

ANTIOXIDATIVE PARAMETERS IN THE SEEDLINGS OF *HORDEUM VULGARE* IN RESPONSE TO PRIMING SEEDS WITH DIATOMITE SOLUTION UNDER SALT STRESS

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Abstract

In modern agriculture, salt stress is one of the major problems. The aim of the study was to investigate the effect of priming seeds of *Hordeum Vulgaris* with diatomite on physiological, photosynthetic and stress related aspects in laboratory conditions. The sterilized seeds were primed with three concentrations of diatomite suspensions: 1) 5g DTM/100 ml, 2) 10 g DTM / 100 ml H₂O, 3) 20 g DTM/100 ml. If the concentration of salt is high in the soil it will give the high osmotic potential of soil as a result and water deficit will be in plants. Moreover, a high concentration of salt could cause nutritional disequilibrium and, as a result, the production of reactive oxygen species was increased and plant growth and biomass were decreased as a stress condition was 200 mM of NaCl solution and after that their biochemical contents were determined. Diatomite may act to alleviate salt stress in the plant. This is achieved by decreasing the permeability of plasma membranes and maintenance of cell form and structure due to the increasing of antioxidative enzymes SOD and CAT. Diatomite offset the negative impacts of NaCl stress due to the increased tolerance of plants to NaCl salinity by enhancement of chlorophyll content and photosynthetic activity.

Key words: Agriculture, Antioxidants, Botany, Diatomites, Silicon.

Introduction

Using natural rocks against salt and drought stresses is very actual? (Liang *et al.*, 2003; Liu and Xu, 2007; Xie *et al.*, 2015; Shahini *et al.*, 2023b). Diatomites naturally occur in sedimentary rock primarily composed of fossilized remains of freshwater diatoms. It is a recyclable, prolonged, reusable and environmentally harmless soil fertilizer. It can be used as an aerator for the root of plants, also minimize leaching and run off thus increase soil water retention and reducing watering (Bahatska *et al.*, 2023; Karches, 2018). Hereinafter, diatomite can promote stronger, healthier, higher-yielding plants that ripen quickly and get self-resistance against abiotic and biotic stresses (Kruger, 2006; Jessen, 2007; Abdalla, 2010). It is composed of silicon dioxide (87-90%) in a soluble form available to plants and a small amount of trace elements.

It was reported that silicon could increase salt tolerance in many agricultural crops like wheat (Gurmani *et al.*, 2013; Ahmad, 2014), rice (Gong *et al.*, 2006; Kim *et al.*, 2013), barley (Liang *et al.*, 2015; Dehtiarova, 2023). However, the significant role of silicon was not documented for the majority of plants. It was reported that silicon uptake by many plant species can provide various benefits such as improved salt and drought tolerance. The profitable role of silicon is more evident for graminaceous plants (Epstein & Bloom, 2005; Ma & Yamaji, 2006).

Studies have shown that leaf transpiration of some plants is reduced considerably by silicon application (Shen *et al.*, 2022). It has been postulated that this effect might be due to the reduction in transpiration rate through cuticular layers thickened by Si deposits (Helaly *et al.*, 2017; Shahini *et al.*, 2023a). It was demonstrated by researchers, that application of silicon might enhance the resistance and tolerance of higher plants to both abiotic

and biotic stresses (Shetty *et al.*, 2011; Egamberdieva *et al.*, 2022). Additionally, silicon can improve photosynthetic pigment and dry matter content, and reduce the permeability of plasma membranes in wheat plants exposed to salt stress (Shen *et al.*, 2010; Brazhnikova *et al.*, 2022). These studies have also indicated that silicon can enhance the activities of Superoxide dismutase (SOD), catalase CAT, and peroxidase (POD) enzymes that decrease oxidative damage during stress (Tuna *et al.*, 2008). It was found by Shetty *et al.*, (2011), that in miniature roses, silicon application induced gene expression and primed the accumulation of several phenol groups compared with the no-silicon control.

It was documented in previous reports that the application of silicon could improve the resistance of plants to environmental stress, but the underlying molecular mechanisms of this effect remain unknown. Priming seeds with silicon is one of the major techniques, which may improve abiotic stress tolerance in plants (Hameed *et al.*, 2013). The aim of the study was to investigate the effect of priming seeds of *Hordeum vulgare* with diatomite on physiological, photosynthetic and stress related aspects in laboratory conditions. In this paper, plant seeds were primed with three concentrations of diatomite from the territory of Kazakhstan (Mugalzhar region) then were grown in salt water to investigate the effect of silicon in mitigating the adverse effects of salt.

Material and Methods

Barley seeds were sterilized in 0.5% potassium permanganate solution (10 minutes) and rinsed three times with distilled water. The sterilized seeds were divided into six groups; the first group was not primed (control) and

without salt stress condition, the second with distilled water (H₂O) + salt stress, third group with 0,1mM Na₂SiO₃+salt stress, the others with diatomite suspension: fourth with 5g DTM/100 ml H₂O+salt stress, fifth 10 g DTM / 100 ml H₂O+salt stress and sixth group with 20 g DTM /100ml H₂O+salt stress. It was found that diatomite suspensions at concentrations of 5 g, 10 g, 20 g / 100 ml were optimal for the priming seeds (Nayekova *et al.*, 2018; Nayekova *et al.*, 2020). The diatomite solutions were sterilized in autoclave under 1 ATM. 120°C.

Ten barley grains were planted in Petri dishes and watered with ordinary distilled water in the same amount. In order to avoid contact with air in the aquatic environment, the seeds should sit at the bottom of the water and were tested for salinity. Salt conditions were simulated with a 200 mM NaCl solution (strong salinity). Their growth and development in an ash-salted environment were determined.

