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Full Length Article

Rhizobacteria with ACC-deaminase Activity Improve Nutrient Uptake, Chlorophyll Contents and Early Seedling Growth of Wheat under PEG-induced Drought Stress

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Abstract

Drought stress is the leading constraint impairing the wheat growth across the globe. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing plant growth promoting rhizobacteria (PGPRs) has the potential to mitigate the negative effects of drought stress on crops. This study was carried out to investigate the role of newly evolved ACC-deaminase containing PGPRs in improving the early wheat growth under polyethylene glycol (PEG) induced drought stress. Out of 45 strains isolated from wheat rhizosphere, 23 strains were found as drought-tolerant PGPRs, which were able to grow on 20% PEG containing Dworkin and Foster (DF) media. Among these 23, the 4 most effective ACC-deaminase producing PGPRs were selected, identified and characterized. Among these 4 strains, *Leclercia adecarboxylata* and *Agrobacterium fabrum* were newly reported and *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* were earlier reported drought tolerant ACC-deaminase PGPRs. Wheat seeds inoculated with above mentioned strains of PGPRs and un-inoculated dry seeds were grown in hydroponic culture under three levels of PEG-induced drought stress *i.e.*, 0 (-0.05 MPa), 10 (-0.23 MPa) and 20% (-0.78 MPa). PEG-induced drought stress, 20% PEG level was more severe, which decreased root and shoot lengths and dry weights, and seedling dry weight due to decreased chlorophyll contents and low uptake of nitrogen, phosphorus and potassium. However, seeds inoculation with PGPRs, especially newly reported strains *L. adecarboxylata* and *A. fabrum*, substantially improved seedling growth under drought stress due to elevated nutrients uptake and high chlorophyll contents. More wheat growth, under optimal and less than optimal conditions, subjected to *L. adecarboxylata* and *A. fabrum* application was linked with their higher ACC-deaminase and IAA production potential. In conclusion, newly reported drought tolerant ACC-deaminase containing PGPRs *L. adecarboxylata* and *A. fabrum* have the potential to improve early wheat growth under drought stress due to higher ACC-deaminase and IAA production potential.

Keywords: ACC-deaminase; Growth hormones; Molecular identification; PGPRs; Polyethylene glycol 6000

Introduction

In recent times scarcity of water due to change in the climatic conditions is a serious threat to sustainable crop production (Sivakumar, 2011; Hussain *et al*., 2018). Out of 75% available water for humans, 10-30% is consumed by plants as transpiration in both irrigated and rainfed agriculture (Wallace, 2000). The demand for irrigation water is expected to increase ~10% in year 2050s and ~14% in 2080s (Wada *et al.*, 2013) which is already 70% of the global water consumption (Abdullah, 2006). Drought susceptibility covers more than one half of the earth every year (Wilhite, 2000). It is a most critical abiotic factor that disturbs the biochemical and physiological processes in crops leading to a considerable decrease in crop yield (Farooq *et al.*, 2014; Hussain *et al*., 2018).

Wheat (*Triticum aestivum* L.) is a widely cultivated cereal crop which fulfils up to 20% food requirements of daily human diet (Bos *et al.*, 2005). It is also a staple diet for Pakistani residents. According to the FAO report, 1/5th part of the worldwide wheat production is traded which makes it an important economic crop (Kao *et al.*, 2015). It is expected that up to 2020s, wheat demand will be enhanced at the rate of 1.6%/annum (Ortiz *et al.*, 2008). Among several other factors, drought stress is the leading constraint limiting wheat productivity around the globe

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(Farooq *et al*., 2014, 2015); as both vegetative and reproductive stages of wheat are equally sensitive to drought stress (Farooq *et al*., 2014; Tack *et al*., 2014; Hussain *et al*., 2016a). Early drought stress substantially impairs emergence, early stand establishment, root land shoot growth and relative water contents in wheat (Abido and Zsombik, 2018). It also results in reduction of chlorophyll contents (Nikolaeva *et al.,* 2010) due to poor uptake of nitrogen in wheat (Shabbir *et al.,* 2015).

