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Serendipita indica improve seed germination and seedling growth of *Lolium multiflorum* Lam. through amelioration of osmotic adjustment, nutrient accumulation and Na^+/K^+ homoeostasis under salinity conditions

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Abstract: A pot experiment was carried out to determine the effect of *Serendipita indica* on the salt response of *Lolium multiflorum* Lam. Although the salinity decreased the root colonisation of *S. indica* by 28.34%, successful colonisation of *S. indica* increased the seed germination rate, fresh weight, leaf relative water content and chlorophyll content by 28.09, 59.01, 38.78 and 28.80%, respectively, compared with uncolonised seedlings. Under salinity, leaf malondialdehyde content, leaf relative electrical conductivity, as well as Na^+ content and Na^+/K^+ ratio in leaves and roots of *S. indica*-colonised seedlings were decreased by 33.99, 33.31, 63.40% and 47.42, 85.66 and 55.88%, respectively, compared with uncolonised seedlings. Meanwhile, compared with uncolonised seedlings under salinity, the contents of proline in leaves, N, P and K^+ in leaves and roots of the *S. indica*-colonised seedlings were increased by 47.47, 45.69 and 30.05%, and 41.77, 19.51, 19.18 and 155.00%, respectively. These results indicate that *S. indica* colonisation confers salt tolerance in *L. multiflorum* seedlings by enhancing osmotic adjustment *via* actively accumulating proline and K^+, increasing the uptake of nutrients such as N and P, and improving Na^+/K^+ homoeostasis. The study would provide a new idea for the combined application of salt-tolerant plants and symbiotic microorganisms in the ecological restoration of saline-alkali lands.

Keywords: salt stress; growth performance; osmoregulation substances; ionic homeostasis; nutrients uptake

Soil salinity is one of the major abiotic factors threatening sustainable agriculture and food security worldwide (Mukhopadhyay et al. 2021). Excessive accumulation of mineral salts in the soil causes ion imbalance, disturbs the absorption of nutrients, and leads to osmotic stress, nutritional deficiency, ion toxicity and oxidative damage to plants. Meanwhile, salinity toxicity triggers a wide variety of plant responses, including morphological, physiological, biochemical and molecular changes, which eventually affect seed germination, seedling growth and establishment.

Various approaches are being applied to mitigate salt stress, including developing salt-resistant cultivars, flushing of soils, leaching of unnecessary soluble salts from the upper soil to the deep soil, reducing

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salt by harvesting salt-accumulating aerial plant parts, and etc. (Tarolli et al. 2024). The application of plant rhizosphere growth-promoting microorganisms (PGPM) has been well recognised as a safer and sustainable approach for plant growth promotion and production in modern agriculture (Gupta et al. 2022, Díaz-Urbano et al. 2023). Plant growth promotion and alleviation of stresses derived from plant-PGPM interactions were mainly attributed to the well-development root system of plants, the regulation of biosynthesis of plant growth-promoting compounds, the enhanced nutrient availability, the improved water relation, the regulation of ion homeostasis in plants, the accumulation of osmolyte, the up-regulation of antioxidative enzymes, and the activation of systemic resistance (Goswami and Deka 2020, Liu et al. 2022, Sharaya et al. 2023).

Serendipita indica is a mycorrhiza-like root endophytic fungus with a wide range of host plants. Previous studies proved that *S. indica* can improve the host plant's tolerance or resistance to adversities including biotic and abiotic stresses (Boorboori and Zhang 2022, Saleem et al. 2022, Wan et al. 2024). It is predicted to have great potential for application in modern agriculture under the condition of global environmental change. However, its application in the ecological restoration of saline-alkali land is less. *Lolium multiflorum* Lam. is widely used as a vegetative cover to improve the ecological restoration in the Yellow River delta region, Shandong, China. Considering the beneficial effects of both, we propose that the combined application of *S. indica* and *L. multiflorum* may play a greater role in the ecological restoration of the saline-alkali lands. Based on the previous research during which the well establishment of mutualistic symbiosis between *L. multiflorum* and *S. indica* and its favourable effect on the survival and growth of the seedlings have been observed (Liu et al. 2019), the present experiment was carried out to investigate the effect of seed-priming with the spore suspensions of *S. indica* on the seed germination, the morphological and physiological performance of *L. multiflorum* seedling under NaCl-induced salt stress condition.

The present research aimed to explore the potential effect of *S. indica* application on the improvements of *L. multiflorum* in seed germination and seedling growth by stimulating osmoregulation, improving the availability of nitrogen and phosphorus, and regulating the balance of sodium and potassium ions under salinity conditions. The results of our study would provide a new insight into understanding the host plants' salt resistance or tolerance mechanism induced by *S. indica* and a new idea for the combined application of salt-tolerant plants and symbiotic microorganisms in the ecological restoration of saline-alkali lands.

MATERIAL AND METHODS

Plant material and fungus strain. Seeds of *L. multiflorum* and *S. indica* (Preservation No. CGMCC3.17686) were obtained from Xinrui Seed Industry Limited Company (Jiangsu, China) and China General Microbiological Culture Collection Center (Beijing, China), respectively.

Experimental design and treatments. The present experiment was a factorial experiment in a completely randomised design with two factors (*S. indica* × NaCl). Fungus was applied at two levels, including *S. indica*-uninoculation (control) and *S. indica*-inoculation. NaCl was applied at two levels, including NaCl-absent (control) and NaCl-present with 150 mmol/L.

S. indica inoculation was implemented by seed-priming according to the method of Khademian et al. (2019). The sterilised seeds for *S. indica*-uninoculated (control) and *S. indica*-inoculation treatment were inoculated with 150 mL of sterilised distilled water and resulted in spore suspensions of *S. indica* in a rotary shaker (26 ± 1 °C, 150 rpm, THZ-C-1, Guowang Ltd, Jiangsu, China) for 6 h in dark (according to Liu et al. 2019), respectively. For each treatment, 15 seeds were sown into 250 g sterilised substrate mixed with local topsoil (haplic luvisols): sand: grass peat (3:2:2, *v*:*v*:*v*, pH = 6.57) in a cubic pot (7.0 cm). Six pots for each treatment. All the pots were placed on a shelf in the greenhouse (18–25 °C, 65–80% relative humidity, 36°40'N, 117°00'E).

