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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF SOYBEAN SEEDLINGS (*GLYCINE MAX* L.) TO α-TOCOPHEROL TREATMENT UNDER SALT STRESS

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Background. Salinity is one of the most important factors affecting the growth and productivity of plants. It creates stressful conditions for legumes at the initial phases of ontogenesis. The prospects of exogenous α-tocopherol usage to increase the salt resistance of *Glycine max* L. were studied. The aim of the work was to study the influence of salt stress and α-tocopherol on the growth of soybean and indicators characterizing the antioxidant system functioning.

Materials and Methods. Soybean seedlings of the Oksana variety were the objects of our study. Seeds of the control group were soaked in distilled water, seeds of the experimental groups – in α-Toc acetic solutions in concentrations (0.01; 0.1; 0.5; 1.0 g/L). A 100 mM sodium chloride solution was used to create salinity. The samples of cotyledons, hypocotyls and primary roots of 10-day-old soybean were used for determination of biochemical parameters. Germination of seeds, raw weight, length of soybean hypocotyls and roots, content of TBA-reactive substances, carbonyl groups of oxidatively modified proteins, proline, catalase and ascorbate peroxidase activity were determined.

Results. It was established that salinity caused inhibition of soybean seed germination and growth. Pretreatment of soybean seeds with α-Toc (0.1 and 0.5 g/L) effectively increased germination and improved growth indicators of soybean. An increase in TBA-reactive substances and oxidatively modified proteins in hypocotyls and roots of salt-stressed plants was recorded. α-Toc reduced the level of peroxidation and oxidatively modified proteins of soybean seedlings under salinity. Proline synthesis increased during the development of the salt stress reaction, and its accumulation is an adaptive response of soybean plants. The pretreatment of α-Toc caused a significant increase of proline and stimulated catalase and ascorbate peroxidase activity in soybean seedling tissues under salt stress. However, a high concentration of α-Toc (1.0 g/L) slowed down the activity of antioxidant enzymes.

Conclusions. Our study suggests the participation of α-Toc in the formation of legumes salt resistance. The α-Toc pretreatment of soybean seeds improved germination and enhanced growth processes, normalized the oxidative state of the salt-stressed soybean seedlings by inhibiting peroxidation and reducing the degree of oxidatively modified proteins, stimulating the activity of antioxidant enzymes, and increasing the content of proline.

Keywords: salinity, *Glycine max* L., growth, TBA-reactive substances, protein's oxidative modification, proline, antioxidant enzymes

INTRODUCTION

Abiotic stress corresponds to any environmental factor that can limit plant growth and productivity. Salinity in water or soil, specifically in arid or semi-arid regions, is a major factor commonly found in the southern steppe zone of Ukraine. Over 800 million hectares of world land are affected by high levels of salinity, 20–33 % of which is used for agricultural purposes (Imran, *et al.*, 2021). In Ukraine, saline soils occupy 1.71 million ha, of which 848.2 thousand ha are arable land. Salinization can be the result of natural causes and is due to anthropogenic activities (Mohanavelu *et al.*, 2021). Salinity and excessive uptake of ions (Na^+, Cl, SO_4^{2-}) exerts significant effects on plants at a physiological level by hampering their ability to absorb water. In plants experiencing salt stress, there is a decrease in transpiration and photosynthesis rates, as well as issues like ion toxicity, membrane instability, and mineral deficiency, leading to the inhibition of enzymes and metabolic pathways, disruption of nitrogen assimilation, and nodule formation (Balasubramaniam *et al.*, 2023; Kolesnikov *et al.*, 2023; Mykhalkiv *et al.*, 2023). When salt levels exceed tolerable limits, detrimental effects on germination, growth, productivity, and crop yields occur (Massange-Sánchez *et al.*, 2021; Pyda *et al.*, 2021). Oxidative damage occurs due to elevated reactive oxygen species (ROS) levels stemming from salt stress, and may cause structural damage, ultimately leading to plant demise (Ahmad *et al.*, 2019). To cope with the detrimental impacts of salinity, plants activate various physiological and biochemical mechanisms, including the synthesis of compatible solutes and osmoprotectants, as well as antioxidative metabolic responses (Hasanuzzaman *et al.*, 2021; Cordea & Borsai, 2021).

Soybean (*Glycine max* L.) is considered to be one of the major oilseed, high-protein and economically important leguminous crops. To date, the world production of soybeans is almost 352 million tons (Sabagh *et al.*, 2019). The role of soybeans in increasing soil fertility is unsurpassed due to their symbiosis with nodule bacteria, as a result of which they accumulate 150–200 kg/ha of nitrogen.

Soybean is a medium salt-resistant crop which can withstand salinity only up to an average level (0.25–0.3% or 0.5–0.6 mS/m^2). A high concentration of easily soluble salts in the root layer of the soil leads to a decrease in plant productivity from 30 to 100 % (Isayenkov & Maathuis, 2019; Imran *et al.*, 2021).

Most researchers considered the germination and seedling stages of soybean to be the most sensitive to salt stress. (Açikbaş *et al.*, 2023). A number of researchers reported that treatment of legume seeds and seedlings with NaCl salinity reduced their germination, overall growth and severely affected the basic growth and physiological parameters such as fresh weight, dry weight of roots, shoots and number of leaves, leaf area index, and relative water content. It led to chlorophyll loss and slower transportation of photosynthetic components (Isayenkov & Maathuis, 2019; Roychoudhury *et al.*, 2021; Massange-Sánchez *et al.*, 2021).

