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Effect of a growth regulator on the salt resistance of soybean Zheng 196 at the seeding stage

Abstract. Soil salinity is an increasingly pressing issue for global agriculture. Of the 230 million hectares of irrigated farmland worldwide, 20% is affected by salinity, and this percentage continues to rise annually due to improper irrigation practices. Consequently, advancing research into salt tolerance presents an appealing and cost-effective solution for addressing this challenge. The main goal of this study was to examine the effectiveness of a growth regulator in increasing salt

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tolerance in soybeans at the seedling stage. A hydroponic experiment on soybeans was conducted at the Henan Institute of Science and Technology, Xinxiang, China. The seedlings were grown in an artificial climate chamber. Superoxide dismutase activity was measured using a reference method involving nitrotetrazolium blue chloride, while ascorbate peroxidase activity was measured using a standard assay. The use of growth regulators has been shown to mitigate the damage caused by salt stress in plants. In this study, the ability of the growth regulator to alleviate salt stress during the seedling stage of the soybean variety Zheng 196 was evaluated through analysis and measurement of antioxidant enzymes and malondialdehyde. This process provided indirect verification of its role in enhancing the salt resistance of soybean seedlings. The activities of ascorbate peroxidase and catalase were found to increase significantly at a salt concentration of 100 mmol/L, reaching 30% and 35.96%, respectively, while malondialdehyde levels were significantly reduced by 33% under the same conditions. These results offer valuable insights for the development of new growth regulators and scientifically support their feasibility in addressing the current trend of increasing soil salinity

Keywords: *Glycine max* (L.); amino regulator; salt stress; enzyme activity; seedling leaves

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the world's most important oil crop and a high-protein grainfeeding crop. The soybean industry has economic, and political attributes, making it and is of great importance to a country's food security, economic growth, and social stability. This strategic significance is highlighted in the study of V. Borovyk *et al.* (2024). Soybean is a leguminous plant native to East Asia, specifically China and is now widely cultivated across the globe, as indicated by S. Kalenska *et al.* (2022).

P. Jia *et al.* (2021) stated that soil salinisation and drought stress are significant abiotic factors affecting crop yield. According to T. Eryiğit *et al.* (2022) and K. Saravanan *et al.* (2023), environmental degradation and unsustainable cultivation practices are exacerbating soil salinisation, which in turn affects the quality and yield of soybeans. There is a dynamic equilibrium between the production and elimination of reactive oxygen species generated by plants during life processes. Salt stress disrupts this equilibrium, as noted by M. Osman *et al.* (2021). The accumulation of reactive oxygen species, such as superoxide anions and hydrogen peroxide, induces membrane lipid peroxidation, increasing membrane permeability, damaging the membrane system, and causing severe harm to plants. The use of growth regulators and hormones can improve plant salt resistance, mitigate salt stress, and promote plant growth, as highlighted by S. Butenko *et al.* (2022).

According to I. Didur (2023), one of the most effective measures to increase soybean grain production while reducing anthropogenic pressure on ecosystems and conserving energy resources – especially given the exceptionally high cost of mineral fertilisers – is the optimal use of biological intensification factors. These include exploiting symbiotic potential as a cost-effective natural nitrogen source. In recent years, there has been growing scientific and practical interest in the use of biological fertilisers and foliar treatments with various mechanisms of action. These treatments enhance plants' ability to utilise available resources, thereby improving productivity. Thus, it is highly relevant to study not only the efficiency of seed inoculation but also the creation of optimal growth and development conditions for plants by combining inoculation with foliar feeding. This combined approach amplifies the effects of bioactive substances, enhances photosynthetic activity and symbiotic productivity, and ultimately contributes to higher yields.

"Amino VG-Antistress" is a liquid fertiliser containing a high concentration of free amino acids (of vegetable origin) that are quickly absorbed by plant tissues. Amino acids play an important role in vegetative growth and the accumulation of nutrients for protein synthesis. The special composition of this growth regulator promotes balanced growth and accelerates plant recovery from chemical and mechanical damage.

The presence of the anti-stress agent glycine betaine helps plants overcome abiotic stresses and improves metabolic processes within the plant. Amino acids, as the fundamental components of biological functional macromolecular proteins and important plant metabolites, play a key role in nitrogen metabolism and stress resistance (Didora *et al.*, 2019). Glycine betaine can mitigate the inhibitory effects of stress on plants and enhance their stress tolerance. Studies conducted by H. Deng *et al.* (2024) have shown that betaine can regulate the osmotic pressure of plant cells, remove reactive oxygen species, and maintain the stability of biological membranes. Research has also demonstrated that betaine significantly enhances the drought and salt tolerance of plants by stabilising the structure and activity of key enzymes under stress conditions. Furthermore, it improves photosynthetic efficiency and growth by protecting the photosystem and ensuring optimal water balance within plant tissues.

