Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression

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Abiotic stresses such as salt and drought represent adverse environmental conditions that significantly damage plant growth and agricultural productivity. In this study, the mechanism of the plant growth-promoting rhizobacteria (PGPR)-stimulated tolerance against abiotic stresses has been explored. Results suggest that PGPR strains, Arthrobacter protophormiae (SA3) and Dietzia natronolimnaea (STR1), can facilitate salt stress tolerance in wheat crop, while Bacillus subtilis (LDR2) can provide tolerance against drought stress in wheat. These PGPR strains enhance photosynthetic efficiency under salt and drought stress conditions. Moreover, all three PGPR strains increase indole-3-acetic acid (IAA) content of wheat under salt and drought stress conditions. The SA3 and LDR2 inoculations counteracted the increase of abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylate (ACC) under both salt and drought stress conditions, whereas STR1 had no significant impact on the ABA and ACC content. The impact of PGPR inoculations on these physiological parameters were further confirmed by gene expression analysis as we observed enhanced levels of the TaCTR1 gene in SA3-, STR1- and LDR2-treated wheat seedlings as compared to uninoculated drought and salt stressed plants. PGPR inoculations enhanced expression of TaDREB2 gene encoding for a transcription factor, which has been shown to be important for improving the tolerance of plants to abiotic stress conditions. Our study suggest that PGPR confer abiotic stress tolerance in wheat by enhancing IAA content, reducing ABA/ACC content, modulating expression of a regulatory component (CTR1) of ethylene signaling pathway and DREB2 transcription factor.

Introduction

Abiotic stresses such as salinity and drought are common adverse conditions that significantly affect agricultural productivity worldwide. Therefore, it is very necessary to develop sustainable strategies to improve plant’s tolerance against abiotic stress conditions. Plant growth and sustainability in natural environments are influenced by a plethora of soil microorganisms constantly interacting with plants, and comprehensive research has been

Abbreviations – ABA, abscisic acid; ACC, 1-aminocyclopropane-1-carboxylate; IAA, indole-3-acetic acid; PGPR, plant growth-promoting rhizobacterium; qRT-PCR, quantitative reverse transcription-polymerase chain reaction.

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conducted to identify the specificity of such interactions between plant and soil microorganisms (Berendsen et al. 2012, Averill et al. 2014).

Plant growth-promoting rhizobacteria (PGPR) represent one such class of microbes that have been documented to promote plant growth and yield. Either as biofertilizers or control agents, PGPR have been widely used in many agricultural crops to enhance their growth and protect them against various stress conditions (Barnawal et al. 2012, Bharti et al. 2013, Nadeem et al. 2014). Numerous mechanisms such as phosphate solubilization, biological nitrogen fixation and siderophore production and others have been shown to be involved in improving plant growth through enhanced nutrient uptake. Moreover, it has been shown that PGPR modulates the plant hormone status as one of their efficient mechanisms to improve plant tolerance under stress conditions. PGPR can modify plant hormone status by producing auxins and cytokinins (Costacurta and Vanderleyden 1995, Dodd et al. 2010) or by reducing plant ethylene levels through the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses the ACC (immediate or by reducing plant ethylene levels through the bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyses the ACC (immediate ethylene precursor) into α-ketobutyrate and ammonia (Honna and Shimomura 1978, Glick et al. 1998).

Acc deaminase-containing rhizobacteria have been shown to promote plant growth during environmental stresses that stimulate stress-mediated ethylene production (Mayak et al. 2004a, 2004b, Glick et al. 2007, Belimov et al. 2009).

PGPR have been reported to have multidimensional effects on plants (Jiang et al. 2012). It has been indicated that irrespective of mechanisms mediating plant and PGPR interaction, the basic mode of communication involves overall impacts on plant signaling pathways (Puga-Freitas and Blouin 2015). Plant hormones play a critical role in assisting plants to adapt to various environmental conditions by mediating growth and development, nutrient allocation and source/sink transitions (Peleg and Blumwald 2011). Beneficial microbes have an ability to manipulate plant hormone balance for plant protection under stress conditions (Planchamp et al. 2015). They may even exploit plant hormone crosstalk by modifying shoot hormone concentrations and their root-to-shoot signaling for improving plant physiological processes, growth and development under salt and drought stress (Dodd et al. 2010).

