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The endophytic fungus *Piriformospora indica* enhances *Arabidopsis thaliana* growth and modulates Na⁺/K⁺ homeostasis under salt stress conditions

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Highlights

- Colonization by *Piriformospora indica* improves *Arabidopsis thaliana* growth under salt stress.
- *Piriformospora indica* colonized roots showed higher expression of the ion channels *HKT1*, *KAT1* and *KAT2*.

- *Piriformospora indica* colonization leads to a lower Na^+/K^+ ratio, which may be a result of increased *KAT1* and *KAT2* expression and explain the enhanced salt tolerance.

Abstract

The mutualistic, endophytic fungus *Piriformospora indica* has been shown to confer biotic and abiotic stress tolerance to host plants. In this study, we investigated the impact of *P. indica* on the growth of *Arabidopsis* plants under normal and salt stress conditions. Our results demonstrate that *P. indica* colonization increases plant biomass, lateral roots density, and chlorophyll content under both conditions. Colonization with *P. indica* under salt stress was accompanied by a lower Na^+/K^+ ratio and less pronounced accumulation of anthocyanin, compared to control plants. Moreover, *P. indica* colonized roots under salt stress showed enhanced transcript levels of the genes encoding the high Affinity Potassium Transporter 1 (HKT1) and the inward-rectifying K^+ channels KAT1 and KAT2, which play key roles in regulating Na^+ and K^+ homeostasis. The effect of *P. indica* colonization on *AtHKT1;1* expression was also confirmed in the *Arabidopsis* line *gll-HKT::AtHKT1;1* that expresses an additional *AtHKT1;1* copy driven by the native promoter. Colonization of the *gll-HKT::AtHKT1;1* by *P. indica* also increased lateral roots density and led to a better Na^+/K^+ ratio, which may be attributed to the observed increase in *KAT1* and *KAT2* transcript levels. Our findings demonstrate that *P. indica* colonization promotes *Arabidopsis* growth under salt stress conditions and that this effect is likely caused by modulation of the expression levels of the major Na^+ and K^+ ion channels, which allows establishing a balanced ion homeostasis of Na^+/K^+ under salt stress conditions.

1. Introduction

Soil salinity leads to growth retardation in plants and is one of the major challenges in modern agriculture [1]. High salt concentrations in soil decrease the ability of plants to up-take water and nutrients and disrupt the ionic and osmotic equilibrium in the cell [2]. Elevated levels of sodium ions in cells hamper important biochemical mechanisms required for plant growth and survival [3]. In particular, sodium accumulation changes cellular Na^+/K^+ ratios and reduces the availability of potassium ions required for the activities of various enzymes and for the regulation

of osmotic pressure and stomatal closure [4, 5]. Excess Na^+ ions also compete with K^+ ions and can replace them in binding to a number of cytosolic enzymes and proteins, disrupting cellular metabolism in roots and leaf tissues [4]. Cellular K^+ concentration is a crucial parameter in determining plant salinity stress tolerance [6, 7].

Tolerance to high salt concentrations results from combined activities of several pathways in different cellular compartments [8], and plants have developed diverse mechanisms that allow tolerating a high level of sodium ions at the tissue and cellular level [9]. These include the production of various osmolytes, protective metabolites, and proteins, as well as changes in ion homeostasis and Na^+ transport regulation. Osmolytes like proline, glycine-betaine, trehalose, mannitol or sorbitol reverse the osmotic effect of salinity and are involved in radical detoxification and damage repair of chloroplasts [10, 11]. Protective enzymes, such as superoxide dismutase (SOD), peroxidases, oxidoreductases, catalases, and glutathione S-transferases detoxify and scavenge free oxygen radicals and counteract cell death and senescence [12, 13].

Plants control Na^+ homeostasis through a variety of membrane proteins, antiporters, nonspecific cation channels, anion transporters, ABC-type transporters, Na^+ and K^+ transporters, plasma membrane and vacuolar ATPases and aquaporins [14]. In roots, Na^+ exclusion is mainly carried out by the Salt Overly Sensitive (SOS) signaling pathway in which the SOS3/SOS2 complex activates the Na^+/H^+ antiporter SOS1 for sodium efflux [15, 16]. However, export of sodium ions is not restricted to the surface of roots but is also involved in their redistribution throughout the plant [17]. Sequestration of sodium ions in vacuoles is facilitated by members of the Na^+/H^+ exchanger (NHX) family to mitigate toxic concentrations of cytosolic Na^+ and enhance K^+ uptake [18].

