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Short Communciation

Rhizosphere bacteria containing ACC-deaminase conferred drought tolerance in wheat grown under semi-arid climate

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Abstract

Certain rhizobacteria have the potential of lowering endogenous ethylene levels in plants because of their 1-aminocyclopropane-1-carboxylate (ACC)-deaminase activity and promoting root growth. This mechanism is of great agronomic significance under stress environments, which are known to induce accelerated production of ethylene. Thirty rhizobacteria were isolated from rhizosphere of wheat (Triticum aestivum L.) growing in the Southern Punjab, a semi-arid region of Pakistan. Rhizobacteria were screened for ACC-deaminase activity and their potential to confer drought tolerance in wheat crop. Results of laboratory study revealed that selected rhizobacteria lowered endogenous ethylene levels in the rhizosphere as measured by Gas Chromatograph. Axenic studies showed that inoculation increased root-shoot length, root-shoot mass and lateral root number of the inoculated plants by 141, 44, 196, 52 and 30%, respectively, over control. Better-developed roots because of inoculation with plant growth promoting rhizobacteria (PGPR) helped plants, a better crop stand that enhanced moisture and nutrient feeding volume resulting-in improved growth and yields of wheat crop. Two-year multi-location field trials inferred optimum yields with low delta water in semi-arid climate by PGPR containing ACC-deaminase. The enzyme ACC-deaminase probably lowered harmful ethylene levels which partially eliminated drought stress consequently utilizing soil moisture from lower profiles through proliferated roots.

Key words: ACC-deaminase, drought tolerance, rhizobacteria, wheat

Rhizosphere is a zone immediately adjacent to the plant root and a nutrient rich environment due to the release of sugars, amino acids, organic acids, isoflavonoids, plant hormones and enzymes. This high concentration of nutrients makes the rhizosphere a site of complex and intense microbial activity (Pinton *et al*., 2001). Rhizosphere bacteria colonizing the rhizosphere and conferring beneficial effects are called plant growth promoting rhizobacteria (PGPR) (Kloepper *et al*., 1989). Recently, few reports have been published on PGPR as elicitors of tolerance to abiotic stresses such as drought, salt and nutrient deficiency or excess (Glick, 2005; Arshad *et al*., 2008; Zahir *et al*., 2009). Shahzad *et al.* (2010) demonstrated that rhizobacteria having trait ACC-deaminase can facilitate plant growth to overcome abiotic/ deleterious effects.

Ethylene (C_2H_4) an important plant-signaling molecule involves in many plant functions including seed germination, root hair development, root nodulation, flower senescence, leaf abscission and fruit ripening. Ethylene is produced by a two-step process that consists of enzymatic conversion of S-adenosyle methionine (SAM) to ACC followed by the conversion of ACC to ethylene, which is catalyzed by ACC-oxidase (Arshad and Frankenberger, 2002). Ethylene production in plants is increased upon exposure to biotic and abiotic stresses, including extreme temperature, drought, anaerobic conditions, wounding, herbivory and infection by viral, bacterial and fungal pathogens (Yang *et al*., 2008). It has been investigated that certain microbes contain an enzyme ACC-deaminase which hydrolyses ACC, the precursor of ethylene into ammonia and α-ketobutyrate, thereby reducing the levels of ethylene which can inhibit plant growth (Shaharoona *et al*., 2006). Therefore, it is most likely that auxin (IAA) and ACC-deaminase function together to stimulate root elongation (Shah *et al.*, 1998) and ultimately the plant growth. The rapid establishment of roots, either by elongation of the primary roots or by proliferation of lateral and adventitious roots is advantageous for a number of reasons. Firstly, it increases the ability of seedling to anchor in the soil and to obtain water and nutrients from a broader environment, enhancing their chances of survival (Patten and Glick, 2002). Secondly, the rapid elongation of roots minimizes the opportunity for pathogen infection because the root

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quickly passes through the pathogen court allowing pathogens, especially fungi, insufficient time to infect the plant (Conn, 2005).