This work is focused on phytohormonal and physiological responses upon these PGPR treatments under abiotic stress conditions to find out the mechanism of protection induced by PGPR to protect wheat plants from salt and drought stress conditions. In this study, the preliminary mechanism underlying wheat salt stress tolerance driven by PGPR strains, Arthrobacter protophormiae (SA3) and Dietzia natronolimnaea (STR1) has been investigated. Furthermore, we have also explored the mechanism of another PGPR strain, Bacillus subtilis (LDR2) assisted wheat plant tolerance against drought conditions. We have also summarized difference between abiotic stress tolerance mechanisms driven by ACC deaminase containing (LDR2 and SA3) and ACC deaminase lacking (STR1) PGPR.

**Materials and methods**

**Bacterial culture and inoculations**

The PGPR strains Dietzia natronolimnaea STR1 (GenBank Accession no. KJ413139) (Bharti et al. 2016), Arthrobacter protophormiae SA3 (GenBank Accession no. AKF736951) (Barnawal et al. 2014) and Bacillus subtilis LDR2 (GenBank Accession no. JX996197) (Barnawal et al. 2013) were obtained from the microbial culture collection of Microbial Technology Department, CSIR-CIMAP, Lucknow. The culture LDR2 was maintained on nutrient agar medium, whereas STR1 and SA3 were maintained on nutrient agar supplemented with 0.5 M NaCl. The bacterial strains STR1 and SA3 grew profusely at 0.5 M NaCl, 28°C at neutral pH (pH 7.0), whereas LDR2 could tolerate 25% polyethylene glycol (PEG)-10,000 under similar conditions (Barnawal et al. 2013). Bacterial inoculations of wheat seedlings under hydroponic conditions were performed prior to PEG and NaCl applications. Bacterial treatments were prepared in 25 ml nutrient broth in orbital flask shaking incubator at 0.14 g maintained at 28°C for 24 h. For wheat seedling inoculation, bacteria were harvested by centrifugation (4000 g, 15 min, 15°C), washed once with 0.85% NaCl and resuspended in 25 ml of 0.01 M MgSO₄ [approximately 10⁵ colony-forming units (cfu) ml⁻¹]. Then, 1 ml of bacteria suspension (in 0.01 M MgSO₄) was mixed into 49 ml of plant growth medium (half-strength Hoagland nutrient solution). For control, the 1 ml of 0.01 M MgSO₄ solution without PGPR was added into 49 ml of plant growth medium.

**Planting assay and stress conditions**

Wheat (Triticum aestivum) cv. HD2285 seeds were surface sterilized for 5 min in a 3% sodium hypochlorite solution containing 0.1% Triton X-100, then washed with sterile distilled water four times. After washing, seeds were germinated and grown hydroponically with the help of germination sheet rolls kept in 250 ml glass beakers filled with Hoagland nutrient solution (one-half strength) under cool-white-fluorescent light (100 µmoles photon m⁻² s⁻¹) in 14 h/10 h light/dark photoperiod at
24°C. From the day of germination, saline stress was applied for 12 days. Hoagland nutrient solution was exchanged every day. For plants subjected to salt stress, sodium chloride was added to the Hoagland nutrient solution to obtain a final concentration of 100 mM. For conferring drought stress to wheat seedlings, the half-strength Hoagland’s nutrient solution was supplemented with 10% PEG-10,000. The following conditions were maintained for seedlings: Control (no stress, no PGPR); Salt (100 mM NaCl, no PGPR); Drought (10% PEG, no PGPR); SA3 (no stress, inoculated with SA3); SA3 + Salt (100 mM NaCl with SA3); STR1 (no stress, inoculated with STR1); STR1 + Salt (100 mM NaCl with STR1); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2).

**Plant growth measurement**

At harvest (12 days post inoculation), shoot dry weight, shoot length, root dry weight and root length from 10 seedlings were determined. Dry weights were measured after drying seedlings in an oven at 70°C (Bharti et al. 2016).