High Affinity Potassium Transporters (HKT), located in xylem parenchyma and root epidermal cells, are involved in controlling Na^+ transport through plant tissues [19, 20]. HKT1, a high affinity K^+ transporter, was first isolated and described in wheat roots [21] and was later shown to mediate Na^+/K^+ transport when expressed in *Xenopus* oocytes [22]. In Arabidopsis, AtHKT1;1 functions as a selective Na^+ transporter [23] and is important in retrieving Na^+ from the xylem in the roots, reducing Na^+ transport to the shoots [24, 25]. Inward-rectifying K^+ channels, such as AKT1 and KAT1, play a major role in alleviating Na^+ accumulation in plant tissues by mediating K^+ uptake and transport into plant cells [26, 27]. The K^+ channels of the AKT/KAT

subfamily are differentially expressed in root and leaf tissues and show high selectivity for K^+ over other monovalent cations [28].

Symbiotic interactions of plants with beneficial microbes such as plant growth promoting bacteria (PGPB) and endophytic fungi can alleviate abiotic stress and increase the tolerance of plants to adverse growth conditions [29, 30]. Since the development of salt tolerant crops by either conventional breeding or contemporary genetic engineering techniques is, if achievable, a long-term and costly process, the use of microorganisms that mitigate abiotic stress hold promise as a strategy to improve plant growth and crop productivity under salt stress conditions [31, 32]. *P. indica*, a plant root endophytic fungus, mimics the capabilities of arbuscular mycorrhizal fungi in supporting the growth of host plants [33]. *P. indica* can colonize plant roots of barley, Chinese cabbage, maize, rice, sorghum, tobacco, tomato [34], and Arabidopsis [35]. However, unlike mycorrhizal fungi, *P. indica* can grow in axenic culture.

The symbiosis between *P. indica* and roots of colonized plants has been shown to improve plant tolerance to different abiotic and biotic stresses [36]. *P. indica* has been reported to alter plant specialized metabolites, increase nutrient uptake, and promote plant growth [37, 38]. In addition, *P. indica* improves drought tolerance to Arabidopsis and barley [39,40] and was shown to alleviate salt stress in barley and rice by increasing the activity of detoxifying enzymes and photosynthetic pigment content in colonized plants [41,42]. Recently it has been shown that *P. indica* *PiHOG1* gene plays an important role in conferring salinity tolerance to rice plant [43].

In this study, we investigated the impact of *P. indica* colonization on the growth of Arabidopsis plants under normal and salt stress conditions. Our data show that *P. indica* colonization of Arabidopsis plants increases plant growth both under normal and salt stress conditions. Several plant growth parameters, such as fresh and dry mass of the plant, lateral roots density and chlorophyll content, were improved in *P. indica* colonized plants compared to control plants. Our data also point to changes in the ion channel expression as a likely reason for the improved growth under salt stress conferred by *P. indica*.

2. Materials and Methods

2.1. Plant material and growth conditions

Arabidopsis thaliana Columbia (Col-0), *gll* (SAIL_1149_D03) and *gll-HKT1::AtHKT1;1* no. 17-3 (kindly provided by Dr. Paul M Hasegawa, Purdue University) [44] lines were used in

this study. *Arabidopsis thaliana* Col-0 is the wild type and *gll* (SAIL_1149_D03), is a T-DNA insertion line that contains a T-DNA integration between the first and second exon of the *GLI* gene and *gll-HKT1::AtHKT1;1* is the overexpression line in *gll* background in which *Arabidopsis* HKT1 native promoter fused to *AtHKT1;1*. Seeds were sterilized and stratified on the ½ MS medium containing 1% sucrose for two days at 4 °C in the dark. Plates were placed vertically in the plant growth chamber at 22/18 °C day/night cycle under long day conditions (16h light/ 8h dark) at 60% relative humidity. For growing plants in soil, seven-day-old germinated seedlings from plates were transplanted to pots containing Agro-Max soil (Agro Genesis Pte. Ltd. www.agro-genesis.com) and grown under the long day conditions (16h light/ 8h dark) at 60% relative humidity in a growth chamber. No additional mineral fertilizers were added to plants in the pot.

2. 2. Fungal material and root colonization

P. indica was propagated on KM medium [45] and incubated at 22-24 °C in the dark for two weeks. For liquid culture, 500 ml Erlenmeyer flasks with 250 ml liquid KM medium were used and inoculated with five fungal plugs (5 millimeters in diameter reach) and incubated for ten days at 28 °C at 150 rpm on a rotary shaker. Co-cultivation of *P. indica* with roots of *Arabidopsis* plants was carried out using a modified PNM medium as described [46]. For *P. indica* inoculation in soil experiment, a liquid culture of *P. indica* was harvested by centrifugation and mycelium was washed three times with sterilized water. One gram of fresh mycelium was added into 100 ml of sterilized water and fragmented by blending for one minute. Two ml of mycelium suspension was directly injected to root rhizosphere at three and seven days after transplanting, while water was used as a mock treatment in soil experiment. The mycelium suspension contained 1.2×10^6 spores per ml.

