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Protective effects of exogenous L-proline on germination and early seedling development of soybean under osmotic stress

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ABSTRACT

Salinity is a major abiotic stress limiting soybean germination and seedling establishment by impairing water uptake, metabolism, and early growth. Exogenous application of L-proline has emerged as a potential strategy to improve plant tolerance to saline conditions. Soybean seeds (GH 5933 IPRO) were treated with four concentrations of L-proline (0, 0.04, 0.08, and 0.12 g kg^{-1} seed) and subjected to four osmotic potentials (0.0, −0.3, −0.6, and −0.9 MPa) induced by NaCl. Germination, seed vigor, seedling growth, and dry matter accumulation were evaluated in a completely randomized factorial design. Salinity significantly reduced seed vigor from approximately 92% under non-saline conditions to about 45% at −0.9 MPa, while the percentage of normal seedlings declined from approximately 95% to 47%. Application of 0.12 g kg^{-1} L-proline increased seed vigor under severe stress to approximately 74% and improved the percentage of normal seedlings to about 75%, compared with untreated seeds. The proportion of hard and dead seeds under severe salinity decreased from approximately 50% in untreated seeds to about 17% following L-proline treatment. Root length increased from 3.6 to 4.5 cm, whereas shoot length increased from 1.9 to 2.4 cm with increasing L-proline concentration. Dry matter accumulation also increased, reaching approximately 0.31 g $seedling^{-1}$ at the highest L-proline dose under non-saline conditions, while biomass losses under saline stress were partially alleviated. These findings demonstrate that exogenous L-proline enhances osmotic adjustment, improves germination and seedling vigor, stimulates early growth, and reduces seed mortality under salt stress, highlighting its potential as a sustainable strategy for improving soybean establishment in saline environments.

Key words: *Glycine max*, Osmotic adjustment, Plant vigor, Saline stress, Seed germination

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most important agricultural crops worldwide due to its high protein and oil content and its broad utilization in food, feed, and biofuel production (Hartman *et al.,* 2011). Brazil is currently the world's largest soybean producer; however, several production regions are increasingly exposed to abiotic stresses such as drought and soil salinity, which significantly impair crop establishment and productivity (CONAB, 2025; IBGE, 2025). Moreover, climate change is expected to intensify the frequency and severity of these environmental constraints, reinforcing the need for effective strategies to enhance crop tolerance and ensure sustainable agricultural production (Zhang *et al.,* 2022).

Among abiotic stresses, salinity is considered one of the primary factors limiting seed germination and seedling establishment. Elevated salt concentrations reduce soil osmotic potential, thereby restricting water uptake and impairing the metabolic processes required for germination. Additionally, excessive accumulation of salts induces ionic toxicity, nutritional imbalance, oxidative stress, and disruption of cellular homeostasis (Hosseinifard *et al.,* 2022; Hasanuzzaman *et al.,* 2018). In soybean, salt stress negatively affects germination rate, seed vigor, seedling growth, photosynthetic activity, and biomass accumulation, ultimately reducing crop productivity (Medina *et al.,* 2023). Likewise, drought stress compromises several physiological and biochemical processes, including membrane stability, stomatal conductance, and nutrient transport, particularly during the early stages of plant development (Zhou *et al.,* 2023).

Because seed germination and seedling establishment represent highly sensitive phases of the plant life cycle, the development of strategies capable of improving stress tolerance during these stages is essential. In this context, seed treatment with biostimulants and osmoprotective compounds has emerged as a promising and sustainable approach for mitigating the negative effects of such abiotic stresses (Moukhtari *et al.,* 2020). Among these compounds, L-proline has attracted considerable scientific interest because of its multifunctional role in plant stress tolerance mechanisms.