**Estimation of Fv/Fm, net CO₂ assimilation, stomatal conductance and transpiration rate**

Fv/Fm, net CO₂ assimilation, stomatal conductance and transpiration rate were measured using CIRAS-3 portable photosynthesis system attached with CFM-3 chlorophyll fluorescence module (PP Systems model CI-310 PPS; CID Inc., Vancouver, WA, Canada). Maximum photosynthetic efficiency of photosystem II (Fv/Fm) was calculated by measuring chlorophyll fluorescence. CO₂ synthesis was maintained for seedlings: Control (no stress, inoculated with STR1); STR1 (no stress, inoculated with LDR2); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2).

**ACC content measurement**

ACC (1-aminocyclopropane-1-carboxylate) concentrations in seedling tissues were measured (Madhaiyan et al. 2007). A total of 1 g of root sample was frozen immediately by dipping into liquid nitrogen and ground. ACC extraction was done by using 5 ml of 80% methanol containing BHT (2 mg l⁻¹) as an antioxidant and incubated at room temperature for 45 min. Centrifugation of samples was done at 2000 g at 20°C for 15 min and resuspension of pellet was prepared in 4 ml of 80% methanol and centrifuged. The supernatants were combined and evaporated to dryness in vacuum by using rotatory evaporator. ACC levels were determined as described below (Wachtler et al. 1999). Resuspension of residues were prepared in 2 ml distilled water, and then the upper aqueous phase (0.5 ml) extracted with dichloromethane was mixed with 0.1 ml HgCl₂ (80 mM) in test tubes and sealed with rubber septa. Then 0.2 ml sodium hypochlorite solution was injected into the tubes, shaken and incubated for 8 min. A volume 1 ml of the gaseous portion was taken and assayed for ethylene by gas chromatography (Barnawal et al. 2012).

**Quantitative reverse transcription-polymerase chain reaction**

Total RNA from wheat seedlings were extracted from the shoots of 12-days-old plants using TRIZOL reagent (Sigma-Aldrich Inc. MO). RNA preparation was treated with RNase-free DNase I (TaKaRa Bio Dalian Co., Ltd) to remove genomic DNA contamination. Approximately, 3 μg of total RNA was then synthesized into cDNA using a first strand cDNA synthesis kit (Thermo Scientific). A volume of 1 μl of the 1:10 diluted cDNA was subjected to real-time quantitative polymerase chain reaction (qPCR) using SYBR Green dye and gene specific primers. Gene specific primers for DREB2 (forward: 5′-CGGAGAT GCAGCTTCTGTATT-3′, reverse: 5′-GATCTGCAGCG ACGGCTA TT-3′), AUX/IAA1 (forward: 5′-CAACGG CTCACACATTGCTCA-3′, reverse: 5′-AGATCCAC CTTG CGCAGGTA-3′), CTR1 forward: 5′-GCTGCTTCTGT
GAATCCTGTGG-3’, reverse: 5’-ATCCAAATGCTTGAA AACGAA-3’) and Actin (forward: 5’-CGAAGCTTCA GTT GCCCGACA-3’, reverse: 5’-ACCATACCCAGA TCGAGACA-3’) were used in quantitative reverse transcription-polymerase chain reaction (qRT-PCR). PCR conditions were 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s each and annealing/extension at 60°C for 1 min each using Applied Biosystems StepOnePlus™ Real-Time PCR System. The relative expression of genes was calculated using 2−ΔΔCt method with Actin as an endogenous control.

Statistical analysis
The collected data were statistically analyzed using ANOVA with the help of software IBM SPSS PASW Statistics 18. Means and standard errors for growth parameters were calculated for 10 replicate values. Significant differences among treatments were analyzed on the basis of ANOVA, and means were calculated using Duncan’s test under a significance level of P ≤ 0.05.

