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Staphylococcus sciuri SAT-17 improved the growth of salt stressed maize (*Zea mays* L.) by modulated expression of stress responsive genes and anti-oxidative defence mechanisms

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Plant growth promoting rhizobacteria (PGPRs), being the chief components of rhizosphere microbiota, are highly beneficial for plant growth and production. PGPR mediated salt stress tolerance is an intricate process which is governed by plant-bacterial interactions at molecular level. In an earlier study, positive impact of *Staphylococcus scuiri* SAT-17 inoculation on maize growth and physiology, under saline conditions, has been reported. To further elucidate this interaction at molecular level, salt tolerant and sensitive (FH-988 and FH-1137) maize genotypes were raised with or without inoculation of SAT-17 in the absence or presence of 120 mM NaCl. Expression analysis of various salt responsive genes (*NHX1, H*$^+$*-PPase, SOS1, HKT1*, *Cat1* and *APX1*) was carried out. Nutrient acquisition and translocation patterns along with few biochemical parameters were also studied. The results indicated that Na^+ vacuolar sequestration and enhanced antioxidant enzyme activities might have accounted for the better salt tolerance potential of genotype FH-988 under salt stress. In contrast, genotype FH-1137 exhibited reduced Na^+ vacuolar sequestration and antioxidant enzyme activities, thereby resulting in severe Na^+ toxicity and oxidative damage under salt stress. Inoculation of SAT-17 ameliorated the salinity damage by maintaining optimum nutrient root-shoot translocation which in turn resulted in better Na^+ homeostasis and reactive oxygen species scavenging. The results highlighted the contribution of several ion transporters, SOS pathway and antioxidant machinery in imparting salt stress tolerance in maize. The findings can be useful for devising strategies for cultivation of salt sensitive maize genotypes in saline areas thereby contributing in sustainable agricultural development.

Keywords: Antioxidants, gene regulation, ion homeostasis, Na^+/H^+ transporters, plant growth promoting rhizobacteria.

INTRODUCTION

Climate change has aggravated the adversity of abiotic stresses and negatively affected the agricultural production in this anthropocene era. Along with this, high salt concentration in land and water has changed fertile lands to deserted fields ultimately resulting in barren lands (Ilangumaran and Smith, 2017). The salt effected area is increasing alarmingly, 10 % by every year, and may occupy more than half of the arable land till 2050 (Shrivastava and Kumar, 2015). Being sessile organisms, plants are more prone and cannot extricate the detrimental effects of salinity (Zhou *et al.*, 2017). High salt concentration initially decreases soil water potential, thereby causing less water absorption by the roots (Munns and Tester, 2008, Hill *et al.*, 2016). Prolonged salt exposure results in ionic toxicity which impairs metabolic pathways, photosynthetic reduction and ultimately affecting crop yield. Plants avoid high Na^+ accretion in their shoots by minimizing its influx or maximizing its efflux. This is carried out at organ, tissue as well as cellular level by the help of diverse Na^+ transporters, responsible for maintaining ion homeostasis (Hill *et al.*, 2013) by reducing Na^+ loading into the xylem, restraining Na^+ and regulating vacuolar compartmentalization. These transporters mainly include members of SOS pathway, NHX and HKT families (Shabala *et al.*, 2014). The *HKT* gene family includes Na^+ (class I) and Na^+/K^+ (class II) transporters in various plants (Hill *et al.*, 2013). Tonoplast localized NHX1 sequester excess Na^+ into the vacuole, while plasma membrane located SOS1 pumps Na^+ in apoplast. These antiporters are powered by electrochemical H^+-gradient created by tonoplast H^+-

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pyrophosphatase/cellular membrane H^+-ATPase (Bharti *et al.*, 2016).

Glycophytes, in particular, are extremely sensitive to elevated Na^+ concentrations (Kronzucker and Britto, 2011). From roots, Na^+ moves into xylem through transpirational stream towards plant leaves. Leaf Na^+ accumulation is also facilitated by its restricted redistribution to roots (Hill *et al.*, 2016). As leaf is the prime hub of major metabolic processes, therefore it must be shielded from Na^+ prone injury. Hence, under saline conditions, leaf Na^+ should be recirculated to assure normal metabolic processes (Assaha *et al.*, 2017). HKT and SOS1, present in monocot sheath, assist in decreasing Na^+ quantity in leaf by its retrieval from xylem into associated parenchyma cells (Horie *et al.*, 2012). Furthermore, Na^+ vacuolar sequestration is also carried out primarily by antiporters of the NHX family. Interestingly, another promising alternate mechanism for Na^+ sequestration through vesicle trafficking has been suggested in the recent past (Garcia de la Garma *et al.*, 2015).