One of the obvious effects of osmotic or ionic stress resulting from salt exposure, as reported in many plant studies, is the increasing levels of ROS ultimately leading to cellular damage through the oxidation of lipids, proteins, and nucleic acids. Soybean seedlings grown in salinity accumulated H_2O_2, products of lipid peroxidation and protein oxidation, increased in the activity of antioxidant enzymes catalase (CAT), ascorbic acid oxidase, superoxide dismutase (SOD), and peroxidase (POX). Thereby, it reduced the negative impact on germination and post-germination growth stages (Silva *et al.*, 2021). D. B. Shelke (2019) noted a significant increase in Na^+ content at 100 and 200 mM NaCl induced salinity while the content of K^+, Ca^{2+}, and Mg^{2+}, and the activity of SOD, CAT and POX decreased significantly. Salinity also reduces nodulation in soybean, thus affecting nitrogen fixation efficiency, and ultimately the yield (Sabagh *et al.*, 2019; Chung *et al.*, 2020; Sheteiwy *et al.*, 2021). It was shown, that proline, amino acids, sugars, and secondary metabolites content increased under the different rate of salinity stress in four soybean cultivars (Nguyen *et al.*, 2020; Begum *et al.*, 2022).

Nowdays, the attention of researchers is attracted by drugs that would ensure the formation of adaptive properties of crops (Tryhuba *et al.*, 2019; Kolesnikov *et al.*, 2019; Sadiq *et al.*, 2019). Tocopherol is a lipophilic membrane-bound cellular antioxidant found in all photosynthetic organisms. α-Toc has pronounced membranotropic and antioxidant properties and is capable of stabilizing cell membranes, inhibiting the formation of active oxygen metabolites and lipid peroxidation, influencing the activity of the enzymatic antioxidant chain (Bano *et al.*, 2021; Ali *et al.*, 2022).

It should be noted that the effect of exogenous α-Toc on the adaptability of legumes has not been sufficiently elucidated, and the use of a similar substance of natural origin is promising in view of the Green Deal trends in agriculture. A number of studies have shown the effectiveness of the tocopherol treatment of tomatoes, beans, flax, wheat, rice, maize under conditions of salt stress due to its effect on plant growth, the formation of generative organs and the yield of crops (Badr *et al.*, 2021; Al-Omar *et al.*, 2020; Sofy *et al.*, 2020). The application of α-Toc alleviated oxidative damage by reducing lipid peroxidation and decreasing lipoxygenase activity. The up-regulation of antioxidant systems protected soybean from the damaging effects of ROS. Moreover, the content of ascorbate, reduced glutathione and a-tocopherol increased significantly (Alnusairi, 2022). Growth promoting effects of 200 $mg \cdot L^{-1}$ α-Toc have also been observed in *Vicia faba* L. plants (Semida *et al.* 2014). Exogenous α-Toc (100 $mg \cdot L^{-1}$) reduced the content of malondialdehyde (MDA), proline in the leaves and roots and decreased electrolytes leakage due to the presowing treatment of pea seeds and folia application. Catalase and glutathione peroxidase activities were decreased, whereas the ascorbate and glutathione pool was accumulated under the α-Toc influence during vegetative period. α-Toc increased chlorophyll content, leaf area index, and netto photosynthesis of crops. The use of α-Toc increased the biological yeild of peas by 11 % (Kolesnikov, 2014).

I. H. Khamees AL-Kareemawi and A. G. Muhmood AL-Kazzaz (2019) reported that a spray of α-Toc significantly increased the activity of antioxidant enzymes and accumulation of ascorbic acid, phenolics, accessory pigments, calcium, potassium, and magnesium compared with untreated wheat plants. Moreover, soaking of sunflower seeds with α-Toc in various concentrations helped mitigate the adverse effects of salinity (Lalarukh *et al.*, 2020). S. A. Orabi and M. T. Abdelhamid (2016) also found an important role of α-Toc in alleviating the deleterious effects of seawater salinity on *Faba beans*. α-Toc enhanced antioxidant enzyme activity and the levels of proline, carotenoids, and mineral ions (Ca^{2+} and K^{+}) along with a decrease in MDA contents. H. El-Bassiouny & M. S. Sadak (2015) treated flax cultivars with α-Toc (400 $mg \cdot L^{-1}$) as a foliar spray and found enhanced total soluble carbohydrates, free amino acids, proteins, and photosynthetic pigment contents, proline, and nucleic acids, but significantly reduced lipid peroxidation, and activities of polyphenol oxidase, and peroxidase under saline conditions (2000, 4000 and 6000 $mg \cdot L^{-1}$). The studies clearly show that foliar application of α-Toc during salinity stress provides enhanced tolerance by stabilizing biochemical processes, thereby resulting in compensation of biomass/seed yield production (Sadiq *et al.*, 2019; Orabi & Abou-Hussein, 2019; Hasanuzzaman *et al.*, 2022).

The study of soybean responses to α-Toc treatment under salt stress during seedling stages is important for understanding the mechanism of the stress damage and for finding techniques to increase resistance. The results of this study will contribute to the improvement of agrotechnology for growing soybeans in saline conditions with a reduction in crop loss.

The aim of this work was to evaluate the influence of exogenous tocopherol in concentration rate (0.1–1.0 g/L) on the soybean seedlings growth parameters, lipid peroxidation, the degree of protein oxidative modification, proline content, catalase, and ascorbate peroxidase activities under salt stress. The study provides essential information regarding germination and growth requirements, and investigates tolerance of soybean to salt stress.