The free amino acids and betaine contained in the investigated regulator are known to have a significant impact on the salt resistance of soybeans. However, there are limited reports on the effects of this regulator on the salt resistance of soybean seedlings. In this study, the growth regulator was applied to soybean seedlings exposed to different levels of salinity stress. The experimental investigation focused on the effects of the regulator on antioxidant enzyme activity in soybean seedlings, revealing its impact on salt resistance. The study aimed to identify changes in the enzymatic activity of soybean seedlings treated with the growth regulator under varying salinity conditions. Demonstrating the influence of the regulator on enzymatic activity provides a scientific basis for increasing soybean resistance to salinity.

MATERIALS AND METHODS

The objective of the research was to evaluate changes in the enzymatic activity of soybean seedlings treated with a growth regulator under varying levels of environmental salinity. The focus of the study included *Glycine max* L. (variety Zheng 196), a plant growth regulator ("Amino VG-Antistress"), abiotic stress (salinity), and enzyme activity (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase). The experiment was conducted in Xinxiang, China, from March to May 2023. "Amino VG-Antistress" was provided by the Greenhas Group, Italy, while the soybean variety Zheng 196 was obtained from the Henan Academy of Agricultural Sciences, China. The research was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China.

Soybean seeds were grown in 7×7 cm nutrient pots filled with vermiculite. The growth conditions were set as follows: 16 hours of light with an intensity of 8,000 lumens, and day/night temperatures of 25°C/18°C. Once the seedlings developed two leaves and one cotyledon, they were subjected to stress treatment by irrigating them with sodium chloride (NaCl) solutions at concentrations of 50 mmol/L, 75 mmol/L, and 100 $mmol \cdot L^{-1}$. The control group (CK) was irrigated with distilled water, while the experimental group (VG) received the growth regulator.

To minimise variability in antioxidant enzyme content across different plant parts, all samples were collected from the third or fourth fully expanded leaves at the base of the plant, while root samples were taken from the tips of the taproots. Superoxide dismutase (SOD) activity was measured using the method of C. Beauchamp & I. Fridovich (1971), with absorbance recorded at 560 nm. Peroxidase (POD) activity was analysed using guaiacol as a substrate, following the established protocol, with absorbance measurements taken at 470 nm within 3 minutes. Catalase (CAT) activity was determined using a modified version of Neto's method, which calculates based on the rate of H_2O_2 decomposition, measured at 240 nm. Ascorbate peroxidase (APX) activity was evaluated using the method of Y. Nakano & K. Asada (1981), with absorbance recorded at 290 nm. Lipid peroxidation, indicated by malondialdehyde (MDA) content, was measured using the thiobarbituric acid (TBA) method. The reaction mixture was incubated at 95°C for 30 minutes, then rapidly cooled in an ice bath. Following centrifugation at 10,000 g for 20 minutes, the absorbance of the supernatant was recorded at 450 nm, 532 nm, and 600 nm.

Statistical analysis was conducted using SPSS 22 (IBM, Armonk, New York, USA). Duncan's multiple range test was used to identify significant differences, with a significance level of $P < 0.05$. Multivariate analysis of variance (ANOVA) was also applied.

RESULTS AND DISCUSSION

As demonstrated by the findings of P. Dang *et al.* (2024), the active oxygen scavenging system is coordinated by superoxide dismutase, catalase, ascorbate peroxidase, and other substances. The results of this study indicated that the use of the "VG-Antistress" regulator significantly improved the content of superoxide dismutase in soybean seedlings (Fig. 1). With increasing in salt concentrations, the SOD content in soybean seedling leaves gradually increased, and the VG group exhibited higher levels than the CK group. A two-factor analysis of variance was conducted (F_{CK} is the F value for salt concentration, and F_{VG} is the F value for the growth regulator), yielding $F_{CK} = 30.84$, $p = 0 < 0.01$. The main effect of salt concentration was significant, indicating that increasing salt concentration had a pronounced impact on the SOD content of soybean seedling leaves. Similarly, $F_{VG} = 18.40$, $p = 0 < 0.01$, revealed that the growth regulator also had a significant effect, highlighting its substantial influence on SOD content in soybean seedling leaves.