Most of the plants demonstrate molecular and cellular level responses towards drought stress (Kaur and Asthir, 2017). The roots of plants usually show first response against drought by sending signals through abscisic acid for reduction in stomatal conductance (Jiang and Zhang, 2002; Nezhadahmadi *et al.,* 2013). In this way, drought stress results in decreased CO_2 diffusion inside leaves due to poor conductance of mesophyll which results in photosynthesis impairment (Flexas *et al.,* 2008).

Furthermore, drought stress enhances the synthesis of ethylene in plants by stimulating the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (Dubois *et al.,* 2018). Less elongation and radial swelling of the stem are primary indications of higher ethylene accumulation (Abeles *et al.,* 1992). At early stages of crop growth, higher level of ethylene decreases the supply of energy and water at the imbibition phase (Taiz and Zeiger, 2010; Aroca, 2012). Other negative effects of higher ethylene concentration include stomatal closure, evoking of physiological responses, higher transpiration, inhibition of signalling pathway of abscisic acid and less nitrogen fixation (Tamimi and Timko, 2003; Tanaka *et al.,* 2005).

Now a days, scientists are working on various strategies for the protection and improvement of crop productivity under drought stress in all over the world. Among these strategies, inoculation of plant growth promoting rhizobacteria (PGPRs) is a unique strategy for overcoming drought stress (Hussain *et al.,* 2016b, 2018; Saikia *et al.,* 2018). The plant root system is a habitat for millions of PGPRs that form a complex ecological community and affects the growth and productivity of crops (Berg, 2009; Schmidt *et al.,* 2014). These PGPRs can secrete multiple metabolites and enzymes to mitigate biotic and abiotic stresses (Ngumbi and Kloepper, 2016). As water uptake ability of plants is dependent on their roots elongation, inoculation of drought resistant PGPRs can improve 40% root system of plants (Marasco *et al*., 2013). In addition, PGPRs group containing ACC-deaminase is the most remarkable in enhancing the resistance against drought and to promote the health of plants (Shakir *et al.,* 2012; Cherif *et al.,* 2015). ACC-deaminase is a polymeric enzyme which is dependent on pyridoxal 5-phosphate (PLP) (Karthikeyan *et al*., 2004). It is established that the PGPRs containing ACC-deaminase can reduce accumulation of ethylene by breaking cyclo-propanoid amino acid ACC (ethylene precursor), into intermediate compounds, ammonia and α-ketobutyrate (Glick *et al.,* 1999).

The use of ACC-deaminase containing PGPRs in improving drought tolerance of crops including wheat is well reported (Zafar-ul-Hye *et al*., 2014; Hussain *et al*., 2016b, 2018). However, the isolation, identification and characterization of new drought tolerant PGPRs strains from wheat rhizosphere is a pragmatic option to search more efficient strains to induce drought tolerance in wheat. Therefore, this study was conducted to evaluate the efficacy of two newly isolated strains *i.e*., *L adecarboxylata* and *A. fabrum* with high ACC-deaminase and IAA production potential to improve the nutrient uptake, chlorophyll contents and early seedling growth of wheat under drought.

Materials and Methods

Collection of Rhizosphere Soils

Soils of wheat rhizosphere were collected from two different sites *viz.,* Old Shujabad Road (30.11° N and 71.43° E) and Akram Abad (30.16° N and 71.29° E) in Multan, Pakistan and brought into the laboratory. A spatula was used to remove the adhering rhizospheric soil from wheat roots which was homogenized manually for isolation of PGPRs.