Two days after sowing, salt treatment was applied at a concentration of 150 mmol/L NaCl (according to our preliminary experiment) in the sterilised deionised water for both inoculated and un-inoculated seedlings. The controls were grown in the absence of NaCl. The experimental treatment began on 6 May and was terminated on 28 May 2022. During the period of our experimental treatment, all seedlings were watered every other day with 50 mL 150 mmol/L NaCl solution or sterilised water and supplied twice a week with 50 mL sterilised 50% Hoagland's solution (pH = 6.5). The solutions are freely drained from the bottom of the pots. All pots were rotated weekly to avoid edge effects.

Determination of seed germination. One week after sowing, the cumulative number of germinated seeds in each pot of different treatments was recorded, and the seed germination rate (GR) was calculated according to the following formula:

$$GR\,(\%) = \frac{NG}{NS} \times 100$$

where: NG and NS – number of seeds germinated and sowed, respectively.

Root colonisation measurement. At the end of the experiment, seedlings of each pot from the four treatments were analysed for root colonisation by ink-vinegar (5%, *v*/*v*) staining according to the method of Liu et al. (2019). Root colonisation rate (RCR, %) was defined as one hundred times the number of root segments colonised with *S. indica* and the total number of root segments observed.

Growth measurement. At the end of the experiment, seedlings under all given treatments were harvested and were divided into shoot and root portions. The length of the shoots and roots of all seedlings in each repeating pot were measured with a ruler (0.01 cm) and were averaged. Total values of shoot fresh weight (SFW), root fresh weight (RFW) and total fresh weight (FW) of seedlings in each repeating pot were measured and divided by the number of seedlings in the corresponding pot.

Determination of leaf relative water content and chlorophyll content. According to the method of Liu et al. (2019), leaf relative water content (RWC) and chlorophyll content (C_{chl}) were determined by the gravity method and spectrophotometer method, respectively. RWC was calculated as follows:

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

where: FW, TW and DW – leaves' fresh weight, turgid weight (after rehydrating leaves for 24 h), and dry weight (after oven-drying for 48 h at 70 °C), respectively.

0.1 g chopped fresh leaf material was used to extract chlorophyll in 20 mL 80% (*v*/*v*) acetone for 72 h in the dark at 4 °C. Absorbances at 647 nm and 664 nm were determined with a UV-/vis spectrophotometer (UV-2401PC, Shimadzu, Japan) and used to calculate C_{chl} (mg/g FW).

Measurement of malondialdehyde content. Malondialdehyde (MDA) was extracted by homogenising 1.0 g fresh leaf in 10 mL of 0.6% (*w*/*v*) thiobarbituric acid in 10% (*v*/*v*) trichloracetic acid. Absorbance at 450, 532, and 600 nm was determined with a UV-/vis spectrophotometer (UV-2401PC, Shimadzu, Japan) and used to calculate MDA content (μmol/g FW).

Measurement of relative electrical conductivity. The electrical conductivity of leaves was measured using conductivity meters (DDS-307A, YOKE, Shanghai, China) and relative electrical conductivity (REC) was calculated as follows:

$$REC(\%) = \frac{EC_1}{EC_2} \times 100$$

where: EC_1 and EC_2 – electrical conductivity of leaves being incubated at room temperature for 12 h and after being boiled for 30 min, respectively.

Measurement of proline content. Proline was extracted by homogenising 0.5 g fresh leaf in 10 mL of 3% (*w*/*v*) aqueous sulfosalicylic acid. Absorbance at 520 nm was determined with a UV-/vis spectrophotometer (UV-2401PC, Shimadzu, Japan) and used to calculate proline content (μmol/g FW).

Element analysis. At the end of the experiment, K^+ and Na^+ contents in the leaves and roots of *L. multiflorum* were determined according to the method described by Hassani et al. (2019) and Khalid et al. (2018). 0.2 g of powder of the dried root or leaf materials was acid digested in a digestion furnace by mixing with 4 mL HNO_3 (97.5%) and 1 mL $HClO_4$ (72.0%) and heated to 220 °C for 20 min. The resulting mixture was extracted with 5 mL HNO_3 and adjusted to the final volume of 250 mL of distilled water. The contents of K^+ and Na^+ were determined by inductively coupled plasma spectrometry (ICP, ThermoFisher, ICAP7600, Waltham, USA).

For determination of N and P content, 0.2 g of powder of the dried root or leaf materials was acid digested in a digestion furnace by mixing with 5 mL H_2SO_4 (98.0%) and gradually heated to 200 °C and kept for 30 min. During the digestion, 5–6 drops of H_2O_2 (30.0%) were added to catalyse the reaction. After cooling down to room temperature, the resulting solution was transferred to a 50 mL volumetric flask and diluted with distilled water to volume. The contents of P and N were determined according to the molybdovanado phosphate method and the micro-Kjeldahl method, respectively. The unit of element contents was expressed as mg/g DW. Element analyses were carried out by the Instrumental Analytical Center of Shenyang Agricultural University (Shenyang, China).

Statistical analysis. SPSS-13.0 (Windows version, SPSS Inc., Chicago, USA) was used for statistical analysis. All values were expressed as the means ± standard deviation. Analyses of two-way variance (ANOVA) were used to evaluate the effects of

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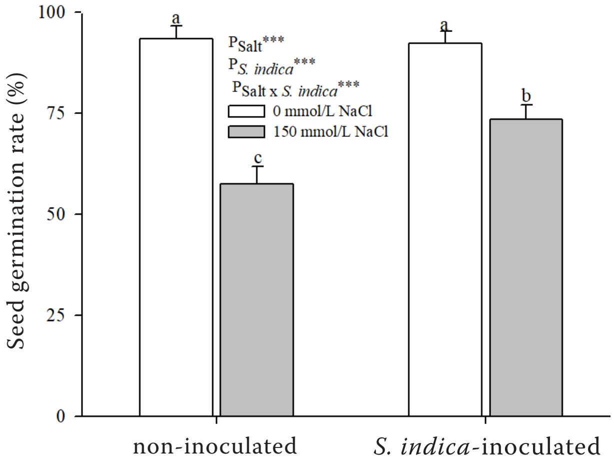