2. 3. Salt treatments

Five-days-old germinated seedlings were transferred to PNM medium containing 100 mM NaCl, while 0 mM NaCl was used as a control. For soil experiment, 15 days after transplanting, trays of inoculated and non-inoculated plants were soaked in water contains 75 mM NaCl for three days. On the fourth day, trays were soaked in 150 mM NaCl and harvested after seven days for downstream analysis. To evaluate the impact of *P. indica* on *Arabidopsis* growth under salt stress,

soil-grown seedlings were inoculated with a liquid culture of *P. indica*. Different salt stress tolerance determining parameters like plant growth, biomass, ion homeostasis were quantified.

2. 4. Root architecture and monitoring of *P. indica* colonization

Primary root length and lateral root density were determined in seedlings grown on PNM medium with *P. indica* for seven days under normal and salt stress conditions. Pictures of seedlings were scanned using a EPSON 3170 scanner at 600 dpi and analyzed using ImageJ (NIH Image).

Root segments of colonized plants were stained with the chitin-specific dyes WGA-AF 488 (Molecular Probes, Karlsruhe, Germany) as described [47]. For counterstaining, ProLong® Gold Antifade Mountant with DAPI (4',6-Diamidino-2-phenylindole) was used to stain intact nuclei according to manufacturer's protocol (Life technology). Confocal fluorescence images were recorded on a multichannel TCS SP2 confocal microscope (Leica, Bensheim, Germany). WGA-AF 488 was excited with a 488 nm laser line and detected at 500–540 nm; while DAPI was excited with a 350 nm laser line and detected at 460 nm.

2. 5. Chlorophyll and anthocyanin quantification

Half gram fresh leaves from *P. indica* colonized and non-colonized plants grown at 150 mM NaCl and 0 mM control plants were collected and extracted in *N,N*-Dimethylformamide at 4 °C in darkness (overnight). The concentration of chlorophyll was determined as described [48]. Anthocyanin was measured as described [49]. Briefly, seedlings were soaked in 600 µl 1% HCl, diluted in methanol (v/v) for acidification, and incubated for 24 h at 4 °C. After addition of 400 µl ddH₂O and 1 ml chloroform, the anthocyanin contents in aqueous phase were measured at 530 nm and 657 nm and calculated using equation: $(A_{530} - A_{657}) / Fw$.

2. 6. Determination of Na⁺ and K⁺ concentrations

Arabidopsis plants were harvested and dried at 80 °C in oven for 24 hours. Dried tissue was digested in 1% HNO₃ overnight at 60 °C [8]. Na⁺ and K⁺ concentrations were determined with a flame photometer, Model 425 (Sherwood Scientific LTD, Cambridge, UK).

2. 7. Nucleic acid extraction and quantitative real-time PCR analysis

Genomic DNA of *Arabidopsis* roots and *P. indica* was extracted from ~100 mg root material using Plant DNeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was extracted using Qiagen RNeasy Kit, and one μg of total RNA was treated with DNaseI and reverse-transcribed to cDNA using oligo (dT) 20 primer and Super Script III reverse transcriptase (Invitrogen, Paisley, UK) following the manufacturer's instructions. Quantitative Real-time PCR was performed using a Biorad Thermocycler model Step One Plus (BioRad; www.biorad.com). For amplification, Absolute SYBR Green Mater Mix (Applied Biosystems; www.lifetechnologies.com) was used in a final volume of 20 μl . The Cyclor was programmed as follows: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 1 min and then 95 °C for 15 s. The $2^{-\Delta\text{Ct}}$ method was used to calculate the difference in expression of chosen genes. All experiments were repeated three times, and mean values, and standard errors were determined from the three independent biological replicates. The significance of differences between data sets was evaluated using paired student's *t-test*. PCR primer sequences are listed in Supplementary Table S1.

2. 8. Statistical analysis

The Student's *t-test* was used for all experiments that displayed a normal distribution based on the Kolmogorov-Smirnov test. The significance of differences between data sets was evaluated using paired student's *t-test*.

