

Exogenous Spermidine Enhances Salt Tolerance in *Nicotiana rustica*: Physiological Mechanisms and Implications for Phytoremediation

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ABSTRACT

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Salt stress disrupts plant physiology by impairing photosynthesis, osmotic balance, and ion homeostasis. This study evaluated the effect of exogenous spermidine (Spd, 1 mM) on *Nicotiana rustica* exposed to moderate (100 mM NaCl) and severe (200 mM NaCl) salinity. Salt stress significantly reduced chlorophyll a, carotenoids, protein contents, and biomass, while inducing proline and soluble sugar accumulation and disturbing K^+/Na^+ and NO_3^-/Cl^- balances. Spd application markedly alleviated these effects under moderate salinity, restoring chlorophyll a and protein levels, sustaining biomass production, limiting excessive proline and sugar accumulation, and reducing Na^+ and Cl^- toxicity while maintaining K^+ and NO_3^- uptake. However, its protective effects were largely absent under severe stress, with only marginal improvements in biomass and osmolyte regulation. These findings indicate that spermidine confers substantial tolerance to moderate but not severe salinity, basically through stabilization of photosynthesis, preservation of protein metabolism, and regulation of ion homeostasis.

Keywords: Ion homeostasis, *Nicotiana rustica*, osmolytes, phytoremediation, polyamines, salt stress, spermidine

Soil salinization is a major abiotic constraint threatening agricultural productivity in Tunisia, where nearly 30% of irrigated lands are affected (Munns and Tester 2008). In arid and semi-arid regions such as Sfax and Gabes, groundwater salinity frequently exceeds 5 g/L, necessitating the adoption of salt-tolerant

crops and biostimulants. *Nicotiana rustica*, which is well-adapted to marginal soils, represents a promising crop for both phytoremediation and biomass production (Kabir et al. 2024).

Polyamines, such as spermidine (Spd), play essential roles in plant stress responses by stabilizing membranes, scavenging reactive oxygen species (ROS), and regulating ion transport (Groppa and Benavides 2008). Despite growing interest, the application of polyamines in Tunisian agriculture remains largely unexplored. We hypothesize that exogenous spermidine can enhance salt tolerance in *N. rustica*,

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with its efficacy being dependent on the severity of the stress.

Salt stress disrupts plant physiology through osmotic imbalance, ion toxicity (especially Na^+ and Cl^-), nutrient deficiencies, and oxidative stress, which collectively inhibit growth and reduce yield (Zhu 2016). In response, plants activate adaptive mechanisms, such as the accumulation of osmoprotectants (e.g. proline, glycine betaine, and soluble sugars) and the regulation of ion transport systems to maintain cellular homeostasis (Flowers and Colmer 2008). Among the biochemical mediators of salt tolerance, polyamines, including putrescine, spermidine, and spermine, have garnered attention for their role in stress mitigation (Gill and Tuteja 2010). Specifically, spermidine functions as both a signaling molecule and an antioxidant, contributing to membrane stability, ROS scavenging, and ion channel regulation (Groppa and Benavides 2008). Previous studies have demonstrated that exogenous spermidine application enhances salt tolerance in various crops by improving photosynthetic performance, reducing oxidative damage, and optimizing ion homeostasis (ElSayed et al. 2018, Raziq et al. 2022).

Despite advances in understanding polyamine mediated stress tolerance, the mechanisms by which spermidine mitigates salt stress in *N. rustica* remain unclear. This species is recognized for its phytoremediation potential due to its vigorous growth and metal accumulation capacity. This study investigates the physiological and biochemical responses of *N. rustica* to salt stress under hydroponic conditions, with and without spermidine supplementation. Plants were exposed to two concentrations of NaCl (100 and 200 mM). Parameters including growth, ion homeostasis (K^+ , Na^+ , Cl^- , NO_3^-), and metabolic adjustments (proline and soluble sugars)

were measured. By elucidating spermidine's protective role, this research aims to contribute to sustainable strategies for enhancing crop salt tolerance and to promote the use of *N. rustica* in phytoremediation and saline agriculture (Zhou et al. 2005).

MATERIALS AND METHODS

Plant cultivation.

Seedlings were initially cultivated in a ¼-strength Hoagland's nutrient solution (Hoagland and Arnon, 1950). Following leaf emergence, they were transferred to 2-liter containers, with six seedlings per container. After 8-9 days, once the cotyledons were fully developed, the seedlings were irrigated with a ½-strength Hoagland's solution for three days before being transferred to a full-strength solution.

The hydroponic growth was conducted under controlled environmental conditions with an artificial light intensity of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, a 16/8-hour light/dark photoperiod, a daytime temperature of 25°C , and relative humidity of 70% (day) and 90% (night). The nutrient solutions were continuously aerated using air pumps and replaced every three days (Epstein and Bloom 2005). After 30 days of growth, salt stress was induced by adding NaCl at two concentrations (100 mM and 200 mM). These salt treatments were applied either alone or in combination with 1 mM spermidine. All treatments were maintained for 12 days.

Biomass assessment.

Plants were harvested and separated into shoots (leaf blades) and roots. Roots were rinsed three times with distilled water and blotted dry using filter paper. Fresh weight (FW) was recorded immediately, after which samples were then oven-dried at 60°C for 72 hours to determine dry weight (DW) (Jones 2001).

Chlorophyll content.

Chlorophyll pigments were extracted from 100 mg of fresh leaf tissue by homogenization in 80% (v/v) acetone, following the method of Lichtenthaler and Wellburn (1983). The samples were incubated for 7 days at 4°C and then centrifuged at 1500 × g for 10 min. The absorbance (Abs) of the supernatant was measured at 663 nm, 645 nm, and 460 nm using a spectrophotometer. Chlorophyll a (Chl a) and carotenoid (Car) concentrations were calculated according to the following equations:

$$* \text{Chl a} = (12.7 \times \text{Abs } 663) - (2.69 \times \text{Abs } 645),$$

$$* \text{Car} = [5 \times \text{Abs } 663] - ((\text{Chl a} \times 3.19) + (\text{Chl b} \times 130.3))/20,$$

and results are expressed in µg/g FW.

