Research Square

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Growth-stimulating and anti-stress activity of lipids of the seed of *Zygophyllum oxianum Boriss*.under salt stress

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Abstract

It is known that the lipid components of plant cells play an important role in protecting plants from the influence of stress factors. Changes in membrane lipids in response to salinity are observed in many plant species, including halophytes and glycophytes. Lipid messenger molecules are involved in many important processes of the adaptive mechanism that allow plants to cope with salt stress.

The aim of the work was the studying of the lipids of the seeds of the plant *Zygophyllum oxianum Boriss.* (family of *Zygophyllaceae*) and their effect on the growth of wheat shoots grown under saline conditions, as well as the effect of plant lipids on the lipid composition of wheat seedlings.

As a result of the study, we found that pre-sowing treatment of wheat and cucumber seeds with neutral components of *Zygophyllum moxianum* seed lipids in low doses stimulates the growth and development of seedlings of these crops under normal conditions and under the influence of salt stress, promotes the formation of total lipids and the biosynthesis of eicosatrienoic and arachidonic acids in wheat seedlings grown under saline conditions.

Full Text

Salt-affected soils are a widespread issue globally and pose a significant challenge for humanity. Current estimates suggest that over 1100 million hectares of cultivated land worldwide are affected by salinization to varying degrees [1]. Among the various types of salt that cause salinization, sodium chloride is the most common and has the strongest adverse impact on plants [2].

In south-western Uzbekistan, the Hungry steppe, Karakalpakstan, Northern Turkmenistan, and Tajikistan, degraded medium and highly saline lands make up a significant portion of the irrigated land in the area, ranging from 18.2% to 55% [3]. The deleterious effects of salinity on these lands are evident in the decline of various plant functions, resulting in a productivity decrease of 25% or more [4, 5, 6].

Under extreme conditions, growth regulators are essential in regulating cellular homeostasis. Pre-sowing seed treatment with plant growth regulators is one of the most effective and environmentally safe methods for enhancing the adaptive capacity of cultivated plants to adverse environmental conditions, such as salinity, water deficit, and drought. This approach is widely used in various crop cultivation techniques [7, 8, 9].

Plant biochemical studies have revealed that plants produce their own protective substances as a response to adverse environmental conditions. These protective substances can be isolated from natural sources and applied to crops to enhance their stability and productivity. Developing new plant protection products based on these biologically active substances of plant origin, known as "biorational pesticides," is an urgent task.

The lipid components of plant cells are known to play a crucial role in protecting plants from the effects of stress factors [10]. Alterations in membrane lipids have been detected in various plant species, including halophytes and glycophytes, in response to salinity. Lipid messenger molecules are involved in several critical processes of the adaptive mechanism that enable plants to withstand salt stress [11].

Biorational growth regulators have been developed from lipid extracts or the sum of lipophilic compounds extracted from higher plants. For instance, a new growth stimulant and plant immunity inducer against fungal, bacterial, and viral diseases, called "biofungistim," has been derived from the larch wood *Larix sibírica* (family of *Pinaceae*) [12]. This product enhances the yield and quality of grain (wheat, barley, oats) and vegetable (potatoes, onions, garlic) crops. Its primary constituents include biologically active lipid and phenolic compounds, such as diterpenes, fatty acids, diterpene hydrocarbons, alcohols, phytosterols, phenolic acids, phenol alcohols, and other compounds.

Extracts derived from the greenery of the *Ábies sibírica* tree (family Pinaceae) have been utilized to create preparations with growth-regulating and fungicidal properties. The drug "verva" contains water-soluble salts of triterpene acids and lipophilic neutral components as active substances. *A. sibírica* also contains polyprenols, essential oil, and vitamins [13]. This composition has a complex and effective impact on plants, accelerating the ripening process of vegetable crops, increasing yields, and reducing their susceptibility to diseases without affecting the quality of vegetables. Another agent with fungicidal properties has been developed, which enhances the yield of cereals, legumes, and vegetables while reducing their susceptibility to fungal diseases. This agent consists of a mixture of saturated fatty acids (FA) with carbon chain lengths of 14:0-34:0 and unsaturated fatty acids with chain lengths of C18-20 (predominantly oleic 18:1 and linoleic 18:2 acids), as well as phytosterols, polyprenols with an admixture of mono- and sesquiterpene compounds [14].