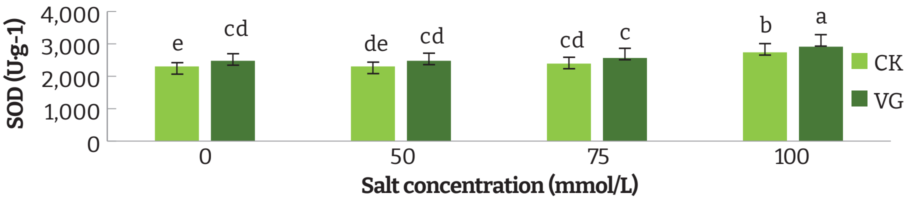

Figure 1. Effect of the growth regulator on superoxide dismutase (SOD) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

Different salt concentrations and the application of the regulator significantly influenced changes in SOD content, with levels in the VG group consistently higher than those in the CK group. Under treatments with 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L salt concentrations, the SOD content in soybean seedlings increased by 6.86%, 6.83%, 6.59%, and 5.78%, respectively, following the application of the regulator. The regulator also substantially increased the content of ascorbate peroxidase in soybean seedlings (Fig. 2).

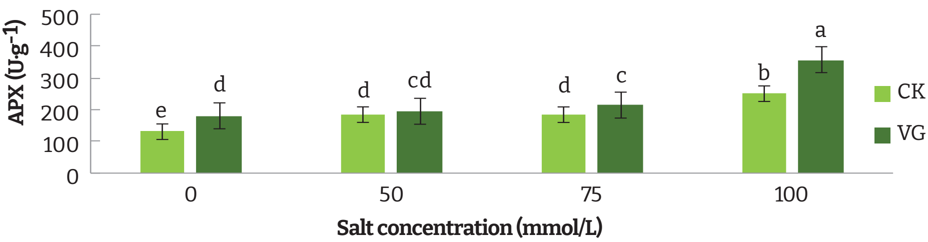

Figure 2. Effect of the growth regulator on ascorbate peroxidase (APX) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

As the concentration of salt increased, ascorbate peroxidase activity gradually increased, and with the application of the regulator, the APX activity was higher than that of the control group. After conducting a two-factor analysis of variance, $F_{CK} = 45.29$, $p = 0 < 0.01$, indicating that the main effect of salt concentration was significant and that increasing salt concentration had a substantial impact on the content of ascorbate peroxidase in soybean seedling leaves. $F_{VG} = 27.08$, $p = 0 < 0.01$, further demonstrated that the main effect of the growth regulator was significant, confirming that the use of the regulator VG significantly influenced the content of ascorbate peroxidase in soybean seedling leaves. Under salt concentrations of 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L, ascorbate peroxidase activity increased by 27.72%, 5.41%, 14.08%, and 30.00% following the use of the regulator, respectively. The application of the regulator notably increased the content of catalase in soybean seedlings (Fig. 3).

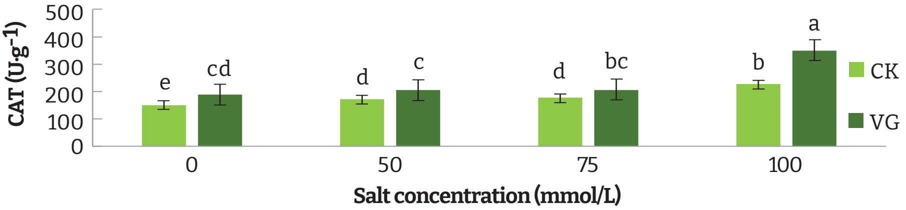

Figure 3. Effect of the growth regulator on catalase (CAT) under different salt stress conditions
Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China
Source: authors' development

As the salt concentration increased, the activity of catalase also increased. While the growth regulator was applied, CAT activity was higher than that observed in the control group. Following a two-factor analysis of variance, $F_{CK} = 29.93$, $p = 0 < 0.01$, indicated that the main effect of salt concentration was substantial, showing that increasing salt concentration significantly influenced the content of catalase in soybean seedling leaves. $F_{VG} = 35.02$, $p = 0 < 0.01$, showed that the main effect of the regulator had a significant effect, demonstrating its notable influence on catalase content. Under salt concentrations of 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L, the regulator increased catalase activity by 20.14%, 16.89%, 15.62%, and 35.96%, respectively.

