various stressors,

with a statistically positive increase in fresh leaf and root biomass, also leaf area using Si NPs.

Previous studies [65] indicate that the root/shoot ratio increases with drought, as plants tend to grow roots and absorb more water over a larger root surface, resulting in reduced shoot growth. This trend was also observed in our study, with a significant increase in root/shoot ratio in drought-affected peas (Figure 1c), but the application of SiO_2 NPs at 50 ppm reduced the negative effects of drought stress on the root/shoot ratio. These results confirm that the application of SiO_2 NPs can strongly affect plant development under drought exposure.

5. Conclusions

In conclusion, watering or foliar spraying solutions containing 50 ppm SiO_2 NPs on pea plants is an efficient way to improve their resistance to drought stress. Favorable effects of SiO_2 NPs on pea growth efficiency under drought conditions have been attributed to the activation of the enzymatic and non-enzymatic antioxidative system to eliminate ROS and enhance relative water content, specific leaf area, and decreased root/shoot level. Based on the results obtained, the use of SiO_2 NPs is recommended for managing the drought stress in pea plants and even in other agricultural plants.

Author Contributions: Conceptualization, R.S., A.B., G.S. and J.M.; methodology, R.S., A.B., L.R., G.S., M.U. and J.M.; validation, A.B., G.S., M.U. and J.M.; formal analysis, R.S.; investigation, R.S.; resources, L.R., G.S. and J.M.; writing—original draft preparation, R.S.; writing—review and editing, A.B., L.R., G.S. and J.M.; visualization, R.S.; supervision, J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: R.S. is thankful to the Lithuanian Research Centre for Agriculture and Forestry for the opportunity to work with nanoparticles and for donated green pea "Respect" seeds. The authors are also grateful to Joana Bendoraitienė from the Kaunas University of Technology for measuring the size and zeta potential of nanoparticle suspension.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Spinoni, J.; Barbosa, P.; Bucchignani, E.; Cassano, J.; Cavazos, T.; Christensen, H.J.; Christensen, B.O.; Coppola, E.; Evans, J.; Geyer, B.; et al. Future global meteorological drought hot spots: A study based on CORDEX data. *J. Clim.* **2020**, *33*, 3635–3661. [CrossRef]
2. Chhipa, H.; Joshi, P. Nanofertilisers, Nanopesticides and Nanosensors in Agriculture. *Sustain. Agric. Rev.* **2016**, *20*, 247–282.
3. Zulfiqar, F.; Navarro, M.; Ashraf, M.; Akram, A.N.; Munne-Bosch, S. Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Plant Sci.* **2019**, *289*, 110270. [CrossRef]
4. Gomez, A.; Narayan, M.; Zhao, L.; Jia, X.; Bernal, R.; Lopez-Moreno, L.M.; Peralta-Videa, J. Effects of nano-enabled agricultural strategies on food quality: Current knowledge and future research needs. *J. Hazard. Mater.* **2020**, *401*, 12391–12398. [CrossRef]
5. Rui, Y.; Gui, X.; Li, X.; Liu, S.; Han, Y. Uptake, transport, distribution and bio-effects of SiO_2 nanoparticles in Bt-transgenic cotton. *J. Nanobiotechnol.* **2014**, *12*, 1–15. [CrossRef]
6. Tarafdar, J.C.; Xiong, Y.; Wang, W.N.; Quinl, D.; Biswas, P. Standardization of size, shape and concentration of nanoparticle for plant application. *Appl. Biol. Res.* **2012**, *14*, 138–144.
7. Mittal, D.; Kaur, G.; Singh, P.; Yadav, K.; Ali, S.A. Nanoparticle-based sustainable agriculture and food science: Recent advances and future outlook. *Front. Nanotechnol.* **2020**, *2*, 10. [CrossRef]
8. Nadmint, P.P.; Dong, Y.D.; Sayer, C.; Hay, P.; Rookes, J.E.; Boyd, B.J.; Cahill, D.M. Nanostructured liquid crystalline particles as an alternative delivery vehicle for plant agrochemicals. *ACS Appl. Mater. Interfaces* **2013**, *5*, 1818–1826. [CrossRef] [PubMed]
9. Santiago, M.; Pagay, V.; Stroock, D.A. Impact of electroviscosity on the hydraulic conductance of the bordered pit membrane: A theoretical investigation. *Plant Physiol.* **2013**, *163*, 999–1011. [CrossRef]
10. Meychik, R.N.; Nikolaeva, I.J.; Yermakov, P.I. Ion exchange properties of the root cell walls isolated from the halophyte plants (*Suaeda altissima* L.) grown under conditions of different salinity. *Plant Soil.* **2005**, *277*, 163–174. [CrossRef]
11. Sun, D.; Hussain, H.I.; Yi, Z.; Siegele, R.; Cresswell, T.; Kong, L.; Cahill, D.M. Uptake and cellular distribution, in four plant species, of fluorescently labeled mesoporous silica nanoparticles. *Plant Cell Rep.* **2014**, *33*, 1389–1402. [CrossRef]
12. Luyckx, M.; Hausman, J.F.; Guerriero, G.; Lutts, S. Silicon and plants: Current knowledge and technological perspectives. *Front. Plant Sci.* **2017**, *8*, 411. [CrossRef]

13. Tripathi, D.K.; Singh, S.; Singh, V.P.; Prasad, S.M.; Chauhan, D.K.; Dubey, N.K. Silicon nanoparticles more efficiently alleviate arsenate toxicity than silicon in maize cultivar and hybrid differing in arsenate tolerance. *Front. Environ. Sci.* **2016**, *4*, 46. [CrossRef]
14. Zahedi, S.M.; Moharrami, F.; Sarikhani, S.; Padervand, M. Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress. *Sci. Rep.* **2020**, *10*, 17672. [CrossRef]
15. Shabbaj, I.I.; Madany, M.M.Y.; Tammar, A.; Balkhyour, M.A.; AbdElgawad, H. Silicon Dioxide Nanoparticles Orchestrate Carbon and Nitrogen Metabolism in Pea Seedlings to Cope with Broomrape Infection. *Environ. Sci. Nano* **2021**, *8*, 1960–1977. [CrossRef]
16. Tantawy, S.A.; Salama, M.Y.A.; El-Nemr, A.M.; Abdel-Mawgoud, R.A.M. Nano silicon application improves salinity tolerance of sweet pepper plants. *Int. J. Chemtech Res.* **2015**, *8*, 11–17.
17. Tripathi, D.K.; Singh, S.; Singh, V.P.; Prasad, S.M.; Dubey, N.K.; Chauhan, D.K. Silicon nanoparticles more effectively alleviated UV-B stress than silicon in wheat (*Triticum aestivum*) seedlings. *Plant Physiol. Biochem.* **2017**, *110*, 70–81. [CrossRef] [PubMed]
18. Rizwan, M.; Ali, S.; Rehman, Z.M.; Malik, S.; Adrees, M.; Qayyum, F.M.; Alamri, A.S.; Alyemeni, N.M.; Ahmad, P. Effect of foliar applications of silicon and titanium dioxide nanoparticles on growth, oxidative stress, and cadmium accumulation by rice (*Oryza sativa*). *Acta Physiol. Plant* **2019**, *41*, 1–12. [CrossRef]
19. Gowayed, H.M.; Al-Zahrani, S.H.; Metwali, M.E. Improving the salinity tolerance in potato (*Solanum tuberosum*) by exogenous application of silicon dioxide nanoparticles. *Int. J. Agric. Biol.* **2017**, *19*, 183–192.
20. Tripathi, D.K.; Singh, V.P.; Prasad, S.M.; Chauhan, D.K.; Dubey, N.K. Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in *Pisum sativum* (L.) seedlings. *Plant Physiol. Biochem.* **2015**, *96*, 189–198. [CrossRef]
21. Attia, A.E.; Elhawat, N. Combined foliar and soil application of silica nanoparticles enhances the growth, flowering period and flower characteristics of marigold (*Tagetes erecta* L.). *Sci. Hortic.* **2021**, *282*, 110015. [CrossRef]
22. Meier, U. *Growth Stages of Mono- and Dicotyledonous Plants: BBCH-Monograph Entwicklungsstadien Mono- und Dikotyler Pflanzen*; Biologische Bundesanstalt für Land- und Forstwirtschaft in Berlin und Braunschweig, Ed.; Blackwell Wissenschafts-Verlag: Berlin, Germany; Wien, Austria, 1997; ISBN 9783826331527.
23. Baris, D.H.; Weatherley, E.P. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci.* **1962**, *24*, 519–570. [CrossRef]
24. Ainsworth, E.A.; Gillespie, K.M. Estimation of Total Phenolic Content and Other Oxidation Substrates in Plant Tissues Using Folin–Ciocalteu Reagent. *Nat. Protoc.* **2007**, *2*, 875–877. [CrossRef] [PubMed]
25. Re, R.; Pellegrini, N.R.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
26. Sharma, P.O.; Bhat, K.T. DPPH antioxidant assay revisited. *Food Chem.* **2009**, *113*, 1202–1205. [CrossRef]
27. Benzie, I.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a aeasure of "Antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef]
28. Velikova, V.; Yordanov, I.; Edreva, A. Oxidative stress and some antioxidant system in acid rain treated bean plants: Protective role of exogenous polyammines. *Plant Sci.* **2000**, *151*, 59–66. [CrossRef]
29. Heath, R.L.; Packer, L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **1968**, *125*, 189–198. [CrossRef]
30. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
31. Dhindsa, S.R.; Plumb-Dhindsa, P.; Thorpe, A.T. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* **1981**, *32*, 93–101. [CrossRef]
32. Aebi, H. Catalase in vitro. *Meth. Enzymol.* **1984**, *105*, 121–126. [CrossRef]
33. Nakano, Y.; Asada, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **1981**, *22*, 867–880. [CrossRef]
34. Sofo, A.; Tuzio, C.A.; Dichio, B.; Xiloyannis, C. Influence of water deficit and rewatering on the components of the ascorbate–glutathione cycle in four interspecific Prunus hybrids. *Plant Sci.* **2005**, *169*, 403–412. [CrossRef]
35. Kumar, S.; Soukup, M.; Elbaum, R. Silicification in grasses: Variation between different cell types. *Front. Plant Sci.* **2017**, *8*, 438. [CrossRef]
36. Maurel, C.; Boursiac, Y.; Luu, T.D.; Santoni, V.; Shahzad, Z.; Verdoucq, L. Aquaporins in plants. *Physiol. Rev.* **2015**, *95*, 1321–1358. [CrossRef]
37. Rao, G.B.; Susmitha, P. Silicon uptake, transportation and accumulation in rice. *J. Pharmacogn. Phytochem.* **2017**, *6*, 290–293.
38. Guntzer, F.; Keller, C.; Meunier, D.J. Benefits of plant silicon for crops: A review. *Agron. Sustain. Dev.* **2012**, *32*, 201–213. [CrossRef]
39. Sangster, A.G.; Hodson, M.J.; Tubb, H.J. Silicon deposition in higher plants. In *Studies in Plant Science*; Datnoff, L.E., Snyder, G.H., Korndörfer, G.H., Eds.; Elsevier: Amsterdam, The Netherlands, 2001; Volume 8, pp. 85–113. [CrossRef]
40. Roduner, E. Size matters: Why nanomaterials are different. *Chem. Soc. Rev.* **2006**, *35*, 583–592. [CrossRef] [PubMed]
41. Siddiqui, M.H.; Al-Whaibi, M.H. Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.). *Saudi J. Biol. Sci.* **2014**, *21*, 13–17. [CrossRef]
42. Palocci, C.; Valletta, A.; Chronopoulou, L.; Donati, L.; Bramosanti, M.; Brasili, E.; Baldan, B.; Pasqua, G. Endocytic pathways involved in PLGA nanoparticle uptake by grapevine cells and role of cell wall and membrane in size selection. *Plant Cell Rep.* **2017**, *36*, 1917–1928. [CrossRef]

43. Zeng, Y.; Himmel, M.E.; Ding, S.Y. Visualizing chemical functionality in plant cell walls. Biotechnol. *Biofuels* **2017**, *10*, 1–16. [CrossRef]
44. Darlington, T.K.; Neigh, M.A.; Spencer, T.M.; Guyen, T.O.; Oldenburg, S.J. Nanoparticle characteristics affecting environmental fate and transport through soil. Environ. *Toxicol. Chem.* **2009**, *28*, 1191–1199. [CrossRef] [PubMed]
45. Schrick, B.; Hydusky, W.B.; Blough, L.J.; Mallouk, E.T. Delivery vehicles for zerovalent metal nanoparticles in soil and groundwater. *Chem. Mater.* **2004**, *16*, 2187–2193. [CrossRef]
46. Kumar, A.; Dixit, K.C. Methods for Characterization of Nanoparticles. In *Advances in Nanomedicine for the Delivery of Therapeutic Nucleic Acids*; Nimesh, S., Chandra, R., Gupta, N., Eds.; Woodhead Publishing: Cambridge, UK, 2017; pp. 43–58. [CrossRef]
47. Clogston, J.D.; Patri, A.K. Zeta potential measurement. *Methods Mol. Biol.* **2011**, *697*, 63–70. [CrossRef]
48. Kinraide, T.B.; Wang, P. The surface charge density of plant cell membranes (σ): An attempt to resolve conflicting values for intrinsic σ. *J. Exp. Bot.* **2010**, *61*, 2507–2518. [CrossRef]
49. El-Naggar, M.E.; Abdelsalam, N.R.; Fouda, M.M.; Mackled, M.I.; Al-Jaddadi, M.A.; Ali, H.M.; Siddiqui, M.H.; Kandil, E.E. Soil application of nano silica on maize yield and its insecticidal activity against some stored insects after the post-harvest. *Nanomaterials* **2020**, *10*, 739. [CrossRef]
50. Zhu, J.Z.; Wang, H.; Yan, B.; Zheng, H.; Jiang, Y.; Mirand, R.O.; Rotello, M.V.; Xing, B.; Vachet, W.R. Effect of surface charge on the uptake and distribution of gold nanoparticles in four plant species. *Environ. Sci. Technol.* **2012**, *46*, 12391–12398. [CrossRef]
51. Alsaeedi, A.; El-Ramady, H.; Alshaal, A.T.; Almohsen, M. Enhancing seed germination and seedlings development of common bean (*Phaseolus vulgaris*) by SiO_2 nanoparticles. *Egypt. J. Soil Sci.* **2017**, *54*, 407–415.
52. Alsaeedi, A.; El-Ramady, H.; Alshaal, T.; El-Garawany, M.; Elhawat, N.; Al-Otaibi, A. Silica nanoparticles boost growth and productivity of cucumber under water deficit and salinity stresses by balancing nutrients uptake. *Plant Physiol. Biochem.* **2019**, *139*, 1–10. [CrossRef]
53. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.* **2014**, *2*, 53. [CrossRef]
54. Garg, N.; Manchanda, G. ROS generation in plants: Boon or bane? *Plant Biosyst.* **2009**, *143*, 81–96. [CrossRef]
55. Sarker, U.; Oba, S. Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of Amaranthus tricolor. *Sci. Rep.* **2018**, *8*, 1–12. [CrossRef]
56. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [CrossRef]
57. Miller, J.N.; Rice-Evans, A.C. Factors influencing the antioxidant activity determined by the ABTS•+ radical cation assay. *Free Radic. Res.* **1997**, *26*, 195–199. [CrossRef]
58. Behboudi, F.; Tahmasebi Sarvestani, S.; Kassaee, M.Z.; Modares Sanavi, S.A.M.; Sorooshzadeh, A. Improving growth and yield of wheat under drought stress via application of SiO_2 nanoparticles. *J. Agric. Sci. Technol.* **2018**, *20*, 1479–1492.
59. Noctor, G.; Veljovic-Jovanovic, S.; Driscoll, S.; Novitskaya, L.; Foyer, H.C. Drought and oxidative load in the leaves of C3 plants: A predominant role for photorespiration? *Ann. Bot.* **2002**, *89*, 841–850. [CrossRef]
60. Ayala, A.; Munoz, F.M.; Arguelles, S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell. Longev.* **2014**, *2014*, 360438. [CrossRef] [PubMed]
61. Grela, R.E.; Samolinska, W.; Kiczorowska, B.; Klebaniuk, R.; Kiczorowski, P. Content of minerals and fatty acids and their correlation with phytochemical compounds and antioxidant activity of Leguminous seeds. *Biol. Trace Elem. Res.* **2017**, *180*, 338–348. [CrossRef] [PubMed]
62. Hussain, B.; Lin, Q.; Hamid, Y.; Sanaullah, M.; Di, L.; Khan, B.M.; He, Z.; Yang, X. Foliage application of selenium and silicon nanoparticles alleviates Cd and Pb toxicity in rice (*Oryza sativa* L.). *Sci. Total Environ.* **2020**, *712*, 136497. [CrossRef]
63. Rehman, U.S.; Bilal, M.R.; Rana, M.R.; Tahir, N.M.; Shah, N.M.K.; Ayalew, H.; Yan, G. Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought. *Crop Pasture Sci.* **2016**, *67*, 712–718. [CrossRef]
64. Mateos-Naranjo, E.; Andrades-Moreno, L.; Davy, A.J. Silicon alleviates deleterious effects of high salinity on the halophytic grass Spartina densiflora. *Plant Physiol. Biochem.* **2013**, *63*, 115–121. [CrossRef]
65. Xu, W.; Cui, K.; Xu, A.; Nie, L.; Huang, J.; Peng, S. Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta Physiol. Plant.* **2015**, *37*, 9. [CrossRef]

Journal of Soil Science and Plant Nutrition (2022) 22:801–814
https://doi.org/10.1007/s42729-021-00692-w

ORIGINAL PAPER

The Effects of Nano-copper, -molybdenum, -boron, and -silica on Pea (*Pisum sativum* L.) Growth, Antioxidant Properties, and Mineral Uptake

Rūta Sutuliené[1] · Lina Rageliené[2] · Pavelas Duchovskis[1] · Jurga Miliauskiené[1]

Received: 28 June 2021 / Accepted: 9 November 2021 / Published online: 15 November 2021

Abstract
Before applying nanoparticles (NPs) for outdoor use, their effects on plants must be verified in a controlled chamber. Molybdenum (Mo) is important for nitrate uptake and boron (B) is necessary for peas (*Pisum sativum* L.) growth, copper (Cu) is important for cellular redox state and silicon (Si) makes the plant tissue stronger. However, it is not clear what concentrations would be beneficial or toxic to pea if used Mo, B, Cu, and Si in the form of NPs. This experiment aimed to determine the concentration-dependent effects of silica (SiO_2), Cu, Mo, and B NPs on pea growth, antioxidant properties, and mineral uptake. The study was performed in a controlled environment chamber in 16-h daylight cycles to evaluate the significance of different concentrations of SiO_2, CuO, Mo, and B NPs for the response of pea plants. In this study were performed growth, non-destructive measurements, chemical antioxidant efficiency (total phenolic compounds, DPPH, ABTS), lipid peroxidation, and hydroxide amount analyzed, also macro- and microelement uptake was evaluated. The most effective and statistically reliable effects were obtained using the following NPs suspension concentrations: SiO_2 – 100, 50 ppm; CuO – 12.5 ppm; Mo – 100, 50 ppm; B – 12.5 ppm. The experiment showed that Mo and SiO_2 NPs had a positive effect at all concentrations, and B and CuO NPs at 100 ppm were toxic to peas. It has been found that SiO_2, Cu, Mo, B NPs can have both positive and negative effects on pea growth, antioxidant system, and mineral uptake depending on the concentration.

Keywords Nanoparticles · Field pea · Antioxidant activity · Oxidative stress

1 Introduction

The purpose of nanoparticles (NPs) in agriculture is to reduce the number of common chemicals, reduce losses in fertilizer use, and increase yields by regulating the use of pesticides and fertilizers (Prasad et al. 2017). However, the actual effects of nanomaterials on plants depend on their composition, concentration, size, surface load, and physicochemical properties, in addition to the sensitivity of plant species (Ma et al. 2015). Silicon (Si), copper (Cu), molybdenum (Mo), and boron (B) are trace elements beneficial to plants, but effects as NPs have been poorly studied.

✉ Rūta Sutuliené
ruta.sutuliene@lammc.lt

[1] Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Kaunas str. 30, LT-54333 Babtai, Lithuania

[2] Faculty of Natural Sciences, Vytautas Magnus University, Vileikos str. 8, LT-44404 Kaunas, Lithuania

Silica (SiO_2) NPs exhibit great potential in agriculture on plant growth and development. It may work better in alleviating different abiotic stresses than bulk material (Rastogi et al. 2019). This was confirmed by the scientists (Tripathi et al. 2015) who studied the effects of Si NPs (75–125 nm, 10μM concentration) on pea seedlings in response to chromium (Cr (VI)) stress and found that SiO_2 NPs regulated the antioxidant defense system in pea under Cr (VI) stress.

The effects of copper oxide (CuO) NPs on peas have been studied in several studies (Ochoa et al. 2017; Nair and Chung 2015). In research (Ochoa et al. 2017), pea plants were cultivated to full maturity in soil amended with CuO NPs (74.3% NPs of 10–100-nm size was in the suspension) at concentrations 0, 50, 100 mg kg^{-1}, and indoleacetic acid (IAA) at 10 and 100 μM and was found that interaction of IAA (10 μM) and CuO NPs (50 mg kg^{-1}) reduced stem and pod biomass of pea plants. Another study (Nair and Chung 2015) observed that the effects of CuO NPs on pea germination depended on applied CuO NPs concentration; toxicity to peas was observed by using 100, 200, 400, and 500 mg L^{-1} CuO

NPs, resulting in significantly reduced length of plant shoots and roots, and increased generation of reactive oxygen species (ROS) and lipid peroxidation.

Molybdenum (Mo) is one of the essential elements required for nitrogen metabolism in rhizobium bacteria in pea roots. There is only one scientific article on the effects of Mo NP on chickpeas [8]. Researchers stated that Mo NPs suspension (100–250nm; 8 mg L^{-1}) stimulated the formation of a nodule and increased their number by two times compared to control plants (Mo NPs untreated plants) (Taran et al. 2014).

Boron (B) is one of the key nutrients for optimal crop growth, development, yield, and quality (Brown et al. 2002). To our knowledge, the effect of B on pea seedlings in NP size has not yet been studied. The effects of B NPs (0.6 mg kg^{-1}) on soybean have been studied, indicating that B NPs affected soybean growth, yield, and grain micronutrient quality under drought conditions (Dimkpa et al. 2017). In addition, B NPs are more effective than bulk for soybean dry weight (Dimkpa et al. 2019).

Based on several studies, the beneficial role of SiO_2, CuO, Mo, and B NPs in plants is not well documented and the effects on green pea are not yet well known. Peas are a widely grown crop. The major pea producers are China, India, the USA, France, and Egypt. Besides, pea seeds are high in protein and a good source of vitamins (Khan et al. 2016). Legume crops are incorporated into agricultural systems to improve soil fertility, plant growth, and limit the use of chemical fertilizers. Pea plants have the beneficial property to form nodules on their roots, in which *Rhizobium* bacteria fix nitrogen from the air and thus enrich the soil. Thus, peas play a significant role in crop rotation, and the overall economic value in the production system and were therefore chosen as model plants. This study aimed to explore the effects of different concentrations of SiO_2, CuO, Mo, and B nanoparticles on green pea (*Pisum sativum* L.) concerning growth traits; chlorophyll; and nitrogen balance index, antioxidant defense system, and regulation of mineral elements. It was hypothesized that NPs of SiO_2, CuO, Mo, and B will have a significant effect on pea plants, causing a different response depending on the concentration of NPs applied.

2 Materials and Methods

2.1 Preparation of Nanoparticle Suspension

Silica (SiO_2) NPs (particle size: 20–30nm; purity: 99%), copper oxide (CuO) NPs (particle size: 25–55nm; purity: 99.95%), molybdenum (Mo) NPs (particle size: 35–45 nm; purity: 95%), and boron (B) NPs (particle size: 100nm; purity: 99.9%) were used for this experiment (US Research Nanomaterials, Inc, Houston, TX, USA). The NPs with concentrations of 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm were suspended in deionized water and ultrasonically dispersed for 60 min. The NPs size and suspension stability were measured using Delsa™ Nano Submicron Particle Size (Beckman Coulter Instruments. Corporation, Fullerton, California) and Zeta Potential device (Dispersion Technology Inc., Bedford Hills, New York, USA).

The obtained data showed that all prepared NPs suspensions were strongly anionic and stable, also monodispersed according to the polydispersity index (PDI) (Table 1).

2.2 Plants and Growth Conditions

The research was carried out in a walk-in controlled environment growth chamber (4 × 6 m, h = 3.2 m) located at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Before sowing, green pea (*Pisum sativum* L. cv. Respect, Maribo Seed International ApS, Denmark) seeds were sterilized in 5% sodium hypochlorite solution for 15 min to assure surface sterility (Lehotai et al. 2011) and rinsed gently with deionized water several times. Then, seeds were soaked in water for 24 h. Sixteen pots were used for each treatment. The 3 seeds were sown in each 500-mL plastic pot which was treated as a single biological replication. Plants were seeded and grown in peat substrate, pH 6 (Profi 1, JSC Durpeta, Lithuania) for 30 days. The average amounts of nutrients (mg L^{-1}) in the substrate were as follows: N, 110; P_2O_5, 50; K_2O, 160. The average amounts of microelements (mg L^{-1}), Fe (4.5), Mn (0.5), Cu (0.1), B (0.02), Mo (0.03), and Zn (0.04), were also present. Electrical conductivity (EC) varied between 2.0 and 2.5 mS cm^{-1} (± 0.03 mS cm^{-1}). The growth conditions were as follows: 16-h photoperiod, 20 ± 2/16 ± 2 °C day/night ambient air temperature, 60 % relative air humidity, ~220 µmol m^{-2}

Table 1 Properties of NPs suspensions in DI water (pH=7.05) zeta potential, results represent the mean ± standard deviation, polydispersity index, and presents of nanoparticles between 1 and 100 nm

	Suspension of NPs of 50 ppm concentration			
	CuO	Mo	SiO_2	B
Zeta potential (ζ; mV)	−26.68 ± 0.631	−24.92 ± 0.314	−20.64 ± 0.333	−28.54 ± 0.359
Polydispersity index (PDI)	0.211	0.218	0.34	0.237
NPs size 1-100 nm in suspension, %	55	68	70	50

s^{-1} photon flux density of photosynthetically active radiation (PAR) provided by high-pressure sodium (HPS) lamp (SON-T Agro, 400 W; Philips, Somerset, NJ, USA). At the 14 BBCH growth stage, when plants unfolded their 3–4 true leaf or had 3–4 tendrils developed (14 days after sowing) (Meier 1997), peas were watered with 100 mL of SiO_2, CuO, Mo, or B NPs suspension containing different concentrations. After that, all plants were watered daily with deionized water and no additional fertilizer or nutrient solution was added. To minimize the potential effect of the growth chamber on plant response, randomization and regular spatial rearrangement of 10 pots per treatment were applied. Others were left unrotated for avoiding border effects. At the end of the experiment when plants reached the 31 BBCH growth stage (beginning of stem elongation), the peas were harvested for biometric, non-destructive measurements of leaf chlorophyll and nitrogen balance (NBI) indexes, and biochemical analyses.

2.3 Biometric and Non-destructive Measurements

For biometric measurements, the shoots were separated from the roots, and then shoot height and root length and fresh and dry weight were determined for randomly selected 10 plants per treatment ($n = 10$). The fresh biomass was measured with an electronic scale (Mettler Toledo AG64, Columbus, OH, USA), and dry biomass was determined following forced-air convection drying at 105°C to a constant dry weight (VENTICELL 222, MBT, Czech Republic). Non-destructive measurements of leaf chlorophyll and nitrogen balance (NBI) indexes in the fully developed leaves (10 plants per treatment, $n = 10$) were performed using a chlorophyll and flavonoid meter (Force-A Dualex® 4 Scientific, Ocala, FL, USA).

2.4 Antioxidant Properties and Total Phenolic Compounds

The extracts used to determine the total phenolic content and antioxidant activity were prepared by grinding 0.5 g of fresh sample with liquid nitrogen and diluting with 5 mL of 80% methanol. After 24 h, the samples were centrifuged for 10 min at 3000 rpm (Hermle Z300K, Germany), extracts were filtered through cellulose filters, and the supernatant was used for further analyses. All biochemical analysis was performed in 3 biological replications.

The total content of phenolic compounds was determined as gallic acid equivalents. A 250 µL aliquot of the sample extract was mixed with 250 µL of 10% (w/v) Folin-Ciocalteu reagent, 500 µL of 1 M Na_2CO_3 solution, and 2 ml of distilled water. After incubation for 20 min in the dark, the absorbance was measured at 765 nm (Ainsworth and Gillespie 2007) (M501, Spectronic Camspec Ltd., UK). The total phenolic compounds quantity mg g^{-1} was calculated from the calibration curve of the gallic acid.

Antioxidant properties of pea leaves were evaluated as the ABTS (2,2 -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (2-diphenyl-1-picrylhydrazyl), radical-scavenging activities.

The ABTS radical cation was obtained by incubating the 2 mM ABTS stock solution (0.112 g in 100 mL deionized water) with 400 µL $K_2S_2O_8$ (0.1982 g in 10 ml deionized water) in the dark for 24 h. Thereafter, 50 µL of the prepared sample was mixed with 2 ml of ABTS solution (ABTS stock solution was diluted 1:7), and the absorbance was measured after 11 min (plateau phase) at 734 nm (M501, Spectronic Camspec Ltd., UK). The ABTS scavenging activity of green pea extracts was calculated as the difference between the initial absorbance and after reacting for 10 min. It was expressed as ABTS mmol scavenged per 1 g of fresh weight (µmol g^{-1} FW). Methanol was used as the blank solution (Re et al. 1999).

For the DPPH assay, a stable 0.1268 µM DPPH (2,2-diphenyl-1-picrylhydrazyl radical) solution was prepared in methanol. Subsequently, 1 mL of the DPPH solution was transferred to a test tube and mixed with 100 µL of the diluted green pea extract with 400 µl methanol. The absorbance was scanned at 515 nm (M501, Spectronic Camspec Ltd., UK) while reacting for 16 min. The free radical scavenging capacity was expressed as µmol of DPPH radicals scavenged per 1 g of fresh weight (µmol g^{-1} FW) (Sharma and Bhat 2009).

2.5 Oxidative Stress Markers

The extracts used to determine the concentration of H_2O_2 and lipid peroxidation in pea tissue were prepared by grinding 0.1 g of fresh sample with liquid nitrogen and diluting with 4 mL of 0.1% TCA (trichloroacetic acid). After centrifugation for 10 min at 3000 rpm, the supernatant was used for further analyses.

Five hundred microliters of the supernatant were added to 1000 µL of 1 M potassium iodide (KI). The absorbance of the mixture was scanned at 390 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., UK). The content of H_2O_2 was determined from a standard curve prepared with known concentrations of H_2O_2 and expressed in fresh weight (µmol g^{-1} FW) (Velikova et al. 2000).

For the measurement of lipid peroxidation in pea leaves, the thiobarbituric acid (TBARS) test was used. The TBARS test determines malondialdehyde (MDA) content in pea leaves samples as the end product of lipid peroxidation. Five hundred microliters of the supernatant was added to 1000 µL 0.5% (w/v) thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The mixture was incubated in boiling water for 30 min. The reaction stopped after the samples have cooled.