A sum of polyprenols, phytosterols, and tocopherols extracted from the leaves of *Gossýpium hirsútum* (family *Malvaceae*) exhibited a growth-stimulating effect. This preparation was named "Uchkun" [15]. Treating seeds of wheat, corn, tomatoes, and cucumbers with an aqueous emulsion of the drug before sowing accelerates plant development and increases their resistance to drought [16]. Furthermore, treating tomato plants *Lycopersicon esculentum* infected with *Tuta absoluta* with the preparation "Uchkun" in combination with an extract from the plant *Haplophyllum perforatum* increases the area of the tomato leaf surface by reducing the intensity of leaf damage caused by the pest, increases the content of photosynthetic pigments in damaged leaves, and increases the number of fruit elements [17]. Polyprenols extracted from the leaves of the plant *Alcea nudiflora* (family of *Malvaceae*) were found to possess insecticidal activity against some insect pests, including *Macrosiphum euphorbiae* and *Callosobruchus maculatus* [18].

Salt-tolerant plants have significant practical value, and comprehensive research and development of halophytes over the last 25-30 years represents a crucial and rapidly developing scientific area. Halophytes have been found to produce various secondary metabolites, including flavonoids, alkaloids, tannins, terpenoids, antioxidants, and more [19, 20]. However, in-depth chemical analysis of the lipids and

fatty acids of halophyte plants has only recently been conducted, and there is limited information available in the literature regarding the composition and biological activity of these compounds.

Previous studies have demonstrated that soaking seeds of cultivated plants with lipid components derived from certain halophytic plants can mitigate the inhibitory effects of sodium chloride stress. The growth-stimulating and stress-protective activities of neutral lipids, glycolipids, phospholipids, and fatty acids (FAs) derived from the seeds of the hyperhalophytic plant *Chenopodium album* (family of *Amaranthaceae*) were examined [21]. It was found that neutral substances (NS) and FAs from neutral plant lipids promote the growth and development of wheat and cotton seedlings under saline conditions, thereby minimizing the negative impact of salt stress.

The stress-protective activity of lipid components derived from the hemihalophyte *Amaranthus retroflexus* (family of *Amaranthaceae*) has been investigated. Pre-sowing soaking of wheat and cucumber seeds in 0.001 and 0.0001% concentrations of HB, FA, and methyl esters of FA helps to alleviate the inhibitory effects of sodium chloride stress, as evidenced by the activation of growth and the accumulation of fresh and dry weight in seedlings. However, neutral lipids did not demonstrate any protective effects [22].

The aim of this work was to explore the lipids in the seeds of the plant *Zygophyllum oxianum Boriss*. (family of *Zygophyllaceae*) and their effect on the growth of wheat shoots cultivated under saline conditions, as well as the impact of plant lipids on the lipid composition of wheat seedlings.

Zygophyllum oxianum Boriss. is a perennial herbaceous plant that is endemic to Central Asia [23]. It grows on slightly and moderately saline soils in the lower reaches of the Amu Darya, Kyzylkum, Ustyurt, and in the Aral Sea region of Uzbekistan. *Z. oxianum* is considered a good ameliorant, and it is used as an anti-infective, anti-rheumatic, and anti-diabetic agent in Uzbek folk medicine [24].

Mature seeds of *Z. oxianum* were collected in the Jizzakh region of Uzbekistan in 2021 and were used for the study. To isolate the neutral lipids (NL), the seeds were crushed and extracted with gasoline (bp72-76 °C) using a Soxhlet apparatus, and the resulting extract was evaporated on a rotary evaporator. The seeds were found to contain 5.1% (dry weight) of NL. The component composition of the NL was determined by thin-layer chromatography on silica gel, with the addition of 13% gypsum in the solvent system hexane: diethyl ether 7:3 and 8:2. 50% H_2SO_4 was used as a spot developer, with subsequent heating. The NL composition was found to contain carotenoids, the biologically active isoprenoid hydrocarbon squalene, fatty acid esters with aliphatic alcohols, triterpenols, and phytosterols, as well as free fatty acids, triterpenols, and phytosterols.