The experiment was carried out in laboratory conditions at an average day / night temperature of 20/18°C, relative air humidity from 50 to 55% and ambient light. Samples were collected after 7 days. To measure growth criteria, 70 plants were selected, and 30 other samples were taken to determine chlorophyll a, b and carotenoids, free proline, superoxide dismutase, hydrogen peroxide, malondialdehyde and catalase. Morphological traits including the length of the root and stems of each plant was measured and images were obtained. Statistical analysis. Analyses of variance (ANOVA) for all the variables were carried out using SAS analysis. The significance of differences was assessed by p-value ($p \leq 0.05$).

Determination of the concentration of chlorophyll a, b and carotenoids. The concentration of chlorophyll a and b and carotenoids was determined by the spectrophotometric method (spectrophotometer SF-2000, weight of the sample of raw leaves – 0.1 g, solvent – 100% acetone) according to the equations:

$$Ca = 9.784D662 - 0.99D644$$

$$Cb = 21.426D644 - 4.650D662$$

$$Ca + Cb = 5.134D662 + 20.436D644$$

$$CK = 4.695D440.5 - 0.268Ca + b$$

where D440.5, D644, D662 – optical density at wave lengths of 440.5, 644 and 662 nm, respectively; Ca, Cb, Ck – concentration of chlorophyll a, chlorophyll b and carotenoids in raw leaves of the objects of study (mg / l) (Lichtenthaler and Wellburn, 1983).

Determination of the content of free proline in plant root samples was carried out according to the classical method of Bates (1973). A weighed portion of plant material (0.5–1 g of raw leaves) was homogenized in 2 ml of sulfosalicylic acid. Then it was centrifuged for 20 min (15000g). To determine proline, 2 ml of acidine hydrin reagent and 2 ml of glacial acetic acid were added to the 2 ml supernatant. The reagent mixture was heated in a water bath at 100°C for 1 hour. The reaction was stopped by immersing the tubes in cold water. Then the reaction mixture was extracted with isopropyl alcohol and the optical density was determined in a DR3900 spectrophotometer (Germany) at a wavelength of 520 nm.

To determine the activity of superoxide dismutase (SOD), the method of Beauchamp & Fridovich (1971) was used. Plant material 0.5 g was ground on ice in a mortar with a small amount of 1.5-2 ml of 0.1 M phosphate buffer pH 7.8 with the addition of glass sand. The homogenate was transferred into a centrifuge tube, washing the mortar with a small (0.5 ml) amount of buffer. The homogenate was centrifuged for 20 min. To start the enzymatic reaction, 0.05 ml of riboflavin was added to all three tubes, after which the second and third tubes are in the dark. The optical density of the contents of all three tubes was determined at a wavelength of 560 nm using a spectrophotometer. This method is based on the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (Parida *et al.*, 2004).

The H₂O₂ content was determined as described by Alexieva *et al.*, (2001). Shoot samples were extracted with cold acetone. 3 ml of the extract was mixed with 1 ml of 0.1% titanium dioxide in 20% H₂SO₄, and the mixture was centrifuged at 6000g for 15 min. The intensity of yellow staining of the supernatant was assessed at 415 nm. The H₂O₂ concentration was determined using a calibration curve plotted in the range of 100-1000 nm H₂O₂. The main statistical characteristics were: the arithmetic mean (X), the standard deviation (a) and the error of the mean (Sx). The results were statistically processed using MS Excel 2007 (Microsoft, USA). Significance was determined using analysis of variance (ANOVA).

The lipid peroxidation level was determined as the content of malondialdehyde (MDA) using the thiobarbituric acid reaction as described by Ma *et al.*, (2016). The activity of catalase was determined by Aebi (1984).

Results and Discussion

After a one-week time of growing barley on filtered paper in Petri dishes, in which unprimed seeds were taken as a control, then were primed with distilled water, and with Na₂SiO₃ solutions – 0.1 mM and primed with different concentrations of diatomite (5g, 10g, 20g / 100ml H₂O). After 3 days of germination, the highest concentration of 200 mM NaCl was added to the seed growth medium. Subsequently, when seeds were primed with various concentrations (5 g DTM / 100 ml H₂O, 10 g DTM / 100 ml H₂O, 20 g DTM / 100 ml H₂O), an enhancement in the overall height of the seedlings, including their roots and shoots, was noted. It was seen significant root growth after priming the seeds with a diatomite suspension (Fig. 1).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