Plant growth promoting rhizobacteria (PGPR) hamper salt uptake in plants by confining cations in their exopolysaccharide matrix, changing root architecture by extended rhizosheaths, and modulating gene expression profiles of ion transporters. PGPR assist in maintaining K^+/Na^+ ratios and ion homeostasis by minimizing leaf Na^+ accretion and by boosting Na^+ exclusion through roots by upregulation of ion transporters (Ilangumaran and Smith, 2017). Under saline conditions, enhanced Na^+ exclusion along with K^+ uptake has been examined in *Zea mays* (L.) after *Azotobacter* inoculation (Rojas-Tapias *et al.*, 2012). In *Arabidopsis thaliana, Burkholderia phytofirmans* PsJN inoculated plants exhibited enhanced tolerance to salt stress. Transcriptomic analysis revealed altered expression pattern of various ion transporters, including *SOS1, AKT1, NHX2*, and *HKT1*, in inoculated plants which might accounts for improved salt tolerance (Pinedo *et al.*, 2015). Reduced Na^+ accumulation and enhanced expression of *PtSOS1* and *PtHKT1* genes in *Puccinellia tenuiflora* has also been observed after *Bacillus subtilis* GB03 inoculation under salt stress (Niu *et al.*, 2016).

Staphylococcus sciuri SAT-17 has been isolated from Kallar grass rhizosphere and its positive effects, in terms of improved plant growth and physiology under induced saline conditions, has been studied in maize genotype FH-922 (Akram *et al.*, 2016). The present study was planned to further elucidate plant-microbe interactions and unravel the changes induced by strain SAT-17 by studying the expression of *NHX1, SOS1, H^+-PPase, HKT1, CAT1, APX1* genes in a salt-tolerant (FH-988) and salt-sensitive (FH-1137) genotype. The molecular responses of each of the genotype were also correlated with various physiological attributes like chlorophyll and H_2O_2 content, lipid peroxidation, and antioxidative enzyme activities including catalase and peroxidase. The study results improved our current understanding of plant-microbe interactions under abiotic stress conditions. Based on the results of the current study, experiments can be planned and executed involving lipid profiling, functional genomics, and expression analysis of different variants of ion transporters (e.g. class I and II of HKT transporters) in maize plants having contrasting salt tolerance potential. Results of the current study and future experiments shall lead to formulation of SAT-17 as a potential bio-fertilizer for application in agriculture fields making cultivation of salt-sensitive maize genotypes possible in saline areas.

MATERIALS AND METHODS

***Culture Conditions of SAT-17*:** The bacterial strain *Staphylococcus sciuri* SAT-17 was used in this study (Akram *et al.*, 2016). SAT-17 was grown in LB medium in a shaking incubator at 37 °C for 10 h. For inoculation, bacterial cells were suspended in deionized water to obtain a final concentration of OD_{600} = 1.0.

Plant Material and Salt Stress Treatment: Seeds of two *Z. mays* genotypes, FH-988 and FH-1137, were taken from Ayub Agriculture Research Institute (AARI) Faisalabad. Experimental soil was obtained from agriculture fields located at University of the Punjab Lahore. Its texture was classified as clay loam having 0.70 g kg^{-1} total nitrogen, 5.7 mg kg^{-1} available phosphorus, 179 mg kg^{-1} available potassium, 1.34% organic matter and a pH of 7.4. After surface sterilization, seeds were soaked in SAT-17 culture or distilled water for 30 min. The soil was treated with SAT-17 culture or ddH_2O as described in an earlier study (Akram *et al.*, 2016). Pots were filled using 8 kg/pot soil and bacterial treated seeds were sown (8 seeds/pot) in soil containing SAT-17 culture, while distilled water imbibed seeds were sown in untreated soil. The seedlings were thinned to five per pot after emergence. After 15 days of thinning, 120 mM salt stress was applied by using NaCl (Rafiq *et al.*, 2020). Plants were irrigated according to the moisture requirement. The experiments were carried out in a greenhouse employing a completely randomized design (CRD) with four replicates for each treatment.

