Molecular and physiological analysis of drought stress responses in *Zea mays* treated with plant growth promoting rhizobacteria

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Abstract

Our research intended to appraise the performance of two different *Pseudomonas* strains on *Zea mays* L. (cv. B73) under drought stress and non-stress conditions. Plants were inoculated with *P. putida* KT2440 (Pp) and *P. fluorescens* (Pf1) followed by sampling at 0, 3rd, and 6th day after imposition of drought stress (DAS). Both strains demonstrated significant improvement in root length, protein content, chlorophyll content, and root and shoot fresh masses as compared to uninoculated drought stressed plants. Real-time quantitative PCR analysis revealed that drought stress responsive genes, i.e., the cold-related dehydrin 410 gene, *WRKY18*, and major facilitator superfamily were significantly down-regulated by Pf1 and Pp inoculation under drought stress condition on 6 DAS. Similarly, the down-regulated transcript abundance of lipoxygenase genes in inoculated plants on 6 DAS showed the role of Pf1 and Pp in scavenging reactive oxygen species under drought stress conditions. Among the selected jasmonic acid pathway responsive genes, maize protease inhibitor and 12-oxo-phytodienoate reductase 7 (*OPR7*) also revealed a potential role of these rhizobacteria under drought stress conditions. Seed inoculation of both strains significantly down-regulated the expression of *OPR7* gene under stress conditions. Our results advocate the complex growth promotion effects of both selected rhizobacterial strains and amelioration of the drought by modulating the expression of drought stress responsive genes.

Additional key words: chlorophyll, gene expression, maize, protein, *Pseudomonas fluorescens*, *Pseudomonas putida*.

Introduction

Plants are affected by various stressful environmental conditions and drought stress is one of the most destructive abiotic stresses in arid or semiarid regions. Global warming has also accelerated the harshness and frequency of drought and consequent reduction of food production (Vinocur and Altman 2005). The crop production and food security is also facing serious problem due to salinity and drought stresses (Munns and Tester 2008). Drought stress usually affects various biochemical and physiological processes like respiration, photosynthesis, ion uptake, nutrient metabolism, and translocation and hence reduces plant growth (Farooq et al. 2008, Jaleel et al. 2008). Drought stress response in plants is a very complex trait and it depends upon several factors such as genotype, severity of the stress, stress duration, environment, and developmental stage. The lack of water in the substrate...
causes limited nutrient acquisition leading to indirect detrimental effects on plant growth (Benabdellah et al. 2011). Another indirect effect is production of reactive oxygen species (ROS), which reduce plant growth by lipid peroxidation, protein denaturation, and DNA mutation. For survival and resilience, plants evolve defensive strategies in response to certain stresses (Bohnert et al. 2006) resulting in production of several detoxifying and defensive enzymes, and compatible solutes (Molinari et al. 2007, Ray et al. 2016). The antioxidant enzymes scavenge free radicals and help in stress compensation and growth promotion (Zakikhani et al. 2012, Dourado et al. 2013, Nogueiro et al. 2015).