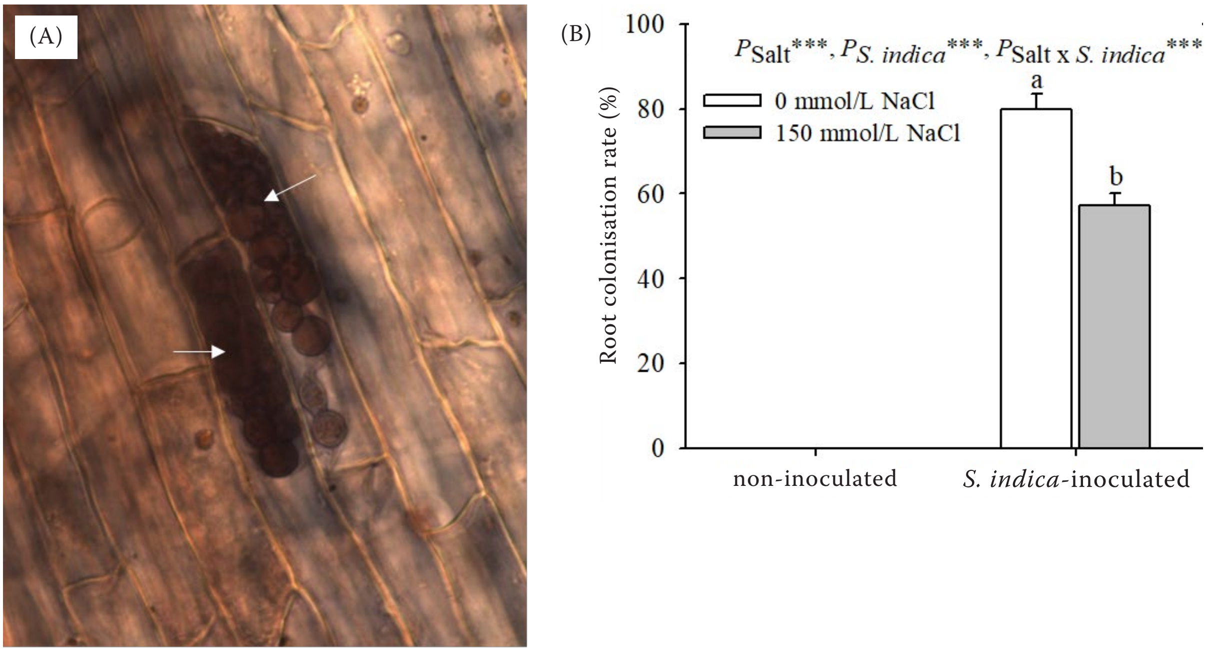
Figure 1. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on seed germination rate of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S.\ indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect; $^{***}P \leq 0.001$

S. indica inoculation and saline stress on seed germination, root colonisation, and morphological and physiological indexes of *L. multiflorum* seedlings. Tukey's honestly significant difference (*HSD*) post hoc test ($P \leq 0.05$) was performed to test the statistical differences for the same parameter among seedlings. Figures were drawn using SigmaPlot 10.0 (Windows version, Systat Software Inc., San Jose, USA).

RESULTS

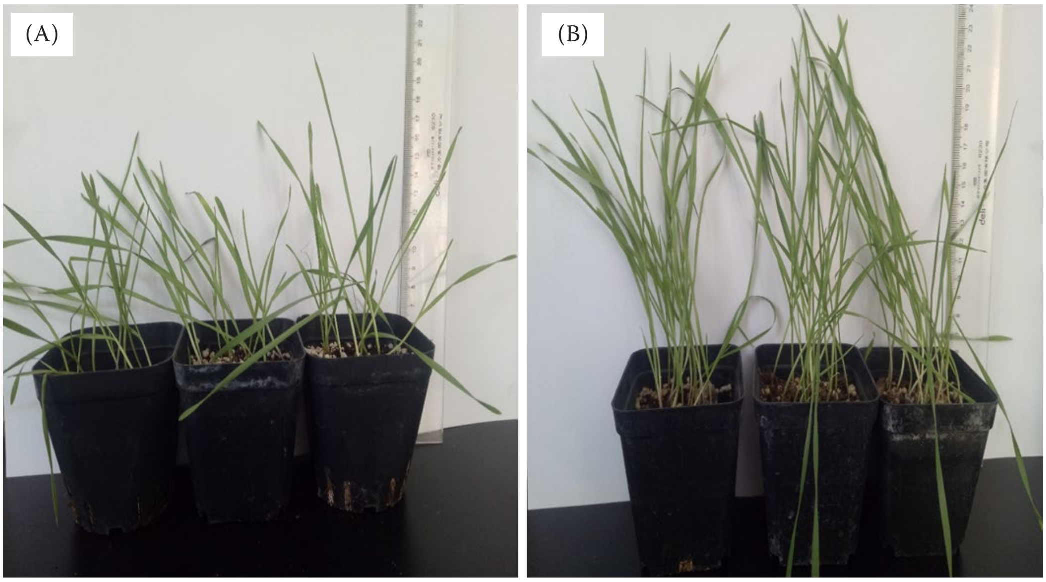
Seed germination. *S. indica* inoculation had no significant effect on GR under non-saline conditions, while it significantly promoted seed germination under salinity conditions (Figure 1). GR of non-inoculated seeds and *S. indica*-inoculated seeds under salinity conditions decreased by 38.55% and 20.37%, respectively, compared with the corresponding seeds under non-salt conditions. Under salinity stress, *S. indica* inoculation increased GR by 28.09% compared with non-inoculated seeds.

Root colonisation. *S. indica* successfully colonised the root system of *L. multiflorum* seedlings, and the chlamydospores were clearly observed intracellularly in the root cortex (Figure 2A). The salt application had a significant influence on the RCR of *L. multiflorum* seedlings. 79.95% of inoculated seedlings were observed to be successfully infected by *S. indica* under NaCl-absent conditions, while the



S. indica chlamydospores in the root cortex

Figure 2. (A) *Serendipita indica* chlamydospores in the root cortex of *Lolium multiflorum* seedlings and (B) the effect of NaCl-induced salinity on root colonisation rate of *L. multiflorum* seedlings. Arrow indicated *S. indica* spores. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S.\ indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect. $^{***}P \leq 0.001$



non-inoculated seedlings *S. indica*-inoculated seedlings

Figure 3. Effect of root colonisation by *Serendipita indica* on seedling growth of *Lolium multiflorum* under salt stress condition

application of 150 mmol/L NaCl decreased RCR by 28.34% (Figure 2B).

Growth. Obviously, the successful colonisation of *S. indica* alleviated the inhibition effect of salinity on the growth of *L. multiflorum* seedlings (Figure 3). Also, the results in Table 1 revealed that *S. indica* inoculation had a positive effect on biomass accumulation and length elongation under both non-saline and saline conditions. Under non-saline conditions, *S. indica* inoculation improved SFW, RFW, FW, SL and RL by 29.00, 23.02, 27.66, 26.59 and 47.31%, respectively, in comparison with the un-inoculated seedlings. Saline application reduced biomass accumulation and length elongation of both non-inoculated and *S. indica*-inoculated seedlings, while non-inoculated seedlings showed a larger decrease in SFW (44.75%), RFW (35.71%), FW (42.91%), and SL (22.57%) than *S. indica*-inoculated ones (SFW, RFW, FW and SL was decreased by 35.56, 4.52, 28.89 and 20.31%, respectively). RL of non-inoculated and *S. indica*-inoculated seedlings was reduced by 18.27% and 13.87%, respectively, under saline conditions.