***Gene Expression Analysis*:** After 48 h of imposed salt stress, plants were harvested (Chen *et al.*, 2016). RNA extraction was performed from plant shoots by hot borate method with slight modifications (Wan and Wilkins, 1994, Khurshid *et al.*, 2017). RNase-free DNase (Thermo Fisher Scientific) was used to wipe out genomic DNA contamination. RevertAid Reverse Transcriptase (Thermo Fisher Scientific) first strand cDNA synthesis kit was used to synthesize cDNA. PCR conditions were 30 sec at 95 °C, leading to 40 cycles of 95 °C for 5 sec, annealing at 52 °C and extension at 60 °C for 30 sec each. Primers used are enlisted in Supplementary Table. Tubulin was taken as internal constitutive reference transcript for normalization of cDNA quantities and control plants

served as calibrator. Relative expression was calculated using normalized cycle threshold values by analyzing recorded fluorescent signal intensities on a Thermo Scientific PikoReal Real-Time PCR System (Livak and Schmittgen, 2001).

***Study of Physiological Attributes*:** Following physiological parameters were analyzed after harvesting (60 days after germination) the plants (Kifle and Laing, 2016).

***Estimation of Plant Biomass and Chlorophyll Content*:** Plant fresh weight was measured at the time of harvesting. For dry weight measurements, plant samples (roots and shoots) were incubated at 110 °C for 24 h. For chlorophyll content estimation, fresh leaf powder (0.5 g) was mixed with 80% acetone (Arnon, 1949). Centrifugation was carried out, for 15 min, at 10,000×g and absorbance of supernatant was monitored at 663 nm, 645 nm, and 470 nm by using Shimadzu UV-1601 spectrophotometer (Arnon, 1949).

***Estimation of Na^+ and K^+ Content*:** Plant samples (roots and shoots) were incubated at 120 °C for 24 h. Afterwards, 0.5 g of samples were added in digestion flasks having conc. H_2SO_4 and incubated overnight. After adding 35% H_2O_2, flasks were placed at 350 °C until the digestion mixture becomes transparent (Wolf, 1982). The solution mixture was filtered after adjusting final volume to 150 mL. Atomic absorption spectrophotometer (Hitachi, Model 7JO-8024, Tokyo, Japan) was used for determination of ionic (Na^+, K^+) content of plant samples.

***Estimation of H_2O_2 Content and Lipid Peroxidation*:** Plant leaves (0.5 g) were macerated in 0.1% trichloroacetic acid (TCA) leading to centrifugation for 20 min at 8,000×g. For H_2O_2 content determination, 1 mL of supernatant was mixed with 2 mL of potassium iodide (1 M) and 1 mL of phosphate buffer (10 mM). Absorbance of reaction mixture was assessed at 390 nm (Velikova *et al.*, 2000). In order to estimate lipid peroxidation level, above obtained supernatant was diluted with equivalent volume of 20% TCA having 0.5% thiobarbituric acid. Reagent mixture was incubated, for 40 min, at 95 °C. Absorbance was monitored at 532 and 600 nm after ice quenching (Heath and Packer, 1968).

***Estimation of Catalase and Peroxidase Activities*:** Fresh leaf powder (0.5 g) was mixed with 50 mM phosphate buffer of pH 7.0. After centrifugation for 15 min at 10,000×g, enzyme activities were determined using the supernatant. Catalase activity measurement was based on determining the rate of H_2O_2 content degradation by using Aebi's method (1984). Peroxidase activity determination was based on oxidation reaction between guaiacol and H_2O_2 (Chance and Maehly, 1955). Absorbance change, at 240 nm, was monitored for 2 min, with 20 sec interval. Absorbance change of 0.01 per min was interpreted as one unit of enzyme activity.

***Statistical Analysis*:** Data was analyzed by applying ANOVA appropriate for CRD by using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA). Bonferroni correction test was used to compare the differences among treatment means ($P < 0.05$).

RESULTS

***Effect on Plant Biomass and Chlorophyll Content*:** SAT-17 inoculation response under salt stress was observed in salt tolerant (FH-988) and salt sensitive genotypes (FH-1137) by analyzing several parameters, including plant fresh and dry weight along with chlorophyll content. Both FH-988 and FH-1137 genotypes showed reduction in fresh weight (~30%) under salt stress which was improved by SAT-17 inoculation (~15%) (Table 1). In terms of dry weight, salt stress as well as SAT-17 inoculation did not affect FH-988 genotype. On the other hand, in FH-1137 genotype, 25% reduction (4.3 g) in dry weight was observed by salt stress imposition which was ameliorated (up to 23%) by SAT-17 inoculation (5.3 g) (Table 1). Chlorophyll content of control plants (29.1 mg g^{-1} FW) of FH-988 genotype was reduced to 26.6 mg g^{-1} FW after salt stress (8%). Under similar conditions, chlorophyll content of FH-1137 genotype was reduced (40%) to 18.7 mg g^{-1} FW in comparison to its respective control (31.5 mg g^{-1} FW). After SAT-17 inoculation, chlorophyll content was increased to 28 mg g^{-1} FW and 25.1 mg g^{-1} FW in FH-988 (5%) and FH-1137 (34%) genotypes respectively. However, under non stress conditions, SAT-17 did not impart any significant difference on plant chlorophyll content (Table 1).

