***Pak. J. Bot.*, 58(3) March, 2026.**

ARTICLE **DOI: http://dx.doi.org/10.30848/PJB2026-3(19)**

INFLUENCE OF ALANINE AND SORBITOL ON SOYBEAN GROWTH, NUTRIENT ASSIMILATION AND ANTIOXIDANT ACTIVITY IN SALT-AFFECTED SOIL

PING HUANG[1], WENWU ZHANG[2], SHOUCHENG HUANG[2*] AND AIRONG LIU[2*]

[1]College of Chemistry and Materials Engineering, Anhui Science and Technology University, Bengbu 233000, China
[2]College of Life and Health Science, Anhui Science and Technology University, Fengyang 233100, China

*Corresponding author's email: huangsc@ahstu.edu.cn; liuar@ahstu.edu.cn

Abstract

Salinity stress has been proved to have detrimental effects to plant growth and development. This experiment investigated the effects of alanine (AL, 2 mM) and sorbitol (SB, 1.5%) on soybean growth in salt-affected soil. Four treatments were set: control, AL, SB, AL+SB. Results demonstrated that both AL and SB had positive effects, especially the combined application of AL+SB exhibited largest mitigation effects to salt stress. Under this treatment, the plant height (54.79%), stem dry weight (22.62%), leaf dry weight (30.54%) and root dry weight (20.08%) increased significantly compared to the control. In addition, the contents of chlorophyll a, chlorophyll b, and total chlorophyll were enhanced by 17.00%, 100.00%, and 45.60%, respectively, while the electrolyte leakage reduced by 23.06%, suggesting improved membrane stability. Furthermore, the treatment promoted the accumulation of K^+ and Ca^{2+} in leaves and stems. SOD, POD, CAT and APX activities were significantly reduced in this treatment, suggesting a lower oxidative burden. The findings underline that application of 2 mM alanine and 1.5% sorbitol as well as their combination is a potential integrated strategy for enhancing soybean growth, photosynthesis, and stress resilience in saline soils.

Key words: Salt stress; Soybean; Alanine; Sorbitol; Nutrient assimilation; Antioxidant activity.

Introduction

Soil salinity is the second most significant cause of land degradation after soil erosion, posing a major challenge to global agriculture (Huang *et al.*, 2024). It reduces the arable land and reduced agricultural productivity, contributing to a 10–25% loss in food production and expanding desertification across nearly 1 billion hectares worldwide (Ashraf & Chen, 2023). Salinity stress disrupts plant growth by inducing osmotic stress, ion toxicity, and nutrient imbalances, all of which impair cellular functions and hinder photosynthesis. This damage weakens plant membranes and triggers the production of reactive oxygen species (ROS), further exacerbating stress-related harm (Kaya *et al.*, 2023). Effective management of soil salinity is essential for maintaining arable land and ensuring sustainable agricultural output to meet the food demands of a growing global population (Saeed *et al.*, 2023).

Sorbitol acts as an osmo protectant and a translocatable carbohydrate, enhancing plant growth and soil health (Pleyerová *et al.*, 2022). It helps maintain cellular water balance, stabilizes membranes, and protects plants from oxidative stress, leading to improved photosynthesis and biomass production (Liang *et al.*, 2012). Additionally, sorbitol facilitates nutrient transport, particularly in phloem loading and unloading, ensuring efficient distribution of essential elements (Li *et al.*, 2023). In the soil, sorbitol promotes microbial activity by serving as an energy source for beneficial microbes, which enhance nutrient cycling and organic matter decomposition. This dual role of sorbitol in plant physiology and soil ecosystem improvement makes it a valuable tool for sustainable agriculture (Yu *et al.*, 2023).

Alanine plays a vital role in enhancing plant growth and soil health by serving as a key metabolite in nitrogen assimilation and stress response pathways (Alfosea-Simón *et al.*, 2020). It facilitates nitrogen transport between plant tissues, promoting protein synthesis and cell division, which contribute to increased biomass accumulation. Alanine also supports root development by improving nutrient uptake efficiency, particularly for essential elements like potassium and calcium (Tiong *et al.*, 2021; Parthasarathy *et al.*, 2019). In the soil, Alanine serves as a carbon and nitrogen source for beneficial microbes, stimulating microbial activity that enhances nutrient cycling (Agrawal *et al.*, 2024). This microbial interaction improves soil fertility, structure, and overall plant resilience against environmental stresses.

