# Beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 induces plant salt tolerance through spermidine production

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## ABSTRACT

The inoculation of plants with plant growth-promoting rhizobacterium (PGPR) has been an effective strategy for enhancing plant salt tolerance to diminish the loss of agricultural productivity caused by salt stress, but the signal transmitted from bacteria to the plant under salt stress is poorly understood. In this study, the salt tolerance of *Arabidopsis thaliana* and *Zea mays* was enhanced by inoculation with *Bacillus amyloliquefaciens* SQR9. Using dialysis bags with different molecular weight cutoffs, we sorted through the molecules secreted by SQR9 and found that spermidine is responsible for enhancing plant salt tolerance. An SQR9 △*speB* mutant deficient in spermidine production failed to induce plant salt tolerance. However, the induction of plant salt tolerance was disrupted by mutating genes involved in reduced glutathione (GSH) biosynthesis and the salt overly sensitive (SOS) pathway in *Arabidopsis*. Using quantitative real time polymerase chain reaction (qRT-PCR), this study demonstrated that spermidine produced by SQR9 leads to increased *GS* and *GR* gene expression, leading to increased levels of GSH, which is critical for scavenging reactive oxygen species (ROS). SQR9-derived spermidine also upregulates the expression of *NHX1* and *NHX7*, which sequesters $Na^+$ into vacuoles and expels $Na^+$ from the cell, thereby reducing ion toxicity.


**Keywords:** Plant growth-promoting rhizobacterium, *Bacillus amyloliquefaciens* SQR9, spermidine, salt stress, glutathione, salt overly sensitive.

## INTRODUCTION

Salt stress is one of the most critical agricultural problems (Shabala and Cuin 2007). Excess salinity imposes nutrient deficiency, osmotic stress, oxidative stress, and ion toxicity on plants and consequently leads to inhibition of plant growth and even death (Chinnusamy et al. 2006). It is therefore necessary to improve plant salt tolerance to enhance agricultural production.

Plant tolerance to salt stress is mainly mediated through hormone regulation, osmotic homeostasis, detoxification, and ionic homeostasis (Deinlein et al. 2014). High salinity disrupts plant phytohormone homeostasis, and hormone regulation is necessary for controlling plant growth, stomatal closure, and the expression of several phytohormone-dependent genes (Dinneny et al. 2008; Geng et al. 2013). The accumulation of osmoprotectants reduces water loss, maximizes water uptake, and mitigates osmotic stress induced by salinity (Székely et al. 2008; Upadhyay et al. 2012). Antioxidants can scavenge reactive oxygen species (ROS), which would be overproduced during salt stress and cause plant programmed cell death, thereby protecting the plant from the damage caused by salt stress (Meyer et al. 2007; Mittler and Blumwald 2015; Moschou et al. 2008;). Moreover, some active transporters in the membrane, such as NHXs and HKTs, can aid in salt tolerance by transporting $Na^+$ out of the cytoplasm (Deinlein et al. 2014; Zhu, 2001).

Several bacteria that colonize plant roots were found to benefit plants through complex interactions and were therefore termed plant growth-promoting rhizobacteria (PGPR). Plant roots can release specific compounds, such as malic acid, to attract PGPR (Fan et al. 2012; Rudrappa et al. 2008). Under stress, PGPR can induce chemical and physical changes in the plant to help plants against abiotic stress, called induced systemic tolerance (Dimkpa et al. 2009; Ma et al. 2014; Yang et al. 2009).

Volatile organic compounds (VOCs) emitted from *Bacillus subtilis* GB03, have been reported to trigger induced systemic tolerance in plants (Zhang et al. 2008; Zhang et al. 2010). VOCs emitted from GB03 were able to facilitate shoot to root $Na^+$ recirculation and reduce $Na^+$ import in *Arabidopsis* roots by regulating the transcription of the high-affinity $K^+$ transporter 1 (HKT1) in shoots and roots of

*Arabidopsis*. Under osmotic stress, phosphoethanolamine N-methyltransferase (PEAMT) expression was upregulated by VOCs emitted from GB03, which increased glycine betaine and choline synthesis in *Arabidopsis*, helping the plants tolerate osmotic stress via an abscisic acid (ABA)-independent pathway (Liu and Zhang 2015; Zhang et al. 2010). Other molecules produced by PGPR strains confer plant tolerance to abiotic stress include 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which degrades the ethylene precursor ACC (Dimkpa et al. 2009; Glick et al. 2007; Mayak et al. 2004), trehalose, which regulates carbon and nitrogen metabolism (Suárez et al. 2008), and indole-3-acetic acid (IAA) and cytokinin, which regulate genes involved in stress tolerance by maintaining plant hormone homeostasis (Dodd and Perez-Alfocea 2012; Yang et al. 2009). In short, organic compounds produced by PGPR regulate plant photosynthesis, hormone homeostasis, osmotic homeostasis, cell membrane integrity, detoxification and ionic homeostasis to confer plant salt tolerance.

The mechanisms allowing plants to tolerate salt stress are very complicated. New mechanisms and genes involved in plant salt tolerance are constantly reported. Thus, other unknown mechanisms involved in PGPR-induced plant salt tolerance may be existed. Our previous studies revealed that *Bacillus amyloliquefaciens* SQR9, a well studied PGPR strain, enhanced plant salt tolerance by regulating phytohormone, osmolyte, antioxidants and ion content in plants (Chen et al. 2016). In contrast with the known mechanisms by which PGPR induce plant salt tolerance, this study showed that spermidine produced by SQR9 confers plant salt tolerance in the two following ways: by regulating glutamine synthetase (GS) and glutathione reductase (GR) expression to increase reduced glutathione (GSH) levels, thus alleviating ROS damage, and by regulating $Na^+/H^+$ antiporters in the tonoplast (NHX1) and $Na^+/H^+$ antiporters in the plasma membrane (NHX7) expression and activating salt overly sensitive (SOS) proteins to control $Na^+$ content in plants.

## RESULTS

### The molecular weight of the SQR9-secreted signal that confers plant salt tolerance is between 100 and 500 Da.

Under salt stress, SQR9 inoculation increased the number of *Arabidopsis* lateral roots

but not primary root length (Fig. S1) and enhanced plant growth (Fig. 1). SQR9 inoculation increased photosynthetic rate and reduced ROS (e.g. $H_2O_2$) content (Fig. S2). Previous studies showed that SQR9 conferred plant salt tolerance by inhibiting the increase of ABA, reducing $Na^+$ content, enhancing total soluble sugar (TSS), GSH content as well as catalase (CAT) and peroxidase activity in the plant (Chen et al. 2016). However, the signal released by SQR9 that affects plant salt tolerance pathways was not clearly determined. To identify the molecular signal produced by SQR9 and the pathways affected by this signal, SQR9 was wrapped in dialysis bags of different molecular weights and tested for the ability to induce plant salt tolerance. This allowed us to determine the range of the molecular weight of the signal. The fresh weight and height of plants inoculated with SQR9 were higher than plants inoculated with inactive SQR9 (negative control). The biomass of plants treated with SQR9 wrapped in dialysis bags with a MWCO greater than or equal to 500 Da was higher than the negative control (Fig. 1A, B, C). There was no significant difference between plants treated with SQR9 wrapped in 100 MWCO dialysis bags and the negative control. The results obtained with *Arabidopsis* were similar to those obtained with maize. We checked the growth of SQR9 in 100 Da dialysis bag and 500 Da dialysis bag in rhizosphere and they showed similar growth curves (Fig. S3). Leaf chlorophyll was tested as an indicator of plant phenotype, low leaf chlorophyll content was used as indicator of impaired plant. The trend of leaf chlorophyll content was similar to plant biomass (Fig. 1D). These results indicate that the molecular weight of the SQR9-derived signal that effectively confers plant salt tolerance is between 100 and 500 Da.

**The signal molecule confers plant salt tolerance by regulating GSH and $Na^+$ contents**

Our previous study showed that many physiological indexes of plants inoculated with SQR9 are affected, including ABA, TSS, CAT, and peroxidase activity, as well as GSH and $Na^+$ content (Chen et al. 2016). To confirm which primary mechanisms for plant salt tolerance were induced by the secreted signal (100-500 Da), the physiological indicators of various pathways that could be affected by SQR9

inoculation were measured in the presence or absence of SQR9-secreted molecules between 100 to 500 Da. GSH content in maize and *Arabidopsis* inoculated with SQR9 or SQR9 wrapped in 500 Da dialysis bags was significantly higher than plants treated with inactive SQR9 or SQR9 wrapped in a 100 Da MWCO dialysis bag (Fig. 2A, B). However, ABA content and TSS content in both *Arabidopsis* and maize were not affected by SQR9-secreted molecules between 100 to 500 Da (Fig. S4A, B, C, D). In *Arabidopsis*, CAT activity was higher in plants inoculated with SQR9 wrapped in 500 Da dialysis bags than in that with 100 Da dialysis bags, however, same experiments in maize did not show significant difference between 100 Da and 500 Da treatment (Fig. S4E, F). Furthermore, the $Na^+$ content in maize and *Arabidopsis* inoculated with SQR9 or SQR9 wrapped in 500 Da MWCO dialysis bags was significantly decreased compared to plants inoculated with inactive SQR9 or SQR9 wrapped in a 100 Da MWCO dialysis bag (Fig. 2C, D). However, the $Na^+$ content in maize shoot (stem and leaf) inoculated with SQR9 or SQR9 wrapped in 100 and 500 Da MWCO dialysis bags was significantly decreased compared to plants inoculated with inactive SQR9 (Fig. 2C). This result indicated that one or more compounds with the molecular weight smaller than 100 Da were involved in reducing $Na^+$ content in shoot. These results revealed that an SQR9-secreted molecule with a molecular weight between 100 and 500 Da confers plant salt tolerance by enhancing the GSH content in plant, reducing the $Na^+$ content in *Arabidopsis* and root of maize, and enhancing CAT activity in *Arabidopsis*.

**Verification of the major plant salt tolerance pathway induced by SQR9**

To confirm the roles of CAT, GSH and ion homeostasis in SQR9-induced plant salt tolerance, *Arabidopsis* mutants deficient in catalase, GSH metabolic pathway and the SOS pathway were inoculated with SQR9. The *cat2-1* mutation plant deficient in catalase activity, showed salt tolerance responding to SQR9 inoculation as well as the wild type plant (Fig. S5). The *gs* and *gr* mutations deficient in glutamate-cysteine ligase and glutathione reductase, respectively, were sensitive to salt stress in presence of SQR9, indicating that reduced glutathione is involved in SQR9-dependent salt stress tolerance (Fig. 3, S6). Mutation in *hkt1*, which is involved in $Na^+$ circulation

from shoot to root and $Na^+$ transportation into plant cell, did not alter the response of plants to SQR9 under salt stress (Fig. 3, S6). *NHX1* and *NHX7* (*SOS1*) encode $Na^+$ transporters located at the vacuolar membrane and plasma membrane, respectively, and control the $Na^+$ dynamic in plant cells. Deletion of either of these two genes resulted in the loss of SQR9-induced plant tolerance to salt stress. In addition, mutation of *sos2* or *sos3*, which aid in regulating the $Na^+$ content in the cytoplasm, resulted in the loss of SQR9-induced plant tolerance to salt stress (Fig. 3, S6). GSH metabolic pathway was related to ROS but not $Na^+$ content in plant, thus the $Na^+$ content of *gs* and *gr* mutants was still reduced significantly with inoculation of SQR9. Meanwhile, the $Na^+$ content of *hkt1* mutants was reduced by SQR9 significantly, which was similar with that of wild type plants. However, there is no significant difference between the $Na^+$ content of *sos1*, *sos2*, *sos3* and *nhx1* mutants inoculated with SQR9 and control under salinity (Fig. S7). These results showed that GSH synthesis (controlled by GS, GR) and the SOS pathway (controlled by NHX1, SOS1, SOS2 and SOS3) are necessary for SQR9 to trigger plant salt tolerance.