Water scarcity in the root zone for such a longer period which hampers crop growth is called drought. It imposes serious reduction in crop yield and is one of the greatest limitations faced by present day agriculture. Intergovernmental Panel on Climate Change (2001) observed a continuous increase in global temperature and aridity because of glass house effect. Non-conventional biotechnological approaches must be opted to combat water shortages to grow crops. Drought stress has extensively associated with elevated release of endogenous ethylene by the plant (Mayak *et al*., 2004). The impact of drought stress on ethylene synthesis is an area to be searched because the accelerated ethylene levels could be responsible for growth inhibition, premature senescence and abscission induced by water deficits (Morgan *et al*., 1990). One way to overcome the limitations of plant growth by drought stress could be to promote root growth and allowing water uptake from the deeper soil profiles to maintain leaf water status, a feed-forward response to soil drying (Reid and Renquist, 1997). The other way could be the lowering of the stress-induced ethylene.

Patten and Glick (2002) while working on mechanisms of plant growth by PGPR observed biosynthesis of auxins (improving root growth), siderophores and ACC deaminase (lowering growth-inhibiting levels of ethylene) as major mechanisms of plant growth. Bashan and de-Bashan (2005) in "Encyclopedia of Soils in the Environment" have narrated "Inhibition of plant ethylene synthesis" as a major mechanism of plant growth promotion resulting-in increased root length by many PGPR communities.

The Southern Punjab comprising the districts of Multan, Vehari, Kanewal, Bahawalpur etc, lies between longitudes 71^o and 73^o East and latitudes 29^o and 30^o North in semi-arid region of Pakistan. The mean annual rainfall ranges from 125-150 mm with hot and long summer (110 ± 10^o F) and mild and short winter (43^o F generally frost free). The soils of the area have formed mainly in alluvial deposits of the Sutlej and Beas Rivers and to some extent alluvial deposits of the Chenab River. Parent material is mixed calcareous alluvium derived from Himalayas with pH value of 8.2-8.4 (Akram *et al*., 1969).To get sustainable and economical yields out of these soils and the environment, isolation and use of novel bacteria capable of conferring drought tolerance in wheat (with cotton-wheat rotation in the area) was the task of the study.

Isolation and screening

Thirty rhizobacteria were isolated from wheat crop grown in the Southern Punjab (districts of Vehari and Khanewal) by using glucose peptone agar medium (GPAM) as described by Wollum-II (1982). The cultures selected were numbered as SBW_1, SBW_2,…, SBW_{30}.

ACC-metabolism assay (qualitative) was carried out as described by Jacobson *et al*. (1994) to characterize the isolates for their ability to use ACC as sole nitrogen source. The isolates were grown on two nitrogen sources (ACC and ammonium sulphate) and one N-free source (magnesium sulphate), to observe the growth rate of isolates, for ACC substrate in parallel to ammonium sulphate. Isolates were categorized into three groups, as strains with higher (> 0.7), medium (0.5-0.69) and lower (< 0.5) ACC-metabolism rate depending upon their O.D value at 550 nm for ACC substrate as compared to ammonium sulphate. Two rhizobacteria (SBW_{17} and SBW_{27}) were selected on the basis of their higher ACC-deaminase activity and auxin production potential for further experimentation. Rhizobacteria varied in their auxin producing capabilities (Anjum *et al*., 2011)

Characterization under axenic conditions

All the thirty isolates were characterized for root colonization, ACC-deaminase, (data being published separately) and growth promotion as described by Shaharoona *et al*. (2006). For growth promotion, seeds were surface sterilized and grown in Petri plates for seven days after germination (three seeds per plate in six repeates). Data on number of lateral roots, root-shoot mass (oven dry basis) and root -shoot length (Area Meter CI-203 CID 9 nc) were recorded.

Field experiment

Field experiments were conducted at two sites, i.e. Agronomic Research Station, Khanewal and Adaptive Research Farm, Vehari. Physicochemical characteristics of the soils were determined before conducting the field trial (Table 1). Wheat seeds (Lasani-2008) were obtained from Wheat Research Institute, Faisalabad and treated with mix culture of selected (SBW_{17} and SBW_{27}) ACC-deaminase containing PGPR isolates in 1:1 ratio on volume basis.