Table 1. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on growth characteristics of *Lolium multiflorum* seedlings

Inoculation treatment	NaCl concentration (mmol/L)	SFW	RFW	FW	SL	RL
		(g/pot)			(cm)	
Non-inoculated	0	4.38 ± 0.25^{b}	1.26 ± 0.06^{b}	5.64 ± 0.27^{b}	20.65 ± 1.26^{b}	11.33 ± 0.57^{c}
	150	2.42 ± 0.18^{d}	0.81 ± 0.04^{c}	3.22 ± 0.26^{d}	15.99 ± 1.45^{c}	9.26 ± 0.63^{d}
S. indica-inoculated	0	5.65 ± 0.38^{a}	1.55 ± 0.08^{a}	7.20 ± 0.25^{a}	26.14 ± 1.92^{a}	17.02 ± 0.73^{a}
	150	3.64 ± 0.17^{c}	1.48 ± 0.05^{a}	5.12 ± 0.21^{c}	18.83 ± 1.32^{b}	14.66 ± 0.87^{b}
Two-way ANOVA						
P_{salt}		***	***	***	***	***
$P_{S.\ indica}$		***	***	***	***	***
$P_{\text{salt} \times S.\ indica}$		**	*	ns	ns	*

Values are means ± standard deviation of six replicates. Different letters in the same column indicate significant differences among different treatments for the same parameter at $P \leq 0.05$; *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ns – no significant effect; SFW – shoot fresh weight; RFW – root fresh weight; FW – total fresh weight; SL – shoot length; RL – root length

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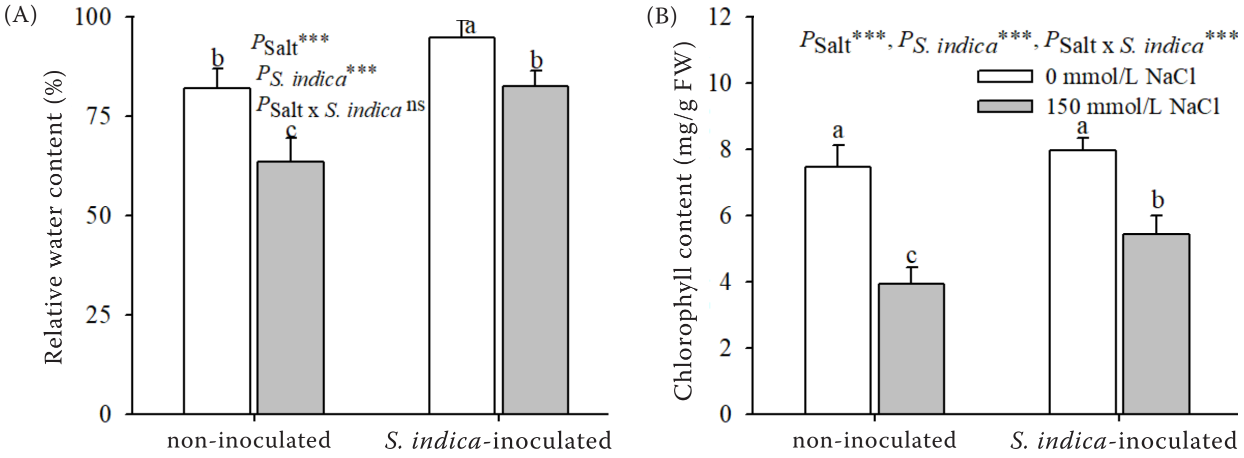

Figure 4. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on (A) leaf relative water content and (B) chlorophyll content of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S. indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect; $^{***}P \leq 0.001$; ns – no significant effect; FW – fresh weight

Leaf relative water content and chlorophyll content. Regardless of colonisation, both RWC and C_{chl} of the observed seedlings were significantly reduced by salinity stress; seedlings without *S. indica* inoculation showed a much larger reduction (RWC and C_{chl} were reduced by 47.52% and 22.36%, respectively) than the *S. indica*-inoculated ones (RWC and C_{chl} were reduced by 31.66% and 12.95%, respectively) (Figure 4). Under non-saline conditions, in comparison with uncolonised seedlings, *S. indica* colonisation significantly increased RWC by 15.81% but had no significant effect on C_{chl}. Under salinity conditions, *S. indica* colonisation significantly increased RWC and C_{chl} by 38.78% and 28.80%, respectively, compared with uncolonised seedlings.

Malondialdehyde content. Salt treatment significantly aggravated lipid peroxidation as manifested by the significant increase of MDA content in *L. multiflorum* seedlings regardless of *S. indica* colonisation (Figure 5A). Compared with seedlings under non-saline conditions, MDA content in the non-inoculated seedlings and *S. indica*-inoculated seedlings induced by salt stress increased by 139.69% and 72.05%, respectively. Under non-saline conditions, *S. indica* inoculation had no significant influence on MDA content, while it significantly decreased

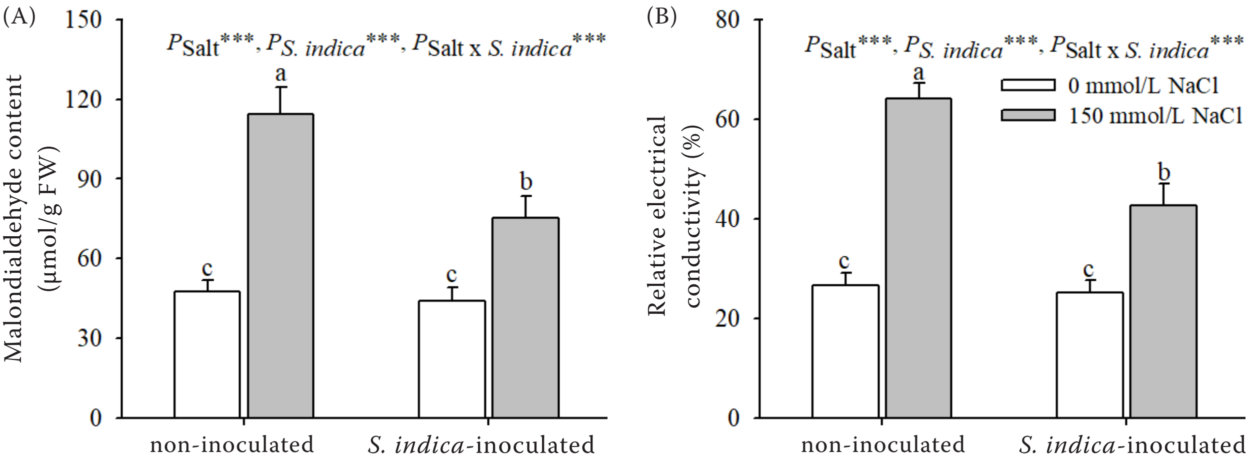

Figure 5. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on (A) malondialdehyde content and (B) relative electrical conductivity of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S. indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect. $^{***}P \leq 0.001$; FW – fresh weight

MDA content by 33.99% compared with uncolonised seedlings under saline conditions.