In order to ameliorate drought stress effects, researchers utilize different genomics and transgenic strategies in plants (Thudi et al. 2014). Modern biotechnological strategies have played a vital role in improving the drought stress tolerance in plants (Lu et al. 2013). With the advantage of plant breeding and genetic engineering, development of drought tolerant cultivars is also an effective approach to increase the crop yield and water use efficiency but genetically modified plants as a whole could not find a good place (Wahid et al. 2007). Symbiotic interactions of microbes with plants can help them survive a variety of biotic and abiotic stresses. Beneficial plant-microbe interactions can mitigate the stress effects in plants significantly (Marulanda et al. 2006). Stressed plants are dependent on plant growth promoting rhizobacteria (PGPR) that help in enhancing their metabolic activities to withstand the stress (Kavamura et al. 2013). The plant inoculation with such beneficial microbial activities helps in the growth promotion, stress tolerance, survival, and adaptation to changing climate conditions (Liddycoat et al. 2009, Marulanda et al. 2009, Benabdellah et al. 2011). Under drought stress, abscisic acid (ABA) biosynthesis increases and its catabolism is decreased eliciting the signals for closure of stomata in order to minimize the water loss and enhance the root network for maximizing the nutrients uptake (Bray 2002, De Smet et al. 2006), whereas ethylene production rises. Such effects of PGPR have also been attributed to a reduction of the ethylene production to compensate the normal ABA level in plants and ultimately protect them against damaging effects of drought stress (Arzanesh et al. 2011). Many previous studies have also shown the involvement of jasmonates in drought stress. Application of jasmonates, i.e., jasmonic acid (JA) or methyl jasmonate ameliorates water stress by increasing the antioxidative capacity of plants (Bandurska et al. 2003). Drought stress affects several genes and their products in plants at transcriptional, post-transcriptional, and translational levels. However, the knowledge of molecular mechanisms elucidating plant-PGPR interactions in rhizosphere is scarce and needs attention (Nautiyal et al. 2013). Therefore, we used two different Pseudomonas strains (Pseudomonas putida KT2440 and Pseudomonas fluorescens Pf1) to study their effects on Zea mays L. (cv. B73) growth under drought and non-stress conditions. We studied the gene expression of different drought stress related, defense related, and JA related genes to understand underlying biochemical and physiological changes in the host plant elicited by selected PGPR. This study can boost the practical applicability of such biotechnological strategies especially in upcoming climate change.

Materials and methods

Bacterial inoculation: Both Pseudomonas fluorescens Pf1 and P. putida KT2440 strains obtained from the Department of Plant Sciences, Pennsylvania State University, PA, USA were grown on Luria-Broth (LB) medium at a temperature of 30 ± 1 °C on an incubator shaker (150 rpm) for 36 h. The cultures were then spun (at 6000 g and 4 °C for 10 min). Pellets were washed thrice and absorbance of the culture was set at 1 at 600 nm = 10^-3 - 10^-6 colony forming units (CFU) cm^-3 (Bhuvaneswari et al. 1980).

Plant growth and drought stress conditions: A pot experiment was conducted in order to compare the efficacy of two PGPR strains for maize growth improvement under drought stress condition. Maize Zea mays L. (cv. B73) seeds obtained from the Department of Plant Sciences, the Pennsylvania State University, PA, USA, were surface sterilized by soaking them in 1 % (m/v) sodium hypochlorite for 3 min and then in 70 % (v/v) ethanol for 1 min. The seeds were rinsed in distilled autoclaved water for three times and were incubated in bacterial suspension (P. putida KT2440 and P. fluorescens Pf1) or in distilled water (control) for 2 h. The seeds were then sown in cylindrical shaped pots and after one week of germination, the seedlings were reduced to one per pot. Each pot contained ~2 kg of the sterilized growth media containing a 1:1 mixture of soil (Hagerstown silt loam) and potting mix (commercial plant growth substrate, organic vigoro, Home Depot, USA). in a greenhouse, the average day/night temperatures were 22—28 / 14—17 °C, a 16-h photoperiod, an irradiance of 250 µmol m^-2 s^-1, and an air humidity of 60 %. Pots were arranged randomly with five biological replicates of each treatment. In order to maintain optimal soil moisture, almost equal amount of water was given to each pot for 21 d followed by drought stress for one week. The plants were used for all in situ measurements as growth parameters followed by sampling on 0, 3rd, and 6th day after drought stress imposition (DAS).

Assessment of growth parameters: Maize leaf samples were randomly collected on 0, 3, and 6 DAS with five biological replicates for each treatment group. Plant height was measured from the base of the plant to the tip of longest leaf. Root and shoot fresh masses and root length were measured from respective plants and the samples were dried at 60 °C for 72 h to determine dry masses. Samples for RNA extraction, cDNA synthesis, and PCR were immediately frozen at -80 °C. A chlorophyll meter (SPAD 502 Plus, Minolta, Tokyo, Japan) was used to assess the chlorophyll content of the leaf tissue (Markwell 1999). Third or fourth leaf was used for total protein content measurement by method of Lowry et al. (1951) using bovine serum albumin as a standard.
In the case of relative water content (RWC) measurements, the 3\(^{rd}\) or 4\(^{th}\) leaf of maize plants was selected. After excision, the leaf was weighed (FW) and soaked in distilled water at 4 °C in the dark for 24 h to measure its fresh mass. The leaf was carefully dried with tissue paper and its turgid weight (TW) was measured. Then, it was oven dried at 72 °C for 48 h to measure dry weight. The RWC was calculated as previously described (Teulat et al. 2003) using a formula 

\[
\text{RWC [\%]} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100.
\]