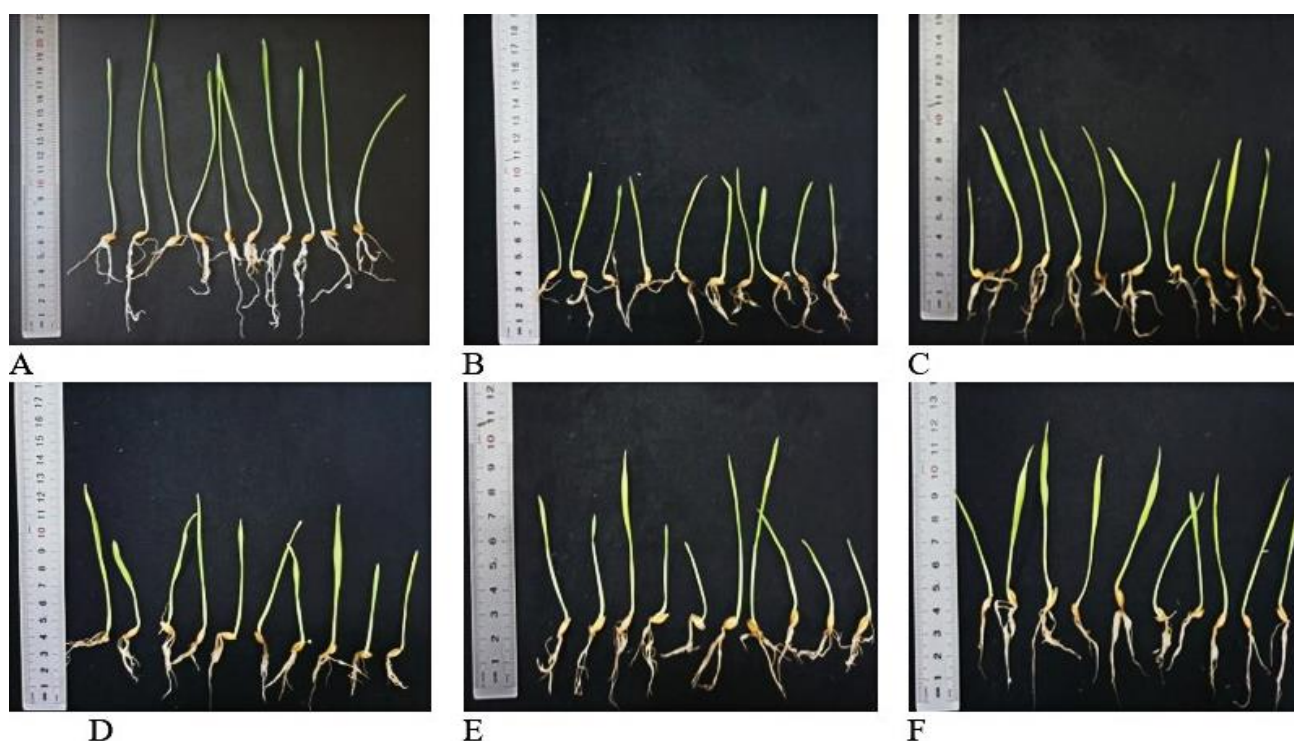


Fig. 1. Effect of seed priming with an increase in diatomite concentration on the growth of roots and shoots.

In accordance with the results shown in Figure 1, a positive effect of presowing priming of barley seeds in a suspension of diatomite at various concentrations on morphogenesis under salt stress conditions was established. This is demonstrated in the length of the roots and leaves of seedlings. The datum of morphometric measurements is shown in (Fig. 2).

In the control, with the presence of NaCl (salt stress conditions), the inhibition of development was observed (barley seedlings). The average length of the leaves and roots of Control 2 did not exceed 3.4 and 6 cm, the plants were very weak after salt stress, the leaves were twisted, as could be seen from the photographs in Figure 1. However, in the samples with seed treatment in a suspension of diatomite, inhibition of growth and development of seedlings were reduced. In three such variants, the length of the roots of the seedlings turned out to be higher by 0.8–1.0 cm compared to the control ($p \leq 0.05$), a similar stem length was noted ($p \geq 0.05$). The stem diameter after priming seeds with this suspension was also 0.05 mm larger than in the control. Seed priming with Na_2SiO_3 (0.1mM/l) also positively affected the growth and development of barley seedlings under stress conditions: as could be seen from the results, the average root length is 3.6 cm, and the stem length is 7 cm. Pre-sowing seed priming in suspension diatomite 20% led to the maximum growth of barley seeds compared to the control.

Among the photosynthetic pigments, the levels of chlorophyll (a) (Chla) and (b) (Chlb) were determined, as well as the level of carotenoids ($\mu\text{g/g}$ wet weight), which were able to prevent damage caused by the formation of triplet chlorophyll and singlet oxygen (Fig. 2). When plants are exposed to stress, chloroplasts are one of the first cell organelles to be negatively affected. They transmit the salinity signal along the retrograde pathway, changing the expression of their genes (Mero *et al.*, 2023).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mm (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H_2O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H_2O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H_2O). Experiments were carried out in three to four replicates (Fig. 3).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mm (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H_2O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H_2O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H_2O). Experiments were carried out in three to four replicates.

The datum in Figure 3 showed that priming with diatomite suspension increased dry matter accumulation under salt stress. NaCl applied in nutrient solution resulted in a considerable decrease in dry matter accumulation (Control 2). Using the silicon and diatomite as a priming solution did not affect dry matter accumulation under no salt stress, indicating that silicon in nutrient solution alleviated the growth inhibition induced by added NaCl (Yunyk & Harbar, 2022). These results are similar to the experiments of Corrado (2023).

In the control variant (control 1), the content of Chla and Chlb were 1.1 $\mu\text{g/g}$, 0.6 $\mu\text{g/g}$, respectively. Under salt stress, the amount of photosynthetic pigments was decreased (Control 2). Thus, the content of Chla and Chlb was 1.0 $\mu\text{g/g}$, 0.5 $\mu\text{g/g}$. A decrease in the content of carotenoids was also

noted, in control 1 carotenoid was $-0.4 \mu\text{g/g}$, and in salt stress (Control 2) it was $-0.3 \mu\text{g/g}$. However, barley seedlings after seed treatment with various concentrations of diatomite and seed priming with sodium silicate (0.1mM/l) showed an increase in photosynthetic pigments, and a decrease in inhibition due to salt stress ($p \leq 0.05$). All test samples showed a higher content of Chla and Chlb and carotenoids compared to the control sample (Control 1), which was not subjected to salt stress (Fig. 4).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