Then, the samples were centrifuged at 10 000×*g* for 5 min, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (M501, Spectronic Camspec Ltd., UK). The value for non-specific absorbance at 600 nm was subtracted (Heath and Packer 1968). The amount of MDA–TBA complex (red pigment) in leaves was calculated:

$$C_{MDA} = (A532 - A600)/E_{MDA} \quad (1)$$

C_{MDA} concentration of MDA, μM
A532, A600 absorbance at wavelengths
E_{MDA} MDA extinction coefficient 155 mM^{-1} cm^{-1}

2.6 Determination of Macro- and Microelements

The macro- and microelements quantity in pea leaves, stem, and roots were determined using the microwave digestion technique combined with inductively coupled plasma optical emission spectrometry. Complete digestion of dry plant material (0.3 g) was achieved with 8 mL 65% HNO_3 using a microwave digestion system Multiwave GO (Anton Paar GmbH, Graz, Austria). The digestion program was as follows: (1) 170 °C reached within 3 min, digested for 10 min; (2) 180 °C reached within 10 min, digested for 10 min. After, full digestion samples were diluted to 50 mL with deionized water. The elemental profile was analyzed by an ICP–OES spectrometer (Spectro Genesis, SPECTRO Analytical Instruments, Kleve, Germany). The operating conditions employed for ICP-OES determination were 1300 W RF power, 12 L min^{-1} plasma flow, 1 L min^{-1} auxiliary flow, 0.8 L min^{-1} nebulizer flow, and 1 mL min^{-1} sample uptake rate. The analytical wavelengths chosen were P I 213.618 nm, K I 766.491 nm, S I 182.034 nm, Ca II 445.478 nm, Mg II 279.079 nm, Fe II 259.941 nm, Zn I 213.856 nm, Mn II 259.373 nm, and Cu I 324.754 nm. The calibration standards were prepared by diluting a stock multi-elemental standard solution (1000 mg L^{-1}) in 6.5% (v/v) nitric acid and by diluting stock phosphorus and standard sulfur solutions (1000 mg L^{-1}) in deionized water. The calibration curves for all the studied elements were in the range of 0.01–400 mg L^{-1}. The contents of macro- and microelements in the dry weight of pea are presented (Viršilė et al. 2020).

2.7 Statistical Analysis

All the values were expressed as mean ± standard deviation. Data were analyzed using the analysis of variance (ANOVA) test followed by Tukey HSD at $p \leq 0.05$ to identify significant differences between control peas that have not been affected by NPs and plants that were exposed to different NPs concentrations. Pearson's correlation coefficient was used to determine relationships between antioxidant activity assays. All statistical analyses were performed using XLSTAT (Xlstat, Addinsoft, Paris, France, 2020).

3 Results

3.1 The Effects of Nanoparticles on the Pea Growth Parameters

Plants subjected to CuO, SiO_2, B, Mo NPs at different concentrations showed distinct morphological responses (Table 2). Peas irrigated with 12.5 ppm CuO NPs suspension had the most positive effect on plant shoot height (40%), root length (104%), aboveground fresh (87%) and dry biomass (47%), root fresh (109%), and dry biomass (56%) compared to control plants (watered with water). Using the 100 ppm of Mo NPs, it had the greatest positive effect on pea shoot height (48%), root length (85%), aboveground fresh (83%), dry biomass (61%), root fresh (122%), and dry biomass (72%) compared to control plants. The suspension of B NPs had a similar effect to CuO NPs, as the 12.5 ppm concentration had the greatest positive effect on pea shoot height (57%), root length (92%), aboveground fresh (95%), dry biomass (116%), root fresh (104%), and dry biomass (41%) compared to control plants. Suspension of SiO_2 NPs had a comparable effect to Mo NPs, as concentrations of 100 and 50 ppm had the greatest positive effect on pea shoot height (44%), root length (75%), aboveground fresh (109%), dry biomass (135%), root fresh (119%), and dry biomass (136%) compared to control.

Chlorophyll and flavanol index showed statistically significant differences between plants irrigated with different NPs suspensions (Table 3). Both indices was most dependent on applied NPs concentration on pea plants; CuO at 12.5 ppm, Mo at 50ppm, B at 12.5ppm, and SiO_2 at 50ppm increased chlorophyll index by 85%, 76%, 90%, and 100% and flavanol index by 37%, 28%, 44%, and 45%, accordingly. CuO (12.5), Mo (50ppm), B (12.5ppm), and SiO_2 (50ppm) NPs had a statistically significant effect on the NBI, increasing it by 38%.

3.2 The Effects of NPs on the Antioxidative System in Pea Leaves

In pea plants, a significant effect on phenolic compounds was found under all applied NPs within the different concentrations, except for SiO_2 under 12.5 ppm (Fig. 1). The strongest statistically significant effects on DPPH radical scavenging activity (Fig. 2) were found using SiO_2 (100 ppm), CuO (12.5 ppm), Mo (50 ppm) NPs; it was increased by 35%, 12%, 34% respectively. However, B NPs did not have a statistically significant difference in DPPH radical scavenging activity in pea leaves. The greatest statistically significant impact on ABTS radical scavenging activity (Fig. 3) in pea

Table 2 Effects of NPs 100, 50, 25, 12.5 ppm suspension on pea shoot height, root length, fresh and dry shoot, and root biomass compared to control 0 ppm. Mean values within columns followed by different letters differ significantly at $p < 0.05$ (n=10) according to Tukey (HSD) test

NPs	Concentration, ppm	Shoot height, cm	Root length, cm	Aboveground fresh biomass, g	Aboveground dry biomass, g	Root fresh biomass, g	Root dry biomass, g
CuO	100	20.34 d	8.26 b	1.240 c	0.217 d	0.469 bc	0.037 c
	50	23.28 c	8.44 b	1.868 b	0.323 b	0.501 b	0.055 b
	25	26.34 b	9.32 a	2.353 a	0.361 a	0.668 a	0.058 b
	12.5	28.14 a	9.10 a	2.472 a	0.353 a	0.763 a	0.069 a
	0	20.02 d	4.46 c	1.322 c	0.240 c	0.365 c	0.044 c
Mo	100	29.64 a	8.26 a	2.429 a	0.388 a	0.809 a	0.076 a
	50	27.66 b	7.26 b	2.220 b	0.380 a	0.667 b	0.065 b
	25	24.70 c	7.42 b	1.525 d	0.304 b	0.577 b	0.058 b
	12.5	24.64 c	6.94 c	1.672 c	0.259 c	0.627 b	0.063 b
	0	20.02 d	4.46 d	1.322 e	0.240 c	0.365 c	0.044 c
B	100	19.34 e	5.46 c	1.364 c	0.270 d	0.341 d	0.043 b
	50	20.44 c	4.98 d	1.502 c	0.366 b	0.444 c	0.044 b
	25	24.30 b	7.26 b	2.112 b	0.323 c	0.598 b	0.053 ab
	12.5	30.34 a	8.58 a	2.578 a	0.518 a	0.696 a	0.061 a
	0	20.02 d	4.46 e	1.322 c	0.240 d	0.365 d	0.044 b
SiO_2	100	28.66 a	9.20 a	2.857 a	0.501 b	1.049 b	0.089 b
	50	28.84 a	9.20 a	2.769 a	0.564 a	1.166 a	0.105 a
	25	28.20 a	9.10 a	2.698 a	0.465 c	0.782 c	0.082 c
	12.5	25.70 b	7.84 b	2.396 b	0.388 d	0.624 d	0.073 d
	0	20.02 c	4.46 c	1.322 c	0.240 e	0.365 e	0.044 e

plants was found using SiO_2 (100 ppm), CuO (12.5 ppm), Mo (50 ppm), and B (50 ppm) NPs, which increased by 22%, 17%, 25%, and 19% respectively compared to control plants.

The highest and positive correlation between the content of phenolic compounds, DPPH and ABTS scavenging activity, was found in pea plants exposed to SiO_2 NPs suspension ($r_{(DPPH)} = 0.954$, $p < 0.001$; $r_{(ABTS)} = 0.964$, $p < 0.001$) (Table 4). The lowest but still positive correlation between the content of phenolic compounds, DPPH and ABTS scavenging activity, was found in pea plants exposed to B NPs suspension ($r_{(DPPH)} = 0.590$, $p < 0.05$; $r_{(ABTS)} = 0.435$ not significant).

3.3 The Effects of NPs on the Oxidation System in Pea Leaves

The results showed that the accumulation of H_2O_2 in the pea leaves was mostly significantly lower or comparable to the control plants, and these effects depended mainly on the applied NPs and concentration (Fig. 4). It is worth noting that the accumulation of H_2O_2 in pea leaves was significantly induced when plants were exposed to B NPs and CuO NPs at 100 ppm and SiO_2 NPs at 12.5 ppm.

The level of damage related to oxidative stress was determined by monitoring the differences in lipid peroxidation in terms of MDA formation. Figure 5 shows a significant difference in MDA concentration in the pea leaves of all treatments. The results indicate that the plant treated with 100 ppm B NPs had significantly higher MDA content compared to control plants, while the lowest MDA content was the 12.5 ppm and 25 ppm B NPs-treated plants. For Mo NPs results, 50 ppm and 100 ppm treatments to the plants had reduced MDA content compared to untreated plants. Comparing the effects of the four concentrations of CuO NPs suspension, it was obvious that the lowest applied concentration (12.5ppm) showed the lowest MDA content in pea leaves. All the treated plants with SiO_2 NPs show a lower MDA content when compared to the untreated plants.

3.4 NPs Effect on Macroelements (Phosphorus (P), Potassium (K), Calcium (Ca), magnesium (Mg)) and Microelements (Sulfur (S), Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Molybdenum (Mo), Boron (B)) of Peas Leave, Stem, and Root

SiO_2, CuO, Mo, and B NPs had the greatest statistically significant effect on pea leaf trace elements. They increased the amount of Ca and Mg in pea leaves compared to control plants. Moreover, the amount of Ca, K, Fe, Zn, and Mn was increased in pea stems. Furthermore, it was observed that the use of SiO_2 and CuO NPs

Table 3 Non-destructive measurement parameters in green peas leave, chlorophyll and flavanol index, nitrogen balance index (NBI). Different letters indicate statistically significant differences between means according to the Tukey (HSD) test at the confidence level $p = 0.05$ (n=10).

NPs	Concentration, ppm	Chlorophyll index	Flavanol index	NBI
CuO	100	22.842 c	1.030 a	23.116 b
	50	27.884 b	1.026 a	27.248 ab
	25	31.488 ab	1.089 a	29.098 ab
	12.5	34.224 a	1.053 a	32.500 a
	0	18.503 c	0.768 b	24.154 b
Mo	100	31.889 a	1.126 a	28.369 b
	50	32.584 a	0.983 b	33.217 a
	25	25.129 b	0.877 bc	28.715 b
	125	26.039 b	0.888 bc	29.274 b
	0	18.503 c	0.768 c	24.154 c
B	100	26.285 b	1.036 a	25.531 b
	50	24.726 b	0.820 b	30.177 a
	25	25.129 b	0.877 bc	28.715 b
	12.5	35.238 a	1.106 a	32.118 a
	0	18.503 c	0.768 b	24.154 b
SiO_2	100	34.369 ab	1.152 a	29.806 ab
	50	37.084 a	1.117 a	33.762 a
	25	34.258 ab	1.071 a	32.467 a
	12.5	33.369 bc	1.078 a	32.223 a
	0	18.503 c	0.768 b	24.154 b

increased the S content in the leaves and roots by 3 times. Mo NPs increased P, Ca, Mg, and Fe two times; besides, a strong accumulation of Mo content in pea roots was determined (Table 5). B NPs affected the amount of Ca, Fe, and Mn in pea roots by 36%, 38%, and 476%, respectively (Table 6). Exposure of peas to 100 ppm CuO NPs (Table 7) increased the copper content 14 times in the leaves, 4 times in the stem, and decreased 10 times in the roots. SiO_2 NPs affected the accumulation of macronutrients in pea leaves and stems more than in roots (Table 8).

4 Discussion

The physicochemical interaction of NPs with plants in terms of their energy and surface charge results in the modification of specific membrane surface proteins, transporters, and receptors (Juárez-Maldonado et al. 2019). Inside the leaves, there is a biological membrane with hydrophobic and hydrophilic components and unequal distribution of negative charges (lignin surface potential and cellulose fibers are −45 and −15 mV, respectively) (Mittal et al. 2020). NPs with a zeta potential between −10 and +10 mV are approximately neutral, and NPs with a zeta potential greater than +30 mV or less than −30 mV are considered strongly cationic and anionic, respectively, and stable due to particles and particle repulsion (Clogston and Patri 2011). According to International Standards Organizations (ISOs), polydispersity index values <0.05 are more common for monodisperse samples and values > 0.7 are common for a wide (e.g., polydisperse) particle distribution (ISO standards ISO 22,412:2017 and ISO 22,412:2017). Researchers reported that the zeta potential of CuO NPs is −34.4 ± 0.5 mV in suspension in deionized water at pH 7 when NPs were purchased at 10–100nm in primary size (Keller et al. 2018; Adeleye et al. 2014; Hong et al. 2014). In this research, the zeta potential of CuO NPs was found to be −26.68 ± 0.6 mV in suspension in deionized water when NPs were purchased at 25–55nm in primary size. The zeta potential of SiO_2 NPs was obtained to be −40 mV by other scientists (El-Naggar et al. 2020); meanwhile, the zeta potential of SiO_2 NPs was −20.64 ± 0.3 in our study. Unfortunately, there are no scientific articles with already performed measurements of zeta potential using Mo and B nanoparticles. The negative value showed to be an anionic type of suspensions in all studied systems.

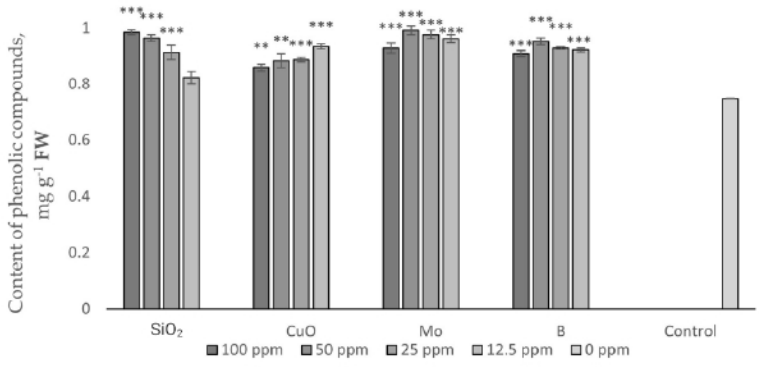


Fig. 1 Effects of SiO_2, CuO, Mo, B NPs on the content of phenolic compounds in pea leaves. Data are means ± SE of 3 replicates, $n = 3$, Tukey (HSD): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with control

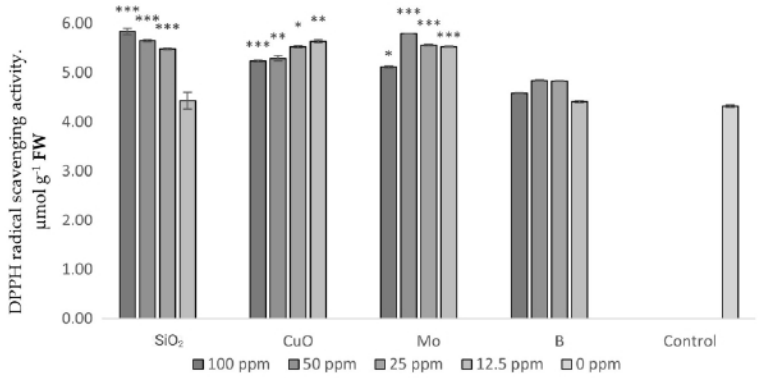


Fig. 2 Effects of SiO_2, CuO, Mo, B NPs on the DPPH radical scavenging activity. Data are means ± SE of 3 replicates, $n = 3$, Tukey (HSD): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with control

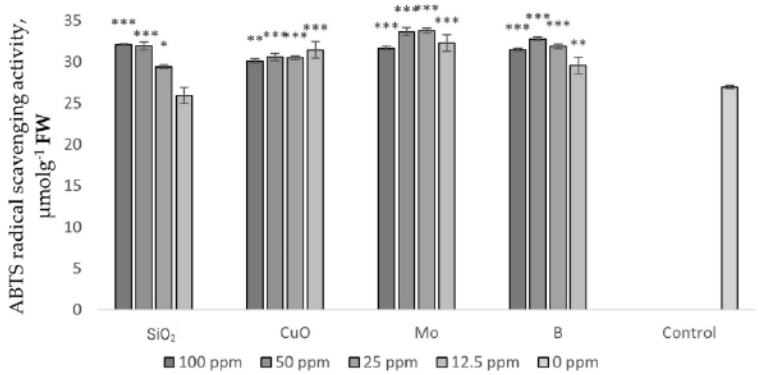


Fig. 3 Effects of SiO_2, CuO, Mo, B NPs on the ABTS radical scavenging activity. Data are means ± SE of 3 replicates, $n = 3$, Tukey (HSD): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with control

By participating in electron transfer, NPs induce various responses in plants, increase the activity of plant enzymes, promote the conversion of nitrates to ammonia, intensify plant respiration and photosynthesis processes, synthesize enzymes and amino acids, enhance carbon, and nitrogen nutrition, and thus directly affect plant mineral nutrition.

The effects of CuO NPs on plants are extensively studied compared to other NPs researched in this study. Comparing the results of other authors (Nair and Chung 2015), it is observed that the effect of CuO NPs on plants is similar; the higher the CuO concentration, the higher the toxicity determined in peas, starting at 100 ppm. Toxic effects were confirmed in soybean by elevated levels of lipid peroxidase and peroxide in plants at concentrations of 100 ppm CuO NPs and higher (Yusefi-Tanha et al. 2020). A reduction in soybean yield was also observed with the use of CuO NPs at concentrations of 50–100 ppm (Ochoa et al. 2017). Does the question, therefore, arise as to what effect could a lower concentration of CuO NPs have on plants? The experiment shows that using a concentration of 12.5 ppm can produce a positive response both in the antioxidant system and in reducing oxidative stress such as lipid peroxidation and peroxide content in peas. Researchers have found (Ogunkunle et al. 2018; Yusefi-Tanha et al. 2020) that using CuO NPs up to 25 nm has a stronger effect on lipid peroxidation and peroxide concentration than using 60–80 nm CuO NPs, so using smaller particles would be beneficial to reduce the peroxide concentration in pea leaves. By the way in agreement with the data of other authors (Ogunkunle et al. 2017, 2018), it can be seen from the macro-microelements that the higher the concentration of CuO NPs the more it accumulates in pea leaves and roots. Cu belongs to the micronutrients that are faced with the problem of poor bioavailability; though this element is essential for the vital function of plants, the use of Cu as an NPs size could have an appropriate solution. However, it should be mentioned that excess Cu can affect plants as heavy metal and can therefore be harmful to plants and increase the level of oxidative stress.

Mo is imported into plants by special molybdenum transporters. Mo is biologically inactive unless it is complexed by a specific prosthetic group (except bacterial nitrogenase). This Mo cofactor (Moco) after biosynthesis is distributed to

Table 4 Pearson's correlation coefficients (r) for the relationships between antioxidant assays and phenolic contents ($^{*}p$ <0.05; $^{**}p$<0.01; $^{***}p$<0.001)

Control	Phenols	ABTS	DPPH
Phenols	1		
ABTS	0.890**	1	
DPPH	0.970**	0.753**	1
CuO NPs	Phenols	ABTS	DPPH
Phenols	1		
ABTS	0.694*	1	
DPPH	0.793*	0.683*	1
B NPs	Phenols	ABTS	DPPH
Phenols	1		
ABTS	0.435	1	
DPPH	0.590*	0.754*	1
SiO_2NPs	Phenols	ABTS	DPPH
Phenols	1		
ABTS	0.964***	1	
DPPH	0.954***	0.927***	1
Mo NPs	Phenols	ABTS	DPPH
Phenols	1		
ABTS	0.727*	1	
DPPH	0.837**	0.635*	1

both Mo-enzyme families of Moco-binding proteins (sulfite oxidase (SO), nitrate reductase (NR), mitochondrial amidoxime reducing component (mARC)) and Mo-enzymes (aldehyde oxidase (AO), xanthine dehydrogenase (XDH)). The most important Mo-enzyme for plant survival is cytosolic NR, which catalyzes the first step in nitrate uptake (Kaufholdt et al. 2017; Mendel and Schwarz 2011). The conversion of nitrates to nitrites is essential for pea plant growth and development as shown by studies with Mo NPs (Taran et al. 2014). The results of our research showed a statistically significant increase in pea aboveground biomass, shoot height, and root height as compared to control plants. It is also important to note that higher antioxidant activity and lower levels of H_2O_2 and MDA in peas were found as plants were treated with 50 and 100 ppm Mo NPs. Similar changes were observed in another study [8] showing increased activity of the chickpea antioxidant system, particularly increased catalase activity at 8 ppm Mo NPs.

In our study, all mineral uptakes in pea plants were significantly affected by the Mo NPs treatments. In leaves, Mo NPs induced the accumulation of P, K, Ca, Mg, and in stems; Fe also contributes to these elements. In the roots, the largest accumulation was observed of all identified elements, as well as an exceptional effect was observed in the increase of Cu and Fe content compared to the effect of other used NPs. Also, the application of Mo NPs to pea plants induced the accumulation of Mo in pea leaves, stems, and roots.

One of the main functions of B in plants has been described as its ability to produce esters with rhamnogalacturonan II (RGII). The formation of this borate ester is necessary to perform cell wall functions and maintain structure (Ryden et al. 2003). Ninety percent of all B entering the plant is carried to the plant cell wall (Goldbach and Wimmer 2007). Plasma membranes of pea root nodules and uninfected pea root cells also contain RGII glycoproteins, but these proteins do not occur in B-deficient cells, suggesting that borate stabilizes them in peri bacteroid and plasma membranes (Bolaños et al. 2001). B is also required for the regulation of glycoproteins, which are necessary as indicators for the differentiation of bacteria into a nitrogen-fixing form (Bolaños et al. 2004). The results of this study showed that low concentrations of B NPs have a positive effect on pea plant growth parameters and increased efficiency of the antioxidant activity was observed. When higher concentrations of B NPs were used, an increase in MDA and H_2O_2 levels in pea leaves was observed. H_2O_2 plays an important role in plants, especially because photosynthesis provides an additional source compared to non-photosynthetic organisms. H_2O_2 is one of the signals of the state

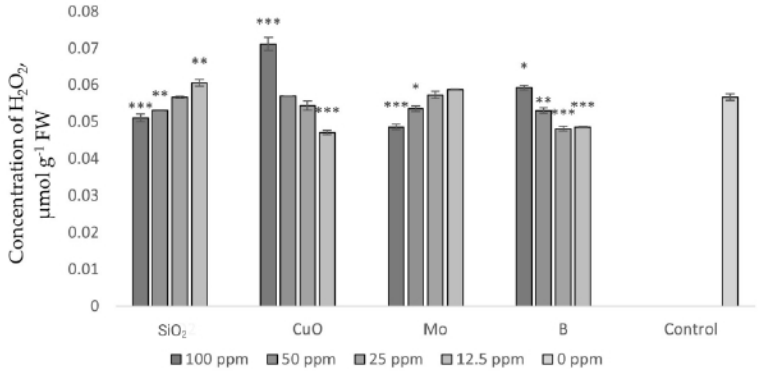


Fig. 4 Effects of SiO_2, CuO, Mo, B NPs on the peroxide amount. Data are means ± SE of 3 replicates, $n = 3$, Tukey (HSD): $^{*}p$ <0.05; $^{**}p$<0.01; $^{***}p$<0.001 compared with control

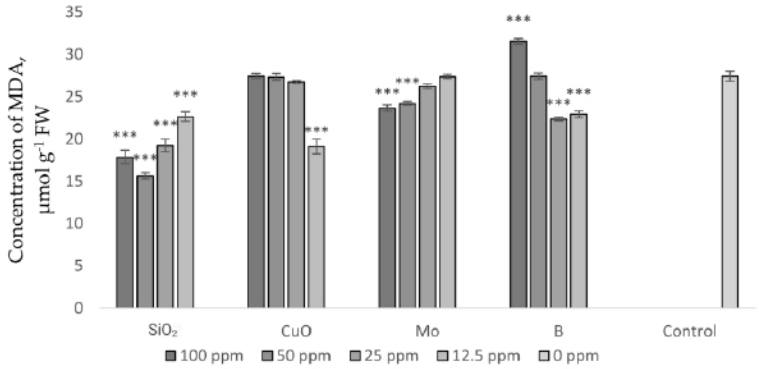


Fig. 5 Effects of SiO_2, CuO, Mo, B NPs on the MDA amount. Data are means ± SE of 3 replicates, $n = 3$, Tukey (HSD): $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with control

Table 5 Effects of Mo NPs suspension on macroelements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg)) and microelements (sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), boron (B)) of peas leave, stem, and root compared to control 0 ppm. Mean values within columns followed by different letters differ significantly at $p < 0.05$ according to Tukey (HSD) test (n=3)

Treatment Mo NPs	Macroelements, mg g^{-1} DW				Microelements, µg g−1 DW						
Leaves	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	3.897 a	15.953 a	40.871 a	10.761 a	0.736 b	104.827 a	19.366 a	48.636 b	8.803 a	26.408 a	6.382 a
50 ppm	2.952 b	11.001 b	23.268 b	6.629 b	0.443 c	89.566 c	10.983 b	37.068 c	5.107 b	8.512 b	1.098 c
25 ppm	1.545 c	6.166 c	17.426 c	4.751 d	0.298 d	41.099 d	3.214 e	20.999 d	2.786 bc	5.033 c	0.429 d
12.5 ppm	1.308 d	4.054 d	12.636 d	3.113 e	0.253 e	31.394 e	3.552 d	8.353 e	3.936 b	4.512 c	1.440 c
0 ppm	1.607 c	11.333 b	10.138 e	5.545 c	4.515 a	94.868 b	7.905 c	97.233 a	1.186 e	1.858 d	4.534 b
Stem	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	2.523 a	9.108 b	20.743 a	5.808 a	0.326 b	80.251 a	9.049 c	29.635 a	6.391 ab	28.077 a	4.298 a
50 ppm	1.629 b	9.472 a	18.125 b	5.621 a	0.314 b	64.176 b	11.375 a	16.583 c	6.286 ab	11.840 b	3.772 a
25 ppm	1.620 b	7.451 d	12.770 c	4.319 b	0.260 d	58.838 b	9.545 b	14.800 d	7.611 a	10.573 c	0.967 c
12.5 ppm	1.359 c	6.198 e	20.869 a	5.787 a	0.282 c	51.886 bc	1.526 d	24.907 b	4.524 b	8.596 d	0.491 c
0 ppm	1.069 d	7.725 c	12.787 c	4.220 b	0.970 a	39.531 c	1.019 e	11.931 e	2.333 c	2.694 e	1.547 b
Roots	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	2.020 a	19.477 a	20.643 b	7.331 a	2.378 b	7217.571 a	31.399 a	180.463 a	34.253 a	22.766 c	2.253 c
50 ppm	1.911 b	16.868 b	23.189 a	7.233 a	2.372 b	4894.413 b	26.005 b	129.406 b	26.623 b	17.263 c	5.250 c
25 ppm	1.641 d	8.873 d	16.873 c	5.117 c	2.321 c	7061.15 a	6.371 d	20.979 c	8.081 c	158.103 a	24.263 a
12.5 ppm	1.941 b	11.773 c	18.975 b	5.876 b	2.336 c	7093.522 a	15.216 c	26.428 c	10.322 c	110.892 b	17.849 b
0 ppm	1.859 c	15.999 b	9.293 d	5.788 b	2.607 a	2975.111 b	7.037 d	121.188 b	21.241 b	19.807 c	7.688 c

of photosynthesis and stomatal movements (Smirnoff and Arnaud 2019). Therefore, chlorophyll and flavanol indices were not adversely affected even at 100 ppm B NPs concentration, but shoot height and root length of pea were lower than control plants. The suspension of B NPs was observed to have a positive effect on Ca and Mg in pea leaves, K, Ca, and B in stems, Ca, Mg, S, Fe, Mn, and B in roots.