Mineral ion contents (Na⁺, K⁺, and Cl⁻).

Ion content analysis was performed on 25 mg of dried, ground leaf tissue. The tissue was digested in 25 mL of 0.5 N sulfuric acid. Sodium (Na⁺) and potassium (K⁺) concentrations in the digest were measured using a flame photometer (Corning). Chloride (Cl⁻) content was determined by argentometric titration according to the Buchler-Cotlove method (Yemm and Willis 1954, Chapman and Pratt, 1961). All results are expressed in µmol·g⁻¹ DW.

Nitrate content.

Nitrate was extracted in cold 0.1 N sulfuric acid and quantified by colorimetric analysis according to the method of Henriksen and Selmer-Olsen (1970).

Total soluble proteins.

Soluble protein content was determined by the Bradford assay (Bradford, 1976). A 25 µL aliquot of extract was mixed with 975 µL of 5 × diluted Bradford reagent. After color development, absorbance was read at

595 nm. Results are expressed in mg. g⁻¹ FW.

Soluble sugars.

Soluble sugars were extracted from 25 mg of dried tissue using 5 mL of 80% ethanol and incubated at 70°C for 30 min (McCready et al. 1950). After centrifugation (6000 rpm, 15 min), 25 µL of the supernatant was mixed with 5 mL of anthrone reagent and boiled at 100°C for 10 min. After cooling on ice, absorbance was measured at 640 nm. Quantification was done using a glucose standard curve (0.1 g/L) (Staub 1963).

Proline content.

Proline content was estimated according to Bates et al. (1973). Fresh tissue (100 mg) was homogenized in 1.5 mL of 3% sulfosalicylic acid at 4°C and centrifuged at 14,000 rpm for 20 min. The supernatant (500 µL) was mixed with 500 µL of 3% SSA, 1 mL of acid ninhydrin, and 1 mL of glacial acetic acid. The mixture was incubated at 100°C for 20 minutes and cooled to 4°C. The chromophore was extracted using 2 mL of toluene. After mixing and resting for 1 hour, absorbance was read at 520 nm. Results are expressed as µmol/g FW.

Statistical analysis.

A two-way analysis of variance (ANOVA) was performed using XLSTAT software (version 2015) to evaluate the main effects of NaCl concentration (0, 100, and 200 mM) and spermidine treatment (0 or 1 mM), as well as their interaction, on all measured physiological parameters. When the ANOVA indicated significant differences ($p < 0.05$), post-hoc comparisons of means were conducted using Tukey's Honest Significant Difference (HSD) test at a 5% significance level.

RESULTS

Effects of salt stress and spermidine on photosynthetic pigments.

A two-way ANOVA revealed highly significant effects of both salt stress and spermidine treatment on photosynthetic pigment concentrations in *N. rustica* ($p < 0.001$). Chlorophyll a (Chl a) content in control plants was $0.85 \mu\text{g}\cdot\text{g}^{-1}$ FW. Exposure to 100 mM NaCl caused a significant 41% reduction in Chl a to $0.50 \mu\text{g}\cdot\text{g}^{-1}$ FW ($p < 0.001$). This decline was significantly alleviated by treatment with 1 mM spermidine, which restored Chl a content to $0.64 \mu\text{g}\cdot\text{g}^{-1}$ FW ($p < 0.05$ compared to 100 mM NaCl), statistically

indistinguishable from the control ($p > 0.05$). Under severe salt stress (200 mM NaCl), Chl a content further decreased to $0.48 \mu\text{g}\cdot\text{g}^{-1}$ FW, with spermidine application failing to exert a significant protective effect ($0.29 \mu\text{g}\cdot\text{g}^{-1}$ FW; $p > 0.05$ vs 200 mM NaCl alone). Carotenoid concentrations exhibited a similar pattern, declining from $0.26 \mu\text{g}\cdot\text{g}^{-1}$ FW in controls to $0.22 \mu\text{g}\cdot\text{g}^{-1}$ FW under 100 mM NaCl; spermidine treatment restored carotenoids to $0.28 \mu\text{g}\cdot\text{g}^{-1}$ FW ($p < 0.05$ vs 100 mM NaCl). At 200 mM NaCl, carotenoid levels were $0.205 \mu\text{g}\cdot\text{g}^{-1}$ FW and spermidine had no significant effect ($0.215 \mu\text{g}\cdot\text{g}^{-1}$ FW; $p > 0.05$) (Fig. 1)

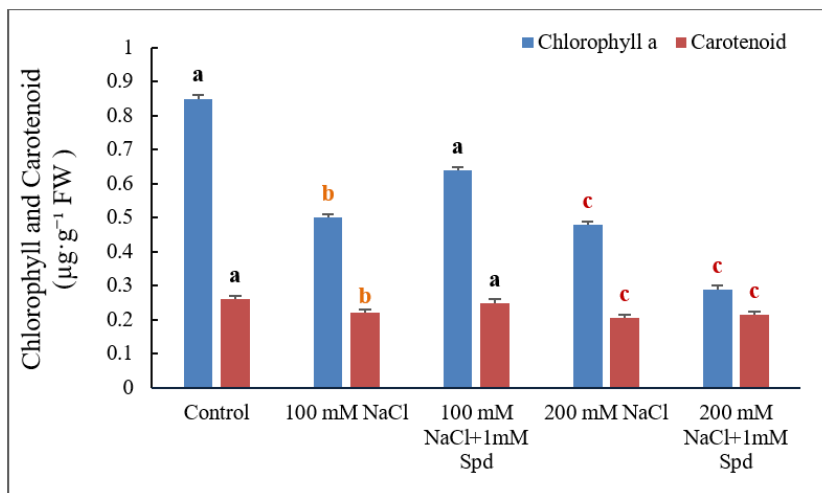


Fig. 1. Effect of 1 mM spermidine on chlorophyll a and carotenoid contents of *Nicotiana rustica* under NaCl stress. Values represent means \pm SD ($n = 3$). Different letters labelling each bar (with same color) indicate significant differences according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

Effects of salt stress and spermidine on biomass composition.

