



Piriformospora indica improves salinity stress tolerance in *Zea mays* L. plants by regulating Na⁺ and K⁺ loading in root and allocating K⁺ in shoot

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Abstract

Piriformospora indica is known as a fungus that can easily colonize a wide range of plants and enhance host's growth and tolerance to abiotic stresses, including salinity. The mechanistic basis behind this phenomenon remains poorly understood. This work was aimed to fill in this gap and reveal mechanisms enhancing salinity tolerance in maize roots colonised by *P. indica*. A range of agronomic and physiological characteristics were compared between inoculated and non-inoculated maize plants under 0/100/200 mM NaCl conditions. The impact of *P. indica* inoculation on root's cytosolic K⁺ retention ability were also assessed using micro-electrode ion flux estimation technique. The results showed that inoculated plants had higher biomass, higher stomatal conductance, lower K⁺ efflux from roots and higher potassium content in shoots than non-inoculated plants under salt stress. Collectively, the results indicated that the beneficial effects of inoculation on plant performance under saline conditions were mainly attributed to the improved stomata operation associated with higher rate of K delivery into the shoots.

Keywords *Piriformospora indica* · Salinity stress · Potassium loading · Micro-electrode ion flux estimation

Abbreviations

ABA	Absciscic acid
AM	Arbuscular mycorrhizal
BSM	Basic salt medium
Ci	Intercellular CO ₂ concentration
DW	Dry weight

FW	Fresh weight
Gs	Stomatal conductance
MDA	Malondialdehyde
MIFE	Micro-electrode ion flux estimation
PGPR	Plant growth-promoting rhizobacteria
<i>P. indica</i>	<i>Piriformospora indica</i>
Pn	Net photosynthetic rate
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
Tr	Transpiration

Ping Yun and Le Xu have contributed equally to this work.

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Introduction

As a major agricultural problem, soil salinity affects most of arable land in arid regions. More than 800 million hectares of the total land surface suffers from soil salinization, significantly impacting on growth and productivity of agricultural crops (Shelden et al. 2016).

A number of approaches have been used to transform the saline-alkali land through fresh water irrigation and chemical modification, which however resulted in the huge cost and relatively minor beneficial effect. Attempts to improve the salt tolerance of crops through conventional breeding

programmes have been also met with a rather limited success, due to physiological and genetic complexity of the salt tolerance trait. Also modest results were obtained from using an in vitro selection; marker-assisted breeding; the use of transgenic plants; interspecific hybridization; and using halophytes as alternative crops (Ismail and Horie 2017).

The naturally occurring plant growth-promoting rhizobacteria (PGPR) could result in a remarkable growth promotion and nutrient uptake under salinity stress in many plants (e.g. tomato, Balliu et al. 2015). Lately, the discovery of *Piriformospora indica* which belongs to the basidiomycete order Sebaciniales suggested that this species could easily colonize a wide range of higher plants, increasing grain yield, and protecting their hosts from a large variety of abiotic stresses including drought (Zhang et al. 2017a, b), salinity (Ghaffari et al. 2016) and cold injury (Murphy et al. 2014). They have been also shown to be efficient in enhancing plant tolerance to biotic stresses (Waller et al. 2005), and assisting hosts in nutrients uptake (Achatz et al. 2010).

The mechanisms behind the above beneficial effects of *P. indica* on abiotic stress tolerance remain elusive. It was shown that *P. indica* affected the carbohydrate metabolism, nitrogen metabolism, and ethylene biosynthesis pathway in salt-affected plants; also, production of antioxidant enzymes that ameliorate damage of plants under salinity stress was enhanced (Waller et al. 2005; Ghaffari et al. 2016). It was also shown that *P. indica* could protect wheat plants from the detrimental effects of salinity by influencing the uptake of water, photosynthetic pigment contents and proline accumulation in seedlings (Zarea et al. 2012). Similarly, *P. indica* rescued growth diminution of rice seedlings under salt stress through enhanced photosynthetic pigment content and proline accumulation (Jogawat et al. 2013). Li et al. (2017) demonstrated that tolerance to salinity stress conferred by *P. indica* in *Medicago truncatula* was via accumulation of osmoprotectants, stimulating antioxidant enzymes and the expression of defence-related genes.