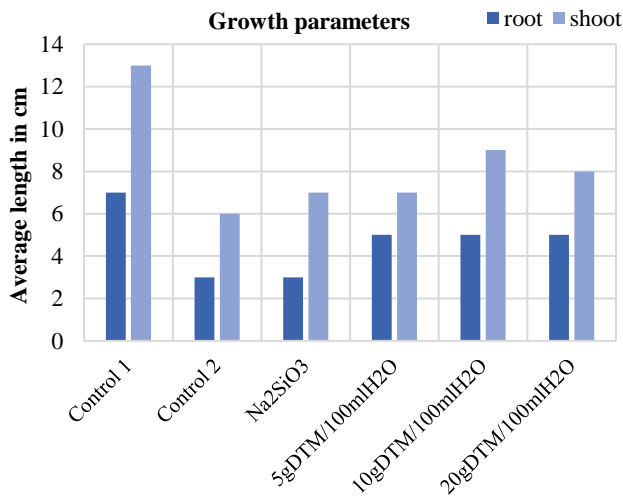


Fig. 2. Effect of seed priming with an increase in the concentration of diatomite on the growth of roots and shoots.

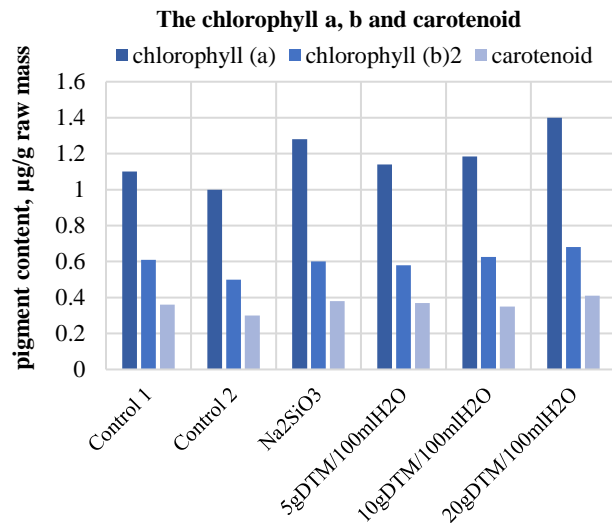


Fig. 4. The effect of seed priming with an increase in the concentration of diatomite on the content of chlorophyll a, b and carotenoid.

An increase in the physiological parameters of test samples under salt stress had a morphological justification compared to Control 2, for example, there was an increase in the content of photosynthetic pigments and carotenoids in the wet mass. Thus, it is assumed that due to the stimulating effect of Si compounds on photosynthetic pigments, there is an increase in plant biomass under salt stress (Lopushniak *et al.*, 2021).

In the control variant (Control 1), the content of free proline was $0.03 \mu\text{M/g}$ in the stems and this is used as an indicator of the level of resistance to stress. Under salt stress, the content of free proline was increased (Control 2) – in the roots $0.25 \mu\text{M/g}$ and in the stems $0.37 \mu\text{M/g}$. In experimental samples (Na₂SiO₃, 5g DTM/100 ml H₂O, 10g DTM/100ml H₂O, 20g DTM/100ml H₂O) under salt stress inhibition of proline accumulation, resulted in a decrease in its content, both in roots and in stems (Fig. 5). The accumulation of free proline during the treatment of seeds with different concentrations of diatomite showed similar datum.

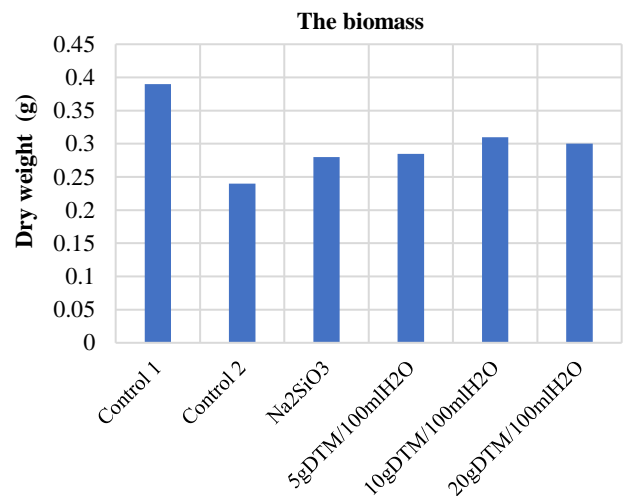


Fig. 3. Effect of seed priming with an increase in the concentration of diatomite on the biomass (Dry weight).

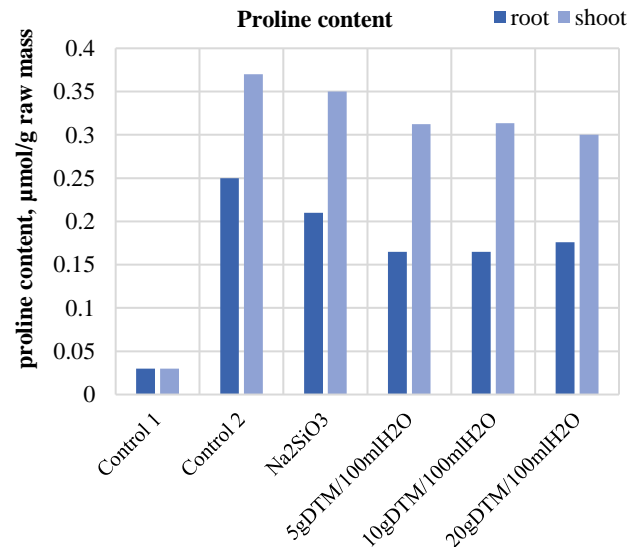


Fig. 5. Effect of priming with diatomite on proline content.