**The SQR9 produced signal that confers plant salt tolerance is spermidine**

To identify the signal responsible for conferring plants tolerance to salt stress, the 100-500 Da SQR9 secretions were extracted with different organic solvents. Under salt stress, the plant fresh weight increased when treated with SQR9 secretions from the aqueous, ethyl ether, and especially chloroform extracts (Fig. 4). However, the root fresh weight had no significant difference and even significantly decreased when treated with the SQR9 secretion extracted with ethyl acetate and n-pentane, respectively (Fig. 4). These results reveal that, the key signal molecule displayed the highest solubility is in chloroform phase, but was insoluble in ethyl acetate and n-pentane. The SQR9-derived molecules between the molecular weights of 100 and 500 Da were classified. Base on the knowledge of chemicals with activity to affect plant physiology, IAA, gibberellin acid 3 (GA3), trans-zeatin (ZT), trehalose, and some types of polyamines were selected as potential signals with the molecular weight between 100 and 500. These potential signals were tested for solubility in the aforementioned extraction reagents. IAA, GA3, ZT and trehalose are poorly soluble in

chloroform. However, polyamines displayed the greatest solubility in chloroform compared with the other reagents; as a consequence, they are considered to be potential signals secreted by SQR9 for conferring plant salt tolerance. Polyamines produced by SQR9 were detected by high-performance liquid chromatography (HPLC). Spermidine was detected in both extracellular and intracellular samples of SQR9, and the content was 26.4 μM and 29.9 μM in the bacterial culture ($OD_{600}$=1), respectively, while spermine was not detected (Fig. 5A, B, C). The *Bacillus amyloliquefaciens* SQR9 genome was searched for genes related to polyamine synthesis, and *speB* was found to be the key gene in polyamine biosynthesis (Fig. 5D). The *speB* gene was knocked out, and the mutant strain was complemented with the entire *speB* gene. The mutation of *speB* caused a deficit in extra- and intracellular spermidine production. The extracellular and intracellular spermidine contents for mutant complemented strain Δ*speB* (c-Δ*speB*) were 22.2 and 26.6 μM in the bacterial culture ($OD_{600}$=1), respectively (Fig. 5C). There is no significant difference between the growth rate of Δ*speB*, c-Δ*speB* and wild type SQR9 strains in either LB medium or MS medium (with or without 100 mM NaCl) (Fig. S8). These results revealed that *speB* gene knock out did not affect SQR9 growth.

**The SQR9 produced spermidine induced plant salt tolerance**

To study the role of spermidine in inducing plant salt tolerance, plants growing on plates were treated with different concentrations of spermidine under salt stress. The optimal concentration of spermidine that effectively enhances plant salt tolerance is between 1 and 10 μM (Fig. 6A). The *speB* gene was key gene of synthesis of putrescine and spermidine (Fig. 5D). To determine whether spermidine was the key SQR9 signal, dicyclohexylamine (DCHA), an inhibitor of spermidine synthase, was used to inhibit the production of spermidine by SQR9 (Fig. 6B) (Biondi et al. 1988). The addition of DCHA resulted in an inability of SQR9 to confer plant salt tolerance (Fig. 6C). Furthermore, the mutant strain Δ*speB* did not aid plants against salt stress, but promoted *Arabidopsis* growth in the absence of salt stress (Fig. 6D, S9). As expected, complementation strain c-Δ*speB* conferred plant salt tolerance the same as the wild type SQR9 (Fig. 6E). Moreover, the deficiency of *speB* mutant strain in

conferring plant salt tolerance was rescued by purified spermidine (5 μM) (Fig. S10). These results indicate that SQR9 produced spermidine plays a vital role in inducing the plant salt tolerance.

**Spermidine confers plant salt tolerance through regulating GSH and $Na^+$ contents**

As we found that the 100-500 Da compounds secreted by SQR9 affect plant GSH and SOS pathway to induce plant salt tolerance in this study, we would like to check whether spermidine functions through regulating GSH and $Na^+$ content. Transcription levels of *GS, GR, NHX1,* and *NHX7* were examined in *Arabidopsis* upon inoculation or addition of SQR9, spermidine production deficient mutant, the complemental strains, or the pure spermidine. The *GS* and *GR* genes encode key enzymes involved in glutathione biosynthesis and reduction pathways. *NHX1* and *NHX7* are the key genes involved in reducing $Na^+$ content in the cytoplasm. Transcription of *GS*, *GR*, *NHX1*, and *NHX7* in *Arabidopsis* was upregulated upon treatment with either pure spermidine or upon inoculation with wild type SQR9 compared with plants inoculated with inactive SQR9 (Fig. 7). Inoculation with the mutant strain Δ*speB*, did not lead to a significant increase in *GS*, *GR*, *NHX1*, and *NHX7* transcription. These results indicate that SQR9 produced spermidine mainly regulates the expression of *GS* and *GR* genes, which in turn enhanced the GSH content in plants. These data further suggest that enhanced transcription of *NHX1* and *NHX7* by SQR9-derived spermidine might be involved in the mechanism by which SQR9 protects plants against salt stress.

To ensure the independence of spermidine functions on other pathways like HKT1, the responses of the mutant plants to spermidine were investigated. Results showed that spermidine induced *hkt1* mutant plant tolerance to excess salt, however, spermidine could no longer induce the tolerance of plant deficient in *SOS1 SOS2*, *SOS3*, *NHX1*, *GR* or *GS* (Fig. 8). These results are consistent with the influence of SQR9 on Arabidopsis mutants and confirm that spermidine induces plant salt tolerance through SOS pathway and GSH metabolism (Fig. 3, 8).

## DISCUSSION

Our previous study has demonstrated that SQR9 maintains photosynthesis, reduces $Na^+$ content, enhances antioxidant and TSS content, as well as inhibits ABA accumulation in plants to help plants tolerate salt stress (Chen et al. 2016). In this research, we identified the major signal produced and secreted by SQR9, which conferred plant salt tolerance, is spermidine. Moreover, we found that spermidine conferring plant tolerance to salt stress is dependent of regulating SOS pathway and GSH synthesis. While, CAT, TSS and ABA in *Arabidopsis* and maize are not necessary for the induced tolerance to salt stress by SQR9 produced spermidine. However, SOS pathway and GSH synthesis are both necessary for SQR9-induced plant salt tolerance. In the present study, SQR9 produced spermidine was shown to be the major signal that induced plant salt tolerance (Fig. 6E). The bacterially produced spermidine may induce systemic tolerance to salt stress by regulating the GSH content and $Na^+$ dynamics at the transcriptional level in plant. Overproduction of ROS induced by salt stress serves as signal to regulate $Na^+$ homeostasis and causes damage to plants (Moschou et al. 2008; Deinlein et al. 2014). And we suppose that the excess ROS caused by salt stress would not be reduced at the early stage of inoculation of SQR9. After salt stress for 14 days, ROS content would be reduced by the bacterial produced spermidine due to the accumulation of GSH (Fig. 2, 8, S2).

Furthermore, spermidine regulates expression of NHX1, the $Na^+$ transporters located at the vacuolar membrane, and SOS proteins to sequester $Na^+$ into vacuoles and expel $Na^+$ from the cell, thereby, $Na^+$ toxicity would be alleviated (Fig. 2, 8, 9). In the present study, the $Na^+$ contents of *sos* mutant plants are relatively higher compared with other reports (24 hours) due to the longer treating time with salt (14 days) (Zhu et al. 1998; Zhang et al. 2008). Zhu et al. (1998) reported that $Na^+$ content in *sos1* is significantly lower than that in *sos2* and *sos3* in the presence of salt stress, but we did not detect the significant difference. The reason of the inconsistence might due to the prolonged treating time with salt, which leads damage of *sos1* (Shi et al. 2002). It is reported that SOS3 is necessary for SOS pathway in roots, but not in shoot (Quan et al. 2007; Deinlein et al. 2014). However, we found that both root and shoot of *sos3* showed deficiency in response to SQR9 under salt stress. It is believed that root of

*sos3* would be damaged by salt stress in the presence of SQR9, and consequently, the shoot would be affected by the impaired root. That would be the reason of why the decrease of shoot biomass and increase of $Na^+$ content in shoot of *sos3* could not be released by inoculation of SQR9.

Studies on induction of systemic tolerance by *Bacillus subtilis* GB03 showed that VOCs emitted by GB03, such as 2,3-butanediol and acetoin (Farag et al., 2013), are the major compounds that help plants to tolerate abiotic stress (Zhang et al. 2008). Interestingly, the signal transduction in conferring plant tolerance in the interaction between *Bacillus amyloliquefaciens* SQR9 and plant is quite different with that in *Bacillus subtilis* GB03-plant interaction, as VOCs emitted by SQR9 did not significantly improve plant salt tolerance (Fig. S11). The VOCs emitted from GB03 reduce $Na^+$ content in plants under salt stress by affecting the transcription of HKT1, a $K^+$ transporter located in the plasma membrane of *Arabidopsis* that regulates $Na^+$ uptake and shoot-to-root $Na^+$ recirculation in *Arabidopsis* (Zhang et al. 2008). In addition, the SOS3, the protein involved in the regulation of several ion transporters respond to salt stress, is not required for the GB03 to induce plant tolerance of salt stress (Zhang et al. 2008). In contrast, we found HKT1 is not required for the spermidine induced *Arabidopsis* tolerance to salt stress. But the NHX1 and SOS proteins are essential for exerting the function of SQR9 produced spermidine on plant salt tolerance. It is known that osmotic stress and ion toxicity are always exhibited in plant under salinity conditions (Zhu 2001; Deinlein et al. 2014). GB03 upregulated the expression of *PEAMT* to enhance choline and glycine betaine content, thereby helping *Arabidopsis* tolerate osmotic stress (Zhang et al. 2010). However, SQR9 does not upregulate *PEAMT* expression (data not shown).

In plants, the polyamines (PAs) protect plants from salt stress, especially spermidine and spermine. The PAs metabolic route interacts with several metabolisms, such as biosynthesis of glutathione, nitrogen metabolism, SOS signal pathway, and the others (Alcázar et al. 2010; Kusano et al. 2007, 2008). Previous studies have revealed that polyamines play a critical role in plant tolerance to abiotic stress (Takahashi and Kakehi 2010; Yamaguchi et al. 2006). *Arabidopsis* double

knockout-mutant plant (acl5/spms), which could no longer produce spermine, are highly sensitive to salt, whilst exogenous synthetic spermine mitigates plant salt hypersensitivity (Yamaguchi et al. 2006). TSP-16, the spermidine synthase overexpress transgenic plants showed upregulated expression of various genes related to stress and enhanced plant salt tolerance (Kasukabe et al. 2004).

Spermidine can be synthesized by several *Bacillus* sp. (Burrell et al. 2010; Sekowska et al. 1998; Xie et al. 2014). *Bacillus subtilis* OKB105 produces spermidine as a growth promoting substance and promotes *Nicotiana tabacum* growth by regulating expansin expression and reducing ethylene content in plant (Xie et al. 2014). Xie *et al.* (2014) believe that the growth promotion of *Nicotiana tabacum* by OKB105 is achieved by the fact that bacterial spermidine enhanced the biofilm formation of OKB105 and thereby enhancing the bacterial ecological fitness. The new finding of this study is that bacterially produced spermidine is more than a growth promoting substance, it is also a critical molecule that induce maize and *Arabidopsis* salt tolerance (Fig. 6E).

In conclusion, this study demonstrates that spermidine produced by SQR9 is the key molecule that induces plant salt tolerance, accumulation of GSH and regulation of the SOS pathway are required during this process (Fig. 9). As a consequence of SOS regulation, $Na^+$ is sequestered into vacuoles and expelled from the cell to reduce $Na^+$ toxicity. In addition, GSH content is enhanced to reduce ROS damage during salt stress. However, the mechanism by which plants sense SQR9 produced spermidine, are still not clear, and these questions merit further investigation in future studies.

## MATERIALS AND METHODS

### Strains and plant culture conditions

*Bacillus amyloliquefaciens* SQR9 (China General Microbiology Culture Collection Center, CGMCC accession no. 5808) was cultured at 30 °C with shaking at 170 rpm for 10 h in Luria-Berta (LB) medium (5 g $l^{-1}$ yeast extract, 10 g $l^{-1}$ tryptone, 10 g $l^{-1}$ NaCl; pH 7.0-7.2). One culture was adequately hyperthermia inactivated twice and used as a “dead cell” negative control. The cultures were centrifuged at 6000 rpm for 10 min. The cells were suspended and diluted with double-distilled water (DDW) to a

final concentration of $5\times10^6$ CFU $ml^{-1}$.