Isolation, Purification and Selection of Drought Tolerant Isolates

Serial dilutions with distilled water (10^{-1} to 10^{-7}) were made by taking 1.0 g homogenized rhizospheric soils. For the isolation of PGPRs containing ACC deaminase, Dworkin and Foster (DF) minimal salt media was prepared having ACC as main source of nitrogen (Dworkin and Foster, 1958). For the growth of PGPRs, incubation in an automated chamber (HP400S, Ruihua Co., Ltd., Wuhan, China) was done at 25ºC for 48 h. There were 45 bacterial colonies which were initially isolated as described by Jalili *et al.* (2009). For the purpose of purification, all the isolates were grown multiple times on DF media. The purified strains were grown on DF media in which 20% polyethylene glycol (PEG, Biotechnology grade purchased from ShangHai Biochem. Co., Ltd., Shanghai, China) was added to induce artificial drought stress. The isolates which were able to grow in the presence of 20% PEG in DF media were selected as drought tolerant PGPRs. Out of forty-five, 23 wheat rhizosphere isolated strains were selected as drought-tolerant PGPRs which were able to grow on 20% polyethylene glycol (PEG) containing DF media. Among these twenty-three, the most effective four strains were selected for further studies.

Biochemical Characterization of Effective PGPRs

The ACC-deaminase activity of four selected PGPRs trains was examined following procedure stated by El-Tarabily (2008) and Honma and Shimomura (1978). The production of indole acetic acid (IAA) by drought tolerant PGPRs was examined with and without L-tryptophan (L-TRP; Sigma, Shanghai, China) as described by Glickmann and Dessaux

(1995). Isolates were grown on Pikovskaya's medium (Pikovskaya, 1948) to evaluate the ability to solubilize the phosphorus. The potassium solubilizing activity of isolates was examined following the method described by Setiawati and Mutmainnah (2016). The characteristics of selected drought tolerant ACC deaminase containing PGPRs is provided in Table 1.

Identification of PGPRs

After confirmation of ACC deaminase production and other plant growth promoting traits of PGPRs, molecular identification of most efficient drought tolerant ACC deaminase producing PGPRs was carried out through 16S rRNA gene sequencing by using PCR primers 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' and 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'. The gene sequencing primers were 907R 5' (CCG TCA ATT CMT TTR AGT TT) 3' and 785F 5' (GGA TTA GAT ACC CTG GTA) 3'. By using BLAST analysis, 16S rRNA gene sequences were aligned and relationships were deduced (Siddikee *et al.,* 2010). The most effective drought tolerant ACC deaminase containing rhizobacterial strains were identified as *Leclercia adecarboxylata*, *Agrobacterium fabrum*, *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* (Fig. 1). For confirmation of AcdS gene responsible for the production of ACC deaminase, NCBI gene bank was consulted that confirmed that *B. amyloliquefaciens* (NCBIa), *A. fabrum* (NCBIb) and *P. aeruginosa* (NCBIc) have AcdS gene while work is yet continued on *L. adecarboxylata*. However, biochemical analysis confirmed that *L. adecarboxylata* has ability to produce ACC deaminase (Table 1).

Experimental Details

A hydroponic glass jar (3-inch diameter, 6-inch length) experiment was conducted on wheat under axenic conditions in the laboratory of Soil Microbiology and Biochemistry, Department of Soil Science, Bahauddin Zakariya University (BZU), Multan, Pakistan. Seeds of wheat variety Galaxy-2013 (obtained from BZU research farm, Multan, Pakistan) were selected and subjected to surface sterilization by dipping in $HgCl_2$ (0.1%) for 5 min. After that, seeds were washed three times with autoclaved water (Sadiq and Ali, 2013). On each sterilized filter papers (Whatman's No. 40), three healthy seeds were placed on which respective PGPRs *i.e., Leclercia adecarboxylata, Agrobacterium fabrum, Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* inocula were poured while un-inoculated seeds were taken as control. Inoculated and un-inoculated seeds were subjected to three levels of PEG (0, 10 and 20%), mixed in water to induce artificial drought stress of -0.05, -0.23 and -0.78 MPa, respectively (Piwowarczyk *et al.,* 2014). The experiment was laid out following completely randomized design with factorial arrangement and replicated three times.

Finally, the seeds were rolled in 2 filter papers and placed in a sterilized glass jar in such a way that seeds were not under submerged conditions. Twenty mL water level was maintained in each jar throughout the experiment. All the macro and micronutrients were applied four times; at every 5th day in the form of 5 mL Hoagland solution (Hoagland and Arnon, 1938) starting from sowing.

