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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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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 *gl1-HKT:AtHKT1;1* that expresses an additional *AtHKT1;1* copy driven by the native promoter. Colonization of the *gl1-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

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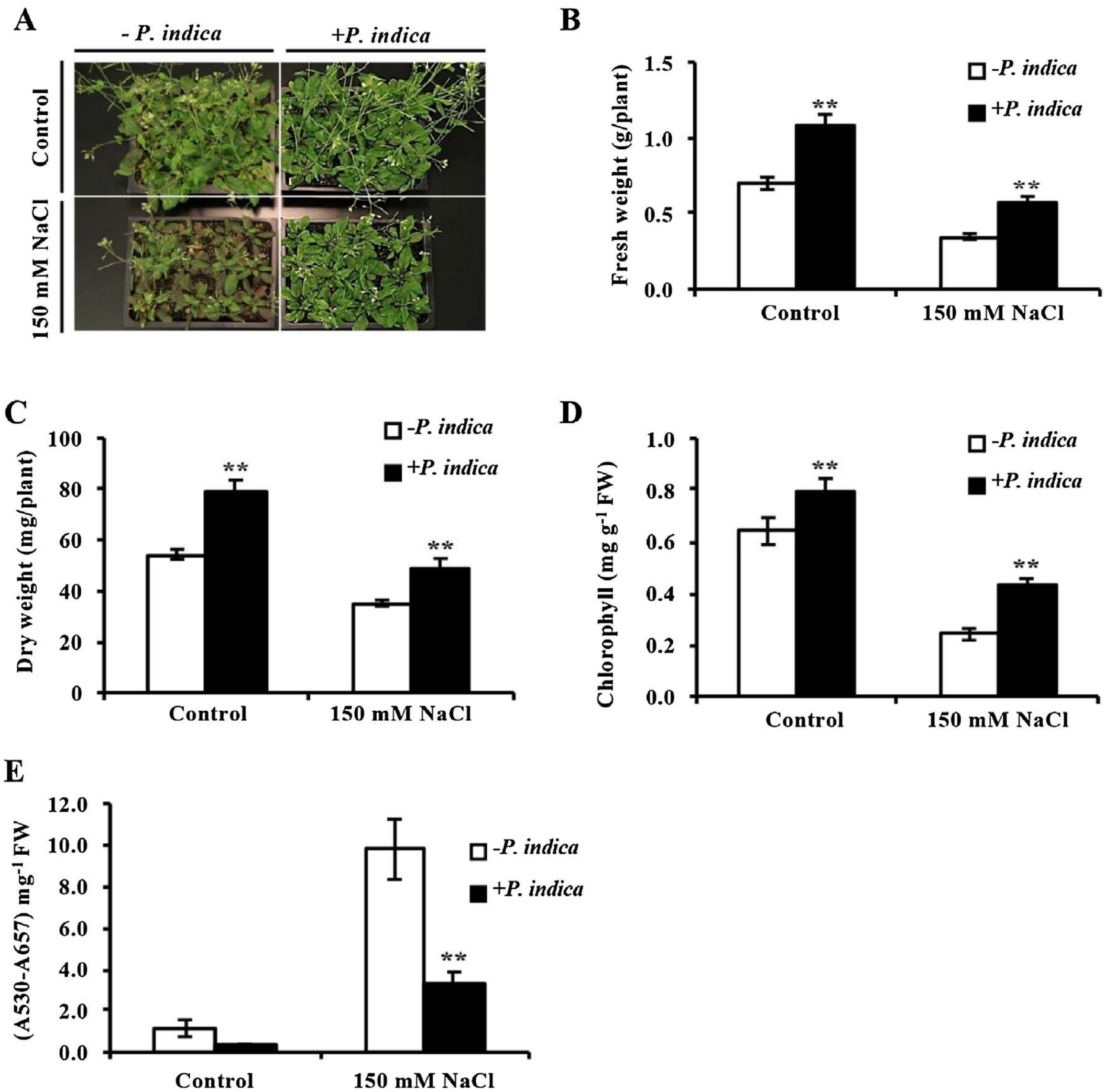

Fig. 1. Impact of *P. indica* colonization on Arabidopsis (Col-0) growth in soil in the presence and absence of 150 mM NaCl. The appearance of inoculated and control plants after two weeks growth with or without salt stress is shown in (A), fresh weight in (B), dry weight in (C), total chlorophyll content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

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