After treatment with a 10% ethanol solution of KOH at boiling for 1 hour, lipophilic NSs and fatty acids were isolated from the NL. The content of NSs was found to be 6.88% (by weight of NL), and their composition is presented in Table 1.

Table 1. Composition of neutral substances of *Zygophyllum oxianum* seeds

Components Content	% by weight
Aliphatic hydrocarbons	17.30
Carotenoids (provitamin A)	0,035
Isoprenoid hydrocarbon squalene	5,10
Triterpenols, aliphatic alcohols	34.60
Phytosterols	40.65
Unidentified Components	2.80

Based on the provided information, Table 1 reveals that the neutral substances (NIs) found in Zygophyllum oxianum seeds mainly consist of phytosterols, triterpenols, aliphatic alcohols, and hydrocarbons. The fatty acids were transformed into methyl esters using diazomethane and subsequently subjected to analysis using a GC system, specifically the Agilent 6890 N chromatograph. The GC analysis was carried out under the following conditions: employing a flame ionization detector, utilizing a capillary column measuring 30 m x 0.32 mm with an HP-5 stationary phase, employing helium as the carrier gas, and utilizing a temperature program of 150 - 270°C.

Table 2. Composition of fatty acids in neutral lipids from Zygophyllum oxianum seeds, determined through GC analysis, presented as a percentage of mass.

Fatty acid	Content	Fatty acid	Content
12:0	Footprints	trans-18:1n9	1.19
14:0	0.28	18:2 n6	76.26
15:0	Footprints	20:0	0.66
16:0	5.06	20:1	0.80
16:1	0.30	22:0	0.40
17:0	0.16	24:0	0.17
18:0	1.49	$\sum$ saturated FA	8.06
18:1n9+ 18:3n3	13.39	$\sum$ saturated FA	91.94

Table 2 shows that the FAs in *Z. oxianum* lipids include 15 components, with 18:2n6 linoleic acid being the dominant component, comprising more than 76% of the total FAs. The sum of oleic and linolenic acids (18:1n9 + 18:3n3) is also present in notable amounts.

The study evaluated the responses of cultivated plants to the effects of growth regulators under stress, as determined by growth indicators such as the linear dimensions of the shoot and root. The effect of NS of *Z. oxianum* on the germination of seeds and the development of wheat (*Triticum aestivum L.*) variety "Tatiana" and cucumber (*Cucumis sativus*) variety "Orzu" seedlings was examined in laboratory conditions using a known methodology [25]. Control seeds were soaked in water, and experimental seeds were soaked in a solution of NS of *Z. oxianum* at concentrations of 0.01%, 0.001%, and 0.0001% for 18 hours. The synthetic growth regulator Floroxan was used as a reference. After soaking, the seeds were placed on filter paper in Petri dishes, and tap water and 1% sodium chloride solution were added. The seeds were then germinated for 5 days at a temperature of 25-27°C, after which the length of the roots and height of the stem part of wheat and cucumber seedlings were measured.

The results indicate that when using NS at a concentration of 0.0001% on wheat culture under normal conditions, the length of the underground and aboveground parts of the plant exceeded that of the control by 24.3% and 30.4%, respectively. On cucumber seedlings, the length of the underground and aboveground parts of the plant exceeded that of the control by 44.5% and 22.9%, respectively. Figures 1 and 2 demonstrate that NS at a concentration of 0.0001% under normal conditions have a stimulating effect on wheat seedlings, showing an increase of up to 30% compared to the control. On cucumber seedlings, NS at a concentration of 0.0001% showed a stimulating effect up to 44.5% compared to the control.

The study investigated the impact of Z. oxianum NS on the growth of wheat seedlings when cultivated under salt stress conditions. The results indicate that the maximum activity was demonstrated by NS at a concentration of 0.0001%. The length of wheat roots (3.97 cm) was 60.73% higher than the control, while the stems (3.05 cm) were 60.5% longer than the control. Seeds treated with Floroxan increased the length of wheat roots and stems by 48.2% and 72.1%, respectively. These findings are illustrated in Figure 3.

The investigation of Z. oxianum NS's impact on cucumber seedling growth under salt stress (depicted in Figure 4) revealed that at a concentration of 0.0001%, the root length (2.94 cm) was 51.5% longer than the control, while the stem length (1.39 cm) was 54.4% longer than the control.