MATERIALS AND METHODS

Plant material, experimental procedures. The soybean seedlings (*Glycine max* L.) of Oksana variety were used as a model object of our study. The Oksana medium-ripe variety (originator: Institute of fodder of the National Academy of Agrarian Sciences of Ukraine, Vinnytsa) is distinguished by ecological plasticity necessary for cultivation in the Steppe zone. The seeds have high productivity and the germination rate is 94 %. The weight of 1000 seeds is 138.9 g.

Soybean seeds were placed at growth chamber conditions with a temperature regime of 23±1 °C in the dark. Three days after sowing, the germinated seeds were grown at 16/8 hours photoperiod, 60 % relative humidity (RH) and 100 $mmol\ m^{-2}s^{-1}$ of photosynthetic photon flux density provided by fluorescent lamps. Seeds were germinated according to the International Seed Testing Association (ISTA) protocol. For each treatment, 250 seeds were placed on five 90 mm diameter Petri dish (50 seeds on each dish). Two layers of filter paper were moistened with 5 mL of incubation medium (ISTA, 2014).

The design of the experiment included 6 groups. One group (control) was used for control where soybean seeds were germinated on water. A 100 mM sodium chloride solution was used to create salinity medium, in which the seeds of other groups were germinated for 10 days. Such concentration of sodium chloride solution produces an osmotic potential -0.5 MPa.

Before sowing, the soybean seeds of the absolute control group and saline control were soaked in distilled water. The soybean seeds of α-tocopherol (α-Toc) treated groups were soaked in a solution containing solubilized α-Toc acetate in concentrations of 0.01 g/L, 0.1 g/L; 0.5 g/L; 1.0 g/L with the addition of 0.001% dimethylsulphuroxide (DMSO). The α-Toc dose range was chosen based on our previous studies and data from other studies (Kolesnikov, 2014; Badr *et al.*, 2021; Alnusairi, 2022). A 10% oil solution of DL-α-Toc acetate ("Technolog", Ukraine) was used to prepare an α-Toc emulsion with the help of a nonionic emulsifier Twin 80 (oxyethyl ether of fat acid). The resulting emulsion was diluted with water and DMSO to the required concentration.

Growth measurement. The germination percentage (GP), was measured on the 7th day after sowing (DAS). The calculation of laboratory germination was made using the ratio (%) of the total number of seeds that were taken for germination to the number of not germinated seeds. The swollen, rotten, and abnormally germinated seeds are referred to as not germinated ones (ISTA, 2014). This index was calculated using the formula:

$$\mathrm{GP} = \frac{50 - n}{50} \cdot 100\,\%,$$

where, n – number of seeds that did not germinate in 7 days, un.

The seedling vigor index was obtained by multiplying the seedling length by the germination percentage and calculated using the formula:

$$\text{SVI} = \text{Seedling lenth (cm)} \cdot \text{GP (\%)}$$

The raw weight (RW, g) of primary roots and seedlings was quantified using analytical scales on the 10th DAS. Hypocotyls length (cm) was recorded as the distance from the root neck to the place where the cotyledons attach to the hypocotyl (Hakizimana, 2000). Cotyledons, hypocotyls and primary roots were used for determination of biochemical parameters on the 10th DAS.

TBA-reactive substances (TBARS) determination. Plant samples were homogenized in the porcelain mortar at the presence of 100 mmol tris-HCl buffer (pH 7.8) at a ratio 1:9 (v/v) at temperature 0–4 °C. The tissue homogenate was centrifuged for 10 min (8000 g) and the supernatant liquid was analyzed. Plant homogenate with a volume of 1.0 mL was incubated in the boiled water bath with 3.0 mL of 0.5% thiobarbituric acid (TBA) on 20% trichloroacetic acid (TCA) solution for 30 min. After that, the reaction mixture was placed on ice and then centrifuged for 10 min at 5000 g. The supernatant was used for photometric analyses. The absorbance of supernatant was read at 532 nm ("Unico UV-2800") subsequent to subtraction of non-specific absorption at 600 nm. The level of TBARS was measured according to MDA concentration, which was calculated using its extinction coefficient 155 $\mathrm{mM^{-1}cm^{-1}}$ and expressed in $\mu\mathrm{M \cdot g^{-1}}$ of raw tissue (Dhindsa *et al.*, 1981).

Determination of protein oxidative modification. The degree of protein oxidative modification was evaluated by the content of carbonyl groups. Firstly, 12.5 mM of 2,4-dinitrophenylhydrazine (DNPH) with 2.5 M HCl was added to the sample of homogenized plant tissue. The samples were precipitated with 10% TCA after the incubation at room temperature for 1 hour. Then washing procedure was performed, and final pellets were dissolved in 6 M urea with trifluoroacetic acid pH 2.3. The formation of 2,4-dinitrophenylhydrazone was recorded at the wavelength of 370 nm ("Unico UV-2800"), and the level of carbonyl groups in oxidatively modified protein (OMP) was calculated using the molar extinction coefficient of 21000 $\mathrm{M^{-1}cm^{-1}}$ and expressed in μmol·mg-1 protein (Levine *et al.*, 2000).

Proline determination. Proline contents ($\mu g \cdot g^{-1}$) were measured using the rapid colorimetric method by L. S. Bates *et al.* (1973). Proline was extracted from 1.0 g of raw tissue by grinding in 10 mL of 3% (v/v) sulfosalicylic acid. The mixture was then centrifuged at 4000 g for 10 min. In a test tube, 2 mL of the supernatant followed by 2 mL of freshly prepared acid-ninhydrin solution and 2 mL of acetic acid was placed. The tube was incubated in a boiled water bath for 1 hour and the reaction was terminated in an ice-bath. Then the reaction mixture was extracted with 4 mL of toluene and shaken for 20 sec. After the separation, the toluene phase was collected into a test tube and its absorbance was read at 520 nm. Proline concentrations were determined from a standard curve prepared using analytical grade proline.