***Ion Homeostasis Effect on Na^+ and K^+ Content*:** In salt tolerant genotype (FH-988), root Na^+ content of control plants was 9.1 mg g^{-1} DW. Salt stress imposition resulted in elevated Na^+ content (18.5 mg g^{-1} DW) which was decreased to 15.1 mg g^{-1} DW after SAT-17 inoculation. While in salt sensitive genotype (FH-1137), Na^+ content in roots of control plants (8.7 mg g^{-1} DW) was increased to 22.1 mg g^{-1} DW under salt stress. However, a remarkable decrease in the content (66%) was observed after SAT-17 inoculation (Table 1). Almost similar trend was noticed while measuring Na^+ content in plant shoots (Table 1). Salt stress resulted in decreased K^+ content in roots and shoots. In FH-988 genotype, control plants were having 18.3 mg g^{-1} DW of K^+ in shoots, which was decreased to 10% by salt stress (16.4 mg g^{-1} DW). However, SAT-17 inoculation compensated the decrease in salt stressed plants (19.1 mg g^{-1} DW). In FH-1137 genotype, under salt stress, shoot K^+ content was decreased to 31% (10.3 mg g^{-1} DW) as compared to control (15.1 mg g^{-1} DW). After SAT-17 inoculation, this decrease was reduced to only 6% (14.2 mg g^{-1} DW). Almost similar trend was noticed in plant roots (Table 1).

***Effect on Ion Transporter Genes*:** Gene expression profiles of *NHX1* and *HPPase* revealed almost similar expression pattern under salt stress and salt bacterial interactions. In salt tolerant genotype (FH-988), salt stress could not impose negative effects on the expression pattern of *NHX1* and *HPPase* genes. However, expression of both genes was reduced (2.5 fold compared to control) in salt sensitive genotype (FH-1137). After SAT-17 inoculation, enhanced