However, effects of salinity are broader than merely osmotic stress and increased ROS production. Specific ion toxicity becomes a dominant component after prolonged salinity exposures (Munns and Tester 2008), and disturbance to K^+ homeostasis is also a key determinant of differential salinity stress tolerance, at both intra- and inter-specific level (Shabala et al. 2016; Shabala 2017). Ion toxicity caused by excessive Na^+ and Cl^- accumulation decrease the uptake of essential nutrients like phosphorus (P), potassium (K), nitrogen (N), and calcium (Ca) (Zhu 2001; Munns and Tester 2008). Moreover, Na^+ toxicity is often associated with its competitive nature with K^+ for binding to essential sites (Cuin et al. 2008). Also, salinity stress alters plant hormonal status and, specifically, ABA production. ABA is a major driving force behind stomatal closure that is essential in reduction of water evaporation under salt stress (Yang

et al. 2014). ABA may also regulate other physiological and biochemical processes under salt stress, including those related to ionic homeostasis. It was shown, for example, that ABA contributes to a higher ratio of K^+/Na^+ , by enhancing expression of genes encoding ion transporters and proton pumps which help in the Na^+ compartmentation and selective absorption of K^+ and thus maintains the normal K^+/Na^+ ratio (Janicka and Klobus 2007). However, not much is known about effects of *P. indica* on the functional expression and activity of transporters regulating plant Na^+ and K^+ homeostasis, and contributing mechanisms can be only speculated by making analogies with a mycorrhizal colonization.

Mycorrhizal colonization has also been shown to enhance K^+ absorption and prevent Na^+ translocation to shoot tissues, so mycorrhizal plants often have a higher K^+/Na^+ ratio and a lower shoot Na^+ concentration under saline stress conditions (Sharifi et al. 2007). The only similar report for *P. indica* was a recent study by Abdelaziz et al. (2017) who showed that colonized *Arabidopsis* roots had lower Na^+/K^+ ratio and enhanced transcript levels of the genes encoding the high affinity potassium transporter 1 (HKT1) and the inward-rectifying K^+ channels KAT1 and KAT2, compared to non-inoculated plants under salt stress.

In this work, we hypothesised that *P. indica* might regulate maize salt tolerance by similar mechanisms and aimed to gain a better understanding of how *P. indica* impacts plant ion homeostasis. Our specific focus was on root ion loading, one of the key traits conferring salinity stress tolerance in plants (Shabala et al. 2010; Bose et al. 2014).

Materials and methods

Plant materials and fungal culture

Grains of maize variety Huayu 11 were obtained from Hubei Jingchu Seed Industry Co., Ltd. Grains were surface sterilized with 2% NaClO for 10 min, and rinsed under running tap water for 1 h to remove any traces of NaClO. Grains were germinated on a tissue paper at 27 °C in a growth chamber under a dark condition for 2 days. *P. indica* was cultured on *Aspergillus* liquid media at 28 °C in the dark with shaking at 150 rpm for 12 days.

Root inoculation

The seedlings were inoculated by immersing them into 1% (w/v, 1 g filtered mycelium in 100 mL water) fungal mycelium; the control was inoculated with the autoclaved inoculum. All seedlings were planted into cylindrical pots (radius 14 cm, height 7 cm) filled with 1 kg sterilized sand (water content 25%) and grown in a growth chamber with watering 150 mL water every 2 days. A relative humidity of

55% and a temperature of 25 °C were provided, and daily illumination was 16 h. Roots of inoculated and non-inoculated plants were collected randomly from three different seedlings to check *P. indica* colonization after fortnight of inoculation. The extent of colonization was examined under a light microscope (Nikon CX41-72C02) as described by Jogawat et al. (2013). Briefly, washed root samples were soaked in 10% KOH solution overnight, acidified with 1 M hydrochloric acid for 20 min, and stained with 0.04% Trypan blue for 40 min, then examined under the microscope. The intracellular chlamydospores in roots were used as proof for colonization.

Experimental protocols

The experiment contained six treatments (2×3): inoculated/non-inoculated *P. indica* × 0/100/200 mM NaCl, which included: 0 mM NaCl + *P. indica*, 100 mM NaCl + *P. indica*, 200 mM NaCl + *P. indica*, 0 mM NaCl – *P. indica*, 100 mM NaCl – *P. indica*, 200 mM NaCl – *P. indica* (Fig. S1a).

Twelve plants of each treatment were grown, and the experiment was replicated three times. Seedlings were transplanted from sand into 1/2 Hoagland solution once colonization was examined. Two days after transplantation, salinity treatment has commenced by adding appropriate amounts of NaCl to Hoagland solution. The treatment lasted for 5 days. Agronomical and physiological characteristics were measured at the end of the treatments.

Agronomical assessment

The maize root surface area, root volume, total leaf area, fresh weight (FW) and dry weight (DW) of shoot and root of inoculated and non-inoculated plants were investigated after 5 d of salt treatments. Root surface area and root volume were assessed by WinRHIZO system (Regent Instruments, Canada). Leaf area was calculated by using $0.75 \times \text{length} \times \text{maximum widths}$ (Jin et al. 2016). The material was dried at 80 °C in an oven, to achieve a constant weight before the dry weight assessment.