Determination of catalase activity. Catalase activity (EC 1.11.1.6) was determined by measuring the rate of H_2O_2 conversion to O_2 at room temperature and the result was presented in $mcatal \cdot mg^{-1}$ protein (Aebi, 1984). Plant homogenate with a volume of 0.1 mL, obtained as previously described, was added to 2.0 mL of 0.03% H_2O_2 and incubated for 5 min. The reaction was terminated by 1.0 mL of 4% ammonium molybdate solution. The absorbance of the colored complex was read at 410 nm. CAT activity was calculated using its extinction coefficient 22400 $mM^{-1}cm^{-1}$. The protein content in plant homogenate was measured by O. H. Lowry *et al.* (1951) with a help of Pholine reagent.

Determination of ascorbate peroxidase activity. Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was determined by measuring the rate of ascorbate oxidation in the presence of H_2O_2. The plant homogenate was centrifuged in a phosphate buffer (pH 7.8) for 10 min at 10,000 rpm. The supernatant was transferred to a test tube and kept on ice to prevent loss of activity. Enzyme activity was determined at a temperature of 37 °C. Then, 2.25 mL of phosphate buffer, 0.1 ml of ascorbic acid solution, 0.1 mL of hydrogen peroxide solution, 0.05 mL of EDTA solution were added to the cuvette. The reaction was started by adding 0.5 mL of the supernatant. The mixture was quickly shaken and the change in optical density at 290 nm was measured on a spectrophotometer. APX activity was expressed in μmol of ascorbate ($\varepsilon = 2.8\ M^{-1}cm^{-1}$) per 1 mg of protein (Nakano & Asada, 1981).

Statistical analysis. Statistical analysis of the results was carried out using SPSS software. All measurement represents the means and standard error (±SE) of five replicas. The data were analyzed using ANOVA test. Statistically significant differences between means were compared at the 0.05 probability level using Duncan's test. Pearson's correlation test was conducted to determine the correlations between parameters (Gomez & Gomez, 1984).

RESULT AND DISCUSSION

The effect of salt stress and α-Toc on germination and growth. The germination of soybean seeds significantly decreased under conditions of sodium chloride salinity (**Table 1**). Soybean seeds germination for 7 days showed that pre-sowing α-Toc treatment caused changes in biometric indicators. The laboratory germination of soybean seeds increased by 9 % under α-Toc treatment at a concentration of 0.1 g/L and by 7 % under α-Toc treatment at a concentration of 0.5 g/L compared with salt stressed plants. Under the pre-treatment with α-Toc at higher concentrations (1.0 g/L), there was no increase in germination of soybean seeds, and it remained at the level of germination of seeds germinated in a 100 mM NaCl solution.

Table 1. **Germination percentage of seeds, raw weight and length of soybean seedlings and roots under α-Toc and salinity treatment**

Salinity, mM NaCl	α-Toc, g/L	Germination, %	Raw weight/100 un., g		Length, cm		SVI
			seedling	root	hypocotyl	root	
0	0	77.5±2.7	49.21±1.51	19.24±0.88	6.70±0.41	7.35±0.13	515±35
100	0	57.0±2.7^a	37.38±1.66^a	12.73±0.69^a	2.82±0.17^a	4.94±0.10^a	158±14^a
100	0.01	60.2±3.2^a	36.84±1.54^a	13.05±1.01^a	3.56±0.28^a	5.01±0.15^a	213±28^{ab}
100	0.10	66.0±1.7^{ab}	43.58±1.42^{ab}	16.56±0.78^{ab}	4.83±0.25^{ab}	6.25±0.18^{ab}	322±29^{ab}
100	0.50	64.1±1.7^{ab}	42.12±1.44^{ab}	15.30±0.65^{ab}	4.65±0.18^{ab}	5.77±0.11^{ab}	304±26^{ab}
100	1.00	58.05±2.44^a	39.04±1.61^a	14.64±0.56^{ab}	4.02±0.21^{ab}	4.98±0.13^a	243±21^{ab}

None: ^{a} – significant compared to control (H_2O treatment) ($P < 0.05$); ^{b} – significant compared to salinity control (100 mM NaCl treatment) ($P < 0.05$)

The main indicator of the plants vitality is the growth of their biomass. The raw weight of seedlings and roots of soybeans germinated in a 100 mM NaCl solution was lower than in the control group. α-Toc (0.01 g/L) did not affect the raw weight of soybean seedlings. A significant increase in the raw weight of 10-days seedlings was recorded by 16.6 % and 12.7 % and soybean roots by 30.0 % and 20.2 % under pre-treatment with α-Toc solutions at concentrations of 0.1 g/L and 0.5 g/L, respectively. However, α-Toc at higher concentrations (1.0 g/L) promoted soybean biomass growth under salt stress conditions, but with lower efficiency.

A decrease in the length of hypocotyls and roots was observed under the influence of salinity factor. The length of soybean hypocotyls germinated in a 100 mM NaCl solution decreased by 2.38 times, and the root length – by 1.49 times compared to the parameters of absolute control seedlings. However, the length of hypocotyls increased by 1.26–1.71 times and the length of roots – by 1.01–1.27 times under α-Toc pre-treatment in all studied concentrations under salinity conditions and compared to untreated seeds that germinated in a 100 mM NaCl solution. α-Toc at a higher concentration (1.0 g/L) did not cause an increase in the length of soybean roots.