Salinity stress significantly decreased the biomass of *N. rustica* (Two-way ANOVA, $p < 0.001$). Exposure to 100 mM NaCl reduced leaf and root fresh weight by 39% and 48%, respectively, compared to the control ($p < 0.001$). The application of 1 mM spermidine (Spd)

significantly mitigated this reduction ($p < 0.01$ vs. 100 mM NaCl), restoring leaf and root fresh weights to 93% and 98% of control values, respectively. A similar protective effect was observed for dry matter accumulation; Spd application significantly improved leaf and root dry weights to approximately 91% and 98% of the control ($p < 0.05$).

In contrast, under severe salt stress (200 mM NaCl), the protective effect of Spd was limited and not statistically significant ($p > 0.05$): leaf

fresh weight increased modestly from 44% to 55% of control, and root fresh weight from 37% to 55% (Fig. 2).

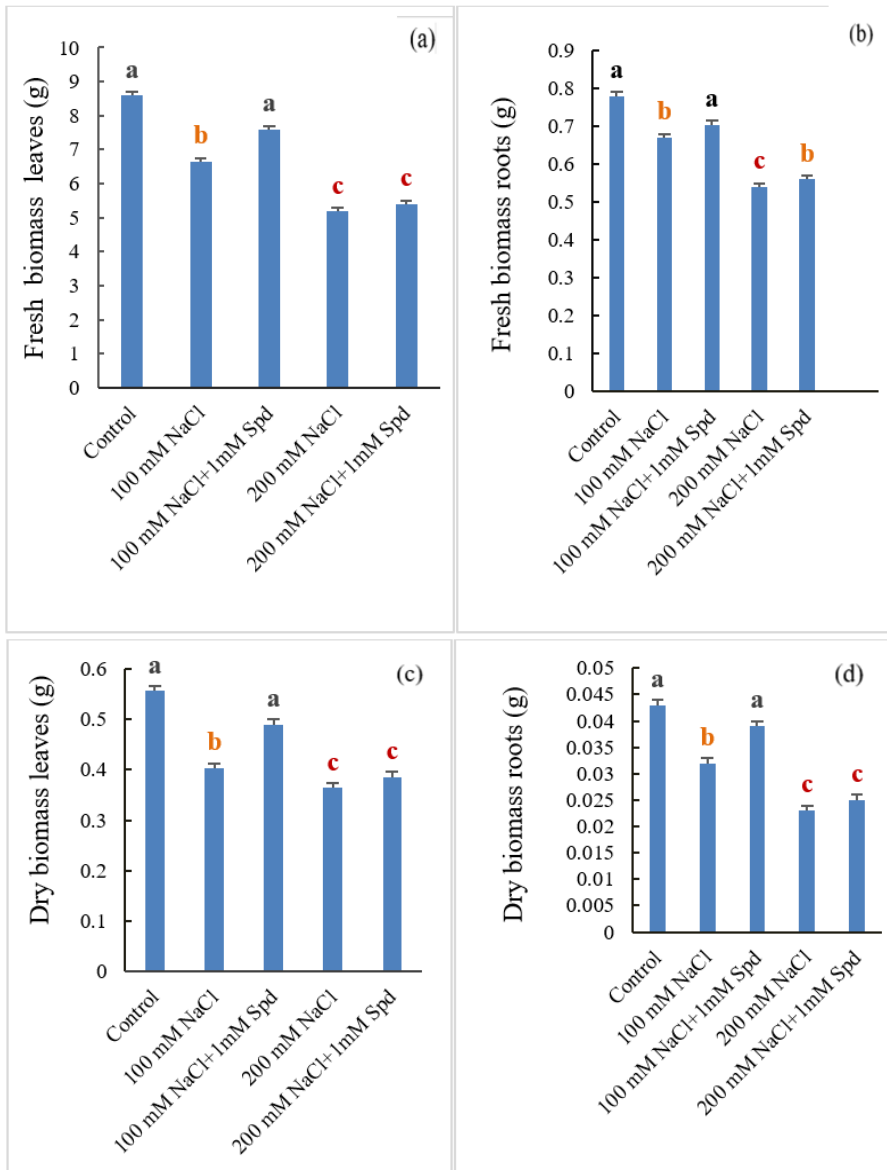


Fig. 2. Effect of 1 mM spermidine on fresh (a, b) and dry biomass (c, d) production in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means \pm SD (n = 3).

Effect of spermidine and salt stress on proline content.

Proline content showed a highly significant response to salt stress and spermidine treatment with tissue-specific differences (Two-way ANOVA, $p < 0.001$). In leaves, 100 mM NaCl induced a 13-fold proline accumulation, rising from 0.07 mg·g⁻¹ FW in controls to 0.982 mg·g⁻¹ FW ($p < 0.001$). Spermidine application at this salinity reduced leaf proline by 8% to 0.906 mg·g⁻¹ FW ($p < 0.05$ vs. 100 mM NaCl). In contrast, root

proline decreased by 87% under 100 mM NaCl to 0.099 mg·g⁻¹ FW ($p < 0.001$ vs. control), with spermidine having no significant effect (0.09 mg·g⁻¹ FW; $p > 0.05$). At 200 mM NaCl, leaf proline remained elevated (0.22 mg·g⁻¹ FW, a 3.1-fold increase; $p < 0.001$), and spermidine treatment caused a non-significant decrease to 0.21 mg·g⁻¹ FW ($p > 0.05$). Root proline at 200 mM NaCl returned to control levels (0.22 mg·g⁻¹ FW; $p > 0.05$), unaffected by spermidine (Fig. 3)