Physiological assessment

The chlorophyll content (SPAD values) was measured daily after commencement of salinity treatment from the third leaf by using chlorophyll meter (SPAD-502, Konica, Japan). The malondialdehyde (MDA) content was determined at the end of the treatment as described elsewhere (Fan et al. 2015). Briefly, 0.3 g leaf tissue were ground with 5 mL trichloroacetic acid (TCA), then a mixture of 3 mL supernatant liquid and 3 mL thiobarbituric acid (TBA) was heated for 15 min by using a boiling water bath to complete the colour reaction. The absorbance was measured at 532 nm (A_{532}),

600 nm (A_{600}) and 450 nm (A_{450}) using a spectrophotometer (Shimadzu UV-1800, Japan). The MDA concentration of the colour reaction product was calculated as $C_{\text{MDA}} (\mu\text{mol/L}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$, and the MDA content of the sample was calculated using an equation: $\text{MDA} (\mu\text{mol/g FW}) = ((C_{\text{MDA}} \times 6 \text{ mL}/1000)/3 \text{ mL}) \times 5 \text{ mL}/0.3 \text{ g}$. The gas exchange was measured by LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA) from the same leaf as SPAD measurements. Measurements were conducted on a daily basis, between 10 am and 12 pm, under constant light conditions provided by the built-in lighting system.

Electrophysiology

After *P. indica* inoculation for 2 weeks, seedlings were transplanted from the sand into the aerated hydroponic solution (500 μM KCl and 100 μM CaCl_2) and kept for 2 days. Then, net K^+ fluxes were measured from mature root zone (20 mm from the root tip) in response to transient NaCl treatment using non-invasive microelectrode MIFE technique (UTas Innovation, Hobart, Australia), as described previously (Shabala et al. 2003). In brief, microelectrodes were pulled out using PE-22 puller (Narishige, Tokyo, Japan), dried in an oven and silanized with tributylchlorosilane (Cat 90794, Sigma-Aldrich, Australia). The prepared electrode blanks were backfilled with 200 mM KCl and electrode tips front-filled with potassium selective ionophore (Cat 99311, Sigma-Aldrich, Australia). Electrodes were mounted on a micromanipulator (MMT-5, Narishige, Tokyo, Japan) and calibrated in a set of three calibration solutions (250, 500 and 1000 μM KCl). For measurements, the electrode tip was positioned 45 μm away from the root surface. A computer-controlled stepper motor moved the electrode between two positions—45 μm (M1) and 85 μm (M2)—from the root surface, in a square-wave manner (half-cycle 6 s). The potential difference between the two positions were recorded and converted into electrochemical potential difference by CHART software (Newman 2001) using a calibrated Nernst slope. MIFEFLUX software was used to calculate net ion fluxes using cylindrical geometry profile (Newman 2001).

The ion flux measuring protocol was as described by Cuin et al. (2008). Briefly, a seedling was immobilised in a 35 mL Petri dish filled with 27 mL of Basic Salt Medium (BSM: 500 μM KCl and 100 μM CaCl_2) solution and adapted for 1 h. The steady fluxes were measured in the BSM solution for 5 min. Then 3 mL of NaCl stock solution was applied to bring final NaCl concentration to either 100 mM or 200 mM, and transient K^+ fluxes were recorded for a further 40 min. The first 60 s after NaCl stock addition to the measuring chamber were discarded to comply with non-stirred layer conditions required for MIFE measurements (Shabala and Hariadi 2005). This period appears as a gap in all figures.

Plant elemental analysis

The third leaves were harvested from 12 plants of each treatment and dried in an oven at 75 °C to obtain stable weight. For analysis, 0.5 g of dried leaf or root samples were powdered and digested in 5 mL $\text{H}_2\text{SO}_4\text{--H}_2\text{O}_2$, and then the reaction solutions were diluted to 100 mL. The potassium and sodium contents were detected using flame atomic absorption spectrometry (SuZhou FP640) modified from the method of Jiang et al. (2016). The sample ion content was calculated using a standard calibration curve taking into account a dilution factor.

Data analysis

The relative value (treatment value/control value) was calculated to estimate difference between inoculated (+ *P. indica*) and non-inoculated (– *P. indica*) plants under salinity stress. Data were analysed using two-way analysis of variance in SPSS version 17 (SPSS Inc., Chicago, USA).

Results

Inoculation examination

Chlamydospores of *P. indica* were observed in inoculated seedling's lateral roots (Fig. S1c), and no colonization was observed in roots which were inoculated by autoclaved mycelium (Fig. S1b). The inoculation with life culture of *P. indica* had beneficial effects on both shoot (Fig. S2) and root growth (Fig. S3) of plants grown at extreme salinities (200 mM NaCl).