Maize (*Zea mays*) seeds of cultivar "Jingtian" were sterilized and germinated in a growth chamber. Three-day-old seedlings were transplanted into sterilized 1/4 Hoagland medium (Hoagland and Arnon 1938) and grown at 25 °C in a growth chamber with a 16-h light/8-h dark photoperiod. The medium was shaken at 50 rpm for 2 h every day and replaced every two days (Chen et al. 2016).

*Arabidopsis thaliana* (col-0) seeds were surface sterilized and then placed on 1/2 Murashige and Skoog (MS) media (pH 5.7) containing 1.5% sucrose and 0.8% agar (Murashige and Skoog 1962). T-DNA insertion mutants were obtained from The Arabidopsis Information Resource (TAIR; http://www.arabidopsis.org) and identified in the SALK and GABI-KAT databases. The mutants used in this study were as follows: *gs*, *gr*, *nhx1*, *sos1*, *sos2*, *sos3*, and *hkt1* (Table 1). *Arabidopsis* mutant seeds from the $T_2$ progeny were sterilized and germinated as described above. Gene specific primer pairs and T-DNA specific primers were used for PCR screening to identified T-DNA insertions.

**Inoculation of strains**

*Arabidopsis* seeds were vernalized at 4 °C for 2 days in the dark and then incubated at 22 °C in a growth chamber with a 16-h light/8-h dark photoperiod. Seven-day-old seedlings were transplanted to 1/2 MS solid medium with or without 100 mM NaCl. To measure the impact of SQR9 on plant tolerance, each plate was inoculated with 2 µl of active SQR9 or dead cell suspension. The *nhx1*, *sos1*, *sos2*, and *sos3* mutants were transplanted onto medium with or without 50 mM NaCl. Ten plates were included in each treatment and 6 plants were included in each plate to control for variability. In each plate, the left three plants were mutants and the right three plants were wild type.

Ten-day-old *Arabidopsis* seedlings were detached from 1/2 MS agar plates and transplanted onto 1/4 MS liquid medium. Thirteen-day-old *Arabidopsis* and maize seedlings were treated with or without 100 mM NaCl and inoculated with bacterial suspensions in different molecular weight cut-off (MWCO) dialysis bags (100, 500, 1000, 2000, 5000 MWCO). In this experiment, bacteria cells were suspended with 1/4

MS medium and 1/4 Hoagland medium, respectively. The mediums were shaken at 50 rpm for 2 h every day and replaced every two days. And bacterial suspension was changed every two days. The experiments were repeated 3 times. For each treatment, 48 *Arabidopsis* and 21 maize seedlings were included as replicates.

**Determination of plant biomass**

The plant fresh weight was measured by an analytical scale. Plant height and root length were measured with a ruler. Lateral roots were observed and counted with a stereomicroscope.

**Determination of leaf chlorophyll, reduced glutathione, and $Na^+$ content**

Chlorophyll content (SPAD value) was measured using a chlorophyll meter (SPAD-502, Japan) (Ling et al. 2011). The leaves from different plants used for detect were in the same location of plant. For each treatment, ten independent samples were measured.

The enzymatic recycling method used to measure GSH was previously described (Airaki et al. 2015; Griffith 1980).

To determine $Na^+$ content, whole plants were washed with deionized water and dried at 70 °C for 2 days. All plant samples were homogenized and filtered using a filter with a 1 mm pore size. The $Na^+$ in the plants was extracted as described by Zhang and quantified using an inductively coupled plasma spectrograph (Zhang et al. 2008).

**Fractionation of bacterial secretions**

SQR9 was cultured in Landy medium (Landy et al. 1948) at 30 °C for 36 h. Filtered SQR9 secretions and Landy medium (negative control) were freeze dried and then dissolved in 50 ml of sterile distilled water. Then, the 100 to 500 Da SQR9 secretions and Landy medium were collected using 100 Da and 500 Da MWCO dialysis bags. The 100 to 500 Da SQR9 secretions (50 ml) and Landy medium were partitioned with 50 ml of ethyl acetate, and the ethyl acetate phase was separated. Then, the aqueous phase was fractionated with 50 ml of ethyl ether, and the ethyl ether phase was separated. The aqueous phase was fractionated with 50 ml of n-pentane, and the n-pentane phase was separated. Finally, the aqueous phase was fractionated with 50

ml of chloroform, and the chloroform and aqueous phases were separated. Each organic phase was dried under nitrogen gas. This process resulted in five types of fractions: ethyl acetate, ethyl ether, n-pentane, chloroform, and aqueous fractions. The SQR9 secretion without fractionation (total secretion sample) was also kept.

**Construction of a *Bacillus amyloliquefaciens* SQR9 mutant strain**

To confirm the contribution of SQR9-derived spermidine on plant salt tolerance, the *speB* gene was completely deleted by double cross-over (Zhou et al. 2017; Yan et al. 2008). The sequences of erythromycin were obtained from pAX01 plasmid. Two partial sequence fragments of the *speB* gene were amplified from SQR9 DNA. Then, the recombinant fragments were fused and transformed into the SQR9 strain to generate Δ*speB* mutation. To complement the Δ*speB* mutant, the entire *speB* gene was amplified from *Bacillus amyloliquefaciens* SQR9 chromosomal DNA and ligated into the pNW33 plasmid to obtain pNW33-*speB*, which was transformed into the Δ*speB* mutant to get the complementary strain of mutant Δ*speB* (c-Δ*speB*). The primers used to construct these mutants are shown in Table S1.

**Measurement of spermidine**

The bacterial strains were cultured in Landy medium at 30 ℃ for 36 h. The fermented cultures were centrifuged at 6000 rpm for 10 min at 4 °C. The bacterial cells were treated with lysozyme and suspended in 0.1 M HCl then sonicated. Pure perchloric acid was added to culture filtrates or lysates to obtain a 5% (w/v) solution for 1 h at 4°C. The samples were centrifuged at 10000 rpm for 30 min. Seventy microliters of benzoyl chloride was added to 5 ml of supernatant. Then, each sample was neutralized with 10 ml of 2 M NaOH, vortexed for 20 s and incubated at 37 °C for 30 min. Fifteen milliliters of a saturated NaCl solution and ethyl ether were added to each sample. The organic phase was dried using nitrogen and resuspended in 200 µl of methanol. The sample was detected by HPLC system equipped with a UV detector set at 254 nm and a C18 column (Waters). The mobile phase was 60% methanol, the flow rate was 0.7 ml $min^{-1}$ and the column temperature was 30 °C.

To detect the role of spermidine in plant salt tolerance, different concentrations of exogenous spermidine were added to MS medium with 100 mM NaCl, and DCHA

(10 mM), an inhibitor of polyamine biosynthesis, was added to the bacterial growth medium. Each treatment had six plates containing six plants each.

**Transcription analysis**

RNA was extracted from plants after 1 day of treatment. Plant tissue was flash frozen in liquid nitrogen and then extracted using the Qiagen RNeasy plant mini kit (Qiagen, Valencia, CA, U.S.A.). The transcript levels were quantified by reverse transcription polymerase chain reaction using the PrimeScript RT reagent Kit (TAKARA Biotechnology (Dalian) Co., LTD). Quantitative real-time polymerase chain reactions (qRT-PCR) were performed with SYBR® *Premix EX Taq*™ (TaKaRa) using an ABI 7500 Cycler (Applied Biosystems, Germany). Transcription levels of *GS* (encoding glutamine synthetase), *GR* (encoding glutathione reductase), *NHX1* (encoding $Na^+/H^+$ antiporters in the tonoplast) and *NHX7* (encoding $Na^+/H^+$ antiporters in the plasma membrane) were measured using *ACT* (encoding actin) as an internal reference. For each treatment, three biological replicates are included. The primers used in qRT-PCR are shown in Table S1. The specificity of the amplification was verified by melting-curve analysis and agarose gel electrophoresis. The relative transcription levels were calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

**Statistical analysis**

The data among different treatments were subjected to analysis of variance (ANOVA). Duncan's multiple range tests ($P < 0.05$) was employed to determine differences among means. Statistical significance was analyzed by t-test and SPSS version 18.0 (SPSS, Inc., Chicago, IL).

**Accession numbers**

The sequences of genes used in this article can be found in National Center for Biotechnology Information (NCBI) or TAIR data library under the following accession numbers: *Act* (At3g18780), *NHX1* (At5g27150), *NHX7* (At2g01980), *GR* (At3g24170), and *GS* (At5g35630).

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## SUPPLEMENTARY DATA

The following supplementary data are available.

**Figure S1.** The plant primary root length and lateral root number.

**Figure S2.** The photosynthetic rate and $H_2O_2$ concentration of plants.

**Figure S3.** Growth of *Bacillus amyloliquefaciens* SQR9 in 100 Da and 500 Da dialysis bags.

**Figure S4.** Effects of *Bacillus amyloliquefaciens* SQR9 secretions on physiological indicators of plants.

**Figure S5.** Effects of *Bacillus amyloliquefaciens* SQR9 on the response of *cat2-1* mutant to salt.

**Figure S6.** The phenotype of different *Arabidopsis* mutants inoculated with *Bacillus amyloliquefaciens* SQR9 under salt stress.

**Figure S7.** The $Na^+$ content of mutants under salt tress.

**Figure S8.** The growth curves of different strains.

**Figure S9.** The phenotype of *Arabidopsis* inoculated with different strains.

**Figure S10.** Effects of *Bacillus amyloliquefaciens* SQR9, ΔspeB and 5 μM spermidine (spd) on Arabidopsis under salinity.

**Figure S11.** Effects of VOCs secreted by *Bacillus amyloliquefaciens* SQR9 on plants.

**Table S1.** DNA primers used in this study

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636

637 **Table 1.** *Arabidopsis* mutants used in this study

| Mutants | Stock name | Locus | Function |
|---|---|---|---|
| *gs* | SALK_081530C | At4g23100 | Encodes the enzyme glutamate-cysteine ligase |
| *gr* | SALK_104319C | At3g24170 | Encodes glutathione-disulfide reductase |
| *nhx1* | SALK_034001C | At5g27150 | Encodes $Na^+/H^+$ antiporters in the tonoplast |
| *sos1* | SALK_092851C | At2g01980 | Encodes $Na^+/H^+$ antiporters in the plasma membrane, SOS1 |
| *sos2* | SALK_000367C | At5g01820 | Encodes protein kinase, SOS2 |
| *sos3* | SALK_110426C | At4g17615 | Encodes calcineurin B-like protein, SOS3 |
| *hkt1* | CS476306 | At4g10310 | Encodes high-affinity $K^+$ transporter 1 |
| *cat2-1* | SALK_076998 | AT4G35090 | Encodes catalase 2 |

638

639 **Figure captions**

640 **Figure 1.** Effects of *Bacillus amyloliquefaciens* SQR9 secretions of different
641 molecular weights on plant salt tolerance. (A) Representative images of plants treated
642 with SQR9 wrapped in different molecular weight cut off dialysis bags (5000, 2000,
643 1000, 500 and 100 Da). (B) Quantification of maize fresh weight and plant height. (C)
644 Quantification of *Arabidopsis* fresh weight and plant height. White and black bars
645 represent plant fresh weight and plant height, respectively. (D) Chlorophyll content
646 (SPAD value) was measured. Salt concentration is 100 mM NaCl. SQR9 treatment
647 means plants inoculated by SQR9 without dialysis bag (positive control). CK means
648 negative control. LS5000, LS2000, LS1000, LS500 and LS100 mean living SQR9
649 wrapped in 5000, 2000, 1000, 500 and 100 Da dialysis bags, respectively. White and
650 black bars represent maize and *Arabidopsis*, respectively. Mean values in the same
651 column with different letters indicate significant differences (Duncan's least
652 significant difference, $P \leq 0.05$, n= 21 for maize, n= 48 for *Arabidopsis*).
653
654 **Figure 2.** Effects of *Bacillus amyloliquefaciens* SQR9 secretions of different
655 molecular weights on reduced glutathione (GSH) content and $Na^+$ content of plants.
656 (A) GSH content and (C) $Na^+$ content in maize were measured after maize was treated
657 with *Bacillus amyloliquefaciens* SQR9 secretions of different molecular weights
658 under salt stress for twenty days. (B) GSH content and (D) $Na^+$ content were
659 measured in *Arabidopsis* after being similarly treated for fourteen days. 100 mM CK
660 means plants treated with inactivated SQR9 under 100 mM NaCl, which is negative
661 control. 100 mM SQR9 means plants inoculated with SQR9 under 100 mM NaCl,
662 which is positive control. Different letters indicate significant differences between
663 treatments (Duncan's least significant difference test, $P \leq 0.05$, n=5, mean ± standard
664 deviation).
665
666 **Figure 3.** Effects of *Bacillus amyloliquefaciens* SQR9 on the response of *Arabidopsis*
667 mutants to salt stress. The first five mutants (*sos1*, *sos2*, *sos3*, *nhx1*, *hkt1*) are missing
668 genes related to ion balance in the plant. The last two mutants (*gr* and *gs*) are involved

in reduced glutathione synthesis. Different *Arabidopsis* mutants inoculated with SQR9 or heat-inactivated SQR9 as a control. Plant shoot fresh weight (A) and root fresh weight (B) were measured under salt stress for 14 days. Black and white bars represent SQR9 and heat-inactivated SQR9 control treatments, respectively. The values are the mean ± standard deviation from 48 replicates. The col means wild type *Arabidopsis*. The *sos1*, *sos2*, and *sos3* mutants are treated with 50 mM NaCl. The *nhx1*, *hkt1*, *gr*, and *gs* mutants are treated with 100 mM NaCl. Asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants (t test, $P \leq 0.05$), double asterisks (**) represent $P \leq 0.01$.