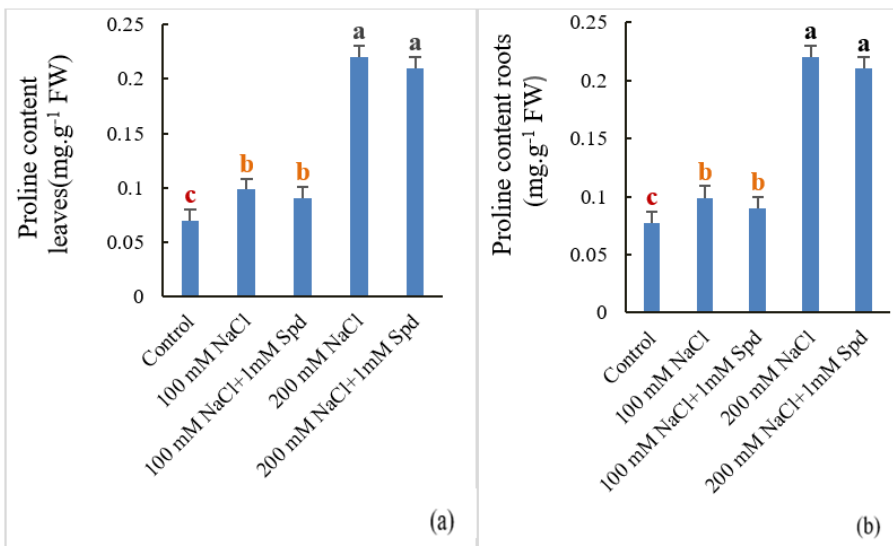


Fig. 3. Effect of 1 mM spermidine on proline content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means \pm SD (n = 3).

Effect of spermidine and salt stress on soluble sugar content in leaves and roots.

Salt stress significantly elevated soluble sugar accumulation in *N. rustica* ($p < 0.05$). In leaves, sugar content increased from 84 $\mu\text{mol}\cdot\text{g}^{-1}$ FW in controls to 93 $\mu\text{mol}\cdot\text{g}^{-1}$ FW under 100 mM NaCl (not statistically significant, $p > 0.05$) and further to 139 $\mu\text{mol}\cdot\text{g}^{-1}$ FW under 200 mM

NaCl ($p < 0.01$). Roots exhibited a more pronounced response, with sugar concentration rising from 12 $\mu\text{mol}\cdot\text{g}^{-1}$ FW in controls to 135 and 156 $\mu\text{mol}\cdot\text{g}^{-1}$ FW under 100 and 200 mM NaCl, respectively ($p < 0.001$ for both). Application of 1 mM spermidine substantially mitigated sugar accumulation at moderate salinity (100 mM NaCl), reducing sugar levels to 81 $\mu\text{mol}\cdot\text{g}^{-1}$ FW in leaves (not significantly

different from controls, $p > 0.05$) and $106 \mu\text{mol}\cdot\text{g}^{-1}$ FW in roots ($p < 0.05$ compared to 100 mM NaCl alone). Conversely, under severe salinity (200 mM NaCl), spermidine displayed limited efficacy,

only slightly reducing sugar content to $135 \mu\text{mol}\cdot\text{g}^{-1}$ FW in leaves and $151 \mu\text{mol}\cdot\text{g}^{-1}$ FW in roots, with no significant difference relative to untreated stressed plants ($p > 0.05$) (Fig. 4)

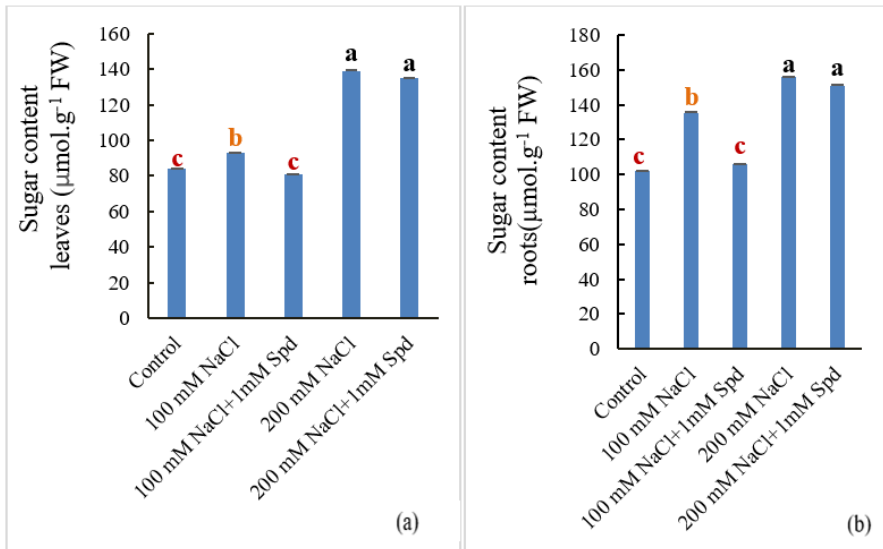


Fig. 4. Effect of spermidine (1 mM) on soluble sugar content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means \pm SD (n = 3).

Effect of spermidine and salt stress on soluble protein content in leaves and roots.

