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RESEARCH ARTICLE

Spermidine (Spd) as a modulator of osmotic, redox and ion homeostasis in common bean seedlings under salinity stress: Physiological, biochemical and molecular aspects

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ABSTRACT

Spermidine (Spd), a naturally occurring molecule in plants, is being investigated for its potential to improve the bean's tolerance to salinity stress. This study explores how common beans (*Phaseolus vulgaris* L.) can be helped to thrive in salty environments. This research involved a group receiving regular watering with half strength Hoagland's solution (HS), and other group watered with a modified form of the same solution containing 75 mM NaCl to stimulate salt stress. Seedlings were treated with foliar applications of Spd (0, 0.5, 1 mM) at five intervals: 15, 20, 25, 30, and 35 d after sowing. Applying Spd significantly reduced damage caused by salt and protected essential pigments for photosynthesis (total chlorophyll by 33% and carotenoids by 57% over than the Spd-untreated plants under saline conditions). Additionally, 1 mM Spd enhanced the antioxidant enzyme activities, i.e., superoxide dismutase, catalase, and ascorbate peroxidase by 15%, 55% and 4.6% and promoted the buildup of beneficial compounds like proline, free amino acids and sugars. Remarkably, exogenous Spd application reduced Na^+ accumulation while improving K^+ content in common beans under salinity stress. Additionally, it enhanced the cell membrane stability index (CMSI) during stressful situations, the maximum CMSI (94.31%) was recorded with 1 mM Spd under non-stress conditions, whereas the minimum CMSI (72.97%) was registered under salinity without Spd. Under salinity condition, the real-time quantitative PCR (qRT-PCR) results demonstrated a significant increase in the expression of the vacuolar-localized Na^+/H^+ antiporter protein (NHX1), and the multifunctional osmotic protection protein (Osmotin) in comparison to the control. Furthermore, common bean treated with Spd showed significantly higher expression levels of *SOS1*, *NHX1*, and *Osmotin*, under normal and salinity conditions, with the highest gene expression observed under 1 mM spermidine treatment under saline conditions. Spermidine at 1 mM notably decreased malondialdehyde levels compared to 0.5 mM (10.68 *vs*. 12.11 nmol g^{-1} FW). Overall, the findings propose that Spd may be an effective tool for cultivating common beans in saline areas, potentially paving the way for increased food production in challenging environments.

Key words: Chlorophyll content, common bean, gene expression, *Phaseolus vulgaris*, photosynthesis, salinity stress, spermidine.

INTRODUCTION

Abiotic stress factors cause a significant decrease in the growth parameters of several plants, the main two factors are drought (AlKahtani et al., 2021; Arafa et al., 2021) and salinity (El Nahhas et al., 2021; Abdou et al., 2023). Salinity is a key environmental element that seriously impacts the growth and development of plants, limiting agricultural output and quality (Aboryia et al., 2022). Additionally, certain mulching methods and fertilizers have altered the soil's physical and chemical properties, worsening secondary soil salinization, this severely affected crop productivity and quality (Alkhateeb et al., 2024). The common bean (*Phaseolus vulgaris* L.), being glycophytic, demonstrates high sensitivity to salt, where soil salt levels of 2 $dS \cdot m^{-1}$ and below negatively impact yield production (El-Beltagi et al., 2023).

As the quantity of salt grows over time, two primary impairments emerge in separate stages: Osmotic stress and specific ion toxicity. These abnormalities then result in secondary pressures, such as oxidative stress and nutritional problems. Under salinity stress, there is a decrease in the absorption of water and nutrients, impairment of membrane function, and disturbance of important biological activities such as respiration, photosynthesis, and protein synthesis (Al-Shammari et al., 2023). Salt tolerance refers to the ability of a plant's genetic makeup to alleviate the negative impacts of salinity, such as ion toxicity, and oxidative stress. This ability helps minimize yield losses in the plant (Balasubramaniam et al., 2023).

Polyamines are a class of basic organic compounds containing N, specifically putrescine, spermidine, and spermine. These chemicals are found in a wide range of living species. They serve as agents that protect or communicate within the plant, playing a crucial role in enhancing the plant's ability to withstand non-living stresses (Korbas et al., 2022). The production of polyamine in plants was recorded under abiotic stresses such as salinity, it can protect the chloroplast from oxidative damage.