As shown in **Table 1**, there is a statistically significant difference ($P < 0.05$) between the salinity treatment in terms of the seedling vigor index. The highest SVI – 515 – was observed at soybeans of control group, while the lowest value was determined for salt treatment group as 158. An α-Toc treatment of soybean seeds resulted in a statistically significant increase in the seedling vigor index. The highest SVI was determined under 0.1 and 0.5 g/L α-Toc treatment, it exceeded the corresponding indicator in soybean plants not treated with α-Toc under salinity conditions by two times.

The effect of salt stress and α-Toc on the TBARS content and oxidative modification of proteins. Salinity stress induced oxidative damage of cell membrane assessed by an increase in lipid peroxidation in soybean plant as compared with unstressed control plants. An increase in the content of TBARS was noted in soybean seedlings 10 DAS under salt stress (**Table 2**).

Pre-treatment of soybean seeds with α-Toc in concentrations of 0.1, 0.5 and 1.0 g/L reduced the TBARS content in soybean hypocotyls by 12.9–18.4 % ($P \leq 0.05$), and by 14.5–18.1 % ($P \le 0.05$) in roots. At the same time, the level of peroxidation in α-Toc treated soybean plants was at the level of the control group that germinated in water without chloride salinity. It should be noted that accumulation of TBARS was not observed in soybean cotyledons under chloride salinity 10 DAS. However, in soybean cotyledons under the influence of α-Toc, there was a slight decrease in the TBARS content by 8 %, compared with samples obtained from the seedlings group of soybean untreated with α-Toc.

Table 2. **TBA-reactive substances and carbonyl group OMP content in cotyledons, hypocotyls and roots of 10-day-old soybean plants under α-Toc and salinity treatment**

Salinity, mM NaCl	α-TPh, g/L	TBARS, μmol MDA·g^{-1} RW			OMP, μmol·mg^{-1} protein		
		cotyledon	hypocotyl	roots	cotyledon	hypocotyl	roots
0	0	24.0±0.3	24.3±0.1	19.6±0.4	9.3±0.3	17.4±0.7	16.4±1.6
100	0	21.2±0.3[a]	29.4±0.7[a]	22.7±0.4[a]	7.6±0.2[a]	32.6±1.2[a]	25.2±1.4[a]
100	0.01	20.1±0.5[a]	28.5±1.1[a]	22.3±0.5[a]	7.5±0.2[a]	29.5±0.8[a]	23.4±1.3[a]
100	0.10	19.6±0.7[a]	25.6±0.9[b]	18.6±0.6[b]	7.2±0.2[a]	21.3±0.7[ab]	18.2±1.1[b]
100	0.50	22.8±0.5	24.0±1.0[b]	18.9±0.6[b]	8.1±0.3[a]	23.5±0.9[ab]	19.7±0.9[ab]
100	1.00	21.8±0.6[a]	24.8±1.1[b]	19.4±0.5[b]	8.5±0.2[b]	26.1±0.5[ab]	22.6±0.9[ab]

Note: [a] – significant compared to control (H_2O treatment) ($P < 0.05$); [b] – significant compared to salinity control (100 mM NaCl treatment) ($P < 0.05$)

It should be noted that soybean is a high-protein crop, therefore oxidative modification of proteins negatively affects their use in plastic metabolic processes under stress. Thus, an increase in the content of carbonyl groups OMP in soybean seedlings and roots was recorded by almost 2 times under the influence of salt stress. α-Toc in concentrations of 0.1 g/L and 0.5 g/L effectively reduced the degree of OMP in the hypocotyls by 34.7 % and 27.9 % ($P < 0.05$), respectively, and in the roots by 27.8 % and 21.8 % ($P < 0.05$) compared to plants germinated on saline medium and untreated with α-Toc. The content of carbonyl groups OMP was reduced by 18.3 % in soybean cotyledons under salt stress. However, pre-treatment with α-Toc did not lead to significant changes in the content of carbonyl groups OMP in cotyledons compared to the untreated group of soybean seedlings germinated in a 100 mM NaCl solution.

The effect of salt stress and α-Toc on the proline content. Proline belongs to the so-called "stress" amino acids. The data in **Table 3** obviously indicate that elevated salinity levels caused significant increase in soybean seedling proline content, as compared with control plants (plants germinated with water).

The proline content significantly increased under salinity conditions in cotyledons by 2.7 times, in hypocotyls by 1.74 times, and in soybean roots by 1.62 times ($P < 0.05$) compared to plants of the control group. In addition, α-Toc treatments induced proline content in stressed plants as well as in unstressed plants relative to their corresponding controls. The content of proline increased gradually in all studied tissues of salt-stressed soybeans as the concentration of α-Toc increased. The most effective accumulation of proline in salt-stressed soybean seedlings was observed under α-Toc pre-treatment

(1.0 g/L). Thus, α-Toc (1.0 g/L) increased the content of proline in soybean cotyledons, hypocotyls, and roots by 1.6, 1.8, and 2.15 times (P <0.05), compared to α-Toc untreated salt-stressed soybean plants.