Relative electrical conductivity. The relative permeability of the membrane in *L. multiflorum* seedlings, regardless of *S. indica* colonisation, was significantly increased by saline application, as manifested by the significant increase of REC (Figure 5B). Compared with seedlings under non-saline conditions, REC of non-inoculated seedlings and *S. indica*-inoculated seedlings under salinity increased by 141.16% and 69.51%, respectively. Under non-saline conditions, no significant difference in REC was observed among non-inoculated and *S. indica*-inoculated seedlings. Differently, *S. indica* inoculation significantly reduced REC by 33.31% compared with uncolonised seedlings under saline conditions.

Proline content. Proline content in leaves of both non-inoculated and *S. indica*-inoculated seedlings was increased under saline conditions compared with

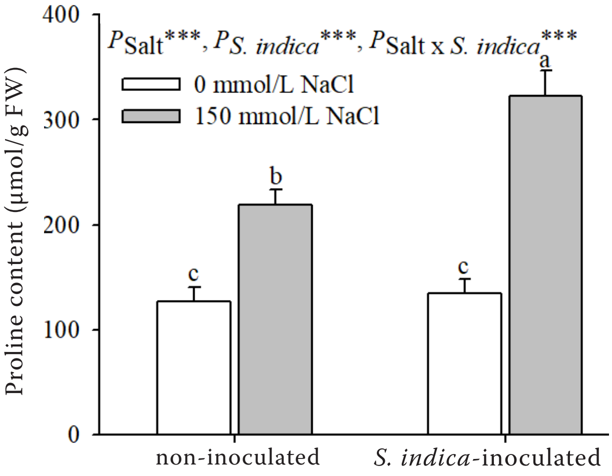

Figure 6. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on proline content of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S.\ indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect. $^{***}P \leq 0.001$; FW – fresh weight

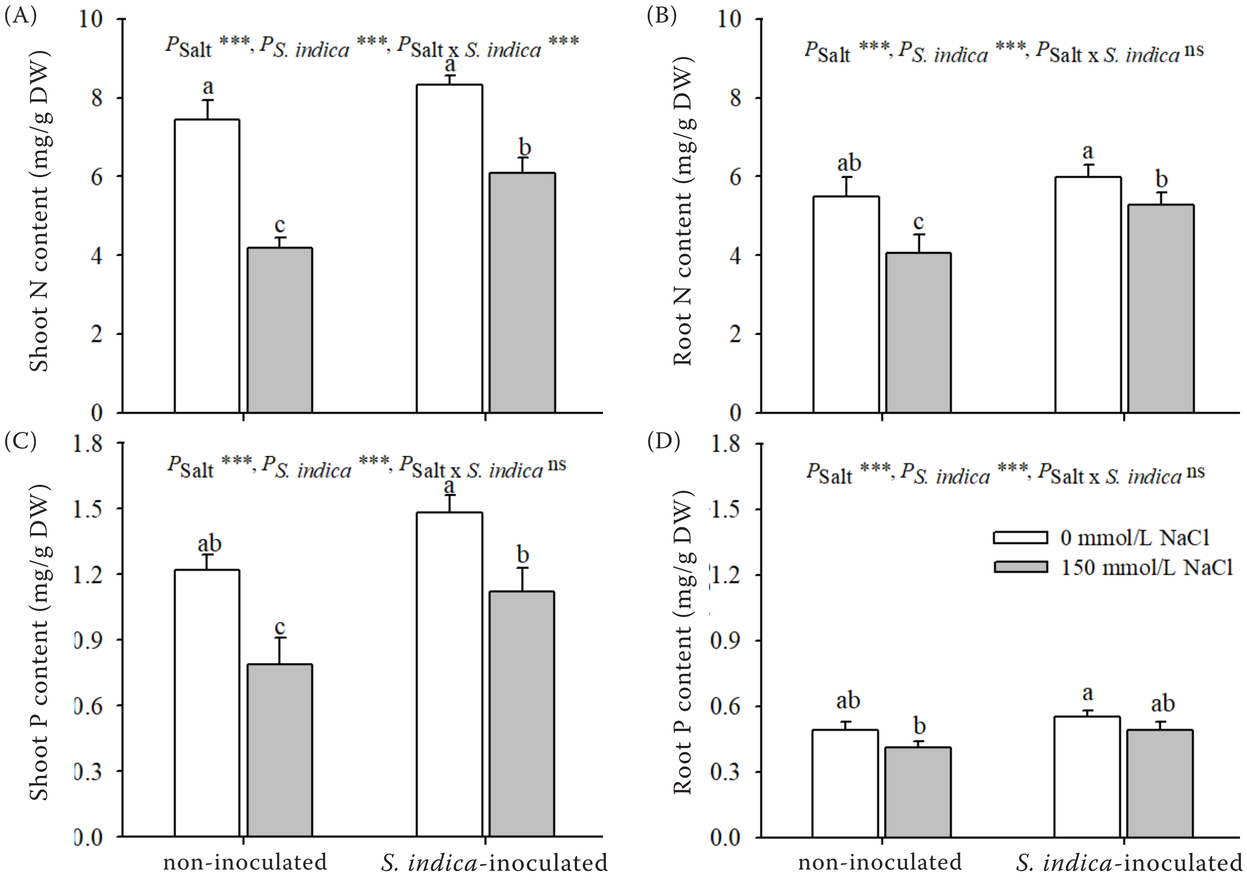

Figure 7. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on nitrogen (N) and phosphorus (P) content in shoot and root of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S.\ indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect. $^{***}P \leq 0.001$; ns – no significant effect; DW – dry weight

the seedlings under non-saline conditions (Figure 6). No significant difference in proline content was observed among non-inoculated and *S. indica*-inoculated seedlings under non-saline conditions, while proline content in *S. indica*-inoculated seedlings (322.58 μmol/g FW) was significantly higher than that of non-inoculated seedlings (218.75 μmol/g FW) under saline condition. Salt treatment significantly increased proline content by 71.81% and 139.09% in non-inoculated and *S. indica*-inoculated seedlings, respectively.