**Real-time quantitative PCR:** Ribonucleic acid was extracted from three biological replicates for each day and treatment group by grinding leaf samples in liquid nitrogen using GenoGrinder 2000. TRIzol (Invitrogen, Carlsbad, USA) was used as an extraction reagent as recommended by the manufacturer (1 cm\(^3\) TRIzol for 0.1 g leaves). Quantification of RNA was done using a Nanodrop ND 2000 spectrophotometer (Thermo Fisher Scientific, San Jose, USA). Genomic DNA was digested by DNase treatment of the extracted RNA (Biolabs, New England, USA). Total extracted RNA (1 ug) was used as a template for the synthesis of cDNA with the help of high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, USA) as recommended. Fast start universal SYBR Green Master Mix (Roche Applied Science, IN, USA) was used for the real-time PCR using a 7500 Fast real-time PCR system (Applied Biosystems). The PCR was conducted by using the conditions: 50 °C for 2 min and 95 °C for 10 min; 95 °C for 30 s and 60 °C for 1 min repeated in 35 cycles; 72 °C for 10 min; and finally a dissociation stage. Sequences of all primers used in this study are listed in Table 1 Suppl. ZmActin was used as a reference gene because it is well known for the normalization (Stürzenbaum and Kille 2001). Transcript abundance was calculated by 

\[
\Delta Ct = C_{\text{target}} - C_{\text{reference}}
\]

and values were expressed as 

\[
2^{-\Delta\Delta Ct}.
\]

Fold changes in transcripts were analyzed and compared between inoculated and control plants for each harvest time and treatment group.

**Statistical analysis:** For all the molecular and physiological parameters, we used two way ANOVA by using JMP Pro 13 (SAS Institute Inc, Cary, NC, USA) unless otherwise mentioned to test the effect of drought stress, PGPR inoculation, and correlation between drought stress and...
PGPR inoculation at $P < 0.05$ for each sampling time. We used the Tukey HSD test for multiple comparisons of least squares means to test the effect of PGPR inoculation in the presence and absence of drought stress at each specific time point for all treatments.

**Results**

Different parameters including plant height, root length, root and shoot fresh and dry masses, relative water content, protein content, and chlorophyll content were measured on 0, 3 and 6 DAS for evaluation PGPR effects.

There were no significant differences in plant height among different treatments. Our study revealed only some tendency that on 3 and 6 DAS, both Pf1 and Pp strains helped plants to increase their height under stress and no-stress condition as compared to non-inoculated control plants (Fig. 1B). On 0 DAS, Pp inoculation more increased root length as compared to Pf1 inoculation and control. Under drought stress both strains significantly increased root length as compared to drought alone. On 3 DAS drought stress showed significant effects on root length whereas PGPR inoculation and their interactions were not statistically significant. Drought stress significantly reduced root length in all variants as compared to control. There was no significant difference observed for root length among different treatments for 6 DAS. Our data revealed that on 3 DAS, both Pf1 and Pp strains inoculation helped plants to ameliorate drought stress by increasing the root length as compared to non-treated control plants (Fig. 1C).

As concerns RWC, no statistically significant differences were observed among different treatments for 0, 3, and 6 DAS. Our data shows that on 3 and 6 DAS, Pf1 and Pp strains slightly improved RWC as compared to non-treated control plants, but the results are not statistically significant (Fig. 1D).

Drought stress significantly decreased protein content on 6 DAS. The effects of PGPR inoculation and the interaction between drought stress and PGPR inoculation were not found statistically significant. Both Pf1 and Pp were found to be effective for ameliorating the effect of drought stress on the protein content on 6 DAS as compared to non-inoculated control plants (Fig. 1A).

Drought stress significantly decreased chlorophyll content on 3 and 6 DAS. The PGPR inoculation and the interaction between drought stress and PGPR inoculation were insignificant. No significant differences among
treatments were observed on 0 DAS as expected. More importantly, both Pf1 and Pp inoculations were found to be effective in the significant amelioration of drought stress and also under control conditions on 3 DAS as compared to non-inoculated control plants (Fig. 1E).