Spermidine (Spd) plays a role in removing reactive oxygen species (ROS) and regulating the redox balance in plants (Zhang et al., 2016). In addition, when plants are exposed to high levels of salt, polyamines can protect them by collecting chemicals that help regulate osmotic pressure, such as soluble sugars or proline. The buildup of soluble chemicals has a crucial role in regulating ion channels, which in turn serves to maintain the internal balance of potassium (K^+) and sodium (Na^+) in plants. This procedure safeguards the integrity of the membrane and the activity of enzymes (Rohman et al., 2017). Furthermore, previous research has revealed that cucumber seedlings, when subjected to salt stress and treated with Spd, demonstrated improved photosynthetic efficiency. In addition, the production of tetrapyrrole was recorded with a higher ability to enhance the protein biosynthesis in the treated samples compared to those treated only with NaCl, as reported by Sang et al. (2016). The common bean, a prominent vegetable crop on a global scale, experiences substantial salt stress as a result of being cultivated in inadequately watered and partially saline circumstances. Spermidine can enhance the ability of different crops to withstand non-living environmental factors by increasing their ability to perform photosynthesis and enhancing the effectiveness of antioxidant enzymes. The impact of externally applied spermidine in reducing the harm induced by salt stress on common bean is yet uncertain. The objective of this research was to study the potential impacts of externally applied spermidine on common bean subjected to salinity stress.

MATERIALS AND METHODS

Growth and treatments

Before planting, common bean seeds (*Phaseolus vulgaris* L.) 'Bronco' were disinfected on the surface using a 0.5% NaOCl solution for a period of 3 min. Afterwards, the seeds were washed four times with distilled water. The seeds were sown in a greenhouse at normal temperatures (25 ± 5 °C). The plastic pots, which were dyed in a shade of dark grey, had a diameter of 13 cm and a volume of 700 cm^3, then filled with washed sand (Table 1). The seeds were sown in separate containers, with one plant per container, and were regularly irrigated with a diluted Hoagland's solution (HS) on a daily basis. The seedlings, which were of uniform size and shape and had reached the age of 2 wk, were divided into two primary categories. One group received regular watering with normal form of HS, whereas the other one was watered with a modified form of Hoagland's solution that

contained 75 mM NaCl in order to cause salt stress. Each primary category was subdivided into three subcategories to achieve spermidine (Spd) concentrations of 0, 0.5, and 1 mM in the pots. The seedlings were treated with five doses of foliar application (Spd) at certain intervals of 15, 20, 25, 30, and 35 d after planting. Every plant received a consistent application of a 10 mL solution on each time. Tween-20 was used at a dosage of 0.05% (v/v) in all treatments. The plants were given a further 5 d to mature before they were gathered for the aim of examining different growth, biochemical, and molecular characters (BBCH 22). The experimental design was a completely randomized design (CRD) with three replicates. The pots number was 108, which was obtained by multiplying the two salinity levels by the three Spd levels, and then multiplying that by the six pots and three replicates.

To growth parameters, upon reaching 40 d of age, two pots were taken from each replicate and the fresh shoot and root systems were promptly weighed.

Table 1. Physical and chemical properties of sand soil.

Parameter	Value
pH	7.1
EC	0.25
SiO_2	96.87
Al_2O_3	2.16
Fe_2O	0.62
MgO	0.04
CaO	0.23
Na_2O	0.06

Determination of H_2O_2 and lipid peroxidation

The quantification of hydrogen peroxide (H_2O_2) was recorded according to Velikova et al. (2000), with numerous adjustments. To summarize, 0.2 g leaf tissue was crushed into a fine powder using 4 mL tri-chloroacetic acid (TCA). The mixture was then spun at a speed of 10000 revolutions per minute at 4 °C for 15 min; 0.5 mL residual liquid obtained after centrifugation was mixed with an equal volume of potassium iodide (1 mM) and potassium phosphate buffer (10 mM, pH 7). The yellow colour's intensity was recorded at 390 nm. The quantification of malondialdehyde (MDA) was conducted utilizing the thiobarbituric acid (TBA) technique. This entailed quantifying the absorbance of the crimson hue that manifested at wavelengths of 535 and 600 nm. The correction approach described by Heath and Packer (1968) was used to compensate for any unspecified turbidity.

Cell membrane stability index

Eight discs of fresh leaves (1.8 cm) diameter, were submerged in 10 mL deionized water and left to incubate for a period of 24 h on a shaker. The electrical conductivity (EC) of the contents (EC1) were determined using EC meters subsequently (DOH-SD1, TC-OMEGA, St-Eustache, Canada). Afterwards, the samples were subjected to autoclaving at a temperature of 120 °C for a period of 20 min to measure the EC2 values. Cell membrane stability index (CMSI) was determined as the following:

$$MSI = \left[1 - \left(\frac{EC1}{EC2}\right)\right] X100$$

where EC1 is the measurement obtained from the EC-meter for 10 leaf discs that were incubated in 10 mL deionized water for a period of 24 h. Meanwhile, EC2 refers to the measurement of EC using an EC-meter on the leaf discs after their membranes have been damaged in the autoclave at 120 °C for 20 min.