Growth parameters under salinity stress

The visual analysis showed that leaves of inoculated seedlings (+ *P. indica*) were bigger and more straight under salinity stress compared with non-inoculated plants (– *P. indica*). We also observed differences in roots with inoculated seedlings having more rootlets and longer roots under NaCl conditions compared with non-inoculated plants. Overall, all agronomical characteristics of inoculated plants such as fresh weight (FW), dry weight (DW) and root characteristics such as root volume were higher than in *P. indica* free plants under salinity stress (Fig. 1). This indicates that non-inoculated plants have more morphologically reduction than inoculated plants under NaCl condition.

Influence of *P. indica* on leaf MDA, SPAD content and gas exchange under salt condition

A mild salinity stress did not result in a substantial damage to membranes, with MDA content increasing only by approximately 10% in colonized plants (Fig. 2b). However,

more severe 200 mM NaCl treatment led to the two-fold MDA increase compared with control. In both cases, the inoculated plants (+ *P. indica*) showed lower level of lipid peroxidation with MDA content in the *P. indica* colonized plants (+ *P. indica*) being lower than in *P. indica* free plants (– *P. indica*) (Fig. 2b). The above membrane damage was also reflected in the amount of the chlorophyll loss (Fig. 2a).

Salinity stress reduced leaf gas exchange characteristics in a dose-dependent manner (Fig. 2c–f). Specifically, relative stomatal conductance (G_s) and transpiration (Tr) of *P. indica*-free plants were 20% less than in *P. indica*-inoculated plants under 100 mM NaCl stress; however, the similar reduction was 40% when NaCl concentration increased to 200 mM (Fig. 2c, d). Significant (at $P < 0.05$) differences in G_s and Tr have been found between inoculated and non-inoculated plants under both 100 and 200 mM treatments, but colonized plants have always shown smaller reduction in Tr , G_s and intercellular CO_2 concentration (C_i) than non-inoculated plants (Fig. 2c–e). As a result, the net CO_2 assimilation rate in inoculated plants treated with 200 mM NaCl was 2.75-fold higher than in non-inoculated (Fig. 2f).

Elements and ion flux measurement

Salinity stress resulted in a massive accumulation of Na in both roots (Fig. 3a) and shoots (Fig. 3b). Root Na content was not dramatically different between 100 and 200 mM treatment while plant shoots treated with 200 NaCl accumulated three-fold more Na (Fig. 3b). Remarkably, plant inoculation with *P. indica* led to a reduction of Na accumulation in the root (Fig. 3a) but increased its content in the shoot (Fig. 3b), suggesting control of Na^+ loading in root by *P. indica*.

The opposite scenario was observed for K^+ content in shoots. Here, K content in roots exposed to 200 mM NaCl treatment was decreased by 4.5–5-fold compared with 100 mM treatment (Fig. 3d). The inoculated plants had less K^+ in roots compared with their non-inoculated counterparts (Fig. 3d) under both 100 or 200 mM NaCl conditions but had much higher K content in the shoots (Fig. 3c).

We then checked whether lower K content in inoculated roots is related to their inability to keep K^+ upon exposure to salinity (MIFE data Fig. 3e, f). This was not the case. An acute salinity stress induced root K^+ efflux in a dose-dependent manner (Fig. 3e, f). However, the magnitude of K^+ loss in inoculated plants was only half of that compared to non-inoculated counterparts. Taken all together (Fig. 3c, d), results suggested that inoculated plants are capable to regulate K loading in root and deliver sufficient quantities of K to the shoot (more than non-inoculated plants).