Salt stress induced a pronounced and statistically significant reduction in total soluble protein content in *N. rustica* ($p < 0.001$). In leaves, protein concentration decreased markedly from $2.82 \text{ mg}\cdot\text{g}^{-1}$ FW in control plants to $0.85 \text{ mg}\cdot\text{g}^{-1}$ FW and $0.36 \text{ mg}\cdot\text{g}^{-1}$ FW under 100 mM and 200 mM NaCl treatments, respectively ($p < 0.001$). A similar decline was observed in roots, where protein content dropped from $2.42 \text{ mg}\cdot\text{g}^{-1}$ FW in controls to $0.74 \text{ mg}\cdot\text{g}^{-1}$ FW and $0.35 \text{ mg}\cdot\text{g}^{-1}$ FW under moderate and severe

salinity stress, respectively ($p < 0.001$). Application of 1 mM spermidine significantly alleviated protein degradation at 100 mM NaCl , restoring leaf protein levels to $2.60 \text{ mg}\cdot\text{g}^{-1}$ FW and root protein to $2.49 \text{ mg}\cdot\text{g}^{-1}$ FW, both statistically comparable to controls ($p > 0.05$). However, under severe salt stress (200 mM NaCl), spermidine's protective effect was minimal, with protein contents remaining low at $0.53 \text{ mg}\cdot\text{g}^{-1}$ FW in leaves and $0.52 \text{ mg}\cdot\text{g}^{-1}$ FW in roots, showing no significant improvement compared to untreated stressed plants ($p > 0.05$). (Fig. 5).

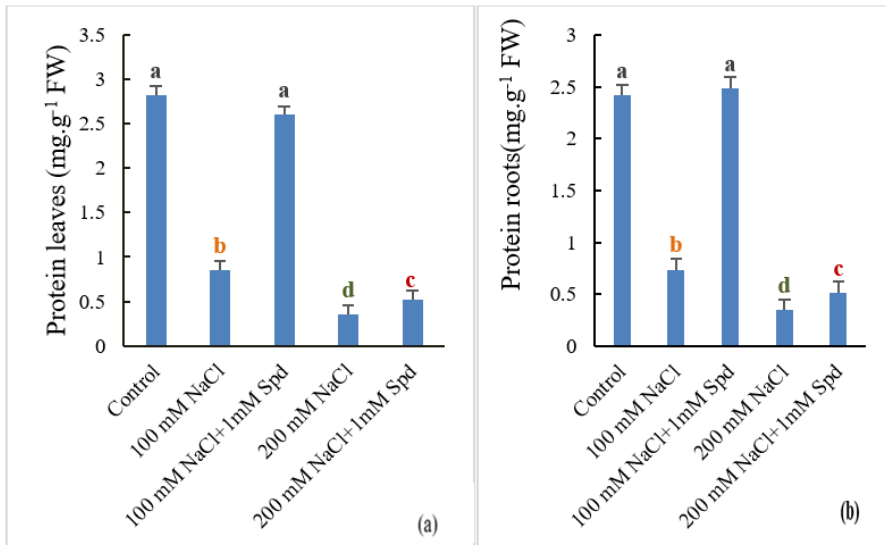


Fig. 5. Effect of spermidine (1 mM) on protein content in leaves and roots of *Nicotiana rustica* under NaCl stress. Values represent means \pm SD (n = 3).

Effect of spermidine and salt stress on ion content (Na^+ , K^+ , Cl^- , NO_3^-) in leaves and roots.

Salt stress significantly perturbed ion homeostasis in *N. rustica* ($p < 0.05$), while exogenous spermidine (Spd) application exerted a salinity-dependent protective effect. Under moderate salt stress (100 mM NaCl), Spd markedly alleviated ionic imbalances. In particular, Spd reduced the accumulation of toxic ions in leaves, lowering Na^+ content from 8598 to 693 $\mu\text{mol}\cdot\text{g}^{-1}$ DW and Cl^- content from 8425 to 825 $\mu\text{mol}\cdot\text{g}^{-1}$ DW ($p < 0.05$). Moreover, Spd contributed to the maintenance of essential ions: root K^+

content was preserved at levels comparable to the control (5763 vs. 6215 $\mu\text{mol}\cdot\text{g}^{-1}$ DW), and leaf NO_3^- concentration was fully restored to 301.23 $\mu\text{mol}\cdot\text{g}^{-1}$ DW, which was statistically indistinguishable from the control (329.35 $\mu\text{mol}\cdot\text{g}^{-1}$ DW; $p > 0.05$).

Conversely, under severe salt stress (200 mM NaCl), the protective effect of Spd was negligible. Na^+ and Cl^- levels remained elevated (e.g., root Na^+ : 14068 $\mu\text{mol}\cdot\text{g}^{-1}$ DW with Spd vs. 14060 $\mu\text{mol}\cdot\text{g}^{-1}$ DW without Spd; $p > 0.05$), while K^+ and NO_3^- concentrations exhibited no significant improvement ($p > 0.05$) (Table 1).

Table 1. Effect of spermidine (Spd) application on ion contents (Na⁺, Cl⁻, K⁺, and NO₃⁻, expressed in $\mu\text{mol g}^{-1}$ DW) in leaves and roots of *Nicotiana rustica* under salt stress

Ion	Plant organs	Control	100 mM NaCl	200 mM NaCl	100 mM NaCl + 1 mM Spd	200 mM NaCl + 1 mM Spd
K ⁺	Leaves	9654±36,769a	5864±170,41c	4213±63,639d	8954±72,831b	4542±219,203d
	Roots	3214±0,707c	2305±205,768d	6215±137,17a	5763±24,416b	3423±50,028c
Na ⁺	Leaves	–	8598±1,41b	16179±26,21a	693± 27,18c	16695±87,45a
	Roots	–	6541±3,54b	14060±36,68 a	795±22,32c	14068±45
NO ₃ ⁻	Leaves	329,35±6,84a	195,695±11,79b	132,54±0,70c	301,23±11,79a	139,32±21,27c
	Roots	520,32±10,60a	386,36±12,72b	253,24±21,92c	510,65±11,31a	235,36±6,15c
Cl ⁻	Leaves	–	8425±18,38b	12956±493,560a	825±21,920 c	11672±309,00a
	Roots	–	5324±86,974b	8654±14,007a	562±17,483c	8745±29,813a

Values represent means ± SD (n = 3). Different letters within each row indicate significant differences according to one-way ANOVA followed by Tukey's test ($p < 0.05$).