Determination of antioxidant enzymes activities

The leaf tissue of common bean plants (0.5 g) was finely crushed and mixed with 4 mL sodium phosphate buffer (pH 7.0) that contained 1% (w/v) polyvinylpyrrolidone (PVP) and 0.1 mM EDTA. The homogenate was centrifuged at 10000 revolutions per minute for 15 min. The liquid that remained above the sediment, referred

to as the supernatant, was gathered and utilized as the enzyme extract. The enzyme extract preparation process was carried out within a temperature range of 0-4 °C. The enzyme extract was analysed for soluble protein content using the Bradford (1976) technique. Superoxide dismutase (SOD) activity was evaluated at 560 nm, following the method outlined by Beyer and Fridovich (1987). Catalase (CAT) activity was quantified by monitoring the decrease in absorbance of H_2O_2 at 240 nm according to Cakmak et al. (1993). The functionality of guaiacol peroxidase (G-POX) was assessed by quantifying its ability to transform guaiacol into tetraguaiacol. This transformation was determined by observing the increase in absorbance at 470 nm (Dias and Costa, 1983). The ascorbate peroxidase (APX) activity involved measuring the decrease in ascorbate levels at 290 nm, according to the method of Nakano and Asada (1981).

Determination of leaf relative water content and osmotic molecules

The estimation of leaf relative water content (RWC) was conducted according to Smart and Bingham (1974). In summary, 10 leaf fresh discs were accurately measured (FW) and soaked in distilled water for 1 h to achieve turgidity (TW). Subsequently, the leaf discs were subjected to oven-drying at 80 °C for 24 h in order to measure the dry weight (DW). The formula for calculating RWC is as follows:

$$\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

where FW represents the fresh weight, DW represents the dry weight, and TW represents the weight of leaf discs when they are fully hydrated. Glycine was quantified as free amino acids (FAA) using the ninhydrin reagent, following the technique described by Hamilton and Van Slyke (1943). The quantification of proline was performed by the ninhydrin reagent, following the methodology defined by Bates et al. (1973). The quantification of total soluble sugars was performed using the phenol-sulfuric acid method (Chow and Landhäusser, 2004).

Na, K and Ca determination

The flame photometric method (flame photometer model Jenway, UK Cole-Parmer, Vernon Hills, Illinois) developed by Havre (1961) was used to determine the amounts of Na, K, and Ca.

Salt responsive genes expression

Total mRNA was extracted from fresh leaves (0.5 g) of different treatments using Total RNA extraction kit (Sigma-Aldrich) according to the manufacturer's protocol.

The RNA was isolated and quantified by spectrophotometry (CT-2200, Chrom Tech, Apple Valley, Minnesota, USA). Then the purified RNA was analysed on 1% agarose gel. Reverse transcription of RNA was performed. The reaction mixture contained 10 as oligo dT primer (10 pmol μL^{-1}), 2.5 µL 5X buffer, 2.5 µL $MgCl_2$, 2.5 µL 2.5 mM dNTPs, 4 µL from oligo (dT), 0.2 µL (5 units μL^{-1}) reverse transcriptase (Promega, Germany) and 2.5 µL RNA. The RT-PCR amplification was performed in a thermal cycler PCR, programmed at 42 °C for 1 h and 72 °C for 20 min.

The reverse transcription-polymerase chain reaction (RT-PCR) amplification was done in a thermal cycler PCR at 42 °C for 1 h and 72 °C for 20 min. To convert 5 µg total RNA into complementary cDNA, a reaction mixture of 2.5 µL including 2.5 mM dNTPs, 2.5 µL $MgCl_2$, 1.0 µL oligo dT primer (10 pmol μL^{-1}), 2.5 µL 5X buffer, and 0.2 µL reverse transcriptase (5 units μL^{-1}) was used (Promega, Gutenbergring, Germany). The RT-PCR was amplified in a thermal cycler PCR for 1.5 h at 42 °C and for 20 min at 80 °C. Rotor-Gene 6000, (Generi Biotech, Trebes, Czech Republic) was used to perform RT-PCR on 1 µL diluted cDNA in triplicate. We used primers of the vacuolar-localized protein (NHX1), the plasma membrane Na^+/H^+ antiporter protein of the salt overly sensitive gene (*SOS1*), Osmotin, and the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene for gene expression analysis. SYBR Green Master Mix (Merck, Rahway, New Jersey, USA) was used for analysis. The experiment used a 20 µL reaction volume. The 20-µL reaction mixture includes 2 µL template, 10 µL SYBR Green Master Mix, 2 µL reverse primer, 2 µL forward primer, and sterile distilled water. Specific settings were used for PCR experiments: It was heated to 95 °C for 15 min, then 40 times at 95 °C for 30 s and 60 °C for 30 s. The cycle thresholds (CTs) of each sample were used to calculate ΔCT values by subtracting the target gene CT value from the β-Actin gene CT value. The gene expression was assayed using the 2-ΔΔCt method (Livak and Schmittgen, 2001).

Statistics

The SAS (1988) (SAS Institute, Cary, North Carolina, USA) program performed one-way ANOVA. Significant differences between means were determined using LSD's multiple range test ($P \leq 0.05$) on means ± SD from three replicates.