**Figure 4.** The effects of various SQR9 secretion extracts on plant biomass under salt stress. SQR9 secretions and Landy medium (control) were extracted with aqueous, ethyl acetate, ethyl ether, n-pentane, and chloroform phases. The values indicate the changes induced by SQR9 secretions extracted with different phases compared to the Landy medium control. Asterisk (*) indicates statistically significant differences between the control and plants treated with SQR9 secretions (t test, $P \leq 0.05$, n=3, mean ± standard deviation); double asterisks (**) represent $P \leq 0.01$.

**Figure 5.** High-performance liquid chromatography (HPLC) analysis of extracellular (A) and intracellular (B) spermidine from *Bacillus amyloliquefaciens* SQR9, Δ*speB*, and c-Δ*speB* strains. (C) Extracellular and intracellular spermidine content from SQR9, Δ*speB* and c-Δ*speB* strains was quantified. (D) The figure shows the polyamine biosynthetic pathway in *Bacillus amyloliquefaciens* SQR9. The values indicate the mean ± standard error from 3 replicates. Different letters above the columns indicate statistically significant differences (Duncan's least significant difference test, $P \leq 0.05$).

**Figure 6.** (A) Plant salt tolerance was affected by different concentrations of synthetic spermidine. Salt concentration is 100 mM NaCl. CK means negative control. (B) Spermidine concentration of culture filtrates of wild type SQR9 and SQR9 treated

699 with DCHA. (C) Effect of dicyclohexylamine (DCHA), an inhibitor of spermidine
700 synthase, on plant salt tolerance. The fresh weight of *Arabidopsis* was measured after
701 fourteen days of treatment under salt stress. The fresh weight of *Arabidopsis*, treated
702 with *Bacillus amyloliquefaciens* SQR9, *ΔspeB*, c-Δ*speB* strains and the
703 heat-inactivated SQR9 control, was measured under non-stress (D) and salt stress
704 conditions (E). White and black bars represent shoot and root fresh weights,
705 respectively. Multiple statistical analyses were performed in groups labeled with same
706 capital letter. Different lowercase letters above the columns indicate statistically
707 significant differences (Duncan's least significant difference test, $P \leq 0.05$, n= 48).
708
709 **Figure 7.** Real-time quantitative polymerase chain reaction analysis of *GS, GR, NHX1,*
710 and *NHX*7 transcript levels in Arabidopsis in response to *Bacillus amyloliquefaciens*
711 SQR9, Δ*speB*, c-Δ*speB* strains, and 5 μM spermidine (spd) under salt stress with 100
712 mM NaCl. CK and 0-CK mean plants in response to inactive SQR9 under salt stress
713 and in the absence of salt stress, respectively. The transcription levels were measured
714 after plants were treated for 1 day under salt stress and compared to the control.
715 Different letters indicate significant differences between treatments (t test, n= 3, $P \leq$
716 0.05).
717
718 **Figure 8.** Effects of 5 μM spermidine (spd) on the response of *Arabidopsis* mutants to
719 salt tolerance. Shoot fresh weight (A) and root fresh weight (B) of *Arabidopsis* were
720 tested under salt stress for 14 days. White and black bars represent negative control
721 and spermidine (spd) treatments, respectively. The col-50 and col-100 represent wild
722 type treated with 50 mM and 100 mM NaCl, respectively. The *sos1*, *sos2*, and *sos3*
723 mutants are treated with 50 mM NaCl. The *nhx1*, *hkt1*, *gr*, and *gs* mutants are treated
724 with 100 mM NaCl. The values are the mean ± standard deviation from 48 replicates.
725 Asterisk (*) indicates statistically significant differences between control plants and
726 plants treated with spd (t test, $P \leq 0.05$).
727
728 **Figure 9.** *Bacillus amyloliquefaciens* SQR9 induce systemic tolerance to salt stress in

plants. Spermidine produced by SQR9 enhances reduced glutathione (GSH) content, thus reducing reactive oxygen species (ROS) content, and regulates the salt overly sensitive (SOS) pathway to sequester $Na^+$ into vacuoles and expel $Na^+$ from the cytoplasm to confer plant salt tolerance.

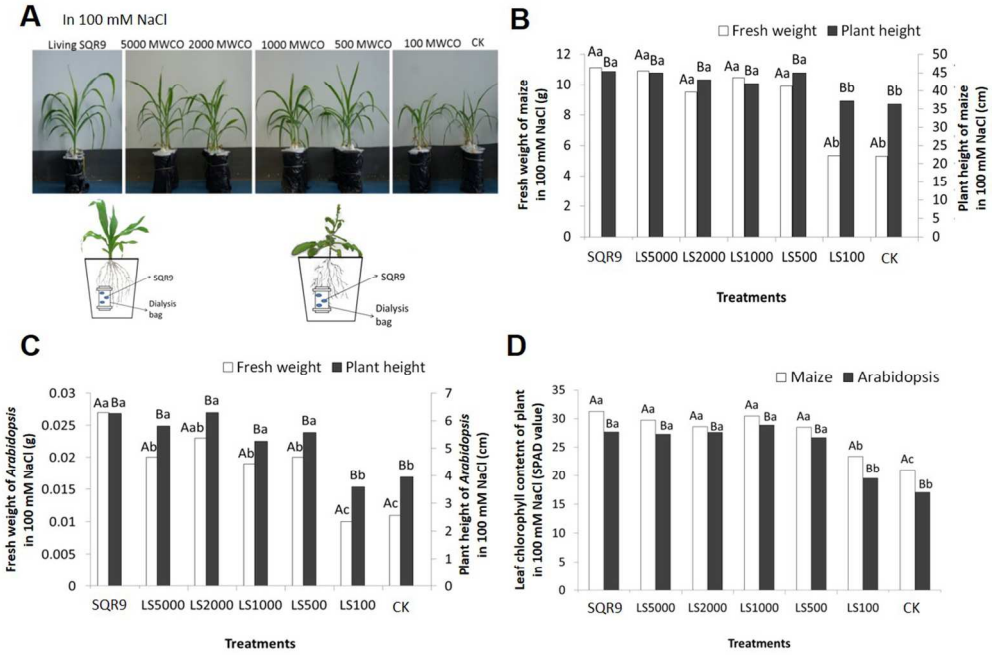


Figure 1. Effects of Bacillus amyloliquefaciens SQR9 secretions of different molecular weights on plant salt tolerance. (A) Representative images of plants treated with SQR9 wrapped in different molecular weight cut off dialysis bags (5000, 2000, 1000, 500 and 100 Da). (B) Quantification of maize fresh weight and plant height. (C) Quantification of Arabidopsis fresh weight and plant height. White and black bars represent plant fresh weight and plant height, respectively. (D) Chlorophyll content (SPAD value) was measured. Salt concentration is 100 mM NaCl. SQR9 treatment means plants inoculated by SQR9 without dialysis bag (positive control). CK means negative control. LS5000, LS2000, LS1000, LS500 and LS100 mean living SQR9 wrapped in 5000, 2000, 1000, 500 and 100 Da dialysis bags, respectively. White and black bars represent maize and Arabidopsis, respectively. Mean values in the same column with different letters indicate significant differences (Duncan's least significant difference, $P \leq 0.05$, n= 21 for maize, n= 48 for Arabidopsis).

Fig. 1
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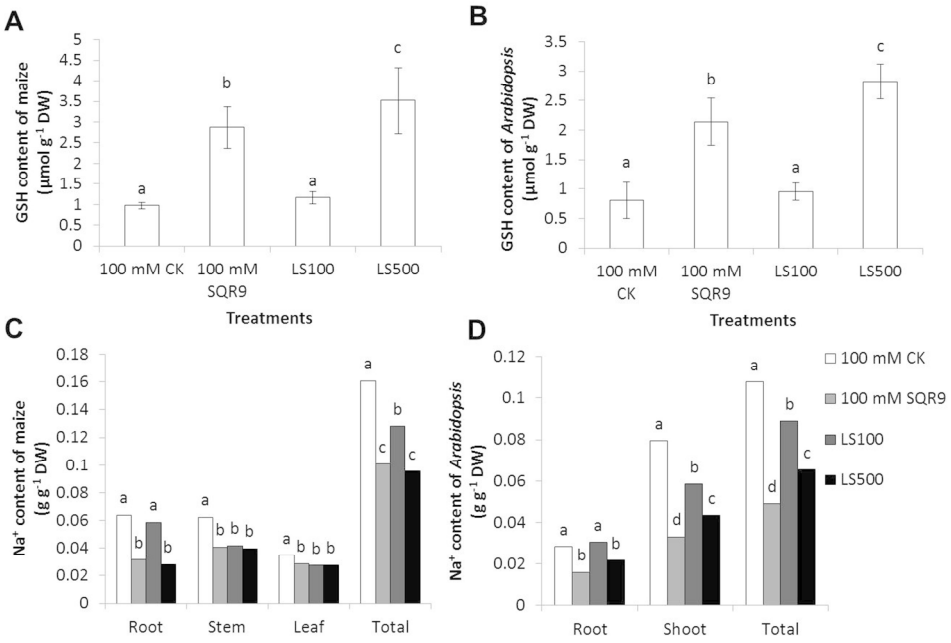


Figure 2. Effects of Bacillus amyloliquefaciens SQR9 secretions of different molecular weights on reduced glutathione (GSH) content and Na+ content of plants. (A) GSH content and (C) Na+ content in maize were measured after maize was treated with Bacillus amyloliquefaciens SQR9 secretions of different molecular weights under salt stress for twenty days. (B) GSH content and (D) Na+ content were measured in Arabidopsis after being similarly treated for fourteen days. 100 mM CK means plants treated with inactivated SQR9 under 100 mM NaCl, which is negative control. 100 mM SQR9 means plants inoculated with SQR9 under 100 mM NaCl, which is positive control. Different letters indicate significant differences between treatments (Duncan's least significant difference test, $P \leq 0.05$, n=5, mean ± standard deviation).