DISCUSSION

This study demonstrates that spermidine (Spd) significantly alleviates the adverse effects of salt stress in *N. rustica*, albeit dependent on stress severity. At moderate salinity (100 mM NaCl), Spd enhanced physiological performance, while at higher salinity (200 mM NaCl), its protective effects were largely ineffective. This aligns with the established role of polyamines as modulators of plant stress responses, with efficacy influenced by stress intensity and duration (ElSayed et al. 2022, Saha et al. 2015).

Salt stress notably reduced chlorophyll a and carotenoid contents, consistent with oxidative damage to thylakoid membranes caused by ionic toxicity (Wang et al. 2024). Spd's partial restoration of pigment levels under moderate stress indicates its role in stabilizing chloroplast membranes and photosystem II complexes, potentially through binding to negatively charged phospholipids and protecting chlorophyll-binding proteins from oxidative degradation (Wang et al. 2024, Yaakoubi

et al. 2014). This stabilization likely supported increased photosynthetic efficiency and near-complete recovery of biomass at 100 mM NaCl, consistent with reports in cucumber and tomato under similar conditions (ElSayed et al., 2022). Under severe stress (200 mM NaCl), pigments declined drastically despite Spd application, reflecting irreversible chloroplast damage and impaired pigment biosynthesis pathways (Wang et al., 2024) (Figs. 1, 2).

Notably, the recovery of dry weight consistently exceeded that of fresh weight (e.g., root dry weight improved by 18%), suggesting Spd enhanced carbon assimilation and biomass formation more than water retention under high salinity. This is consistent with findings in other species, where polyamines were shown to significantly improve photosynthetic carbon fixation, chlorophyll biosynthesis, and dry matter accumulation under saline conditions (Hossain, et al., 2025).

Osmotic adjustment, a critical tolerance mechanism, involves proline and soluble sugars accumulation to maintain

cellular turgor (Hmidi et al. 2018, Khare et al. 2022). In *N. rustica*, proline levels increased in leaves but decreased in roots under moderate salt stress, indicating tissue-specific osmotic responses. Spd marginally reduced leaf proline accumulation, implying alleviation of osmotic and oxidative stress and thus reduced osmolyte demand. Roots accumulated soluble sugars under stress, with Spd suppressing excessive sugar buildup, suggesting improved carbon allocation toward growth rather than osmolyte storage (ElSayed et al. 2022, Khare et al. 2018). These findings suggest that spermidine contributes to osmotic adjustment by modulating soluble sugar accumulation effectively under moderate salt stress but has reduced protective capacity at higher salinity levels. However, Spd's modulation of osmolytes failed under severe salt stress, demonstrating compromised osmotic adjustment capacity (Hmidi et al. 2018) (Figs. 3, 4)

A significant protective effect of Spd was observed on soluble protein content under moderate salinity. Salt stress degraded proteins by impairing synthesis and increasing proteolysis, but Spd restored protein levels close to controls, likely by enhancing ribosome stability and reducing protease activity (ElSayed et al. 2022, Hossain et al. 2025). These findings indicate that spermidine effectively preserves protein synthesis and stability under moderate salinity but loses efficacy under severe ionic stress. This protein preservation explains better dry matter recovery compared to fresh weight, marking improved metabolic stability. Under severe salinity, Spd could not prevent protein loss, indicating irreversible damage to translational mechanism (ElSayed et al. 2022) (Fig. 5)

Ionic homeostasis was also better maintained with Spd at moderate salt

stress, through reduced Na^+ and Cl^- accumulation and sustained K^+ and NO_3^- uptake (Pottosin et al. 2014, Saha et al. 2015 ;). Polyamines regulate ion transport by modulating H^+ -ATPase and Na^+/H^+ antiporter activities, reducing toxic ion influx and promoting selective ion uptake (Saha et al. 2015). This preservation of favorable K^+/Na^+ ratios and nutrient uptake support photosynthetic and protein synthesis processes. Under severe stress, Spd failed to mitigate ion imbalances, reflecting limited protective capacity against ionic toxicity (Pottosin et al. 2014). All Spd effects are summarized in Fig. 6.

The results in table 1 demonstrate that Spd effectively modulates ion transport and alleviates ionic toxicity under moderate salinity; however, its protective mechanisms are insufficient to counteract the ionic stress imposed by severe salt conditions (Saha et al., 2015).

This study conclusively demonstrates that exogenous spermidine enhances salt tolerance in *N. rustica* under moderate salinity conditions (100 mM NaCl), a concentration relevant to real-world irrigation practices such as those in Tunisian coastal aquifers. The protective effects of spermidine arise from a complex interplay of mechanisms, including the preservation of photosynthetic efficiency via antioxidant activity, improved carbon assimilation and biomass allocation, restoration of osmotic balance by reducing stress-induced osmolyte accumulation, and critical maintenance of selective ion homeostasis that minimizes Na^+ and Cl^- toxicity while sustaining essential nutrient uptake. However, this protective capacity significantly declines at higher salinity levels (200 mM NaCl) due to the disruption of ionic regulation, establishing a threshold beyond which spermidine alone is insufficient. These findings underscore the potential of spermidine as a priming agent for crops facing moderate

salinity stress and point to the need for future research into combinatory treatments involving compatible solutes, phytohormones, or nutrient supplements to extend tolerance to more severe salt stress

conditions. Such integrative approaches could enhance the practical application of spermidine in agricultural systems affected by salinity.