RESULTS

Fresh weight, total chlorophyll, and carotenoid

Treatments NaCl and spermidine affected fresh weight of shoot and root, as well as the overall growth rate (Figure 1A). Exogenous application of Spd notably enhanced the fresh weight of both root and shoot under 0 and 75 mM NaCl conditions. Moreover, Spd significantly improved the growth rate under control and salinity conditions. Common bean seedlings cured with 1 mM Spd demonstrated the highest fresh weight and growth rates for both shoots and roots under both saline stress and non-saline stress conditions. Total chlorophyll rates were significantly influenced via NaCl and Spd treatments. Raised water salinity lessened chlorophyll rates compared to control conditions, whereas Spd treatment increased chlorophyll rates regardless of salinity stress (Figure 1B). Particularly, 1 mM Spd notably increased chlorophyll values under non-stress (3.17 mg g^{-1} FW) condition compared to control (2.46 mg g^{-1} FW); in addition, 1 mM Spd increased the chlorophyll values under stress condition (2.46 mg g^{-1} FW) compared to 75 NaCl without using Spd (1.24 mg g^{-1} FW).

Application of Spd also enhanced carotenoid content under all conditions checked (Figure 1C). Increasing NaCl concentration decreased carotenoid content; however, data showed that increasing Spd concentrations counteracted this effect, giving rise to enhance the carotenoid content. Overall, exogenous Spd application enhanced the levels of fresh and dry weight, growth rate, total chlorophyll, and carotenoid contents, indicating its possibility to improve common bean seedlings growth under both control and salinity conditions.

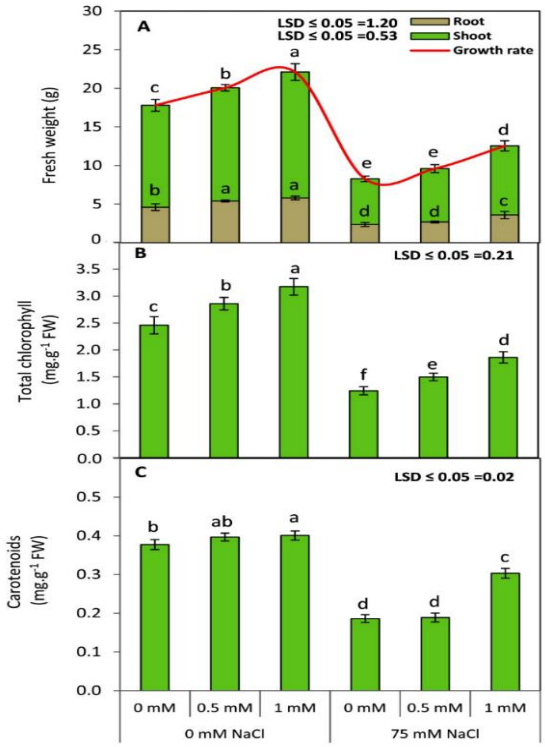

Figure 1. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the vegetative growth (A), total chlorophyll (B) and carotenoids (C) of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

Relative water content and cell membrane stability index

Salinity stress and Spd application significantly influenced RWC (Figure 2A). The application of Spd notably increased RWC under both stress and non-stress conditions. Moreover, 1 mM Spd treatment had a significant impact on RWC under stress (80.56%).and non-stress conditions (94.31%).

Salinity levels, and Spd application also had significant impacts on cell membrane stability index (CMSI). The highest CMSI value was documented with 1 mM under non-stress conditions (94.31%) contrariwise, the lowest value recorded under salinity conditions (72.97%) without using Spd. Notably, exogenous Spd treatment enhanced CMSI under salinity conditions (Figure 2B).

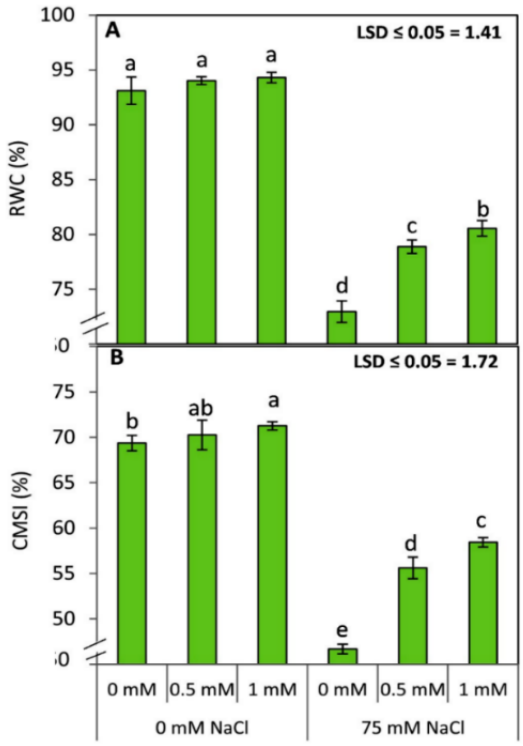

Figure 2. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the leaf relative water content (RWC) (A), and cell membrane stability index (CMSI) (B) of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

Hydrogen peroxide and malondialdehyde

Under salinity stress conditions, data recorded a common increase in both of H_2O_2 and MDA levels. The H_2O_2 recorded highest levels under stress conditions. However, Spd treatment notably reduced H_2O_2 levels under stress conditions (Figure 3A).