In order to ensure the positive effect of PGPR colonization in maize roots under drought stress and non-stress conditions, we assessed the root and shoot fresh and dry masses. For fresh root mass, no significant differences were observed among different treatments on 0 DAS. On 3 DAS, the drought stress and PGPR inoculation showed significant effects, whereas the interaction between drought stress and PGPR inoculation were not found significant. The Pp inoculation increased the root fresh mass as compared to Pf1 and control on 3 DAS. The root fresh mass under drought stress was also higher after Pp inoculation than under Pf1 inoculation and drought alone. On 6 DAS, a significant decrease in the root fresh mass of drought stressed plants was observed as compared to non-stress plants. Our study shows that both Pf1 and Pp inoculations were useful for ameliorating stress by increasing the root fresh mass (Fig. 2).

In the case of shoot fresh mass, we noticed significant negative effects of drought stress on 0 and 3 DAS, whereas the effect of PGPR inoculation and their interactions were not significant. On 6 DAS, drought stress and PGPR inoculation showed significant effects, while their interactive effect was not significant. According to our observations, both Pf1 and Pp helped in mitigating the drought stress effect on 6 DAS (Fig. 2B).

For root dry mass no significant effects of drought stress, PGPR inoculation, and their interaction were observed among different treatments on 0, 3, and 6 DAS (Fig. 2C). For shoot dry mass, we observed significant effects of drought stress, whereas PGPR inoculation and the interactions between drought stress and PGPR inoculations were not statistically significant. Shoot dry mass of drought stressed plants was not different from other treatments on 0, 3, and 6 DAS. Shoot dry mass of drought stressed plants was significantly lower than of Pp inoculated plants on 0, 3, and 6 DAS and lower than of non-stressed non-inoculated control plants and non-stressed PGPR inoculated plants on 3 and 6 DAS. There was no difference in shoot dry mass of stressed inoculated plants and stress non-inoculated plants on 0, 3, and 6 DAS (Fig. 2D).

In order to check the effect of PGPR inoculation on drought responsive gene expression under drought stress, the effect of PGPR inoculation and their interactions were not significant. On 6 DAS, drought stress and PGPR inoculation showed significant effects, while their interactive effect was not significant. According to our observations, both Pf1 and Pp helped in mitigating the drought stress effect on 6 DAS (Fig. 2B).

For root dry mass no significant effects of drought stress, PGPR inoculation, and their interaction were observed among different treatments on 0, 3, and 6 DAS (Fig. 2C). For shoot dry mass, we observed significant effects of drought stress, whereas PGPR inoculation and the interactions between drought stress and PGPR inoculations were not statistically significant. Shoot dry mass of drought stressed plants was not different from other treatments on 0, 3, and 6 DAS. Shoot dry mass of drought stressed plants was significantly lower than of Pp inoculated plants on 0, 3, and 6 DAS and lower than of non-stressed non-inoculated control plants and non-stressed PGPR inoculated plants on 3 and 6 DAS. There was no difference in shoot dry mass of stressed inoculated plants and stress non-inoculated plants on 0, 3, and 6 DAS (Fig. 2D).

In order to check the effect of PGPR inoculation on drought responsive gene expression under drought stress,
we assessed the relative transcript abundance of some important genes in PGPR inoculated and non-inoculated maize plants under stressed versus non-stressed conditions. Significant up-regulation of Z. mays COR410 gene (a dehydrin gene family member associated with freezing tolerance) expression was found in plants under prolonged drought stress (6 DAS). Interestingly, Pf1 inoculation repressed the COR410 expression 2 - 3 folds, whereas Pp inoculation by 3 - 4 folds on 6 DAS. No significant difference of COR410 expression was observed among different treatments on 0 DAS (Fig. 3A).

The Dhn1 (dehydrogenase 1) gene also belongs to the dehydrin family that responds to water deficiency in several plants. No significant differences in its expression was observed among different treatments on 0 and 3 DAS. At 6 DAS, effects of drought stress, PGPR inoculation, and their interactions were found statistically significant. The Pf1 inoculation induced a gradual up-regulation of Dhn1 transcription on 3 and 6 DAS and no significant differences in the Pp inoculated plants was observed in all the treatments and time points. Our results show that Dhn1 expression is induced in maize under drought stress, and P. fluorescens inoculation helped in relative enhanced expression during prolonged stress at 6 DAS (Fig. 3B).