Content of phosphorus and nitrogen. Under non-saline conditions, *S. indica* inoculation had no significant influence on N and P content in shoots and roots compared with the non-inoculated seedlings. Under saline conditions, *S. indica*-inoculated seedlings showed significantly higher content of N in shoots and roots and P content in shoots than the non-inoculated seedlings (Figure 7). Salinity significantly decreased N content in root and shoot of non-inoculated seedlings by 43.89% and 26.05%, respectively, the decrements of which were larger than that of the *S. indica*-inoculated seedlings (N content in shoot and root decreased by 26.98% and 11.71%, respectively). Under salinity conditions, P content in the shoot of non-inoculated seedlings and *indica*-inoculated seedlings decreased by 35.25% and 24.32%, respectively, while no significant difference in root P content was observed between non-inoculated seedlings and *indica*-inoculated seedlings.

Content of Na^+, K^+ and Na^+/K^+ ratio. In comparison with the non-saline, 150 mmol/L NaCl led to more accumulation of Na^+ in roots and shoots of all seedlings; the non-inoculated seedlings (257.31% and 184.79%, respectively) exhibited a higher increase than the *S. indica*-inoculated seedlings (79.89% and 149.36%, respectively) (Figure 8A, B). Under both normal and saline conditions, non-inoculated seedlings had significantly higher Na^+ content in shoots and roots than the *S. indica*-inoculated seedlings.

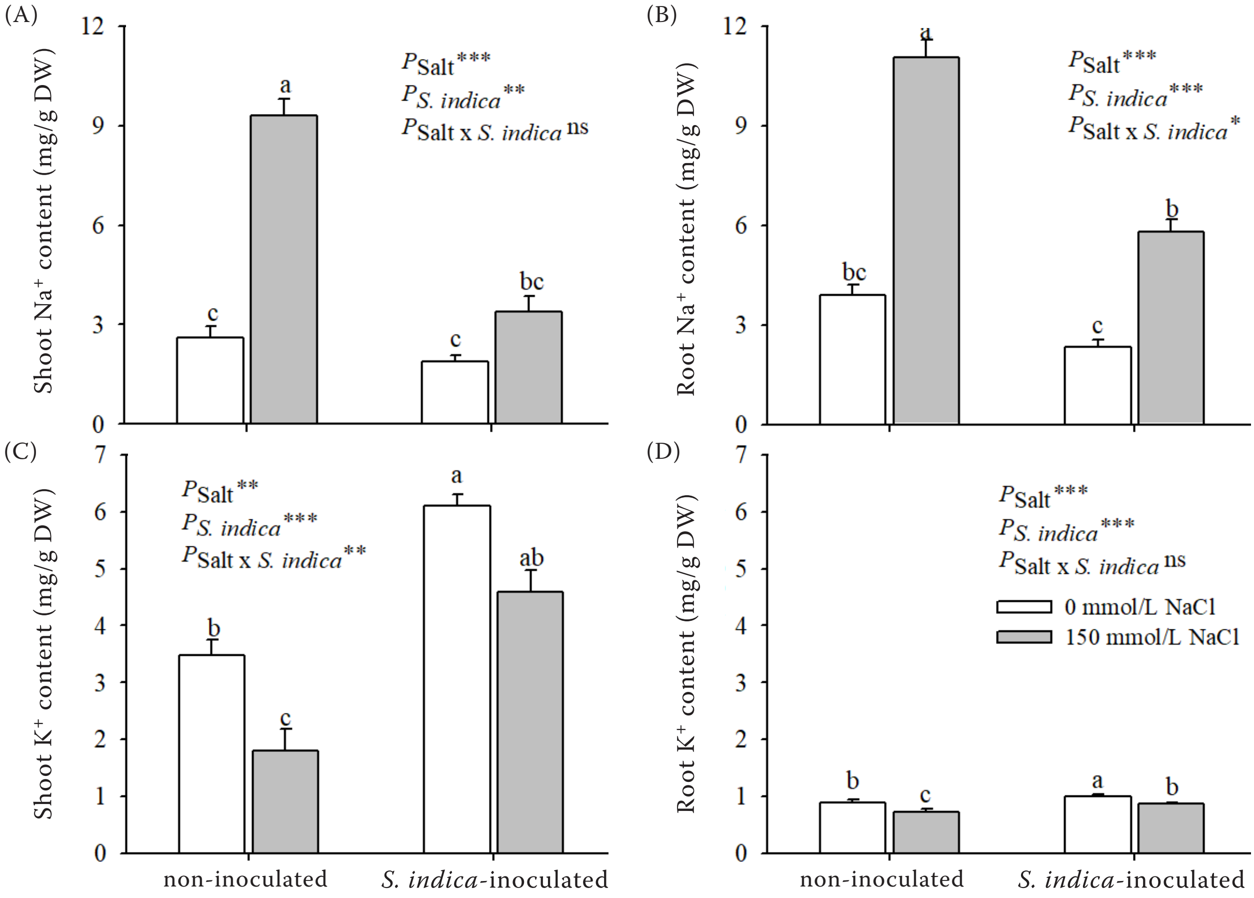

Figure 8. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on Na^+ and K^+ content in shoot and root of *Lolium multiflorum* seedlings. Different letters indicate significant differences among different treatments at $P \leq 0.05$. P_{salt}: salt effect; $P_{S. indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect; $^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$; ns – no significant effect; DW – dry weight