SiO_2 NPs have a positive effect on plant growth; increase their biomass, anatomy, and physiology; modify tissue differentiation; activate defense systems, and help to adapt to stressful conditions (Luyckx et al. 2017). The effects of SiO_2 NPs are widely studied in different plants; but to our knowledge, there is only one research on the effects of these NPs on lentils and soybeans from the legume plant family. It has been reported that the nano-sized mixture of SiO_2 and TiO_2 increased the nitrate reductase activity of soybean (*Glycine max*), enhanced the absorption and utilization of water and fertilizers, stimulated the antioxidant system, and accelerated germination and growth (Lu et al. 2002). The concentration of 60.08 ppm SiO_2 NPs significantly mitigated the negative effects of salt stress on germination, shoot and root length, seedling weight, mean germination time in lentil seedlings, and seedling vigor indices (Janmohammadi et al. 2015), promoting the antioxidant system of soybeans,

Table 6 Effects of B NPs suspension on macroelements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg)) and microelements (sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), boron (B)) of peas leave, stem, and root compared to control 0 ppm. Mean values within columns followed by different letters differ significantly at $p < 0.05$ according to Tukey (HSD) test (n=3)

Treatment B NPs	Macroelements, mg g^{-1} DW				Microelements, µg g−1 DW					
Leaves	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	3.073 a	10.098 d	23.255 d	5.158 e	0.783 a	70.943 d	5.331 c	29.026 d	1.256 c	2.001 b	1.929 d
50 ppm	2.732 b	10.918 c	25.218 a	6.535 c	0.545 d	79.723 c	2.147 d	41.959 b	4.148 a	2.537 a	11.421 a
25 ppm	2.553 c	10.901 c	25.190 a	6.764 b	0.699 c	87.903 b	9.525 a	30.220 c	1.305 c	2.563 a	4.297 c
12.5 ppm	3.035 a	11.756 a	24.768 b	7.088 a	0.742 b	93.364 b	5.191 c	28.339 d	3.157 b	2.525 a	1.797 d
0 ppm	1.607 d	11.333 b	10.138 c	5.545 d	0.515 d	94.868 a	7.905 b	97.233 a	1.186 c	1.858 b	4.534 c
Stem	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.003 e	7.731 b	12.651 e	4.195 d	0.305 d	39.956 c	1.006 d	10.431 d	1.208 d	2.385 b	19. 759 a
50 ppm	1.260 c	7.719 b	14.279 c	4.462 c	0.457 c	50.103 b	6.808 a	12.685 b	1.175 d	2.498 ab	1.574 d
25 ppm	1.408 b	8.166 a	14.744 b	4.984 b	0.311 d	67.360 a	1.949 c	17.869 a	6.606 a	2.166 b	6.943 c
12.5 ppm	1.441 a	7.396 c	17.380 a	5.768 a	0.502 b	67.335 a	2.149 b	12.655 b	4.298 b	2.229 b	9.405 d
0 ppm	1.069 d	7.725 b	12.787 d	4.220 d	0.970 a	39.531 c	1.019 d	11.931 c	2.333 c	2.694 a	1.547 c
Roots	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.943 b	15.587 c	5.255 e	6.170 c	1.478 b	4221.563 b	6.874 a	117.043 ab	8.098 b	9.416 b	18.411 b
50 ppm	2.533 a	16.794 a	8.542 c	6.442 b	1.376 c	3357.684 d	2.165 b	114.313 b	14.475 ab	9.064 b	13.741 c
25 ppm	1.892 b	16.174 b	5.902 d	6.782 a	1.471 b	3780.957 c	7.363 a	103.916 c	11.044 b	9.538 b	10.404 d
12.5 ppm	2.503 a	14.690 d	11.109 a	5.133 e	1.124 d	4816.125 a	2.549 b	121.256 a	8.764 b	9.560 b	36.078 a
0 ppm	1.859 b	15.999 b	9.293 b	5.788 d	2.607 a	2975.111 e	7.037 a	121.188 a	21.241 a	19.807 a	7.688 e

Table 7 Effects of CuO NPs suspension on macroelements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg)) and microelements (sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), boron (B)) of peas leave, stem, and root compared to control 0 ppm. Mean values within columns followed by different letters differ significantly at $p < 0.05$ according to Tukey (HSD) test ($n=3$)

Treatment CuO NPs	Macroelements, mg g^{-1} DW				Microelements, µg g−1 DW						
Leaves	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.774 d	10.138 c	22.741 a	7.462 a	6.021 a	55.703 d	0.996 c	25.445 e	13.993 a	1.051 b	0.608 e
50 ppm	1.994 b	10.441 b	15.697 c	5.452 e	4.546 c	45.781 e	0.352 d	30.731 c	7.243 b	0.914 bc	1.899 b
25 ppm	1.879 c	11.467 a	21.135 b	6.691 c	5.007 b	70.072 c	4.700 b	28.393 d	3.933 c	0.624 c	1.007 d
12.5 ppm	2.125 a	11.220 a	22.608 a	6.858 b	4.358 c	90.982 b	4.249 b	32.558 b	4.355 c	0.637 c	1.381 c
0 ppm	1.607 e	11.333 a	10.138 d	5.545 d	4.515 c	94.868 a	7.905 a	97.233 a	1.186 d	1.858 a	4.534 a
Stem	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.262 a	9.863 a	15.337 a	5.449 b	2.503 b	42.700 bc	2.842 a	11.693 c	5.233 d	0.517 b	1.421 a
50 ppm	1.215 b	6.613 d	10.970 d	4.058 e	3.007 a	52.464 b	1.064 c	10.493 d	6.995 c	0.836 b	0.228 d
25 ppm	0.965 e	7.812 c	13.298 b	4.732 c	2.527 b	65.627 a	2.555 b	10.307 e	8.721 a	0.617 b	1.145 b
12.5 ppm	1.088 c	8.998 b	15.580 a	6.165 a	2.984 a	53.511 b	3.024 a	13.105 a	8.317 b	0.588 b	0.924 c
0 ppm	1.069 d	7.725 c	12.787 c	4.220 d	0.970 c	39.531 c	1.019 c	11.931 b	2.333e	2.694 a	1.547 a
Roots	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.940 b	20.067 b	9.035 b	8.120 a	9.116 a	1948.500 e	7.357 a	51.500 e	2.405 b	1.141 c	4.939 d
50 ppm	1.976 a	15.083 d	5.424 e	5.960 d	6.210 d	3405.684 a	1.145 c	82.136 c	1.312 c	1.249 b	7.846 b
25 ppm	1.805 d	21.459 a	6.475 d	7.412 b	7.860 b	3172.238 b	0.969 c	78.832 d	1.242 c	1.332 b	8.851 a
12.5 ppm	1.810 d	19.829 b	7.057 c	6.608 c	6.721 c	2792.113 d	3.765 b	87.297 b	1.305 c	1.685 b	6.191 c
0 ppm	1.859 c	15.999 c	9.293 a	5.788 e	2.607 e	2975.111 c	7.037 a	121.188 a	21.241 a	19.807 a	7.688 b

Table 8 Effects of SiO_2 NPs suspension on macroelements (phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg)) and microelements (sulfur (S), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), molybdenum (Mo), boron (B)) of peas leave, stem, and root compared to control 0 ppm. Mean values within columns followed by different letters differ significantly at $p < 0.05$ according to Tukey (HSD) test (n=3)

Treatment SiO_2 NPs	Macroelements, mg g^{-1} DW				Microelements, µg g−1 DW					
Leaves	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	3.239 b	12.226 a	22.537 b	6.286 b	1.373 d	77.914 c	5.290 c	34.783 b	3.954 b	1.684 b	9.373 a
50 ppm	3.335 a	9.651 e	23.122 a	6.494 a	1.662 b	73.659 d	3.870 e	32.383 c	4.549 a	1.561 b	9.492 a
25 ppm	2.496 c	10.648 c	20.695 c	6.369 ab	1.563 c	91.719 b	9.402 a	29.202 d	1.537 c	1.673 b	7.685 b
12.5 ppm	2.296 d	10.185 d	19.653 d	6.081 c	1.429 d	66.054 e	4.874 d	32.058 c	4.062 b	1.312 c	9.624 a
0 ppm	1.607 e	11.333 b	10.138 e	5.545 d	4.515 a	94.868 a	7.905 b	97.233 a	1.186 a	1.858 a	4.534 c
Stem	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.567 a	9.026 b	15.896 a	4.847 a	0.854 d	50.378 b	10.746 a	13.778 c	2.234 b	1.543 b	8.033 b
50 ppm	1.354 d	7.470 e	14.747 b	4.841 a	0.896 c	55.198 b	8.629 b	15.063 a	2.451	1.328 bc	4.545 c
25 ppm	1.434 c	9.397 a	12.391 e	4.504 b	0.885 c	95.673 a	5.617 d	11.892 d	3.4066 a	1.195 c	10.577 a
12.5 ppm	1.491 b	7.973 c	14.496 c	4.486 b	1.042 a	50.335 b	8.466 c	14.356 b	1.235 c	1.481 bc	1.552 d
0 ppm	1.069 e	7.725 d	12.787 d	4.220 c	0.970 b	39.531 c	1.019 e	11.931 d	2.333 b	2.694 a	1.547 d
Roots	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu	Mo	B
100 ppm	1.717 d	15.169 c	6.919 b	6.154 b	2.696 a	3581.036 c	6.637 c	91.028 c	1.532 b	3.355 b	1.167 c
50 ppm	1.871 b	14.771 d	6.100 c	6.653 a	2.316 d	4147.903 a	8.246 a	87.196 d	1.818 b	3.636 b	7.467 a
25 ppm	1.904 a	16.522 a	5.469 d	5.967 c	2.331 d	3037.381 d	3.556 e	115.883 b	1.185 b	2.462 c	2.188 b
12.5 ppm	1.810 c	16.583 a	6.165 c	6.068 bc	2.415 c	4060.693 b	4.762 d	84.242 e	0.519 c	3.550 b	0.087 d
0 ppm	1.859 b	15.999 b	9.293 a	5.788 d	2.607 b	2975.111 d	7.037 b	121.188 a	21.241 a	19.807 a	7.688 a

including the activity of SOD, POD, and CAT enzymes (Shen et al. 2010). However, previous studies suggest that the higher SiO_2 NPs concentrations should be used with caution as the inhibitory effect of these NPs (120.16 ppm) was found for lentils germination (Janmohammadi et al. 2015). Our study indicates that treatments with SiO_2 at higher concentrations (50 and 100 ppm) increased antioxidant activity and reduced H_2O_2 content and MDA. In addition, the height of pea shoots, root length, and biomass accumulation was also increased as well as chlorophyll and flavanol indices. There was observed a particular effect on the accumulation of macroelements in pea plants. The content of P, K, Ca, and Mg in pea leaves and stems increased.

Despite the potential benefits mentioned above, the use of nanotechnologies in the agricultural sector is relatively limited and has not yet entered the market compared to other industries. Given the possible practical application of NPs in the agricultural sector, a preliminary cost-benefit analysis should be considered before placing NP-related products on the market, with a proper risk assessment. More extensive research is planned to conduct with greenhouse-grown peas to harvest maturity using CuO, Mo, B, and SiO_2 NPs, and it would be valuable to delve into abiotic stress reduction using various NPs.

5 Conclusions

Nanotechnology is a promising area in agronomy to reduce fertilizer levels. The use of nano-sized particles as fertilizer needs to be thoroughly analyzed, as their effects may depend not only on concentration, surface area, and size but also on the species of plant. In this experiment, studies of pea plants exposed to nanoparticles of silica, copper, molybdenum, and boron revealed that both positive and negative effects could occur. According to biometric indices, non-destructive measurements of leaf chlorophyll and nitrogen balance, chemical antioxidant efficiency analyses, and oxidative stress biomarkers, the most effective and statistically reliable effects were obtained using 100, 50 ppm concentrations of silica and molybdenum nanoparticles as well as using 12.5 ppm concentration of copper oxide and boron nanoparticles. Besides this study, it showed that higher concentrations of copper oxide and boron nanoparticles can induce oxidative stress and be toxic to pea plants.

Acknowledgments R.S. is grateful to the Lithuanian Research Centre for Agriculture and Forestry for the conditions provided for working with nanoparticles and for donated field pea "Respect" seeds. The authors are also appreciative to Joana Bendoraitienė from the Kaunas University of Technology for measuring the size and zeta potential of nanoparticle suspension.

CRediT authorship contribution statement Ruta Sutulienė: conceptualization, methodology, investigation, formal analysis, writing—original draft, visualization. Lina Ragelienė: methodology, resources, writing—review and editing. Pavelas Duchovskis: conceptualization, methodology, resources. Jurga Miliauskienė: conceptualization, methodology, resources, writing—review and editing, supervision.

Declarations

Competing Interest The authors declare no competing interests.

References

Adeleye AS, Conway JR, Perez T, Rutten P, Keller AA (2014) Influence of extracellular polymeric substances on the long-term fate, dissolution, and speciation of copper-based nanoparticles. Environ Sci Technol 48:12561–12568. https://doi.org/10.1021/es5033426

Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2:875–877. https://doi.org/10.1038/nprot.2007.102

Bolaños L, Cebrián A, Redondo-Nieto M, Rivilla R, Bonilla I (2001) Lectin-like glycoprotein PsNLEC-1 is not correctly glycosylated and targeted in boron-deficient pea nodules. MPMI 14:663–670. https://doi.org/10.1094/MPMI.2001.14.5.663

Bolaños L, Redondo-Nieto M, Rivilla R, Brewin NJ, Bonilla I (2004) Cell surface interactions of *Rhizobium* bacteroids and other bacterial strains with symbiosomal and peribacteroid membrane components from pea nodules. MPMI 17:216–223. https://doi.org/10.1094/MPMI.2004.17.2.216

Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J, Hu H, Pfeffer H, Dannel F, Römheld V (2002) Boron in plant biology. Plant Biol 4:205–223. https://doi.org/10.1055/s-2002-25740

Clogston JD, Patri AK (2011) Zeta potential measurement. In: McNeil SE (ed) Characterization of nanoparticles intended for drug delivery. Humana Press, Totowa, NJ, pp 63–70

Dimkpa CO, Bindraban PS, Fugice J, Agyin-Birikorang S, Singh U, Hellums D (2017) Composite micronutrient nanoparticles and salts decrease drought stress in soybean. Agron Sustain Dev 37:5. https://doi.org/10.1007/s13593-016-0412-8

Dimkpa CO, Singh U, Bindraban PS, Adisa IO, Elmer WH, Gardea-Torresdey JL, White JC (2019) Addition-omission of zinc, copper, and boron nano and bulk oxide particles demonstrate element and size -specific response of soybean to micronutrients exposure. Sci Total Environ 665:606–616. https://doi.org/10.1016/j.scitotenv.2019.02.142

El-Naggar ME, Abdelsalam NR, Fouda MMG, Mackled MI, Al-Jaddadi MAM, Ali HM, Siddiqui MH, Kandil EE (2020) Soil application of nano silica on maize yield and Its insecticidal activity against some stored insects after the post-harvest. Nanomaterials 10:739. https://doi.org/10.3390/nano10040739

Goldbach HE, Wimmer MA (2007) Boron in plants and animals: is there a role beyond cell-wall structure? J Plant Nutr Soil Sci 170:39–48. https://doi.org/10.1002/jpln.200625161

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hong J, Rico CM, Zhao L, Adeleye AS, Keller AA, Peralta-Videa JR, Gardea-Torresdey JL (2014) Toxic effects of copper-based nanoparticles or compounds to lettuce (*Lactuca sativa*) and alfalfa (*Medicago sativa*). Environ Sci Process Impacts 17:177–185. https://doi.org/10.1039/C4EM00551A

Janmohammadi M, Sabaghnia N, Ahadnezhad A (2015) Impact of silicon dioxide nanoparticles on seedling early growth of lentil (*Lens culinaris* medik.) genotypes with various origins. Agric For 61 https://doi.org/10.17707/AgricultForest.61.3.02

Juárez-Maldonado A, Ortega-Ortíz H, Morales-Díaz AB, González-Morales S, Morelos-Moreno Á, Cabrera-De la Fuente M, Sandoval-Rangel A, Cadenas-Pliego G, Benavides-Mendoza A (2019) Nanoparticles and nanomaterials as plant biostimulants. IJMS 20:162. https://doi.org/10.3390/ijms20010162

Kaufholdt D, Baillie C-K, Meinen R, Mendel RR, Hänsch R (2017) The molybdenum cofactor biosynthesis network: in vivo protein-protein interactions of an actin associated multi-protein complex. Front Plant Sci 8:1946. https://doi.org/10.3389/fpls.2017.01946

Keller AA, Huang Y, Nelson J (2018) Detection of nanoparticles in edible plant tissues exposed to nano-copper using single-particle ICP-MS. J Nanopart Res 20:101. https://doi.org/10.1007/s11051-018-4192-8

Lambreva MD, Lavecchia T, Tyystjärvi E, Antal TK, Orlanducci S, Margonelli A, Rea G (2015) Potential of carbon nanotubes in algal biotechnology. Photosynth Res 125:451–471. https://doi.org/10.1007/s11120-015-0168-z

Lehotai N, Pető A, Bajkán S, Erdei L, Tari I, Kolbert Z (2011) In vivo and in situ visualization of early physiological events induced by heavy metals in pea root meristem. Acta Physiol Plant 33:2199–2207. https://doi.org/10.1007/s11738-011-0759-z

Lin D, Xing B (2007) Phytotoxicity of nanoparticles: Inhibition of seed germination and root growth. Environ Pollut 150:243–250. https://doi.org/10.1016/j.envpol.2007.01.016

Lu C, Zhang C, Wen J, Wu G, Tao M (2002) Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanis. In: Soybean Science

Luyckx M, Hausman J-F, Lutts S, Guerriero G (2017) Silicon and plants: current knowledge and technological perspectives. Front Plant Sci 8https://doi.org/10.3389/fpls.2017.00411

Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Sci Total Environ 408:3053–3061. https://doi.org/10.1016/j.scitotenv.2010.03.031

Meier U (1997) Biologische bundesanstalt für land- und forstwirtschaft in Berlin und Braunschweig (eds) Growth stages of mono- and dicotyledonous plants: BBCH-Monograph = Entwicklungsstadien mono- und dikotyler Pflanzen. Blackwell-Wiss.-Verl, Berlin Wien

Mendel RR, Schwarz G (2011) Molybdenum cofactor biosynthesis in plants and humans. Coord Chem Rev 255:1145–1158. https://doi.org/10.1016/j.ccr.2011.01.054

Mittal D, Kaur G, Singh P, Yadav K, Ali SA (2020) Nanoparticle-based sustainable agriculture and food science: recent advances and future outlook. Front Nanotechnol 2:579954. https://doi.org/10.3389/fnano.2020.579954

Nair PMG, Chung IM (2015) The responses of germinating seedlings of green peas to copper oxide nanoparticles. Biol Plant 59:591–595. https://doi.org/10.1007/s10535-015-0494-1

Ochoa L, Medina-Velo IA, Barrios AC, Bonilla-Bird NJ, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2017) Modulation of CuO nanoparticles toxicity to green pea (*Pisum sativum, Fabaceae*) by the phytohormone indole-3-acetic acid. Sci Total Environ 598:513–524. https://doi.org/10.1016/j.scitotenv.2017.04.063

Ogunkunle CO, Folarin O, Olorunmaiye ES, Fatoba PO (2017) Bioaccumulation of copper and tissue partitioning of selected mineral nutrients in German round pea (Kleine Rheinlanderin) under the influence copper nanoparticles exposure. Ann Food Sci Technol 18:653–660

Ogunkunle CO, Jimoh MA, Asogwa NT, Viswanathan K, Vishwakarma V, Fatoba PO (2018) Effects of manufactured nano-copper on copper uptake, bioaccumulation and enzyme activities in cowpea grown on soil substrate. Ecotoxicol Environ Saf 155:86–93. https://doi.org/10.1016/j.ecoenv.2018.02.070

Prasad R, Bhattacharyya A, Nguyen QD (2017) Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Front Microbiol 8:1014. https://doi.org/10.3389/fmicb.2017.01014

Rastogi A, Tripathi DK, Yadav S, Chauhan DK, Živčák M, Ghorbanpour M, El-Sheery NI, Brestic M (2019) Application of silicon nanoparticles in agriculture. 3 Biotech 9:90. https://doi.org/10.1007/s13205-019-1626-7

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26:1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Ryden P, Sugimoto-Shirasu K, Smith AC, Findlay K, Reiter W-D, McCann MC (2003) Tensile properties of arabidopsis cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-Borate Complexes. Plant Physiol 132:1033–1040. https://doi.org/10.1104/pp.103.021873

Sharma OP, Bhat TK (2009) DPPH antioxidant assay revisited. Food Chem 113:1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Shen X, Zhou Y, Duan L, Li Z, Eneji AE, Li J (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. J Plant Physiol 167:1248–1252. https://doi.org/10.1016/j.jplph.2010.04.011

Smirnoff N, Arnaud D (2019) Hydrogen peroxide metabolism and functions in plants. New Phytol 221:1197–1214. https://doi.org/10.1111/nph.15488

Taran NY, Gonchar OM, Lopatko KG, Batsmanova LM, Patyka MV, Volkogon MV (2014) The effect of colloidal solution of molybdenum nanoparticles on the microbial composition in rhizosphere of *Cicer arietinum* L. Nanoscale Res Lett 9:289. https://doi.org/10.1186/1556-276X-9-289

Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK (2015) Silicon nanoparticles (SiNp) alleviate chromium (VI) phytotoxicity in *Pisum sativum* (L.) seedlings. Plant Physiol Biochem 96:189–198. https://doi.org/10.1016/j.plaphy.2015.07.026

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Viršilė A, Miliauskienė J, Haimi PJ, Laužikė K, Samuolienė G (2020) The comparison of constant and dynamic red and blue light irradiation effects on red and green leaf lettuce. Agronomy 10:1802. https://doi.org/10.3390/agronomy10111802

Wrigley CW, Corke H, Seetharaman K, Faubion J (2015) Encyclopedia of food grains. Elsevier Science, San Diego

Yusefi-Tanha E, Fallah S, Rostamnejadi A, Pokhrel LR (2020) Particle size and concentration dependent toxicity of copper oxide nanoparticles (CuONPs) on seed yield and antioxidant defense system in soil grown soybean (Glycine max cv. Kowsar). Sci Total Environ 715:136994. https://doi.org/10.1016/j.scitotenv.2020.136994

SANTRAUKA

ĮVADAS

Žemės ūkio pasėliams vis didesnį neigiamą poveikį daro smarkios liūtys, sausros, ekstremalios temperatūros, stiprūs vėjai ir naujų tipų patogenų plitimas, atsirandantis dėl besikeičiančio klimato (Shahzad ir kt., 2021; Rivero ir kt., 2022). Sausros stresas yra vienas labiausiai paplitusių aplinkos veiksnių, ribojančių pasėlių produktyvumą (Basu ir kt., 2016; Liliane ir kt., 2020). Agrometeorologinės sausros atsiranda sumažėjus kritulių kiekiui, o tai nulemia dirvožemio išdžiūvimą. Tokios sausros poveikis augalams priklauso nuo jos trukmės, dirvožemio tipo ir savybių (Seleiman ir kt., 2021). Sausros stresas neigiamai paveikia fotosintezę, nes augalai mažindami vandens praradimą per transpiraciją, uždaro žioteles. Tai sulėtina fotosintezės intensyvumą, sumažėja tarpląstelinio CO_2 kiekis ir maistinių medžiagų pasisavinimas, dėl ko augaluose vyksta morfologiniai ir biocheminiai pakitimai (Khan ir kt., 2018; Gambetta ir kt., 2020; Kapoor ir kt. , 2020; Ozturk ir kt., 2021). Vienas iš pagrindinių augalų atsakų ir redokso balanso sutrikimo ląstelėje sukėlėjų yra suintensyvėjusi aktyvių deguonies junginių (ADJ) gamyba. ADJ sukelia oksidacinį stresą augalų ląstelėse (Yang ir kt., 2021). Augaluose išsivysčiusi antioksidacinė sistema padeda jiems prisitaikyti ir išgyventi nepalankiomis aplinkos sąlygomis (Basu ir kt., 2016; Kapoor ir kt., 2020), o oksidacinių ir redukcinių reakcijų balansas padeda išlaikyti redokso homeostazę juose. Dėl to augaluose susiformuoja fermentinė ir nefermentinė antioksidantų sistema. Siekiant sumažinti žalingą neigiamų aplinkos veiksnių poveikį augalams, gali būti naudojamos išorinės priemonės, tokios kaip įvairių nanodalelių (ND) panaudojimas, tikslingai sustiprinant antioksidacinės sistemos veiklą (Kandhol at el., 2022; Ghani et al., 2022; Ahmad ir kt., 2022). Pastaraisiais dešimtmečiais buvo diskutuojama apie ND taikymą, siekiant pagerinti įvairių pasėlių atsparumą nepalankiems aplinkos veiksniams ir augalų mineralinės mitybos optimizavimą, tačiau iki šiol vis dar trūksta pagrįstų mokslinių žinių apie ND naudą augalų augimui ir derliui. Be to, vis dar nėra pilnai ištirtas įvairių ND poveikis aplinkai, augalų oksidacinio streso biožymenims ir antioksidacinės sistemos aktyvumui.

Nanodalelės – medžiagos, kurių dydis iki 100 nm, pasižyminčios unikaliomis formomis, paviršiaus krūviu ir dideliu paviršiaus plotu (Modena ir kt., 2019; Raval ir kt., 2019), o šios savybės nulemia jų poveikį aplinkai. Tiriant ND poveikį augalams, būtina ištirti ND suspensijų savybes, įvertinti skirtingus pritaikymo būdus, tokius kaip augalų laistymą ar purškimą (Tarafdar ir kt., 2012; Mittal ir kt., 2020). Ypač svarbu parinkti optimalią naudojamų ND koncentraciją, kuri kiekvienai augalų rūšiai gali skirtis ir sukelti priešingą efektą, nei tikimasi.

Sėjamasis žirnis (*Pisum sativum* L.) – vienas populiariausių ankštinių šeimos augalų, itin jautrus drėgmės trūkumui (Nadeem ir kt., 2019). Nano- dydžio mikroelementų molibdeno (MoO_3), boro (B_2O_3), vario (CuO) ir silicio (SiO_2) poveikio sėjamųjų žirnių tyrimams buvo parinkti atsižvelgiant į jų svarbą augalų fiziologijai. MoO_3 ir B_2O_3 yra ypač svarbūs žirniams būdingoms gumbelių bakterijoms ir jų diferenciacijai į azotą fiksuojančią formą. SiO_2 yra svarbus reguliuojant oksidacinį stresą ir aktyvuojant antioksidacinę sistemą, o CuO pasižymi kaip elementas naudojamas prieš patogenus. Atsižvelgiant į jų naudą žirniams, yra būtina ieškoti naujų, efektyvesnių šių mikroelementų pritaikymo būdų, todėl šiame moksliniame darbe pirmą kartą ištirtas nano- dydžio molibdeno, boro, silicio ir vario poveikis žirnių augalams. Šis darbas pasižymi tiek moksline, tiek praktine reikšme, nes tokių inovatyvių technologijų pritaikymas agronomijoje gali padėti išsaugoti derlių esant nepalankioms aplinkos sąlygoms, apsaugoti aplinką nuo sunkiųjų metalų susidarymo dirvoje, sumažinant naudojamų trąšų normas.

Hipotezė. Tikėtina, kad nanodalelėmis stiprinama fermentinė ir nefermentinė antioksidacinė sistema neutralizuoja žalingo oksidacinio streso poveikį sausros veikiamuose žirnių augaluose.

Tyrimų tikslas. Parinkti tinkamiausią SiO_2, MoO_3, B_2O_3, CuO nanodalelių koncentraciją ir taikymo būdą sėjamiesiems žirniams (*Pisum sativum* L.) sustiprinant jų atsparumą sausrai ir kompleksiniam sunkiojo metalo vario ir sausros poveikiui.

Tyrimų uždaviniai:

1. Nustatyti efektyviausias SiO_2, MoO_3, B_2O_3, CuO nanodalelių koncentracijas žirnių morfologiniams parametrams, poveikį oksidaciniam stresui ir antioksidacinei sistemai.
2. Ištirti agrometeorologinės sausros ir SiO_2, MoO_3, B_2O_3, CuO nanodalelių poveikį žirnių oksidaciniam stresui ir antioksidacinei sistemai.
3. Įvertinti kompleksinį aplinkos veiksnių (agrometeorologinės sausros, sunkiojo metalo Cu ir SiO_2, MoO_3, B_2O_3, CuO nanodalelių) poveikį žirnių oksidaciniam stresui ir antioksidacinei sistemai.
4. Įvertinti ND skirtingų panaudojimo būdų efektyvumą, stiprinant žirnių antioksidacinės sistemos veiklą ir atsparumą nepalankiems aplinkos veiksniams.

Disertacijos ginamieji teiginiai:

1. Pritaikius optimalias SiO_2, MoO_3, B_2O_3, CuO nanodalelių suspensijų koncentracijas sėjamiesiems žirniams, augantiems normalaus drėgnio substrate, galima suaktyvinti

antioksidacinės ir oksidacinės sistemos aktyvumą, kuri teigiamai veikia morfologinius parametrus ir padidina augalų produktyvumą.

2. Sėjamųjų žirnių atsparumą agrometeorologinei sausrai padidina SiO_2, MoO_3, B_2O_3, CuO nanodalelės. Nanodalelių suspensijos praturtina augalų mineralinę mitybą bei suaktyvina fermentinius ir nefermentinius antioksidantus, kurie sumažina oksidacinių biožymenų kiekį bei turi teigiamą poveikį sėjamųjų žirnių morfologiniams parametrams ir derliui.
3. SiO_2, MoO_3, B_2O_3 nanodalelių suspensijos padidina sėjamųjų žirnių atsparumą kompleksiniam agrometeorologinės sausros ir sunkiojo metalo vario poveikiui stiprinant antioksidacinę sistemą silpninant oksidacinio streso poveikį bei didinant augalų tolerancijos indeksą variui. CuO nanodalelių poveikis veikia sinergiškai su pertekliniu vario kiekiu padidindamas oksidacinių biožymenų kiekį ir sumažindamas produktyvumą sėjamuosiuose žirniuose.
4. Nanodalelių SiO_2, MoO_3, B_2O_3, CuO suspensijų poveikis antioksidacinei sistemai, morfologiniams parametrams ir mikro- ir makro- elementų akumuliacijai ir derliui sėjamuosiuose žirniuose priklauso nuo jų stabilumo, dydžio, paviršiaus krūvio ir poveikio būdo.

Mokslinio darbo naujumas. Tyrimas suteikia naujų žinių apie SiO_2, CuO, MoO_3, B_2O_3 nanodalelių poveikį žirnių augalų oksidacinio streso biožymenims, antioksidacinei sistemai, makro- ir mikroelementų pokyčiams, kai augalai augo normalaus drėgnio substrate, drėgnio trūkumo sąlygomis bei buvo veikiami kompleksinio sausros ir sunkiojo metalo vario streso. Nustatyta, kad nanodalelių suspensijos gali turėti tiek teigiamą, tiek neigiamą poveikį, kuris priklauso nuo taikomų dalelių savybių, koncentracijos ir pritaikymo metodo. Ištirtas SiO_2, CuO, MoO_3, B_2O_3 nanodalelių dzeta potencialas parodė, kad visos suspensijos buvo stabilios ir anijoninės, o nano-dydžio dalelių buvo nustatyta visose vandeninėse suspensijose. Atrinktos optimaliausios teigiamą poveikį žirnių oksidacinio streso biožymenims, antioksidacinei sistemai, makro- ir mikroelementų pokyčiams ir produktyvumui turėjusios nanodalelių koncentracijos: 50 ppm – SiO_2, CuO, MoO_3, 12,5 ppm – B_2O_3. Taip pat nustatyta, kad purškimas su CuO ir B_2O_3 nanodalelėmis žirnių produktyvumą veikia efektyviau nei laistymas, o augalų laistymas su MoO_3 nanodalelių suspensija buvo efektyvesnis nei purškimas.

Praktinė darbo reikšmė. Metalo pagrindu pagamintų ND poveikio žirniams tyrimai praturtina žinias apie ND naudą ar riziką augalams, padeda įtraukti platesnę ND įvairovę ir plėsti nanotechnologijų naudojimą augalininkystėje, tuo pačiu prisidedant prie bendros žemės ūkio tobulinimo praktikos. Visa tai turi didelę vertę agronomijos mokslo raidai. Be to, disertacinis

darbas turi ir socialinę naudą, nes tokių tyrimų plėtra padėtų patenkinti augalinio maisto poreikius visuomenėje ir prisidėtų prie augalinės kilmės produktų kokybės gerinimo.

Disertacijos apimtis ir struktūra. Disertaciją sudaro įvadas, literatūros analizė, tyrimo metodų aprašymas, rezultatų analizė, aptarimas, išvados, panaudotos literatūros sąrašas ir publikacijų, išleistų kartu su bendraautoriais, sąrašas ir priedai. Literatūros analizė, tyrimo metodikos aprašymas ir rezultatai iliustruojami 15 paveikslų ir 21 lentele. Bibliografijoje yra 295 šaltiniai, disertacijos apimtis 155 puslapiai.

2. TYRIMO OBJEKTAS, SĄLYGOS IR METODAI

2.1 Tyrimo objektas

Sėjamasis žirnis (*Pisum sativum* L.)

2.2 Nanodalelių suspensijos paruošimas. Eksperimentams buvo naudojamos silicio dioksido ((SiO_2) dalelių dydis: 20–30 nm; grynumas: 99%), vario oksido ((CuO) dalelių dydis: 25–55 nm; grynumas: 99,95%), molibdeno trioksido ((MoO_3) dalelių dydis: 35–45 nm; grynumas: 95%) ir boro ((B_2O_3) dalelių dydis: 100 nm; grynumas: 99,9%) nanodalelės (ND) įsigytos US Research Nanomaterials (Inc, Hiustonas, TX USA). ND suspenduotos dejonizuotame vandenyje (12,5; 25; 50; 100 ppm) ir ultragarsu (37 kHz) disperguotos 60 min. ND dydis ir suspensijos stabilumas išmatuoti Delsa ™ nano submikrono dalelių dydžio (Beckman Coulter Instruments, Corporation, Fullerton, Kalifornija) ir dzeta potencialo (Dispersion Technology Inc., Bedford Hills, Niujorkas) matuokliais. Gauti duomenys parodė, kad visos paruoštos ND suspensijos buvo stipriai anijoninės ir stabilios, taip pat monodispersinės pagal polidispersiškumo indeksą (PDI) (1 lentelė).