Table 3. **Proline content in cotyledons, hypocotyls and roots of 10-day-old soybean plants under α-Toc and salinity treatment**

Salinity, mM NaCl	α-Toc, g/L	Proline, μg·mg^{-1} protein		
		cotyledon	hypocotyl	roots
0	0	0.34±0.02	0.70±0.06	0.93±0.10
100	0	0.82±0.06^{a}	1.22±0.10^{a}	1.51±0.08^{a}
100	0.01	0.95±0.05^{a}	1.43±0.09^{a}	1.71±0.11^{a}
100	0.10	1.26±0.06^{ab}	1.87±0.12^{ab}	2.04±0.14^{ab}
100	0.50	1.07±0.07^{a}	2.06±0.15^{ab}	2.68±0.18^{ab}
100	1.00	1.32±0.08^{ab}	2.16±0.14^{ab}	3.25±0.25^{ab}

Note: ^{a} – significant compared to control (H_2O treatment) (P <0.05); ^{b} – significant compared to salinity control (100 mM NaCl treatment) (P <0.05)

The effect of salt stress and α-Toc on the antioxidant enzymes activity. Table 4 shows the effect of salinity and α-Toc on CAT and APX activity. CAT and APX activity in the soybean seedlings exposed to salt stress was significantly reduced as compared with seedlings not exposed to salt stress.

Table 4. **Catalase and ascorbate peroxidase activity in cotyledons, hypocotyls and roots of 10-day-old soybean plants under α-Toc and salinity treatment**

Salinity, mM NaCl	α-Toc, g/L	CAT, mcatal·mg^{-1} protein			APX, μmol·mg^{-1} protein		
		cotyledon	hypocotyl	roots	cotyledon	hypocotyl	roots
0	0	64.3±0.8	232.0±2.3	185.4±2.0	0.26±0.03	0.42±0.03	0.51±0.04
100	0	45.1±0.2^{a}	173.3±2.2^{a}	141.5±3.2^{a}	0.23±0.02^{a}	0.59±0.03^{a}	0.82±0.03^{a}
100	0.01	43.1±0.4^{a}	192.2±2.5^{ab}	149.6±1.8 ^{a}	0.24±0.02	0.65±0.03^{ab}	0.87±0.04^{ab}
100	0.10	55.3±0.4^{ab}	225.4±2.0^{ab}	167.4±1.7^{ab}	0.21±0.01^{a}	0.70±0.04^{ab}	0.93±0.03^{ab}
100	0.50	52.7±0.5^{ab}	231.7±1.9^{b}	180.0±1.8^{b}	0.23±0.02^{a}	0.76±0.03^{ab}	0.95±0.04^{ab}
100	1.00	48.5±0.3^{a}	244.4±2.7^{ab}	199.8±3.0^{ab}	0.20±0.01^{a}	0.62±0.02^{a}	0.85±0.03^{a}

Note: ^{a} – significant compared to control (H_2O treatment) (P <0.05); ^{b} – significant compared to salinity control (100 mM NaCl treatment) (P <0.05)

Salt stress inhibited CAT activity in soybean cotyledons by 29.9 %, in hypocotyls by 25.3 %, and in roots by 23.7 % on the 10th DAS. Exogenous α-Toc in the studied range of concentrations stimulated CAT activity in the soybean seedlings tissues under conditions of salt stress. Moreover, a direct correlation between CAT activity and α-Toc concentration was observed. Thus, CAT activity was the most stimulated by 41 % in the hypocotyls and by 41.2 % in soybean roots under α-Toc pretreatment (1.0 g/L), and by 22.6 % in the cotyledons under the influence of 0.1 g/L α-Toc compared to the salt-stressed soybeans control.

APX activity increased in hypocotyls and soybean roots by 1.4 and 1.6 times under salt stress, respectively (**Table 4**), whereas APX activity was inhibited by 11.5 % in cotyledons due to salt stress. Pre-treatment of soybean seeds with α-Toc increased APX activity, exceeding its activity in soybean hypocotyls by 5–29 % and in roots by 4–16 % compared to the corresponding indicators in salt-stressed control seedlings. The α-Toc seed treatment, however, limited the effect of salinity on CAT and APX activity in the soybean plants. The most effective stimulation of APX activity in the soybean seedlings was observed under 0.5 g/L α-Toc pre-treatment. No significant changes in APX activity were recorded in soybean cotyledons under the influence of α-Toc in saline conditions.

Soil salinity is an environmental stress that can reduce seed germination, plant growth, plant yield and plant constituency. It was found in this study that in response to 100 mM NaCl-induced salinity, the germination of soybean seeds decreased. Inhibited soybean seedling growth induced by salinity occurs as a result of limited water uptake caused by a reduction in hydrolysis and translocation of nutrient reserves, thereby reducing seedling vigor (Parveen *et al.*, 2016). An increased Na^+ and Cl^- uptake reduces seed germination rate and seedling RW (Kumar, 2017). It is known that salt stress causes suppression of the cell elongation phase (Balasubramaniam *et al.*, 2023). Salinity, in our case, reduced root and seedlings length and RW, and seedlings were affected more adversely than roots which finds confirmation in the study by S. Açikbaş *et al.* (2023).

Tocopherols play a vital role in plant adaptation to stress conditions such as drought, salinity, extreme temperature, radiation, and toxic metals. Plant species vary in their tocopherol-induced stress adaptation in terms of their susceptibility and the putative metabolic functions involved (Sadiq *et al.* 2019; Hasanuzzaman *et al.*, 2022). The germination of soybean seeds pretreated with α-Toc before application of the salt stress was, however, very similar to control seeds not exposed to a salt stress. The α-Toc pretreatment stimulation of soybean germination has been observed in other plants (Hasanuzzaman *et al.*, 2022). Comparison of germination, root and hypocotyl length and RW under different α-Toc concentrations showed that pretreatment with 0.1 g/L and 0.5 g/L α-Toc is the most effective. Pretreatment of seeds with α-Toc (0.5 g/L) produced the highest levels of seedling vigor index at 100 mM salt concentration. The exogenous application of α-Toc at a concentration increased to 1.0 g/L did not improve soybean salinity resistance in terms of growth processes compared to its lower concentrations. This can be explained by the fact that α-Toc in high concentrations probably shows a pro-oxidant effect (Bano *et al.*, 2021). A high level of positive correlation between the germination of soybean seeds and its growth indicators in the range of 0.94–0.98 ($P < 0.05$) was revealed.