Results
PGPR-assisted wheat plants tolerate salt and drought stress
Salt and drought stress conditions reduced wheat seedling growth as observed in terms of shoot dry weight, root dry weight, shoot and root length. Significant differences for shoot dry weight as well as root dry weight were found among nonstressed and stressed plants (salt and drought) without PGPR treatments. Under salt stress conditions, treatment of wheat seedlings with PGPR strains, SA3 (A. protophormiae) and STR1 (D. natronolimnaea) significantly enhanced biomass measured as shoot and root dry weight and length in comparison to uninoculated seedlings (Fig. 1A–D). In the same manner, under drought conditions, the PGPR strain LDR2 (B. subtilis) significantly enhanced biomass in comparison to uninoculated seedlings (Fig. 1A–D). Taken together, these results suggest that PGPR strains, SA3 and STR1 can help wheat seedlings in tolerating salt stress, while LDR2 strain can assist wheat seedlings against drought tolerance.

SA3, STR1 and LDR2 inoculations improve plant photosynthetic parameters under salt and drought stress
Salt or drought stress conditions reduced photosynthetic efficiency of wheat seedlings, as observed by significantly reduced photosynthetic parameters such as maximum photosynthetic efficiency of photosystem II (Fv/Fm), net CO2 assimilation, stomatal conductance and transpiration rate (Fig. 2). We observed that wheat seedlings inoculated with SA3, STR1 and LDR2 strains always had higher Fv/Fm value under unstressed conditions. However, SA3 and STR1 inoculations significantly enhanced Fv/Fm value under salt stress conditions as compared to uninoculated stressed seedlings. Similarly, LDR2 assisted wheat seedlings showed higher Fv/Fm value under drought stress compared with uninoculated stressed wheat seedlings (Fig. 2A). Net CO2 assimilation rate was observed to be higher in SA3-, STR1- and LDR2-inoculated seedlings. Net CO2 assimilation was reduced as a result of the drought and salt stress conditions as compared to control seedlings. PGPR inoculations, however, enhanced the rate of CO2 assimilation significantly as compared to uninoculated seedlings after exposure to either salt or drought stresses (Fig. 2B). During stress exposure, a marked decrease in the stomatal conductance across the measurement period was observed. STR1 and SA3 inoculated seedlings recorded higher values of stomatal conductance. Stress exposed wheat seedlings inoculated with PGPR recorded values intermediate to nonstressed and uninoculated stressed seedlings indicating that SA3 and STR1 can improve stomatal conductance under salt stress. On the other hand, LDR2 treatment improved stomatal conductance as compared to uninoculated drought stressed seedlings (Fig. 2C). Transpiration rates lowered by approximately 70 and 65% when exposed to drought and salt stress in comparison to control seedlings. The significant improvement in transpiration rate was observed in salt stressed seedlings when they were treated with SA3 and STR1 in comparison to uninoculated stressed seedlings. Similarly, LDR2 showed higher transpiration rate under drought stress than uninoculated control seedlings (Fig. 2D). Taken together, these results remarkably illustrate the role of these PGPR strains in enhancing photosynthetic efficiency under salt and drought stress conditions.

Effect of SA3, STR1 and LDR2 inoculations on plant IAA content and expression of the AUX/IAA1 gene
Salt or drought stress conditions reduced IAA contents in wheat seedlings (Fig. 3A). Under nonstress conditions, we observed enhanced IAA contents in SA3, STR1 and LDR2 inoculated seedlings. It was increased by 29, 75 and 35% in SA3, STR1 and LDR2 inoculated seedlings as compared to uninoculated seedlings. STR1 and SA3 inoculation enhanced IAA content 150% as compared to uninoculated salt stressed seedlings (Fig. 3A). Under drought stress conditions, LDR2 inoculation enhanced IAA content in wheat seedlings by approximately 80%,
Fig. 1. Effect of PGPR treatments on (A) shoot dry weight, (B) shoot length, (C) root dry weight, (D) root length of wheat plants under salt and drought stress conditions 12 days post inoculation. Control (no stress, no PGPR); Salt (100 mM NaCl, no PGPR); Drought (10% PEG, no PGPR); SA3 (no stress, inoculated with SA3); SA3 + Salt (100 mM NaCl with SA3); STR1 (no stress, inoculated with STR1); STR1 + Salt (100 mM NaCl with STR1); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2). Values are mean of 10 replicates ± SE of means. Different letters indicate statistically significant differences between treatments (Duncan’s multiple range test $P < 0.05$).
Fig. 2. Effect of PGPR treatments on photosynthetic parameters: (A) Fv/Fm, (B) CO2 assimilation rate, (C) stomatal conductance, (D) transpiration rate of wheat plants under salt and drought stress conditions at different interval of times after germination. Control (no stress, no PGPR); Salt (100 mM NaCl, no PGPR); Drought (10% PEG, no PGPR); SA3 (no stress, inoculated with SA3); SA3 + Salt (100 mM NaCl with SA3); STR1 (no stress, inoculated with STR1); STR1 + Salt (100 mM NaCl with STR1); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2). Values are mean of 10 replicates ± SE of means.