Figure 3B shows that the MDA concentration increased in response to increased NaCl concentration, while, were afterwards decreased via using Spd treatment. Under salinity conditions, the lessening in MDA was considerably superior by 1 mM Spd treatment was used compared to 0.5 mM (10.68-12.11 nmol g^{-1} FW) respectively.

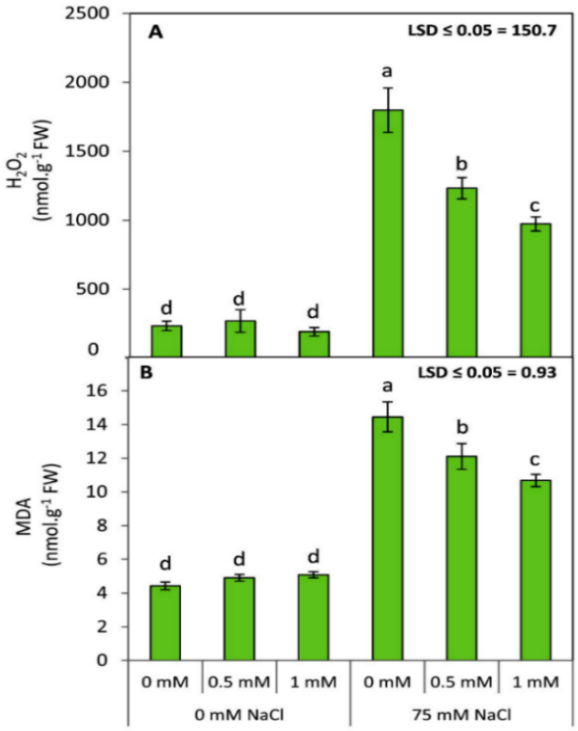

Figure 3. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the concentration of hydrogen peroxide; H_2O_2 (A), and the lipid peroxidation rate as indicated by the concentration of malondialdehyde; MDA (B) in the leaves of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

Proline, free amino acids, and soluble sugars

Common bean' proline content significantly increased under salinity stress; whit the maximum proline content was observed in seedling subjected to salt treatments (Figure 4A). Spermidine treatment was highly effective in increasing proline levels, and this effect was more pronounced particularly in plants grown at 0.5 and 1 mM Spd in combination with salt stress. These findings suggested that exogenous application of Spd might drastically modulate the proline level under saline conditions. From Figure 4B, data showed that free amino acids (FAA) content was affected by both salinity and Spd. Regardless of the NaCl and Spd treatments, FAA levels increased under salinity stress. The highest levels of FAA were recorded in common bean plants treated with 75 mM NaCl and 1 mM Spd (757.72 µg g^{-1} FW). In addition, total soluble sugars also increased by rising salinity concentration (Figure 4C). Data illustrated that Spd enhanced the total soluble sugar content, demonstrating that using Spd treatment might notably alter the total soluble sugar content in common bean plants under stress conditions.

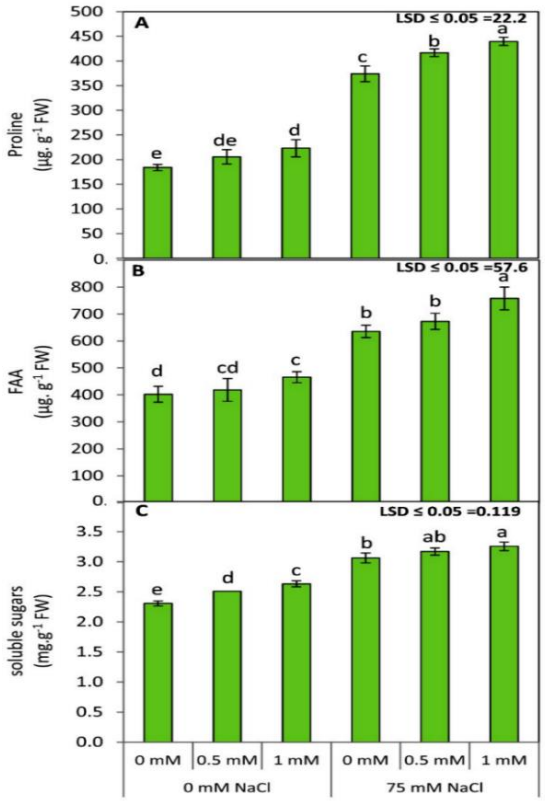

Figure 4. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the concentration of proline (A), and free amino acids (FAA) (B) and total soluble sugars (C) in the leaves of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