Soybean (*Glycine max*), originated from China with over 5000 years history, is one of the most important economic crops in China. However, salt stress constitutes the major restriction for soybean yield. As a typical moderate salt resistant plant, soybean growth will be substantially hindered when the salt concentration is over 5.0 dS/m (Munns &Tester, 2008). Zhang *et al.*, (2025) found that, under NaCl stress, the soybean seedlings exhibited significant shorter plant height and stem diameter, lower chlorophyll contents, whereas much more reactive oxygen species (ROS) such as O_2^- and H_2O_2 (Zhang *et al.*, 2025). The current study plans to explore the potential of alanine and sorbitol to mitigate the salt stress on soybean plants. The hypothesis is that applying alanine and sorbitol combined as an amendment could mitigate the adverse effects of salt stress on soybean, potentially enhancing their growth and productivity. By addressing this knowledge gap

Received: 05-09-2025 | Revised: 17-11-2025 | Accepted: 25-11-2025 | Online: 24-12-2025

and proposing an environmentally friendly solution to mitigate the adverse impacts of salt-affected soil on soybean cultivation, this study contributes to the broader goal of ecosystem and crop preservation.

Material and Methods

Experimental design: A pot experiment was carried out in the research area of Anhui Science and Technology University. Commercial soybean seeds were purchased from a Chinese local verified seed supplier. Seeds were cleaned with 75% ethanol and deionized water after being sterilized with a 5% NaClO solution. In pots with 30 kg of soil, ten seeds were sown, and five uniformly grown seedlings were kept alive by thinning. Routine water and fertilizer management were carried out for soybean seedlings. To ascertain the physio-chemical characteristics of the soil, a random sample was collected from the study region (Table 1). The following were the properties of the soil:

Table 1 Soil physio-chemical properties.

Parameter	Value	Unit
ECe	7.33*	dS/m
pH	8.08	–
Total nitrogen	0.02	%
Soil organic matter	0.47	%
Extractable potassium	121	µg/g
Available phosphorus	6.22	µg/g
Exchangeable sodium (ENa)	112	µg/g
Texture	Clay loam	–

*Showing moderate salt stress. Values represent the mean of three technical replicates from a composite soil sample

Treatments: Four foliar spray treatments (control, 2 mM AL, 1.5% SB, and 2 mM AL + 1.5% SB) in 3 replications. Two sprays were made after four weeks of germination, with a 15-day gap in each foliar.

Plant height and dry biomass: Plant height was measured using an analytical balance. Samples were subjected to an oven at 65°C for 72 hours to measure the dry biomass of leaves, stems and roots.

Chlorophyll contents: Utilizing Arnon's method (Arnon, 1949), we extracted the chlorophyll from fresh leaves using an 95% ethanol, and we measured absorbance at 665/649 nm.

Antioxidants: SOD, POD, CAT and APX detection were conducted according to the typical methods (Dhindsa, 1982; Hori, 1997; Aebi, 1984;Nakano & Asada, 1981).

Electrolyte leakage: In this study, test tubes filled with 20 milliliters of deionized water were filled with uniform leaf segments that had been taken from a steel cylinder. An EC meter that had been previously calibrated was used to assess the initial electrical conductivity (EC1) after a 24-hour incubation period at 25°C. The final electrical conductivity (EC2) was measured after a 20-minute immersion in a 100°C water bath (Lutts *et al.*, 1997).

$$\text{Electrolyte Leakage (\%)} = (EC1 / EC2) \times 100$$

Nutrients analysis: The samples were collected and digested for measurement of K and Ca contents, using a flame photomete method(Donald & Hanson,1998). Briefly, oven-dried soybean leaf and stem samples (0.1 g of powdered dry material) were digested using a H_2SO_4-H_2O_2 mixture. The digestion was carried out until the solution became clear and colorless, after which the digests were cooled and quantitatively transferred to volumetric flasks. For K determination, the diluted digest was aspirated directly into a flame photometer operated in flame emission mode, with measurements taken at a wavelength of 766.5 nm. For Ca determination, lanthanum chloride ($LaCl_3$) was added to both standards and samples at a final concentration of 0.1–0.5% (w/v) as a releasing agent. All samples and standards were measured in triplicate, and instrument stability was monitored by repeated analysis of quality control standards after every 10 sample readings.