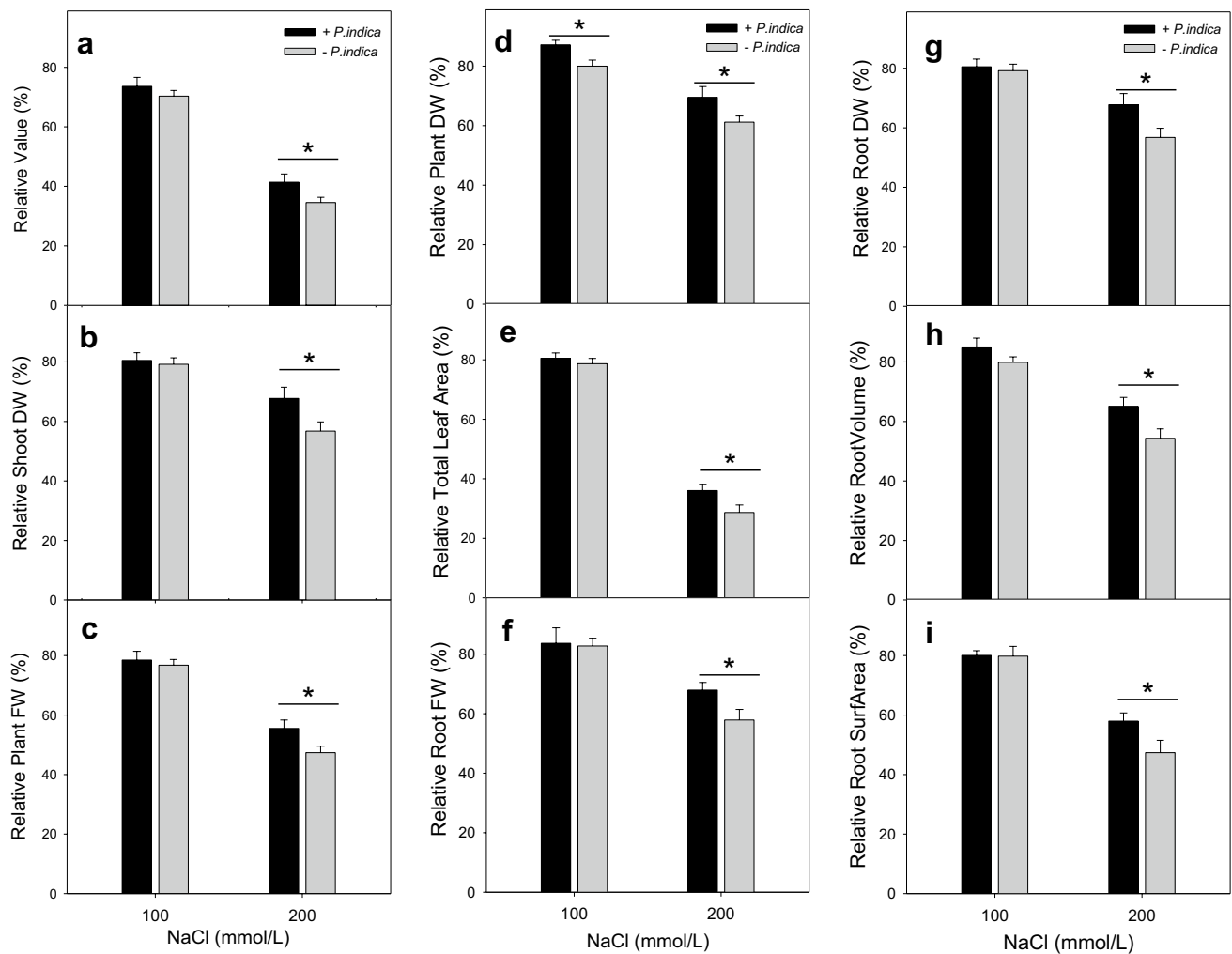


Fig. 1 Effect of *P. indica* on agronomical and root characteristics of *Z. mays* under salt treatment (expressed in relative units as percentage of control): **a** relative shoot fresh weight (FW), **b** relative shoot dry weight (DW), **c** relative plant fresh weight, **d** relative plant dry

weight, **e** relative total leaf area, **f** relative root fresh weight (FW), **g** relative root dry weight (DW), **h** relative root volume, **i** relative root surface area. Mean \pm SE (n = 5). *Significant at $P < 0.05$

Discussion

The biggest advantage of *Piriformospora indica* compared with arbuscular mycorrhizal (AM) fungi is that *P. indica* can be easily cultured on various synthetic media, also it can colonize a very wide range of plants. Furthermore, *P. indica* can promote growth and ameliorate salt stress that caused damage of hosts as previously described (Ghaf-fari et al. 2016; Gill et al. 2016; Vahabi et al. 2016). The findings of this work are consistent with earlier reports and also demonstrate the beneficial effects of *P. indica* inoculation in maize. In addition, we demonstrate that maize seedlings colonized by *P. indica* had enhanced several vital parameters including growth, photosynthesis, and ion uptake under salinity stress compared with non-inoculated plants.

Chlorophyll content (SPAD data, Fig. 2a) was affected by salinity in inoculated plants only at the very severe stress while the difference in the gas exchange characteristics (Gs and Tr, Fig. 2c, d) was observed even at a mild stress level. Specifically, the SPAD values of non-inoculated plant leaf was only half of those in inoculated plants after 200 mM NaCl treatment for 5 days. Impacts to Gs and Tr were even higher with values in the non-inoculated plants being 60% lower under 200 mM NaCl and 13–20% lower under 100 mM NaCl than in inoculated plants. These indicate that the beneficial effects of inoculation on plant performance (biomass) were mainly attributed to the improved stomata operation. Indeed, both Gs and Tr of inoculated plants were significantly different from those in non-inoculated plants ($P < 0.01$) under salinity stress, which means the *P. indica*-colonized plants can still