The WRKY, a major transcription factors family of plants, has a regulatory function (Eulgem and Somssich 2007) in several plant processes including coping with biotic and abiotic stresses. In response to several stress stimuli, the plants start recruiting various WRKYs for downstream cascade regulations. We selected WRKY18 gene to study its involvement in drought stress response and interactive role in case of selected PGPR. For WRKY18 gene on 0 DAS, the model predicted significant effects of PGPR inoculation and the interaction between drought stress and PGPR inoculation, whereas drought stress did not show any significant difference. Significant up-regulation of WRKY18 transcripts was observed when treated with Pp as compared to Pf1 on 0 DAS. Similarly, for 3 DAS, significant effects of PGPR inoculation and their interaction were observed, while effect of drought stress was not significant. On 6 DAS, significant effects of drought stress and PGPR inoculation were observed, whereas their interaction was not found to be statistically significant. There was significant up-regulation of WRKY18 transcription in drought-stressed plants as compared to other treatments on 6 DAS. Interestingly, our data show the evidence of a reduced basal level of WRKY18 transcription in the presence of drought stress in comparison to control samples. This might be due to less availability of motifs or domains required for the binding of WRKY TFs under our conditions.

Fig. 4. Effects of plant growth promoting rhizobacteria (PGPR) on selected lipogenase pathway genes LOX1 (A), LOX3 (B), LOX5 (C), and LOX6 (D) of control maize plants (C) or under drought stress (D). Plants were inoculated with Pseudomonas fluorescens (Pf1) or P. putida (Pp). Means ± SEs, n = 5. Bars sharing common letters are not statistically different from each other at a 95 % confidence level.
experimental conditions (Fig. 3C).

The major facilitator super (MFS) family, a group of secondary transporters, was significantly affected by drought stress, PGPR inoculation, and their interactions already on 0 DAS. Similarly, we observed significant effects of these treatments on 3 DAS, whereas on 6 DAS, PGPR inoculation and the interaction between drought stress and PGPR inoculation were found statistically significant, but the effect of drought stress was not significant. Interestingly, both Pf1 and Pp strains seem to be effective in controlling the membrane transport under prolonged stress (6 DAS) as evident by reduced MFS transcription in inoculated plants under drought stress (Fig. 3D).

The ZmLOX1, ZmLOX3, ZmLOX5, and ZmLOX6 belong to lipoygenases (LOXs) that respond to dehydration stress in plants (Lyons et al. 2013). Significant up-regulation of LOX1 transcription was observed in plants under drought treatment as compared to control on 6 DAS only. Though small up-regulation of LOX1 genes expression was found after PGPR inoculation on 0 DAS, no significant effects of treatments were found on 3 DAS (Fig. 4A). Similarly, significant up-regulation of LOX3 expression was observed in drought-stressed plants as compared to control on 6 DAS. This upregulation was higher than that in drought-stressed inoculated plants. No significant differences were observed in expression of LOX3 gene among different treatments on 0 and 3 DAS (Fig. 4B). No significant differences in LOX5 and LOX6 expression among different treatments were observed on 0 DAS (Fig. 4C,D). Drought stress alone or in combination with inoculation significantly increased LOX5 expression on 3 and 6 DAS (Fig. 4C). The LOX6 gene responded by up-regulating significantly in drought stressed non-inoculated maize plants up to 14-folds on 3 DAS followed by reduction on 6 DAS. These up-regulations were higher than those in drought-stressed inoculated plants (Fig. 4D).

Our data show that, as compared to non-inoculated drought stressed plants, PGPR inoculation might have had a stress amelioration effect on the maize plants in order to withstand the severity of the stress, but this effect was variable for different growth parameters, which may be dependent on the strain used, plant variety, and severity and duration of stress.