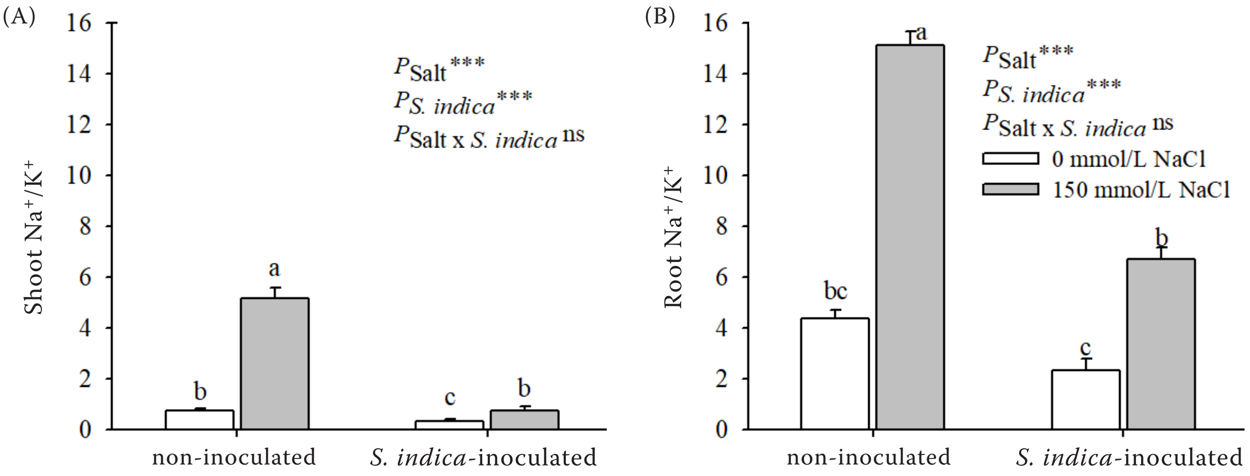

Figure 9. Effect of NaCl-induced salinity and *Serendipita indica* inoculation on Na^+/K^+ ratio in shoot and root of *Lolium multiflorum* seedlings. Different lowercase letters in the same figure indicate statistically significant differences among different treatments for the same parameter at $P \leq 0.05$ based on Tukey's *HSD* post hoc test. P_{salt}: salt effect; $P_{S.\ indica}$: *S. indica* effect; P_{salt} × *S. indica*: salt × *S. indica* effect. $^{***}P \leq 0.001$; ns – no significant effect

Saline stress reduced K^+ content in the shoots and roots of the seedlings, and the *S. indica*-inoculated seedlings (24.88% and 13.00%, respectively) showed a lower decrease than the non-inoculated seedlings (48.28% and 17.98%, respectively) (Figure 8C, D). Under non-saline and saline conditions, *S. indica*-inoculated seedlings exhibited significantly higher K^+ content in shoots and roots than that of the non-inoculated seedlings.

Under both non-saline and saline conditions, *S. indica*-inoculated seedlings showed a significantly lower Na^+/K^+ ratio in roots and shoots compared with the non-inoculated seedlings (Figure 9). The saline application significantly increased the Na^+/K^+ ratio in shoots and roots, and the *S. indica*-inoculated seedlings (Na^+/K^+ ratio in shoots and roots increased by 138.71% and 186.70%, respectively) showed a lower increase than that of the non-inoculated seedlings (Na^+/K^+ ratio in shoots and roots increased by 588.00% and 247.25%, respectively).

DISCUSSION

Salinity stress decreased root colonisation of *S. indica*. *S. indica* can successfully colonise with roots of *L. multiflorum* (Liu et al. 2019), although salt application in this study showed significantly negative effects on root colonisation of *L. multiflorum* seedlings. The reduction of *S. indica* root colonisation induced by salt stress has also been reported in many other species, which might be attributed to the inhibitory effects of salinity on the growth of fungal hyphae and chlamydospores (Lanza et al. 2019) and the destructive effects of salt stress on photosynthesis along with reduction in supply of carbon from plant to fungus (Ghorbani et al. 2018, Abdelaziz et al. 2019).

***S. indica* colonisation improved seed germination and seedling growth under salinity.** *S. indica* has been considered as a growth promoter of host plants under normal and stress conditions (Saleem et al. 2022). In the present study, regardless of fungal colonisation or not, salinity stress greatly inhibited seed germination and seedling growth of *L. multiflorum*. Seed priming with *S. indica* significantly enhanced the GR of *L. multiflorum* under salt stress conditions, although no significant effect was found under non-saline conditions. Also, a significant growth-promotion effect induced by *S. indica* colonisation was observed as manifested by the great improvement in SFW, RFW, FW, SL and RL under both non-saline and saline conditions. The promoted response on growth induced by *S. indica* under salinity conditions has been widely reported in other plants (Ghorbani et al. 2018, Khalid et al. 2018, Abdelaziz et al. 2019).

***S. indica* colonisation increased leaf chlorophyll content and relative water content of *L. multiflorum* seedlings under salinity.** Chlorophyll is the most effective compound of photosynthetic pigments for harvesting light and producing reducing powers; any reduction in C_{chl} would lead to structural changes in the chloroplast, which directly affects photosynthetic capacity (Wang and Grimm 2021). In our study, 150 mmol/L NaCl induced-salinity stress led to a significant decrease in C_{chl} of the studied *L. multiflorum* seedlings regardless of *S. indica* colonisation, which mainly due to the peroxidation of chlorophyll, the activity inhibition of enzymes involved in chlorophyll biosynthesis, and the reduction of nutrients absorption (Stenbaek and Jensen 2010, Ghorbani et al. 2018). The application of the *S. indica* resulted in a significant increase in C_{chl} of the salt-stressed seedling, suggesting an improvement in photosynthetic potential and, hence, great growth and biomass accumulation (as manifested above). Mycorrhiza could increase the absorption of mineral elements such as nitrogen, magnesium, iron, manganese, copper, and zinc, which are the essential constituent elements of chlorophyll or the activator of certain enzymes in chlorophyll biosynthesis (Stenbaek and Jensen 2010).

Water plays a crucial role in plant growth and development, including supplying plant nutrition, participating in photosynthesis, regulating plant body temperature, maintaining cell structure and function, and helping plants carry out material transport. Salinity is well known to reduce water uptake of roots and water transfer to another part of the plants, even in osmotically well-adjusted plants (Ghorbani et al. 2018, Khademian et al. 2019). In our study, NaCl induced-salinity stress led to a significant decrease in RWC of the studied *L. multiflorum* seedlings regardless of *S. indica* colonisation, which could cause stomatal closure and help to maintain the water balance of the stressed plants (Khademian et al. 2019). Symbiotic association between *S. indica* and *L. multiflorum* seedlings resulted in a smaller reduction of RWC than non-inoculated ones, suggesting promotion in water uptake, leaf water potential and hence improvement of gas exchange and growth (as manifested above).