Fig. 2

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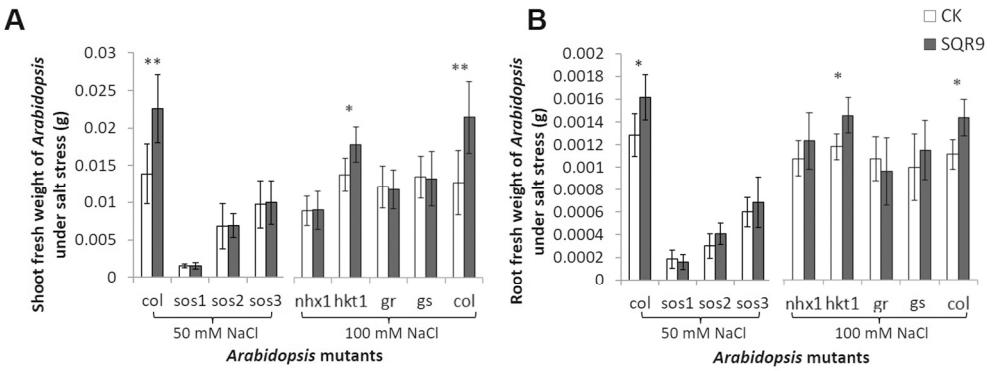


Figure 3. Effects of Bacillus amyloliquefaciens SQR9 on the response of Arabidopsis mutants to salt stress. The first five mutants (sos1, sos2, sos3, nhx1, hkt1) are missing genes related to ion balance in the plant. The last two mutants (gr and gs) are involved in reduced glutathione synthesis. Different Arabidopsis mutants inoculated with SQR9 or heat-inactivated SQR9 as a control. Plant shoot fresh weight (A) and root fresh weight (B) were measured under salt stress for 14 days. Black and white bars represent SQR9 and heat-inactivated SQR9 control treatments, respectively. The values are the mean ± standard deviation from 48 replicates. The col means wild type Arabidopsis. The sos1, sos2, and sos3 mutants are treated with 50 mM NaCl. The nhx1, hkt1, gr, and gs mutants are treated with 100 mM NaCl. Asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants (t test, $P \leq 0.05$), double asterisks (**) represent $P \leq 0.01$.

Fig. 3
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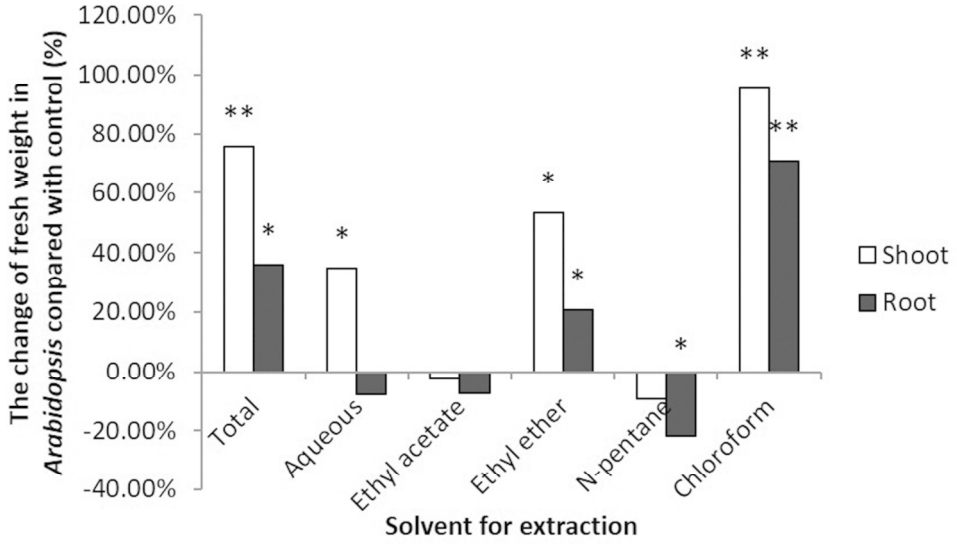


Figure 4. The effects of various SQR9 secretion extracts on plant biomass under salt stress. SQR9 secretions and Landy medium (control) were extracted with aqueous, ethyl acetate, ethyl ether, n-pentane, and chloroform phases. The values indicate the changes induced by SQR9 secretions extracted with different phases compared to the Landy medium control. Asterisk (*) indicates statistically significant differences between the control and plants treated with SQR9 secretions (t test, $P \leq 0.05$, n=3, mean ± standard deviation); double asterisks (**) represent $P \leq 0.01$.

Fig. 4

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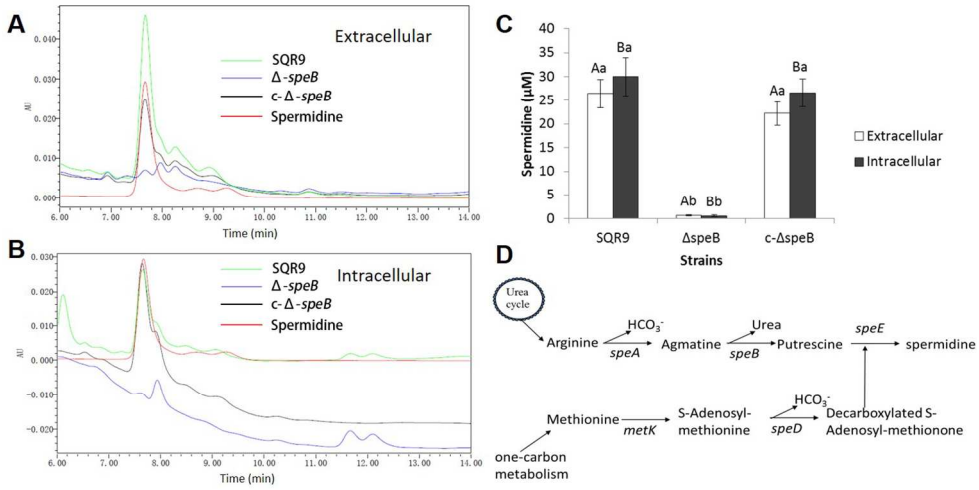


Figure 5. High-performance liquid chromatography (HPLC) analysis of extracellular (A) and intracellular (B) spermidine from Bacillus amyloliquefaciens SQR9, ΔspeB, and c-ΔspeB strains. (C) Extracellular and intracellular spermidine content from SQR9, ΔspeB and c-ΔspeB strains was quantified. (D) The figure shows the polyamine biosynthetic pathway in Bacillus amyloliquefaciens SQR9. The values indicate the mean ± standard error from 3 replicates. Different letters above the columns indicate statistically significant differences (Duncan's least significant difference test, $P \leq 0.05$).

Fig. 5

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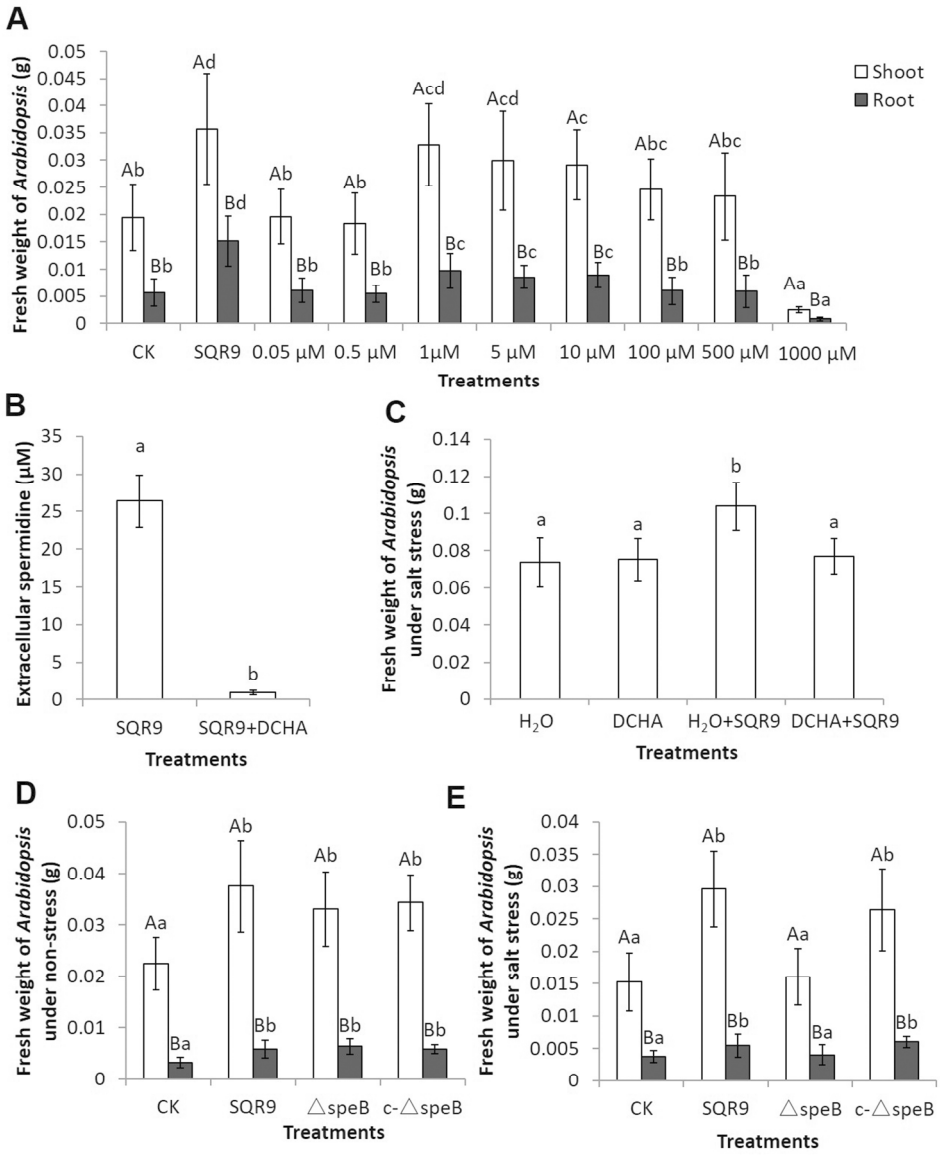


Figure 6. (A) Plant salt tolerance was affected by different concentrations of synthetic spermidine. Salt concentration is 100 mM NaCl. CK means negative control. (B) Spermidine concentration of culture filtrates of wild type SQR9 and SQR9 treated with DCHA. (C) Effect of dicyclohexylamine (DCHA), an inhibitor of spermidine synthase, on plant salt tolerance. The fresh weight of Arabidopsis was measured after fourteen days of treatment under salt stress. The fresh weight of Arabidopsis, treated with Bacillus amyloliquefaciens SQR9, ▵speB, c-▵speB strains and the heat-inactivated SQR9 control, was measured under non-stress (D) and salt stress conditions (E). White and black bars represent shoot and root fresh weights, respectively. Multiple statistical analyses were performed in groups labeled with same capital letter. Different lowercase letters above the columns indicate statistically significant differences (Duncan's least significant difference test, $P \leq 0.05$, n= 48).

Fig. 6
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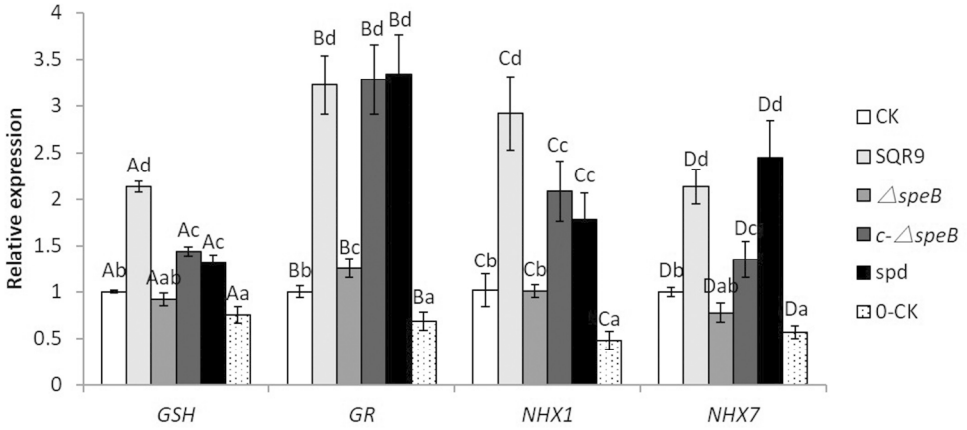


Figure 7. Real-time quantitative polymerase chain reaction analysis of GS, GR, NHX1, and NHX7 transcript levels in Arabidopsis in response to Bacillus amyloliquefaciens SQR9, ΔspeB, c-ΔspeB strains, and 5 μM spermidine (spd) under salt stress with 100 mM NaCl. CK and 0-CK mean plants in response to inactive SQR9 under salt stress and in the absence of salt stress, respectively. The transcription levels were measured after plants were treated for 1 day under salt stress and compared to the control. Different letters indicate significant differences between treatments (t test, n= 3, $P \leq 0.05$).