Proline is a compatible osmolyte naturally accumulated in plants under adverse environmental conditions and is closely associated with tolerance to salinity, drought, temperature extremes, and oxidative stress (Szabados and Savouré, 2010; Trovato *et al.,* 2019). Beyond its role in osmotic adjustment, proline contributes to the stabilization of proteins and cellular membranes, protection of the photosynthetic apparatus, maintenance of cellular redox balance, and scavenging of reactive oxygen species (ROS) (Liang *et al.,* 2013; Sharma *et al.,* 2011). Furthermore, proline metabolism is strongly linked to stress-responsive signaling pathways and the regulation of genes involved in plant adaptation to adverse environmental conditions (Verslues and Sharma, 2010). Recent studies have demonstrated that exogenous application of proline can substantially improve plant tolerance to abiotic stresses (Hosseinifard *et al.,* 2022; Mariano-da-Silva *et al.,* 2025a; Mariano-da-Silva *et al.,* 2025b; Medina *et al.,* 2023; Moukhtari *et al.,* 2020; Zhou *et al.,* 2023). Collectively, these findings highlight the potential of L-proline as an environmentally friendly and sustainable alternative for mitigating stress-induced damage during early plant development.

Therefore, the present study evaluated the effects of seed treatment with exogenous L-proline on the physiological performance of soybean seeds subjected to salt stress. Specifically, the study investigated whether L-proline could improve seed vigor, germination, seedling growth, and biomass accumulation under saline conditions, while mitigating the detrimental effects of salinity during seedling establishment.

MATERIALS AND METHODS

The experiment was conducted at the Seed and Grain Laboratory of the Federal University of Fronteira Sul, Chapecó campus. Soybean seeds of the cultivar GH 5933 IPRO (Golden Harvest, 2025) were obtained from a commercial production field as farm-saved seeds and had not received any prior treatment.

A completely randomized factorial design was adopted, with four replications and two experimental factors (Pimentel-Gomes, 2000). The first factor consisted of four osmotic potentials (0.0, -0.3, -0.6, and -0.9 MPa), while the second factor comprised four L-proline concentrations (0, 0.04, 0.08, and 0.12 g kg^{-1} seed). Seeds were separated into four batches of 800 g and stored in transparent plastic bags. Each batch received an aqueous solution containing the respective L-proline concentration. After solution application, air was injected into the bags, which were vigorously shaken to ensure uniform coating of the seeds. Subsequently, the seeds were dried under shade at 25 ± 2°C for 24 h (Marcos Filho, 2015).

Salinity stress was simulated using sodium chloride (NaCl) solutions prepared according to the calibration curve proposed by Braccini *et al.* (1996), generating osmotic potentials of 0.0, -0.3, -0.6, and -0.9 MPa. Based on the classification of Ayub *et al.* (2020), these osmotic potentials correspond respectively to non-saline, low-salinity, moderately saline, and highly saline soils, considering that soils with electrical conductivity above 4 mmhos cm^{-1} are classified as saline.

Each L-proline-treated seed lot was subdivided into four sublots of 200 g. Seeds were distributed on eight sheets of Germitest paper, with 50 seeds per sheet. The papers were moistened with NaCl solution in a volume equivalent to three times the dry weight of the paper. The sheets were then rolled and maintained in a germination chamber under constant temperature (25 ± 1°C) and photoperiod conditions (Brasil, 2009; Sá *et al.,* 2011).

Germination was evaluated on the fifth and eighth days after sowing, and results were expressed as percentages (Brasil, 2009; Sá *et al.,* 2011). On the eighth day, shoot and primary root lengths were measured using a millimeter ruler, and results were expressed in centimeters. Dry matter accumulation was determined separately for shoots and roots of normal seedlings. Plant material was weighed, transferred to paper bags, and dried in a forced-air oven at 80 ± 2°C for 24 h (Sá *et al.,* 2011). After cooling at room temperature, samples were reweighed, and dry matter content was expressed in grams.