Ion homeostasis

The ratio of Na^+/K^+ in plants has often been utilized as a crucial indicator of plant resilience to salinity. To evaluate the impact of salinity stress and Spd treatment, the levels of K^+ and Na^+ in common beans were assessed with salinity stress and Spd treatment. The Na^+ concentration experienced a notable increase under salinity stress in comparison to the 75 mM NaCl treatment, while the addition of exogenous Spd effectively mitigated this salt-induced rise in Na^+ levels (Figure 5A). Conversely, the K^+ concentration reached its lowest point under 75 mM NaCl but showed a substantial increase when exogenous Spd was applied under salinity stress (Figure 5B). Additionally, the Na^+/K^+ ratio was determined. As depicted in Figure 5C, the Na^+/K^+ ratio significantly increased under salinity stress conditions but was significantly reduced by the application of exogenous Spd under conditions of salinity stress. These findings suggests that exogenous Spd modulates ion homeostasis under salinity stress conditions by diminishes Na^+ uptake while boosting K^+ absorption.

Antioxidant enzymes

The antioxidant enzyme activities in common beans were assessed under saline conditions. As illustrated in Figure 6, exposure to salinity notably enhanced the activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and guaiacol peroxidase (G-POX) compared to the control. Notably, the exogenous application of Spd further increased the activities of CAT, SOD, APX, POD, and G-POX in the stressed common beans, indicating a significant improvement in the antioxidant response.

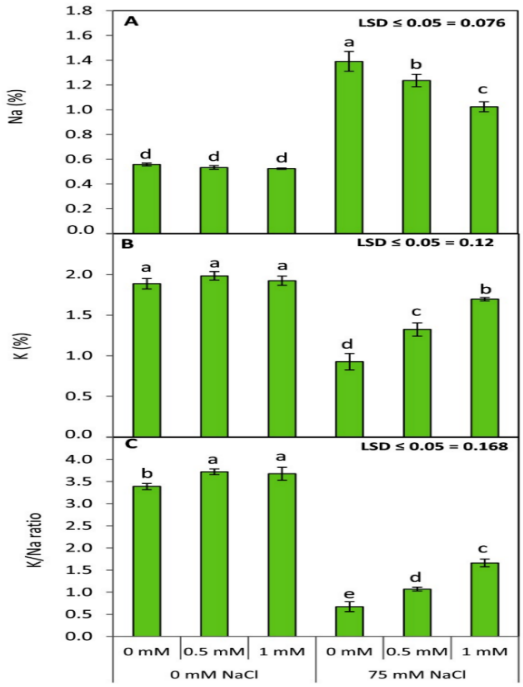

Figure 5. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the concentration of Na (A), K (B) and K/Na ratio (C) in the leaves of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

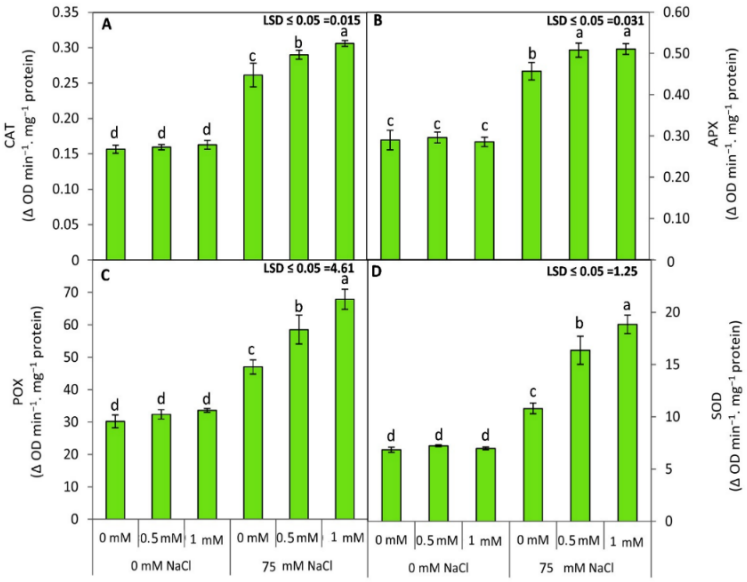

Figure 6. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the activities of antioxidant enzymes including catalase (CAT) (A), ascorbate peroxidase (APX) (B), guaiacol peroxidase (G-POX) (C) and superoxide dismutase (SOD) (D) in the leaves of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

Impact of spermidine on the relative expression of *SOS1*, *Osmotin* and *NHX1*

The qRT-PCR analysis (Figure 7) showed that *SOS1*, *NHX1*, and *Osmotin* were significantly up-regulated ($P \leq 0.05$) in saline-stressed plants compared to non-saline stressed plants. Notably, Spd-treated plants exhibited considerably greater expression levels of *NHX1*, *SOS1*, and *Osmotin* under both saline stress and non-saline stress conditions relative to untreated common bean plants. Among all treatments, the application of 1 mM Spd under saline conditions consistently resulted in the most significant upregulation of gene expression for *NHX1*, *SOS1*, and *Osmotin.*