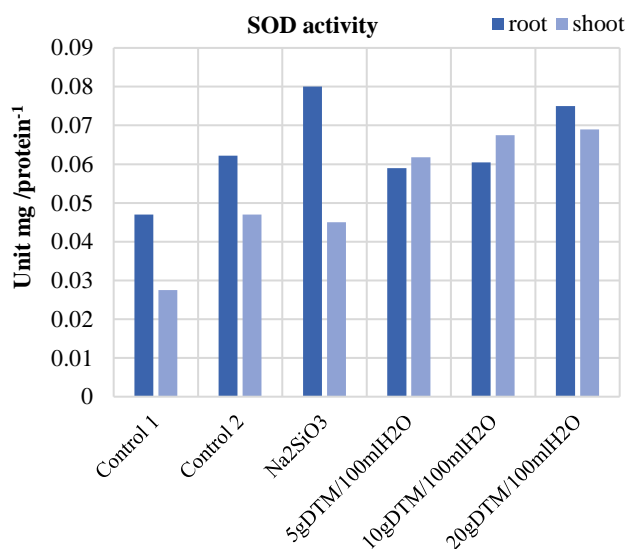


Fig. 6. Effect of priming with diatomite on SOD content.

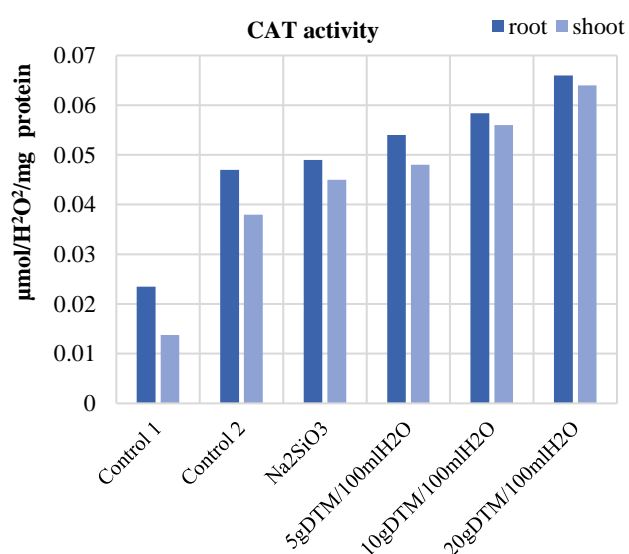


Fig. 7. Detection of total CAT in plants.

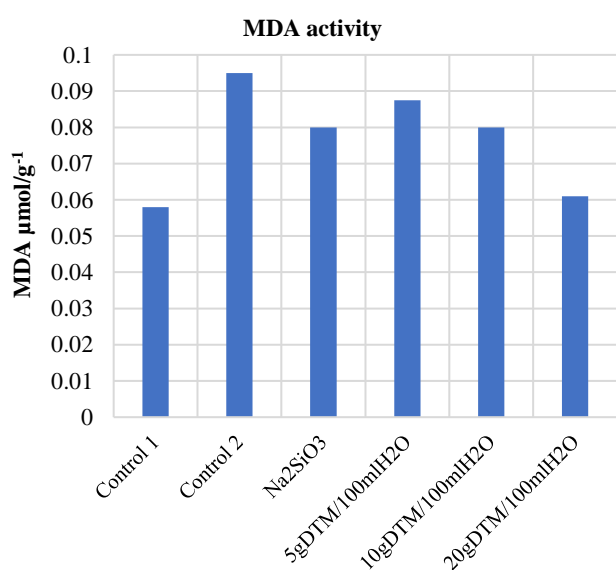


Fig. 8. Detection of total MDA in plants.

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

Dryness negatively affects light and dark reactions in the chloroplast, which causes the formation of ROS, for example, H₂O₂, superoxide radical, and singlet oxygen (Krauss & Nies, 2015). Any abiotic stress causes hyperproduction of H₂O₂ in a plant cell's peroxisomes, chloroplasts and mitochondria. Reactive oxygen species (ROS) react with epigenetic modifiers and hormones in order to control plant development processes and an alternative factor regulating growth at the molecular level (Kong *et al.*, 2018). The level of ROS is controlled by plant homeostasis and usually acts as regulator for growth and development (Todorova *et al.*, 2021; Malik *et al.*, 2023). Oxidative damage at the cellular and molecular level accumulates due to ROS, which subsequently leads to a decrease in plant productivity (Honglin *et al.*, 2019; Vranova *et al.*, 2002). It noted also that the products of spatial regulation of ROS are factors that control the development of plants, due to the ability to react with other signaling molecules, including phytohormones, in coordinating these physiological reactions (Salazar-Sarasua, 2022). ROS induced membrane damage is the main cause of plant cell toxicity. The content of H₂O₂ in the stems of seedlings of barley seeds, treated with suspensions with various concentrations of diatomite and with monosilicic acid 1.5 mM/l, showed a lower level of its content compared to Control 2. This gives us reason to suppose that primed seed has a positive effect on the oxidative status of plants. Primed seed with diatomaceous earth at a concentration of 20g DTM/100ml H₂O approximates the oxidative status of seedlings when adding NaCl to the medium to the status of plants not subject to salt stress.

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates (Fig. 6).

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added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

Seed priming with three concentrations of diatomite led to an increase in the SOD activity in both shoot and root value by almost 1.5 times compared to the control (Control 2). SOD are key enzymes that function as the first line of antioxidant defense of cells and have the ability to convert highly active superoxide radicals into H₂O₂, protecting against the toxic effects of ROS generated during normal cellular metabolic processes or as a result of exposure to various stressful environmental conditions (Fig. 7).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

CAT is a heme-containing enzyme whose main target is hydrogen peroxide. In the presence of catalase, H₂O₂ is converted into water and oxygen. The rate of enzymatic breakdown of peroxide reaches approximately 44,000 molecules per second. After seed priming with various concentrations of diatomite and seed priming with sodium silicate (0.1mM/l) showed an increase in the content of CAT and a decrease in inhibition due to salt stress ($p \leq 0.05$) was observed (Fig. 8).