Fig. 7

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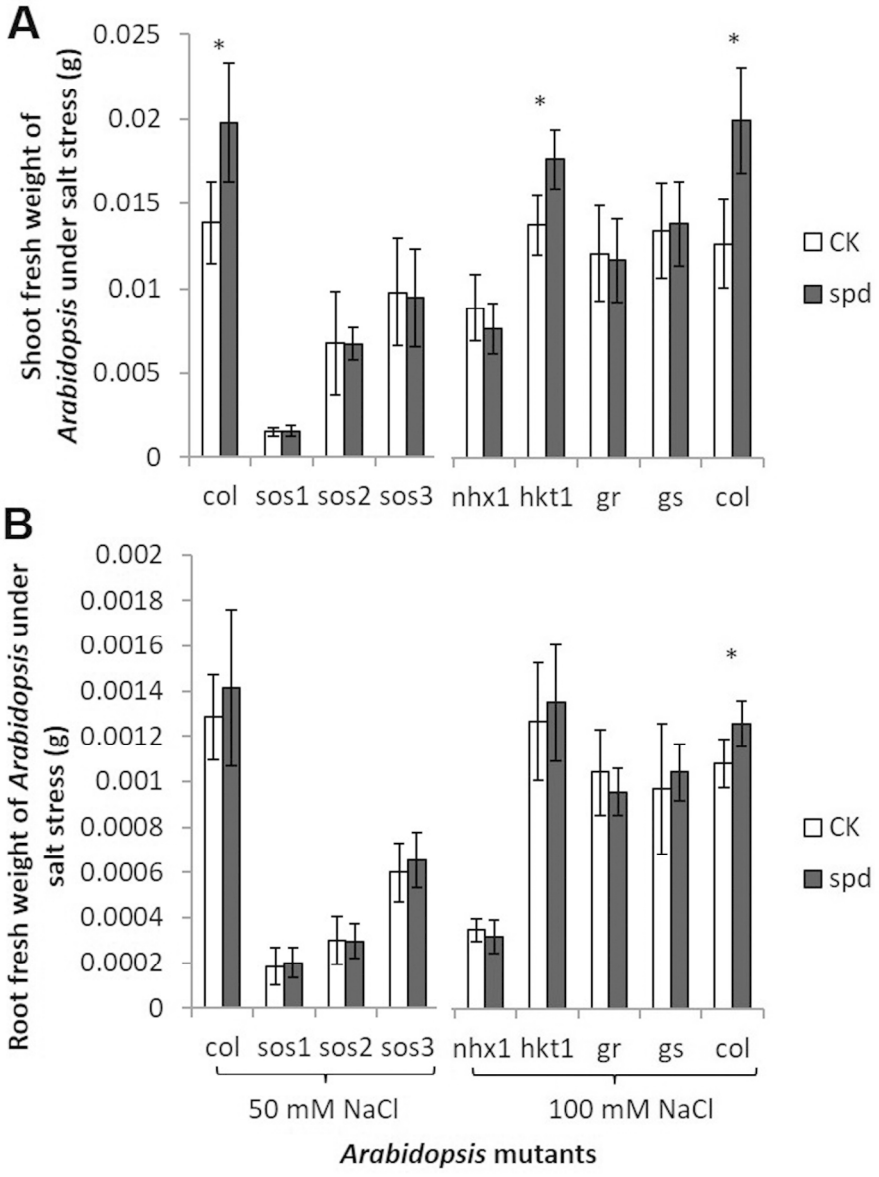


Figure 8. Effects of 5 μM spermidine (spd) on the response of Arabidopsis mutants to salt tolerance. Shoot fresh weight (A) and root fresh weight (B) of Arabidopsis were tested under salt stress for 14 days. White and black bars represent negative control and spermidine (spd) treatments, respectively. The col-50 and col-100 represent wild type treated with 50 mM and 100 mM NaCl, respectively. The sos1, sos2, and sos3 mutants are treated with 50 mM NaCl. The nhx1, hkt1, gr, and gs mutants are treated with 100 mM NaCl. The values are the mean ± standard deviation from 48 replicates. Asterisk (*) indicates statistically significant differences between control plants and plants treated with spd (t test, $P \leq 0.05$).

Fig. 8

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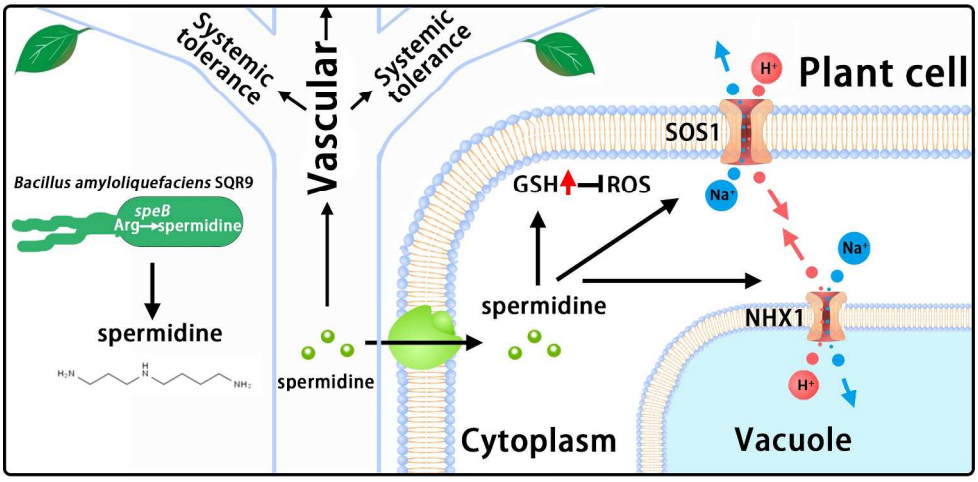


Figure 9. Bacillus amyloliquefaciens SQR9 induce systemic tolerance to salt stress in plants. Spermidine produced by SQR9 enhances reduced glutathione (GSH) content, thus reducing reactive oxygen species (ROS) content, and regulates the salt overly sensitive (SOS) pathway to sequester Na+ into vacuoles and expel Na+ from the cytoplasm to confer plant salt tolerance.
Fig. 9
226x112mm (300 x 300 DPI)

**Figure S1.** The plant primary root length and lateral root number.

Fig. S1

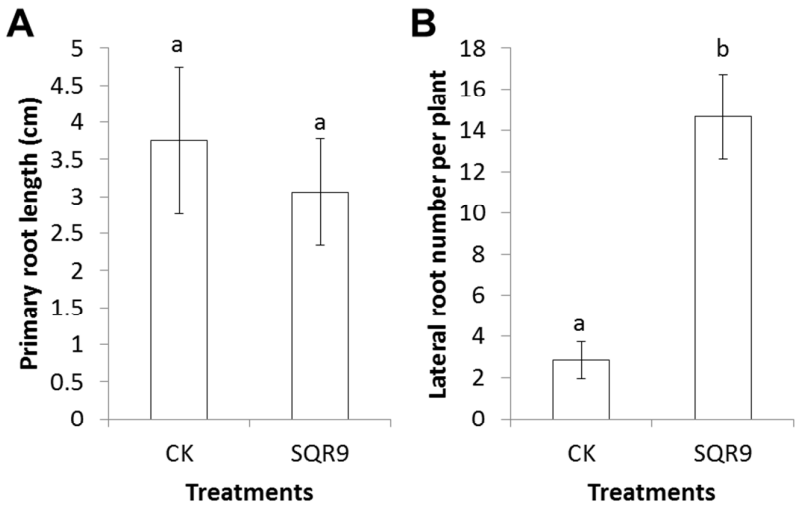


**Figure S1.** The effects of *Bacillus amyloliquefaciens* SQR9 on primary root length (A) and lateral root number (B) of *Arabidopsis* under salt stress. Salt concentration is 100 mM NaCl. CK means negative control. The values indicate the mean ± standard error from 10 replicates. Different letters above the columns indicate statistically significant differences.

**Figure S2.** The photosynthetic rate and $H_2O_2$ concentration of plants.

Fig. S2

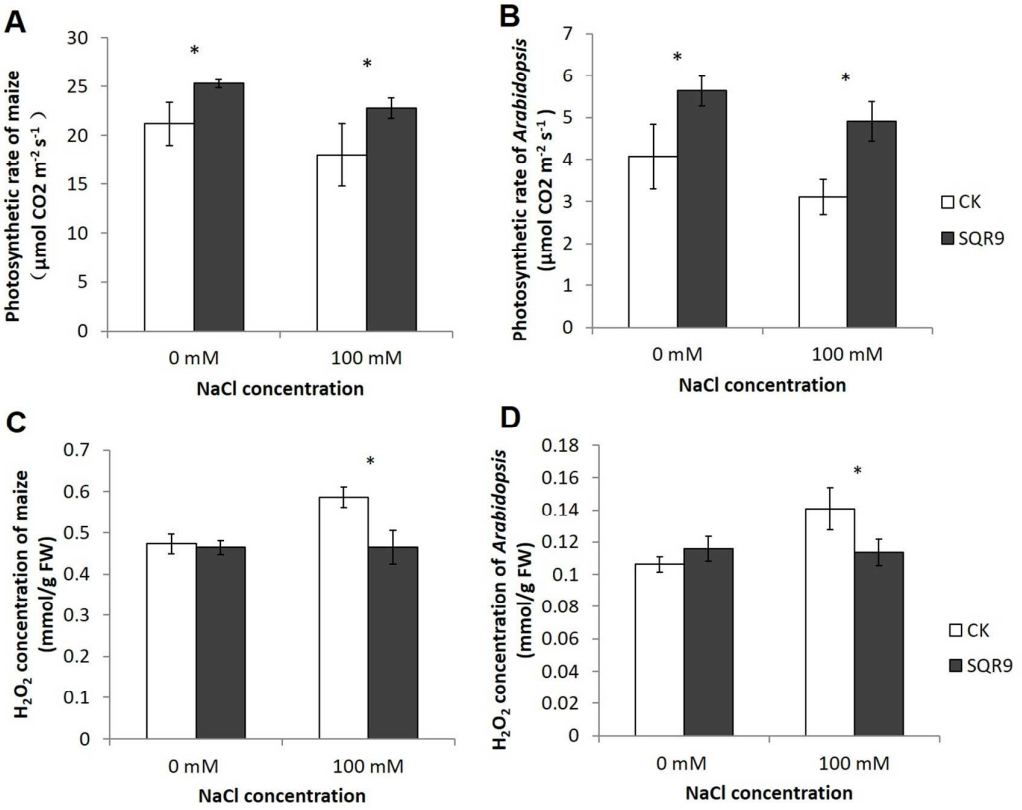


**Figure S2.** The effects of *Bacillus amyloliquefaciens* SQR9 on photosynthetic rate of (A) maize and (B) *Arabidopsis*, as well as $H_2O_2$ concentration of (C) maize and (D) *Arabidopsis*. CK means negative control. White and black bars represent plant inoculated with control and SQR9, respectively. The values are the mean ± standard deviation from 5 replicates. Asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants (t test, $P \leq 0.05$).

**Figure S3.** Growth of *Bacillus amyloliquefaciens* SQR9 in 100 Da and 500 Da dialysis bags.

Fig. S3

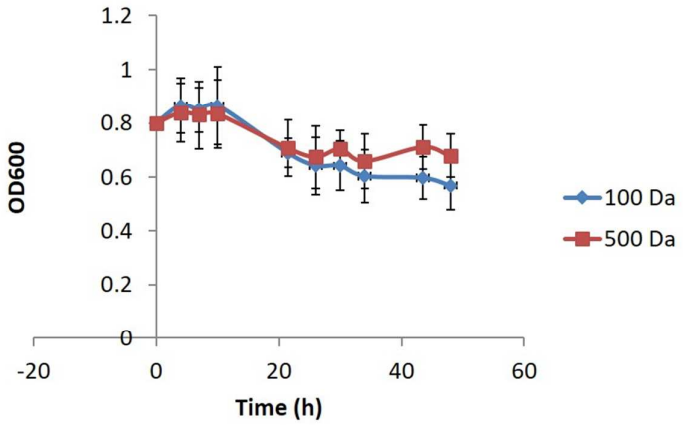


**Figure S3.** The growth of *Bacillus amyloliquefaciens* SQR9 in 100 Da and 500 Da dialysis bags. Blue and red lines represent SQR9 wrapped in 100 Da and 500 Da dialysis bags, respectively. The values are the mean ± standard deviation from 6 replicates.