Growth and Chemical Analysis of Plants

Wheat seedlings were grown for 21 days in jars containing solutions of PEG. Root and shoot lengths of all three plants in each jar were measured and averaged. Likewise, root and shoot dry weight and seedling dry weight was recorded following standard procedures. For determination of dry weight, shoot and root samples were oven dried at 70^{o}C for 48 h.

For nitrogen determination in wheat shoot, H_2SO_4 digestion was done by using digestion mixture (Jones *et al.,* 1991). After that, distillation of samples was performed on Kjeldahl's distillation apparatus (Schouwenberg and Walinge, 1978). For determining phosphorus and potassium concentration in wheat shoot, digestion of samples was done with HNO_3-$HClO_4$ diacid mixture (Chapman and Pratt, 1961). Phosphorus was analyzed in digested samples by a yellow colour method on spectrophotometer at 420 nm wavelength (Jones *et al.,* 1991). Potassium was determined on flame photometer by following method described by Nadeem *et al.* (2013).

Chlorophyll Contents

Chlorophyll contents in fresh leaves were extracted by using acetone (80%) as described by Arnon (1949). Absorption of each sample was taken on a spectrophotometer (HITACHI U-2000, Beijing, China) at 663 and 645 nm wavelength. The chlorophyll a, chlorophyll b and total chlorophyll were finally calculated by using the equations as follows:

$$\text{Chlorophyll a (mg/g f.wt)} = \frac{12.7(OD\,663) - 2.69(OD\,645)V}{1000(W)} \quad (1)$$

$$\text{Chlorophyll b (mg/g f.wt)} = \frac{22.9(OD\,645) - 4.68(OD\,663)V}{1000(W)} \quad (2)$$

$$\text{Total Chlorophyll (mg/g f.wt)} = \text{Chlorophyll a + Chlorophyll b} \quad (3)$$

Where,

V = final volume made

W = gram of fresh leaf sample

f.wt = fresh weight

Statistical Analysis

Statistical analysis of wheat morphological traits and biochemical attributes was done using standard statistical procedures (Steel *et al.,* 1997) on SPSS 18.0 software. All the treatments were compared using two-way ANOVA followed by LSD test at $p \leq 0.01$.

Results

PGPRs Characteristics

Selected drought-tolerant PGPRs strains *viz.*, *L. adecarboxylata*, *A. fabrum*, *B. amyloliquefaciens* and *P. aeruginosa* were able to produce indole acetic acid (IAA) with and without L-tryptophan (L-TRP; Sigma, Shanghai, China; Table 1). The *L. adecarboxylata* produced 291% higher IAA as compared to *B. amyloliquefaciens* in the presence of L-tryptophan (Table 1). The phosphorus and potassium solubilizing activity was the maximum in *L. adecarboxylata* while minimum in *A. fabrum*. In case of ACC deaminase production, performance of *A. fabrum* was best among all the studied drought-tolerant ACC deaminase containing PGPRs (Table 1).

Growth Attributes

Different levels of PEG and inoculation with PGPRs had significant effect on root and shoot lengths, dry weights and seedling dry weight of wheat; while their interaction was non-significant in this regard (Table 2). Under drought stress, 20% PEG level in particular, wheat seedlings observed minimum root and shoot lengths, dry weights and seedling dry weight against the maximum values of these traits recorded under 0% PEG solution (Table 2). Nonetheless seed inoculation with different PGPRs, *L. adecarboxylata* and *A. fabrum*, significantly improved these traits compared with un-inoculated seeds (Table 2). Maximum increase of 40.4% in shoot length was noted as compared to control where *L. adecarboxylata* was applied as an inoculum. In case of root length, *A. fabrum*, *B. amyloliquefaciens* and *P. aeruginosa* remained statistically alike to each other and performed significantly best as compared to control (Table 2). Maximum increase of 115% in root length was noted as compared to control where *B. amyloliquefaciens* was applied as an inoculum. Likewise inoculation with *L. adecarboxylata* and *A. fabrum* improved root and shoot dry weights against control (Table 2). Seed inoculation with *L. adecarboxylata* observed 36 and 60% more shoot and root dry weights of wheat (Table 2). In case of seedling dry weight, *L. adecarboxylata* and *A. fabrum* performed significantly better than control. Maximum increase of 37.5% in seedling dry weight was noted as compared to control where *L. adecarboxylata* was applied as an inoculum (Table 2).