Jasmonic acid pathway has a potential role in drought stress through some known and some unexplored cross talks. In order to explore this, the changes in transcription of some JA pathway genes were also assessed. Transcription of a maize protease inhibitor (MPI) showed an insignificant difference among different treatments on 0 and 3 DAS. On
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6 DAS, the effects of drought stress and the interaction between drought stress and PGPR inoculation were significant, whereas the effect of PGPR inoculation was not significant. Our data suggests the positive role of PGPR in ameliorating the effect of drought stress (Fig. 5A). For ribosome inactivating protein 2 (RIP2) gene expression, no significant effects of drought stress, PGPR inoculation, and their interactions were observed on 0 and 6 DAS. However, on 3 DAS, there were significant effects of drought stress, PGPR inoculation, and the interaction between drought stress and PGPR inoculation. The highest RIP2 expression was observed in drought-stressed plants inoculated with Pp (Fig. 5B). Plant oxo-phytodienoate reductases (OPR) are usually expressed under different environmental stimuli inducing JA, but their biochemical or physiological roles are still unclear. No significant differences in expression of OPR7 were observed on 0 and 3 DAS. On 6 DAS, drought stress and PGPR inoculation showed significant effects, whereas the interaction between drought stress and PGPR inoculation was not found statistically significant. A higher up-regulation of OPR7 transcription was observed in drought-stressed non-inoculated plants as compared to inoculated ones (Fig. 5C). Hydrogen peroxide lyase (HPL) gene is thought to play a role in mediating plant defense responses. No significant effects of drought stress, PGPR inoculation and their interaction were observed in case of HPL transcript among different treatments on 0 and 3 DAS. Only PGPR inoculation showed a significant effect on 6 DAS: HPL expression in Pf1 inoculated treated plants was highest (Fig. 5D).

**Discussion**

The PGPR play important roles in plant growth, development, and physiology. They promote the growth of the plants by various mechanisms like production of siderophores, antibiotics, phytohormones, by phosphate solubilization in substrate, and by nitrogen fixation. They help plants to withstand different stress conditions by activation of defense pathways and downstream signaling. The Pp and Pf1 strains are known as an important and largest group of rhizobacteria with plant growth promotion properties, potentially known as biocontrol (Kloeper et al. 1992). In current study, the maize treated with Pp and Pf1 under drought stress showed increased protein content, root length, chlorophyll content, fresh root and shoot fresh and dry masses relative to non-inoculated plants.

It has been reported that plants reduce the biosynthesis of certain proteins and increase the biosynthesis of defensive proteins to overcome drought stress trauma in plants, which is also dependent on the intensity and duration of stress. Despite the defensive proteins being produced in response to stress conditions, the total soluble protein content decreases gradually with intensity and duration of stress due to a decrease in the rate and efficiency of photosynthesis (Mohammadkhani and Heidari 2008). Another reason for a reduced protein content can be the accelerated rate of protein degradation due to enhanced protease activity and activities of other catabolic enzymes, which is characteristic for oxidative stress induced by drought stress. (Moran et al. 1994). Our study also exhibits similar results for protein in drought treated plant groups when compared with control groups. However, this effect was ameliorated significantly on 6 DAS in the PGPR inoculated plant group under drought stress condition.

Plants under drought stress tend to decrease the water loss with the help of different defense strategies, whereas simultaneously increase the nutrient uptake by enhancing the root network. The occurrence of *Pseudomonas sp.* in and around the root system of cereals, vegetables and their beneficial effects upon inoculation has been well studied. In our study, a significant decrease in root length of drought treated plant groups was observed started on 3 DAS as compared to the control treatment. Both PGPR strains significantly ameliorated the drought stress by increasing the root length as compared to non-inoculated drought stressed control plants. The promotion in growth parameters might be due to the synthesis of bacterial growth hormones, e.g., indole acetic acid and gibberellins (Khalid et al. 2004) resulting in increased nutrient uptake in plants. Because of rapid colonization of the roots, these rhizobacteria are widely used as soil inoculants in many crops for growth promotion.

Drought stress can easily be judged by RWC (Fisher et al. 2000), as it is significantly reduced in leaves (Nayyar and Gupta 2006). Stressful conditions can be mitigated in the presence of PGPR due to their growth promoting effects on the physiology and molecular biology of the plants (Dodd et al. 2010). In this study, we did not observe any difference in RWC of both inoculated and non-inoculated maize plants exposed to the drought stress as compared to control plants.