as compared to uninoculated seedlings (Fig. 3A). These results demonstrate the role of PGPR strains in enhancing IAA content under salt and drought stress.

Furthermore, we examined the effect of PGPR strains on the expression of AUX/IAA1 gene encoding for transcriptional repressor of auxin-responsive genes (Li et al. 2015) under salt and drought stress conditions. We observed an upregulation of AUX/IAA1 expression under salt and drought stress conditions (Fig. 3B). Under nonstress condition, SA3, LDR2 and STR1 inoculated plants displayed higher AUX/IAA1 expression as compared to uninoculated seedlings. Under salt stress, the seedlings inoculated with STR1 and SA3 displayed downregulation of AUX/IAA1 gene expression in comparison to uninoculated salt stressed seedlings. Similarly, the LDR2 inoculated drought stressed seedlings showed reduced AUX/IAA1 gene expression as compared to uninoculated drought stressed seedlings (Fig. 3B). Taken together, these results suggest that PGPR modulates both IAA synthesis and the auxin signaling pathway.

Effect of SA3, STR1 and LDR2 inoculations on plant ACC and ABA content

We observed enhanced ACC content under salt as well as drought conditions (Fig. 4A). The LDR2 and SA3 strains are ACC deaminase-containing rhizobacteria, while STR1 does not have ACC deaminase activity (Barnawal et al. 2013, Barnawal et al. 2014, Bharti et al. 2016). Under nonstress condition, no significant variation of ACC content was observed between untreated and SA3/STR1/LDR2 inoculated seedlings. Under salt stress
condition, ACC content was decreased in SA3 inoculated seedlings as compared to uninoculated seedlings, whereas no significant difference was observed STR1 inoculated seedlings and uninoculated seedlings. Under drought stress, ACC content was reduced in LDR2 treated seedlings as compared to uninoculated seedlings (Fig. 4A).

We observed increased ABA content under salt as well as drought conditions (Fig. 4B). Under nonstress condition, no significant variation of ABA content was observed between untreated and SA3/LDR2 inoculated seedlings. As compared to control plants, the ABA content was increased in STR1 inoculated wheat seedlings under no stress condition (Fig. 4B). Under salt stress condition, ABA content was decreased in SA3 inoculated seedlings as compared to uninoculated seedlings, whereas no significant difference was observed STR1 inoculated seedlings and uninoculated seedlings. Under drought stress, ABA content was reduced in LDR2 treated seedlings as compared to uninoculated seedlings (Fig. 4B).

**SA3, STR1 and LDR2 treatments modulate expression of the gene encoding CONSTITUTIVE TRIPLE RESPONSE1 (TaCTR1)**

We examined the effect of PGPR on the expression of the gene encoding CTR1, which is a regulatory component of the ethylene signaling pathway that modulates stress related changes in plants (Zhao et al. 2016). The relative expression level of TaCTR1 was found to be downregulated in the wheat seedlings subjected to drought or salt stress as compared to nonstressed control seedlings (Fig. 5). Nonstressed seedlings inoculated with SA3, STR1 and LDR2 maintained a higher TaCTR1 expression levels in comparison to the uninoculated control seedlings. Under salt and drought stress conditions, we observed that SA3, STR1 and LDR2 inoculated seedlings display higher expression of TaCTR1 as compared to uninoculated drought and salt stressed seedlings (Fig. 5). Under salt and drought stress, the recovered expression of TaCTR1 gene in PGPR inoculated seedlings demonstrates PGPR involvement in improving stress tolerance.