1 lentelė. SiO_2, B_2O_3, CuO, MoO_3 ND suspensijų dejonizuotame vandenyje savybės: dzeta potencialas (vidurkis ± standartinę paklaidą), polidispersiškumo indeksą, nanodalelių dydžio nuo 1 iki 100 nm procentą suspensijoje.

	SiO_2 ND	**B_2O_3 ND**	**CuO ND**	**MoO_3 ND**
Dzeta potencialas (ζ; mV)	-20,64 ± 0,333	-28,54±0,223	-26,68±0,631	-24,92±0,314
Polidispersinis indeksas (PI)	0,34	0,237	0,245	0,218
ND dydis 1–100 nm suspensijoje, %	70%	43%	54%	68%

2.3 Eksperimentų schema

Vizualiai apibendrinta metodika pateikta 1 paveiksle. Prieš sėją žirnių sėklos buvo sterilizuojamos 5% natrio hipochlorito tirpale 15 min., kad būtų užtikrintas paviršiaus sterilumas (Lehotai ir kt., 2011) ir kelis kartus skalaujamos dejonizuotu vandeniu. Tada sėklos 24 valandas mirkytos vandenyje.

Atlikti šie eksperimentai:

Pradinis (pirminis) eksperimentas atliktas siekiant nustatyti tinkamas SiO_2, B_2O_3, CuO, MoO_3 ND koncentracijas ir jų poveikį žirnių augalams. Tyrimas atliktas Lietuvos agrarinių ir miškų mokslo centro Sodininkystės ir daržininkystės institute esančioje kontroliuojamos aplinkos augimo kameroje (4 × 6 m, h = 3,2 m). Po 3 sėklas buvo sėjama į 500 ml plastikinį vazoną, kuris buvo traktuojamas kaip vienas biologinis pakartojimas. Vienam variantui buvo naudojama 16 vazonų. Augalai pasėti ir augo durpių substrate Profi 1 (pH 6, EL 2,0 - 2,5 mS cm^{-1} (± 0,03 mS cm^{-1}), vidutiniai maistinių medžiagų kiekiai (mg L^{-1}) substrate: N, 110; P_2O_5, 50; K_2O, 160, Fe (4,5), Mn (0,5), Cu (0,1), B (0,02), Mo (0,03), ir Zn (0,04), UAB Durpeta, Lietuva) 30 dienų. Žirniai augo esant 16 h fotoperiodui, 20 ± 2/16 ± 2 °C dienos/nakties aplinkos oro temperatūrai, 60 % santykinei oro drėgmei, ~220 µmol m^{-2} s^{-1} fotosintetiškai aktyvios spinduliuotės fotonų srauto tankiui (PAR) naudojant aukšto slėgio natrio (HPS) lempas (SON-T Agro, 400 W; Philips, Somerset, NJ, JAV). Žirniai laistyti 100 ml SiO_2, B_2O_3, CuO, MoO_3 ND suspensijomis, su skirtingomis koncentracijomis (12,5; 25; 50; 100 ppm), kai augalai turėjo 3–4 tikruosius lapus arba suformavo 3–4 ūselius (14 dienų po sėjos, 14 BBCH augimo tarpsniu) (Meier, 2018). Po to, visi augalai kasdien buvo laistomi dejonizuotu vandeniu jų papildomai netręšiant. Siekiant sumažinti galimą augimo kameros poveikį augalų atsakui, buvo taikomas reguliarus 10 vazonų erdvinis pertvarkymas kiekviename variante. Siekiant išvengti krašto efekto, likę 6 vazonai buvo palikti kraštuose ir nejudinami. Eksperimento pabaigoje, augalams pasiekus 31 BBCH augimo stadiją – stiebo ilgėjimo pradžią (Meier, 2018), žirniai nuskinti biometriniams matavimams ir biocheminėms analizėms. Išsamiai ištyrus ir išanalizavus paveiktus augalus, buvo parinktos 12.5; 25; 50 ppm kiekvienos ND koncentracijos, skirtos naudoti tolesniuose eksperimentuose. Šiais eksperimentais buvo siekiama parinkti efektyviausias skirtingų ND koncentracijas drėgmės trūkumo veikiamiems žirnių augalams.

Dvejuose šiltnamiuose (3 × 6 m, h = 2 m; 55°05'08.4"N 23°48'03.5"E, 51 m aukštyje; vidutinio klimato zona šiaurės pusrutulio) dvejų metų pavasario-vasaros laikotarpiu (2019–2020 m.) buvo atlikti žemiau aprašomi eksperimentai.

Eksperimentams žirnių sėklos buvo paruoštos sėjai, kaip aprašyta aukščiau. Dešimt sėklų buvo pasėta į 10 l tūrio plastikinius vazonus (7 vazonai vienam variantui, išdėstyti atsitiktine

tvarka), užpildyti ~ 8 kg dirvožemio mišinio (atitinkamai 7:1 dirvožemio ir perlito tūrio santykis). Dirvožemis buvo sunkus priemolis, pH 7,4 ± 0,1; humuso koncentracija – 3,6±0,1 %; P_2O_5 – 243±8 mg kg^{-1}; K_2O - 348±37 mg kg^{-1}; NH_4^+ – 4 ±0,6 mg kg^{-1}; NO_3^- – 22±0,9 mg kg^{-1}; SiO_2 – 39±0,8 mg kg^{-1}. Praėjus 5 dienoms po sėjos žirnių daigai išretinti iki 7 augalų vazone. Po 16 dienų auginimo kiekvienas žirnių vazonas patręštas 7 g amonio salietra. Žirniai nupurkšti fungicidais, nes veislė 'Respect' yra jautresnė miltligei. Vazonai buvo laistomi vandeniu kasdien iki 80% substrato drėgmės (SD) su graduotu cilindru bei matuojama SD su substrato drėgmės jutikliu (Delta-T prietaisai, HH2 drėgmės matuoklis, Kembridžas, Jungtinė Karalystė) 35 dienas. Augalai augo natūralaus paros ilgio fotoperiodu, vidutinė dienos/nakties temperatūra buvo 22,2/14,4 °C; santykinė oro drėgmė – 58/77 ± 5% prieš sausrą; 10 dienų agrometeorologinės sausros metu vidutinė dienos/nakties temperatūra buvo 25,4/16,6 °C, o santykinė oro drėgmė – 53/75 ± 5%, duomenys buvo matuojami viso eksperimento metu (Termio+ duomenų kaupiklis, Lenkija).

Žirniams pasiekus 40 BBCH augimo tarpsnį (Meier, 2018), jie buvo palaistyti su 100 ± 1 ml vienam vegetaciniam indui arba purškiami augalai iki visiško sušlapimo (apie 14±0,5 ml augalui) tirpalais, kuriuose buvo skirtingos SiO_2, B_2O_3, CuO, MoO_3 ND koncentracijos: 0 (laistyti arba apipurkšti vandeniu, neapdoroti su ND), 12,5 ppm, 25 ppm ir 50 ppm. Po ND panaudojimo, vienos dalies žirnių augalų laistymas buvo sustabdytas ir inicijuotas sausros stresas (30% SD), o kita dalis – laistomi vandeniu, palaikant normalią substrato drėgmę (80% SD). Šie poveikio režimai buvo taikomi 10 dienų. Po to augalai atsitiktinai buvo atrenkami įvertinti jų morfologinius parametrus ir biocheminį atsaką.

Vėlesniuose eksperimentuose siekėme nustatyti, ar ND suspensijos gali apsaugoti augalus nuo kompleksinio agrometeorologinės sausros ir vario, kaip sunkiojo metalo, poveikio.

Šio eksperimento sąlygos: vidutinė dienos/nakties temperatūra 24,2/14,4 °C; santykinė oro drėgmė – 54/75 ± 5% prieš ekspoziciją; 10 dienų sausros metu vidutinė dienos/nakties temperatūra buvo 26,2/17,0 °C, o santykinė oro drėgmė – 50/73 ± 5%. Dirvožemio paruošimas, žirnių sėjimas ir apdorojimas ND buvo toks pats, kaip aprašyta aukščiau. Norint sukelti Cu kaip sunkiojo metalo įtampą žirniuose, į pusės vegetacinių indų substratą buvo pridėta 160 mg kg^{-1} $CuSO_4$ vandeninio tirpalo. Kai žirniai pasiekė 40 BBCH augimo stadiją (Meier, 2018), jie buvo laistomi (100 ± 1 ml viename vazone) arba purškiami iki visiško sudrėkimo (apie 14±0,5 ml $augalo^{-1}$) ND tirpalais, naudojant ankstesnių eksperimentų nustatytas veiksmingiausias koncentracijas: SiO_2 – 50 ppm, CuO – 50 ppm, MoO_3 – 50 ppm, B_2O_3 – 12,5 ppm. Žirnius paveikus ND, vienos dalies augalų laistymas buvo nutrauktas ir pradėtas sausros stresas (30 % SM), o kiti – buvo laistomi vandeniu, kad būtų palaikoma normali dirvožemio drėgmė (80 % SM). Šie režimai buvo taikomi 10 dienų iki derliaus nuėmimo. Augalai buvo atsitiktinai atrenkami pasiekus BBCH 50 augimo stadiją (Meier, 2018) siekiant įvertinti jų morfofiziologinį atsaką.

2.4 Morfologinių parametrų matavimai

Biometriniams matavimams atsitiktinai atrinkta 10 augalų iš varianto (n = 10). Antžeminė žirnio dalis atskirta nuo šaknų, išmatuotas stiebo aukštis ir šaknies ilgis, pasverta augalų žalia biomasė (ŽM) ir sausas svoris (SM) po džiovinimo. ŽM ir SM pasverti elektroninėmis svarstyklėmis (Mettler Toledo AG64, Columbus, OH, JAV). SM nustatytas augalus džiovinant 105°C temperatūroje konvekcinėje džiovinimo spintoje (VENTICELL 222, MBT, Čekija). Pasvėrus antžeminės žirnio dalies ŽM, po 10 augalų iš kiekvieno varianto buvo paliekami 24 valandoms plūduriuoti dejonizuotame vandenyje, po to augalai pasverti nustatyti turgorinei masei (TM), kad būtų galima išskaičiuoti santykinį vandens kiekį (SVK) (Baris ir Weatherley, 1962):

$$\text{SVK}, \% = \frac{(\text{ŽM} - \text{SM})}{(\text{TM} - \text{SM})} \text{x} 100 \,. \qquad (1)$$

Lapų plotas išmatuotas automatiniu lapų ploto matuokliu (AT Delta-T Devices, Wallingford, JK). Apskaičiuotas specifinis lapų plotas (SLP), kur bendras augalo lapų plotas (n=10) padalintas iš antžeminės dalies SM ir išreikštas cm^2 g^{-1}. Šaknų ir antžeminės augalų dalies santykis nustatytas kaip šaknies SM ir antžeminės SM santykis. Žirnių derlius apskaičiuotas pagal Koiter ir Bill Ashton (2021) aprašytą metodiką. Bio-koncentracijos faktorius, translokacijos faktorius ir tolerancijos indeksas apskaičiuoti pagal Zacchini su bendraautoriais pateiktą metodiką (2009).

2.5 Antioksidacinis aktyvumas

Nefermentiniai antioksidantai

Žirnių lapų antioksidacinės savybės įvertintos spektrofotometriškai (spektrofotometru M501, Spectronic Camspec Ltd., UK) kaip DPPH (2-difenil-1-pikrilhidrazilas), ABTS (2,20-azino-bis (3-etilbenzotiazolin-6-sulfonrūgštis)) diamonio druskos) radikalų surišimo geba (Sharma ir Bhat, 2009; Re ir kt., 1999) ir FRAP redukcijos antioksidacinė geba (Benzie ir Strain, 1996). Bendras fenolinių junginių kiekis, išreikštas galo rūgšties ekvivalentu, nustatytas Folin–Ciocalteu metodu (Ainsworth ir Gillespie, 2007) absorbciją matuojant ties 765 nm.

Fermentiniai antioksidantai

Ekstraktai buvo ruošiami iš 0,5 g šviežio mėginio, jį homogenizuojant su skystu azotu ir praskiedžiant 5 mL ekstrahavimo buferiu (100 mM kalio fosfato buferis, pH 7,8, kuriame yra 0,1 mM EDTA). Po 10 minučių centrifugavimo 3000 aps./min. (Hermle Z300K, Vokietija), supernatantas buvo surinktas ir naudojamas fermentinio aktyvumo tyrimams. Visi ekstrakto paruošimo etapai buvo atlikti 4 °C temperatūroje. Šie ekstraktai buvo naudojami

spektrofotometriniams (M501, Spectronic Camspec Ltd., UK) superoksido dismutazės (SOD), katalazės (CAT), askorbato peroksidazės (APX), glutationo reduktazės (GR) ir gvajakolio peroksidazės (GPX) aktyvumui žirnių lapuose nustatyti.

SOD aktyvumas nustatytas pagal formazano mėlio susidarymo slopinimą esant 560 nm bangos ilgiui (Dhindsa et al., 1981) ir išreiškiamas UI mg^{-1} baltymų min^{-1}. APX aktyvumas išmatuotas prie 290 nm bangos ilgio (Nakano and Asada, 1981), naudotas ekstinkcijos koeficientas 2,8 mM/cm, išreikštas µmol AsA mg^{-1} baltymų min^{-1}; GR aktyvumas – pagal oksiduoto glutationo (GSSG) kiekį, kurio koncentracija nustatoma prie 340 nm bangos ilgio (Sofo et al., 2005); naudotas absorbcijos koeficientas 6.22 mM^{-1} cm^{-1}, išreikštas µmol NADPH mg^{-1} baltymų min^{-1}. CAT aktyvumas nustatytas naudojant H_2O_2 kaip substratą prie 240 nm ilgio bangos (Aebi, 1984); naudotas absorbcijos koeficientas 6.22 mM^{-1} cm^{-1}, išreikštas mmol H_2O_2 mg^{-1} baltymų min^{-1}; GPX aktyvumas nustatytas pagal gvajakolio oksidacijos sukeliamą absorbcijos esant 470 nm bangos ilgiui padidėjimą (Kvaratskhelia et al., 1997).

Suminis baltymų kiekis nustatytas Bradfordo metodu prie 595 nm bangos ilgio (Bradford, 1976). Pagal kalibracinę kreivę, kurioje jaučio serumo albumino tirpalas naudotas kaip standartas, išskaičiuotas baltymų kiekis mg mL^{-1}.

2.6 Oksidacinio streso biožymenys

Lipidų peroksidacijai ir vandenilio peroksido (H_2O_2) koncentracijai žirnių lapuose nustatyti naudojami ekstraktai paruošti iš 0,1 g šviežio mėginio sumalant skystu azotu ir atskiedus 4 ml 0,1 % trichloracto rūgšties (TCA). Po 10 minučių centrifugavimo 3000 aps./min. (Hermle Z300K, Vokietija), supernatantas naudotas tolimesnėms analizėms. H_2O_2 matavimams naudotas 1 M kalio jodidas (KI), absorbcija matuota su spektrofotometru (M501, Spectronic Camspec Ltd., JK) esant 390 nm bangai. H_2O_2 kiekis buvo išskaičiuotas iš kalibracinės kreivės ir išreiškiamas µmol g^{-1} ŽM (Velikova ir kt., 2000). Tiobarbitūrinės rūgšties (TBARS) testo pagalba (Heath ir Packer, 1968) nustatytas malondialdehido (MDA) kiekis žirnių lapų mėginiuose, kaip galutinis lipidų peroksidacijos produktas. Absorbcija buvo matuojama esant 532 nm ir 600 nm bangoms, naudojant spektrofotometrą (M501, Spectronic Camspec Ltd., JK). MDA-TBA komplekso (raudonojo pigmento) kiekis lapuose buvo apskaičiuotas ir išreikštas nmol g^{-1} ŽM:

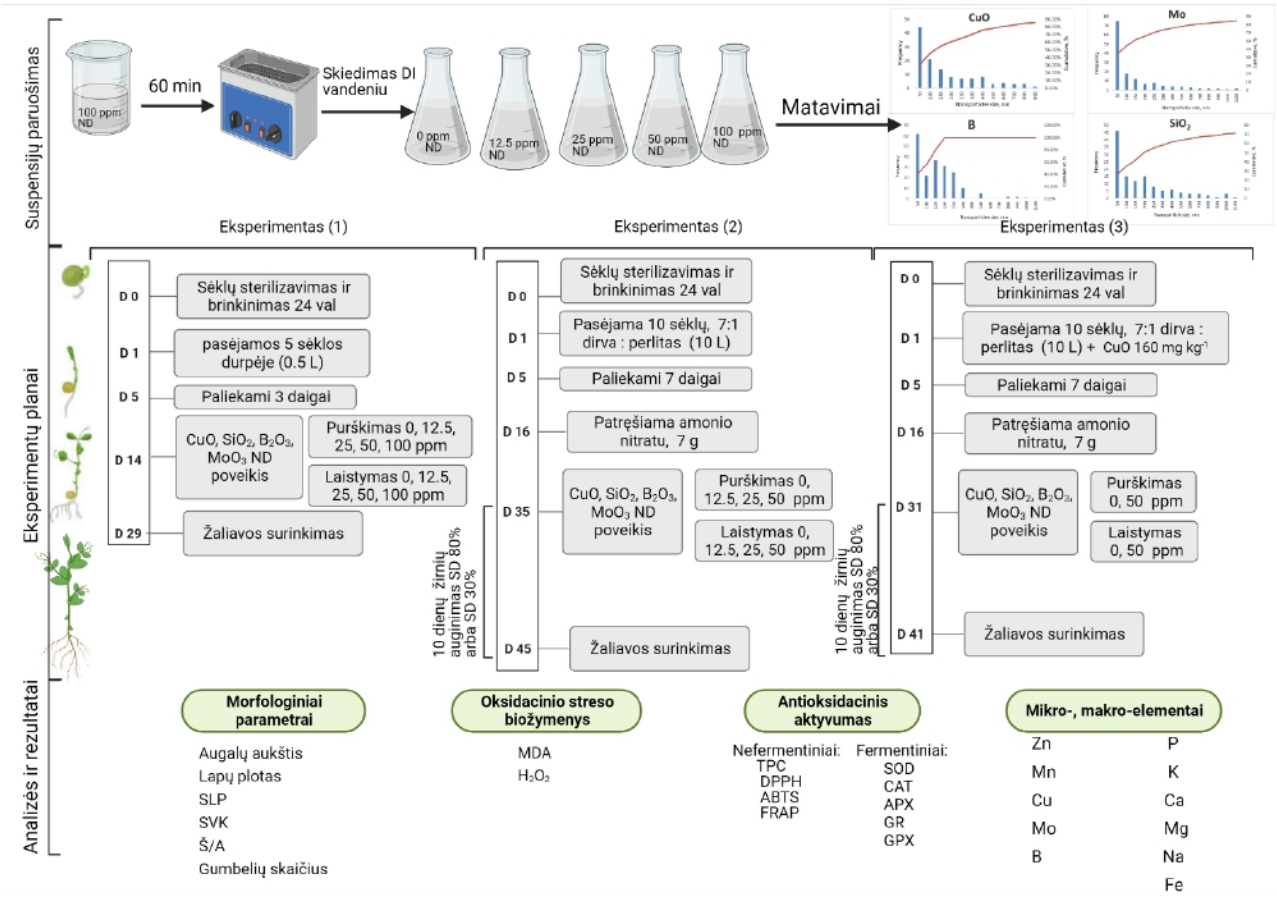
2.7 Makro- ir mikroelementų nustatymas

Makro- ir mikroelementų kiekis žirnių lapuose, stiebe ir šaknyse nustatytas naudojant mikrobangų skaidymo techniką kartu su indukciniu būdu susietos plazmos optinės emisijos spektrometriją. Visiškas sausos augalinės medžiagos (0,3 g) skaidymas gautas naudojant 8 ml

65 % HNO_3 su mikrobangų skaidymo sistema Multiwave GO (Anton Paar GmbH, Gracas, Austrija). Po to mėginiai nufiltruoti praskiesti iki 50 ml ir analizuoti su ICP–OES spektrometru (Spectro Genesis, SPECTRO Analytical Instruments, Kleve, Germany).

2.8 Statistinė analizė

Visos vertės buvo išreikštos kaip vidurkis ± standartinis nuokrypis. Siekiant nustatyti reikšmingus skirtumus duomenys buvo analizuojami naudojant dispersinės analizės (ANOVA) testą Tukey HSD esant $p \leq 0,05$. Analizuojami skirtumai tarp variantų, kai žirniai buvo auginami normaliomis sąlygomis ir atskirai tarp žirnių, kurie buvo auginami sausros sąlygomis. Mikro- ir makroelementų analizės rezultatai taip pat buvo lyginami atskirai tarp kontrolinių – sausros metu augintų žirnių, neapdorotų su ND (SD 30%) ir apdorotų su ND, ir tarp kontrolinių žirnių, augintų normaliomis sąlygomis (SD 80%) neapdorotų su ND ir paveiktų su ND.



1 pav. Tyrimų schema: nanodalelių suspensijų ruošimas, eksperimentų planai, matavimai ir analizės

3. REZULTATAI

Šiame skyriuje analizuojama SiO_2, MoO_3, B_2O_3 ir CuO nanodalelių (ND) poveikis žirnių daigams augusiems optimaliomis sąlygomis (3.1), pilnai išsivysčiusiems žirnių augalams augusiems agrometeorologinės sausros (3.2) bei kompleksinio vario ir agrometeorologinės sausros (3.3) sąlygomis.

3.1 Silicio dioksido, vario oksido, molibdeno trioksido ir boro trioksido nanodalelių poveikis žirnių morfologiniams parametrams ir antioksidacinei sistemai

Siekiant išsiaiškinti, kaip žirnių daigai reaguotų į skirtingos koncentracijos nanodalelių (ND) poveikį juos auginant optimaliomis sąlygomis, buvo atliktas pirmasis eksperimentas. Šiame skyriuje analizuojamas silicio dioksido (SiO_2), vario oksido (CuO), molibdeno trioksido (MoO_3) ir boro trioksido (B_2O_3) ND poveikis žirnių daigų morfologiniams parametrams ir antioksidaciniam aktyvumui. Pateikti rezultatai yra lyginami su augalais, kurie nebuvo paveikti ND.

Nanodalelių poveikis žirnių morfologiniams parametrams

Rezultatai parodė, kad naudojant SiO_2 ND, žirnių aukštis padidėjo 28–45 %, priklausomai nuo didėjančios koncentracijos (2 lentelė). Žirnius palaisčius su SiO_2 ND 12,5, 25, 50 ir 100 ppm suspensijomis, žirnių vidutinė ŽM padidėjo atitinkamai 81, 104, 116 ir 109 %. Didėjanti SiO_2 ND koncentracija tirpale, padidino žirnių SM iki 135%. Augalų palaistymas su 100 ppm koncentracijos suspensija, šaknis pailgino apie 76 %, o su kitomis koncentracijomis - iki 106%. Didėjanti SiO_2 ND koncentracija teigiamai paveikė šaknų ŽM ir SM. Rezultatai patvirtino teigiamą SiO_2 ND poveikį žirnių aukščiui, ŽM ir SM bei šaknų ilgiui esant visoms tirpalo koncentracijoms (1 lentelė).

MoO_3 ND turėjo teigiamą poveikį žirniams, didėjant jų koncentracijai tirpale (2 lentelė). Didesnė MoO_3 ND koncentracija tirpale padidino žirnių aukštį iki 48%, ŽM – iki 61%, o SM – iki 84 %. Šaknų ilgis padidėjo 56–85 %, o jų biomasė padidėjo tiek šviežia 58–121 %, tiek sausa 30–71 % esant 12,5–100 ppm koncentracijoms tirpale.

Nustatyta, kad mažiausia CuO ND koncentracija tirpale turėjo didžiausią teigiamą poveikį žirnio morfologiniams parametrams (2 lentelė). Naudojant 12,5, 25 ir 50 ppm koncentracijas, žirnių aukštis padidėjo 41, 32 ir 16 %, ŽM - 87, 78 ir 41 %, o SM – 47, 50 ir 35 % lyginant su ND nepaveiktais augalai. Šaknų ilgiui reikšmingą poveikį turėjo visos naudotos CuO ND koncentracijos. Didėjančios CuO ND koncentracijos tirpale turėjo teigiamą poveikį šaknų ŽM tačiau didėjant koncentracijai biomasės didėjimas sulėtėjo.

Mažesnės B_2O_3 ND koncentracijos turėjo teigiamą poveikį žirnių morfologiniams parametrams (2 lentelė). Žirnių aukštis padidėjo 52, 21 ir 2 %, ŽM – 95, 60 ir 14 %, o SM –

116, 35 ir 52 %, kai jie buvo palaistyti su 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis. Šaknų ilgis padidėjo 92, 62 ir 11 %, šaknų ŽM – 91, 64 ir 21 % panaudojus aukščiau nurodytas B_2O_3 ND koncentracijas.

2 lentelė. SiO_2, CuO, MoO_3, B_2O_3 ND (12,5; 25; 50; 100 ppm) poveikis P. sativum L. morfologiniams parametrams: augalų antžeminės dalies aukštis, šviežia ir sausa biomasė (ŽM, SM), šaknų ilgis, ŽM ir SM. 0 – kontroliniai augalai, laistomi dejonizuotu vandeniu. Vidutinės reikšmės stulpeliuose su skirtingomis raidėmis reikšmingai skiriasi nuo kontrolinės vertės, kai $p < 0,05$ (n=10) pagal Tukey (HSD) testą

Nanodalelių koncentracija, ppm		Antžeminė dalis			Šaknys		
		Aukštis, cm	ŽM, g	SM, g	Ilgis, cm	ŽM, g	SM, g
H_2O	0	20,0 b	1,3 b	0,240 b	4,5 b	0,365 b	0,044 b
SiO_2	12,5	25,7 a	2,4 a	0,388 a	9,1 a	0,624 a	0,073 a
	25	28,2 a	2,7 a	0,465 a	9,2 a	0,782 a	0,082 a
	50	28,7 a	2,9 a	0,501 a	9,2 a	1,049 a	0,089 a
	100	28,8 a	2,8 a	0,564 a	7,8 a	1,166 a	0,105 a
CuO	12,5	28,1 a	2,5 a	0,353 a	9,1 a	0,763 a	0,069 a
	25	26,3 a	2,4 a	0,361 a	8,3 a	0,668 a	0,058 b
	50	23,3 a	1,9 a	0,323 a	8,4 a	0,501 a	0,055 b
	100	20,3 b	1,2 b	0,217 c	9,3 a	0,469 a	0,037 c
MoO_3	12,5	24,6 a	1,7 b	0,259 b	6,9 a	0,627 a	0,063 b
	25	24,7 a	1,5 b	0,304 a	7,4 a	0,577 a	0,058 b
	50	27,7 a	2,2 a	0,380 a	7,3 a	0,667 a	0,065 a
	100	29,6 a	2,5 a	0,388 a	8,3 a	0,809 a	0,076 a
B_2O_3	12,5	30,3 a	2,6 a	0,518 a	8,6 a	0,696 a	0,061 a
	25	24,3 a	2,1 a	0,323 a	7,3 a	0,598 a	0,053 b
	50	20,4 b	1,5 b	0,366 a	4,9 b	0,444 a	0,044 b
	100	19,3 c	1,4 b	0,270 b	5,5 a	0,341 b	0,043 b

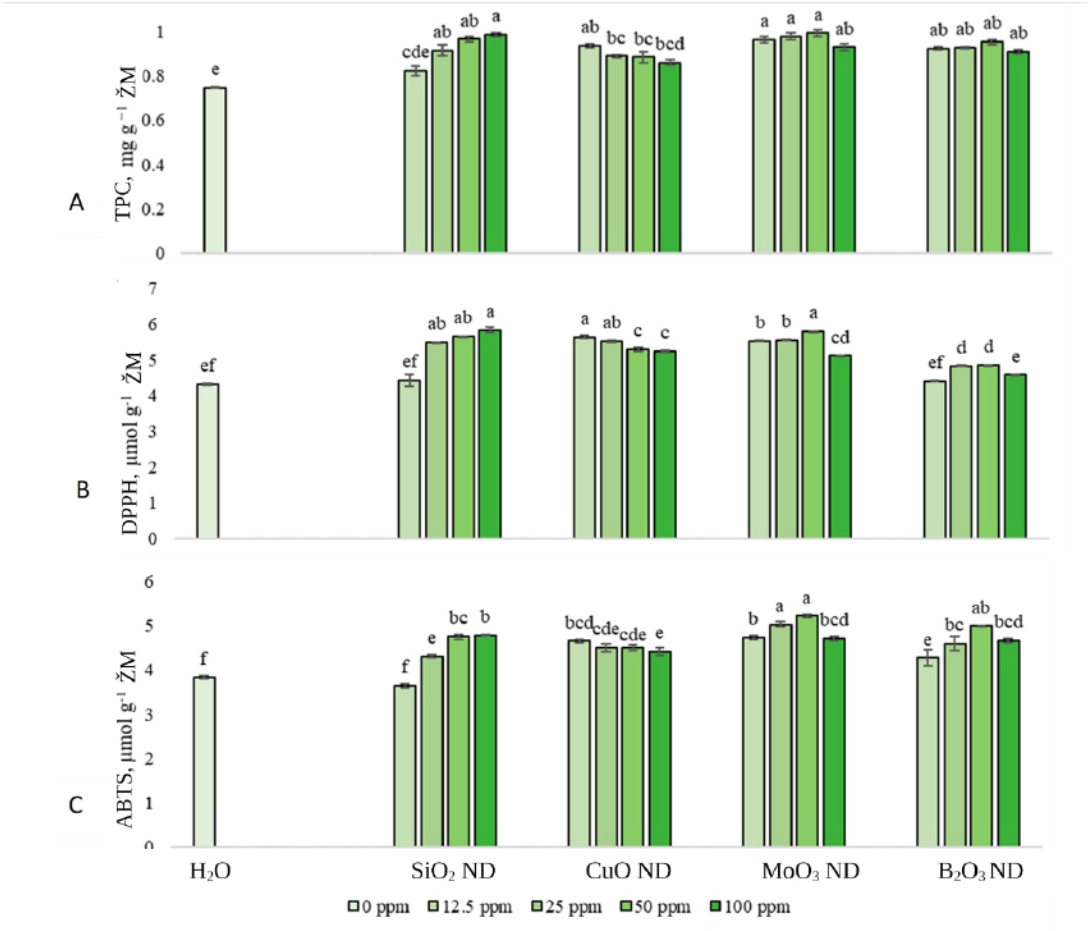
ŽM – šviežia biomasė; SM – sausa biomase

Nanodalelių poveikis žirnių antioksidaciniam aktyvumui

Visos naudotos ND suspensijos paveikė antioksidacinį atsaką žirnių lapuose (2 pav.). Didėjant SiO_2 ND koncentracijai, žirnių lapuose bendras fenolinių junginių (TPC) kiekis padidėjo 10 (12,5 ppm), 22 (25 ppm), 30 (50 ppm) ir 32 % (100 ppm) (2A pav.). Panašus poveikis buvo pastebėtas 2-difenil-1-pikrilhidrazilo (DPPH) ir 2,20-azino-bis (3-etilbenzotiazolin-6-sulfonrūgšties) (ABTS) laisvųjų radikalų surišimo aktyvume (2B, C pav.).