Data were subjected to analysis of variance (F-test, $p \leq 0.05$). Regression analyses were performed at a 95% confidence level to evaluate the effects of osmotic potential and L-proline concentrations. Linear and quadratic models were selected based on orthogonal polynomial regression criteria (Pimentel-Gomes, 2000; Souza, 1998).

RESULTS AND DISCUSSION

The analysis of variance (F-test) revealed a significant interaction between osmotic potential and L-proline concentration for seed vigor, indicating that the response of soybean seeds to L-proline depended on the intensity of saline stress. Partitioning of the interaction showed significant effects of L-proline within the osmotic potentials of -0.3, -0.6, and -0.9 MPa, while osmotic potential significantly affected vigor at all tested L-proline concentrations (Fig. 1A and 1B).

The regression analyses demonstrated that seed vigor progressively declined as osmotic potential decreased, confirming the detrimental effects of saline stress on soybean seed performance. However, increasing concentrations of L-proline mitigated these reductions, particularly under moderate and severe stress conditions. These results reinforce the role of L-proline as an important osmoprotective compound capable of stabilizing cellular metabolism under adverse environmental conditions. According to Szabados and Savouré (2010), proline acts in osmotic adjustment, membrane stabilization, and protection against oxidative damage. In addition, proline metabolism contributes to the oxidative pentose phosphate pathway by generating cytosolic NADPH, an essential reducing agent for stress defense mechanisms (Signorelli, 2016).

For the variable normal seedlings, a significant interaction was also detected between osmotic potential and L-proline doses. The partitioning analysis indicated significant effects of L-proline at osmotic potentials of -0.6 and -0.9 MPa, while osmotic potential affected seedling formation across all L-proline levels (Fig. 1C and 1D).

The results indicate that saline stress severely impaired seed germination and seedling establishment, especially at lower osmotic potentials. Nevertheless, the application of L-proline partially alleviated these effects, resulting in higher percentages of normal seedlings under severe saline conditions. Germination is considered one of the most stress-sensitive stages of the plant life cycle, and reductions in water uptake caused by salinity directly compromise metabolic activation and embryo growth (Hubbard *et al.*, 2012; Mariano-da-Silva *et al.*, 2025b). The protective effect observed in the present study suggests that exogenous L-proline may improve osmotic adjustment during early seedling development.