Salinity stress causes overproduction of ROS that affects the plants leading to ion toxicity, nutritional inadequacy, osmotic stress, and genotoxicity that causes oxidative stress. Increased ROS levels trigger lipid peroxidation in cell membranes, affecting normal cellular activity. Oxidative stress is exacerbated by lipid peroxidation by producing radicals from lipids, which affect proteins and DNA (Bano *et al.*, 2021; Hasanuzzaman *et al.*, 2022). Our data demonstrated that salinity augmented lipid peroxidation levels in hypocotyls and soybean roots, although lipid peroxidation decreased in cotyledons, which can be associated with the slowing down of metabolic processes in the cotyledons and the relocation of plastic substances. Tocopherols inhibit the chain propagation stage in lipid autoxidation, making them an efficient free radical trap (Massange-Sánchez *et al.*, 2021).

The results of our study showed that exogenously applied α-Toc (0.1–1.0 g/L) decreased TBARS content in hypocotyls and roots of soybean plants. Recent studies have also shown that tocopherol plays a positive role under salt-stress conditions by controlling Na^+/K^+ homeostasis and the hormonal balance while minimizing oxidative stress (Begum, 2022). Additionally, it has been demonstrated that the foliar application of α-Toc in onion plants and *Vicia fava* improve salt tolerance with an increase in plant growth and productivity in this condition (Semida *et al.*, 2014).

ROS produced as a result of salt stress can damage proteins in various ways. Direct modification is the process of modifying a protein's function by disulfide bond formation, nitrosylation, carbonylation, and glutathionylation. Through the breakdown of fatty acid peroxidation products, proteins are indirectly altered. The content of carbonyl groups in tissues under oxidative stress is high, which is an indicator of protein oxidation. Such amino acids of plant proteins as tyrosine and cysteine are modified by ROS under the diverse stressors (Bano *et al.*, 2021). In our study, an increase in the content of carbonyl groups OMP in soybean seedlings and roots under the salt stress was marked. The accumulation of free carbonyl content was demonstrated by N. Banik and S. Bhattacharjee (2020) in salt stressed rice. Similarly to our findings, Z. Mushtaq *et al.* (2020) and R. Ahmad *et al.* (2019) showed that increased ROS generation due to salinity leads to lipid peroxidation and protein oxidation. A high significant (P <0.05) correlation was noted in our study between the content of TBARS and the degree of OMP in soybean hypocotyls and roots (r = 0.77–0.84).

Exogenous α-Toc significantly decreased the content of carbonyl groups OMP in soybean seedlings germinated in a 100 mM NaCl solution. Similar results on the reduction of carbonyl groups in plants due to salt stress were reported by H. A. Taie and M. M. Rady (2024).

Previous studies showed that proline involved in osmotic regulation of salt stressed plants, thus playing an important role in the tolerance of plants under salinity stress conditions (Meena *et al.*, 2019), and may be used as a positive approach to reduce the toxicity of Na^+ ions (Shahid *et al.*, 2022). In the present study, proline concentration increased significantly in salt stressed plants compared to the normal control. These results were similar to those of M. Sh. Sadak *et al*. (2019). Accumulation of proline and other aminoacids are considered clear indicators of tolerance to salt stress. The elevation of proline content in the soybean seedlings under NaCl treatment explained the proline contribution to the maintenance of low osmotic potential to enhance water uptake. These findings support the idea that proline accumulation is part of physiological responses of plant to salinity stress (Meena *et al.*, 2019).

The pretreatment with α-Toc led to an increased proline content in cotyledons, hypocotyls and roots of soybean. Moreover, the content of proline in the studied tissues of soybean seedlings increased as the applied concentration of α-Toc increased under salinity. Previous studies have also demonstrated the stimulative effect of α-Toc on proline biosynthesis and accumulation in plants (Kolesnikov, 2014; El-Bassiouny & Sadak, 2015; Hasanuzzaman *et al.*, 2022). α-Toc might, therefore, have a role in regulating the levels of osmoprotectants other than proline and/or in regulating other physiological attributes of salt-stressed plants.

Plants often produce some antioxidant enzymes to avoid oxidative damage under stress conditions and keep concentrations of the reactive oxygen species within a limited and narrow functional range (Akyol *et al.*, 2020; Kolupaev *et al.*, 2023). It is well known that activities of antioxidant enzymes such as catalase or ascorbic acid peroxidase considerably decrease the levels of superoxide and hydrogen peroxide in salt stressed plants (El-Beltagi *et al*., 2020). A high negative correlation was found between the level of peroxidation and CAT activity in hypocotyl tissues (r = -0.94), roots (r = -0.79) and an average positive correlation in soybean cotyledons (r = +0.61). Similar correlations were observed between OMP and CAT activity in soybean seedlings. CAT activity was found to be lower in the cotyledons, hypocotyls and soybean roots under the salt stress. But, APX activity increased in hypocotyls and roots of salt stressed soybean seedlings. As reported by H. S. El-Beltagi *et al.* (2020), a higher activity of antioxidant enzymes caused a decrease in lipid peroxidation under salinity conditions. It appears that APX enzyme showed more significant importance in regulating H_2O_2 at intracellular levels than CAT and enhanced protection against oxidative stress conditions.