DPPH laisvųjų radikalų surišimo aktyvumas padidėjo iki 35 % naudojant SiO_2 ND suspensiją. ABTS laisvųjų radikalų surišimo aktyvumas padidėjo apie 12 %, 24 % ir 25 %, kai žirniai buvo paveikti 25 ppm 50 ppm ir 100 ppm SiO_2 ND suspensijomis.

Nustatyta, kad MoO_3 ND didino TPC kiekį apie 29, 31, 33 ir 24 % po poveikio 12,5, 25, 50 ir 100 ppm MoO_3 ND suspensijomis. (2A pav.). DPPH laisvųjų radikalų pašalinimo aktyvumas intensyviai didėjo esant 12,5 ppm, tačiau aktyvumas padidėjo ne taip stipriai, kai augalai buvo palaistyti su 100 ppm suspensija (2B pav.). ABTS laisvųjų radikalų surišimo aktyvumui taip pat įtakos turėjo MoO_3 ND koncentracijos didėjimas tirpale (2C pav.). Apskritai buvo pastebėta, kad 100 ppm MoO_3 ND suspensija silpniau paveikė TPC kaupimąsi ir antioksidacinį pajėgumą žirnių lapuose nei mažesnės koncentracijos.

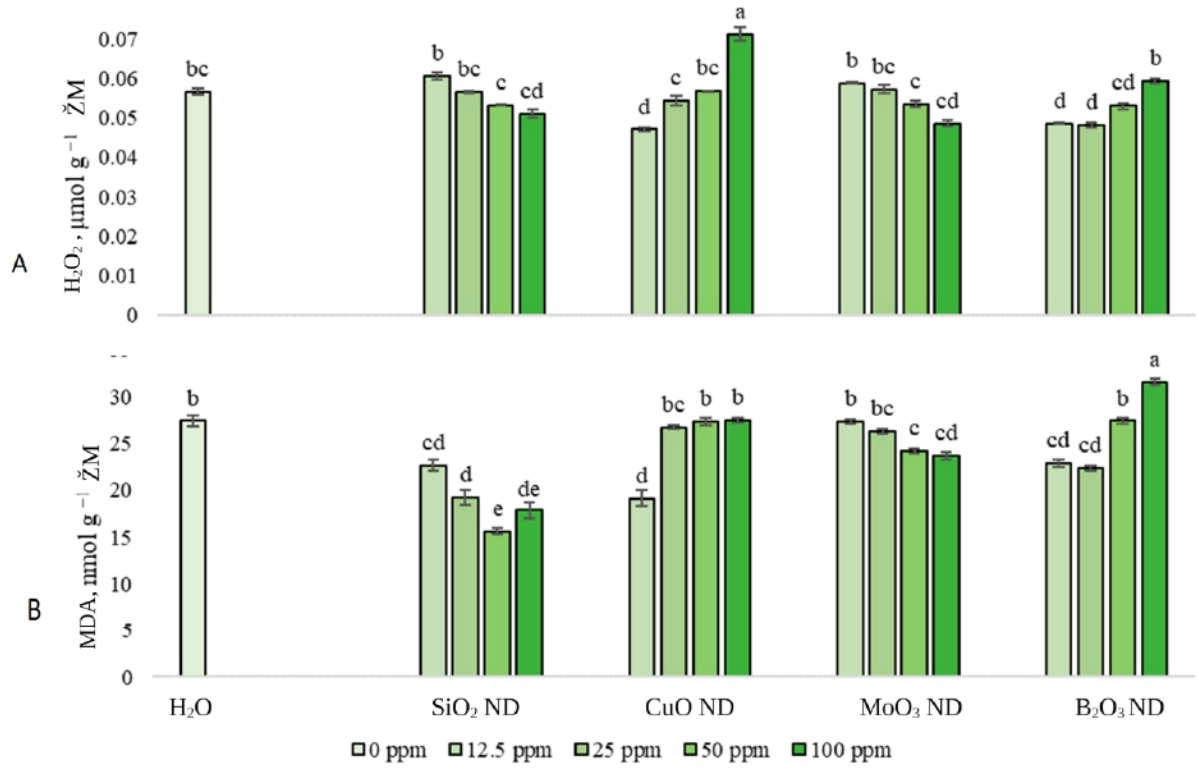


2 pav. SiO_2, CuO, MoO_3 ir B_2O_3 ND (0; 12,5; 25; 50, 100 ppm) poveikis bendram fenolinių junginių kiekiui (A, TPC), DPPH (B) laisvųjų radikalų surišimo aktyvumui, ir ABTS (C) laisvųjų radikalų surišimo aktyvumui sėjamajame žirnyje (P. sativum L.) H_2O – kontroliniai augalai, laistyti dejonizuotu vandeniu. Vertės yra vidurkiai ± SE apskaičiuoti iš devynių pakartojimų. Skirtingos raidės rodo reikšmingus skirtumus pagal Tukey HSD testą ($p < 0,05$)

TPC kiekis žirnių lapuose padidėjo 25, 19, 18 ir 15 % po poveikio su 12,5, 25, 50 ir 100 ppm CuO ND suspensijomis (2A pav.). Taip pat naudotos skirtingų koncentracijų CuO ND suspensijos teigiamai paveikė DPPH ir ABTS laisvųjų radikalų surišimo gebą. (2B, C pav.). Rezultatai parodė, kad didėjant CuO ND koncentracijai suspensijoje, susilpnėjo antioksidantų aktyvumas žirniuose.

12,5, 25, 50 ir 100 ppm B_2O_3 ND koncentracijos stipriai stimuliavo TPC kaupimąsi žirniuose, padidinant jį atitinkamai apie 23, 24, 28 ir 22 % (2A pav.). DPPH laisvųjų radikalų surišimo aktyvumas buvo silpnai paveiktas (2B pav.), o ABTS - B_2O_3 ND suspensijos padidino apie 12, 20, 30 ir 22 % (2C pav.) augalus paveikus 12,5, 25, 50 ir 100 ppm tirpalais.

SiO_2 ND efektyviai sumažino vandenilio peroksido (H_2O_2) ir malondialdehido (MDA) koncentracijas žirnių lapuose (3A, B pav.). H_2O_2 koncentracija žirnių lapuose sumažėjo iki 10 % po poveikio 25, 50 ir 100 ppm SiO_2 tirpalais. Nustatytas nežymus H_2O_2 kiekio padidėjimas po augalų palaistymo su 12,5 ppm SiO_2 ND suspensija. MDA kiekis žirnių lapuose žymiai sumažėjo, palaisčius augalus skirtingų koncentracijų SiO_2 ND tirpalais.



3 pav. SiO_2, CuO, MoO_3 ir B_2O_3 ND (0; 12,5; 25; 50, 100 ppm) poveikis vandenilio peroksido (A, H_2O_2) ir malondialdehido (B, MDA) kiekiui sėjamajame žirnyje (P. sativum L.). H_2O – kontroliniai augalai, laistomi dejonizuotu vandeniu. Vertės yra vidurkiai ± SE apskaičiuoti iš devynių pakartojimų. Skirtingos raidės rodo reikšmingus skirtumus pagal Tukey HSD testą ($p < 0,05$)

MoO_3 ND mažino H_2O_2 kiekį žirnių lapuose, augalus palaisčius 50 ir 100 ppm koncentracijos tirpalais. Taip pat nustatyta, kad lipidų peroksidacija sumažėjo didėjant MoO_3 ND koncentracijai suspensijoje.

12,5 ppm CuO ND suspensija turėjo stipriausią poveikį H_2O_2 (17 %) ir MDA (30 %) kiekiui žirnių lapuose. Šiek tiek silpnesnis poveikis nustatytas augalus paveikus 25 ppm koncentracija. O 100 ppm CuO ND tirpalas reikšmingai didino (26 %) H_2O_2 kiekį žirnių lapuose.

12,5 ir 25 ppm B_2O_3 ND suspensijos efektyviai mažino H_2O_2 kiekį žirnių lapuose. MDA kiekis žirnių lapuose reikšmingai sumažėjo, kai augalai buvo palaistyti 12,5 ir 25 ppm B_2O_3 ND tirpalais. O 100 ppm B_2O_3 ND suspensija, reikšmingai didino MDA kiekį žirniuose.

3.2 Agrometeorologinės sausros ir nanodalelių poveikis žirniams

Šiame skyriuje aptariamas ND poveikis agrometeorologinės sausros paveiktiems žirniams, augintiems esant 30 % substrato drėgniui (SD 30 %) ir optimaliomis sąlygomis esant normaliam 80 % substrato drėgniui (SD 80 %). Išsamiai aptariama ND poveikis morfologiniams parametrams, antioksidacinės sistemos aktyvumui, makro- ir mikroelementų kaupimuisi žirniuose. ND poveikis žirniams buvo ištirtas augalus veikiant skirtingomis ND koncentracijomis bei taikant du poveikio būdus – purškiant augalus ir laistant per šaknis.

3.2.1 Silicio dioksido nanodalelių poveikis žirniams augusiems skirtingomis substrato drėgnio sąlygomis

Nanodalelių poveikis morfologiniams parametrams

Žirnių laistymas 12,5, 25 ir 50 ppm silicio dioksido (SiO_2) ND suspensijomis turėjo reikšmingą poveikį žirnių aukščiui (3 lentelė, SD 80 %) esant pakankamai substrato drėgniui. Be to, laistymas 50 ppm SiO_2 ND suspensija skatino lapų vystymąsi. Augalus purškiant 12,5, 25 ir 50 ppm SiO_2 ND suspensija, jų lapų plotas padidėjo iki 15 %. Santykinis vandens kiekis (SVK) didėjo, kai augalai buvo palaistyti 50 ppm SiO_2 ND suspensija. Po palaistymo 12,5, 25 ir 50 ppm suspensijomis atitinkamai padidėjo šaknų ir antžeminės dalies santykis apie 7, 34 ir 68 %. Specifinio lapų ploto (SLP) skirtumų žirniuose po jų laistymo su skirtingų koncentracijų SiO_2 ND tirpalais nenustatyta, tačiau po nupurškimo su 12,5 ppm – SLA sumažėjo apie 13 %. Nustatytas teigiamas poveikis gumbelių formavimuisi, gumbelių skaičius padidėjo iki 5 kartų, kai žirniai buvo palaistyti SiO_2 ND tirpalais ir augo normaliame substrato drėgnyje. Tuo tarpu žirnius nupurškus SiO_2 ND tirpalais, šaknyse gumbelių skaičius padidėjo nežymiai iki 1,4 karto. Nustatyta, kad augalus paveikus 50 ppm SiO_2 ND suspensija, žirnių derlius gali padidėti iki 40 %.

SiO_2 ND parodė reikšmingą teigiamą poveikį žirnių augalams, kai jie augo substrate, kuriame nėra pakankamai drėgmės (3 lentelė, SD 30 %). Po palaistymo 12,5, 25 ir 50 ppm SiO_2 ND suspensijomis, žirnių aukštis padidėjo apie 23, 20 ir 18 % atitinkamai, o apipurškus – 8, 11 ir 24 %. Lapų plotas padidėjo apie 11 ir 13 %, kai žirniai buvo palaistyti 25 ir 50 ppm tirpalais, bei 12, 18 ir 10 % augalus nupurškus 12,5, 25 ir 50 ppm SiO_2 ND tirpalais. Nustatytas SVK padidėjimas po žirnių palaistymo 12,5 ppm ir 50 ppm SiO_2 ND suspensijomis, kai augalai augo esant 30 % substrato drėgniui. Žirnius paveikus 50 ppm SiO_2 ND tirpalu laistant, reikšmingai padidėjo šaknų ir antžeminės dalies santykis (iki 30 %). Tačiau šaknų ir antžeminės dalies santykis sumažėjo žirnius nupurškus 12,5 ir 25 ppm SiO_2 ND tirpalais. SiO_2 ND nepaveikė žirnių augusių sausros sąlygomis SLP, išskyrus augalus, nupurkštus 25 ppm tirpalu. Sausros paveiktų žirnių šaknyse susiformavo daugiau gumbelių (30 %) juos palaisčius 50 ppm SiO_2 ND suspensija, o nupurškus 12,5, 25 ir 50 ppm tirpalais – 33 %, 50 % ir 180 %. Žirnių augusių drėgmės trūkumo sąlygomis derlius reikšmingai padidėjo (iki 45 %) juos paveikus SiO_2 ND suspensijas.

Nanodalelių poveikis oksidacinio streso biožymenims

SiO_2 ND padidino H_2O_2 koncentraciją žirnių lapuose, palaisčius 12,5 ppm 25 ppm ir 50 ppm, bei nupurškus 12,5 ppm tirpalais, kai augalai augo esant 80 % substrato drėgniui (3 lentelė, SD 80 %). Žinant, kad H_2O_2 augalams reikia nedideliais kiekiais, galima teigti, kad toks padidėjimas yra optimalus augalams ir nesukėlė neigiamo atsako. MDA kiekis žirnių lapuose žymiai sumažėjo, kai žirniai buvo paveikti 50 ppm SiO_2 ND suspensija (3 lentelė, SD 80 %).

Žirniuose paveiktuose sausros streso, H_2O_2 kiekis padidėjo iki pavojingos augalui koncentracijos, sukeliančios ląstelėse disbalansą (3 lentelė, SD 30 %). Juose H_2O_2 koncentracija padidėjo 2,5 karto, lyginant su augalais, augusiais normalaus drėgnio substrate. Tačiau augalų laistymas SiO_2 ND tirpalais sumažino H_2O_2 koncentraciją iki 27 %, o purškimas – 21 %. Žirnių lapuose MDA koncentracija padidėjo daugiau nei 26 %, kai augalai buvo veikiami sausros, palyginti su augusiais 80 % SD. Pažymėtina, kad žirniuose paveiktuose sausros ir SiO_2 ND tirpalų, MDA kiekis žymiai sumažėjo, lyginant su ND nepaveiktais augalais.

Nanodalelių poveikis nefermentiniams antioksidantams

Nustatytas SiO_2 ND slopinamas poveikis žirnių antioksidacinei sistemai, kurie augo 80 % drėgnio substrate (3 lentelė, SD 80 %). TPC kiekis sumažėjo 17 %, augalus palaisčius 12,5 ppm koncentracijos suspensija, o nupurškus SiO_2 ND tirpalais sumažėjo iki 23 %. Statistiškai patikimą slopinantį poveikį FRAP antioksidacinei galiai sukėlė augalų apdorojimas SiO_2 ND suspensijomis. Poveikis DPPH laisvųjų radikalų surišimo aktyvumui buvo daugialypis, po poveikio 25 ppm tirpalu aktyvumas sumažėjo apie 13 %, tačiau po augalų nupurškimo 12,5 ppm

suspensija padidėjo 25 %. ABTS laisvųjų radikalų šalinimo aktyvumas žymiai padidėjo žirniuose, augusiuose normalaus drėgnio substrate, nupurškus 50 ppm SiO_2 ND suspensija.

Priešingi rezultatai gauti tiriant SiO_2 ND poveikį žirnių augalams, augusiems sumažinto drėgnio substrate (3 lentelė, 30 % SD). Žirnių lapuose nustatytas reikšmingas 16, 18 ir 23 % TPC padidėjimas palaisčius, o 20, 25 ir 30 % TPC padidėjimas nupurškus augalus 12,5, 25 ir 50 ppm SiO_2 ND suspensijomis. Rezultatai parodė, kad laistymas 50 ppm SiO_2 ND tirpalu, DPPH laisvųjų radikalų surišimo gebą padidino apie 35 %, o purškimas iki 23 %. FRAP antioksidacinė geba padidėjo iki 20 % augalus palaisčius 25 ir 50 ppm SiO_2 ND ir iki 19 % nupurškus, lyginant su sausra paveiktais žirniais. SiO_2 ND ir sausra ABTS laisvųjų radikalų surišimo aktyvumą padidino 69, 82 ir 86 %, augalus palaisčius 12,5, 25 ir 50 ppm SiO_2 ND tirpalais. Tačiau po nupurškimo tik 50 ppm suspensija padidino ABTS aktyvumą iki 26 %.

Nanodalelių poveikis fermentiniams antioksidantams

12,5 ir 25 ppm SiO_2 ND suspensijos statistiškai patikimai stimuliavo CAT aktyvumą nupurškus žirnius, augusius esant pakankamam substrato drėgniui (3 lentelė, SD 80 %). APX ir GR fermentams nustatytas slopinantis poveikis, APX aktyvumas sumažėjo iki 38 % po poveikio 50 ppm suspensija, o GR aktyvumas – iki 13 %, kai žirniai buvo palaistyti 50 ppm SiO_2 ND suspensija. Ypač stiprus poveikis nustatytas GPX ir SOD fermentų veiklai. GPX aktyvumas padidėjo 9, 8 ir 5 kartus palaisčius žirnius 12,5, 25 ir 50 ppm SiO_2 ND suspensijomis, o po purškimo visos naudotos koncentracijos didino GPX aktyvumą 9 kartus. SOD aktyvumas taip pat padidėjo augalus paveikus SiO_2 ND tirpalais: laistant iki 47 %, purškiant iki 48 %.

Žirniuose augusiuose drėgmės trūkumo sąlygomis, CAT aktyvumas padidėjo 36, 84 ir 153 % palaisčius ir 13, 41 ir 119 % nupurškus augalus 12,5, 25 ir 50 ppm SiO_2 ND suspensijomis (3 lentelė, 30 % SD). Didėjant SiO_2 ND koncentracijai, SOD aktyvumas po laistymo padidėjo iki 37 %, o po purškimo – iki 23 % sausros paveiktuose žirnių augaluose. GR aktyvumui teigiamos įtakos turėjo laistymas 25 ir 50 ppm SiO_2 ND suspensijomis, o nupurškus SiO_2 ND suspensija, sausros paveiktus žirnius, GR aktyvumas padidėjo per 128 %. Pažymėtina, kad buvo nustatytas APX ir GPX aktyvumo sumažėjimas. APX aktyvumas sumažėjo apie 37 % ir 16 % po laistymo 12,5 ir 25 ppm SiO_2 ND suspensijomis. GPX aktyvumo slopinimas (19 %) nustatytas sausros paveiktus žirnius laistant 12,5 ppm SiO_2 ND suspensija, o purškimas slopino aktyvumą iki 30 %.

3 lentelė. SiO_2 ND (12,5; 25; 50 ppm) poveikis sėjamajam žirniui (P. sativum L.) augusiam skirtingo drėgnio substratuose (SD 80 %; SD 30 %), išreiškiamas procentiniu pokyčiu (%), lyginant su kontrole (80 % SD kontrolė reiškia augalus, auginamus SD 80 % ir neapdorotus su ND; SD 30 % kontrolė reiškia sausros paveiktus, bet neapdorotus su ND augalus) poveikio stiprumo žemėlapyje. Statistiškai reikšmingi skirtumai pažymėti pajuodintu šriftu Tukey HSD testą ($p<0,05$)

SiO_2 ND poveikis, ppm	SD 80 %						SD 30 %					
	Laistymas			Purškimas			Laistymas			Purškimas		
	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Augalų aukštis	6	**15**	**21**	4	3	3	**23**	**20**	**18**	**8**	**11**	**24**
Lapų plotas	1	**4**	**31**	**8**	**9**	**15**	0	**11**	**13**	**12**	**18**	**10**
Gumbeliai	**200**	**300**	**460**	100	**140**	100	0	0	**33**	**33**	**50**	**183**
SVK	1	4	**6**	3	2	3	**7**	**7**	2	**3**	**4**	**10**
Š/A	**7**	**34**	**68**	**70**	**23**	**4**	**27**	**14**	**30**	-3	**-8**	16
SLP	-11	-3	-9	**-13**	6	-3	-24	-20	-4	2	**23**	-25
Derlius	-2	-5	**40**	-14	4	**38**	**37**	**37**	**44**	**40**	**35**	**45**
ABTS	-1	2	6	3	1	**8**	**69**	**82**	**86**	-7	5	**26**
DPPH	2	**-13**	-3	**25**	**-15**	5	1	9	**35**	**11**	**19**	**23**
TPC	**-17**	-4	-4	**-21**	**-23**	**-12**	**16**	**18**	**23**	**20**	**25**	**30**
FRAP	**-16**	**-12**	**-19**	**-18**	**-11**	**-28**	3	**20**	**20**	2	**7**	**19**
HP	**26**	**20**	**12**	**78**	0	**-9**	**-15**	**-25**	**-27**	1	**-13**	**-21**
MDA	**15**	**5**	**-9**	**4**	1	**-5**	**-15**	**-18**	**-19**	**-3**	**-9**	**-13**
GR	2	2	**-13**	7	-11	-19	2	**21**	**100**	**46**	**55**	**128**
GPX	**914**	**841**	**525**	**919**	**965**	**912**	-19	0	6	**-29**	**-30**	**-4**
APX	16	-12	**-32**	13	-24	**-38**	**-37**	**-16**	-5	**-65**	**-37**	**-25**
SOD	**49**	**44**	**47**	**42**	**47**	**48**	**5**	**32**	**37**	**7**	**18**	**23**
CAT	15	8	**-16**	**93**	**89**	19	**36**	**84**	**153**	**13**	**41**	**119**

SVK – santykinis vandens kiekis, Š/A - šaknų ir antžeminės dalies santykis, SLP – specifinis lapų plotas, TPC – suminis fenolinių junginių kiekis, HP – vandenilio peroksidas, MDA – malondialdehidas, GR – glutationo reduktazė, GPX – gvajakolio peroksidazė, APX – askorbato peroksidazė, SOD – superoksido dismutazė, CAT – katalazė. , Cu kiekis lapuose, stiebe ir šaknyje. 0 – kontroliniai augalai laistomi dejonizuotu vandeniu, sausros stresas – 30 % substrato drėgmės.

3.2.2 Vario oksido nanodalelių poveikis žirniams augusiems skirtingomis substrato drėgnio sąlygomis

Nanodalelių poveikis žirnių morfologiniams parametrams

4 lentelėje pateikti rezultatai rodo, kad vario oksido (CuO) ND neturėjo įtakos augalų, augusių 80 % SD, aukščiui, SLP ir šaknų bei antžeminės dalies santykiui. Lapų plotas sumažėjo 13 % palaisčius 25 ar 50 ppm CuO ND suspensijomis. Žirnius nupurškus 12,5 ir 25 ppm CuO ND suspensijomis, lapų plotas šiek tiek sumažėjo. SVK sumažėjo, augalus paveikus 50 ppm CuO ND tirpalu. Žirnių, augusių su 80 % substrato drėgme, derliui nebuvo nustatytas reikšmingas pokytis.

Didžiausia CuO ND koncentracija (50 ppm) ženkliai sumažino žirnių aukštį laistant ar purškiant CuO ND jiems augant nepakankamo drėgnio substrate (4 lentelė, 30 % SD). Augalus laistant 25 ir 50 ppm suspensijomis, lapų plotas sumažėjo 14 ir 18 %, o purškiant 25 ppm suspensija – 20 %. SLP sumažėjo 12 % augalus palaisčius 12,5 ir 25 ppm CuO ND tirpalais. CuO ND turėjo teigiamą poveikį gumbelių susidarymui ant žirnių šaknų, kai augalai buvo

paveikti 50 ppm CuO ND koncentracija. Nustatytas derliaus padidėjimas apie 27 % augalus laistant ir 47 % juos purškiant CuO ND lyginant su sausros streso paveiktais augalais.

Nanodalelių poveikis oksidacinio streso biožymenims

CuO ND turėjo stiprų poveikį tiek H_2O_2, tiek MDA kaupimuisi žirnių augaluose, kurie augo pakankamos drėgmės substrate (4 lentelė, SD 80 %). H_2O_2 kiekis padidėjo 70, 41 ir 16 % žirnius laistant 12,5, 25 ir 50 ppm CuO ND suspensijomis, o purškiant – 45, 74 ir 72 %, atitinkamai. MDA kiekis taip pat reikšmingai padidėjo (54 %) žirnius paveikus CuO ND tirpalais. Iš šių rezultatų galime daryti išvadą, kad CuO ND stipriai aktyvuoja lipidų peroksidaciją ir gali sukelti oksidacinį stresą augaluose.

Tačiau CuO ND poveikis H_2O_2 kiekiui augale pasikeitė kartu su sausros poveikiu (4 lentelė. 30 % SD). H_2O_2 kiekis žymiai sumažėjo, žirnius paveikus skirtingų CuO ND koncentracijų tirpalais. Tiek po laistymo, tiek po purškimo CuO ND tirpalais, MDA kiekis žirniuose didėjo iki 66 %.

Nanodalelių poveikis nefermentiniams antioksidantams

Žirnių laistymas 12,5 ppm CuO ND tirpalu žymiai sumažino TPC kiekį, bet nustatytas padidėjimas paveikus 25 ir 50 ppm koncentracijų tirpalais (4 lentelė. 80 % SD). Žirnių purškimas 12,5 ir 25 ppm CuO ND tirpalais, sumažino TPC kiekį 17 ir 10 %, atitinkamai. ABTS laisvųjų radikalų surišimo aktyvumo slopinimas buvo nustatytas palaisčius žirnius 25 ir 50 ppm arba nupurškus 12,5 ir 25 ppm CuO ND suspensijomis, lyginant su kontroliniais augalais. DPPH laisvųjų radikalų šalinimo aktyvumas sumažėjo 36 ir 13 %, kai žirniai buvo palaistyti 12,5 ir 50 ppm CuO ND suspensijomis (4 lentelė. 80 % SD). Tačiau reikšmingas DPPH laisvųjų radikalų šalinimo aktyvumo stimuliavimas nustatytas žirnius nupurškus 25 ir 50 ppm CuO ND suspensijomis. FRAP antioksidacinė geba žirnių lapuose padidėjo iki 3 kartų juos palaisčius ir iki 2 kartų nupurškus CuO ND suspensijomis.

Žirniuose, kurie buvo auginti 30 % SD ir CuO ND buvo nustatyta daugiau teigiamų rodiklių lyginant su augalais augintais. Pavyzdžiui, TPC kiekis žirnių lapuose žymiai padidėjo, kai sausros paveikti žirniai buvo laistomi arba purškiami bet kokia CuO ND koncentracija. Be to, DPPH laisvųjų radikalų surišimo aktyvumas didėjo didėjant CuO ND koncentracijai. ABTS laisvųjų radikalų surišimo aktyvumas buvo stimuliuojamas iki 70 % priklausomai nuo naudotos koncentracijos laistant ir iki 50 % purškiant CuO ND tirpalais. Nustatytas reikšmingas ABTS laisvųjų radikalų surišimo aktyvumo sumažėjimas nupurškus augalus 12,5 ppm CuO ND suspensija. Žirnių lapuose teigiamas poveikis FRAP antioksidacinei galiai pastebėtas augalus paveikus visomis CuO ND koncentracijoms.

Nanodalelių poveikis fermentiniams antioksidantams

Stiprus slopinamasis poveikis CAT ir GR aktyvumui nustatytas žirnių lapuose, kai augalai augo pakankamos drėgmės substrate (4 lentelė. 80 % SD). CAT aktyvumas sumažėjo iki 65 % žirnius paveikus CuO ND suspensijomis. Be to, GR aktyvumas sumažėjo 37, 27 ir 17 %, žirnius palaisčius 12,5, 25 ir 50 ppm CuO ND suspensijomis, atitinkamai. Žymus aktyvumo skatinimas nustatytas APX fermentui, nes palaisčius CuO ND suspensijomis ji padidėjo iki 5,6 karto, o nupurškus – 4,6 karto. GPX aktyvumas reikšmingai padidėjo iki 94 % palaisčius augalus bet kokios koncentracijos CuO ND tirpalu, o purškiant reikšmingą poveikį turėjo tik 25 ppm CuO ND tirpalas. SOD aktyvumas padidėjo, žirnius laistant 25 ir 50 ppm, ir purškiant 12,5 ppm CuO ND tirpalais.

Esant sausrai, žirnių lapuose nustatytas CuO ND neigiamas poveikis GR aktyvumui (4 lentelė. 30 % SD), sumažinant jį iki 93 %. Taip pat slopinantis CuO ND suspensijų poveikis nustatytas GPX aktyvumui. GPX aktyvumo padidėjimas nustatytas tik augalus palaisčius 25 ppm CuO ND suspensija. CAT aktyvumas padidėjo iki 1,8 karto naudojant bet kurią CuO ND koncentraciją. Be to, APX aktyvumas taip pat buvo žymiai paskatintas žirnius paveikus CuO ND suspensijomis. Žirnių laistymas CuO ND reikšmingai padidino SOD aktyvumą (iki 33 %), kuris priklausė nuo naudotos koncentracijos, be to, purškiant 50 ppm tirpalu SOD aktyvumas padidėjo 53 %.

Makro- ir mikroelementų sudėties pokyčiai

Vario (Cu) kiekis žirnių lapuose, augusiuose substrate su pakankamu drėgmės kiekiu, padidėjo 24 % juos palaisčius 50 ppm CuO ND suspensija (4 lentelė. 80 % SD). Cu kiekis žirnių lapuose padidėjo juos paveikus CuO ND tirpalais: 12,5 ppm – 49 %, 25 ppm – 112 %, o 50 ppm – 233 %, ta pati tendencija buvo pastebėta Cu kaupimuisi žirnio stiebe. Nustatytas Cu kiekio padidėjimas žirnių šaknyse 59, 101 ir 159 % , augalus palaisčius, o dar daugiau jo susikaupė – 77, 113 ir 230 % juos nupurškus 12,5, 25 ir 50 ppm CuO ND suspensijomis, atitinkamai.