**SA3, STR1 and LDR2 treatments modulate expression of the gene encoding dehydration responsive element binding2 (DREB2)**

Dehydration responsive element binding (DREBs) proteins are plant transcription factors that play an important role in improving the tolerance of plants to abiotic stress conditions (Lata and Prasad 2011). The expression level of TaDREB2 was found to be upregulated in the wheat seedlings subjected to drought stress or salt stress as compared to nonstressed control seedlings (Fig. 6). Nonstressed seedlings inoculated with SA3, STR1 and LDR2
**Fig. 4.** Effect of PGPR treatments on (A) ACC content, (B) ABA content under salt and drought stress conditions 12 days post inoculation. Control (no stress, no PGPR); Salt (100 mM NaCl, no PGPR); Drought (10% PEG, no PGPR); SA3 (no stress, inoculated with SA3); SA3 + Salt (100 mM NaCl with SA3); STR1 (no stress, inoculated with STR1); STR1 + Salt (100 mM NaCl with STR1); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2). Values are mean of five replicated ± SE. Different letters indicate statistically significant differences between treatments (Duncan’s multiple range test $P < 0.05$).

**Fig. 5.** Effect of PGPR treatments on the expression of *TaCTR1* gene under salt and drought stress conditions 12 days post inoculation. The data represents means of triplicate biological repeats ± SE. Different letters indicate statistically significant differences between treatments (Duncan’s multiple range test $P < 0.05$). Control (no stress, no PGPR); Salt (100 mM NaCl, no PGPR); Drought (10% PEG, no PGPR); SA3 (no stress, inoculated with SA3); SA3 + Salt (100 mM NaCl with SA3); STR1 (no stress, inoculated with STR1); STR1 + Salt (100 mM NaCl with STR1); LDR2 (no stress inoculated with LDR2); LDR2 + Drought (10% PEG with LDR2).

displayed upregulated *TaDREB2* expression in comparison to the uninoculated control seedlings. A higher expression of *TaDREB2* was observed in SA3, STR1 and LDR2 treated seedlings under salt and drought stress conditions as compared to uninoculated drought and salt stressed seedlings (Fig. 6). Under salt and drought stress, the enhanced expression of *TaDREB2* gene in PGPR inoculated seedlings suggests the role of PGPR
in improving stress tolerance of wheat seedlings through DREB2 transcription factor.

**Discussion**

Increased consciousness about the harmful impacts of chemical fertilizers on the environment has led to the development of rhizobacteria as the alternative resource for promoting plant growth and improving soil health under abiotic and biotic stresses. A number of PGPR have been shown to be involved in promotion of plant growth and tolerance against drought and salt stress conditions. Efforts are being taken to elucidate the mechanism of interaction as illustrated through altered plant metabolism at proteomic, transcriptomic or metabolomic levels. The plant molecular responses to abiotic stresses comprise interactions and complex crosstalk with multiple molecular pathways (Takahashi et al. 2004). Salinity and drought stress trigger several early signaling events leading to reactive oxygen species formation (Smekalova et al. 2014). Variations in plant physiological parameters like photosynthesis, stomatal conductance and hormonal balance are good markers of stress in plants. The present study builds on the exhaustive studies indicating that growth hormones are primarily involved and may play an important role both in causing stress induced damage but also alleviating the impact of stress on plant growth.

In the current study, PGPR strains, SA3 and STR1 improve wheat seedling growth and yield in salt stress conditions, while LDR2 strain can improve wheat seedling growth and yield in drought condition. Of the three, the SA3 and LDR2 strains have been documented to possess ACC deaminase-producing ability and have been shown to have positive impact on *Pisum sativum* and *Trigonella* spp. growth, respectively, under abiotic stress conditions (Barnawal et al. 2013, Barnawal et al. 2014).