As noted by O. Laslo & A. Melnychuk (2021), malondialdehyde is a commonly used indicator to measure the degree of oxidative stress, reflecting the extent of membrane lipid peroxidation in plants. Under salt stress, antioxidant enzymes play a crucial role in scavenging plant reactive oxygen species, as observed in the research of C. Accoroni *et al.* (2020). The application of the VG regulator reduced the content of malondialdehyde in soybean seedlings (Fig. 4).

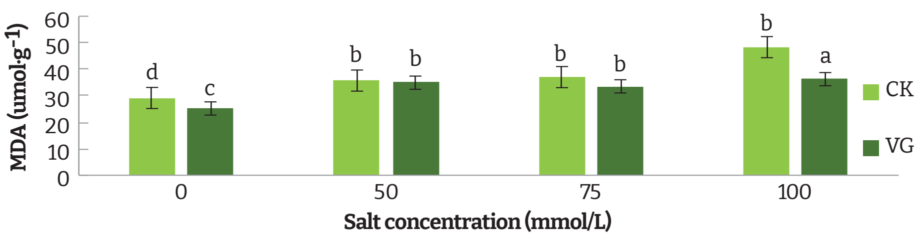

Figure 4. Effect of the growth regulator on malondialdehyde (MDA) under different salt stress conditions
Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China
Source: authors' development

As the salt concentration increased, the malondialdehyde content gradually decreased, and with the application of the regulator, the MDA content was lower than that of the control group. After a two-factor analysis of variance, $F_{CK} = 45.29$, $p = 0 < 0.01$, the main effect of salt concentration was significant, indicating that increasing salt concentration significantly influenced the content of MDA in soybean seedling leaves. $F_{VG} = 27.08$, $p = 0 < 0.01$, therefore, the main effect of the growth regulator was notable, indicating that its use had a significant effect on the content of MDA in soybean seedling leaves. At salt concentrations of 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L, the use of the regulator decreased MDA levels by 15.71%, 2.31%, 10.14%, and 33.33%, respectively.

The research showed that under salt stress, the antioxidant enzyme activity of soybeans increased, as noted by A. Melnyk *et al.* (2022). The plant's antioxidant enzyme system can effectively remove reactive oxygen species generated by metabolic activities in plants (Dudkina & Bondareva, 2019; Hasanuzzaman *et al.*, 2022). Reactive oxygen species and free radicals can cause varying degrees of oxidative damage to plants. According to A. Eliçin *et al.* (2021), the antioxidant enzymes can convert excess reactive oxygen species and free radicals in plants into less toxic or harmless substances, maintaining the balance of reactive oxygen species. Furthermore, increased antioxidant enzyme activity can also indicate that plants have experienced environmental stress. As is known from the results of M. Iqbal *et al.* (2021), membrane lipid peroxidation may occur when plant organs age or are damaged under adverse circumstances. MDA is the final decomposition product of membrane lipid peroxidation, and its content reflects the degree of environmental damage to plants (Hadzovskyi *et al.*, 2020; Zhou *et al.*, 2024).

Under salt stress, soybean superoxide dismutase, ascorbate peroxidase, and catalase activities showed an increasing trend, while MDA content also gradually accumulated (Osman *et al.*, 2021; Saravanan *et al.*, 2023). In this study, the activity of antioxidant enzymes and MDA content gradually increased with higher salt concentrations. This observation aligns with findings from previous studies.

When using the VG growth regulator, the activities of CAT, SOD and APX increased more than in the control group, indicating that the regulator enhanced antioxidant enzyme activity under salt stress and mitigated the damage caused by oxygen-free radicals in soybeans. At a salt concentration of 100 mmol/L, SOD activity increased by 5.78%, with no significant increase at lower concentrations. The maximum increase in APX activity was 30%, while CAT activity increased by up to 35.96%. The content of MDA decreased under different salt treatment conditions following the use of the regulator, indicating that the regulator reduced membrane oxidation. At a salt concentration of 100 mmol/L, the maximum reduction in MDA was 33.33%. It can be speculated that the application of the growth regulator influenced the salt resistance of soybeans, improving their tolerance to salt stress to a certain extent, with the effect being more pronounced at higher salt concentrations.

CONCLUSIONS

In this study, the "Amino VG-Antistress" regulator was evaluated for its ability to improve the salt tolerance of the Zheng 196 soybean variety at the seedling stage. The regulator enhanced the antioxidant capacity of Zheng 196 soybean seedlings and mitigated the effects of salt stress. The effect was most pronounced at a salt concentration of 100 mmol/L, confirming the regulator's ability to improve soybean salt resistance. However, for large-scale field applications and diverse terrains, the effect of this regulator requires further verification due to environmental variability and other uncertain factors.