4 lentelė. CuO ND (12,5; 25; 50 ppm) poveikis sėjamajam žirniui (P. sativum L.) augusiam skirtingo drėgnio substratuose (SD 80 %; SD 30 %), išreiškiamas procentiniu pokyčiu (%), lyginant su kontrole (80 % SD kontrolė reiškia augalus, auginamus SD 80 % ir neapdorotus su ND; SD 30 % kontrolė reiškia sausros paveiktus, bet neapdorotus su ND augalus) poveikio stiprumo žemėlapyje. Statistiškai reikšmingi skirtumai pažymėti pajuodintu šriftu Tukey HSD testą (p<0,05)

CuO ND poveikis, ppm	SD 80 %						SD 30 %					
	Laistymas			Purškimas			Laistymas			Purškimas		
	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Augalų aukštis	-2	0	-4	-6	-4	-8	5	0	**-9**	2	5	**-17**
Lapų plotas	-7	**-12**	**-13**	**-21**	**-19**	8	1	**-14**	**-18**	12	**-20**	-1
Gumbeliai	20	-40	**220**	-60	**120**	**180**	-83	-17	**83**	17	-100	**283**
SVK	2	2	**-5**	-3	-3	**-4**	-1	-7	-15	-3	-4	-9
Š/A	25	-29	-1	62	-20	-36	22	-24	-24	-18	-9	-34
SLP	-26	-24	-17	-24	12	10	**-12**	**-13**	4	13	-7	-3
Derlius	-4	-2	-1	-9	-11	-7	2	1	**27**	7	14	**47**
ABTS	**7**	**-15**	**-45**	**-13**	**-13**	**26**	**55**	**70**	**60**	**-54**	**50**	**48**
DPPH	**-36**	-5	**-13**	-7	**6**	**18**	**17**	**21**	**56**	6	13	**70**
TPC	**-4**	**4**	**4**	**-17**	**-10**	1	**6**	**10**	**27**	**7**	**4**	**15**
FRAP	**177**	**304**	**-13**	**136**	**100**	**190**	**103**	**182**	**224**	**224**	**253**	**220**
HP	**70**	**41**	**16**	**45**	**74**	**72**	**-20**	**-32**	**-23**	**-28**	**-35**	**-37**
MDA	**52**	**54**	**50**	**52**	**77**	**81**	**66**	**51**	**27**	**61**	**62**	**38**
GR	**-37**	**-27**	**-17**	2	**-27**	**-21**	**-70**	**-93**	**-92**	**-68**	**-81**	**-78**
GPX	**90**	**83**	**94**	15	**59**	-4	**-9**	**16**	2	**-23**	**-37**	**-22**
APX	**364**	**556**	**173**	**463**	**320**	**308**	**72**	**25**	**58**	**71**	**19**	**33**
SOD	1	**18**	**21**	**25**	**-5**	**-3**	**33**	**29**	**20**	-3	**-31**	**53**
CAT	**-57**	**-37**	**-63**	**-48**	**-68**	**-65**	**176**	**166**	**128**	**167**	**153**	**153**
Cu (lapai)	1	-11	**24**	**49**	**112**	**233**	-8	-4	-17	**50**	**153**	**419**
Cu (stiebai)	**13**	0	**19**	**50**	**88**	**199**	**14**	**35**	**55**	**69**	**89**	**359**
Cu (šaknys)	**59**	**101**	**159**	**77**	**113**	**230**	**29**	**86**	**94**	**64**	**101**	**166**

SVK – santykinis vandens kiekis, Š/A - šaknų ir antžeminės dalies santykis, SLP – specifinis lapų plotas, TPC – suminis fenolinių junginių kiekis, HP – vandenilio peroksidas, MDA – malondialdehidas, GR – glutationo reduktazė, GPX – gvajakolio peroksidazė, APX – askorbato peroksidazė, SOD – superoksido dismutazė, CAT – katalazė. , Cu kiekis lapuose, stiebe ir šaknyje. 0 – kontroliniai augalai laistomi dejonizuotu vandeniu, sausros stresas – 30 % substrato drėgmės.

Laistymas CuO ND nesukėlė reikšmingų Cu kiekio skirtumų sausros paveiktuose žirnių augalų lapuose (4 lentelė. 30 % SD). Tačiau Cu kiekis lapuose padidėjo iki 55 %, kai sausros veikiami žirniai buvo nupurkšti CuO ND suspensijomis. Sausros paveiktų žirnių šaknyse Cu susikaupė daugiau juos laistant CuO ND tirpalais, lyginant su ND nepaveiktais augalais. Purškimas CuO ND turėjo teigiamą poveikį Cu kaupimuisi visose žirnio dalyse, o Cu kiekis didėjo didėjant CuO ND koncentracijai. Lapuose Cu kiekis padidėjo iki 4 kartų, stiebuose – iki 3,5, šaknyse – iki 1,7 karto.

3.2.3 Molibdeno trioksido nanodalelių poveikis žirniams augusiems skirtingomis substrato drėgnio sąlygomis

Nanodalelių poveikis morfologiniams parametrams

Molibdeno trioksido (MoO_3) ND suspensijos turėjo reikšmingą poveikį žirnių, augusių 80 % drėgnio substrate, morfologiniams parametrams (5 lentelė). Nustatytas padidėjęs žirnių aukštis palaisčius bet kokios koncentracijos MoO_3 ND suspensijomis. 50 ppm MoO_3 ND suspensija paskatino lapų ploto augimą tiek augalus palaisčius (25 %), tiek nupurškus (15 %). 50 ppm MoO_3 ND suspensija turėjo ypatingai stiprų teigiamą poveikį SVK, taip pat šaknų ir antžeminės dalies santykiui. SLP sumažėjo apie 28 % žirniams augant pakankamai drėgname substrate ir juos palaisčius 12,5 ppm MoO_3 ND tirpalu, o nupurškus 12,5 ir 50 ppm suspensijomis, SLP sumažėjo iki 30 %. MoO_3 ND tirpalai turėjo stiprų poveikį gumbelių susidarymui ant šaknų, nes jų skaičius padidėjo 3 ir 5,6 karto palaisčius 25 ir 50 ppm tirpalais ir 1,4 karto didesnis nupurškus 50 ppm MoO_3 ND suspensija, lyginant su kontroliniais augalais. Žirnius nupurškus 50 ppm MoO_3 ND tirpalu pastebėtas reikšmingas derliaus padidėjimas (5 lentelė. SD 80 %).

MoO_3 ND efektyviai sumažino sausros poveikį žirnių morfologiniams parametrams (5 lentelė, 30 % SD). Žirnius palaisčius ar nupurškus MoO_3 ND tirpalais jų aukštis padidėjo iki 40 % ir iki 24 % atitinkamai. MoO_3 ND suspensijos didino augalų lapų plotą, sausros paveiktuose žirniuose. SVK padidėjo 10 %, kai augalai buvo palaistyti 50 ppm MoO_3 ND. Visos MoO_3 ND koncentracijos sumažino SLP. Pastebėta, kad ir sausros sąlygomis buvo nustatytas teigiamas MoO_3 ND poveikis gumbelių formavimuisi. Nustatytas 1,2–5 kartų padidėjimas, kai žirniai buvo laistomi 25 ir 50 ppm, ir 1,2–2 kartų – nupurškus MoO_3 ND. Žirnių, paveiktų sausros, derlius stipriai priklausė nuo naudotos MoO_3 ND koncentracijos, nes padidėjo iki 80 % ir 64 % po palaistymo ir purškimo 50 ppm MoO_3 ND suspensija.

Nanodalelių poveikis oksidacinio streso biožymenims

H_2O_2 kiekis žirnių lapuose padidėjo iki 2 kartų augalus paveikus skirtingų koncentracijų MoO_3 ND suspensijomis (SD 80 %). MoO_3 ND skatino lipidų peroksidaciją žirnių augaluose, augusiuose 80 % SM (5 lentelė. 80 % SD). Tai rodo MDA koncentracijos padidėjimas žirniuose, kai jie buvo paveikti 12,5 ir 25 ppm MoO_3 ND suspensijomis.

Sausros paveiktuose žirniuose H_2O_2 kiekis sumažėjo 8, 12 ir 20 %, augalus palaisčius 12,5, 25 ir 50 ppm MoO_3 ND suspensijomis, bet MDA koncentracija padidėjo 9, 11 ir 25 %,

atitinkamai (5 lentelė. 30 % SD). Purškimas MoO_3 ND suspensijomis turėjo panašų poveikį mažinant H_2O_2 kiekį žirnių lapuose. Sumažėjęs MDA kiekis buvo nustatytas žirniuose, purkštuose 50 ppm MoO_3 ND suspensija, lyginant su augalais, augusiais sausros sąlygomis ir neapdorotais ND.

Nanodalelių poveikis nefermentiniams antioksidantams

Pastebėta, kad MoO_3 ND turi dvejopą poveikį nefermentiniams antioksidantams žirniuose, augusiems 80 % SD (5 lentelė). TPC kiekio sumažėjimas iki 20 % nustatytas augalus palaisčius12,5 ppm MoO_3 ND tirpalu, o nupurškus 12,5 ir 25 ppm tirpalu TPC kiekis sumažėjo 23 ir 15 %, atitinkamai. Žirnių lapuose FRAP antioksidacinė geba žymiai padidėjo augalus palaisčius ar nupurškus bet kokios koncentracijos MoO_3 ND tirpalu. Žirnių palaistymas 25 ir 50 ppm MoO_3 ND tirpalais paskatino DPPH laisvųjų radikalų surišimo aktyvumą 27 ir 36 %, atitinkamai. Purškimas 25 ppm MoO_3 ND suspensija sumažino DPPH laisvųjų radikalų surišimo aktyvumą. ABTS laisvųjų radikalų surišimo aktyvumas buvo slopinamas žirnius palaisčius 12,5 ppm ir nupurškus 25 ppm MoO_3 ND suspensijomis.

Sausros paveiktuose žirniuose, MoO_3 ND poveikis nefermentiniams antioksidantams buvo reikšmingas (5 lentelė. 30 % SD). TPC kiekis sausros paveiktuose žirniuose juos palaisčius MoO_3 ND tirpalais padidėjo iki 37 %, o nupurškus – iki 15 %. ABTS laisvųjų radikalų surišimo aktyvumas žirniuose ypač padidėjo – 60, 67 ir 105 % augalus palaisčius 12,5, 25 ir 50 ppm MoO_3 ND tirpalais, atitinkamai. Be to, MoO_3 ND išpurškimas ant augalų padidino ABTS laisvųjų radikalų surišimo aktyvumą iki 70 %. Rezultatai rodo (5 lentelė. 30 % SD), kad laistant MoO_3 ND DPPH laisvųjų radikalų surišimo aktyvumas padidėjo iki 145 %, o purškiant – iki 81 %. Bet kokios koncentracijos MoO_3 ND poveikis buvo reikšmingas FRAP antioksidacinei galiai žirnių lapuose.

5 lentelė. MoO_3 ND (12,5; 25; 50 ppm) poveikis sėjamajam žirniui (P. sativum L.) augusiam skirtingo drėgnio substratuose (SD 80 %; SD 30 %), išreiškiamas procentiniu pokyčiu (%), lyginant su kontrole (80 % SD kontrolė reiškia augalus, auginamus SD 80 % ir neapdorotus su ND; SD 30 % kontrolė reiškia sausros paveiktus, bet neapdorotus su ND augalus) poveikio stiprumo žemėlapyje. Statistiškai reikšmingi skirtumai pažymėti pajuodintu šriftu pagal Tukey HSD testą ($p<0,05$)

MoO_3 ND poveikis, ppm	SD 80 %						SD 30 %					
	Laistymas			Purškimas			Laistymas			Purškimas		
	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50

Augalų aukštis	**6**	**21**	**25**	**7**	**5**	**17**	**10**	**18**	**40**	**4**	**10**	**24**
Lapų plotas	-3	15	**25**	**-9**	2	**15**	-7	-8	**30**	**12**	**10**	**10**
Gumbeliai	40	**320**	**560**	40	20	**140**	-50	**117**	**533**	-17	**117**	**183**
SVK	**5**	**6**	**8**	**3**	**5**	**7**	13	9	**21**	7	7	10
Š/A	-1	2	22	**33**	**-15**	11	-45	1	23	36	-8	16
SLP	**-28**	-22	-24	**-34**	-6	**-30**	-12	**-30**	**-29**	-12	-18	**-25**
Derlius	**6**	**5**	**6**	1	-12	**7**	**11**	**26**	**80**	**3**	**15**	**64**
ABTS	**-11**	-5	2	-3	**-8**	4	**60**	**67**	**105**	**43**	**65**	**70**
DPPH	5	**27**	**36**	6	**-7**	13	**59**	**53**	**145**	**36**	**76**	**81**
TPC	**-20**	4	4	**-23**	**-15**	1	**20**	**28**	**37**	-1	**22**	**15**
FRAP	**117**	**141**	**202**	**111**	**146**	**190**	**231**	**241**	**242**	**55**	**100**	**142**
HP	**109**	**101**	**122**	**68**	**120**	**65**	**-8**	**-12**	**-20**	**-9**	**-12**	**-30**
MDA	**18**	-2	**13**	**19**	**24**	**5**	**9**	**11**	**25**	**13**	-1	**-17**
GR	**-70**	**-59**	**-56**	-33	**-52**	**-62**	**-56**	**-57**	**-31**	**-88**	**-79**	**-71**
GPX	**161**	**76**	**28**	**110**	**142**	**168**	-11	**43**	**55**	**17**	-2	**56**
APX	**692**	**899**	**607**	**423**	**481**	**748**	**198**	**490**	**622**	**216**	**276**	**422**
SOD	**25**	**65**	**19**	5	**24**	**35**	-2	**-15**	**17**	-10	0	**20**
CAT	-16	-19	-4	**41**	-10	-2	**234**	**215**	**224**	**161**	**191**	**183**
Mo (lapai)	**14**	**27**	**43**	**14**	**26**	**47**	**160**	**234**	**310**	**27**	**421**	**454**
Mo (stiebai)	**10**	**34**	**34**	**29**	**44**	**46**	**201**	**205**	**215**	**114**	**122**	**136**
Mo (šaknys)	**200**	**213**	**360**	**130**	**177**	**184**	**21**	**28**	**69**	**38**	**56**	**73**

SVK – santykinis vandens kiekis, Š/A - šaknų ir antžeminės dalies santykis, SLP – specifinis lapų plotas, TPC – suminis fenolinių junginių kiekis, HP – vandenilio peroksidas, MDA – malondialdehidas, GR – glutationo reduktazė, GPX – gvajakolio peroksidazė, APX – askorbato peroksidazė, SOD – superoksido dismutazė, CAT – katalazė. , Cu kiekis lapuose, stiebe ir šaknyje. 0 – kontroliniai augalai laistomi dejonizuotu vandeniu, sausros stresas – 30 % substrato drėgmės.

Nanodalelių poveikis fermentiniams antioksidantams

Stiprus MoO_3 ND poveikis fermentinių antioksidantų aktyvumui nustatytas augaluose, augusiuose pakankamo drėgnio substrate (80 %) (5 lentelė). CAT aktyvumas padidėjo 41 % žirnius nupurškus 12,5 ppm MoO_3 ND suspensija. 12,5, 25 ir 50 ppm MoO_3 ND koncentracijos sukėlė žymiai didesnį APX aktyvumą lyginant su ND nepaveiktas augalais. Nustatyta, kad GR aktyvumas labai sumažėjo po poveikio MoO_3 ND suspensijomis. MoO_3 ND turėjo stiprų poveikį GPX aktyvumui žirnių lapuose, kuris priklausė nuo koncentracijos ir naudojimo būdo.

Sausros poveikis kartu su MoO_3 ND skatino APX aktyvumą žirnių lapuose (5 lentelė. 30 % SD). Žirnius palaisčius 12,5, 25 ir 50 ppm MoO_3 ND suspensijomis, nustatytas APX aktyvumo padidėjimas iki 6 kartų, o nupurškus iki 4 kartų. Panašūs rezultatai nustatyti ir CAT aktyvumui žirnių lapuose. Žirnius palaisčius 12,5, 25 ir 50 ppm MoO_3 ND tirpalais, CAT aktyvumas padidėjo 2 kartus, o nupurškus CAT aktyvumas padidėjo nuo 1,6 iki 1,9 karto. Žirnių laistymas 12,5, 25 ir 50 ppm MoO_3 ND suspensijomis GR aktyvumą sumažino apie 56,

57 ir 31 %, o purškimas - 88, 79 ir 71 %, atitinkamai. Sausros paveiktuose žirniuose GPX aktyvumas padidėjo iki 43 ir 55 % augalus palaisčius 25 ir 50 ppm MoO_3 ND suspensijomis. GPX aktyvumas padidėjo 17 ir 56 % kai augalai buvo nupurkšti 12,5 ir 50 ppm MoO_3 ND suspensijomis. SOD aktyvumas buvo slopinamas, žirnius palaisčius 25 ppm, ir skatinamas palaisčius ar nupurškus 50 ppm MoO_3 ND suspensija.

Makro- ir mikroelementų sudėties pokyčiai

Mo kiekis žirnių lapuose didėjo didėjant MoO_3 ND koncentracijai suspensijoje (5 lentelė). Žirnių lapuose, augusiuose normaliomis sąlygomis (SD 80 %), ir juos laistant MoO_3 ND nustatytas Mo kiekio padidėjimas iki 43 %, o purškiant - iki 47 %. Panaši tendencija pastebėta ir Mo kiekio kaupimuisi žirnio stiebe. Šaknyse Mo susikaupė iki3,6 karto daugiau, kai žirniai buvo laistomi ir iki 1,8 karto daugiau juos purškiant MoO_3 ND tirpalais.

Sausros ir MoO_3 ND poveikis ypatingai padidino Mo kiekį žirnių lapuose ir stiebuose. Laistymas MoO_3 ND suspensijomis nulėmė iki 3 kartų didesnį Mo kiekį lapuose, o purškimas padidino Mo kiekį iki 4,5 karto.

3.2.4 Boro nanodalelių poveikis žirniams augusiems skirtingomis substrato drėgnio sąlygomis

Poveikis morfologiniams parametrams

Žirnių augusių normalaus drėgnio substrate (80 % SD) aukštis padidėjo 14 ir 27 %, kai jie buvo palaistyti 12,5 ir 50 ppm B_2O_3 ND suspensijomis, o nupurškus – 28 ir 19 %, atitinkamai (6 lentelė, 80 % SD). Lapų plotas taip pat reikšmingai padidėjo, augalus palaisčius 12,5 ir 50 ppm B_2O_3 ND suspensijomis, o nupurškus lapų ploto padidėjimas nustatytas naudojant visas koncentracijas. Taip pat, buvo nustatytas teigiamas B_2O_3 ND poveikis SVK. Augalus nupurškus 12,5 ppm B_2O_3 ND tirpalu, SLP sumažėjo 15 %. Šaknų ir antžeminės dalies santykis statistiškai patikimai padidėjo augalus palaisčius šiomis suspensijomis 12,5 ppm (21 %), 25 ppm (36 %), ir 50 ppm (68 %). Tai rodo, kad augaluose, augusiuose normalaus drėgnio substrate (80 % SD) B_2O_3 ND koncentracijos didėjimas skatino šaknų augimą. Be to, teigiamas poveikis buvo pastebėtas ir augalus nupurškus 12,5 ppm, 25 ppm ir 50 ppm B_2O_3 ND suspensijomis, šaknų ir antžeminės dalies santykis padidėjo 68 %,– 18 %, ir– 34 %, atitinkamai. Rezultatai taip pat rodo, kad B_2O_3 ND teigiamai veikė gumbelių formavimąsi ant augalų šaknų, padidindami jų kiekį iki 5,6 karto žirnius palaisčius ir iki 3,4 juos nupurškus. Žirnių laistymas 50 ppm B_2O_3 ND tirpalu turėjo reikšmingą teigiamą poveikį derliui, o purškiant žirnių derlius padidėjo, kai buvo naudotos, 12,5 ir 25 ppm B_2O_3 ND suspensijos.

B_2O_3 ND stipriai paveikė žirnių augalus, augusius sausros streso sąlygomis (6 lentelė, 30 % SD). Skirtingų koncentracijų B_2O_3 ND suspensija padidino augalų aukštį, kai jie buvo laistomi ar purškiami. Be to, laistant augalus 25 ir 50 ppm B_2O_3 ND suspensijomis, lapų plotas padidėjo atitinkamai 30 ir 40 %. Esant didesnei B_2O_3 ND koncentracijai, statistiškai reikšmingai padidėjo SVK. Sausros paveiktuose žirniuose, šaknų ir antžeminės dalies santykis padidėjo iki 30 % augalus palaisčius ir iki 14 % juos nupurškus B_2O_3 ND tirpalais. 50 ppm B_2O_3 ND koncentracija padidino šaknų gumbelių skaičių 3 kartus augalus palaisčius ir iki 6 kartų - nupurškus. Laistymas 12,5 ir 25 ppm B_2O_3 ND suspensija turėjo teigiamą poveikį žirnių derliui, o purškiant B_2O_3 ND sausros paveiktus žirnius derlius padidėjo iki 16 %.

Nanodalelių poveikis oksidacinio streso biožymenims

Rezultatai rodo, kad B_2O_3 ND poveikis padidino H_2O_2 kiekį augaluose, juos laistant skirtingų koncentracijų tirpalais, kai žirniai augo esant pakankamam substrato drėgniui (6 lentelė, 80 % SD). Nupurškus augalus, nustatytas statistiškai patikimas 65 % H_2O_2 kiekio padidėjimas, kai žirniai buvo paveikti 12,5 ppm B_2O_3 ND koncentracija. Žirnių lapuose nustatytas reikšmingas MDA koncentracijos sumažėjimas, augalus paveikus bet kokia B_2O_3 ND koncentracija.

Sausros ir B_2O_3 ND paveiktuose augaluose nustatytas reikšmingas H_2O_2 ir MDA slopinimas (6 lentelė, 30 % SD). Apipurškus augalus 12,5, 25 ir 50 ppm B_2O_3 ND tirpalais, H_2O_2 kiekis sumažėjo 18, 24 ir 45 %, o palaisčius – 22, 37 ir 9 %, atitinkamai. MDA koncentracija sumažėjo 22, 13 ir 17 %, žirnius palaisčius, o nupurškus – 20, 25 ir 22 % .kai B_2O_3 ND suspensijos buvo šių koncentracijų 12,5, 25 ir 50 ppm.

Nanodalelių poveikis nefermentiniams antioksidantams

Nustatyta, kad tiek laistymas, tiek purškimas B_2O_3 ND sumažino TPC kiekį žirnių lapuose iki 30 % (6 lentelė, 80 % SD). B_2O_3 ND nepaveikė ABTS laisvųjų radikalų surišimo aktyvumo žirniuose. Tačiau nustatyta, kad žirnius nupurškus 25 ir 50 ppm B_2O_3 ND suspensijomis, DPPH laisvųjų radikalų surišimo aktyvumas padidėjo 25 ir 24 %, atitinkamai. Be to, laistymas ar purškimas 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis didino FRAP antioksidacinę galią, kai augalai augo 80 % SD.

Rezultatai parodė(6 lentelė, 30 % SD), kad purškiant sausros paveiktus žirnius 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis turi teigiamą poveikį nefermentiniams antioksidantams, pavyzdžiui TPC kiekis padidėjo iki 18 %, tačiau laistymas 12,5 ppm suspensija žymiai sumažino TPC kiekį. ABTS laisvųjų radikalų surišimo aktyvumas padidėjo iki 73 % augalus laistant ir iki 96 % juos purškiant B_2O_3 ND tirpalais. Panaši tendencija buvo gauta FRAP antioksidacinės galios rezultatuose. Sausros ir 12,5 bei 25 ppm B_2O_3 ND suspensijų laistymas

padidino (20 %) DPPH laisvųjų radikalų surišimo aktyvumą. O nupurkšti 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis augalai su pasižymėjo DPPH laisvųjų radikalų surišimo aktyvumo padidėjimu apie 35, 24 ir 25 %, atitinkamai.

Nanodalelių poveikis fermentiniams antioksidantams

B_2O_3 ND sukėlė CAT, APX, SOD ir GPX aktyvumą žirnių lapuose, kai jie augo normalaus drėgnio substrate (80 % SD) (6 lentelė). APX aktyvumas ypač padidėjo augalus palaisčius B_2O_3 ND suspensijomis, kiek silpnesnį poveikį sukėlė purškimas. CAT aktyvumas padidėjo iki 2 kartų, augalus palaisčius B_2O_3 ND suspensijomis. Purškiant žirnius 12,5, 25 ir 50 ppm B_2O_3 ND tirpalais, CAT aktyvumas padidėjo 1,3, 1,8 ir 2 kartus. SOD aktyvumas padidėjo 41 % augalus laistant ir 46 % juos purškiant B_2O_3 ND tirpalais. GPX aktyvumas išsiskyrė tuo, kad mažesnės koncentracijos laistant turėjo stipresnį teigiamą poveikį, o purškiant – didesnės. 12,5 ir 50 ppm B_2O_3 ND suspensijos, sumažino GR aktyvumą žirniuose.

Stiprų GR aktyvumo sumažėjimą lėmė sausros ir B_2O_3 ND poveikis – laistant jis sumažėjo apie 55 %, o purškiant – 45 % (6 lentelė, 30 % SD). Taip pat buvo nustatytas slopinantis poveikis SOD aktyvumui žirnius palaisčius 25 ir 50 ppm B_2O_3 ND tirpalais, tačiau SOD aktyvumo padidėjimas (36 %) nustatytas paveikus 12,5 ppm B_2O_3 ND suspensija. Be to, SOD aktyvumo padidėjimas 51 %, gautas sausros streso sąlygomis augusius žirnius nupurškus B_2O_3 ND tirpalu. Palaisčius žirnius B_2O_3 ND suspensijomis, APX suaktyvėjo iki 1,4 karto, o nupurškus – iki 8 kartų. GPX aktyvumas sausros paveiktuose žirniuose padidėjo iki 91 % augalus palaisčius B_2O_3 ND tirpalais, CAT aktyvumas ypač padidėjo laistant arba purškiant augalus bet kokios koncentracijos B_2O_3 ND tirpalais.

Makro- ir mikroelementų sudėties pokyčiai

B kiekis žirnių lapuose, augusių pakankamos drėgmės substrate (6 lentelė, 80 % SD), padidėjo 3, 5 ir 12 kartų laistant ir 2, 3 ir 11 kartų juos nupurškus 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis, atitinkamai. B kiekis stiebe padidėjo 10 kartų augalus palaisčius ir iki 17 kartų juos nupurškus B_2O_3 ND tirpalais. Be to, B kiekis padidėjo 3, 7 ir 20 kartų augalų šaknyse, kurie augo normaliomis substrato drėgmės sąlygomis ir palaistyti 12,5, 25 ir 50 ppm B_2O_3 ND suspensijomis, atitinkamai. Žirnius nupurškus B_2O_3 ND tirpalais, B kiekis šaknyse padidėjo iki 3 kartų.

Sausros paveiktus (30 % SD) žirnius nupurškus 25 ir 50 ppm B_2O_3 ND tirpalais, B kiekis lapuose padidėjo 3 ir 5 kartus, atitinkamai. Be to, B kiekis lapuose padidėjo 3 kartus , kai žirniai buvo palaistyti B_2O_3 ND suspensijomis. Po žirnių palaistymo B_2O_3 ND suspensijomis, stiebuose nustatytas žymus (iki 18 kartų) B kiekio padidėjimas.

6 lentelė. B_2O_3 ND (12,5; 25; 50 ppm) poveikis sėjamajam žirniui (P. sativum L.) augusiam skirtingo drėgnio substratuose (SD 80 %; SD 30 %), išreiškiamas procentiniu pokyčiu (%), lyginant su kontrole (80 % SD kontrolė reiškia augalus, auginamus SD 80 % ir neapdorotus su ND; SD 30 % kontrolė reiškia sausros paveiktus, bet neapdorotus su ND augalus) poveikio stiprumo žemėlapyje. Statistiškai reikšmingi skirtumai pažymėti pajuodintu šriftu pagal Tukey testą ($p<0,05$)

B_2O_3 ND poveikis, ppm	SD 80 %						SD 30 %					
	Laistymas			Purškimas			Laistymas			Purškimas		
	12.5	25	50	12.5	25	50	12.5	25	50	12.5	25	50
Augalų aukštis	**14**	7	**27**	**28**	9	**19**	**9**	**26**	**17**	**13**	**20**	**14**
Lapų plotas	**28**	9	**30**	**38**	**25**	**24**	-17	**30**	**40**	-4	1	-6
Gumbeliai	**560**	**200**	**480**	**340**	**240**	**200**	17	**200**	**317**	0	67	**583**
SVK	**2**	**4**	**5**	**6**	**4**	**4**	-1	**10**	**11**	-2	4	**9**
Š/A	**21**	**36**	**68**	**69**	**18**	**34**	**4**	**14**	**30**	**11**	**14**	**5**
SLP	14	-5	-11	-15	-6	-3	-17	-4	-16	-13	-23	**-29**
Derlius	**-21**	2	**20**	**12**	**11**	-1	**16**	**10**	6	**19**	**16**	**14**
ABTS	-7	-11	-6	7	5	0	**73**	**61**	**56**	**92**	**96**	**74**
DPPH	-5	4	7	0	**25**	**24**	**20**	**8**	3	**35**	**24**	**25**
TPC	**-29**	**-22**	**-22**	**-19**	**-21**	**-25**	**-6**	-1	1	**18**	**5**	**15**
FRAP	**106**	**81**	**83**	**132**	**109**	**166**	**194**	**174**	**141**	**217**	**151**	**148**
HP	**119**	**89**	**109**	**65**	10	6	**-22**	**-37**	**-9**	**-18**	**-24**	**-45**
MDA	**-31**	**-25**	**-14**	**-7**	**-15**	**-23**	**-22**	**-13**	**-17**	**-20**	**-25**	**-22**
GR	**-19**	0	**-19**	**-30**	-8	**-32**	**-53**	**-57**	**-54**	**-45**	**-42**	**-47**
GPX	**147**	**136**	14	11	**71**	**153**	-3	-2	**13**	**81**	**91**	**12**
APX	**1657**	**1100**	**1817**	363	**498**	200	**136**	**33**	**46**	**750**	**634**	**522**
SOD	**29**	**41**	**41**	**18**	**46**	**15**	**36**	**-20**	**-29**	**9**	**9**	**51**
CAT	**173**	**107**	**84**	**131**	**175**	**214**	**161**	**159**	**132**	**91**	**69**	**48**
B (lapai)	**18**	**16**	7	8	4	**20**	-3	**36**	**52**	**-18**	**-20**	**-24**
B (stiebai)	**210**	**794**	**1017**	**221**	**1508**	**1670**	**17**	**63**	**83**	-9	-2	5
B (šaknys)	**344**	**735**	**2062**	**87**	**202**	**315**	**47**	**167**	**253**	**61**	**158**	**217**

SVK – santykinis vandens kiekis, Š/A - šaknų ir antžeminės dalies santykis, SLP – specifinis lapų plotas, TPC – suminis fenolinių junginių kiekis, HP – vandenilio peroksidas, MDA – malondialdehidas, GR – glutationo reduktazė, GPX – gvajakolio peroksidazė, APX – askorbato peroksidazė, SOD – superoksido dismutazė, CAT – katalazė. , Cu kiekis lapuose, stiebe ir šaknyje. 0 – kontroliniai augalai laistomi dejonizuotu vandeniu, sausros stresas – 30 % substrato drėgmės.

3.3 Kompleksinio agrometeorologinės sausros ir sunkiojo metalo vario bei nanodalelių poveikis žirniams

Lauko sąlygomis augalai vienu metu gali patirti keletą skirtingų abiotinių įtampų, pavyzdžiui: sumažėjusį vandens prieinamumą sausros metu ir ekstremalią temperatūrą, pernelyg padidėjusį vandens kiekį potvynių metu, sunkiųjų metalų poveikį, padidėjusį dirvožemio kietumą, ribojantį šaknų augimą ir maistinių medžiagų trūkumą. Įvairūs stresai sukelia skirtingą augalų atsaką, todėl prisitaikydami prie nepalankių aplinkos veiksnių augalai naudoja skirtingus aklimatizacijos mechanizmus. Kai augalą vienu metu veikia keletas nepalankių aplinkos

veiksnių, jų poveikis gali sustiprėti ir augalai patiria stipresnį stresą. Šiame skyriuje analizuojams kompleksinis ND, agrometeorologinės sausros ir sunkiojo metalo vario (Cu) poveikis žirnių augalams.