Chlorophyll Contents

Interaction among PGPRs and PEG levels had significant effect on chlorophyll a, b and total chlorophyll contents of wheat seedlings (Table 3). Seedlings obtained from seeds inoculated with *L. adecarboxylata* and *A. fabrum* showed higher chlorophyll a, b and total chlorophyll contents at 0% PEG while un-inoculated seeds and

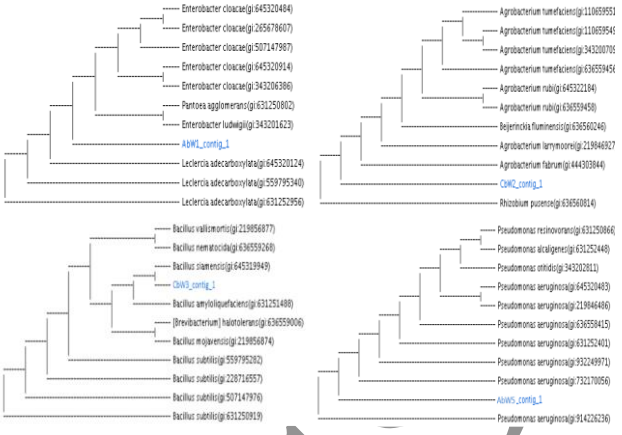

Fig. 1: Phylogenetic tree obtained from 16S rDNA sequence alignment for most effective drought tolerant ACC deaminase producing isolates collected from wheat rhizosphere

inoculated seeds with *B. amyloliquefaciens* had the minimum chlorophyll a, b and total chlorophyll contents at 20% PEG (Table 3). Nonetheless, wheat seedlings obtained from seeds inoculated with *L. adecarboxylata* and *A. fabrum* produced higher chlorophyll a, b and total chlorophyll contents under well-watered (0% PEG) and drought conditions (10 and 20% PEG) compared with un-inoculated control (Table 3). Maximum increase of 142, 123 and 205% in total chlorophyll contents was noted where *A. fabrum*, *L. adecarboxylata* and *A. fabrum* were inoculated at 0, 10 and 20% PEG levels, respectively (Table 3).

Nutrients Uptake

Interactive effect of PGPRs and drought stress (PEG levels) was significant for shoot nitrogen (N), phosphorus (P) and potassium (K) concentration in wheat (Fig. 2, 3 and 4). PEG-induced drought stress substantially reduced NPK uptake while seed inoculation with different PGPRs improved NPK concentration under well-watered and stressed condition compared with control (Fig. 2, 3 and 4). Seeds inoculation with *A. fabrum* observed higher shoot N concertation at 0% PEG while seedlings obtained from un-inoculated seeds recorded the minimum shoot N concentration under 10 and 20% PEG levels (Fig. 2). Maximum increase of 207 and 133% in shoot N concentration compared with control was noted where inoculated seeds with *L. adecarboxylata* were sown with 10 and 20% PEG levels (Fig. 2). Likewise, seed inoculation with *L. adecarboxylata*, *A. fabrum* and *P. aeruginosa* resulted in higher shoot P concentration while un-inoculated seeds grown under 20% PEG had minimum shoot P concentration in wheat (Fig. 3). Moreover 122, 77 and 220% more shoot P concentration was observed in seedlings obtained from inoculated seeds with *L. adecarboxylata*, *B. amyloliquefaciens* and *A. fabrum* sown at 0, 10 and 20% PEG, respectively against un-inoculated control (Fig. 3).