The study then proceeded to examine the effect of pre-sowing treatment of wheat seeds with Z. oxianum NS on the lipid composition of wheat seedling cells grown under salt stress. Pre-sowing treatment involved soaking wheat seeds in a 0.0001% Z. oxianum NS solution for 18 hours. The treated seeds were then planted on canvas and germinated for 10 days, with multiple daily sprays of 1% NaCl saline. Total lipids from 10-day-old seedlings were isolated using the method described by Kates [26], which encompassed crushing fresh seedlings, fixation with hot isopropanol, filtration of the isopropanol extract, and triple extraction of the residue using a 1:1 mixture of chloroform and isopropanol. The combined extracts were subsequently evaporated on a rotary evaporator at a temperature not exceeding 35°C. The yield of total lipids (TL) from seedlings of control seeds was 1.32%, while that of seedlings treated with Z. oxianum NS was 1.6%. Hence, treatment of wheat seeds with Z. oxianum NS stimulates lipid biosynthesis (TL 1.32% → 1.6%).

Fatty acids were isolated from the total lipids, and their composition was determined using GLC, as shown in Table 3.

Table 3. Fatty acid composition of seedlings from untreated (I) and treated with NS of *Z. oxianum* (II) wheat seeds grown under saline conditions, GC, % of mass

Fatty acid	I	II	Fatty acid	I	II
12:0	0,37	0,75	20:0	0,22	0,96
14:0	1,14	1,41	20:1	0,28	1,70
14:1	0,71	0,94	20:2	2,89	-
16:0	37,55	38,49	20:3, 20:4*	-	5,04
16:1	0,64	1,30	22:0	0,20	1,98
17:0	0,37	-	22:2	0,37	-
17:1	0,34	-	24:0	0,38	Footprints
18:0	3,52	4,53	24:1	0,28	-
18:1	8,53	7,80	$\Sigma_{\text{saturated FA}}$	43,75	48,12
18:2	15,54	14,06	$\Sigma_{\text{saturated FA}}$	56,25	51,88
18:3	26,67	21,04			

*Come out with one peak

The results presented in Table 3 indicate that 18 fatty acids are synthesized in the seedlings of untreated wheat seeds, with palmitic acid (37.55%), linolenic acid (26.67%), and linoleic acid (15.54%) being the dominant components. In contrast, the seedlings of treated seeds showed the presence of 15 fatty acids with similar major components, but with the addition of polyunsaturated eicosatrienoic 20:3 and arachidonic 20:4 acids (5.04%) with increased biological activity. Arachidonic acid is a rare component in the lipid composition of higher plants. However, it is known to be an effective plant growth regulator [27]. Arachidonic acid and its metabolites affect the expression of genes responsible for immunity and genes that control the synthesis of plant growth, differentiation, and development factors. It induces systemic resistance in plants to abiotic and biotic damaging factors and diseases [28]. Furthermore, arachidonic acid improves the generative capacity of plants and contributes to their rapid recovery even after damage by hail or pests. It stimulates plant growth, encouraging them to continue vegetation in order to restore their physiological state after damage [29].

Conclusion

The growth-stimulating and anti-stress properties of neutral substances in *Zygophyllum oxianum* seeds can be attributed to the high content of phytosterols and triterpenols, the presence of provitamin A (carotenoids), and the isoprenoid hydrocarbon squalene. Phytosterols are known for their wide range of biological activities and their ability to improve the stability of cell membranes [30]. They exhibit fungicidal, antiparasitic, antibacterial, and antioxidant properties, as well as antiprotozoal activity [31]. Beta-sitosterol, the main component of phytosterols, has been shown to exhibit antimicrobial and antifungal activity [32].

Triterpenoids play an important role in the cell as biotransformers and contribute to better absorption and digestibility of the main active ingredients. They have low toxicity and exhibit antimicrobial activity [33]. Endogenous unsaturated fatty acids and their metabolites can significantly alter the expression and metabolism of plant genes, thereby influencing the outcome of interactions between plants and microbes, as well as plants and herbivores [34]. As mentioned earlier, arachidonic acid induces systemic resistance in plants to abiotic and biotic damaging factors and diseases [28]. Therefore, the presence of these compounds in *Z. oxianum* seeds can explain their growth-stimulating and anti-stress properties, as observed in the study.