A) Control 1 (seeds were poured with distilled water); B) Control 2. NaCl was added at a concentration of 200 mM (the seeds were primed with distilled water) C) NaCl was added at a concentration of 200 mM (the seeds were primed with monosilicic acid 1.5 mM /L) D) NaCl was added at a concentration of 200 mM (seeds were primed with 5g DTM / 100 ml H₂O) E) NaCl was added at 200 mM (seeds were treated with 10 g DTM / 100 ml H₂O) F) NaCl was added at 200 mM (seeds were primed with 20 g DTM / 100 ml H₂O). Experiments were carried out in three to four replicates.

These results were similar to those of Eraslan *et al.* (2008), Kim *et al.* (2014) and Abbas *et al.* (2015) who had reported that the application of silicon, decreased lipid peroxidation and increased SOD, CAT activities in both leaves and roots under salt stress. Also, Xie *et al.* (2015) reported that silicon application increased photosynthetic rate and stomatal conductance.

Conclusions

Seeds were primed with diatomite, which resulted in a decrease in ROS generation MDA, proline, H₂O₂ and enhancing of the activities SOD, CAT, photosynthetic pigment contents under salt stress. An increasing the level of fermentative antioxidants during the salt stress leads to the neutralization of reactive oxygen species and creates advantageous conditions for plant growth and

development. In this research, priming seeds with the three concentrations of diatomite increased photosynthetic pigments under salt stress compared to non-primed seeds of plants. The effect of ROS accumulation in plant cells under stress is lipid peroxidation via oxidation of unsaturated fatty acids, leading to membrane damage and electrolyte leakage. In the present study under salt stress the level of SOD, CAT was increased, but the level of MDA and H₂O₂ was decreased after the priming seeds with diatomite solutions.

Finally, diatomite may act to alleviate salt stress in plants by decreasing the permeability of plasma membranes and maintenance of cell form and structure due to the increasing of antioxidative enzymes SOD and CAT. Diatomite offset the negative impacts of NaCl stress due to the increased tolerance of a plant to NaCl salinity by enhancement of chlorophyll content and photosynthetic activity. The results of this study can be used in the field of botany and agro-industry and can serve as a basis for future studies of the influence of diatomite on other types of plants.

References

- Abbas, T., R.M. Balal, M.A. Shahid, M.A. Pervez, C.M. Ayyub, M.A. Aqueel and M.M. Javaid. 2015. Silicon-induced alleviation of NaCl toxicity in okra (*Abelmoschus esculentus*) is associated with enhanced photosynthesis, osmoprotectants and antioxidant metabolism. *Acta Physiol. Plant.*, 37: 1-15. <http://dx.doi.org/10.1007%2Fs11738-014-1768-5>
- Abdalla, M.M. 2010. Sustainable effects of diatomites on the growth criteria and phytochemical contents of *Vicia faba* plants. *Agric. Biol. J. North America*, 1(5): 1076-1089. <http://dx.doi.org/10.5251/abjna.2010.1.5.1076.1089>
- Aebi, H. 1984. Catalase *In vitro*. *Methods Enzymol.*, 105: 121-126. [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3)
- Ahmad, B. 2014. Interactive effects of silicon and potassium nitrate in improving salt tolerance of wheat. *Int. J. Agric. Res.*, 13: 1889-1899. [https://doi.org/10.1016/S2095-3119\(13\)60639-5](https://doi.org/10.1016/S2095-3119(13)60639-5)
- Alexieva, V., I. Sergiev, S. Mapelli and E. Karanov. 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.*, 24: 1337-1344. <https://doi.org/10.1046/j.1365-3040.2001.00778.x>
- Bahatska, O., V. Melnyk and O. Snarovkina. 2023. Assessment of drought resistance in plants of the genus *Aristolochia* L. *Ukrain. J. Forest Wood Sci.*, 14(3): 8-20. <https://doi.org/10.31548/forest/3.2023.08>
- Bates, L.S., R.P. Waldren I.D. and Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil*, 39: 205-207. <https://doi.org/10.1007/BF00018060>
- Beauchamp, C. and Fridovich, I. 1971. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, 44: 276-287. [http://dx.doi.org/10.1016/0003-2697\(71\)90370-8](http://dx.doi.org/10.1016/0003-2697(71)90370-8)
- Brazhnikova, Y.V., A.I. Shaposhnikov, A.L. Sazanova, A.A. Belimov, T.D. Mukasheva and L.V. Ignatova. 2022. Phosphate mobilization by culturable fungi and their capacity to increase soil P availability and promote barley growth. *Curr. Microbiol.*, 79(8): 240. <https://doi.org/10.1007/s00284-022-02926-1>
- Corrado, C., M.M. Barreca, S. Raimondo, P. Diana, G. Pepe, M.G. Basilicata, A. Conigliaro and R. Alessandro. 2023. Nobiletin and xanthohumol counteract the TNF α -mediated activation of endothelial cells through the inhibition of the NF- κ B signaling pathway. *Cell Biol. Int.*, 47(3), 634-647.