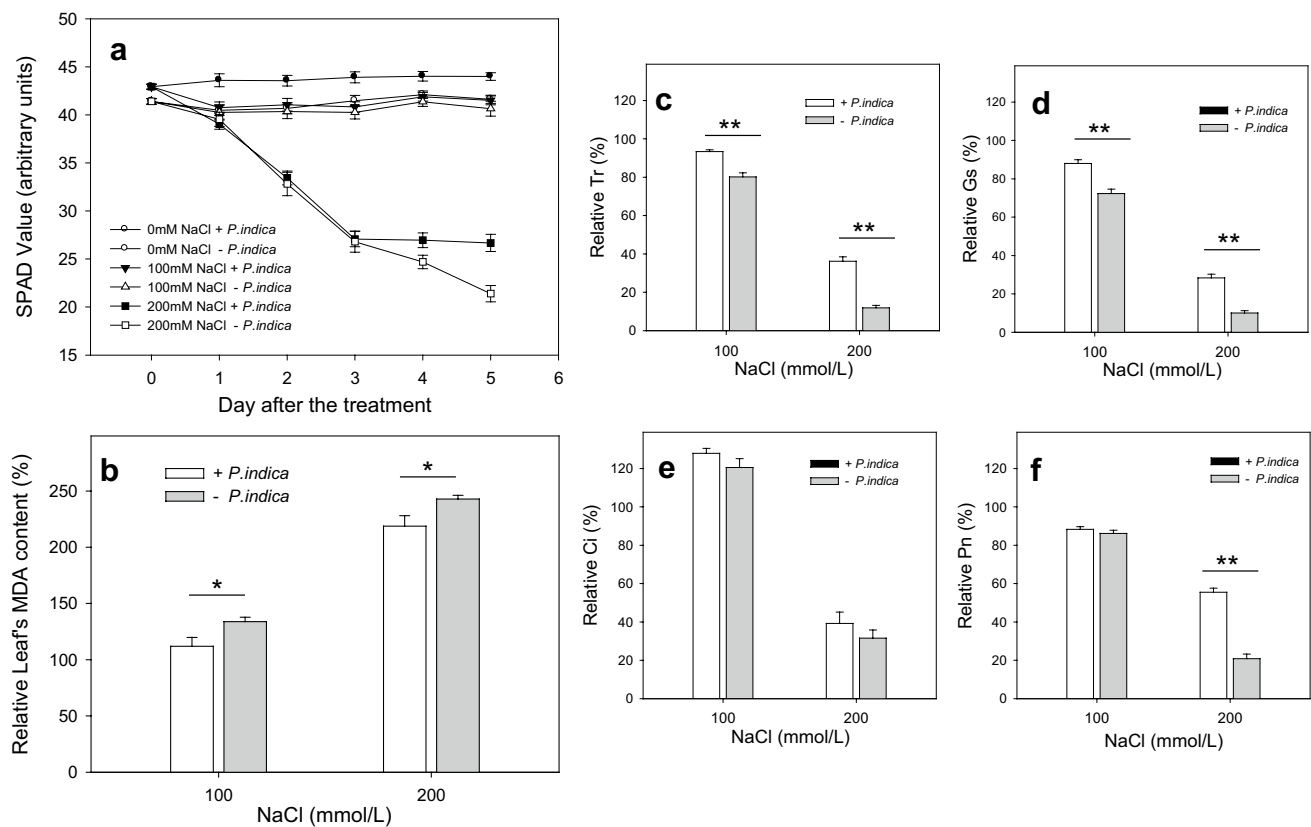


Fig. 2 Effect of *P. indica* on leaf characteristics in *Z. mays* under salt treatment: **a** leaf chlorophyll content (SPAD values), **b** the relative malondialdehyde (MDA) content in leaves, **c** relative transpiration (Tr) (% control), **d** relative stomatal conductance (Gs) (% control), **e**

relative intercellular CO_2 concentration (Ci) (% control), **f** relative net photosynthetic rate (Pn) (% control). Mean \pm SE ($n=5$). *Significant at $P < 0.05$, **significant at $P < 0.01$

maintain higher stomatal conductance and transpiration rate when exposed to salinity.

Salinity stress reduces water availability leading to decline in both Gs and net photosynthetic rate (Pn) (Choudhury et al. 2017), with stomatal closure considered as the primary factor to limit photosynthesis. The decrease in stomatal conductance can be induced by the incapability of guard cells to fully adjust stomatal apertures due to the lack of the fully functional osmotic adjustment (Misra et al. 2015). Salinity stress also results in a significant increase in ABA level (Han et al. 2015) that acts as a signal to the stomata closure. At the early stage of salinity stress, changes in stomatal conductance are also matched by changes in the root hydraulic conductivity. It was shown that tolerant plants have some aquaporin genes up regulated to release the reduction of hydraulic conductance, which can help to maintain high stomatal conductance and photosynthesis to improve plant salt resistance (Liu et al. 2015).