Based on the results of the study, it can be concluded that the pre-sowing treatment of wheat and cucumber seeds with neutral components of *Zygophyllum oxianum* seed lipids in low doses can effectively stimulate the growth and development of seedlings for these crops, both under normal conditions and under the influence of salt stress. This treatment also promotes increased formation of total lipids and biosynthesis of eicosatrienoic and arachidonic acids, especially in wheat seedlings grown under saline conditions. These findings suggest that *Z. oxianum* seeds have great potential as a natural growth promoter and stress reliever for agricultural crops. Further research can explore the possibilities of using *Z. oxianum* seeds for enhancing the growth and productivity of other crops under various environmental stresses.

Declarations

Ethics Approval

This research study titled "Growth-stimulating and Anti-stress Activity of Lipids of the Seed of Zygophyllum oxianum Boriss. under Salt Stress" has been conducted in accordance with ethical guidelines and standards. The study design and experimental procedures involving plant samples and laboratory analyses were reviewed and approved by the appropriate ethical committee at the S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan. Ethical considerations were taken into account to ensure the responsible and humane treatment of the plant materials involved in the study.

Consent to Participate

Not Applicable

Consent for Publication

Not Applicable

Availability of data and materials

All data and materials related to this study are available within the paper. The measurements and observations presented in the paper were obtained using laboratory devices and analytical methods as described in the methods section. The seeds of Zygophyllum oxianum Boriss. were collected from the Jizzakh region of Uzbekistan in 2021. The isolation and characterization of neutral lipids (NL) were performed through extraction with gasoline using a Soxhlet apparatus. The composition of NL was determined through thin-layer chromatography on silica gel, and the content of neutral substances (NS) was quantified. The specific composition and data related to the neutral substances are provided in Table 1 of the paper.

The authors are committed to transparency and scientific rigor. If any additional data or materials are required to reproduce or validate the findings of this study, the corresponding author (Rano Zakirova) can be contacted through the provided email address (ranozakirova@mail.ru). The authors are willing to provide further details, clarification, or additional data upon request to ensure the integrity of the research and facilitate scientific collaboration.

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- Competing interests
- Authors' contributions
- Acknowledgements

Competing Interests

The authors declare that there are no competing interests associated with the research presented in this paper. There are no financial, personal, or professional relationships that could be construed as potentially influencing the results, interpretation, or conclusions of this study.

Authors' Contributions

Each author has contributed significantly to the research and preparation of this manuscript. The specific contributions of each author are as follows:

Gusakova Svetlana: Orchestrated and oversaw the setup of biochemical experiments, closely monitored the experiments, and played a pivotal role in analyzing and summarizing the acquired results.

Zakirova Rano (Corresponding Author): Spearheaded the design and execution of biological studies focusing on the impact of salinization on wheat growth, ensuring rigorous experimental protocols were in place.

Ibotov Shahrukh: Carried out the intricate processes of isolating, fractionating, and identifying lipids, contributing critical expertise to the chemical aspect of the research.

Kurbanova Elvira: Diligently conducted the biological studies, which included plant cultivation, measurement of wheat seedlings, and the application of statistical methods to analyze the resulting data.

Yuldasheva Nigora: Took the lead in extracting non-polar compounds and fatty acids, followed by the comprehensive gas chromatography analysis of the fatty acids, providing essential insights into the chemical composition of the samples.