**Figure S4.** Effects of *Bacillus amyloliquefaciens* SQR9 secretions on physiological indicators of plants.

Fig. S4

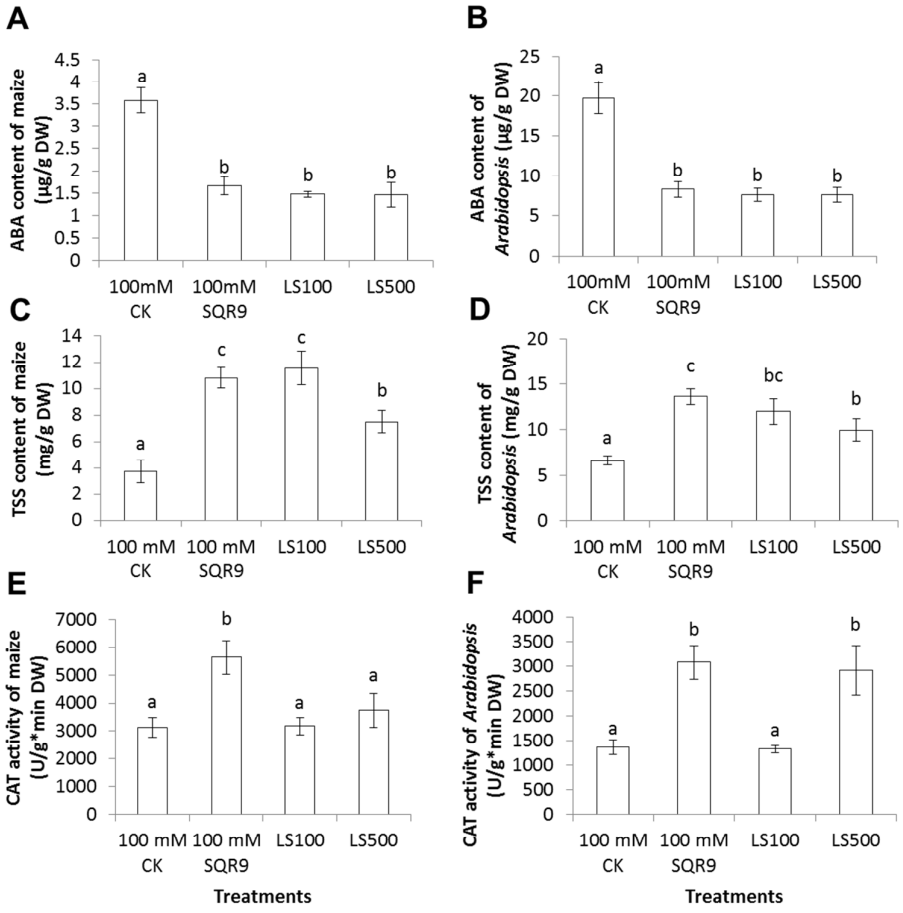


**Figure S4.** The effects of different molecular weights of *Bacillus amyloliquefaciens* SQR9 secretions on abscisic acid (ABA) (A and B), total soluble sugar (TSS) (C and D), and catalase (CAT) (E and F). A, C, and E represent physiological indicators of maize. B, D, and F represent physiological indicators of *Arabidopsis*. Different letters above the columns indicate significant differences between treatments (Duncan's least significant difference test, $P \leq 0.05$, n=5, mean ± standard deviation).

**Figure S5.** Effects of *Bacillus amyloliquefaciens* SQR9 on the response of *cat2-1* mutant to salt.

Fig. S5

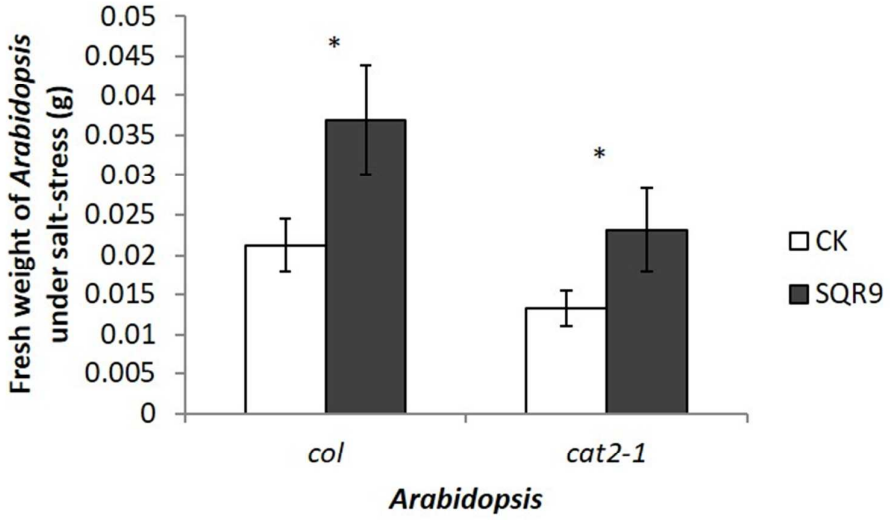


**Figure S5.** The effects of *Bacillus amyloliquefaciens* SQR9 on the response of *cat2-1* mutant to salt tolerance. The shoot fresh weight (A) and root fresh weight (B) of wide type (col) and *cat2-1* mutant were measured. Asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants (t test, $P \leq 0.05$, n=48, mean ± standard deviation).

**Figure S6.** The phenotype of different *Arabidopsis* mutants inoculated with *Bacillus amyloliquefaciens* SQR9 under salt stress.

Fig. S6

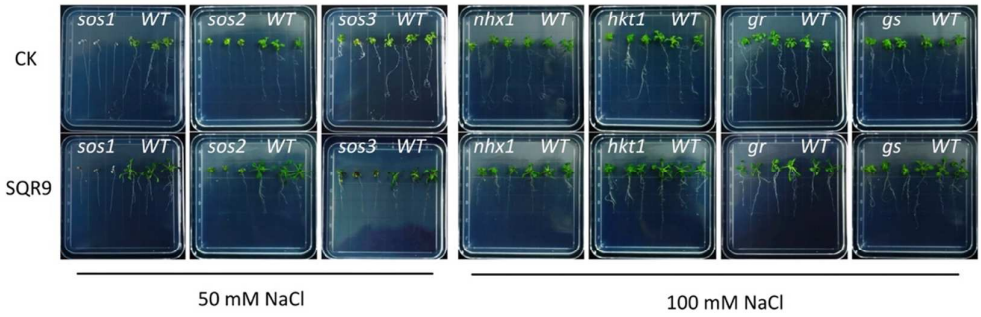


**Figure S6.** The representative images of different *Arabidopsis* mutants inoculated with *Bacillus amyloliquefaciens* SQR9 or heat-inactivated SQR9 as a control under salt stress for 14 days. The WT means wild type *Arabidopsis*. The *sos1*, *sos2*, and *sos3* mutants are treated with 50 mM NaCl. The *nhx1*, *hkt1*, *gr*, and *gs* mutants are treated with 100 mM NaCl.

**Figure S7.** The $Na^+$ content of mutants under salt stress.

Fig. S7

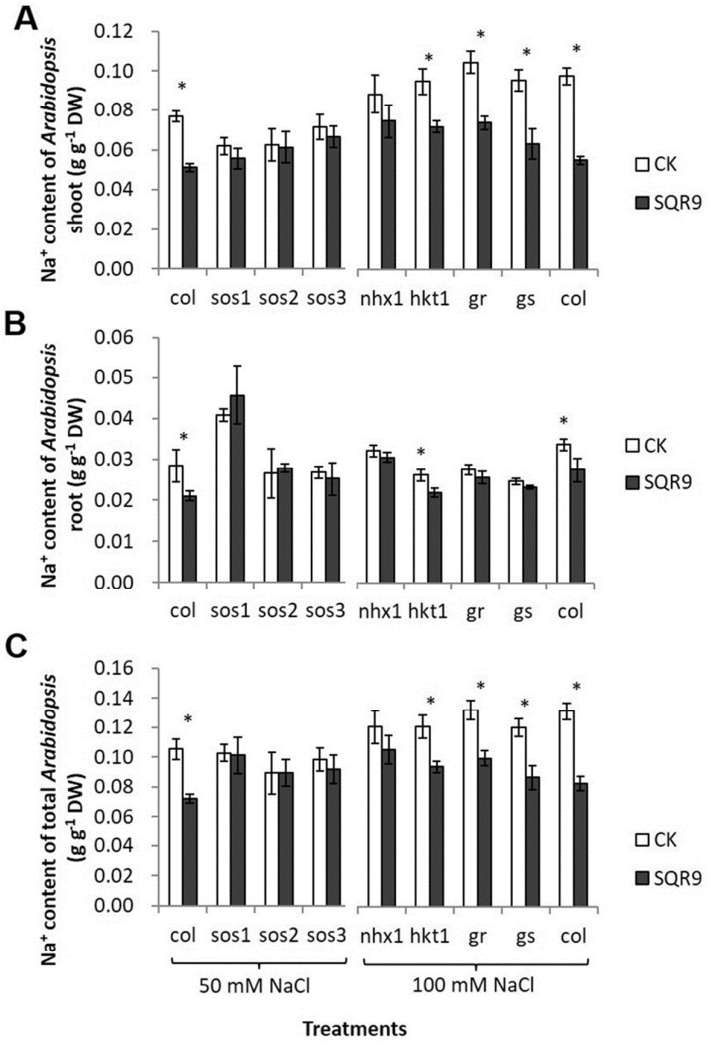


**Figure S7.** The effect of *Bacillus amyloliquefaciens* SQR9 on $Na^+$ content of *Arabidopsis* mutants shoot (A), root (B), and total (C) under 50 mM and 100 mM NaCl. The *sos1*, *sos2*, and *sos3* mutants are treated with 50 mM NaCl. The *nhx1*, *hkt1*, *gr*, and *gs* mutants are treated with 100 mM NaCl. The values are the mean ± standard deviation from 5 replicates. Asterisk (*) indicates statistically significant differences between plants inoculated with SQR9 and control plants (t test, $P \leq 0.05$).

**Figure S8.** The growth curves of different strains.

Fig. S8

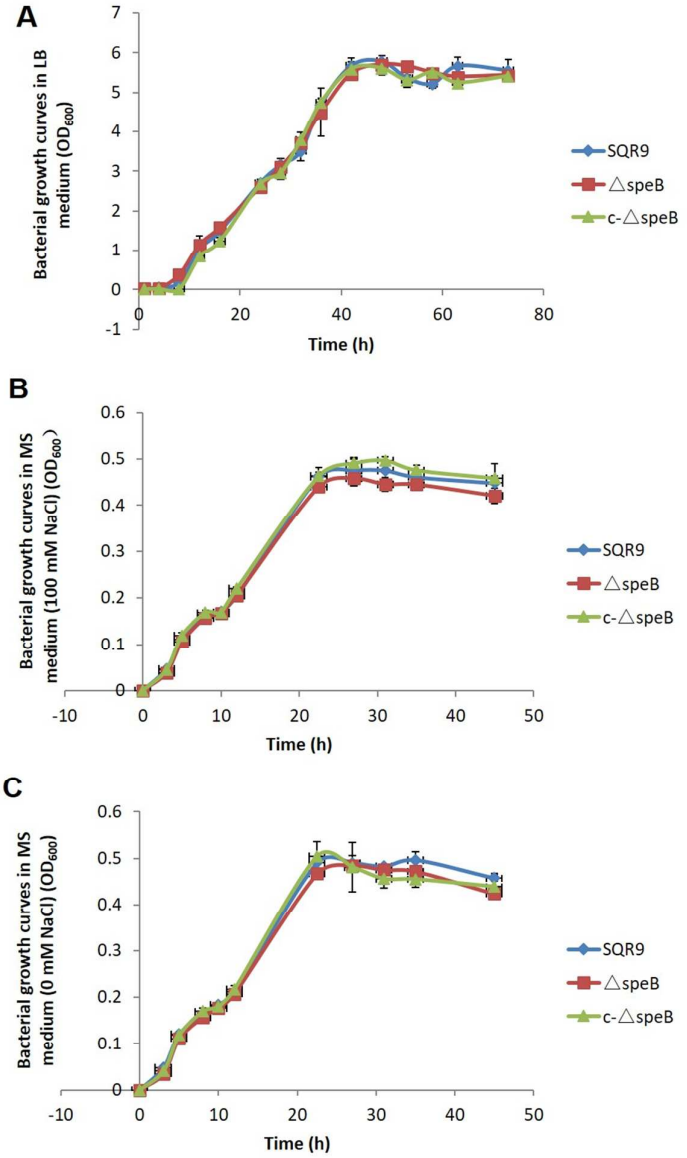


**Figure S8.** The growth curves of *Bacillus amyloliquefaciens* SQR9, Δ*speB* and c-Δ*speB* strains. (A) The growth curves of different strains were detected in LB medium. To measure bacterial growth in plant nutrient solution, the growth curves of different strains were detected in MS medium with 100 mM NaCl (B) or with 0 mM NaCl (C). The values are the mean ± standard deviation from 6 replicates.