The osmotic adjustment can be achieved by either de novo synthesis of compatible solutes (organic osmolytes) or by increased uptake of inorganic ions (K, Na and Cl). As the energy cost of biosynthesis of organic osmolytes may

be 10-fold higher than for uptake of inorganic ions (Oren 1999), it was argued that the latter strategy is more efficient (Shabala et al. 2010). However, Na^+ accumulation in the cytosol results in Na^+ toxicity and should be avoided by an efficient Na^+ sequestration in vacuoles, which is not always the case. Thus, relying on K^+ for the osmotic adjustment in the shoot is the most preferred option.

In this study, the Na^+ content in inoculated maize roots was 17% less than in non-inoculated plants, and the shoot K^+ content in inoculated plants was 25% higher than in non-inoculated plants. This indicates that the inoculated plants were capable to reduce Na^+ load in the roots but at the same time transported much more K^+ to the shoots. As potassium is a key ion mediating stomata movement (Kollist et al. 2014), that may explain 2.5-fold higher Gs values in the inoculated plants treated with 200 mM NaCl compared with the non-inoculated plants. The higher K^+ availability may be thus instrumental for the fully functional stomatal operation, allowing plants to avoid toxicity of Na and, at the same time, conserve a pool of available ATP that otherwise is used for the (expensive) process of de novo synthesis of organic osmolytes.