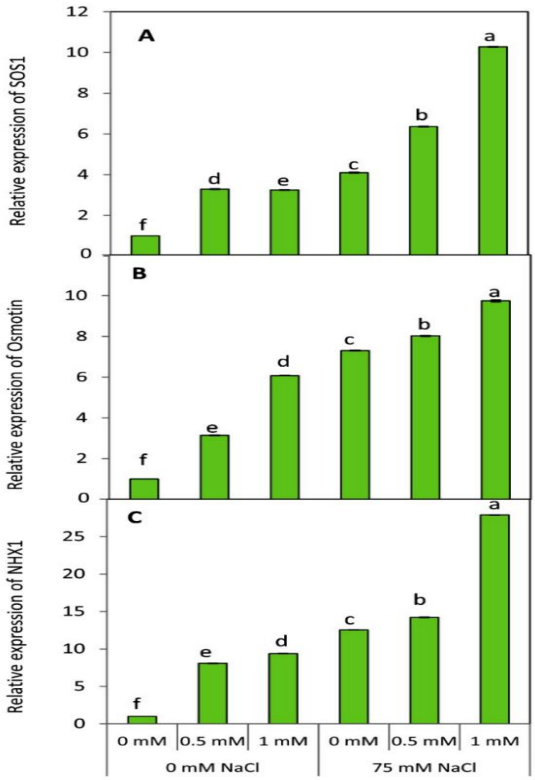

Figure 7. Effect of exogenous spermidine (0, 0.5 and 1 mM) on the relative expression of plasma membrane Na^+/H^+ antiporter protein of salt overly sensitive gene *SOS1* (A), and the multifunctional osmotic protective protein Osmotin (B) and vacuolar-localized Na^+/H^+ antiporter protein NHX1 (C) of snap bean plants grown in the leaves of common bean seedlings grown under non-saline and saline (75 mM, NaCl) conditions. Bars represent standard error (SE) of the means (n = 3). Different letters indicate significant differences among the treatments at $P \leq 0.05$, according to LSD's multiple range test.

DISCUSSION

The sustainable growth of the common bean is significantly hindered by salinity stress; therefore, it is crucial to explore techniques for improving the ability of common beans to tolerate high levels of salt. Polyamines, like spermidine (Spd), have been shown to effectively adjust plant resilience to several stressors (Pálfi et al., 2022). Previous studies have demonstrated that the application of Spd improves the ability of cucumber plants to tolerate high salt levels by adjusting the antioxidant enzymes activity, hence reducing oxidative damage (Wu et al., 2018).

The application of Spd in tomato plants effectively mitigated the adverse impacts of salinity, enhanced the mechanisms for removing reactive oxygen species (ROS), and promoted better regulation of ion balance under salinity stress (Raziq et al., 2022). Our results found that the application of external Spd enhanced the growth of common bean seedlings when they were exposed to salinity-alkalinity stress. Our research indicates that the use of external Spd enhanced the growth of common beans when they were exposed to high levels of salt in the environment. Therefore, this outcome indicates that the application of external Spd treatment effectively reduced the growth inhibition and improved the ability of common bean to tolerate salinity.

Our observations indicate that the presence of salinity resulted in a significant reduction in growth, including both the weight of plant shoots and roots, as well as the rate of growth. Nevertheless, the addition of Spd led to a notable enhancement in growth. Raziq et al. (2022) found that the growth of tomato seedlings under salinity stress can be improved by applying Spd externally.

The present investigation demonstrated that exposure to salinity led to a substantial decline in the levels of chlorophyll and carotenoid in the common bean. Nevertheless, the use of external Spd could potentially mitigate the reduction in photosynthetic pigments caused by salinity. Chlorophyll, being the predominant pigment in the photosynthetic system, has a vital function in the process of photosynthesis and the absorption of light throughout plant development. Therefore, the substance of the subject is intricately connected to the effectiveness of photosynthesis (Li et al., 2015). According to Li et al. (2015), the introduction of external Spd improved photosynthesis in tomato by increasing the amount of chlorophyll. Therefore, we concluded that the application of external Spd predominantly increased the rate of photosynthesis in common bean plants experiencing salinity by improving the levels of photosynthetic pigments.

The ability of externally applied Spd to maintain a high relative water content (RWC) in stressed common bean plants may be due to its involvement in osmotic adjustment, which is helped by an increase in the internal proline content (Duan et al., 2008). Similarly, the application of Spd improves the effectiveness of compatible mycorrhizal symbiosis induction on common bean plants when they are subjected to stressful conditions (El-Beltagi et al., 2023). The capacity of externally applied Spd to maintain RWC and cell membrane stability index (CMSI) in stressed common bean plants may be due to its function in osmotic adjustment, which is helped by an increase in internal proline levels.