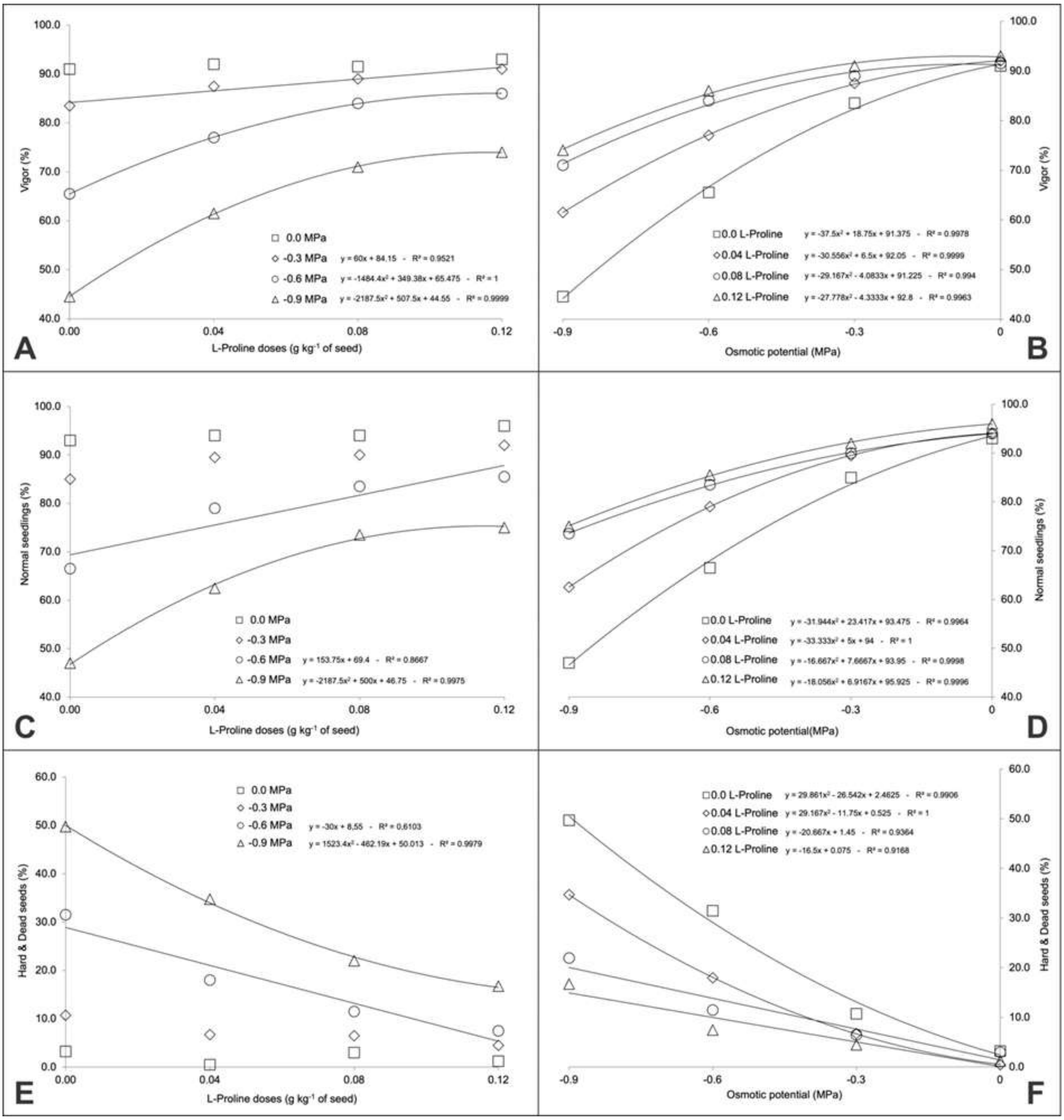

Fig. 1. Effects of osmotic potential induced by NaCl and exogenous L-proline application on the physiological performance of soybean seeds (cv. GH 5933 IPRO)

(A - B) Seed vigor; (C - D) percentage of normal seedlings; and (E - F) percentage of hard and dead seeds as affected by osmotic potentials (0.0, -0.3, -0.6, and -0.9 MPa) and L-proline doses (0, 0.04, 0.08, and 0.12 g kg-1 seed). Regression equations were fitted at a 95% confidence level.

Vol. 28, Issue: 6, p. 1-8, 2026
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Table 1. Mean percentage of germination (% abnormal seedlings) of Glycine max seeds, treated with different doses of L-proline and subject to salt stress

Osmotic potential (MPa)	L-proline doses (g kg^{-1} of seeds)			
	0.0g	0.04g	0.08g	0.12g
0.0	3.75	5.50	3.00	2.75
-0.3	4.25	3.75	3.50	3.50
-0.6	2.00	3.00	5.00	7.00
-0.9	3.25	2.75	4.50	8.25