**Figure S9.** The phenotype of *Arabidopsis* inoculated with different strains.

Fig. S9

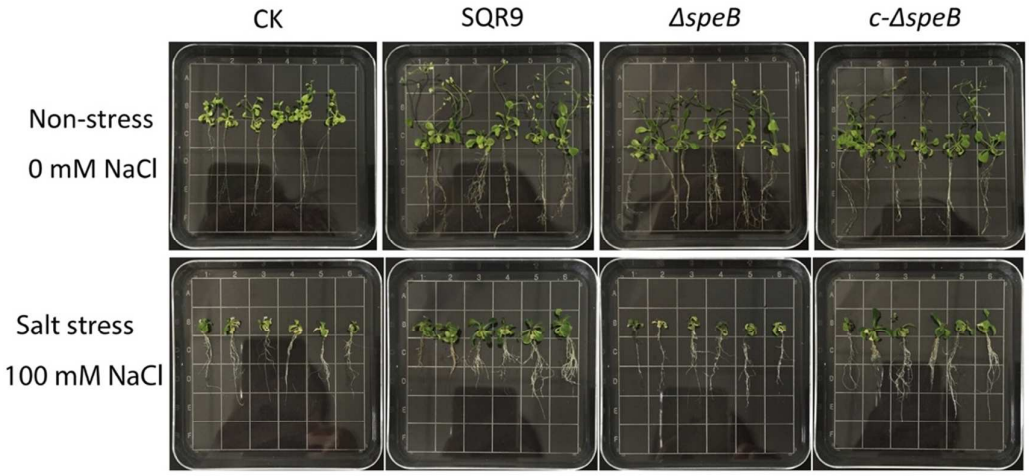


**Figure S9.** The representative images of *Arabidopsis* inoculated with *Bacillus amyloliquefaciens* SQR9, *ΔspeB*, c-Δ*speB* strains and the heat-inactivated SQR9 control under non-stress and salt stress for 14 days.

**Figure S10.** Effects of *Bacillus amyloliquefaciens* SQR9, Δ*speB* and 5 μM spermidine (spd) on *Arabidopsis* under salinity.

Fig. S10

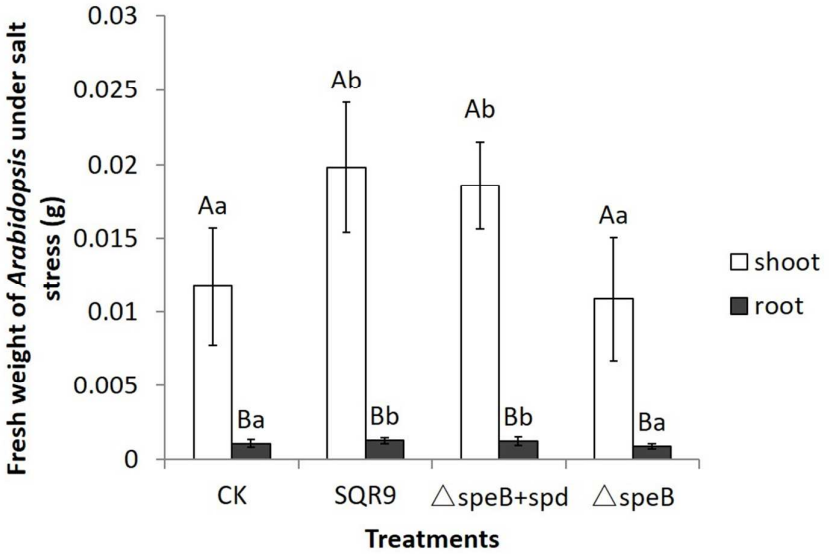


**Figure S10.** The effects of *Bacillus amyloliquefaciens* SQR9, Δ*speB* and 5 μM spermidine (spd) on *Arabidopsis* fresh weight under 100 mM NaCl. Different letters above the columns indicate statistically significant differences ((Duncan's least significant difference test, $P \leq 0.05$, n=36).

**Figure S11.** Effects of VOCs secreted by *Bacillus amyloliquefaciens* SQR9 on plants.

Fig. S11

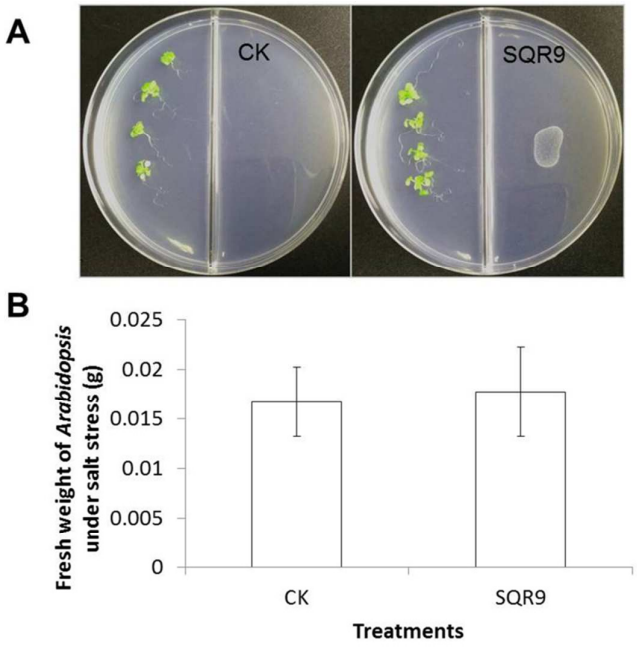


**Figure S11.** The effects of VOCs secreted by *Bacillus amyloliquefaciens* SQR9 on salt treated plants. (A) Representative images of *Arabidopsis* inoculated with control and SQR9 under salt stress. (B) Plant fresh weight was measured under salt stress for 14 days. Salt concentration is 100 mM NaCl. CK means negative control. The values are the mean ± standard deviation from 32 replicates.

**Table S1.** DNA primers used in this study

| Name | Sequence (5'-3') | Use |
|---|---|---|
| qRT-PCR analysis in *Arabidopsis* | | |
| ACT-F | CCTGCCATGTATGTTGCCATT | Internal reference |
| ACT-R | AATCGAGCACAATACCGGTTGT | |
| GS-F | AGTTATGCCTGGACAGTGGG | Detection of expression of GSH |
| GS-R | GGGCTTCAGCCTCAAGAGTT | |
| GR-F | TGCTGCTAGGTTTTCGGCTAAT | Detection of expression of GR |
| GR-R | CCTCAGAGCTAATAGGGTGAAATGG | |
| NHX1-F | TTGGTGGTCTGGTCTCAT | Detection of expression of NHX1 |
| NHX1-R | CTGGTGCGGTAATAGGTAG | |
| NHX7-SOS1-F | AGCAAGGAGACTGGAACA | Detection of expression of NHX7 |
| NHX7-SOS1-R | CGAAGAAGGCGTAGAACA | |
| Construction of mutants | | |
| speB-up-F | TCCCTATACAAGTTGTTGAGCAAGT | |
| speB-up-R | ACACGGCAAGCAAGATGATTCG | Construction of the site-directed |
| speB-down-F | GGGCGGCTTGCGATAAATACTTT | mutant Δ*speB* |
| speB-down-R | ACATTACATACGGAACAGACGGAAT | |
| Erm-speB-F | GCATTTCACGAATCATCTTGCTTGCCGTGTTCTAGAGCAACGTTCTTGCCATTG | |
| Erm-speB-R | TTTGATGAAGCATATTCAGGAAAAGTATTTACTCTTCCTTTTTCAATATTATTG | |
| speB-V-F | TGCTTCTTGCAGCTTCTGTAGGA | |
| speB-V-R | GCATTCCGCCGATCAGTGTCA | |
| c-Δ*speB*-F | CGGGGTACCTTATTTCACAAACCCAAGCAGCAT | Complementation of the mutant |
| c-Δ*speB*-R | CCGGAATTCGTACGGCGCAGGAGCGAATCTT | Δ*speB* |

## MATERIALS AND METHODS

### Determination of photosynthetic rate, $H_2O_2$, ABA, TSS and CAT activity

The photosynthetic rate of leaves was measured with Li-cor6400 analyzer system in the daytime. Photosynthetically active radiation for maize was 1200 μmol $m^{-2}$ $s^{-1}$. Photosynthetically active radiation for *Arabidopsis* was 500 μmol $m^{-2}$ $s^{-1}$.

The $H_2O_2$ was measured with $H_2O_2$ detection kit (A064). The fresh sample (1 g) was homogenized by liquid nitrogen. 0.9% NaCl (4 ml) was added. The sample was extracted sufficiently to be homogenate. The homogenate was centrifuged at 3500 rpm for 15 min at 4 °C. The supernatant was used to detect $H_2O_2$ content.

ABA, TSS, and CAT activity were measured as described by Chen et al. (2016).

### Measurement of growth curve

1% (v/v) of SQR9, Δ*speB* and c-Δ*speB* bacterial liquid were inoculated into LB medium (5 g $l^{-1}$ yeast extract, 10 g $l^{-1}$ tryptone, 6 g $l^{-1}$ NaCl; pH 7.0-7.2) and 1/2 MS containing 1.5 % sugar (0 mM and 100 mM NaCl) without antibiotics in triangular flask respectively. The triangular flask was cultured at 30 °C with shaking at 170 rpm. To measure SQR9 growth in 100 Da and 500 Da dialysis bag, thirteen-day-old maize were cultured in 1/2 Hoagland medium for two days, and root exudates were collected and sterilized. SQR9 suspension, which suspended with 1/2 Hoagland, was wrapped in 100 Da and 500 Da dialysis bag. The dialysis bag containing bacterial suspension was added into root exudate solution. The mediums were cultured at 30 °C with shaking at 80 rpm for two days. The sample was taken to detect absorbance ($OD_{600}$) with spectrophotometer in different times.

### Measurement of colonization of root by bacterium

The maize root were inoculated with *B. amyloliquefaciens* SQR9-gfp, ΔspeB-gfb and c-ΔspeB-gfb by drenching for 2 h with 35 ml of an overnight culture (OD600 nm of 0.5, cultured in LB and resuspended in sterile double-distilled water). The treatments were replicated 8 times. The method used to measure colonization was described by Liu et al. (2014).

### Measurement of effect of VOCs produced by SQR9 on plant

To measure the effect of VOCs produced by SQR9 on plant tolerance, seven-day-old

*Arabidopsis* seedlings were planted on one side of specialized plastic petri dishes that contain a center partition, both sides contained 1/2 MS media with or without 100 mM NaCl. The nonplant side of the petri dish was inoculated with 10 µl of active SQR9 or dead cell suspension.

## LITERATURE CITED

Chen, L., Liu, Y., Wu, G., Veronican Njeri, K., Shen, Q., Zhang, N., and Zhang, R. 2016. Induced maize salt tolerance by rhizosphere inoculation of *Bacillus amyloliquefaciens* SQR9. Physiol. Plant. 158:34–44

Liu, Y., Zhang, N., Qiu, M., Feng, H., Vivanco, J. M., Shen, Q., and Zhang, R. 2014. Enhanced rhizosphere colonization of beneficial *Bacillus amyloliquefaciens* SQR9 by pathogen infection. FEMS Microbiol. Lett. 353:49–56