Silicio dioksido nanodalelių poveikis žirniams, esant kompleksiniam sausros ir vario stresams

SiO_2 ND turėjo stiprų teigiamą poveikį žirniams paveiktiems sausros ir Cu (7 lentelė). Žirnių aukštis, šaknų ir antžeminės dalies santykis, SVK ir derlius padidėjo atitinkamai 24, 86, 24 ir 50 %, augalus palaisčius SiO_2 ND tirpalais esant sausrai ir Cu (LxSxCu). Kai augalus paveikė LxSxCu, DPPH laisvųjų radikalų surišimo aktyvumas ir FRAP antioksidacinė geba žymiai padidėjo, atitinkamai 21 ir 10 %, o TPC kiekis padidėjo apie 21 %. Be to, SiO_2 ND stimuliavo GR ir CAT aktyvumą, atitinkamai 50 ir 67 %, kai augalai augo LxSxCu sąlygomis. Laistymas SiO_2 ND suspensijomis sumažino H_2O_2 ir MDA kiekį lapuose, kai augalai buvo paveikti sausros ir Cu. Taip pat buvo nustatytas bio-koncentracijos faktorius (BCF) padidėjimas tiek lapuose (75 %), tiek šaknyse (94 %), taip pat translokacijos faktorius (Tf) –padidėjo apie 150 %, o tolerancijos indeksas (Ti) – 85 %.

Sausros ir Cu paveiktiems žirniams nustatytas teigiamas SiO_2 ND poveikis, kai jie buvo nupurkšti SiO_2 ND tirpalais (PxSxCu). Augalų aukštis, SVK, šaknų ir antžeminės dalies santykis bei derlius padidėjo atitinkamai 34, 23, 99 ir 44 %. Taip pat SiO_2 ND turėjo teigiamą poveikį antioksidaciniam aktyvumui (ABTS, DPPH, FRAP) ir padidino TPC kiekį žirnių lapuose esant PxSxCu sąlygoms. Tačiau PxSxCu stipriai sumažino fermentų, tokių kaip GPX, APX ir SOD aktyvumą žirniuose. Purškimas SiO_2 ND tirpalais žymiai sumažino H_2O_2 (57) ir MDA (55 %) kiekį žirnių lapuose. Nustatytas BCF padidėjimas tiek lapuose (99 %), tiek šaknyse (81 %), taip pat padidėjo Tf iki 200 %, Ti iki 90 % po poveikio PxSxCu.

7 lentelė. Agrometeorologinės sausros, sunkiojo metalo vario (Cu) ir SiO_2 ND (50 ppm) poveikis sėjamajam žirniui (P. sativum L.). H_2O – kontroliniai augalai nupurkšti arba palaistyti dejonizuotu vandeniu, substrato drėgnis SD) 80 %; sausros stresas – SD 30 %. Rezultatuose pateikiami vidurkiai, o skirtingos raidės pažymi statistiškai patikimus skirtumus tarp variantų pagal Tukey HSD testą ($p < 0,05$)

50 ppm SiO_2 ND poveikis	80 % SD	30 % SD					
	H_2O	H_2O	Cu	Laistymas ND	Purškimas ND	Cu + Laistymas ND	Cu + Purškimas ND
Morfologiniai parametrai							
Augalo aukštis	32,9 a	22,2 cd	20,0 d	28,3 ab	30,4 a	24,9 c	26,8 bc

Lapų plotas	76,9 a	49,9 c	43,9 cd	55,9 b	62,7 ab	44,8 d	50,4 c
Gumbelių sk.	8,3 a	3,7 d	2,0 e	6,0 bc	6,3 b	3,6 d	2,6 de
SVK	80,6 a	41,7 c	29,3 e	47,3 b	45,8 bc	36,3 cd	35,9 cd
Š/A	11,3 a	5,4 bc	4,1 c	6,0 bc	8,9 a	6,8 bc	8,3 ab
SLP	9,5 bc	11,0 ab	13,5 a	8,5 b	9,4 bc	8,4 b	9,6 bc
Derlius	3,4 a	2,4 b	1,8 c	3,3 a	2,6 b	2,7 b	2,6 b
Nefermentinių antioksidantų aktyvumas							
ABTS	113,2 a	103,1 b	96,4 bc	111,4 a	109,1 ab	93,9 c	106,6 a
DPPH	45,1 ab	38,5 c	36,8 cd	45,9 ab	49,2 a	44,5 ab	44,8 ab
TPC	2,7 ab	2,3 bc	1,9 d	2,7 ab	2,9 a	2,3 bc	2,4 b
FRAP	1475,8 a	1158,6 b	939,5 d	1380,3 a	1405,9 a	1027,6 bc	1117,3 bc
Fermentinių antioksidantų aktyvumas							
Gr	0,053 c	0,074 bc	0,084 b	0,041 c	0,027 c	0,126 a	0,094 b
GPX	11,1 c	15,5 b	44,9 a	8,4 d	8,1d	19,3 b	10,6 cd
APX	25,1 e	51,3 b	74,3 a	61,5a	55,6 ab	48,2 cd	34,5 d
SOD	46,8 c	67,2 b	74,5 a	73,9 a	79,7 a	14,7 de	11,1 e
CAT	16,7 d	31,9 c	33,1 c	37,7 ab	15,2 d	55,8 a	43,2 ab
Oksidacinio streso rodikliai							
HP	12,3 e	25,5 ab	26,2 a	21,6 bc	19,6 d	23,1 b	20,3 bc
MDA	54,7 f	62,3 de	97,3 a	89,9 b	76,8 d	83,4 bc	74,7 d
Vario akumuliacija							
BCF (lapai)			0,04 b			0,07 a	0,08 a
BCF (šaknys)			1,6 b			3,1 a	2,9 a
Tf			2,1 b			5,2 a	6,1 a
Ti			62,7 c			116,2 b	119,3 a

BCF – bio-koncentracijos faktorius, Tf – translokacijos faktorius, Ti – tolerancijos indeksas

Vario oksido nanodalelių poveikis žirniams, esant kompleksiniam sausros ir vario stresams

Žirnius veikiant sausrai, Cu ir palaisčius CuO ND tirpalais, nustatytas reikšmingas šaknų ir antžeminės dalies santykio padidėjimas (8 lentelė), be to, reikšmingai sumažėjo lapų plotas ir derlius. Taip pat, nustatytas FRAP antioksidacinės galios ir TPC kiekio sumažėjimas bei statistiškai reikšmingas DPPH ir ABTS laisvųjų radikalų surišimo aktyvumo sumažėjimas žirnių lapuose. CuO ND padidino SOD aktyvumą augaluose paveiktuose sausra ir Cu, o GR, GPX, APX ir CAT fermentų aktyvumo pokyčiams statistinis patikimumas nenustatytas lyginant su augalais paveiktais sausra ir Cu. CuO ND reikšmingai sumažino H_2O_2 kiekį, tačiau MDA

kiekiui žirniuose jokio poveikio nenustatyta. Augalų laistymas CuO ND padidino BCF (13 %) šaknyse, sumažino Tf (33 %) ir neturėjo poveikio Ti.

Kai žirnių augalai buvo nupurkšti CuO ND, gauta daugiau teigiamų rezultatų lyginant su laistymu. Nustatytas SVK padidėjimas iki 44 % augaluose paveiktuose sausra ir Cu bei nupurkštuose CuO ND tirpalais. Šiuose augaluose taip pat nustatytas ABTS laisvųjų radikalų surišimo aktyvumo ir TPC kiekio padidėjimas, o fermentiniams antioksidantams poveikio nenustatyta, be to lapuose žymiai sumažėjo H_2O_2 ir MDA. CuO ND išpurškimas ant augalų neturėjo įtakos lapų ir šaknų BCF bei Ti, tačiau žymiai sumažino Tf.

8 lentelė. Agrometeorologinės sausros, sunkiojo metalo vario (Cu) ir CuO ND (50 ppm) poveikis sėjamajam žirniui (P. sativum L.). H_2O – kontroliniai augalai nupurkšti arba palaistyti dejonizuotu vandeniu, substrato drėgnis SD) 80 %; sausros stresas – SD 30 %. Rezultatuose pateikiami vidurkiai, o skirtingos raidės pažymi statistiškai patikimus skirtumus tarp variantų pagal Tukey HSD testą ($p < 0,05$)

50 ppm CuO ND poveikis	80 % SM	30 % SM					
	H_2O	H_2O	Cu	Laistymas ND	Purškimas ND	Cu + Laistymas ND	Cu + Purškimas ND
Morfologiniai parametrai							
Augalo aukštis	32,9 a	22,2 cd	20,0 d	25,0 b	24,3 b	19,9 d	21,8 cd
Lapų plotas	76,9 a	49,9 b	43,9 c	40,6 cd	46,8 bc	24,3 e	40,2 cd
Gumbelių sk.	8,3 a	3,7 b	2,0 cd	2,1 cd	3,1 c	1,7 d	1,9 cd
SVK	80,6 a	41,7 bc	29,3 d	48,1 b	47,2 b	38,2 c	42,2 bc
Š/A	11,3 a	5,4 bc	4,1 c	10,2 a	6,8 b	8,1 b	6,1 bc
SLP	9,5 cd	11,0 c	13,5 ab	10,0 c	9,2 cd	13,2 b	14,7 a
Derlius	3,4 a	2,4 b	1,8 c	2,4 b	2,7 ab	1,4 de	1,6 cd
Nefermentinių antioksidantų aktyvumas							
ABTS	113,2 a	103,1 ab	96,4 bc	108,7 b	111,5 a	90,7 bc	110,2 a
DPPH	45,1 a	38,5 b	36,8 c	36,6 c	35,9 c	35,2 c	35,3 c
TPC	2,7 a	2,3 bc	1,9 c	2,6 ab	2,6 ab	1,2 d	2,4 b
FRAP	1475,8 a	1158,6 b	939,5 de	1082,9 d	1201,8 c	683,4 f	890,0 e
Fermentinių antioksidantų aktyvumas							
Gr	0,053 c	0,074 b	0,084 ab	0,073 b	0,066 bc	0,094 a	0,083 ab
GPX	11,1 f	15,5 e	44,9 ab	21,4 cd	19,6 cd	47,0 a	37,5 b
APX	25,1 d	51,3 c	74,3 a	63,0 b	63,7 b	70,3 ab	72,3 ab
SOD	46,8 e	67,2 d	74,5 cd	133,3 a	82,6 c	105,8 b	73,8 cd
CAT	16,7 d	31,9 bc	33,1 b	28,0 c	38,5 a	34,0 ab	32,2 bc
Oksidacinio streso rodikliai							

HP	12,3 d	25,5 ab	26,2 ab	24,8 ab	22,4 bc	19,7 c	20,1 c
MDA	54,7 e	62,3 d	97,3 a	84,5 b	81,9 bc	97,1 a	73,3 c
Vario akumuliacija							
BCF (lapai)			0,04 ab			0,05 ab	0,06 a
BCF (šaknys)			1,6 b			1,8 a	1,7 ab
Tf			2,1 a			1,4 c	1,7 b
Ti			62,7 ab			60,2 b	63,0 a

BCF – bio-koncentracijos faktorius, Tf – translokacijos faktorius, Ti – tolerancijos indeksas

Molibdeno trioksido nanodalelių poveikis žirniams, esant kompleksiniam sausros ir vario stresams

9 lentelėje pateikti rezultatai rodo kompleksinio sausros, Cu ir MoO_3 ND poveikį žirniams. Žirnių aukštis padidėjo 29 %, lapų plotas 28 %, gumbelių skaičius 500 %, SVK 44 %, derlius 88 %, šaknų ir ūglių santykis 120 %, o poveikio SLP nenustatyta, kai žirniai buvo veikiami sausros, Cu ir palaistyti 50 ppm MoO_3 ND suspensija (LxSxCu). Taip pat nustatytas reikšmingas ABTS, DPPH ir FRAP verčių padidėjimas. Be to, TPC kiekis žirniuose padidėjo daugiau nei 35 %. Nustatytas GPX ir CAT fermentų aktyvumo padidėjimas žirniuose, kurie buvo veikiami LxSxCu, tačiau nustatytas GR ir SOD aktyvumo sumažėjimas. Be to, žirnių lapuose H_2O_2 ir MDA kiekis sumažėjo apie 22 %. MoO_3 ND padidino Tf iki 44 %, o Ti – 76 %.

Statistiškai patikimi teigiami rezultatai nustatyti gumbelių skaičiui, SVK ir derliui, žirnius veikiant sausra, Cu ir purškiant MoO_3 ND suspensija (PxSxCu). Žirnių lapuose antioksidacinis pajėgumas (DPPH, ABTS ir FRAP) padidėjo iki 13 %, o TPC kiekis – 32 % po poveikio su PxSxCu. Fermentiniams antioksidantams slopinantis poveikis nustatytas tik GR aktyvumui (17 %), o kitų, GPX, APX, SOD ir CAT aktyvumas padidėjo atitinkamai 10, 35, 36 ir 20 %. Nustatyta, kad MDA kiekis žirnių lapuose sumažėjo 23 %. Be to, BCF šaknyse padidėjo apie 25 %, Ti – 13 %, o poveikio Tf nenustatytai.

9 lentelė. Agrometeorologinės sausros, sunkiojo metalo vario (Cu) ir MoO_3 ND (50 ppm) poveikis sėjamajam žirniui (P. sativum L.). H_2O – kontroliniai augalai nupurkšti arba palaistyti dejonizuotu vandeniu, substrato drėgnis SD) 80 %; sausros stresas – SD 30 %. Rezultatuose pateikiami vidurkiai, o skirtingos raidės pažymi statistiškai patikimus skirtumus tarp variantų pagal Tukey HSD testą ($p < 0,05$)

50 ppm MoO_3 ND poveikis	80 % SD	30 % SD					
	H_2O	H_2O	Cu	Laistymas ND	Purškimas ND	Cu + Laistymas ND	Cu + Purškimas ND
Morfologiniai parametrai							
Augalo aukštis	32,9 a	22,2 c	20,0 d	27,8 b	20,0 d	25,8 bc	23,7 bcd

Lapų plotas	76,9 a	49,9 c	43,9 cd	52,1 bc	43,9 cd	56,5 b	43,5 c
Gumbelių sk.	8,3 b	3,7 d	2,0 e	7,0 bc	2,0 d	11,7 a	7,3 bc
SVK	80,6 a	41,7 c	29,3 e	49,4 b	43,0 bc	43,8 bc	33,6 d
Š/A	11,3 a	5,4 bc	4,1 c	6,1 bcd	9,3 ab	8,9 ab	6,1 bc
SLP	9,5 abc	11,0 ab	13,5 a	9,3 bc	14,0 a	9,4 ab	8,5 d
Derlius	3,4 ab	2,4 c	1,8 d	4,2 a	3,8 a	3,4 ab	3,3 ab
Nefermentinių antioksidantų aktyvumas							
ABTS	113,2 a	103,1 bc	96,4 e	110,3 a	96,4 e	105,0 bc	101,8 d
DPPH	45,1 a	38,5 c	36,8 d	42,2 b	36,9 d	42,5 b	41,8 b
TPC	2,7 a	2,3 cd	1,9 d	2,5 b	1,8 e	2,5 b	2,4 bc
FRAP	1475,8 a	1158,6 c	939,5 de	1216,2 b	924,6 de	1184,2 b	1058,2 c
Fermentinių antioksidantų aktyvumas							
Gr	0,053 c	0,074 b	0,084 a	0,089 a	0,070 b	0,072 b	0,070 b
GPX	11,1 c	15,5 bc	44,9 b	15,1 bc	19,6 bc	48,1 a	49,3 a
APX	25,1 d	51,3 c	54,3 c	58,2 b	74,5 a	71,8 ab	72,9 ab
SOD	46,8 e	67,2 c	74,5 b	73,3 b	74,2 b	64,4 cd	101,2 a
CAT	16,7 f	31,9 e	33,1 e	44,4 b	48,9 a	35,3 d	39,6 c
Oksidacinio streso rodikliai							
HP	12,3 e	25,5 bc	26,2 b	21,9 d	26,2 b	30,2 a	25,4 bc
MDA	54,7 e	62,3 c	97,3 a	58,6 d	63,3 c	75,7 b	75,3 b
Vario akumuliacija							
BCF_{Cu} (leaves)			0,04 b			0,06 a	0,05 ab
BCF_{Cu} (roots)			1,6 c			2,3 a	1,8 b
Tf_{Cu}			2,1 b			3,7 a	1,9 b
Ti_{Cu}			62,7 c			131,8 a	112,5 b

BCF – bio-koncentracijos faktorius, Tf – translokacijos faktorius, Ti – tolerancijos indeksas

Boro nanodalelių poveikis žirniams, esant kompleksiniam sausros ir sunkiojo metalo vario stresams

Remiantis 10 lentelėje pateiktais rezultatais, B_2O_3 ND teigiamai veikė kompleksinio streso paveiktų augalų morfologinius parametrus. Palaisčius žirnius, paveiktus sausros ir Cu, B_2O_3 ND suspensijomis (LxSxCu), nustatyta, kad gumbelių skaičius padidėjo 1,5 karto, SVK – 39 %, šaknų ir antžeminės dalies santykis – 75 %, derlius – 56 %. Laistymas 12,5 ppm B_2O_3 ND suspensija, sausros ir Cu paveiktuose žirniuose padidino DPPH laisvųjų radikalų surišimo aktyvumą 18 % ir FRAP antioksidacinę galią 36 %, tačiau ABTS laisvų radikalų surišimo aktyvumui statistiškai reikšmingų rezultatų nenustatyta. TPC kiekis žirnių lapuose padidėjo 34 %. SOD ir CAT aktyvumą teigiamai veikė LxSxCu, o slopinamasis poveikis pasireiškė APX

ir GR fermentams. MDA koncentracija sumažėjo 22 %, o H_2O_2 kiekiui statistiškai reikšmingo poveikio nenustatyta. Nenustatyta statistiškai patikimo poveikio BCF, tačiau Tf po palaistymo B_2O_3 ND tirpalais sumažėjo 14 %, o vario Ti padidėjo 42 %.

Nupurškus žirnius 12,5 ppm B_2O_3 ND suspensija (PxSxCu), žirnių atsparumas sausrai ir Cu padidėjo, nes nustatytas teigiamas poveikis žirnių aukščiui (28 %), SVK (20 %), šaknų ir antžeminės dalies santykiui (64 %) bei derliui (44 %). Derliaus padidėjimą galima sieti su DPPH laisvųjų radikalo surišimo gebos padidėjimu (20 %) ir FRAP antioksidacinės galios padidėjimu (10 %) bei sumažėjusiu MDA kiekiu (38 %) žirnius nupurškus B_2O_3 ND suspensijomis, lyginant su augalais, nepaveiktais ND, bet paveiktais sausros ir Cu. Analizuojant ABTS laisvųjų radikalų surišimo aktyvumą, TPC kiekį, GR aktyvumą ir H_2O_2 kiekį žirnių lapuose, statistiškai patikimų rezultatų nerasta tarp žirnių paveiktų sausra ir Cu ir žirnių paveiktų PxSxCu. Be to, nustatytas stiprus 57 % SOD aktyvumo padidėjimas ir 160 % padidėjęs CAT aktyvumas. Žirnių purškimas 12,5 ppm B_2O_3 ND suspensija paveikė Tf ir Ti, jie padidėjo 33 ir 64 %, atitinkamai.

10 lentelė. Agrometeorologinės sausros, sunkiojo metalo vario (Cu) ir B_2O_3 ND (12,5 ppm) poveikis sėjamajam žirniui (P. sativum L.). H_2O – kontroliniai augalai nupurkšti arba palaistyti dejonizuotu vandeniu, substrato drėgnis SD) 80 %; sausros stresas – SD 30 %. Rezultatuose pateikiami vidurkiai, o skirtingos raidės pažymi statistiškai patikimus skirtumus tarp variantų pagal Tukey HSD testą ($p < 0,05$)

12.5 ppm B_2O_3 ND poveikis	80 % SD	30 % SD					
	H_2O	H_2O	Cu	Laistymas ND	Purškimas ND	Cu + Laistymas ND	Cu + Purškimas ND
Morfologiniai parametrai							
Augalo aukštis	32,9 a	22,2 c	20,0 d	27,2 b	31,2 a	21,0 d	25,6 bc
Lapų plotas	76,9 a	49,9 c	43,9 cd	40,9 c	57,1 b	49,0 c	36,9 d

Gumbelių sk.	8,3 a	3,7 b	2,0 c	5,0 b	7,0 a	5,0 a	3,7 bc
SVK	80,6 a	41,7 b	29,3 d	44,2 bc	38,1 bc	40,7 bc	35,3 bc
Š/A	11,3 a	5,4 bc	4,1 c	7,2 b	5,7 bc	7,2 b	6,7 b
SLP	9,5 bc	11,0 ab	13,5 a	7,4 d	11,1 ab	9,4 bc	8,5 cd
Derlius	3,4 a	2,4 c	1,8 d	2,8 ab	2,7 ab	2,8 ab	2,6 ab
Nefermentinių antioksidantų aktyvumas							
ABTS	113,2 a	103,1 b	96,4 bc	112,3 a	112,1 a	91,9 c	94,9 c
DPPH	45,1 a	38,5 c	36,8 c	43,5 b	47,6 a	43,4 b	43,9 b
TPC	2,7 ab	2,3 cd	1,9 e	2,8 ab	2,9 a	2,6 bc	2,2 de
FRAP	1475,8 a	1158,6 b	939,5 de	979,6 d	1029,4 c	1280,3 a	1033,8 c
Fermentinių antioksidantų aktyvumas							
Gr	0,053 b	0,074 a	0,084 a	0,031 c	0,078 a	0,050 b	0,073 a
GPX	11,1 d	15,5 c	44,9 b	65,2 a	49,4 b	15,1 c	13,6 cd
APX	25,1 f	51,3 d	74,3 a	70,0 ab	57,5 bc	66,5 b	34,2 e
SOD	46,8 e	67,2 d	74,5 cd	100,1 b	117,0 b	146,4 a	116,9 b
CAT	16,7 e	31,9 c	33,1 c	25,2 d	25,8 d	54,8 b	87,4 a
Oksidacinio streso rodikliai							
HP	12,3 c	25,5 ab	26,2 ab	26,4 ab	25,3 ab	23,8 b	26,7 ab
MDA	54,7 f	62,3 e	97,3 a	91,6 b	64,6 d	75,7 c	60,4 e
Vario akumuliacija							
BCF_{Cu} (lapai)			0,04 a			0,03 a	0,04 a
BCF_{Cu} (šaknys)			1,6 b			1,4 bc	1,9 ab
Tf_{Cu}			2,1 b			1,8 c	2,8a
Ti_{Cu}			62,7 c			89,2 b	103,0 a

BCF – bio-koncentracijos faktorius, Tf – translokacijos faktorius, Ti – tolerancijos indeksas

APTARIMAS

Šiame tyrime buvo išaiškintas SiO_2, MoO_3, B_2O_3 ir CuO nanodalelių (ND) poveikis žirnių daigams augusiems optimaliomis sąkygomis, pilnai išsivysčiusiems žirnių augalams augusiems agrometeorologinės sausros bei kompleksinio vario ir agrometeorologinės sausros sąlygomis. Siekiant išsamiai įvertinti ND poveikį žirniams, buvo tiriamas ne tik nefermentinių ir fermentinių antioksidantų atsakas, bet ir kiti rodikliai, bei buvo įvertintas skirtingų ND koncentracijų poveikis augalus purškiant ar laistant. Tyrimai parodė, kad SiO_2, MoO_3, B_2O_3 ir CuO ND teigiamai veikia žirnius ir mažina žalingą agrometeorologinės sausros bei kompleksinių įtempčių poveikį. ND sąveika su augalais lemia fizikinės ir cheminės ND savybės (dydis, paviršiaus krūvis, koncentracija suspensijose) bei pati augalų fiziologija. ND sąveika su

augalais gali lemti specifinių membranos paviršiaus baltymų, receptorių ir transporterių modifikaciją (Juárez-Maldonado ir kt., 2019). Biologinės membranos su hidrofobiniais ir hidrofiliniais komponentais bei netolygiai pasiskirsčiusiais lignino ir celiuliozės pluoštais sukuria netolygų neigiamą paviršiaus krūvį nuo -45 iki -15 mV (Mittal ir kt., 2020). Pastebėta, kad neigiamai įkrautos (neigiamą krūvį turinčios) augalų ląstelių sienelės veikia kaip jonų mainų paviršius, kuris potencialiai skatina katijonų prasiskverbimą. Atsižvelgiant į skirtingus ND pateikimo augalams būdus, pastebėtina, kad kai ND pasiekia augalus per dirvą (šaknis), o dirvožemio dalelės dažniausiai būna neigiamo krūvio, didesnį neigiamą krūvį turinčios ND tokioje dirvoje tampa dar judresnės. Priešingai, teigiamą krūvį turinčios ND lengvai pritraukia neigiamo krūvio dirvožemio paviršiaus daleles. Mažėjant vidutiniam dirvožemio dalelių dydžiui, mažėja ND judrumas. Molio kiekis dirvožemyje gali veikti kaip anijoninis adjuvantas, neleidžiantis kauptis ND ir padidinti jų mobilumą. Todėl labai svarbu ištirti ND suspensijų savybes, kad būtų galima suprasti jų patekimo į augalą ir poveikio mechanizmus. Nanodalelių, kurių zeta potencialas yra nuo –10 iki +10 mV, yra neutralios ir mažiau stabilios nei ND, kurių zeta potencialas didesnis nei +30 mV arba mažesnis nei –30 mV (atitinkamai stiprūs katijonai ir anijonai) (Clogston ir Patri, 2011).

ND gali sukelti įvairias reakcijas augaluose, padidinti fermentų aktyvumą, paskatinti nitratų virtimą amoniaku, suintensyvinti kvėpavimo ir fotosintezės procesus, sintetinti fermentus ir aminorūgštis, sustiprinti anglies ir azoto mitybą ir/arba tiesiogiai paveikti augalų mineralinę mitybą. Tačiau, nepaisant jau minėtų galimų pranašumų, nanotechnologijų pagalba sukurtų produktų naudojimas žemės ūkio sektoriuje yra gana ribotas, lyginant su kitomis pramonės šakomis. Todėl, atsižvelgiant į galimą praktinį ND pritaikymą žemės ūkio sektoriuje, prieš pateikiant su ND susijusius gaminius į rinką, reikėtų atlikti preliminarią kaštų ir naudos analizę, taip pat tinkamai įvertinti riziką aplinkai ir žmonių sveikatai.

SiO_2 ND poveikis žirnių morfologiniams parametrams, oksidacinio streso biožymenims ir antioksidaciniam aktyvumui

Mūsų tyrimuose SiO_2 ND vandeninės suspensijos zeta potencialas buvo -20,64 mV. Kitame moksliniame straipsnyje nurodoma, kad naudojant 10 g kg^{-1} SiO_2 ND, zeta potencialas buvo -40 mV. Tai rodo, kad suspensijos yra stabilios ir anijoninės.

Nustatyta, kad žirnių augalų apdorojimas didesnėmis SiO_2 (50 ir 100 ppm) ND koncentracijomis padidino antioksidacinį aktyvumą ir sumažino H_2O_2 kiekį ir MDA jų lapuose, kai jie augo optimaliomis sąlygomis. Be to, augant normalaus substrato drėgnio sąlygomis, padidėjo žirnių ūglių aukštis, šaknų ilgis bei biomasė. Ypatingas poveikis pastebėtas

makroelementų kaupimuisi žirnių augaluose; žymiai padidėjo P, K, Ca ir Mg kiekis žirnių lapuose ir stiebuose. SiO_2 ND teigiamai paveikė skirtingų augalų rūšių augimą, padidino jų biomasę ir fiziologines savybes, suaktyvino antioksidacinę sistemą, padėjo prisitaikyti prie stresinių sąlygų (Luyckx ir kt., 2017). Apskritai SiO_2 ND poveikis yra plačiai tiriamas skirtinguose augaluose (Lu ir kt., 2007; Janmohammadi ir kt., 2015). Tačiau iš ankštinių augalų šeimos SiO_2 ND poveikis iki šiol buvo ištirtas tik lęšiams ir sojos pupelėms. Nustatyta, kad nano dydžio SiO_2 suspensija padidino nitratų reduktazės aktyvumą, stimuliavo antioksidacinę sistemą, pagerino vandens ir trąšų įsisavinimą bei sojų pupelių (*Glycine max*) daigumą ir augimą (Lu et al. 2007). Ankstesni tyrimai rodo, kad didesnes SiO_2 ND koncentracijas reikia vartoti atsargiai, nes nustatytas neigiamas 120,16 ppm ND koncentracijos poveikis lęšių dygimui (Janmohammadi ir kt., 2015).

Rezultatai rodo, kad vandens trūkumas dirvožemyje sumažino žirnių aukštį, lapų plotą, SLP, SVK ir padidino šaknų ir antžeminės dalies santykį. Tokius gautus rezultatus patvirtina ir kitų tyrėjų atlikti tyrimai (Arafa ir kt., 2021). Khatun ir kt., 2021; Bangar ir kt., 2019). Pažymėtina, kad laistymas SiO_2 ND suspensijomis turėjo didesnį teigiamą poveikį žirniams, augusiems normalaus drėgnio substrate, nei purškimas. Žirniai turi grupę akvaporinų MIP – Si įtraukimo (eng.: influx) transporterių (SiT1 ir SiT2) ir ištekėjimo (eng.: efflux) transporterių (SiT6), esančių centrinėje ir šoninėse šaknyse, palengvinančių Si patekimą į ksilemą ir leidžiančių laisvai judėti augale (Maurel ir kt. al., 2015; Raoi ir Susmitha, 2017). Tačiau sausros atveju medžiagų judėjimas ksilemoje sutrinka, todėl laistymas Si ND tampa mažiau efektyvus. Purškiant ant augalų, SiO_2 ND padengia lapų paviršių dėl ko gali susidaryti papildomas Si sluoksnis, apsaugantis augalus nuo transpiracijos ir turgorinio slėgio pokyčių. Be to, augalus apipurškus mažo dydžio SiO_2 ND, jos gali prasiskverbti pro vaško sluoksnį ir taip laisvai judėti augale. Dėl tokio poveikio augalų purškimas SiO_2 ND tirpalais gali turėti didesnį teigiamą poveikį esant sausros stresui.

Remdamiesi gautais duomenimis, galima teigti, kad SiO_2 ND sėkmingai suaktyvina antioksidacinę sistemą, taip sumažėja oksidacinio streso poveikis sausros paveiktiems žirniams ir išsaugomas jų derlius. SiO_2 ND yra stipriai susijusios su vandens kiekio augaluose palaikymu, tačiau yra tik keli moksliniai straipsniai nagrinėjantys SiO_2 ND ir sausros poveikį augaluose. Pavyzdžiui, braškių nupurškimas SiO_2 ND sausros streso metu padidino CAT, APX, SOD ir GR fermentų aktyvumą, sumažino MDA ir H_2O_2 kiekį (Zahedi ir kt., 2020). Be to, sausros sąlygomis su SiO_2 ND augintuose kviečiuose nustatytas lapų žalumo, santykinio vandens kiekio ir derliaus padidėjimas (Behboudi ir kt., 2018).

CuO ND poveikis žirnių morfologiniams parametrams, oksidacinio streso biožymenims ir antioksidaciniam aktyvumui

Mūsų tyrimuose CuO ND vandeninės suspensijos zeta potencialas buvo –26,68 mV. Kiti tyrėjai nurodė, kad CuO ND zeta potencialas gali būti –34,4 ± 0,5 mV dejonizuoto vandens suspensijoje, kai pH 7, o pirminis ND dydis 10–100 nm (Keller ir kt., 2018; Adeleye ir kt., 2014; Hong ir kt. al. 2015). Tai rodo, kad suspensijos buvo stabilios ir anijoninės.