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Figures

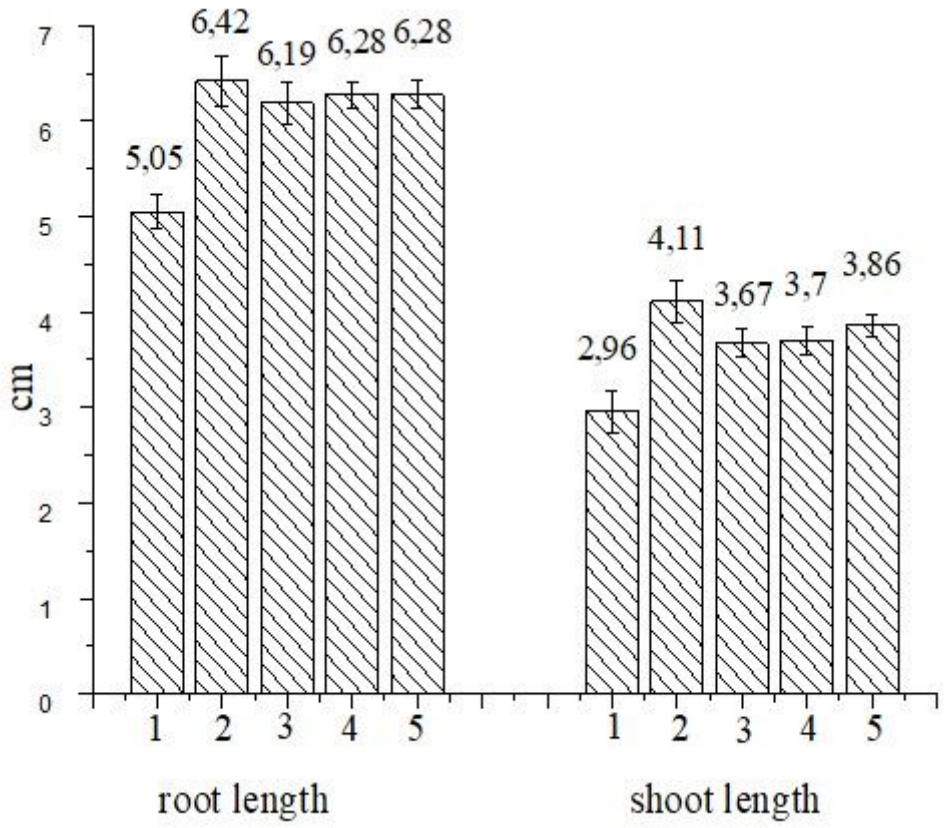


Figure 1

Effect of soaking wheat seeds in a solution of Z. oxianum NS on seedling growth during cultivation under normal conditions.