Statistical analysis

The data was managed using Microsoft Excel software. A two-way analysis of variance (ANOVA) was performed with treatment (four levels: control, AL, SB, AL+SB) as a fixed factor and block (three replicates) as a random factor. Mean comparisons were conducted using Tukey's honestly significant difference (HSD) test at $p<0.05$.

Results

Plant height, and stem/leaf/root dry biomass: After adding 2mM AL, 1.5% SB, and 2mM AL+1.5% SB, a significant increase in plant height (46.78%, 20.45%, and 54.79%), stem dry biomass (17.67%, 10.84%, and 22.62%), root dry biomass (17.39%, 5.29%, and 30.54%) and leaf dry biomass (14.16%, 5.06%, and 20.89%) was observed in comparison to the control. (Fig. 1A, B, C, and D).

Photosynthesis pigments and electrolyte leakage: Adding 2mM AL, 1.5 SB, and 2mM AL+1.5% SB treatment caused a significant increase in chlorophyll a (11.40%, 8.30%, and 17.00%), chlorophyll b (90.82%, 48.03%, and 100%), total chlorophyll (36.22%, 20.72%, and 45.60%), and showed decrease in electrolyte leakage (9.63%, 4.14%, and 23.06%) compared to the control. (Fig. 2A, B, C, and D).

K^+ and Ca^{2+} contents of leaf and stem: As shown in Fig. 3, adding 2mM AL, 1.5% SB, and 2mM AL+1.5% SB demonstrated a rise in leaf K (28.82%, 20.27%, and 47.11%), leaf Ca (72.66%, 33.68%, and 96.71%), stem K (16.40%, 7.82%, and 33.15%), and stem Ca (17.01%, 12.50%, and 21.47%) respectively over the control.

Activities of SOD, POD, CAT and APX: Applying 2mM AL, 1.5% SB, and 2mM AL+1.5% SB caused decrease in SOD activities (19.42%, 9.30%, and 33.50%), POD activities(29.14%, 6.60%, and 48.16%), CAT activities (23.45%, 4.06%, and 32.64%), and APX activities (20.09%, 11.84%, and 25.17%) over the control (Fig. 4A, B, C, and D).

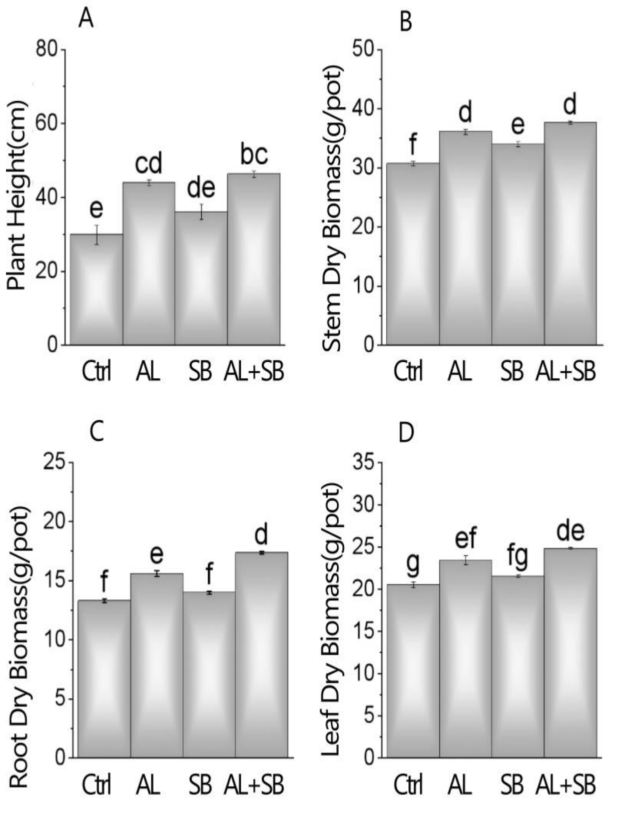

Fig. 1. Effects of each treatment on plant height (A), stem dry biomass (B), root dry biomass(C) and leaf dry biomass(D). The bars represent the mean of three replicates with standard error and the different letters on the bars represented significance at p≤0.05. Same as below.