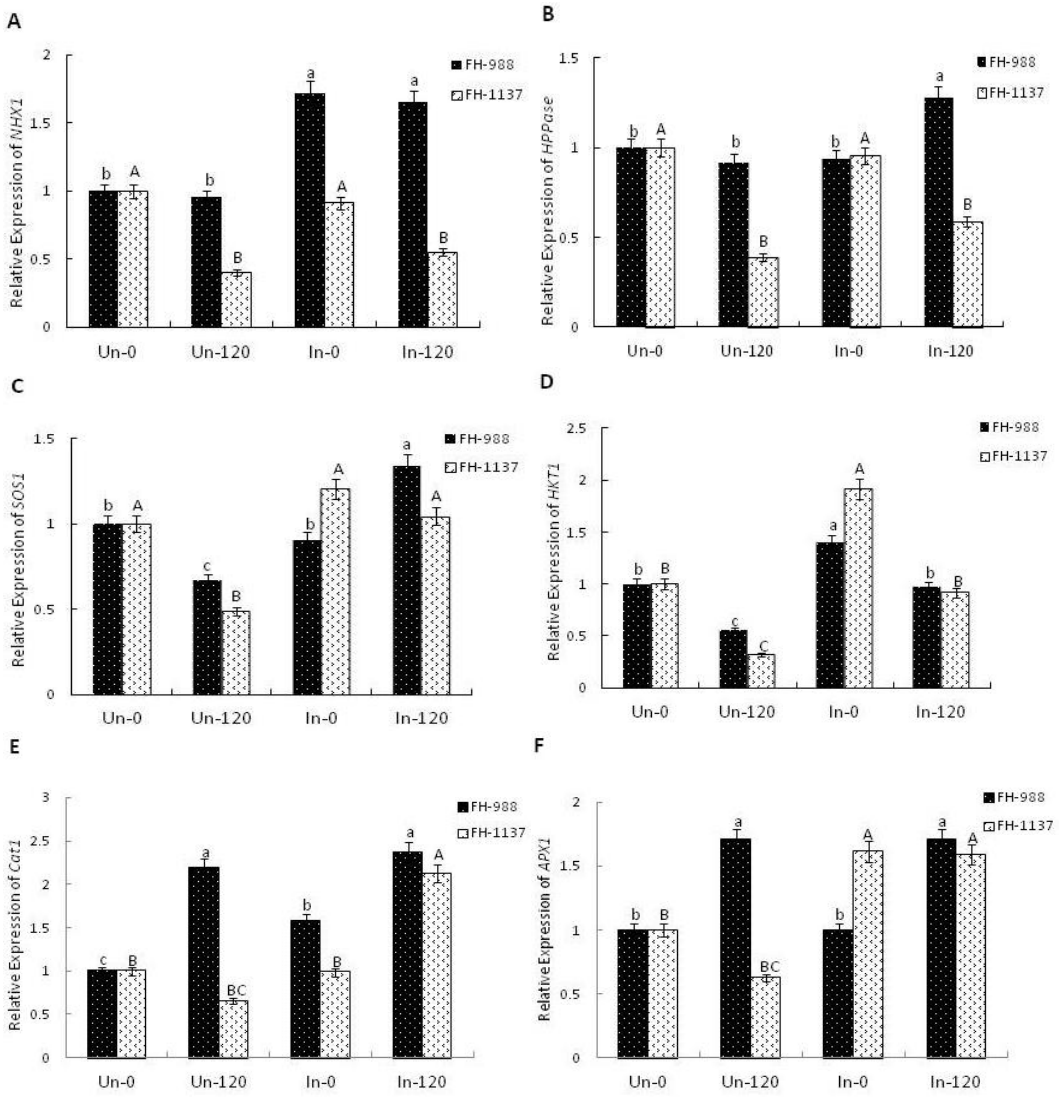

Figure 1. Expression analysis of genes involved in ion homeostasis and H_2O_2 homeostasis in *Zea mays* shoots. (A) *NHX1*, (B) *H^+-PPase*, (C) *SOS1*, (D) *HKT1*, (E) *Cat1*, and (F) *APX1*. Un-0, plants grown in the absence of *S. sciuri* SAT-17 and NaCl; Un-120, plants grown in the absence of *S. sciuri* SAT-17 but in the presence of 120 mM NaCl; In-0, plants grown in the presence of *S. sciuri* SAT-17 but absence of NaCl; In-120, plants grown in the presence of *S. sciuri* SAT-17 and 120 mM NaCl. A significant difference between various treatments is indicated by different lower (FH-988) and upper (FH-1137) case letters.

expression of *NHX1* and *HPPase* genes was observed in FH-988 genotype, while it was remain unchanged in FH-1137 (Fig. 1 A, B).

Expression analysis of *SOS1* revealed down-regulation in gene expression in FH-988 (1.5 fold) and FH-1137 (2 fold) genotypes under salt stress. However, SAT-17 inoculation resulted in ~2 fold enhanced gene expression in both genotypes (Figure 1C). In case of *HKT1*, salt stress resulted in ~2 fold decreased gene expression in FH-988 genotype, which was compensated by SAT-17 inoculation. Similarly, in FH-1137, reduced gene expression (3 folds) by stress conditions was regained after SAT-17 inoculation. Under non stress conditions, SAT-17 inoculation resulted in ~2 fold enhanced gene expression in FH-1137 genotype (Figure 1D).

***H_2O_2 Homeostasis Effect on H_2O_2 Content and Lipid Peroxidation*:** In the present study, the extent of oxidative damage was estimated by H_2O_2 production and lipid peroxidation. Under imposed stress, salt tolerant genotype (FH-988) exhibited a decrease (14%) in H_2O_2 content (10.2 ng g^{-1} FW), while the content was elevated by 50% (15.8 ng g^{-1} FW) in salt sensitive genotype (FH-1137) in comparison to their control plants (Table 1). In both genotypes, H_2O_2

production was not affected by SAT-17 inoculation under control conditions. However, SAT-17 inoculation, under salt stress, resulted in 17% decrease in H_2O_2 content in FH-1137, whereas no significant variation was detected in FH-988. Higher membrane damage (55%), in the form of TBARS, was evident in salt susceptible genotype (FH-1137) (Table 1). However, it was compensated by SAT-17 inoculation.