Nustatyti reikšmingi sausros paveiktų augalų antioksidacinio aktyvumo pokyčiai naudojant CuO ND. Ypatingai stiprus teigiamas poveikis nustatytas CAT, APX ir SOD fermentams. Taip pat CuO ND stipriai paveikė FRAP antioksidacinę galią. Atsižvelgiant į rezultatus galime teigti, kad CuO ND naudojimas nedideliais kiekiais gali padidinti augalų atsparumą ir apsaugoti juos nuo aplinkos įtempčių. Mūsų rezultatai parodė, kad CuO ND suspensijos žymiai sumažino H_2O_2 kiekį žirnių augaluose, bet padidino MDA koncentraciją, tai rodo, kad CuO ND aktyviai dalyvauja lipidų peroksidacijos procesuose. Mokslininkai nustatė, kad H_2O_2 ir MDA kiekis pupelių šaknyse žymiai padidėjo, o poveikis lapuose nenustatytas augalus paveikus 20, 50, 100, 200 ir 500 ppm CuO ND tirpalais (Gopalakrishnan ir kt., 2014). Jie pabrėžė, kad CuO ND paveikė SOD koduojančių genų raišką bei perteklinis Cu kiekis galėjo padidinti H_2O_2 susidarymą pupelių šaknyse. Taip pat nustatyta, kad padidėja ir CAT genų ekspresija naudojant mažesnes CuO ND koncentracijas. APX geno ekspresijos lygis buvo mažesnis, lyginant su CAT genu, todėl galėjo susilpnėti H_2O_2 neutralizavimas. Todėl mokslininkai iškėlė hipotezę, kad CuO ND streso metu suaktyvina augalų antioksidacinius gynybos mechanizmus; nepilnas H_2O_2 pašalinimas galėjo padidinti ROS kiekį pupelių šaknyse, veikiamose CuO ND (Gopalakrishnan ir kt., 2014). Nair ir Chung (2014) ištyrė 0, 0,5, 1, 2, 5, 10, 20, 50 ir 100 mg L^{-1} CuO ND suspensijų poveikį *Arabidopsis* augalams. Jų tyrimas parodė, kad po poveikio padidėjo genų, koduojančių fermentinius ir nefermentinius antioksidacinius gynybos mechanizmus aktyvumas bei padidėjo ROS gamyba. Po metų tas pats mokslininkas ištyrė CuO ND poveikį žirnių augalams. Mūsų išvados sutinka, kad kuo didesnė CuO ND koncentracija, tuo pasireiškia didesnis toksiškumas žirniams, pradedant nuo 100 ppm (Nair ir Chung, 2015). Toksinis poveikis sojų pupelėms patvirtintas nustačius padidėjusią lipidų peroksidaciją ir H_2O_2 kiekį, kai CuO ND koncentracija buvo 100 ppm ir didesnė (Yusefi-Tanha et al. 2020). Taip pat buvo pastebėtas sojų pupelių derlingumo sumažėjimas naudojant 50–100 ppm CuO ND koncentraciją (Ochoa ir kt., 2017).

Mūsų tyrimai parodė, kad CuO ND turėjo teigiamą poveikį antioksidacinei sistemai ir Cu kaupimuisi, kai augalai augo vandens trūkumo sąlygomis ir buvo purškiami per lapus. CuO ND įsisavinimas ir translokacija buvo plačiai ištirti ryžių augaluose (Peng ir kt., 2015). CuO ND

translokacijos metu ištirpęs Cu prijungiamas prie cisteino, citrato ir fosfato ligandų, o dalis Cu (II) redukuojama į Cu (I). Nustatyta, kad CuO ND gali judėti į šaknies epidermį, egzodermą ir žievę ir pasiekti endodermą, tačiau CuO ND sunkiai prasiskverbia pro Kasparijos juostelę (Peng ir kt., 2015). Ji trukdo CuO ND patekti iš šaknų į augalo antžeminę dalį. Tai galėjo lemti mažesnį pasiskirstymą augale ir lokalizuotą poveikį žirniams, kai jie buvo laistomi CuO ND tirpalais. Tai paaiškina mūsų gautus rezultatus, kur CuO ND laistytų žirnių šaknyse Cu kaupimasis buvo didesnis, tačiau lapuose jo kiekis nepadidėjo, o nupurškus augalus Cu kiekis padidėjo visose augalo dalyse.

Moksliniuose straipsniuose, taip pat, yra rašoma apie teigiamą CuO ND poveikį augalams. Pavyzdžiui, salotų purškimas 0, 0,5, 1,0, 2,0, 4 ir 6 ppm CuO ND suspensijomis teigiamai paveikė bendrą fenolinių junginių ir flavonoidų kiekį, antioksidacinį aktyvumą ir chlorofilų kiekį (Gaucin-Delgado ir kt., 2022). Be to, CuO ND inicijavo APX, SOD ir CAT aktyvumą ir žymiai padidino Cu kaupimąsi salotų lapuose. Pomidorų daigus paveikus 10 ppm CuO ND suspensija, žymiai padidėjo šviežia biomasė ir chlorofilų kiekis, taip pat cukraus kiekis, NR ir CAT aktyvumas bei sumažėjo MDA (Singh ir kt., 2017). Kaip matome iš aptartų publikacijų, CuO ND poveikį augalams dažniausiai lemia koncentracija. Atsižvelgiant į tai, kad ND yra daug kartų mažesnės dalelės nei įprastos medžiagos ir turi daug didesnį paviršiaus plotą, todėl augalams naudojama koncentracija turėtų būti sumažinta, kad būtų pasiektas teigiamas atsakas ir išvengta toksiško poveikio.

MoO_3 ND poveikis žirnių morfologiniams parametrams, oksidacinio streso biožymenims ir antioksidaciniam aktyvumui

MoO_3 ND vandeninės suspensijos zeta potencialas buvo -24,92 mV. Remiantis mokslinėmis publikacijomis, MoO_3 ND suspensijos zeta potencialas gali būti -32 mV, patvirtinantis, kad tirpalas yra stabilus ir stipriai anijoninis.

MoO_3 ND reikšmingai skatino gumbelių susidarymą ant žirnių šaknų ir stipriai paveikė nefermentinius ir fermentinius antioksidantus. Išsamiame tyrime apie MoO_3 ND poveikį hidroponikoje augintiems ryžių daigams nustatyta, kad efektyviausia koncentracija buvo 100 ppm, kuri sukėlė hormezės būseną (Sharma ir kt., 2021). Taip pat buvo nustatyta, kad ryžiuose sumažėjo chlorofilų ir karotenoidų kiekis esant didesnei nei 100 ppm MoO_3 ND koncentracijai. Be to, MoO_3 ND ryžiuose padidino MDA koncentraciją ūgliuose, bet sumažino šaknyse. Toks neigiamas poveikis gali būti susijęs su per didelės koncentracijos naudojimu.

Gauti rezultatai rodo, kad SOD aktyvumas sumažėjo žirnių lapuose, paveiktuose MoO_3 ND, o APX, GPX ir CAT aktyvumas padidėjo, tai nulemia greitesnį (aktyvių deguonies junginių neutralizavimą. Yra paskelbta publikacija (Yang ir kt., 2020), kurioje mokslininkai nurodė, kad

pridėjus 1, 10 mg kg^{-1} MoO_3 ND padidino fermentinių antioksidantų, tokių kaip POD, SOD ir CAT, aktyvumą sojų pupelėse, tačiau didesnės koncentracijos (100 ir 1000 mg kg^{-1}) buvo toksiškos. Kitame tyrime buvo naudotos 200 ir 1000 ppm koncentracijų MoO_3 ND suspensijos (Huang ir kt., 2021). Tyrėjai pastebėjo, kad kukurūzų lapai gali sukaupti didesnį Mo kiekį nei kviečių lapai, o tai rodo, kad skirtingos augalų rūšys gali skirtingai reaguoti į skirtingas MoO_3 ND koncentracijas.

Mo nėra biologiškai aktyvus elementas, todėl nedideliais kiekiais jis yra prijungiamas į specifines proteazių grupes. Šis Mo kofaktorius (Moco) yra priskiriamas abiem Moco surišančioms šeimoms: sulfito oksidazei (sulfito oksidazė, nitratų reduktazė (NR), mitochondrijų amidoksimo reduktorius) ir ksantino oksidoreduktazei (aldehido oksidazė (AO), ksantino dehidrogenazė). Svarbiausias Mo surišantis fermentas augalų išlikimui yra citozolinis NR, kuris katalizuoja pirminį nitratų įsisavinimo žingsnį (Kaiser ir kt., 2005; Mendel ir Schwarz 2011). Nitratų pavertimas nitritais yra būtinas augalų augimui ir vystymuisi. Wu (2018) pasiūlė Mo sukeltos oksidacinės tolerancijos sausros streso metu mechanizmo schemą. Pažymima, kad sumažėjus substrato drėgniui, augalų apsaugos sistema aktyvuojama per ABA ir NO signalus. Kaip Mo-fermentų komponentas, Mo stimuliuoja ABA sintezę ir NO gamybą atitinkamai per AO ir NR ir taip reguliuoja oksidacinę toleranciją sausros paveiktuose augaluose (Wu ir kt., 2018). Tačiau neaišku, ar toks pats mechanizmas gali būti pritaikytas naudojant nano dydžio Mo. Pavyzdžiui, pranešama, kad 0,1 ir 1 ppm MoO_3 ND suspensijos aktyvina nitratų reduktazės aktyvumą špinatuose ir padidina chlorofilų kiekį (Abbasifar et al., 2020).). Be to, naudojant 8 ppm Mo ND koncentracijos tirpalą, padidėjo sėjamųjų avinžirnių antioksidacinis aktyvumas (Taran ir kt., 2014). Taip pat, 10, 20, 30, 40 ir 50 ppm koncentracijų MoO_3 ND purškimas ant paprastųjų pupelių augalų lapų žymiai pagerino morfologinius parametrus, tokius kaip šviežia ir sausa biomasė, augalo aukštis ir šaknų ilgis, lyginant su neapdorotais augalais (Osman ir kt., 2020). Esminis šio tyrimo parametras buvo genomo šablono stabilumo procentas (GTS%), tiesiogiai susijęs su DNR pakitimo laipsniu ir DNR atkūrimo bei replikacijos kompetencija. Genominis nestabilumas apima struktūros pokyčius, tokius kaip padidėjęs bazinių porų mutacijų dažnis. Tyrėjai nustatė, kad GTS% vertės sumažėjo, didėjant MoO_3 ND koncentracijai nuo 10 iki 40 ppm, atitinkamai 84,61 – 78,21 %. Be to, mutageninis MoO_3 ND poveikis paprastųjų pupelių DNR sukėlė kelių genų, koduojančių specifinius baltymus, ekspresiją, įjungdamas arba išjungdamas tam tikrus genus daigų ir žydėjimo stadijose (Osman ir kt., 2020). Tai galėjo lemti, kad visos MoO_3 ND koncentracijos reikšmingai paveikė paprastųjų pupelių augalų fiziologines savybes, padidino derlių ir jo kokybę.

Kadangi nedideli MoO_3 ND kiekiai yra veiksmingesni už įprastas molibdeno druskas, jų pritaikymas žemės ūkyje, galėtų padidinti augalų produktyvumą tiek optimaliomis, tiek

nepalankiomis augimo sąlygomis. Mūsų rezultatuose nustatyta, kad naudojant 50 ppm MoO_3 ND suspensiją, esant normaliai dirvožemio drėgniui, žirnių derlius padidėja 6 %, o sausros paveiktų žirnių derlius galimai padidėja iki 80 %. Nors gaminti MoO_3 ND pramoniniu būdu yra brangus procesas, padidintas augalų derlius iš hektaro padengia susidariusį skirtumą ir netgi padidina pelną.

Boro ND poveikis žirnių morfologiniams parametrams, oksidacinio streso biožymenims ir antioksidaciniam aktyvumui

Mūsų tyrimų metu B_2O_3 ND vandeninės suspensijos dzeta potencialas buvo -28,54 mV. Tačiau kiti mokslininkai nustatė, kad B_2O_3 ND vandeninė suspensija su 0,2 % tritono x-100 buvo -30,3 mV (Barreto ir kt., 2021). Tokios dzeta potencialo vertės rodo, kad tirpalai yra stabilūs ir anijoniniai. Be to, šios suspensijos PDI buvo 0,23, o kiti mokslininkai nustatė 0,4 (Barreto ir kt., 2021), tai rodo, kad suspensijos yra monodispersinės.

Mūsų atlikti tyrimai parodė, kad ant žirnių šaknų susidarančių gumbelių skaičius reikšmingai padidėjo po poveikio B_2O_3 ND tiek augalams augant normaliame, tiek esant drėgmės trūkumui substrate. B augale yra transportuojamas ksilemoje, o apie 90 % yra sujungiamas į augalų ląstelių sieneles (Goldbach ir Wimmer 2007). B yra pagrindinis elementas formuojant esterius su ramnogalakturonanu II (RG-II, eng.: rhamnogalacturonan II). Šis borato esteris reikalingas išlaikyti normalioms ląstelių sienelių funkcijoms ir struktūrai (Ryden ir kt., 2003). Optimaliomis sąlygomis, kai dirvoje pakanka B, RGII-glikoproteinai susidaro ir žirnių šaknų gumbelių bei šaknų ląstelių plazminėse membranose. Tačiau esant B trūkumui RGII-glikoproteinai nėra sintetinami, o jų trūkumas destabilizuoja plazminę membraną ir gumbelių susidarymą (Bolaños ir kt., 2001). Taip pat, B, kaip glikoproteinų komponentas, yra būtinas siekiant gumbelinių bakterijų diferenciacijos į azotą fiksuojančią formą (Bolaños ir kt., 2004). Taip pat tiek mūsų rezultatuose, tiek kitų mokslininkų publikacijose nurodoma, kad B gali stimuliuoti tiek fermentinį, tiek nefermentinį antioksidacinį aktyvumą. Daugelyje mokslinių publikacijų pabrėžiama B nauda augalų antioksidacinei sistemai esant įvairioms streso sąlygoms (Alpaslan ir Gunes, 2001; Bonilla ir kt., 2004; Bastías ir kt., 2004), tačiau tik keliuose tyrimuose buvo tirtas B_2O_3 ND poveikis (Dimkpa ir kt., 2019; Zewail ir kt., 2021; Mahmoud ir kt., 2020).

Kompleksinio streso ir ND poveikis žirniams

Mokslininkai pastebi, kad augalus dažniau veikia kompleksiniai neigiami aplinkos veiksniai nei pavieniai. Vis dar yra daug neištirtų galimų streso derinių tarp sausros, druskingumo, karščio bangų, atšalimo, užšalimo, ozono, patogenų, UV, maistinių medžiagų trūkumo, per didelio CO_2 kiekio, per didelio apšvietimo ir sunkiųjų metalų (Suzuki ir kt., 2014).

Vienas iš šio mokslinio darbo tikslų buvo ištirti galimą kompleksinį agrometeorologinės sausros ir sunkiųjų metalų įtempčių poveikį žirniams kartu su skirtingų ND sąveiką. Atliekant skirtingų augalų rūšių kompleksinių įtempčių tyrimus, transkriptominės ir proteominės analizės parodė, kad svarbiausias yra antioksidacinės sistemos atsakas. Suaktyvėjęs antioksidacinis pajėgumas ir/arba sumažėjęs aktyvių deguonies junginių (ROS) kaupimasis yra mechanizmas, veikiantis augaluose, kuris padidina atsparumą kompleksiniam stresui. Tiriant bendrą sausros ir sunkiojo metalo Cu poveikį, rezultatai rodo, kad labiau sumažėja morfologiniai parametrai ir nefermentinių antioksidantų aktyvumas, tačiau stipriau padidėja fermentinių antioksidantų aktyvumas ir oksidacinio streso biožymenų kiekis žirniuose, lyginant augalus paveiktus tik sausra ir žirnius paveiktus sausra bei sunkiuoju metalu Cu. Tokį persidengiantį kompleksinio streso poveikį patvirtina ir kiti mokslinimai. Pavyzdžiui, buvo ištirtas kompleksinis sausros ir sunkiojo metalo Cd poveikis kukurūzų augalams (Naz ir kt., 2021). Šiame tyrime mokslininkai nustatė, kad toks derinys nepaveikė augalų morfologinių parametrų, tačiau žymiai sumažino bendrą chlorofilų, baltymų, karotinoidų kiekį ir santykinį vandens kiekį augaluose. Kombinuotas stresas žymiai padidino oksidacinio streso biožymenų ir suaktyvavo SOD, CAT, APX ir POD fermentinius antioksidantus. Tiek sausra, tiek jos derinys su sunkiojo metalo Cu stresu sumažino žirnių augimą ir lėmė beveik identiškus augalų pokyčius. Poveikis buvo adityvus, abiem stresiniams veiksniams veikiant vienu metu, todėl augalai buvo mažesni ir atsakas į stresą buvo stipresnis nei kiekvienam stresui veikiant atskirai. Tačiau buvo pastebėta išimtys panaudojus ND.

Žirnių augalų purškimas SiO_2 ir B_2O_3 ND buvo veiksmingesnis jiems augant kompleksinio streso sąlygomis, nors prieš tai atliktame tyrime tik su sausros stresu skirtumų tarp ND panaudojimo būdų nebuvo nustatyta. Tai gali būti siejama su neigiamu sunkiojo metalo Cu poveikiu šaknų augimui. Kadangi sausros sąlygomis augalai didžiąją dalį energijos skiria šaknų augimui, kad surastų daugiau vandens, o per didelis Cu kiekis dirvožemyje tai slopino ir trukdė pasisavinti tiek esminius elementus, tiek stabdė šaknų augimą (Kumar ir kt., 2021). Tyrėjai ištyrė, kad SiO_2 ND pagerino kviečių augimo rodiklius, kurie buvo paveikti kompleksinio sausros ir sunkiojo metalo Cd stresų, taip pat SiO_2 ND sumažino Cd koncentraciją skirtinguose kviečių audiniuose, slopino oksidacinį stresą ir padidino chlorofilo kiekį. (Khan ir kt., 2020) Tyrėjai taip pat pažymi, kad SiO_2 ND taikymas gali būti veiksmingas būdas siekiant sumažinti Cd koncentraciją javų grūduose. Mūsų tyrime buvo nustatyti reikšmingi Cu kaupimosi augaluose pokyčiai, žirnius paveikus SiO_2 ND suspensijomis Tiek purškimas, tiek laistymas SiO_2 ND reikšmingai padidino Cu biokoncentraciją žirnių lapuose ir šaknyse, translokacijos faktorių ir tolerancijos indeksą. Tai gali būti susiję su didesniu santykiniu vandens kiekiu ir

padidėjusiu antioksidaciniu aktyvumu augaluose, kurie buvo veikiami kompleksinio streso ir SiO_2 ND.

Įdomūs rezultatai gauti žirniuose, kurie buvo paveikti kompleksinio streso ir B_2O_3 ND, nes šių augalų lapuose MDA koncentracija labai sumažėjo, tačiau MDA koncentracija buvo didžiausia tais atvejais, kai nustatytas Cu perteklius. Kaip minėta aukščiau, tai gali būti susiję su tuo, kad, didžioji dalis B, patenkančio į augalą, yra surišta su augalo ląstelių sienelėmis apsaugant ląsteles nuo lipidų peroksidacijos, kurią gali sukelti per didelis Cu kiekis (Blevins ir Lukaszewski, 1998). Be to, B_2O_3 ND padidino Cu tolerancijos indeksą bei pagrindinių fermentinių antioksidantų SOD ir CAT aktyvumą žirniuose. Siekiant ląstelėse išlaikyti homeostazę SOD katalizuoja superoksido radikalo virtimą į H_2O_2, o CAT H_2O_2 paverčia vandeniu. Toks reikšmingas šių fermentų suaktyvėjimas galėjo padėti žirniams lengviau ištverti kompleksinio streso poveikį.

Žirnių laistymas su MoO_3 ND, esant kompleksiniam stresui, reikšmingai paveikė gumbelių susidarymą, kurį stipriai sumažino sausros ir sunkiojo metalo Cu poveikis. Taip pat nustatytas reikšmingas augalų santykinio vandens kiekio padidėjimas. Dėl to matome padidėjusią Cu bioakumuliaciją šaknyse, bei padidėjusius translokacijos faktorių ir Cu tolerancijos indeksą. Taip pat reikšmingai padidėjo fermentinių antioksidantų GPX, APX, SOD ir CAT aktyvumas, kuris nulėmė oksidacinio streso rodiklių sumažėjimą. Reikia paminėti, kad CuO ND sustiprino stresorių kompleksinį neigiamą poveikį žirniams. Tai rodo, kad CuO ND žirniuose veikia sinergiškai su sunkiuoju metalu Cu.

Šis darbas praplečia žinias apie galimą kompleksinių įtempčių poveikį žirniams. Be to, šis tyrimas išsiskiria savo svarba dėl išsamiai aptariamo kompleksinių įtempčių ir skirtingų ND poveikio žirnių morfologiniams parametrams, derliui, antioksidacinės sistemos aktyvumui bei elementų akumuliacijai.

IŠVADOS

1. Žirnių daigams ir pilnai išsivysčiusiems žirniams, augusiems normalaus drėgnio substrate ar sausros sąlygomis, efektyviausios SiO_2, CuO ir MoO_3 nanodalelių suspensijų koncentracijos buvo 50 ppm, o B_2O_3 – 12,5 ppm.

2. Naudojama SiO_2 nanodalelių suspensija padidino žirnių atsparumą sausrai. Drėgmės trūkumo sąlygomis SiO_2 nanodalelės 27 % sumažino oksidacinių biožymenų koncentraciją ir 30 % padidino bendrą fenolinių junginių kiekį bei nefermentinių antioksidantų aktyvumą žirniuose. Reikšmingas aktyvinantis poveikis nustatytas fermentiniams antioksidantams katalazei (159 %), superoksido dismutazei (37 %) ir glutationo reduktazei (128 %), augalus paveikus 50 ppm SiO_2 nanodalelių tirpalu. Sausros paveiktų žirnių derlius išlaikytas 45 % panaudojus SiO_2 nanodalelių suspensiją.

3. CuO nanodalelės 37 % sumažino vandenilio peroksido, bet 66 % padidino malondialdehido koncentraciją substrato drėgmės trūkumo paveiktuose žirnių augaluose. Didžiausia 50 ppm CuO nanodalelių koncentracija iki 2,5 karto padidino nefermentinių antioksidantų aktyvumą bei paskatino katalazės (167 %) ir askorbato peroksidazės (72 %) aktyvumą žirniuose, augusiuose sausros sąlygomis. Didžiausia Cu akumuliacija žirnių lapuose, stiebe bei šaknyse nustatyta nupurškus augalus 50 ppm CuO nanodalelių suspensija.

4. Panaudojus MoO_3 nanodalelių suspensiją sausros sąlygomis, nustatytas teigiamas poveikis žirnių aukščiui (40 %) ir lapų plotui (30 %) bei iki 30 % sumažėję oksidacinio streso rodikliai. Nustatytas 37 % bendro fenolinių junginių kiekio ir 145 % nefermentinių antioksidantų aktyvumo padidėjimas augaluose paveiktuose sausros ir MoO_3 nanodalelių. MoO_3 nanodalelės 2 kartus padidino katalazės ir askorbato peroksidazės aktyvumą bei 20 % superoksido dismutazės ir 56 % gvajakolio peroksidazės aktyvumą. Didžiausia Mo akumuliacija nustatyta žirnių šaknyse, kai augalai buvo laistomi nanodalelių suspensijomis.

5. B_2O_3 nanodalelės turėjo teigiamą poveikį žirnių aukščiui (26 %), lapų plotui (40 %) ir 2,5 karto padidino antioksidantų aktyvumą. Augaluose paveiktuose sausros ir B_2O_3 nanodalelių 6 kartus suaktyvėjo askorbato ir 91 % gvajakolio peroksidazės bei 51 % padidėjo superoksido dismutazės aktyvumas. B didžiausia akumuliacija nustatyta šaknyse nepriklausomai nuo poveikio būdo. Žirnių, paveiktų sausros ir palaistytų su B_2O_3 nanodalelėmis, stiebuose ir lapuose B akumuliacija vyko intensyviausiai.

6. Tiriant kompleksinį Cu, kaip sunkiojo metalo, ir sausros poveikį žirnių augalams nustatyta, kad stipresnį kelių stresorių sukeliamą oksidacinį stresą slopino naudojamos SiO_2, MoO_3 ir B_2O_3 nanodalelių suspensijos. Jos efektyviai sumažino vandenilio peroksido ir malondialdehido koncentracijas augale bei padidino antioksidacinės sistemos aktyvumą lyginant

su augalais kurie buvo paveikti sausros ir Cu pertekliaus. Laistymas CuO nanodalelėmis sustiprino sausros ir perteklinio Cu neigiamą poveikį augalams. Žirniuose tolerancijos indeksą Cu statistiškai patikimai didino tiek laistymas, tiek purškimas SiO_2, purškimas B_2O_3 ir laistymas MoO_3 nanodalelių suspensijomis.

7. Atsižvelgiant į nanodalelių savybes bei patekimo į augalą būdus, nustatyta, kad laistymas MoO_3 nanodalelių suspensija efektyviau sumažino stresų sukeliamą neigiamą poveikį žirniuose nei purškimas ir išsaugojo 80 % derliaus. B_2O_3 ir CuO nanodalelių suspensijų efektyvumas buvo stipresnis jomis purškiant žirnius, šie poveikiai, atitinkamai, išsaugojo 92 % ir 47 % derliaus. O SiO_2 nanodalelių poveikis pagal taikymo būdą iš esmės nesiskyrė ir derlius buvo apie 40 % didesnis lyginant su augalais paveiktais sausra, bet nepaveiktais nanodalelėmis.

MOKSLINIŲ PUBLIKACIJŲ DISERTACIJOS TEMA SĄRAŠAS

Žurnaluose su Mokslinės informacijos instituto duomenų bazės Web of Science (ISI WOS) citavimo indeksu:

1. Sutulienė, R., Ragelienė, L., Samuolienė, G., Brazaitytė, A., Urbutis, M. and Miliauskienė, J., 2021. The Response of Antioxidant System of Drought-Stressed Green Pea (*Pisum sativum* L.) Affected by Watering and Foliar Spray with Silica Nanoparticles. Horticulturae, 8(1), 35.
2. Sutulienė, R., Ragelienė, L., Duchovskis, P. and Miliauskienė, J., 2022. The Effects of Nano-copper, -molybdenum, -boron, and -silica on Pea (*Pisum sativum* L.) Growth, Antioxidant Properties, and Mineral Uptake. Journal of Soil Science and Plant Nutrition, 22(1), pp.801-814.

Straipsniai kituose periodiniuose mokslo leidiniuose:

1. (Sutulienė, R.) Paulauskaitė, R., Miliauskienė, J. and Ragelienė, L., 2019. Uptake and effect of nanoparticles in plants. Sodininkyste ir Daržininkyste, 38(1/2), pp.31-46.

Pranešimai konferencijose:

1. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2022 rugpjūčio 14-19 d. Stendinis pranešimas: Influence of Silica Nanoparticles on the Enzymatic and Non-Enzymatic Antioxidant Activity of Green Pea (*Pisum sativum* L.) Affected by Drought. 31st International Horticultural Congress (IHC).
2. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2022 balandžio 16-30 d. Stendinis pranešimas: The response of drought-stressed green pea (*Pisum sativum* L.) to boron nanoparticle application. 1st International Electronic Conference on Horticulturae (IECHo).
3. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2022 gegužės 24-27 d. Žodinis pranešimas: Impact of copper oxide nanoparticles on the antioxidant system of drought affected pea. 17 th International Conference of young scientists on energy and natural sciences issues (CYSENI).
4. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2021 gegužės 24-28 d. Žodinis pranešimas: Growth properties of pea plants in response to the complex effects of

different nanoparticles and drought stress.17 th International Conference of young scientists on energy and natural sciences issues (CYSENI).

5. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2020 rugsėjo 2-4 d. Stendinis pranešimas: The effect of CuO, Mo, B and SiO_2 nanoparticles on pea (*Pisum sativum* L.) plants during the agrometeorological drought. Scandinavian Plant Physiology Society PhD Student (SPPS).
6. Sutulienė, R., Ragelienė, L. ir Miliauskienė, J., 2020 gruodžio 17-18 d. Žodinis pranešimas: The Effect of CuO, Mo, B, SiO_2 Nanoparticles and Agrometeorological Drought Stress on Growth Parameters of Pea (*Pisum sativum* L.). International Conference on Agriculture and Horticulture (ICAH).

APIE AUTORĘ

Rūta Sutulienė yra gimusi 1992 kovo 1 d. Marijampolėje, Lietuvoje. 2011 m. sėkmingai baigė Marijampolės „Sūduvos“ gimnaziją. 2015 m. Vytauto Didžiojo universitete, Gamtos mokslų fakultete, įgijo biologijos srities bakalauro mokslinį laipsnį. 2018 m. Vytauto Didžiojo universitete, Gamtos mokslų fakultete įgytas magistro laipsnis taikomosios biochemijos srityje. Nuo 2019 m. iki dabar dirba jaunesniąja mokslo darbuotoja Lietuvos agrarinių ir miškų mokslų centre, Sodininkystės ir daržininkystės institute. 2021 m. dalyvavo trumpalaikėje mokslinėje stažuotėje Antverpeno universitete, Belgijoje ir 2019 m. Krokuvos Agronomijos universitete, Lenkijoje.

PADĖKA

Nuoširdžiai dėkoju mokslinei vadovei dr. Jurgai Miliauskienei ir doc. dr. Linai Ragelienei už kantrybę, skirtą laiką, palaikymą ir patarimus vykdant tyrimus bei rengiant disertaciją. Dėkoju visiems Augalų fiziologijos laboratorijos kolegoms, sugebėjusiems darbo vietą paversti antrais namais, kur visada jautiesi laukiamas. Ačiū prof. habil. dr. Pavelui Duchovskiui išugdžiusiam nuostabias mokslininkes akad. Dr. Giedrę Samuolienę, dr. Akvilę Viršilę, dr. Aušrą Brazaitytę, dr. Viktoriją Vaštakaitę-Kairienę, dr. Kristiną Laužikę, į kurias visada gali kreiptis dėl pagalbos, patarimo, kartu paliūdėti ir pasidžiaugti. Taip pat dėkoju LAMMC SDI už galimybę studijuoti doktorantūros studijose ir skatinimą plėsti akiratį vykstant į stažuotes ir dalyvaujant tarptautinėse konferencijose. Nuoširdžiai dėkoju tėvams ir vyrui už palaikymą ir paskatinimą studijuoti.

Rūta SUTULIENĖ

ENVIRONMENTALLY INDUCED OXIDATIVE STRESS IN GREEN PEA AND ITS MANAGEMENT MEASURES

Doctoral Dissertation

Edited by

Spausdino – Vytauto Didžiojo universitetas
K. Donelaičio g. 58, LT-44248 Kaunas
Užsakymo Nr. 22-177. Tiražas 15 egz. 2022 11 07.
Nemokamai