3. Results

3. 1. *P. indica* improves *Arabidopsis* growth under normal and salt stress conditions

The root endophytic fungus *P. indica* was previously shown to enhance barley and rice growth under normal and salt stress conditions [42,43,50]. To evaluate the impact of *P. indica* on *Arabidopsis* growth, soil-grown seedlings were inoculated with the liquid culture of this fungus. The presence of *P. indica* within *Arabidopsis* roots under high-salt conditions was confirmed by PCR amplification of *P. indica* Intragenic Transcribed Spacer (*Pi* ITS) locus at 10, 15 and 21 days after inoculation (Fig. S1A). *P. indica* chitin-specific staining shows fungal mycelium covering the root surface and penetrating into the cortex five days after inoculation (Fig. S1B). As can be seen in (Fig. 1A), the application of salt stress generally decreased the growth rate of both non-colonized control and colonized plants. Notably, *P. indica* colonization significantly enhanced

plant growth under non-stress and salt-stress conditions, as revealed by fresh mass, dry mass and chlorophyll content measurements (Fig. 1A-E). Moreover, colonized plants were less affected by salt stress than non-colonized plants (Fig. 1A-D). In addition, *P. indica* colonization led to a markedly lower anthocyanin content compared to non-colonized control plants both under normal and salt stress conditions (Fig. 1E).

P. indica also increased Arabidopsis root branching under non-stressed conditions (Fig 2A and B) [51], hence, we investigated whether *P. indica* colonization results in the same alteration under salt stress. The presence of 100 mM NaCl led to a general inhibition of root growth in both colonized and non-colonized Arabidopsis plants grown on PNM agar plates (Fig. 2A-D). However, the negative effect of 100 mM salt on the number of lateral roots was significantly less pronounced in colonized plants compared to non-colonized plants (Fig. 2A-B). Consistent with the improvement of lateral root density, *P. indica* colonization also increased plant fresh mass under both normal and salt stress conditions (Fig. 2C). No difference was observed in primary root length between colonized and non-colonized plants in either normal or salt stressed conditions (Fig. 2D).

3. 2. *P. indica* modulates the transcript levels of *AtHKT1;1*

To investigate the impact of *P. indica* on salt stress tolerance at the molecular level, we measured the transcript levels of several genes known to encode proteins involved in Na⁺ and K⁺ homeostasis and of the abiotic stress marker gene *Relative to Desiccation A (RD29a)* in both colonized and non-colonized Col-0 Arabidopsis plants in the presence and absence of salt stress. Expression of the stress marker gene, *RD29a* is elevated in shoots and roots of salt treated Arabidopsis in the soil, although less markedly in colonized seedlings (Fig. 3A- B). Interestingly, the expression level of *AtHKT1;1* in salt-grown roots was markedly higher in colonized plants than non-colonized plants (Fig. 4A). Notably, *P. indica* colonization in the absence of salt stress did not change the expression level of *AtHKT1;1* in roots (Fig. 4A). Several other ion homeostasis genes such as *NHX1*, *NHX2*, *SOS1*, *SOS2* and *SOS3* showed similar transcript levels in colonized and non-colonized plants when grown in the presence of salt (Fig. S2).

To confirm the results of *AtHKT1;1* expression, we co-cultivated *P. indica* with different Arabidopsis lines (*gll* and *gll-HKT1::AtHKT1;1* [44]) on PNM agar plates in the presence and absence of salt. The line *gll-HKT1::AtHKT1;1* expresses an additional copy of *AtHKT1;1* under the control of the native promoter. Interestingly, we observed over four and eight fold increases in

the expression level of *AtHKT1;1* in the roots of colonized *gll* and *gll-HKT1::AtHKT1;1* seedlings compared to non-colonized control plants (Fig. 4B). Upon *P. indica* colonization, we also observed higher lateral root density in all tested lines under normal and salt stress conditions. Furthermore, this increase in the lateral root density was more pronounced in *AtHKT1;1 (gll-HKT1::AtHKT1;1)* line (Fig. 4C-D).

3. 3. Colonization of *P. indica* alters Na⁺ and K⁺ contents in Arabidopsis

AtHKT1;1 has been shown to be responsible for Na⁺ retrieval from shoots [24] and high expression of *AtHKT1;1* can modulate Na⁺/K⁺ homeostasis. We quantified Na⁺ and K⁺ contents in shoots and roots of WT and *gll-HKT1::AtHKT1;1* plants grown on salt-containing PNM plates. Colonized Arabidopsis seedlings in both lines had a lower Na⁺ and a higher K⁺ content in shoots and roots when grown under salt stress compared to non-colonized plants (Fig. 5A and B). Notably, we observed significantly lower Na⁺ and higher K⁺ content in shoots and roots of colonized *gll-HKT1::AtHKT1;1*, compared to WT plants (Fig. 5A and B). These data indicate that *P. indica* colonization is affecting the Na⁺ / K⁺ in the presence of high salt concentrations.