***S. indica* colonisation alleviated oxidation damage of *L. multiflorum* seedlings under salinity.** The biofilm system is the primary injury site of salt damage. Salt affects various physiological metabolism and enzyme activities by destroying the permeability of the plasma membrane of plant cells, and it affects the growth and development of plants (Goswami and Deka 2020). MDA and REC are widely used to estimate the stability and relative permeability of the plasma membrane of plant cells under stressful conditions (Khademian et al. 2019, Liu et al. 2021). In the present study, salt treatment significantly aggravated oxidative stress on membrane damage, as manifested by the significant increase in MDA content and REC of the studied *L. multiflorum* seedlings. Seedlings colonised by *S. indica* showed smaller increases in both indicators than those of the non-colonised seedlings. The results once again verified the mitigative effect of *S. indica* colonisation on the membrane damage caused by salinity-induced oxidative stress (Boorboori and Zhang 2022).

***S. indica* colonisation increased proline content.** Accumulation of proline, one of the most important osmotic adjustment substances, has been proven to be one of the most prominent strategies of plants in improving salt tolerance, mainly by maintaining cell turgor or osmotic balance, scavenging reactive oxygen species, stabilising membranes to prevent electrolyte leakage, supplying energy, and maintaining chlorophyll level to protect photosynthetic activity (Alagoz et al. 2023). The studied *L. multiflorum* seedlings, regardless of *S. indica* colonisation, responded to salt stress by actively producing and accumulating proline. Seedlings colonised by *S. indica* were more conducive to the accumulation of proline than the non-colonised seedlings. *S. indica*-induced increase in proline of the host plants under salt stress conditions has been widely reported, which indicates that *S. indica* colonisation could enhance host plants' adaption to salinity by prompting osmotic regulation *via* accelerating the accumulation of proline (Hassani et al. 2019, Boorboori and Zhang 2022, Wan et al. 2024).

***S. indica* colonisation enhanced the uptake of nitrogen and phosphorus of *L. multiflorum* seedlings under salinity.** The positive effects of mycorrhizal symbiosis on plant salinity tolerances are greatly related to increased absorption of nutrients and reduced nutrient losses by rhizodeposition (Jacoby et al. 2017, Zhou et al. 2020). In the present study, 150 mmol/L NaCl treatment reduced the N and P concentrations of shoots and roots in both *S. indica*-inoculated and non-inoculated seedlings. Compared to the non-inoculated seedlings, *S. indica*-inoculated seedlings exhibited higher content of N and P. In particular, N and P content in shoots were higher than that in roots of the *S. indica*-inoculated seedlings. The results suggested that *S. indica* helps host plants to absorb more nutrients under salinity conditions and to allocate more N and P to leaves, thereby promoting plant growth. The enhancement of nutrient absorption in the inoculated seedlings was attributed to the improved root system by altering the root architecture and increasing root length by extra-radical hyphae (Aslam et al. 2019, Ghorbani et al. 2019). The increase of P concentration is mainly attributable to the high inorganic phosphate-solubilising capability which can be enhanced by a variety of organic acids and acid phosphatases produced by *S. indica* and the rhizobacteria it interacts or communicates with (Wu et al. 2019). Also, *S. indica* can up-regulate the expression of SiPHO80 (a phosphate-responsive cyclin of *S. indica*) (Loha et al. 2018) and PiHOG1 (yeast HOG1 homologue from *S. indica*) (Jogawat et al. 2016) that are essential for phosphate transport to the host plant. As one of the main components of chloroplasts, the increase of leaf N level is beneficial in promoting photosynthesis rate. Moreover, phosphate can affect N allocation to ribulose bisphosphate carboxylase oxygenase, which is closely related to photosynthetic rate (Wu et al. 2019). Thus, the significant increase in N and P levels in the shoots of *L. multiflorum* seedlings might result in an improvement in photosynthesis rate, thereby causing better growth and high biomass accumulation under salt stress conditions.

***S. indica* colonisation improved K^+/Na^+ homoeostasis of *L. multiflorum* seedlings under salinity.** Under salinity conditions, a large amount of Na^+ will influx into the cytoplasm and result in plant growth retardation. One of the strategies plants used to cope with Na^+ toxicity is to counterbalance the entry of Na^+ ions into cells by increasing intracellular K^+ concentrations (Gupta et al. 2022). Therefore, the Na^+/K^+ ratio is a key indicator of plant salt tolerance. In our study, salinity led to a significant increase in Na^+/K^+ due to the more absorption of Na^+. Compared to the non-inoculated seedlings, *S. indica* application increased K^+ content but decreased Na^+ content and Na^+/K^+ ratio of the salt-stressed seedlings. *S. indica* might induce more accumulation of Na^+ in vesicles, inside vacuole of root cells or in intra-radical fungal hyphae (Khalid et al. 2018, Khademian et al. 2019, Boorboori and Zhang 2022), thereby preventing the toxicity of Na^+ in host plants. K^+ accumulation in the *S. indica*-inoculated seedlings can improve the stomatal regulation function and thus increase the photosynthetic efficiency of the salinity-treated seedlings (Boorboori and Zhang 2022, Wan et al. 2024). Furthermore, K^+ is the essential element involved in osmotic regulation, synthesis of various proteins, and regulation of various enzyme activity. Thus, *S. indica* can improve the tolerance of *L. multiflorum* seedlings to salinity stress by increasing K^+ accumulation, reducing Na^+ absorption and transportation, and improving Na^+/K^+ homoeostasis. The improved K^+/Na^+ homoeostasis is probably related to the expression of Na^+/H^+ antiporter (SOS1) (Abdelaziz et al. 2019), cyclic nucleotide-gated channel (CNGC15) (Ghorbani et al. 2019), and *Serendipita* ENA ATPases (Lanza et al. 2019).

In conclusion, salt stress induced heavy negative effects on seed germination and seedling growth of *L. multiflorum*, which was evidently mitigated by successful colonisation with *S. indica*. *S. indica* alleviated the salt-induced negative effects on *L. multiflorum* seedlings, at least partly, by (1) facilitating the accumulation of osmoregulatory substances, such as proline and K^+, to improve the osmoregulatory ability of host plants; (2) increasing the uptake of nutrients such as N and P to improve photosynthetic capacity; (3) improving Na^+/K^+ homoeostasis to alleviate the detrimental effects of ionic toxicity on membrane stability and photosystem. The results would provide a new idea for the combined application of salt-tolerant plants and symbiotic microorganisms in the ecological restoration of saline-alkali lands.

However, it remains unclear how *S. indica* promote the synthesis of the osmotic adjustment substances, the absorption of nutrient elements, and the ionic equilibrium, which have yet to be explored. Transcriptome sequencing and metabolome analysis can potentially reveal the underlying molecular and metabolic mechanism behind the enhanced salt tolerance of *S. indica*-inoculated *L. multiflorum* seedlings by improvements in osmotic adjustment, nutrient absorption, or ionic equilibrium.

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