- Dehtiarova, Z. 2023. Nutrient regime of the soil depending on the share of sunflower in short-rotational crop. *Ukrain. Black Sea Reg. Agrar. Sci.*, 27(2): 87-95. <https://doi.org/10.56407/bs.agrarian/2.2023.87>
- Egamberdieva, D., B. Alaylar, A. Kistaubayeva, S. Wirth and S.D. Bellingrath-Kimura. 2022. Biochar for improving soil biological properties and mitigating salt stress in plants on salt-affected soils. *Commun. Soil Sci. Plant Anal.*, 53(2): 140-152. <https://doi.org/10.1080/00103624.2021.1993884>
- Epstein, E. and A.J. Bloom. 2005. Mineral nutrition of plants, principles and perspectives. Studies on sodium bypass flow in lateral rootless mutants *lrl1* and *lrl2*, and crown rootless mutant *cr1* of rice (*Oryza sativa* L.). *Plant Cell Environ.*, 33: 687-701.
- Eraslan, F., A. Inal, D.J. Pealbeam and A. Gunes. 2008. Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L.) grown under boron toxicity and salinity. *J. Plant Growth Regul.*, 55: 207-219. <http://dx.doi.org/10.1007/s10725-008-9277-4>
- Gong, H.J., D.P. Randall and T.J. Flowers. 2006. Silicon deposition in root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. *Plant Cell Environ.*, 29: 1970-1979. <https://doi.org/10.1111/j.1365-3040.2006.01572.x>
- Gurmani, A.R., A.N. Bano, H. Khan, M. Jahangir and T.J. Flowers. 2013. Exogenously applied silicate and abscisic acid ameliorates the growth of salinity stressed wheat (*Triticum aestivum* L.) seedlings through Na⁺ exclusion. *Aust. J. Crop Sci.*, 7: 1219-1226.
- Hameed, A., M. Sheikh, A. Jamil and S.M. Basra. 2013. Seed priming with sodium silicate enhance seed germination and seedling growth in wheat (*Triticum aestivum* L.) under water deficit stress induced by polyethylene glycol. *Pak. J. Life Soc. Sci.*, 11: 19-24. <https://doi.org/10.3390/plants11152015>
- Helaly, M.N., H. El-Hoseiny, N.I. El-Sheery, A. Rastogi and H.M. Kalaji. 2017. Regulation and physiological role of silicon in alleviating drought stress of mango. *Plant Physiol. Bioch.*, 118: 31-44. <https://doi.org/10.1016/j.plaphy.2017.05.021>
- Honglin, H., U. Farhan, Z. Dao-Xiu, Y. Ming and Z. Yu. 2019. Mechanisms of ROS regulation of plant development and stress responses. *Front. Plant Sci.*, <https://www.frontiersin.org/articles/10.3389/fpls.2019.00800/full>
- Jessen, L. 2007. Advertising sales business manager. *Ind. News*, 1(250): 629-677.
- Karches, T. 2018. Effect of internal recirculation on reactor models in wastewater treatment. *WIT Transact. Ecol. Environ.*, 228: 145-153. <https://doi.org/10.2495/WP180151>
- Kim, Y.H., A.L. Khan, M. Waqas, J.K. Shim, D.H. Kim, K.Y. Lee and I.J. Lee. 2014. Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. *J. Plant Growth Regul.*, 33: 137-149. <https://doi.org/10.1007/s00344-013-9356-2>
- Kim, Y.H., A.L. Khan, M. Waqas, J.K. Shim, D.H. Kim, K.Y. Lee and I.J. Lee. 2013. Silicon application to rice root zone influenced the phytohormonal and antioxidant responses under salinity stress. *J. Plant Growth Regul.*, <https://link.springer.com/article/10.1007/s00344-013-9356-2>
- Kong, X., H. Tian, Q. Yu, F. Zhang, R. Wang, S. Gao, W. Xu, J. Liu and E. Shani. 2018. PHB3 maintains root stem cell niche identity through ROS-responsive AP2/ERF transcription factors in *Arabidopsis*. *Cell Rep.*, 22(5): 1350-1363. <https://doi.org/10.1016/j.celrep.2017.12.105>
- Krauss, G.J. and D. Nies. 2015. Specialized Plant Metabolites: Diversity and Biosynthesis. *Ecol. Biochem., Environ. Intersp. Interact.* <https://onlinelibrary.wiley.com/doi/10.1002/9783527686063.ch2>
- Kruger, G. 2006. Certification decision for Diatomite de Mozambique code 1959ZA0600R1educ. *South Africa Int.*, 1: 3.
- Liang, X., H. Wang, Y. Hu, L. Mao, L.D. Sun, W. Nan and Y. Bi. 2015. Silicon does not mitigate cell death in cultured tobacco BY-2 – cells subjected to salinity without ethylene emission. *Plant Cell Rep.*, 34: 331-343. <https://doi.org/10.1007/s00299-014-1712-6>
- Liang, Y.C., Q. Chen, Q. Liu, W.H. Zhang and R.X. Ding. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). *J. Plant Physiol.*, 160: 1157-1164. <https://doi.org/10.1078/0176-1617-01065>
- Lichtenthaler, H. and A.R. Wellburn. 1983. Determination of total carotenoid and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 603: 591-593. <https://doi.org/10.1042/bst0110591>
- Liu, Y.X. and X.Z. Xu. 2007. Effects of silicon on polyamine types and forms in leaf of *Zizyphus jujuba* cv. *Jinsi-xiaozao* under salt stress. *J. Nanjing Univ. Technol.*, 31: 27-32.
- Lopushniak, V., H. Hrytsuliak, M. Gumentyk, M. Kharytonov, B. Barchak and T. Jakubowski. 2021. The formation of the leaf surface area and biomass of the miscanthus giganteus plants depending on the sewage sludge rate. *E3S Web of Conf.*, 280: 06009. <https://doi.org/10.1051/e3sconf/202128006009>
- Ma, D., D. Sun C. Wang H. Qin H. Ding Y. Li and T. Guo. 2016. Silicon application alleviates drought stress in wheat through transcriptional regulation of multiple antioxidant defence pathways. *J. Plant Growth Regul.*, 35: 1-10. <https://doi.org/10.1007/s00344-015-9500-2>
- Ma, J.F. and N. Yamaji. 2006. Silicon uptake and accumulation in higher plants. *Trends Plant Sci.*, 11: 392-397. <https://doi.org/10.1016/j.tplants.2006.06.007>
- Malik, M., O. Shpykuliak, S. Kravchenko, L. Malik and V. Yuzhykova. 2023. Development of farms in wartime conditions. *Ekonom. APK*, 30(1): 40-50. <https://doi.org/10.32317/2221-1055.202301040>
- Mero, G., B. Skenderasi, E. Shahini, S. Shahini and E. Shahini. 2023. Main directions of plants integrated protection in the conditions of organic agriculture. *Sci. Hor.*, 26(3): 101-111. <https://doi.org/10.48077/SCIHOR3.2023.101>
- Nayekova, S.K., K.M. Aubakirova, K.K. Aitlessov, V.V. Demidchik and Z.A. Alikulov. 2020. Impact of diatomite priming of seeds of hordeum vulgare in salinity. *EurAsian J. Biosci.*, 14: 705-712.
- Nayekova, S.K., M.Z.M. Alikulov, M.M. Satkanov, Z. Myrzabaeva, K.M. Aubakirova, Sh. E. Arystanova and G. Zh Segizbayeva. 2018. Influence of presowing priming of seeds in the presence of various concentrations of diatomite on the growth and development of barley seedlings (*Hordeum vulgare*). Available at: https://www.researchgate.net/publication/339385003_the_effect_of_pre-sown_priming_of_barley_seeds_in_the_solutions_of_different_salts_in_the_combination_with_diatomite_on_allantoin_content_in_roots_seedlings_under_saline_conditions
- Parida, A.K., A.B. Das and P. Mohanty. 2004. Defence potentials to NaCl in a mangrove. *Bruguiera Parviflora*, differential changes of isoforms of some antioxidative enzymes. *J. Plant Physiol.*, 161: 531-542. <https://doi.org/10.1078/0176-1617-01084>
- Salazar-Sarasua, B., M.J. López-Martín, E. Roque, R. Hamza, L.A. Cañas, J.P. Beltrán and C. Gómez-Mena. 2022. The tapetal tissue is essential for the maintenance of redox homeostasis during microgametogenesis in tomato. *Plant J.*, 112(5): 1281-1297. <https://doi.org/10.1111/tbj.16014>
- Shahini, E., A. Bexolli, O. Kovalenko, N. Markova and Y. Zadorozhnyi. 2023a. Features of growing garden strawberries in open ground conditions. *Sci. Hor.*, 26(7): 106-117. <https://doi.org/10.48077/scihor7.2023.106>