3. 4. *P. indica* colonization modulates transcript levels of K⁺ channels

The observed high K⁺ content may be the result of enhanced inwards K⁺ channel activity. To check whether *P. indica* colonization affects the transcript levels of such channels, we analyzed the expression of the two main K⁺ channels *KAT1* and *KAT2*. Our results indicate differential expression of *KAT1* and *KAT2* in roots and shoots of colonized plants. We observed a two-fold increase in *KAT1* and *KAT2* transcript levels in shoots of colonized *gll-HKT1::AtHKT1;1* plants under salt stress, compared to non-colonized plants (Fig. 6A and B). This induction was much higher in roots of *gll-HKT1::AtHKT1;1* colonized plants (Fig. 6 C and D).

4. Discussion

P. indica has been shown to improve plant growth and to increase plant tolerance to different abiotic and biotic stress factors [36, 39]. Enhancement of plant growth by *P. indica* under salt stress has been reported for several plant species [52, 53], but not for Arabidopsis. Most of these studies are focused on initial salt stress signaling and metabolite production. In addition to initial phase of salt stress that resembles drought stress, a continuous salt stress for a longer period

also results in ion toxicity caused by the accumulation of sodium and chloride ions, which is accompanied by a lower potassium uptake impacting the activity of several enzymes and impeding plant's growth. However, the exact mechanism by which *P. indica* improves plant's growth under persistent salt stress is not understood. Here, we demonstrated that *P. indica* enhanced the growth of Arabidopsis under salt stress and investigated the impact of this fungus on Na⁺ and K⁺ content, and the expression levels of several ion channels which play a central role in Na⁺/K⁺ homeostasis. Our results suggest that *P. indica* colonization modulates the expression levels of the major Na⁺ and K⁺ ion channels *HKT1*, *KAT1* and *KAT2*, which may allow to maintain a balanced ion homeostasis of Na⁺ and K⁺ under salt stress conditions and be a reason for the improved plant growth under salt stress conditions observed upon *P. indica* colonization. *RD29a* is commonly used as stress reporter gene. As a fast response to salt-stress, *RD29a* expression is activated by binding of salt-stress-specific transcription factors to ABA responsive *cis*-acting elements (ABRE) in its promoter [54]. The lower induction of *RD29a* in colonized plants under salt stress indicates that *P. indica* can quickly alleviate salt-stress.

The addition of salt to growth media clearly impaired Arabidopsis growth and photosynthetic capacity. These effects were counteracted by *P. indica* colonization, resulting in a higher biomass and increased chlorophyll content both in non-stressed and stressed plants. The increase in chlorophyll content, stimulated by *P. indica*, under salt stress explains the common observation that *P. indica* colonized plants exhibit greater fresh and dry weight [55]. Previous studies showed that *P. indica* colonization triggers several stress-tolerance mechanisms in host plants [41,42,56], including an increase in the levels of detoxifying and antioxidant-forming enzymes, in addition to several indirect mechanisms [57,58]. For instance, *P. indica* induces leaf dehydroascorbate reductase (DHAR) activity [41] and increases ascorbic acid content in colonized barley roots under salt stress conditions [56]. Such mechanisms may also contribute to the salt stress tolerance observed in Arabidopsis, as indicated by improved growth and by reduced symptoms of stress, such as less anthocyanin accumulation.

The increase of root branching and plant biomass under stress condition is one of the mechanisms to cope with salt stress since it enlarges the surface area for the absorbance of different ions like K⁺ and Ca²⁺ [59]. Consistent with this observation, plants colonized with *P. indica* and exposed to salt stress have more lateral roots, and this higher lateral root density is more pronounced in Arabidopsis plants that have an additional copy of *HKT1*. However, colonization

of *P. indica* did not increase primary root length. The insensitivity of primary root to *P. indica* colonization was also observed previously in Arabidopsis [51].