***Effect on Antioxidant Enzyme Activities*:** Under non stress conditions, no significant variation in antioxidant enzyme activities was observed between control and SAT-17 treated plants of both genotypes. Under salt stress, salt tolerant genotype (FH-988) exhibited an increase (~25%) in catalase (37 U mg^{-1} protein) and peroxidase (24.7 U mg^{-1} protein) activities as compared to their respective controls (Figure 2 A, B). Conversely, in salt sensitive genotype (FH-1137), catalase (20 U mg^{-1} protein) and peroxidase (18 U mg^{-1} protein) activities were reduced (15-25%) under same conditions. However, SAT-17 inoculation resulted in 20-30% enhanced enzyme activities in FH-1137.

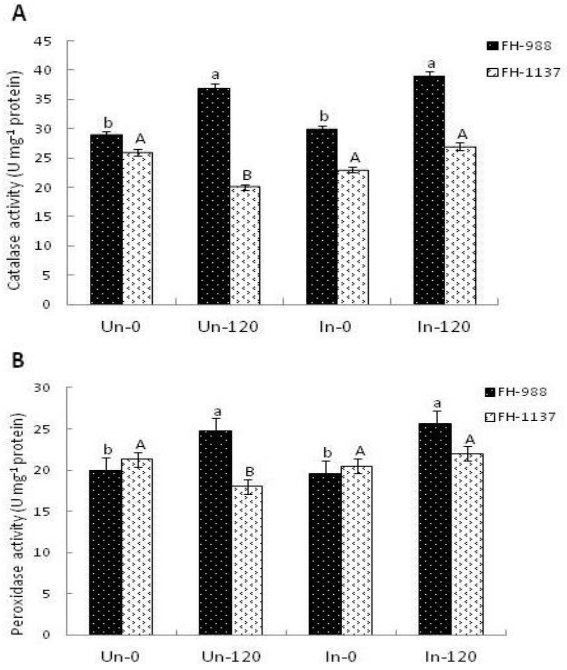

Figure 2. Determination of enzyme activities involved in H_2O_2 homeostasis in *Zea mays* shoots. (A) Catalase and (B) peroxidase activities. Un-0, plants grown in the absence of *S. sciuri* SAT-17 and NaCl; Un-120, plants grown in the absence of *S. sciuri* SAT-17 but in the presence of 120 mM NaCl; In-0, plants grown in the presence of *S. sciuri* SAT-17 but absence of NaCl; In-120, plants grown in the presence of *S. sciuri* SAT-17 and 120 mM NaCl. A significant difference between various treatments is indicated by different lower (FH-988) and upper (FH-1137) case letters.

***Effect on Antioxidant Enzymatic Genes*:** In salt tolerant genotype (FH-988), *Cat1* and *APX1* genes were significantly upregulated (>1.5 fold) under salt stress (Figure 1 E, F). Whereas, gene expression of *Cat1* and *APX1* in salt sensitive genotype (FH-1137) was downregulated (~1.5-fold) under stressed conditions. However, in SAT-17 inoculated salt stressed plants of FH-1137 genotype, gene expression of *Cat1* and *APX1* in FH-1137 was significantly upregulated (~2.5-fold) in comparison to uninoculated salt stressed plants. In FH-988 genotype, no significant variation was seen under similar conditions.

DISCUSSION

Two of the imperative determinants for salt tolerance include the conservation of optimum K^+/Na^+ ratio and the maintenance of optimum Na^+ concentrations in the cytosol (Gupta and Huang, 2014). Under normal circumstances, being one of the most abundant cations, potassium serves as a cofactor in several enzymatic reactions and also involved in regulating membrane potential, osmotic balance, cytosolic pH, stomatal aperture and protein trafficking (Assaha *et al.*, 2017). Its uptake is arbitrated by ion affinity transporters such as AKT1, which assures the ion uptake at the extracellular concentration greater than 10 µM (Nieves-Cordones *et al.*, 2014). Within the cell, vacuolar storage of K^+ is carried out by activity of antiporters like NHX2 (Leidi *et al.*, 2010). However, salt stress increases Na^+ uptake which disturbs K^+/Na^+ ratio and creates ion toxicity. Plant-PGPR interaction is based on multifaceted cross-talks and signaling events as a result of which PGPR can ameliorate negative effects of salt stress, particularly by varying selectivity of various ions through gene expression modulation in stressed plants and ensure an optimum K^+/Na^+ cellular ratio (Hamdia *et al.*, 2004, Ilangumaran and Smith, 2017).