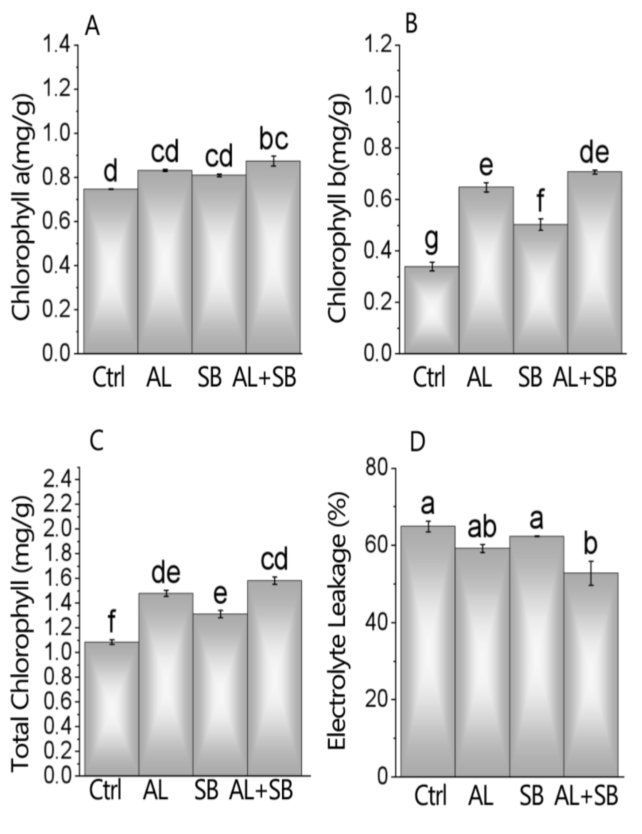

Fig. 2. Effects of each treatment on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and electrolyte leakage (D) of soybean.

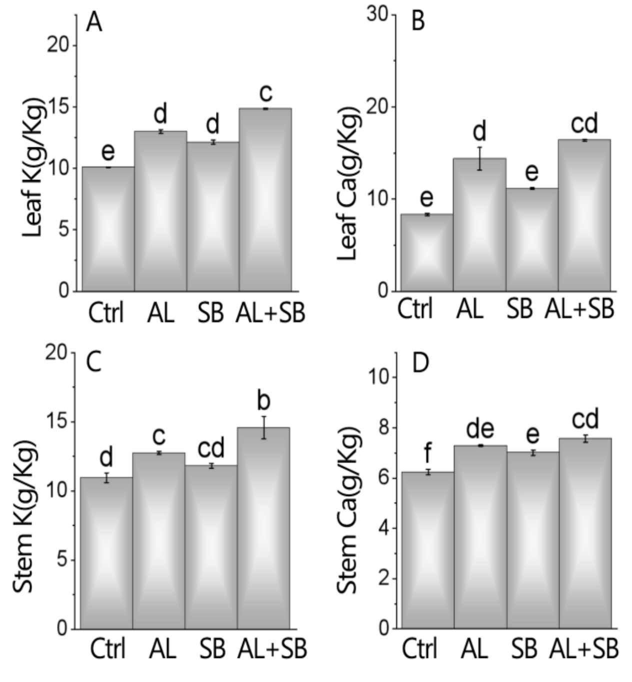

Fig. 3. Effects of each treatment on leaf K (A), leaf Ca (B), stem K (C), and stem Ca (D) of soybean.