It has been shown that barley plants co-cultivated with *P. indica* accumulate higher K^+ and lower Na^+ in leaves under salt stress, compared to control plants [50]. Our results indicate that *P. indica* colonization alters Na^+/K^+ homeostasis of Arabidopsis grown in soil under salt stress conditions. To shed light on the molecular mechanism of this alteration, we investigated the expression of *AtHKT1;1* that plays a major role in Na^+ homeostasis. Our results show that *P. indica* colonization leads to a three-fold higher *AtHKT1;1* transcript levels in roots of colonized plants compared with non-colonized plants. Consistent with this result, expression analysis in the *gll-HKT::AtHKT1;1* showed a correspondingly higher expression of *AtHKT1;1* transcript levels. *AtHKT1;1* has a high affinity for Na^+ [23, 22]. In connection, *AtHKT1;1* induction did not allow Na^+ transport to the shoots and, hence, alleviates the negative effects of salt stress on shoot growth [44]. The capability of soil microbes to confer salt tolerance to Arabidopsis through regulating *AtHKT1;1* transcript level was previously reported [60]. In this respect our results are different from those of Zhang et al. [60] who showed that co-cultivation of GB03 bacteria with Arabidopsis leads to a lower Na^+ content and an increase in *AtHKT1;1* transcript levels in shoots of colonized plants under salt stress condition. However, our results are consistent with the detailed study of cells/tissue specific *AtHKT1;1* expression in Arabidopsis showing that *AtHKT1;1* expression is restricted to root stellar cells and functions in the retrieval of sodium ion from shoots [61]. It has also been shown that induction of *ThHKT1;2* in roots of *Theilungiella salsuginea* (a relative of Arabidopsis) have a major role in salt stress tolerance [59].

The expression of HKTs that are responsible for K^+ homeostasis is induced by salt stress or by K^+ deficiency [62]. Under salt stress, plants become deficient in K^+ and try to compensate this deficiency by increasing the K^+ import capacity or to release K^+ stored in different cell compartments [63, 64]. The measurement of *KAT1* and *KAT2* transcript levels indicates that both of these inward rectifying K^+ channels expression was induced in colonized plants. This induction was more pronounced in *gll-HKT::AtHKT1;1* roots than in wild-type. Thus, the enhancement of plant growth under salt stress conditions may be caused by the combined modulation of the transcript levels of the three channels *HKT1*, *KAT1* and *KAT2*.

Plants can tolerate elevated levels of Na^+ by proper redistribution of Na^+ to different plant organs and cell compartments and if K^+ uptake in the presence of salt stress is well maintained

[65]. However, we cannot exclude the possibility that *P. indica* is helping plants in sodium compartmentalization, though *P. indica* colonization did not impact the transcript levels of the NHXs and SOSs pathway genes (Supplementary Fig. S2). In conclusion, our results indicate that *P. indica* colonization improves plant growth under normal and salt stress conditions, leads to a decrease in Na⁺ content in shoots and roots of colonized plants under salt stress, and alters the Na⁺/K⁺ homeostasis by increasing the *AtHKT1;1* and *KAT1* and *KAT2* transcript levels. It can be assumed that these alterations reduce the adverse effects of high salt concentrations.

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Figures and legends

Figure 1. Impact of *P. indica* colonization on *Arabidopsis* (Col-0) growth in soil in the presence and absence of 150 mM NaCl. The appearance of inoculated and control plants after two weeks growth with or without salt stress is shown in (A), fresh weight in (B), dry weight in (C), total chlorophyll content in (D), and anthocyanin content in (E). Data are mean \pm SE (n=12). ** represent significant difference between treatments at $P < 0.01$ based on *t*-tests in three independent experiments.