Table 1: Characterization of wheat isolated most efficient drought tolerant ACC deaminase containing PGPRs

Source/Attributes	Strains/Values			
Accession number	NR_104933.1	NR_074266.1	FN597644.1	CP012001.1
Closest genus	*Leclercia*	*Agrobacterium*	*Bacillus*	*Pseudomonas*
Closest specie	*Adecarboxylata*	*Fabrum*	*amyloliquefaciens*	*aeruginosa*
P-Solubilization (μg/mL)	26.6 ± 1.04	16.2 ± 1.48	20.9 ± 2.48	22.8 ± 1.36
K-Solubilization (μg/mL)	20.1 ± 1.02	26.7 ± 1.49	23.4 ± 1.92	17.9 ± 1.02
IAA (with L-Tryptophan) (μg/mL)	67.8 ± 2.20	58.8 ± 3.27	17.3 ± 2.34	24.8 ± 1.49
IAA (without L-Tryptophan) (μg/mL)	3.42 ± 0.27	2.43 ± 0.34	1.12 ± 0.6	3.16 ± 0.21
ACCD activity (μmol α-ketobutyrate nmol mg^{-1} protein h^{-1})	304.9 ± 24.1	349.6 ± 21.4	313.2 ± 34.3	245.4 ± 19.5

Table 2: Effect of ACC deaminase containing PGPRs on shoot, root, seedling length and dry weight under PEG induced drought stress

Treatments	Shoot length (cm)	Root length (cm)	Shoot DW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)	Seedling DW (g $plant^{-1}$)
PEG levels (%)					
0	20.2 a	12.7 a	0.18 a	0.015 a	0.20 a
10	19.3 a	10.8 b	0.15 ab	0.013 a	0.16 ab
20	17.1 b	9.20 c	0.14 b	0.010 b	0.15 b
LSD Value $p \leq 0.01$	1.99	1.12	0.04	0.002	0.04
Plant growth promoting rhizobacteria					
Control	15.6 c	6.10 c	0.14 ab	0.010 c	0.15 b
L. adecarboxylata	21.9 a	10.8 b	0.19 a	0.016 a	0.21 a
B. amyloliquefaciens	19.3 b	13.1 a	0.13 b	0.011 c	0.14 b
A. fabrum	19.3 b	11.8 ab	0.17 ab	0.015 ab	0.19 ab
P. aeruginosa	18.3 b	12.8 a	0.14 b	0.012 bc	0.15 b
LSD value at $p \leq 0.01$	2.56	1.44	0.05	0.003	0.05

Means not sharing the same letter, within a column, differ significantly from each other at $p \leq 0.01$
DW = Dry weight; PEG = Polyethylene glycol

Table 3: Interactive effect of ACC deaminase containing PGPRs and drought stress on shoot and root length, shoot and root dry weight of wheat seedlings

PGPRs	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Total Chlorophyll (mg/g)		
	PEG_0	PEG_{10}	PEG_{20}	PEG_0	PEG_{10}	PEG_{20}	PEG_0	PEG_{10}	PEG_{20}
Control	0.32 ef	0.29 e-g	0.13 gh	0.21 ef	0.19 f	0.08 g	0.53 fg	0.47 g	0.21 hi
L. adecarboxylata	0.77 a	0.62 ab	0.23 fg	0.49 a	0.43 ab	0.23 ef	1.26 a	1.05 a-c	0.46 gh
B. amyloliquefaciens	0.55 bc	0.34 d-f	0.05 h	0.38 bc	0.23 ef	0.04 g	0.93 b-d	0.57 fg	0.09 i
A. fabrum	0.78 a	0.51 b-d	0.38 c-f	0.49 a	0.32 cd	0.26 d-f	1.28 a	0.83 c-e	0.64 e-g
P. aeruginosa	0.65 ab	0.43 c-e	0.29 e-g	0.45 ab	0.30 de	0.20 f	1.10 ab	0.73 d-f	0.48 fg
LSD Value $p \leq 0.01$		0.17			0.09			0.25	

Means sharing different letters, within a column or row, are significantly different at $p \leq 0.01$
PEG_0 = 0% Polyethylene glycol 6000; PEG_{10} = 10% Polyethylene glycol 6000; PEG_{20} = 20% Polyethylene glycol 6000

Seeds inoculation with *L. adecarboxylata*, *A. fabrum* and *B. amyloliquefaciens* strains resulted in maximum shoot K concentration at 0% PEG while un-inoculated seeds remained poor in this regard at 10 and 20% PEG (Fig. 4). Maximum increase of 159 and 307% in shoot K concentration was noted with inoculation of *A. fabrum* compared with control at 0 and 10% PEG, respectively (Fig. 4).