Maximum photosynthetic efficiency of photosystem II (Fv/Fm), a photosynthetic parameter, is used to detect stress in plants (Maxwell and Johnson 2000). To assess the changes in the photosynthetic performance of wheat seedlings subjected to drought and salinity stresses and microbial inoculation, we measured the photosynthetic parameters such as, Fv/Fm, net CO₂ assimilation rate, stomatal conductance and transpiration rate. The PGPR strains, SA3, STR1 and LDR2, enhanced the photosynthetic efficiency of wheat seedlings under salt and drought stress conditions. The Fv/Fm value indicates the maximum quantum efficiency of PSII. Abiotic stresses such as drought and salinity reduce the quantum efficiency of PSII photochemistry (Briantais et al. 1996). The results obtained in the present study support the previous observations, which clearly demonstrate negative effects of salinity and drought stress on Fv/Fm ratio. The decline in Fv/Fm values can be corroborated to oxidative damages induced by drought and salt stresses (Iseki et al. 2013). However, PGPR treatments under stress conditions enhance Fv/Fm values as compared to uninoculated stressed seedlings suggesting a role for these PGPR strains in restoring optimal photosynthesis. A process of water conservation generally occurs in plants during drought or salt stress (due to less availability of water), which creates low gaseous exchange rate or stomatal conductance of plant leaves that causes plant tissue damage and death (Rivero et al. 2014). PGPR treatments under stress conditions enhance stomatal conductance as compared to uninoculated stressed control seedlings indicating a role for SA3, STR1 and LDR2 strains in restoring optimal stomatal conductance. Stomata play a critical role in controlling water loss through transpiration and influx of CO₂ for photosynthesis (Hetherington and Woodward 2003). Drought stress reduces photosynthetic rate due to stomatal closure that restricts the diffusion of CO₂ into the leaf (Izanloo et al. 2008). Drought and salt stress both lower the transpiration rate of plant leaves (Mishra et al. 2012). We observed that PGPR treatments maintain the optimal transpiration rate under abiotic stress conditions. Hence, our study remarkably illustrates that these strains are able to maintain optimal photosynthetic parameters and protect wheat plant from these adverse environmental conditions.

Phytohormones play a major role in plant growth and development during plant responses to various environmental conditions. Plants adjust their phytohormonal levels to reduce the harmful effects during stress (Glick 2007). Plant growth promoting microbial inoculation can modulate phytohormonal levels in the plants under environmental stresses (Glick 2012). Microbes alter the plant hormonal balance through secreting growth regulators or by inducing their synthesis within the plant (Cabot et al. 2014). IAA orchestrates a number of physiological processes in plants. Prakash and Prathapanan (1990) have shown that NaCl treatment significantly reduced IAA contents in rice leaves. This indicates that abiotic stress condition could affect IAA contents in plants. The present study shows that IAA content is negatively affected by both salinity and drought stress. However, PGPR inoculations enhanced plant IAA concentrations as a part of plant growth promotion mechanism (Duca et al. 2014, Kurepin et al. 2015). AUX/IAA gene family represents early auxin response genes encoding nuclear proteins acting as transcriptional repressors of auxin-responsive genes (Li et al. 2015). Degradation of AUX/IAA proteins promotes activation of ARF transcription factors and the subsequent expression of auxin.
responsive genes (Hagen and Guilfoyle 2002). Upregulation of certain AUX/IAA1 genes upon salt stress has been observed in Arabidopsis plant (Jiang and Deyholos 2006). We have examined the effect of these PGPR strains on the expression of AUX/IAA1 gene under salt and drought stress conditions. In this study, we found that both salt and drought conditions induce upregulation of the AUX/IAA1 gene. However, STR1 and SA3 treatments under salt stress conditions led to downregulation of Aux/IAA1 gene expression as compared to uninoculated salt stressed plants. Similarly, LDR2 treatment under drought condition led to downregulation of Aux/IAA1 gene expression as compared to un-inoculated drought stressed plants. Taken together, these observations suggest that PGPR modulates the auxin signaling pathway to protect the plant from abiotic stress conditions.