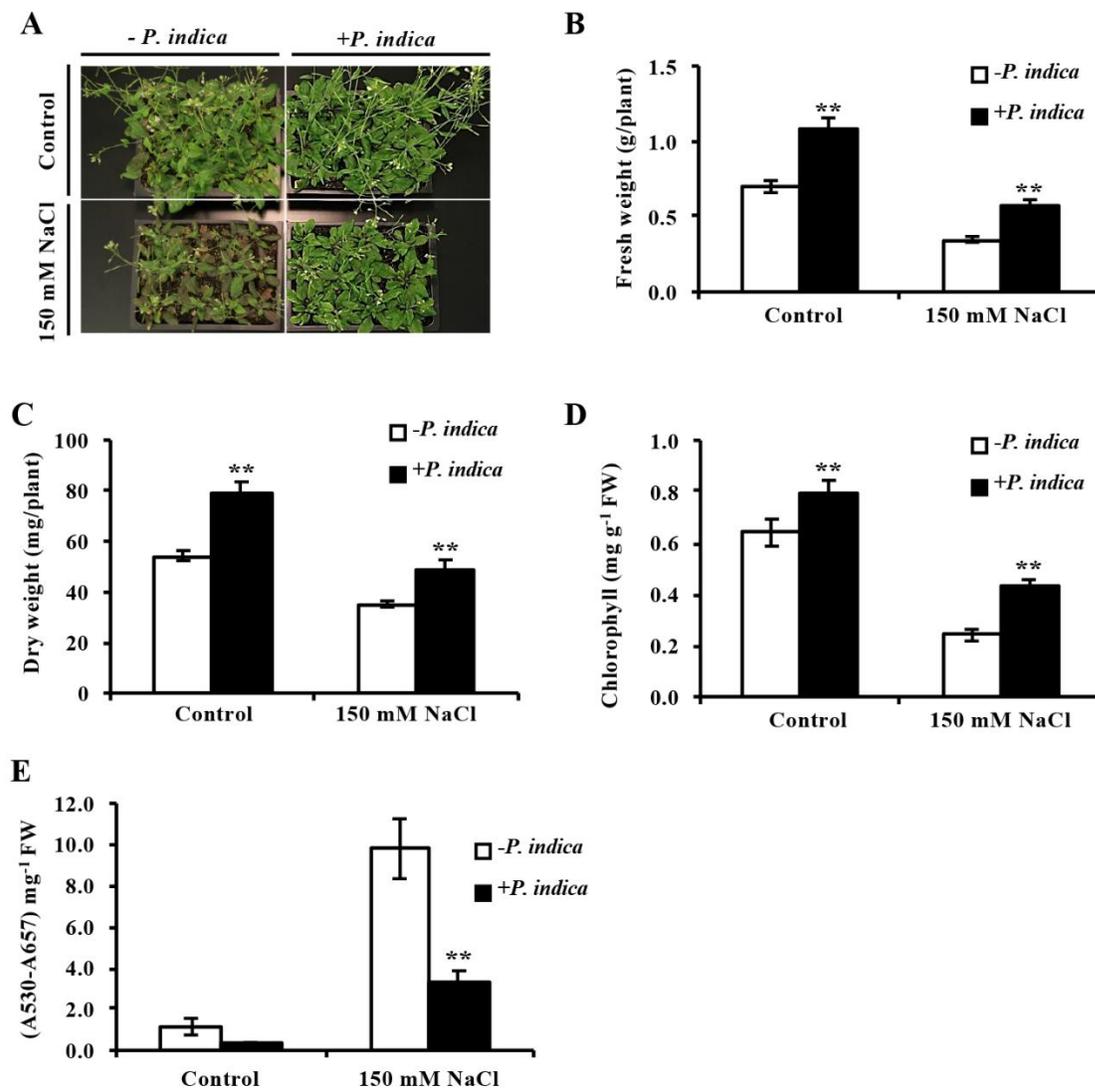


Figure 2. Relative mRNA expressions of *RD29a*. Experiments were performed on shoot and root from three-week-old *Arabidopsis* plants in soil. Relative mRNA expression level of *RD29a* in shoot (A) and root (B). Data are mean \pm SE (n=3). ** represent the significant difference between treatments at $P < 0.01$, by *t*-test, based on 3 independent experiments.

Figure 2.

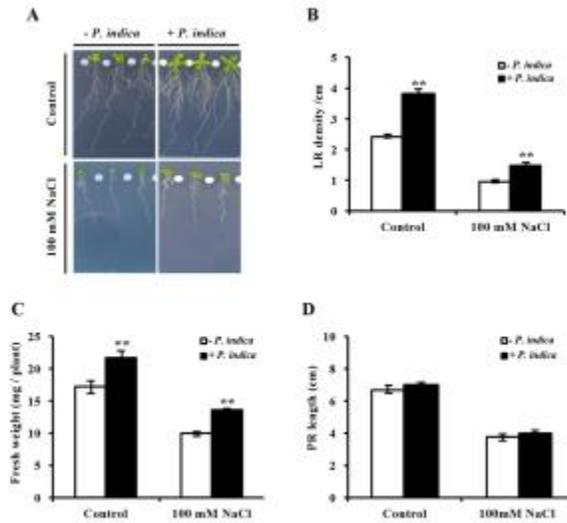


Figure 3. *P. indica* colonization influences root morphology of *Arabidopsis* grown under salt stress. Seedlings were grown vertically for seven days on modified PNM medium containing 0 mM or 100 mM NaCl. The appearance of the seedlings is shown in (A), while lateral root (LR) density in (B), fresh weight in (C) and primary root (PR) length in (D). Data are mean \pm SE (n=16). ** represent the significant differences between treatments at $P < 0.0$ based on *t*-tests in three independent experiments.

Figure 3.