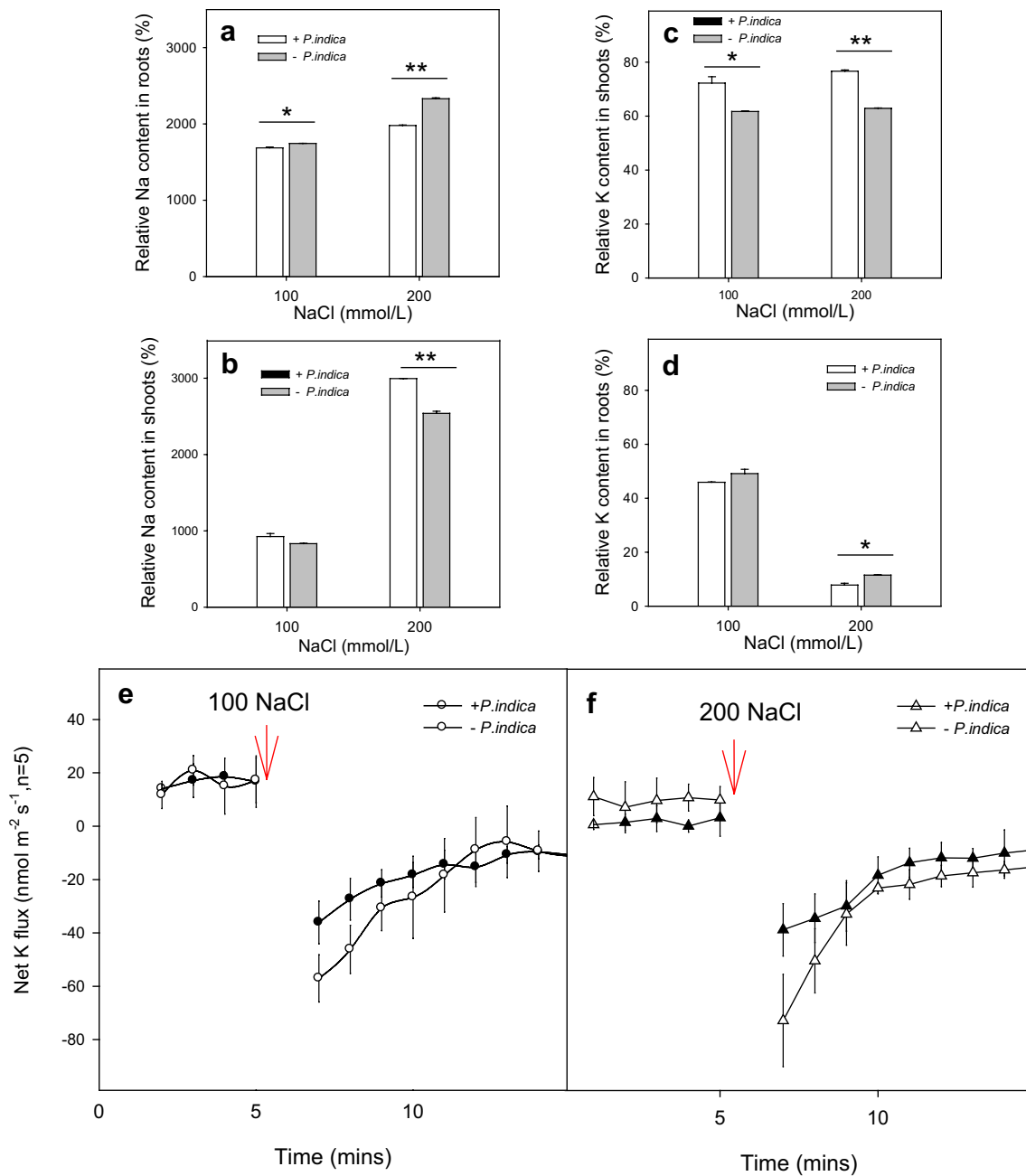


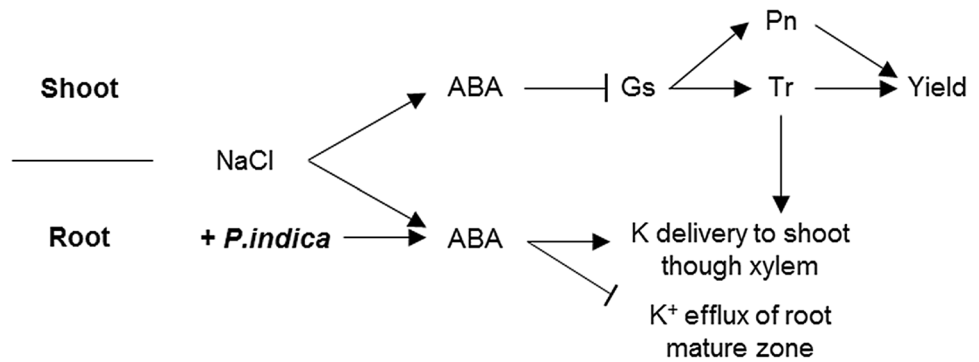
Fig. 3 Effect of *P. indica* on Na⁺/K⁺ content in *Z. mays* under salt treatment: **a** relative Na⁺ content in roots (% control), **b** relative Na⁺ content in shoots (% control), **c** relative K⁺ content in shoots (% control), **d** relative K⁺ content in roots (% control); ion fluxes measured

from root mature zone surface. Transient net K⁺ fluxes of *P. indica* inoculated and non-inoculated *Z. mays* responses to **e** 100 mM NaCl, **f** 200 mM NaCl. Mean \pm SE (n=5). *Significant at $P < 0.05$, **significant at $P < 0.01$

To avoid toxicity of Na⁺ to shoots, plants need to reduce its delivery to photosynthetic tissues. This can be achieved by either increased rate of re-translocation of Na⁺ back to roots via the phloem (Kobayashi et al. 2017), or by controlling the amount of Na⁺ loaded into the xylem (Shabala et al. 2013; Zhu et al. 2016). As the former option represents a “futile cycle”, an efficient control of xylem Na⁺ loading should be preferred. Several ion transporters may impact

on xylem Na⁺ content. This includes Na⁺/H⁺ antiporter (SOS) (Shi et al. 2002) and high-affinity Na⁺/K⁺ permeable transporter (HKT) (Zhang et al. 2017a, b). The most likely candidates for xylem K⁺ loading are HKT, high-affinity K⁺ transporter (HAK) (Wang et al. 2015) and stellar K⁺ outward rectifying channel (SKOR) (Hu et al. 2016). Which of them may be affected by *P. indica* inoculation remain to be studied.

Fig. 4 Suggested interactions of ABA, *P. indica* and a plant under salinity conditions, arrows mean promote, perpendicular lines mean inhibit (see text for details)



ABA is considered as a stress hormone, Roberts and Snowman (2000) suggested that ABA can induce accumulation of K^+ through K^+ channels, which is consistent with our results showing that the roots of inoculated plants had less K^+ efflux compared with non-inoculated plants (Fig. 3e, f), thus indicating that under salinity condition plants inoculated with *P. indica* have higher ability to keep potassium than non-inoculated plants.

As discussed above, maintenance of shoot K^+ content is important for plants in order to retain growth and development under saline condition. Also, K^+ supply to a shoot requires xylem loading. Furthermore, accumulation of K^+ in the xylem vessels of maize and sunflower roots can be induced by ABA (Collins and Kerrigan 1974; Fournier et al. 1987). Research conducted on barely and *Arabidopsis* showed that ABA levels in inoculated roots could be higher than in non-inoculated roots that were attributed to *P. indica*-induced ABA signalling to affect initial host defence (Peskan-Berghofer et al. 2015).

Based on the above results, a testable model is suggested (Fig. 4). Salinity stress increases ABA levels in shoots, leading to stomata closure, in an attempt to save water although causing yield penalties. At the same time, increased ABA level in roots controls xylem parenchyma-based K^+ and Na^+ loading thus controlling their delivery to the shoot, to be used for osmotic adjustment. Plants inoculated with *P. indica* were shown to have elevated ABA levels (Peskan-Berghofer et al. 2015) and thus have an advantage of being more capable in sending K for osmotic adjustment (and stomata re-opening). ABA also restricts Na delivery to the shoot by controlling its loading into the xylem. This model may be used as a guide for the future studies. Also, the molecular identity of these transporters remains a subject for the future work.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to this work.

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