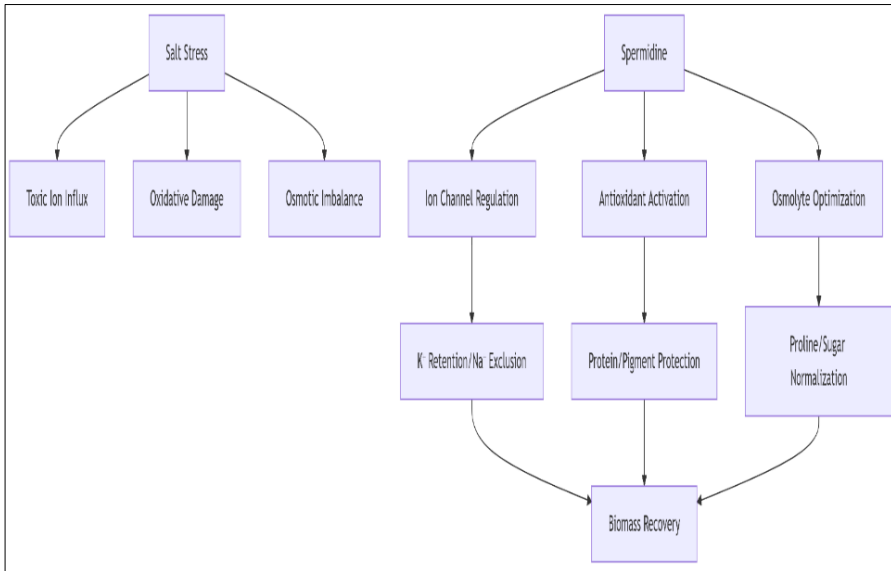


Fig. 6. Mechanisms of spermidine-mediated salt stress tolerance in *Nicotiana rustica*: From ion homeostasis to physiological recovery.

RESUME

Essid I. M'rah S. et Chaffei-Haouari Ch. 2025. La spermidine exogène améliore la tolérance au sel chez *Nicotiana rustica*: Mécanismes physiologiques et implications pour la phytoremédiation. *Tunisian Journal of Plant Protection* 20 (2): 29-41.

Le stress salin constitue l'un des principaux facteurs abiotiques limitant la croissance et la productivité des plantes, en perturbant la photosynthèse, le métabolisme des protéines, l'équilibre osmotique et l'homéostasie ionique. Cette étude a évalué l'effet de l'application exogène de spermine (Spd, 1 mM) sur *Nicotiana rustica* soumis à une salinité modérée (100 mM NaCl) et sévère (200 mM NaCl). Le stress salin a entraîné une réduction significative de la chlorophylle a, des caroténoïdes, de la biomasse et des protéines solubles, tout en induisant une accumulation de proline et de sucres solubles de manière spécifique aux tissus, ainsi qu'une perturbation des rapports K^+/Na^+ et NO_3^-/Cl^- . L'application de Spd sous salinité modérée a significativement atténué ces effets, en restaurant les niveaux de la chlorophylle a et des protéines, en maintenant la production de biomasse, en modulant l'accumulation des osmolytes et en réduisant la toxicité du Na^+ et du Cl^- tout en préservant l'absorption de K^+ et de NO_3^- . En revanche, sous salinité sévère, l'effet protecteur de Spd s'est révélé limité et largement inefficace. Ces résultats suggèrent que la spermidine améliore la tolérance au stress salin modéré principalement en stabilisant la photosynthèse, en préservant le métabolisme des protéines et en régulant l'homéostasie ionique, mais demeure insuffisante pour contrer les dommages induits par une salinité extrême.

الملخص

الصيد، إشراق وصباح مراح وشراز الشافعي-الهوري. 2025. تحسين تحمل نباتات *Nicotiana rustica* للملوحة بواسطة السبيرميدين الخارجي: الآليات الفسيولوجية وانعكاساتها على المعالجة النباتية.

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تُعد ملوحة التربة من أهم القيود البيئية التي تحد من إنتاجية المحاصيل، خاصة في المناطق الجافة وشبه الجافة. ويُعد الإجهاد الملحي أحد أهم العوامل اللاأحيائية التي تحد من نمو النباتات وإنتاجيتها، حيث يؤدي ذلك إلى اختلال عملية التمثيل الضوئي، واضطراب أيض البروتينات، واختلال التوازن الأسموزي والأيوني. هدفت هذه الدراسة إلى تقييم تأثير المعاملة الخارجية لمادة السبيرميدين بتركيز 1 ملليمول على نبات *Nicotiana rustica* المعرض لملوحة معتدلة (100 ملليمول NaCl) وملوحة شديدة (200 ملليمول NaCl). أظهر الإجهاد الملحي انخفاضاً ملحوظاً في الكلوروفيل a والكاروتينات والكتلة الحيوية والبروتينات الذاتية، بالتوازي مع زيادة تراكم البرولين والسكريات الذاتية بـصور خاصة في الأنسجة، بالإضافة إلى اضطراب في نسبة توازن K^+/Na^+ و NO_3^-/Cl^- . ساعدت المعاملة بالسبيرميدين تحت الملوحة المعتدلة على التخفيف من هذه التأثيرات، حيث استعاد النبات مستويات الكلوروفيل a والبروتينات، وحافظ على النمو والكتلة الحيوية، وعدل تراكم المواد الأسموزية، وخفض من سمية Na^+ و Cl^- مع الحفاظ على امتصاص K^+ و NO_3^- . في المقابل، كان تأثيره محدوداً وغير فعال تحت الملوحة الشديدة. تشير هذه النتائج إلى أن السبيرميدين يعزز من قدرة النبات على تحمل الملوحة المعتدلة عبر تثبيت عملية التمثيل الضوئي والحفاظ على أيض البروتين وتنظيم التوازن الأيوني، إلا أنه غير كافٍ للتغلب على الضرر الناجم عن الملوحة القصوى.