Discussion

In this pot study, PEG-induced drought (20% PEG solution) was more severe, which substantially reduced the wheat growth; while seed inoculation with ACC deaminase containing PGPRs, specially *L. adecarboxylata* and *A. fabrum*, substantially counteracted the damaging effects of PEG-induced drought stress on wheat growth (Table 2).

PEG-induced drought stress impaired the root and shoots growth of wheat (Table 2) possibly due to impaired cell division and cell elongation. Both cell division and elongation are the key components of plant growth negatively affected by drought stress (Taiz and Zeiger, 2010; Hussain *et al.*, 2018) leading to decrease in root and shoot length and dry weights as was observed in this study. According to Gargallo-Garriga *et al.* (2014), drought stress deactivates metabolic processes that results in reduction of shoot length. Small uptake of NPK (Fig. 2, 3 and 4) under drought stress was primarily linked with small root growth (Table 2) and decreased water uptake due to elevated osmotic stress. Reduction in nutrients uptake is quite common in crop plants subjected to drought stress due to impaired root system and accelerated osmotic stress (Hussain *et al.*, 2018). Likewise, decreased wheat seedling dry weight in this pot study was linked with small root growth, limited nutrients uptake and decrease in chlorophyll contents (Table 2, 3; Fig. 2, 3 and 4). Drought stress results in elevated production of ethylene which deteriorates the cell membrane integrity by lipid

molecules degradation due to its direct contact with chloroplast that activates chlorophyllase (chlase) gene. This activation of chlorophyllase (chlase) gene severely damage chlorophyll in plants (Matile *et al.*, 1997).

Nonetheless, application of PGPRs, *L. adecarboxylata* and *A. fabrum* in specific, markedly counteracted the damaging effects of drought on wheat growth *i.e*., shoot and root lengths and dry weights and seedling dry weight (Table 2). The reasons behind this growth improvement might be the reduction in endogenous ethylene production due to their higher ACC-deaminase activity and secretion of growth hormone *i.e*., IAA that resulted in better roots elongation and intake of nutrients (Table 2 and Fig. 2, 3 and 4). The proposed mechanism of ACC deaminase functioning by Glick *et al.* (1999) under abiotic stress strengthen our argument of better root elongation and improvement in growth attributes by reduction in endogenous stress ethylene through ACC-deaminase producing *L. adecarboxylata* and *A. fabrum i*noculation (Table 1). According to Glick *et al.* (1999), enzyme ACC-deaminase initially breakdown ethylene precursor, 1-aminocyclopropane-1-carboxylic acid into α-ketobutyrate and NH_3. Reduction in rhizospheric ethylene resulted in the movement of roots accumulated ethylene from the inside of roots to outside in rhizosphere along a concentration gradient. Thus, low accumulation of stress generating ethylene in roots, resulted in significant improvement in root elongation. Similar kind of improvement in plant growth attributes was also documented by many scientists where ACC-deaminase containing PGPRs were inoculated (Naz *et al.*, 2013; Zafar-ul-Hye *et al.*, 2014, 2015). The findings of Xie *et al*. (1996) also supported our argument and suggested that growth hormone IAA is an allied factor, which might also be responsible for an improvement in root elongation. High secretion of IAA by PGPRs significantly enhance root surface area, adventitious and lateral root length (Mohite, 2013).