The rate of photosynthesis in plants usually decreases under drought stress (Kawamitsu et al. 2000) because of reduced stomatal conductance to lower the water loss leading to the reduced CO2 fixation. Also the chlorophyll content and the photosynthetic machinery are affected under drought stress causing the inhibition of photosynthesis (Iurbe-Ormaetxe et al. 1998, Manivannan et al. 2007). Many studies on *Triticum aestivum* (Gajewska and Sklodowska 2007), *Z. mays* (Krantev et al. 2008), *Brassica juncea* (Alam et al. 2007), *Bruguiera gymnorrhiza*, and *Kandelia candel* (Huang and Wang 2010) have reported the decrease in chlorophyll content under stress conditions. Our results are consistent with previous studies indicating that the drought stress significantly reduced the chlorophyll content on 3 and 6 DAS. The PGPR were found to be helpful for the plants by increasing the chlorophyll content on 3 DAS when compared with non-inoculated plants under drought stress.

The drought stress also negatively affected the root and shoot fresh and dry masses, however, PGPR-treated plants had significantly higher root fresh mass on 3 and 6 DAS and shoot fresh and dry masses on 3 DAS when compared with the stressed non-inoculated plants. This might be because of their ability to help the plants in increasing their above-ground and below-ground growth by nutrients acquisition, assimilation, enhanced roots networking and

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metabolites production.

Complex multi-component signaling pathways are triggered in plants in order to restore cellular homeostasis and to promote survival under different abiotic stresses but the knowledge about these molecular mechanisms is still limited. Some drought stress inducible genes respond directly to the stress, whereas some genes respond after the accumulation of ABA. All these genes have protective roles against the stress (Bray 1997). Transcripts of such genes usually accumulate under drought stress conditions. A PGPR inoculation can reduce the severity of the stress by regulating the expression of defense related genes, which can play a role in counteracting the damage caused by dehydration conditions. In order to explore the role of PGPR, we analyzed several genes for their relative expression under given conditions using real-time qPCR.

The ZmCOR410 and Dhn1 belong to the dehydrin gene family that are over-expressed under dehydration conditions in maize plants and due to ABA exposure (Danyuk et al. 1998). In our study, induced expression of E. mays COR410 under drought stress conditions in non-inoculated plants showed evidence of plant cell dehydration due to drought stress at 6 DAS. Induced expression of COR410 gene to several folds is interesting. Characterization of such regulatory genes in signaling pathways may help devise strategies to improve plant-rhizobacterial interactions. In our experiment, P. fluorescens Pf1 and P. putida KT2440 inoculations may have reduced the dehydration damage by down-regulating COR410 expression in PGPR inoculated stressed and PGPR inoculated non-stressed plants as compared to non-inoculated stressed plants to help the plants to withstand stress. Similarly, Dhn1 gene expression was up-regulated almost 13-folds under drought stress in non-inoculated plants on 3 DAS but decreased almost 7-folds on 6 DAS showing turnover of Dhn1 transcription after prolonged drought stress. The Pf1 inoculation induced a gradual up-regulation of Dhn1 gene on 0, 3, and 6 DAS, which was significant on 6 DAS. The Dhn1 gene contribute in osmotic stress tolerance and Pf1 was found to be more efficient for inducing its expression as compared to Pp.

The WRKY transcription factors target downstream genes that are important for detoxification and scavenging ROS like peroxidases, LOXs LOX1, and glutathione-S-transferases (Jiang and Deyholos 2009). Due to the role of WRKY18 in osmotic stress response, ROS scavenging activity becomes likely (Remy et al. 2013). Some changes in transcription of WRKY18 gene in the case of drought stress and inoculations were observed. Non-inoculated drought stressed plants showed up-regulation of WRKY18 gene expression on 0 and 6 DAS compared to PGPR inoculated drought stressed plants and non-inoculated non-stressed plants. The PGPR inoculation might have reduced the effect of drought stress on 6 DAS by lowering the expression of WRKY18 gene.