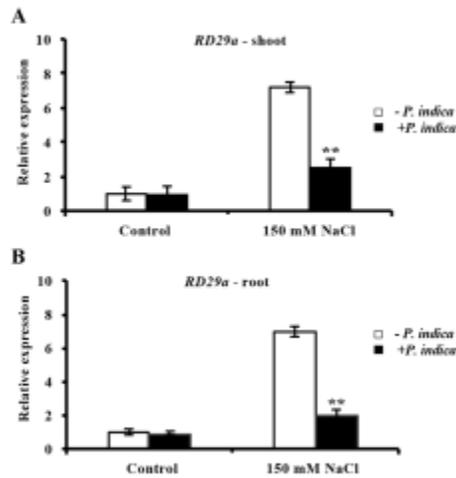


Fig. 4. *AtHKT1;1* relative expression and root architecture of Arabidopsis (Col-0) wild type and the two mutants *gl1* and *HKT1;1-OX* (*gl1-HKT1::AtHKT1;1*) when colonized with *P. indica* under salt stress condition. Seedlings were grown vertically for seven days on modified PNM medium containing 0 mM or 100 mM NaCl. Relative mRNA expressions of *HKT1;1* in roots of Col-0 Arabidopsis (A), *gl1* and *HKT1;1-OX* (*gl1-HKT1::AtHKT1;1* complimentary line) (B). Phenotyping of *gl1* and *gl1-HKT1::AtHKT1;1* seedlings under salt stress condition, with or without *P. indica* (C), Lateral root (LR) density (D). ** represent significant differences between treatments at $P < 0.01$, respectively, based on *t*-tests in three independent experiments.

Figure 4.

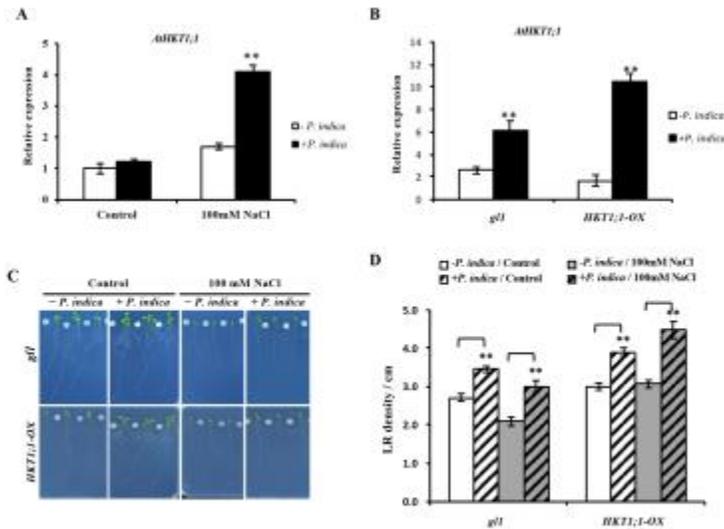


Figure 5. Na⁺ and K⁺ content in shoots (A) and roots (B) of WT (*gll*) and *HKT1;1-OX* (*gll-HKT1::AtHKT1;1*) Arabidopsis seedlings. Seedlings were grown vertically for seven days on modified PNM medium containing 100 mM NaCl with or without *P. indica*. ** represent significant differences between treatments at P<0.01 based on *t*-tests in three independent experiments.

Figure 5.

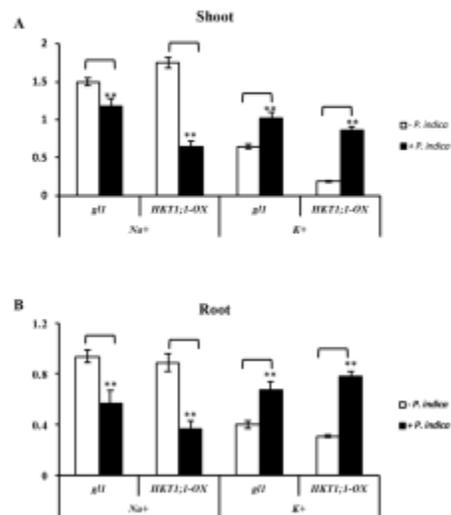


Figure 6. Relative expression of *KAT1* and *KAT2* mRNA level. WT (*gll*) and HKT1;1-OX (*gll-HKT1::AtHKT1;1*) plants grown vertically for seven days on PNM medium containing 0mM or 100 mM NaCl with or without *P. indica*. *KAT1* and *KAT2* expression in shoots (A, B) and roots (C, D). The mRNA levels are expressed as a ratio of the value measured in the colonized plant tissue to that in the corresponding non-colonized control tissue. Data are mean \pm SE (n=3) based on three independent experiments.

Figure 6.