In order to study the strategy adopted by plants of both genotypes to deal with salt stress, with or without SAT-17 inoculation, expression analysis of vacuolar and plasma membrane ion transporters was carried out. The intrinsic salt tolerance mechanisms of both genotypes seem to be different due to distinctive gene expression profiles. Gene expression analysis in FH-988 genotype indicated that there might be coordination of activities between plasma membrane and tonoplast ion transporters. The diminished activity of one was compensated by the optimized activity of other which resulted in maintaining low levels of intracellular Na^+ even under salt stress. It seems that, in salt tolerant genotype (FH-988), relatively less Na^+ toxicity under stress conditions was due to vacuolar compartmentalization of Na^+ ions carried out by NHX1. H^+-PPase activity lead to increased vacuolar acidification, by using low cost pyrophosphate as a substrate, to generate the proton motive force required for NHX antiporter activity. In *Arabidopsis*, vesicle trafficking mechanism has been proposed in Na^+ vacuolar sequestration.

NHX1 located in vesicle membranes regulate Na^+ entry into the vesicles, leading to the movement and fusion of vesicles to vacuoles thereby releasing vesicular load in the vacuole (Hamaji *et al.*, 2009). The relatively better K^+/Na^+ and reduced oxidative damage in FH-988 can also be attributed to NHX1 activity. More vacuolar sequestration of Na^+ in salt tolerant wheat (*Triticum aestivum* L.) varieties than sensitive ones was recorded earlier (Ismail and Horie, 2017).

In this study, it has been found that SAT-17 modulates the gene expression of important ion transporters under salt stress. SAT-17 inoculation assisted FH-988 plants to maintain ion homeostasis by efficient Na^+ compartmentalization in vacuoles (through enhancing gene expression levels of *NHX1* and *H^+-PPase*), by shoot to root Na^+ recirculation (through upregulation of *HKT1*) and by maximizing cellular Na^+ efflux (through upregulation of *SOS1*). It seems that 1-aminocyclopropane-1-carboxylic acid deaminase exhibiting plant beneficial bacteria hampered Na^+ mobilization and its entry into plant roots by secretion of exopolysaccharides (Ashraf *et al.*, 2004) as well as maintained enriched nutrient status of plants by enhanced uptake of macronutrients (Akram *et al.*, 2016) to ensure better ion homeostasis thereby protecting the plants from Na^+ toxicity.

Downregulation of vacuolar as well as plasma membrane ion transporter genes (*NHX1, SOS1, HKT1* and *H^+-PPase)* in FH-1137 under salt stress revealed that FH-1137 could not cope with the ionic stress thereby suffering severe Na^+ toxicity by excessive Na^+ accumulation. This was further verified by high Na^+ and low K^+ content in plant samples (root and shoot) under salt stress. Na^+ accretion is extremely lethal for plants which affects cytosolic K^+ efflux induction, resulting in disturbance of cellular homeostasis and nutrient deficiency leading to cellular death (Munns and Tester, 2008, Cabot *et al.*, 2014). Na^+ influx also depolarizes cellular membranes, which activates K^+ efflux through depolarization activated K^+ channels (Jayakannan *et al.*, 2013). SOS1 serves as the first line of defense to counteract Na^+ influx. Besides, it also facilitates in enhancing K^+ uptake (Fraile-Escanciano *et al.*, 2010). SAT-17 mediated modulation of SOS1, along with the improved K^+/Na^+ ratio, indicated that SAT-17 might help to prevent membrane depolarization by regulating more negative internal potential via minimizing Na^+ influx hence facilitating intracellular K^+ retention. SAT-17 induced activation of *HKT1* can also account for the less Na^+ accretion as HKT1, localized at xylem-symplast boundary, helps to mediate Na^+ unloading from xylem saps (Assaha *et al.*, 2017). It has also been reported that *AtHKT1* is being regulated by emission of bacterial volatiles, enabling *Arabidopsis thaliana* (L.) plants to flourish even under salt stress through decreasing 50% Na^+ (Zhang *et al.*, 2008). Higher *AtHKT1* expression after PGPR *Dietzia natronolimnaea* STR1 inoculation has been ascribed to the improved wheat growth and enhanced salt tolerance (Bharti *et al.*, 2016).