كلمات مفتاحية: إجهاد ملحي، بوليأمينات، توازن الأيونات، سبيرميدين، معالجة نباتية، مواد أسموزية، *Nicotiana rustica*

LITERATURE CITED

- Bates, L.S., Waldren, R.P., and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39: 205-207.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.
- Chapman, H.D., and Pratt, P.F. 1961. *Methods of Analysis for Soils, Plants and Waters*. University of California, Division of Agricultural Sciences, California, USA, 309 pp.
- ElSayed, A.I., Rafudeen, M.S., and Golladack, D. 2022. Polyamines: Bioactive molecules with stress-protective roles in plants under salinity stress. *Environmental and Experimental Botany* 205: 105106. DOI: 10.1016/j.sjbs.2022.02.053
- Epstein, E., and Bloom, A.J. 2005. *Mineral Nutrition of Plants: Principles and Perspectives*, 2nd Edition. Sinauer Associates, Massachusetts, USA, 400 pp.
- Flowers, T.J., and Colmer, T.D. 2008. Salinity tolerance in halophytes. *New Phytologist* 179: 945-963. <https://doi.org/10.1111/j.1469-8137.2008.02531.x>
- Gill, S.S., and Tuteja, N. 2010. Polyamines and abiotic stress tolerance in plants. *Plant Signaling and Behavior* 5: 26-33. <https://doi.org/10.4161/psb.5.1.10291>
- Groppa, M.D., and Benavides, M.P. 2008. Polyamines and abiotic stress: Recent advances. *Amino Acids* 34: 35-45. <https://doi.org/10.1007/s00726-007-0501-8>
- Henriksen, A., and Selmer-Olsen, A.R. 1970. Automatic methods for determining nitrate and nitrite in water and soil extracts. *Analyst* 95: 514-518. <https://doi.org/10.1039/AN9709500514>
- Hmidi, D., Ben Amar, R., and Bouslama, M. 2018. Effect of salinity on osmotic adjustment, proline and soluble sugar contents in plants. *Plant Science Today* 5: 20-29.
- Hoagland, D.R., and Arnon, D.I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular 347*, University of California, Berkeley, California, USA, 32 pp.
- Hossain, M.M., Kordrostami, M., Goto, F., and Rahimi, M. 2025. Spermine treatment improves salinity tolerance in *Plantago major* by altering growth parameters, biochemical profiles and gene expression. *Scientific Reports* 15:

25762. <https://doi.org/10.1038/s41598-025-11903-0>
- Jones, J.B. 2001. *Laboratory Guide for Conducting Soil Tests and Plant Analysis*. CRC Press, Boca Raton, Florida, USA, 363 pp.
- Kabir, M.A., Hossain, M.K., Hossain, M.A., Molla, M.O.F., Khatun, M.S., and Mostofa Jamal, M.A.H. 2024. Impact of water and soil salinity on coastal agriculture in Bangladesh: Insights and mitigation strategies. *American Journal of Multidisciplinary Research and Innovation* 3: 36-48.
- Khare, T., Srivastav, A., Shaikh, S., and Kumar, V. 2018. Polyamines and their metabolic engineering for plant salinity stress tolerance. Pages 339-358. In: *Salinity Responses and Tolerance in Plants, Volume 1: Targeting Sensory, Transport and Signaling Mechanisms*. Springer International Publishing, Cham.
- Lichtenthaler, H.K., and Wellburn, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11: 591-592. <https://doi.org/10.1042/bst0110591>
- McCready, R.M., Guggolz, J., Silveira, V., and Owens, H.S. 1950. Determination of starch and amylose in vegetables. *Analytical Chemistry* 22: 1156-1158.
- Munns, R., and Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* 59: 651-681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>.
- Pottosin, I., and Shabala, S. 2014. Polyamines control of cation transport across plant membranes: Implications for ion homeostasis and abiotic stress signaling. *Frontiers in Plant Science* 5: 154. <https://doi.org/10.3389/fpls.2014.00154>
- Raziq, A., Mohi Ud Din, A., Anwar, S., Wang, Y., Jahan, M.S., He, M., Ling, C.G., Sun, J., Shu, S., and Guo, S. 2022. Exogenous spermidine modulates polyamine metabolism and improves stress responsive mechanisms to protect tomato seedlings against salt stress. *Plant Physiology and Biochemistry* 187: 1-10.
- Saha, J., Brauer, E.K., Sengupta, A., Popescu, S.C., Gupta, K., and Gupta, B. 2015. Polyamines as redox homeostasis regulators during salt stress in plants. *Frontiers in Environmental Science* 3: 21. <https://doi.org/10.3389/fenvs.2015.00021>
- Shabala, S., and Cuin, T.A. 2008. Potassium transport and plant salt tolerance. *Physiologia Plantarum* 133: 651-669. <https://doi.org/10.1111/j.1399-3054.2007.01008.x>
- Staub, A.M. 1963. Extraction and estimation of plant sugars. Pages 51-54. In: *Methods in Enzymology*, Vol. 6. S.P. Colowick and N.O. Kaplan, Eds. Academic Press, New York, USA.
- Wang, X., An, T., Hu, X., and Yu, Z. 2024. Sensitivity and responses of chloroplasts to salt stress: oxidative and ionic stress effects. *Frontiers in Plant Science* 15: 1374086. <https://doi.org/10.3389/fpls.2024.1374086>
- Yaakoubi, K., Da Silva, D., and Amiar, Z. 2014. Protective action of spermine and spermidine against oxidative damage in plant photosystems. *PLoS ONE* 9 (11): e112893. <https://doi.org/10.1371/journal.pone.0112893>
- Yemm, E.W., and Willis, A.J. 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal* 57: 508-514. <https://doi.org/10.1042/bj0570508>
- Zhou, F.G., Chen, B., and Yang, Z.M. 2005. Polyamines regulate H⁺-ATPase activity under salt stress. *Plant Physiology* 144: 1064-1074.
- Zhu, J.K. 2016. Abiotic stress signaling and responses in plants. *Cell* 167: 313-324. <https://doi.org/10.1016/j.cell.2016.08.029>