- Shahini, E., D. Shehu, O. Kovalenko and N. Nikonchuk. 2023b. Comparative analysis of the main economic and biological parameters of maize hybrids that determine their productivity. *Sci. Hor.*, 26(4): 86-96. <https://doi.org/10.48077/scihor4.2023.86>
- Shen, X., Y. Zhou, L. Duan, Z. Li, A.E. Eneji and J. Li. 2010. Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and Ultraviolet-B radiation. *J. Plant Physiol.*, 170: 847-853. <https://doi.org/10.1016/j.jplph.2010.04.011>
- Shen, Z., X. Cheng, X. Li, X. Deng, X. Dong, S. Wang and X. Pu. 2022. Ameliorative effects of silicon against salt stress in *Gossypium hirsutum* L. *BMC Plant Biol.*, 22(1): 390. <https://doi.org/10.1186/s12870-022-03783-7>
- Shetty, R., X. Frette, B. Jensen, N.P. Shetty, J.D. Jensen, J.L.J. Hans, N. Mari-Anne and C.P. Lars. 2011. Silicon induced changes in antifungal phenolic acids, flavonoids, and key phenylpropanoid pathway genes during the interaction between miniature oses and the biotrophic pathogen *Podosphaera pannosa*. *Lant Physil.*, 157: 2194-2205. <https://doi.org/10.1104/pp.111.185215>
- Todorova, D., I. Sergiev, E. Shopova, L. Brankova, J. Jankauskienė, S. Jurkonienė, V. Gavelienė and R. Mockevičiūtė. 2021. Physiological responses of Pea plants to treatment with synthetic auxins and auxin-type herbicide. *Botany*, 27(2): 125-133. <https://doi.org/10.35513/Botlit.2021.2.2>
- Tuna, A.L., C. Kaya, D. Higgs, B. Murillo-Amador, S. Aydemir and A.R. Girgin. 2008. Silicon improves salinity tolerance in wheat plants. *Environ. Exp. Bot.*, 62: 10-16. <https://doi.org/10.1016/j.envexpbot.2007.06.006>
- Vranova, E., D. Inzé and F. Van Breusegem. 2002. Signal transduction during oxidative stress. *Environ. Exp. Bot.*, 53: 1227-1236. <https://doi.org/10.1093/jexbot/53.372.1227>
- Xie, Z., R. Song, H. Shao, F. Song, H. Xu and Y.L. Liu. 2015. Silicon improves maize photosynthesis in saline-alkaline soils. *Sci. World J.*, 2015(10): 245072. <http://dx.doi.org/10.1155/2015/245072>
- Yunyk, A. and L. Harbar. 2022. Efficiency of use of macronutrients by spring rapeseed plants. *Plant Soil Sci.*, 13(1): 67-73. [https://doi.org/10.31548/agr.13\(1\).2022.67-73](https://doi.org/10.31548/agr.13(1).2022.67-73)

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