The MFS family is a group of secondary transporters required for the transport across the cellular membranes using proton gradient as an energy source (Petrasek et al. 2006). Recently, MFS has also been assigned a dual role in Arabidopsis for drought stress tolerance and polar auxin transport through regulating movements of stomata and auxin transport by changing proton and potassium transition (Remy et al. 2013). Our data show a significant up-regulation of MFS gene expression on 3 and 6 DAS in non-inoculated maize plants under drought stress, which was significantly repressed by Pp inoculation on 3 and 6 DAS and by Pf1 inoculation on 6 DAS. These data show that PGPR inoculation might have modulated the expression of these defense genes to help withstand the drought stress.

Current research emphasizes the central role of ROS production in cells as a signaling interface in plant adaptation to salt and drought stresses. The ROS in excess are scavenged particularly through antioxidant metabolites like glutathione, ascorbate, and tocopherols, etc., and through ROS detoxifying enzymes like catalase, superoxide dismutase, ascorbate peroxidase, etc. (Mittler 2002). Lipoxygenases are involved in various physiological processes (Brash 1999). They are non-heme iron containing dioxygenases, which catalyze the conversion of lipids and polyunsaturated fatty acids into hydroperoxy fatty acids, which are further degraded into oxylipins such as jasmonic acid, methyl jasmonic acid, and traumatin (Blée 2002). Oxylipins are known to play an important role in development, senescence, formation of flavor compounds, and defense responses to abiotic and biotic stresses (Williams et al. 2000, Ye et al. 2000, Vellosillo et al. 2007, Yang et al. 2012). We also assessed the expression pattern of the genes involved in LOX pathway, such as LOX1, LOX3, LOX5, and LOX6, to characterize the role of our selected PGPR strains in drought stress amelioration. These genes were up-regulated under drought stress possibly to scavenge ROS so as to overcome the stress. The LOX1, LOX3, LOX5 genes were significantly up-regulated on 6 DAS in non-inoculated drought stressed maize plants, whereas LOX6 gene significant up-regulation started on 3 DAS and also showed a significant up-regulation on 6 DAS. Both Pseudomonas putida KT2440 and P. fluorescens Pf1 inoculations might have helped in withstanding drought stress through ROS scavenging on 6 DAS. P. putida KT2440 was found to be more efficient as compared to P. fluorescens Pf1 for repressing the expression of these genes.

A wide range of abiotic stresses can induce JA signaling like drought, wounding (Doares et al. 1995), osmotic stress (Kramell et al. 1995), and also the extracts of yeasts (Parchmann et al. 1997). Stress-inducible genes play a role both in initial stress responses and also in establishing stress tolerance in plants. Exogenous JA is found to protect the plants from drought induced stress through enhancing the activity of antioxidant enzymes (Nafie et al. 2011). Over-expression of JA pathway related genes in transgenic plants also improves osmotic stress tolerance in addition to showing ABA-hypersensitive phenotypes (Shinozaki and Yamaguchi-Shinozaki 2004). Moreover, drought stress also raises the endogenous JA content in maize root cells (Xin et al. 1997). Our results demonstrate the involvement of MPI, and OPR7 in drought stress response as a defense mechanism leading to stress amelioration. The MPI expression showed a gradual increase in drought.
TREATED WITH RHIZOBACTERIA, might have helped plants in tolerating the drought stress. 

Many studies on plant tolerance to drought showed that PGPR inoculation improves drought tolerance by affecting the transcription of drought stress responsive genes (Sarma and Saikia 2014) and also the phytomorphological balance (Figueiredo et al. 2008). In our current study, drought stress significantly affected the protein and chlorophyll by lowering their content and also decreased root length and shoot and root biomasses. However, PGPR treatment under stress helped plants to improve their growth most probably through nutrient uptake. At the transcriptional level, PGPR mediated down regulation of many drought stress responsive genes, i.e., COR410, WRKY18, and MFS, and also of JA pathway responsive genes, LOX, MPI, and OPR7, might have helped plants in tolerating the drought stress.

The results presented in this work suggest that the P. putida KT2440 and F. fluorescens Pf1 are efficacious PGPR strains against drought stress in maize plants possibly by attenuating the transcript levels of drought stress responsive genes, lipoygenase pathway genes, and JA pathway responsive genes. Collectively, these responses can facilitate the maize plants from desiccation under stressful conditions.

References

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