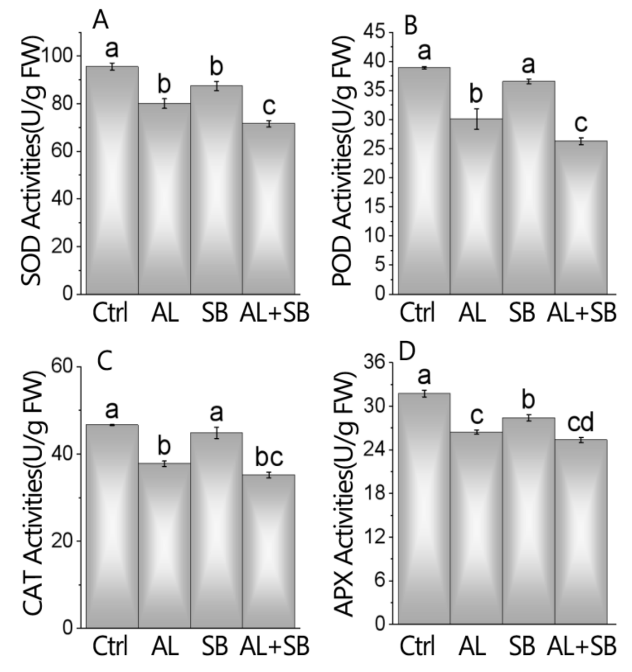

Fig. 4. Effects of each treatment on peroxidase (POD) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C), and ascorbate peroxidase (APX) (D) of soybean.

Discussion

The experiment evaluated the individual or combined mitigation effects of alanine (AL) and sorbitol (SB) on soybean under salinity stress. The results revealed that the application of AL, SB exhibited positive influences, which is consisted with previous findings (Liu *et al*., 2024; Khaliq *et al*., 2023). However, according to the present findings, 2 mM AL+1.5% SB displayed significant combination impacts, promoting plant growth and nutrient uptake, enhancing biomass production, and reducing oxidative damage.

Plants need the essential amino acid alanine for multiple metabolic processes. It is vital for coping with stress while assimilating nitrogen into the system (Lea & Miflin, 1977). This metabolic intermediate enables the exchange of carbon and nitrogen substances between the different organs of the plant(Parthasarathy *et al.*, 2019). The continuous loop between different parts of the plant helps to maintain energy stability as well as establish metabolic equilibrium, especially during environmental stress. Through osmotic adjustment alanine helps plants hold more water for the maintenance of their cellular structure (Alfosea-Simón *et al.*, 2020). Protein synthesis starts through alanine functioning as a precursor agent that drives cell development and tissue growth as shown in the results of increased plant height and biomass accumulation (Pena-Soler *et al.*, 2014). Alanine is involved in the synthesis of membrane transporters and ATP-dependent ion channels. Enhanced nitrogen assimilation and protein synthesis likely promote the expression and activity of K^+ and Ca^{2+} transporters at the root plasma membrane. Additionally, alanine may contribute to energy (ATP) supply via the TCA cycle, supporting active ion uptake (Tiong *et al.*, 2021; Ma *et al.*, 2025).

Sorbitol functions as an osmoprotectant, protecting plant cell structures under stress (Williamson *et al.*, 2002). The osmotic pressure regulation function of sorbitol in cells enables them to manage water equilibrium that reduces the effects of drought and salinity stress. Plants use sorbitol to benefit their energy production and carbon distribution system through carbohydrate transport and energy supply functions (Ochoa-Gómez & Roncal, 2017). Plants benefit from better phloem loading and unloading abilities that sorbitol provides to enhance nutrient and assimilate transport which leads to healthier plant growth. The protective effect of sorbitol on chloroplast membranes and photosynthetic pigments seems to cause increased chlorophyll content in plants (Lo Bianco *et al.*, 2000; Issa *et al.*, 2020). Sorbitol also exhibits antioxidant properties which protect plants against reactive oxygen species through limiting their accumulation levels (Hsu & Kao, 2003). Lower ROS levels reduce the need for enzymatic detoxification because they led to lower activities of SOD, POD, CAT, and APX antioxidant enzymes.

Conclusions

In present experiment, combined effects of 2mM alanine and 1.5% sorbitol showed the greatest improvement in the physiological attributes, photosynthetic efficiency, nutrient assimilation and regulated antioxidant activity of soybean plants in salt-affected soil. It is recommended that 2mM AL+1.5% SB should be applied to achieve better soybean growth in salt-affected pot soil. However, more investigations at the field level are suggested to explore in future researches.