The effective drought tolerant ACC-deaminase containing PGPRs (*L. adecarboxylata* and *A. fabrum*) of current study were also capable to produce IAA with and without L-tryptophan (Table 1). Safronova *et al.* (2006) suggested that colonization of PGPRs increase surface area of roots for nutrients absorption and their ability to solubilize immobile nutrients (*e.g*. phosphorus) which might be one of the reason behind the improvement in shoot nutrients concentration through inoculation of ACC deaminase containing PGPRs. Nonetheless, higher NPK shoot concentration under *L. adecarboxylata* and *A. fabrum* inoculation was linked with higher root growth (Table 2) and their higher P and K solubilizing activity (Table 1). Moreover, higher chlorophyll contents and better uptake of NPK (Table 3 and Fig. 2, 3 and 4) in these treatments might have been translated into higher seedling weight both under normal and PEG-induced drought conditions (Table 2). According to Hassan *et al.* (2015, 2016), better uptake of N, P and K nutrients play a vital role in the improvement of shoot and root dry weight. Stefan *et al.* (2013) also confirmed

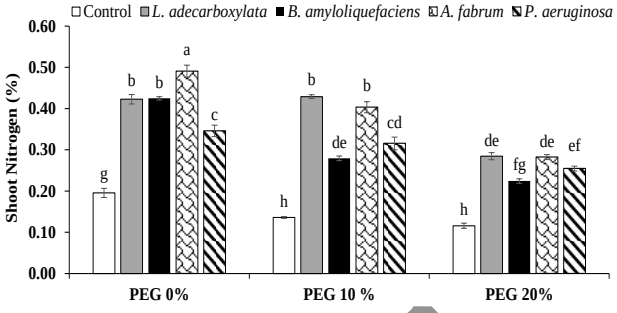

Fig. 2: Effect of ACC deaminase containing PGPRs on wheat shoot nitrogen concentration under drought stress ± SE (standard error). Means sharing the same letter are statistically similar at $p \leq 0.01$

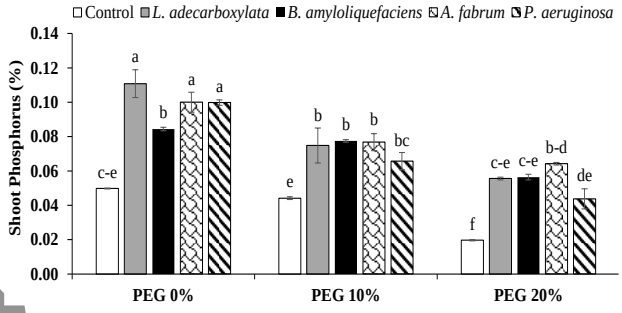

Fig. 3: Effect of ACC deaminase containing PGPRs on wheat shoot phosphorus concentration under drought stress ± SE. Means sharing the same letter are statistically similar $p \leq 0.01$

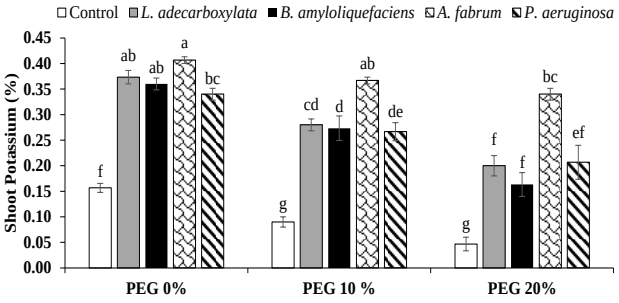

Fig. 4: Effect of ACC deaminase containing PGPRs on wheat shoot potassium concentration under drought stress ± SE. Means sharing the same letter are statistically similar $p \leq 0.01$

our results of improvement in chlorophyll contents as they observed and suggested that secretion of IAA by PGPRs is a co-factor responsible for the improvement in chlorophyll contents of runner bean (*Phaseolus coccineus* L.). However, according to Wu *et al.* (2006) better intake of N and P stimulates the synthesis of chlorophyll contents.

Conclusions

The PEG-induced drought stress impaired the early wheat growth while application of newly reported drought tolerant ACC-deaminase containing PGPRs *i.e., L. adecarboxylata*

and *A. fabrum* counteracted these damaging effects. The improvement in wheat growth under optimal and sub-optimal conditions with PGPRs application was primarily linked with their higher ACC deaminase activity, IAA production and NPK uptake. However, further investigations are needed at field level to introduce *L. adecarboxylata* and *A. fabrum* as new drought tolerant ACC-deaminase containing PGPRs to improve growth of cereal crops.

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