Reactive oxygen species (ROS) are also involved in alteration of ion fluxes; for instance, in Arabidopsis roots, ROS activate K^+ efflux channels thereby leading to K^+ loss (Demidchik *et al.*, 2010). Hence, plants need a strict coordination between ROS synthesis and scavenging, so that stress signal can be properly perceived and retreated (Pinedo *et al.*, 2015). This is facilitated by the activation of antioxidant mechanism. In this study, transcriptional analysis of ROS scavenging enzymatic genes, *Cat1* and *APX1*, revealed the genetic modulation under salt stress as well as SAT-17 inoculation. A synergistic relationship was observed between the gene expression and genotypic salt tolerance potential. The enhanced gene expression along with the enhanced enzyme activities might enable FH-988 genotype to resist salt stress by providing oxidative stress protection as catalase and ascorbate peroxidase are linked to two distinct H_2O_2 scavenging enzymatic categories. Ascorbate peroxidase being significant for precise modulation of ROS for signal transduction, while catalase being important for scavenging of surplus ROS produced under abiotic stresses (Mittler, 2002).

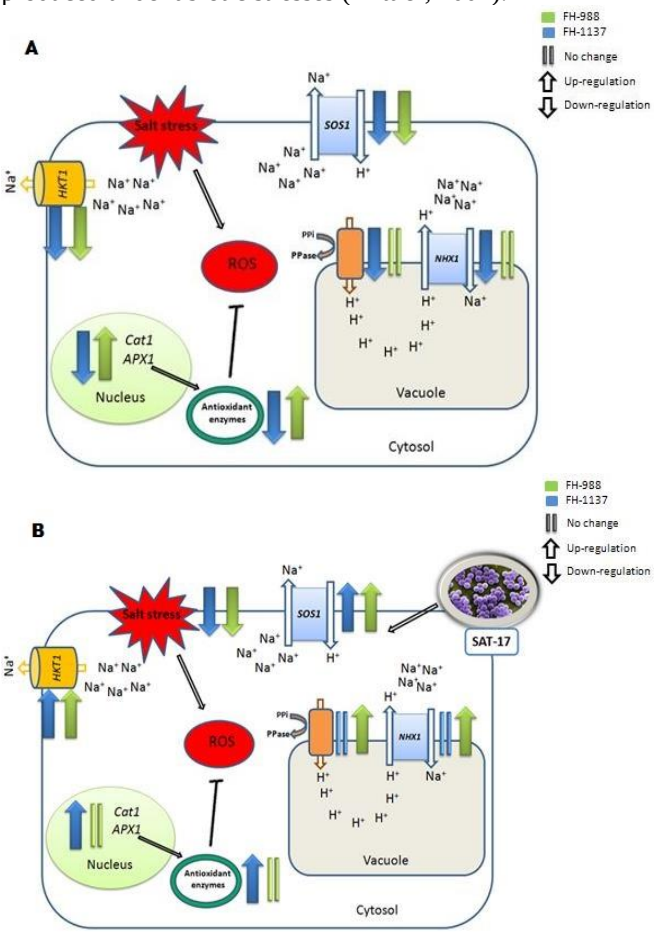

Figure 3. A schematic representation of gene expression modulation under (A) salt stress and (B) *S. sciuri* SAT-17 inoculation in *Zea mays* shoots

Reduced expression of ROS pathway genes in salt sensitive genotype (FH-1137) was further validated by a profound reduction in catalase and peroxidase activities. Under stressed conditions, genotypic dependent response patterns of antioxidant enzymatic genes have been reported in *Cynodon dactylon* (L.) (Hu *et al.*, 2012). Salt sensitive *Z. mays* plants acquired protection from salt induced damage, due to SAT-17 inoculation, via ROS scavenging by accumulating antioxidant enzymes, leading to reduced membrane damage and enhanced chlorophyll content. Our results are in compliance with the earlier findings (Safdarian *et al.*, 2019). Enhanced ascorbate peroxidase activity has also been reported by Bharti *et al*. (2013) in Bacopa (*Bacopa monnieri* L.) due to salt tolerance enhancement by PGPR inoculation. On the whole, gene expression modulation of ion homeostasis and H_2O_2 homeostasis observed under salt stress and SAT-17 inoculation in both genotypes has been summarized in a graphical representation in Figure 3.

***Conclusion*:** Results of this study demonstrated that the better salt tolerance potential of FH-988 genotype can be attributed to vacuolar sequestration of Na^+ and enhanced antioxidant enzyme activities. Furthermore, inoculation of strain SAT-17 resulted in better Na^+ homeostasis in genotype FH-988. Contrarily, salt susceptibility of genotype FH-1137 might possibly be due to its inability to cope with ionic and oxidative stresses. Inoculation of strain SAT-17 probably has improved the growth of FH-1137 by enhancing Na^+ efflux and antioxidant enzyme activities. However, further studies are needed to elucidate the salt stress amelioration potential of SAT-17 under field conditions.

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