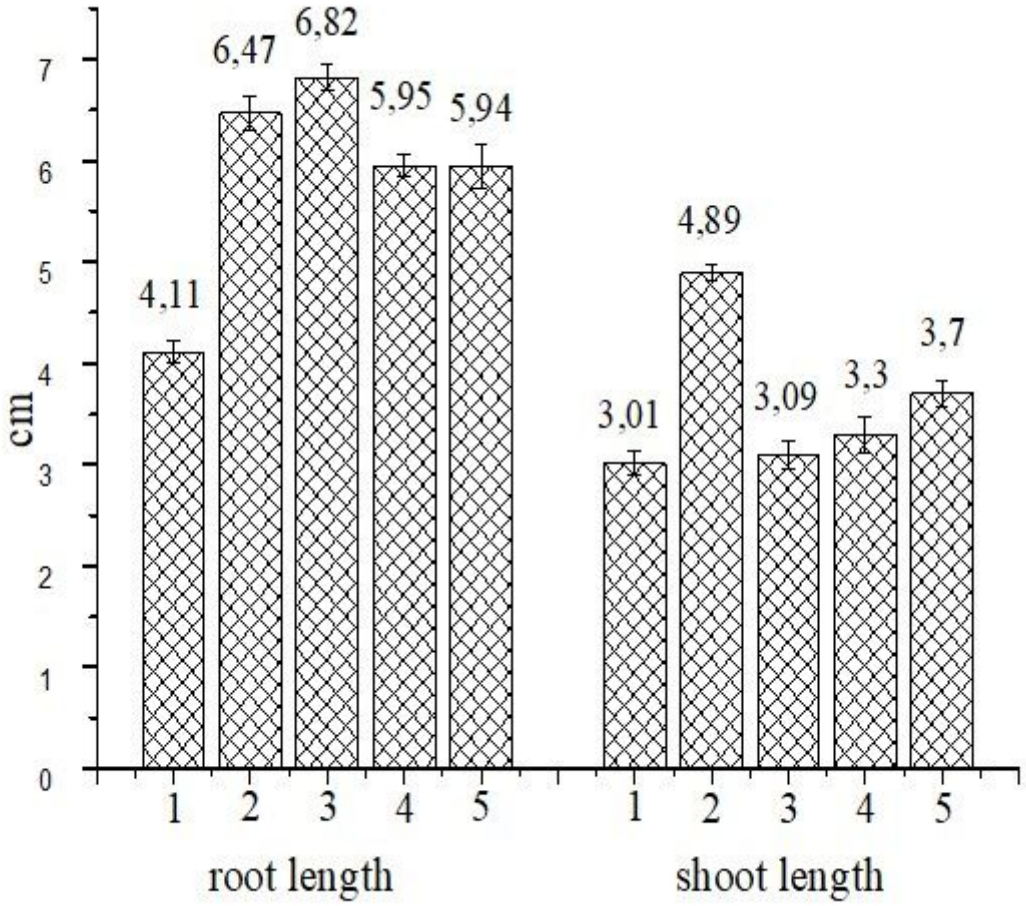


Figure 2

Influence of pickling cucumber seeds in a solution of Z. oxianum NS on seedling growth under salt stress.

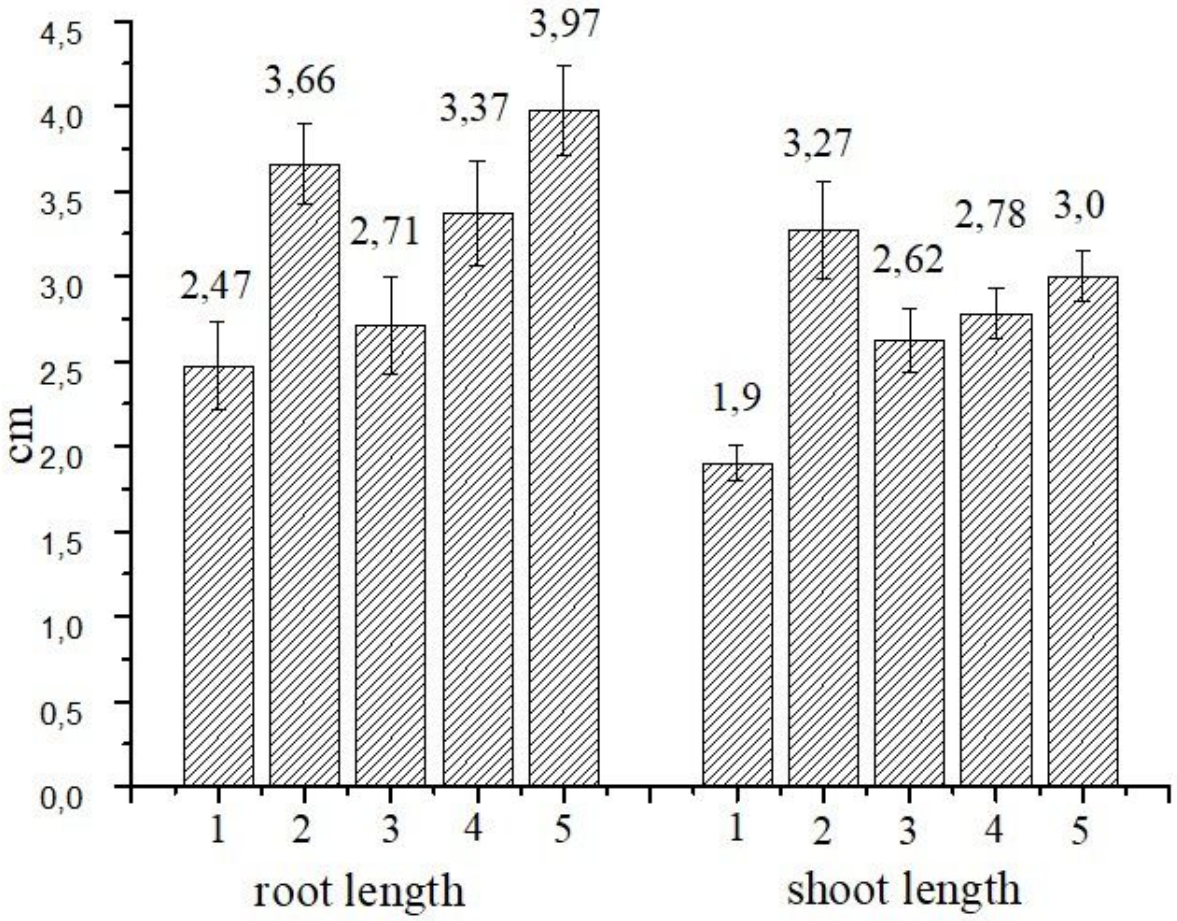


Figure 3

Effect of soaking wheat seeds in a solution of Z. oxianum NS on seedling growth under salt stress.