One common adaptive response to salinity is the buildup of proline. In the common bean, the level of proline buildup caused by salinity was higher under salty conditions. The present investigation found that the addition of external Spd substantially increased the levels of proline in common bean plants subjected to salinity conditions. Elevated proline levels can act as a scavenger for hydroxyl radicals and singlet oxygen. Furthermore, proline acts as a suitable solute that is able to tolerate osmotic stress, while also supplying essential N and C supplies necessary for recovering from stress. Moreover, it participates in stress signal transduction pathways, therefore aiding in the improvement of salt tolerance (El-Shawa et al., 2022).

The presence of salt stress increased the levels of total soluble sugars and free amino acids (FAA) in common bean. Plants treated with salt exhibit a stress management strategy, as seen by an elevation in FAA and total soluble sugars, according to Jameel et al. (2024). Saline stress triggers osmotic stress and ionic toxicity in plants, leading to dehydration of plant cells and disruption of their regular metabolic processes. Salinity stress induces ionic toxicity, causing an excessive buildup of Na^+ in plant cells, this disrupts the Na^+/K^+ ratio and hinders plant growth.

Our study found that the Na^+ concentration significantly rose but the K^+ content remained unchanged under salt stress, leading to an elevated Na^+/K^+ ratio. Spermidine and other polyamines have the ability to control the ion transporters in the plasma membrane via several mechanisms, such as the suppression of Na^+ entering and K^+ exiting electrical currents. Our results displayed that the addition of Spd from an external source enhanced the absorption of K^+ and reduced the sodium uptake (Na^+), resulting in a decrease in the ratio Na^+/K^+. Spermidine serves as both a scavenger of ROS, which helps neutralize free radicals, and an activator of antioxidant enzymes, so boosting their effectiveness (Korbas et al., 2022).

Our findings showed that high salt levels led to increase the enzyme activity of SOD, POD, G-POX, APX, and CAT, these results are in accordance with the findings of Alshammari et al. (2024). Nevertheless, the utilization of external Spd notably increased the levels of activity in all four antioxidant enzymes when compared to the levels reported under salinity conditions. This discovery is consistent with the outcomes documented in a study on citrus seedlings (Khoshbakht et al., 2018). The qRT-PCR molecular studies confirmed that the genes *SOS1*,

NHX1, and *Osmotin* were noticeably up-regulated ($P \leq 0.05$) in plants exposed to saline conditions compared to those not subjected to salt stress (Figure 7). These genes include *SOS1*, *NHX1*, and *Osmotin*. Overexpression of these genes can improve plants' ability to withstand salt stress by controlling the balance between K and Na ions and achieving osmotic equilibrium (Alnusairi et al., 2022). Conversely, plants that were exposed to Spd showed a significant increase in *SOS1*, *NHX1*, and *Osmotin* compared to the plants that were not treated, particularly in saline environments. This response may relate to the role of Spd as a powerful antioxidant and crucial biomolecule in controlling how plants handle salt stress. It suggests that adding Spd from an external source can guard the cells from dying due to oxidative stress by enhancing the plant's natural ability to fight against harmful oxidants.

CONCLUSIONS

In this work, we examined how exogenous spermidine (Spd) affects common bean salinity tolerance. Salinity stress stunted plant development. However, exogenous Spd greatly reduced salt stress-induced growth inhibition. Under salt stress, exogenous Spd increased pigment concentration, decreased oxidative damage, and preserved Na^+/K^+. Under salt stress, exogenous Spd increased proline, free amino acids, and total soluble sugar accumulation. In conclusion, exogenous Spd is essential for common bean salinity stress tolerance. These results establish the groundwork for studying how exogenous Spd reduces salinity damage in common beans, which could help them grow in saline soils.

Author contribution

Conceptualization: M.A.A., N.A.I.A. Methodology: M.A.A.A., N.A.I.A., M.A., N.A.Al., K.A. Software: W.B.Al., A.Al., N.A.I.A., M.F.F. Validation: M.A.A.A., N.A.I.A., M.A. Formal analysis: N.A.I.A., M.A. Investigation: M.A.A., N.A.I.A. Resources: M.A.A.A., N.A.I.A., M.A. Data curation: M.A.A.A., N.A.I.A., M.A., N.A.Al., H.K.A., K.A. Writing-original draft: M.A.A.A., N.A.I.A., M.A., N.A.Al., H.K.A., K.A., W.B.Al., A.Al., N.A.T.Al., A.A.M.A., B.M.Al., S.M.AL, E.S.D., D.B.D., M.F.F. Writing-review & editing: M.A.A.A., N.A.I.A., M.A., N.A.Al., H.K.A., K.A. Supervision: N.A.I.A., M.A. Project administration: M.A.A.A., N.A.I.A., M.A. Funding acquisition: W.B.Al., A.Al., N.A.T.Al., A.A.M.A., B.M.Al., S.M.AL, E.S.D., D.B.D. All co-authors reviewed the final version and approved the manuscript before submission.

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