Data obtained through this study illustrated that CAT and APX activities increased in soybean seedlings tissues with rising α-Toc concentration under salinity stress. While CAT primarily scavenges H_2O_2 in the peroxisomes, APX does the same in the cytosol and chloroplasts. APX uses ascorbic acid as a reducing agent, but ascorbic acid, in turn, is reduced by tocopherol. However, no reliable correlations were found in this study between APX activity on the one hand, and the content of TBARS or OMP on the other hand. Numerous reports show that tocopherols work synergistically with ascorbate and preserve cell redox homeostasis in stressed plants. Boosting the levels of antioxidant enzymes could be linked to the soybean plant's ability to hinder salt absorption and enhance its own adjustment to NaCl toxicity when treated with α-Toc. Alternatively, it could be argued that α-Toc's presence suppresses ROS and boosts the immune response by encouraging the expression of CAT and APX genes in plants (Mehmood *et al*., 2020). These results confirm previous findings of K. Souana *et al*. (2020) who reported that APX and CAT together play a vital protective role in plant tolerance under salinity stress conditions.

Thus, the research results confirm the data of other studies about the effect of exogenous tocopherol on germination and growth of *Glycine max* L., mitigation of biomolecules oxidative damage, and activity of the enzymatic antioxidant system under salinity.

CONCLUSIONS

This study showed that the addition of α-Toc increased salt tolerance in soybean seedlings (*Glycine max* L.). The germination and growth of soybean plants were influenced significantly by salt stress. Salinity induced lipid peroxidation, oxidative modification of proteins in soybean seedlings and a corresponding development of osmolyte content, as well as changes in CAT and APX activity. Application of α-Toc (0.1 g/L and 0.5 g/L) under saline stress has markedly enhanced the soybean germination rate and growth by reducing TBARS content, and the degree of oxidative modification of proteins, strong accumulation of proline, activation of CAT and APX antioxidant enzymes in soybean seedlings organs. The application of α-Toc may thus be a promising and eco-friendly approach to optimizing legumes production in salt environments. The treatment based on α-Toc and its physiological roles also warrants further research.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

Animal Studies: this article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceptualization, [M.K.]; methodology, [M.K; Yu.P.]; validation, [M.K.]; formal analysis, [M.K; Yu.P.]; investigation, [M.K; Yu.P.]; resources, [M.K; Yu.P.]; data curation, [Yu.P.]; writing – original draft preparation, [M.K; Yu.P.]; writing – review and editing, [M.K; Yu.P.]; visualization, [M.K; Yu.P.]; supervision, [M.K.]; project administration, [M.K.].

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ФІЗІОЛОГІЧНІ ТА БІОХІМІЧНІ РЕАКЦІЇ ПРОРОСТКІВ СОЇ (*GLYCINE MAX* L.) ПІД ВПЛИВОМ α-ТОКОФЕРОЛУ ТА ЗА УМОВ СОЛЬОВОГО СТРЕСУ

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Вступ. Засолення є одним із найважливіших чинників впливу на ріст і продуктивність рослин. Це створює стресові умови для зернобобових на початкових фазах розвитку. Досліджено перспективи використання екзогенного α-токоферолу для підвищення солерезистентності рослин *Glycine max* L. Метою роботи було вивчити вплив сольового стресу й α-токоферолу на ріст проростків сої та показники, що характеризують функціонування антиоксидантної системи.

Матеріали та методи. Для досліджень використовували насіння сої сорту Оксана. Насіння контрольної групи замочували у дистильованій воді, насіння дослідних груп – у розчинах α-токоферол ацетату в концентраціях 0,01; 0,1; 0,5; 1,0 г/л. Для створення сольового тла використовували 100 мМ розчин натрію хлориду. Для досліджень відбирали зразки сім'ядоль, гіпокотиль і коренів 10-добових проростків сої. Визначали схожість, сиру масу, довжину проростків і коренів сої, концентрацію ТБК-активних продуктів, вміст карбонільних груп ОМ білків, проліну, КАТ і АПО активності.

Результати. Встановлено, що засолення викликало інгібування проростання насіння сої та ріст. Передпосівна обробка насіння сої α-токоферолом (0,1 та 0,5 г/л) ефективно збільшувала схожість і покращувала ростові показники рослин сої. Зафіксовано зростання ТБК-АП та ОМ білків у гіпокотилях і коренях сої під час дії засолення. α-Токоферол знижував рівень пероксидації та ОМ білків проростків сої за умов засолення. Посилення синтезу проліну відбувалося в ході розвитку сольової стрес-реакції, а накопичення проліну є адаптивною реакцією рослин сої. Разом із тим, застосування екзогенного α-токоферолу викликало суттєве зростання вмісту проліну та стимулювало КАТ і АПО активності у тканинах проростків сої за умов сольового стресу. Проте висока концентрація α-токоферолу (1,0 г/л) уповільнювала роботу антиоксидантних ферментів.

Висновки. Наші дослідження свідчать про участь α-токоферолу в процесах формування солерезистентності зернобобових культур. α-Токоферол під час обробки насіння сої покращував схожість і посилював ростові процеси, нормалізував оксидативний стан проростків сої через гальмування пероксидації та зниження ступеня ОМ білків, стимулювання активності антиоксидантних ферментів і підвищення вмісту проліну в умовах сольового стресу.

Ключові слова: засолення, *Glycine max* L., ріст, ТБК-активні речовини, окисна модифікація білків, пролін, антиоксидантні ферменти

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