The PGPR strains, LDR2 and SA3, are ACC deaminase containing rhizobacteria, while STR1 does not have ACC deaminase activity. As expected, LDR2 and SA3 reduce the ACC content, while STR1 does not affect the ACC content in plants. ACC is the immediate precursor of ethylene (Barnawal et al. 2017). Under salt and drought stress conditions, plants characteristically produce amplified levels of ethylene. Hence, this level of ethylene production limits plants capability of growth and proliferation until stress conditions are removed and ethylene level is reduced. ABA-dependent and ABA-independent signaling pathways have been shown to be involved in plant response to osmotic stress induced by salt, drought and cold. A remarkable increase in ABA content has been observed in plants under either drought or salt stress (Hoad 1975, Chen et al. 2016), which was also evident in our studies. PGPR (LDR2 and SA3) inoculations led to reduction in ABA content in wheat plants under either drought or salt stress conditions, which could be attributed to lower ethylene levels in such plants. It has been shown that ethylene affects ABA synthesis in plants (Rowe et al. 2016); thus, inoculation with ACC deaminase-containing rhizobacteria (LDR2 and SA3) might be affecting plant ABA levels. Applications of LDR2 and SA3 led to reduction of ABA content in stressed plants, suggesting reduced stress intensity in such plants. STR1 strain does not alter either ACC or ABA content in plants suggesting other mechanism are induced to achieve stress tolerance. This work also supports the idea that ACC deaminase-containing rhizobacteria are capable of reducing stress conditions through management of ethylene and ABA levels.

Ethylene is perceived by ethylene receptor related to bacterial two component regulators. Ethylene binding results in the inactivation of the receptors and consequently, downstream protein CONSTITUTIVE TRIPLE RESPONSE1 (CTR1) is inactivated. CTR1 inactivation led to activation of the downstream protein ETHYLENE INSENSITIVE2 (EIN2), a positive component of ethylene signaling pathway and consequent activation of transcription factors, EIN3 and ETHYLENE RESPONSSE FACTOR (ERF) start triggering hundreds of genes and is known as the ethylene responsive gene (Yasumura et al. 2015). We observed that salt or drought stress conditions repress CTR1 expression encoding a regulatory component of the ethylene signaling pathway that modulates stress related changes in plant growth and development. Inoculation of PGPR led to enhancement of expression level of CTR1 under stressed and nonstressed conditions, suggesting that PGPR enhances plant survival by negating stress conditions. DREBs, a class of plant transcription factors that modulate the expression of stress-inducible genes in an ABA-independent manner, play a key role in enhancing the abiotic stress tolerance by interacting with a DRE/CRT cis-element located in the promoter of abiotic stress-responsive genes of plants. Transgenic plants overexpressing several DREB transcription factor showed more tolerance to drought, salt, heat and freezing stress conditions (Lata and Prasad 2011). The present study demonstrates a upregulation of TaDREB2 in PGPR-inoculated plants under stress conditions. In this context, it is worth noting that Enterobacter sp., a PGPR, also enhanced DREB2 expression level in Arabidopsis upon salt treatment (Kim et al. 2014). Taken together, these results suggest that increased expression of DREB2 led to enhanced tolerance against drought and salt in LDR2, STR1 and SA3 treated plants.

This study suggests that ACC deaminase producing rhizobacteria as well as ACC deaminase lacking rhizobacteria are capable of promoting abiotic stress tolerance and plant growth by influencing IAA biosynthesis, auxin signaling pathway and modulating expression of genes encoding CTR1 and DREB2 proteins. We observed the only difference in mode of action used by both class of PGPR is that ACC deaminase producing rhizobacteria are capable of modulating ethylene and ABA content in plants while rhizobacteria lacking ACC deaminase is not able to modulate ethylene and ABA. This work provides implications for applying PGPR strains in providing plants to tolerate abiotic stress.

Author contributions
A. K., A. P., D. B., N. B. conceived and designed this research. D. B., N. B., S. S. P. and C.S.C conducted experiments. A. P., D. B. and N. B. analyzed data. A. P., D. B. and N. B. wrote the manuscript. All authors read and approved the manuscript.
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