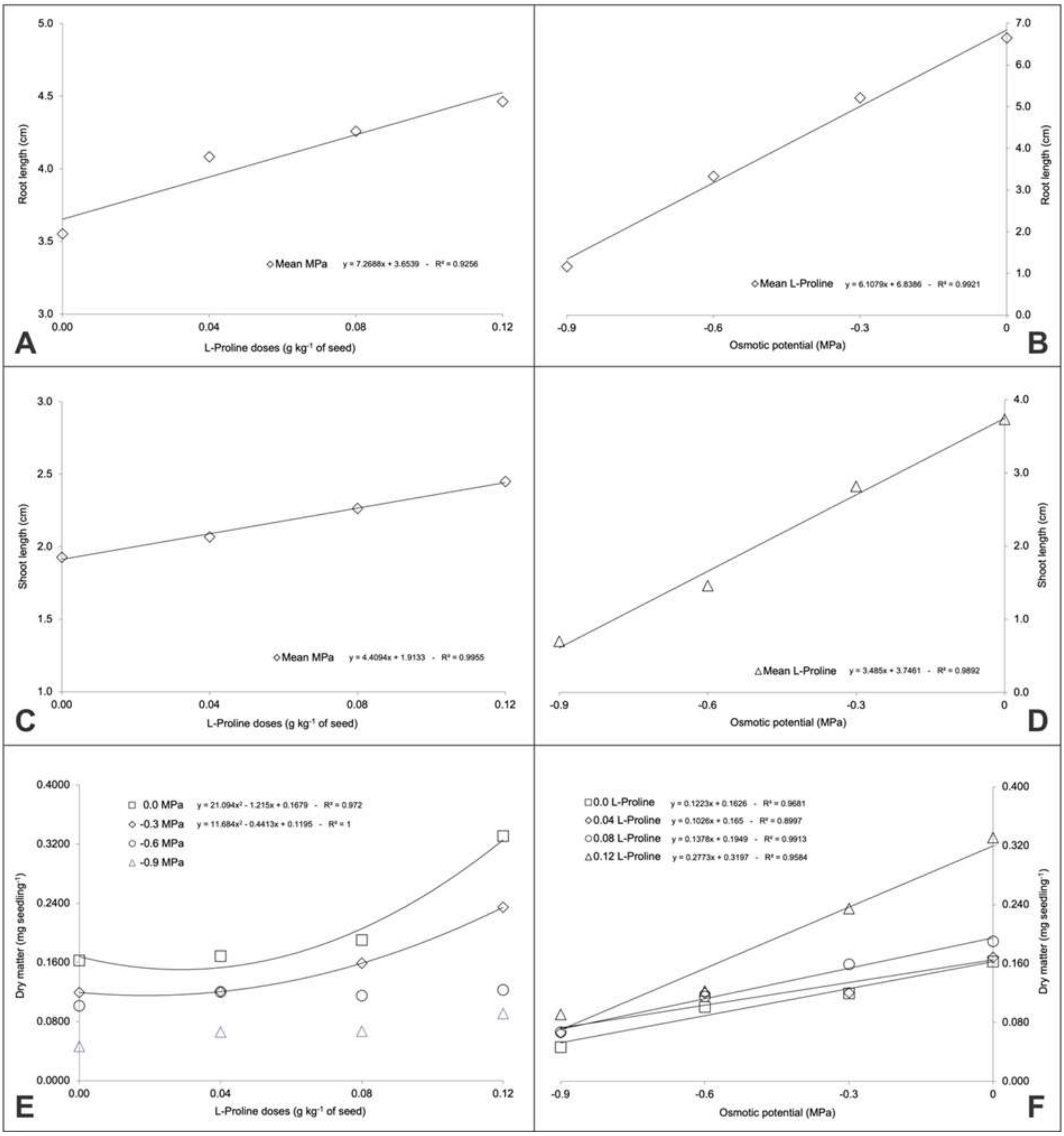

Fig. 2. Effects of osmotic potential induced by NaCl and exogenous L-proline application on soybean seedling growth and biomass accumulation

(A - B) Root length; (C - D) shoot length; and (E - F) dry matter accumulation of soybean seedlings (cv. GH 5933 IPRO) subjected to osmotic potentials of 0.0, -0.3, -0.6, and -0.9 MPa and treated with L-proline doses of 0, 0.04, 0.08, and 0.12 g kg-1 seed. Regression equations were fitted at a 95% confidence level.