Acknowledgment

This work was finally supported by the following projects: the Key Program of Natural Science Foundation for Universities of Anhui Province (2024AH050331), Mechanism of potassium-rich biochar in enhancing soybean growth in cadmium-contaminated soil. This work was also supported by the Industry-University-Research collaboration project:Mechanisms and applications of modified biochar in regulating crop growth (20250578).

Competing Interests: The authors declare no competing interests.

Author Contribution: P.H.; S.H.; A.L; contributed to the conceptualization and design of the study, as well as data collection, analysis, and interpretation. W. Zh.; S.H.; contributed to the statistical analysis; S.H.; interpretation of the data. All authors have reviewed and approved the final version of the manuscript.

References

Aebi, H. 1984. Catalase *In vitro*. *Method Enzym*., 105: 121-126.

Agrawal, N., R.S. Chunletia, A.M. Badigannavar and S. Mondal. 2024. Role of alanine aminotransferase in crop resilience to climate change: a critical review. *Physiol. Mol. Biol. Plants*, 30: 1935-1953.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiol*., 24(1): 1-15.

Ashraf, F. and Y. Chen. 2023. Synergistic effects of biochar and Arbuscular Mycorrhizal fungi on enhancing *Elymus elymoides* growth in saline coastal soil. *Pak. J. Bot.*, 55(SI): 119-126.

Dhindsa, R.S., P.L. Plumb-Dhindsa and D.M. Reid. 1982. Leaf senescence and lipid peroxidation: Effects of some phytohormones, and scavengers of free radicals and singlet oxygen. *Physiol Plant.*, 56(4): 453-457.

Donald, A.H. and D. Hanson. 1998. Determination of potassium and sodium by flame emmision spectrophotometery. In: (Ed.): Kalra, Y. Handbook of Reference Methods for Plant Analysis. 1st edition. Washington, D.C.: CRC Press, 153-155.

Hori, M, H. Kondo, N. Ariyoshi, H. Yamada, A. Hiratsuka and T. Watabe. 1997. Changes in the hepatic glutathione peroxidase redox system produced by coplanar polychlorinated biphenyls in Ah-responsive and-less-responsive strains of mice: mechanism and implications for toxicity. *Environ. Toxicol. Pharmacol.*, 3: 267-275.

Hsu, S.Y. and C.H. Kao. 2003. Differential effect of sorbitol and polyethylene glycol on antioxidant enzymes in rice leaves. *Plant Growth Regul.*, 39(1): 83-90.

Huang, S., P. Huang, M. Hareem, M. Tahzeeb-ul-Hassan, U. Younis and K. Dawar. 2024. Evaluating the hidden potential of deashed biochar in mitigating salinity stress for cultivation of fenugreek. *Sci. Rep*., 14: 141.

Issa, D.B., S.M. Alturki, T.K. Sajyan and Y.N. Sassine. 2020. Sorbitol and lithovit-guano25 mitigates the adverse effects of salinity on eggplant grown in pot experiment. *Agron. Res.*, 18(1): 113-126.

Kaya, C., F. Ugurlar, M. Ashraf, P. Alam and P. Ahmad. 2023. Nitric oxide and hydrogen sulfide work together to improve tolerance to salinity stress in wheat plants by upraising the AsA-GSH cycle. *Plant Physiol. Biochem*., 194: 651-663.

Khaliq, M., K. Nawaz, K. Hussain, M. Javeria, I. Iqbal, N. Arshad, A. Attique, R. Zainab, W.U. Nisa and M. Qurban. 2023. Foliar application of sorbitol is a shotgun approach to alleviate the adverse effects of salinity stress on two varieties of wheat (*Triticum aestivum* L.). *Pak. J. Bot.*, 55(4): 1243-1256.

Lea, P.J. and B.J. Miflin. 1977. Amino acid metabolism. *Ann. Rev. Plant Physiol.*, 28: 299-329.

Li, T., Q. Wei, Y. Cui, H. Tan, H. Zhang and F. Zeng. 2023. Foliar application of organic sorbitol-chelated calcium promotes peanut (*Arachis hypogaea* L.) growth and changes phyllosphere bacterial community structure. *J. Soil Sci. Plant Nutr.*, 24(1): 561-571.