The application of the regulator during the seedling stage of the Zheng 196 soybean variety was investigated for its efficacy in mitigating salt stress. This was achieved through the analysis and measurement of antioxidant enzyme activity and malondialdehyde content. The findings indirectly verified the regulator's effectiveness in enhancing the salt tolerance of soybean seedlings. Under salt concentrations of 50 mmol/L, 75 mmol/L, and 100 mmol/L, the activities of superoxide dismutase, ascorbate peroxidase, and catalase all increased. At a salt concentration of 100 mmol/L, superoxide dismutase activity increased by 5.78%, though this increase was not significant. In contrast, ascorbate peroxidase and catalase activities showed significant increases of 30% and 35.96%, respectively, at the same salt concentration, while malondialdehyde content notably decreased by 33%. This demonstrated that under high salt concentrations, the regulator significantly enhanced the antioxidant capacity of soybean seedlings and reduced membrane oxidation. These findings indirectly reflect the regulator's slow-release effect under salt stress during the soybean seedling stage. The proven enhancement of enzyme activity by the regulator contributed to increased soybean resistance to salinity.

According to the results of the research, it was determined that the use of the growth regulator had a significant impact on changes in enzymatic activity. The main compounds (enzymes) that are indicators of the resistance of plant organisms to increased salinity were studied. The key findings demonstrated the effectiveness of the regulator and highlighted its potential for increasing resistance. Along with this, the results are relevant for scientists seeking to develop substances with similar compositions to create newer, more effective growth regulators with anti-stress properties.

Despite earlier reports on the exogenous application of melatonin in soybean, no studies, to the best of the authors' knowledge, have specifically addressed its effects under salt stress conditions. Furthermore, salt stress can be precisely and reproducibly simulated in laboratory settings. The use of NaCl is a widely accepted approach in studies exploring the impacts of salt stress. The results offer additional evidence supporting the physiological role of melatonin and provide a theoretical foundation for its application in enhancing salt tolerance in agricultural practices. These findings are crucial for advancing the development of new growth regulators and provide scientific evidence supporting their feasibility in addressing the growing challenge of soil salinity. To further build upon these findings, future research should explore the long-term effects of the growth regulator on soybean growth and yield under diverse environmental conditions and examine its potential interactions with other stress factors to develop comprehensive strategies for enhancing crop resilience.

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CONFLICT OF INTEREST

None.

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Вплив регулятора росту на солестійкість сої сорту Чжен 196 на стадії проростання

Анотація. Засолення ґрунтів стає все більш актуальною проблемою для світового сільського господарства. З 230 мільйонів гектарів зрошуваних сільськогосподарських угідь у світі 20 % зазнають впливу засолення, і цей відсоток продовжує щорічно зростати через неправильну практику зрошення. Таким чином, поглиблення досліджень солестійкості є привабливим і економічно ефективним рішенням цієї проблеми. Основна мета роботи – дослідити ефективність впливу регулятора росту на підвищення солестійкості на стадії проростання сої. Гідропонний експеримент із соєю було проведено в Хенанському інституті науки і технологій (м. Сіньсян, Китай). Розсаду вирощували в камері зі штучним кліматом. Було проведено вимірювання рівня супероксиддисмутази в експерименті з використанням нітротетразолію синього хлориду, аналіз пероксидази аскорбінової кислоти. Застосування регуляторів здатне зменшити шкоду сольового стресу для рослин. Після використання регулятора на стадії проростання сої сорту Чжен 196 було досліджено здатність регулятора зменшувати сольовий стрес шляхом аналізу та вимірювання антиоксидантних ферментів та малонового діальдегіду, а також опосередковано підтверджено його позитивний вплив на солестійкість проростків сої. Активність аскорбатпероксидази та каталази значно зростала за концентрації солі 100 ммоль/л, досягаючи 30 % та 35,96 %, тоді як вміст малонового діальдегіду значно знижувався на 33 % за концентрації солі 100 ммоль/л. Ці результати є важливими для розробки нових регуляторів росту та науково доводять доцільність їх застосування за сучасних тенденцій до підвищення засолення ґрунтів

Ключові слова: *Glycine max*. (L.); амінорегулятор; сольовий стрес; активність ферментів; листки проростків