In contrast, no significant interaction or isolated effects of osmotic potential and L-proline doses were observed for abnormal seedlings (Table 1). These findings indicate that the occurrence of abnormal seedlings was not substantially influenced by either salinity levels or proline application. Although saline stress commonly induces ionic toxicity and restricts water absorption through the accumulation of Na^+ and Cl^- ions (Murillo-Amador *et al.,* 2002; Farissi *et al.,* 2011), the formation of abnormal seedlings in this study may have been more strongly associated with intrinsic seed characteristics than with the treatments applied.

For hard and dead seeds, a significant interaction between osmotic potential and L-proline concentration was detected. Significant effects of L-proline were observed under -0.6 and -0.9 MPa, while osmotic potential significantly affected the variable at all L-proline concentrations (Fig. 1E and 1F). The incidence of hard and dead seeds increased with increasing saline stress, confirming the inhibitory effect of low osmotic potential on seed viability. However, higher doses of L-proline reduced the proportion of non-viable seeds, indicating a protective effect against stress-induced damage. This response may be associated with the participation of proline metabolism in energy production and oxidative protection during germination (Kishor and Sreenivasulu, 2014).

For root length, no significant interaction between factors was observed, indicating that the effects of osmotic potential and L-proline occurred independently. However, both factors showed significant individual effects (Fig. 2A and 2B). Root growth decreased progressively under saline stress, reflecting the inhibition of cell expansion and water uptake. Conversely, increasing L-proline doses promoted greater root elongation, suggesting a beneficial effect on early root development. Similar responses were reported by Biancucci *et al.* (2015) and Mariano-da-Silva *et al.* (2025a), who demonstrated that proline and tryptophan stimulate root elongation by regulating cell division.

A similar pattern was observed for shoot length. Although no interaction between the factors was detected, both osmotic potential and L-proline concentrations significantly influenced seedling shoot growth (Fig. 2C and 2D). Salinity reduced shoot development due to osmotic restriction and ionic toxicity, while L-proline application promoted a linear increase in shoot growth. These findings indicate that exogenous proline partially mitigates the harmful effects of saline stress, contributing to improved seedling establishment and vigor, similarly to responses reported for exogenous L-tryptophan in soybean seeds under stress conditions (Mariano-da-Silva *et al.,* 2025a; Mariano-da-Silva *et al.,* 2025b).

Dry matter accumulation also showed a significant interaction between osmotic potential and L-proline doses. Significant effects of L-proline were observed at osmotic potentials of 0.0 and -0.3 MPa, while osmotic potential significantly affected all L-proline levels (Fig. 2E and 2F). Biomass production declined as saline stress intensified, demonstrating the negative effects of reduced water availability on plant growth. However, L-proline application promoted greater dry matter accumulation, particularly under low to moderate stress conditions. These results suggest that exogenous L-proline may enhance biomass production by improving osmotic regulation and maintaining metabolic activity under stress. Similar effects were reported by Teixeira *et al.* (2019), who observed increased root dry matter in plants treated with L-proline under water deficit conditions.

CONCLUSION

Overall, the results demonstrate that saline stress negatively affects soybean seed germination and early seedling development, while exogenous application of L-proline mitigates part of these adverse effects. The protective role of L-proline was particularly evident under moderate and severe osmotic stress, highlighting its potential as a physiological enhancer for improving soybean establishment under saline environments.

From an agronomic and environmental perspective, the use of L-proline represents a sustainable strategy for mitigating the impacts of abiotic stress in soybean cultivation. As a naturally occurring amino acid involved in plant stress metabolism, L-proline offers a low-toxicity and environmentally compatible alternative to synthetic growth regulators or chemical stress mitigators. Its application may contribute to improved seedling establishment, greater tolerance to saline conditions, and enhanced crop performance, especially in areas affected by soil salinization and irregular water availability. These findings reinforce the potential of L-proline as an economically viable and ecologically responsible tool for supporting soybean production under adverse environmental conditions.

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