Liang, D., M. Cui, S. Wu and F. Ma. 2012. Genomic structure, sub-cellular localization, and promoter analysis of the gene encoding sorbitol-6-phosphate dehydrogenase from apple. *Plant Mol. Biol. Report.*, 30(4): 904-914.

Liu, Y., J, Zheng, L. Ge, H. Tang, J. Hu, X. Li, X.Y. Wang, Y. Zhang and Q.H. Shi. 2024. Integrated metabolomic and transcriptomic analyses reveal the roles of alanine, aspartate and glutamate metabolism and glutathione metabolism in response to salt stress in tomato. *Sci. Hort.*, 328: 112911.

Lo Bianco, R., M. Rieger and S.J.S. Sung. 2000. Effect of drought on sorbitol and sucrose metabolism in sinks and sources of peach. *Physiol. Plant*, 108(1): 71-78.

Lutts, S., J.M. Kinet and J. Bouharmont. 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann. Bot.*, 78(3): 389-398.

Ma, W., X. Fang, M. Qiu, M. Hareem, Z. Erden and C.C. Toprak. 2025. Mitigating drought stress in fenugreek through synergistic effects of alanine and potassium-enriched biochar. *BMC Plant Biol.*, 25: 139.

Marina Alfosea-Simón, Silvia Simón-Grao and E.A. Zavala-Gonzalez. 2020. Physiological, nutritional and metabolomic responses of tomato plants after the foliar application of amino acids aspartic acid, glutamic acid and alanine. *Front Plant Sci.*, 11: 581234.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.*, 59: 651-681.

Nakano, Y. and K. Asada. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.*, 22(5): 867-880.

Ochoa-Gómez, J.R. and T. Roncal. 2017. Production of sorbitol from biomass. *Prod. Platf. Chem. from Sustain Resour.*, 265-309.

Parthasarathy, A., M.A. Savka and A.O. Hudson. 2019. The synthesis and role of β-alanine in plants. *Front. Plant Sci.*, 10: 921.

Pena-Soler, E., F.J. Fernandez, M. Lopez-Estepa, F. Garces, A.J. Richardson and J.F. Quintana. 2014. Structural analysis and mutant growth properties reveal distinctive enzymatic and cellular roles for the three major L-alanine transaminases of *Escherichia coli*. *PLoS One*, 9: e102139.

Pleyerová, I., J. Hamet, H. Konrádová and H. Lipavská. 2022. Versatile roles of sorbitol in higher plants: luxury resource, effective defender or something else? *Planta*, 256(1):13.

Saeed, S., A. Ullah, S. Ullah, M.S. Elshikh, J. Noor and S.M. Eldin. 2023. Salicylic acid and α-tocopherol ameliorate salinity impact on wheat. *ACS Omega.*, 8(29): 26122-26135.

Tiong, J., N. Sharma, R. Sampath, N. MacKenzie, S. Watanabe and C. Metot. 2021. Improving nitrogen use efficiency through overexpression of alanine aminotransferase in rice, wheat, and barley. *Front Plant Sci.*, 12:628521.

Tiong, J., N. Sharma, R. Sampath, N. MacKenzie, S. Watanabe, C. Metot and M. Okamoto. 2021. Improving nitrogen use efficiency through overexpression of alanine aminotransferase in rice, wheat, and barley. *Front Plant Sci.*, 12: 628521.

Williamson, J.D., D.B. Jennings, W.W. Guo, D.M. Pharr and M. Ehrenshaft. 2002. Sugar alcohols, salt stress, and fungal resistance: polyols multifunctional plant protection? *J. Amer. Soc. Hort. Sci.*, 127(4): 467-473.

Yu, H., W. Shao, G. Xu, N. Xie, X. Yang and D. Gao. 2023. Soil amendment with sorbitol and mannitol changes the soil microbial community and its enzymatic activities. *J. Soils Sedim.*, 23:1857-1876.

Zhang, Y., S. Liu, X. Liang, J. Zheng, X. Lu, J. Zhao, H. Li, Y. Zhan, W. Teng, H. Li, Y. Han, X. Zhao and Y. Li. 2025. GmFER1, a soybean ferritin, enhances tolerance to salt stress and root rot disease and improves soybean yield. *Plant Biotechnol. J.*, 23(8): 3094-3112.