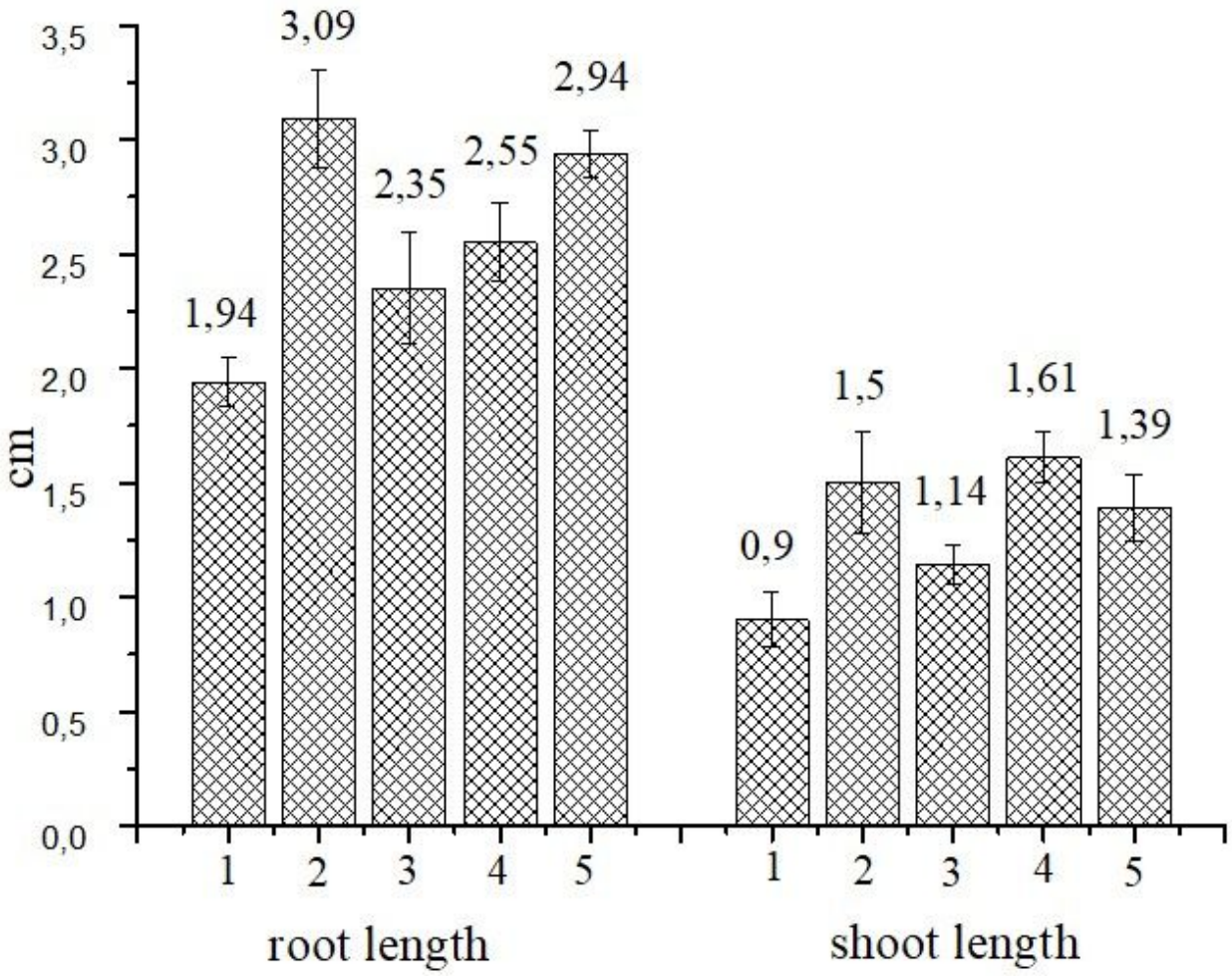


Figure 4

Influence of Z. oxianum NS maceration on cucumber seedling growth under salt stress.