Table 1: Physiochemical characteristics of the soils before conduct of trials

Parameter	ARS Khanewal	ARF Vehari
pH	7.8	8.0
ECe (dS m^{-1})	1.8	2.1
Total N (%)	0.0042	0.0040
Olsen P(mg kg^{-1})	9.5	7.8
Organic matter (%)	0.58	0.45
Texture	Loam	Sandy loam
K (mg kg^{-1})	231	210

Inoculum was prepared in general purpose medium. The selected isolates were cultured in 500 mL conical flask containing 100 mL medium and incubated at 28 ± 1 ^{0}C under shaking at 100 rpm for three days to get an optical density of 0.5 (recorded at 535 nm wavelengths). Inoculated/un-inoculated wheat seeds were sown with single row seed drill keeping row to row distance of 25.0 cm with plot size, 10 x 3 m^2. Each experiment was conducted in randomized complete block design (RCBD) with three repeats. Canal water was used for irrigation. One irrigation was skipped from recommended irrigation schedule at milking stage of wheat to give a drought stress to the crop. Three graded fertilizer levels of NPK viz. 60-50-0, 90-75-0 and 120-100-0 kg ha^{-1} were applied. All P and half N was applied at sowing and remaining half N was applied at tillering stage. Wheat grain yields were recorded at the time of harvest.

Growth parameters i.e. plant height, number of tillers $plant^{-1}$, spike length, root and shoot growth, etc. were recorded by randomly selecting five plants from each treatment in case of field trials (data selectively shown). The data were statistically analyzed (Steel *et al*., 1997) and means were compared by Duncan's multiple range test (Duncan's, 1955).

Data in Table 2 from plate experiment under axenic condition registered highly significant effect of inoculation on wheat seedling growth. Both the PGPR isolates (SBW_{17} and SBW_{27}) selected for field studies significantly improved root-shoot length and biomass of wheat, but the pace of increase was much higher in case of SBW_{27} exhibiting 116.5, 43.2, 151.2, 46.9 and 58.1 percent increased root length, shoot length, root mass, shoot mass and lateral root number over control, respectively.

Wheat response to PGPR (a consortium of SBW_{17} and SBW_{27}) under water stress in field was studied in trials conducted at ARS, Khanewal and ARF, Vehari with graded levels of mineral fertilizer. Data depicted in Table 3 described significantly positive effects of PGPR inoculation on grain yield of wheat at both the locations. PGPR inoculation produced 3755 kg ha^{-1} grain even with one irrigation skip in comparison with 3586 kg ha^{-1} of un-inoculated control at Khaniwal. Wheat crop also responded significantly to fertilizer levels exhibiting 4125 kg ha^{-1} at 120-100-0 kg NPK ha^{-1} in comparison with 3616 kg ha^{-1} at 90-75-0 kg NPK ha^{-1}, which itself was significantly higher than the yield obtained at 60-50-0 kg NPK ha^{-1}. The interactive analysis of fertilizer and inoculum inferred that, though inoculum increased the grain yield at all fertilizer

Table 2: Stimulatory effect of ACC-deaminase containing PGPR on root-shoot growth of wheat in plate experiment under axenic condition

Treatment	Root length	Shoot length	Root mass	Shoot mass	Lateral root Number
	(cm)		Over dry (mg)		
Control	31.5 c*	11.8 b	3.9 c	76.5 b	4.3 c
SBW_{17}	52.4 b	16.6 ab	6.8 b	108.0 a	5.6 a
SBW_{27}	68.2 a	16.9 a	9.8 a	112.4 a	6.8 a

*Mean sharing similar letter (s) in a column do not differ significantly at p = 0.05

Table 3: Wheat yield as affected by PGPR inoculation (ARS, Khanewal and ARF, Vehari)

Treatment NPK (kg ha^{-1})	Grain Yield (kg ha^{-1}) ARS, Khanewal		
	Un-inoculated	Inoculated**	Means
60-50-0	3167 f*	3375 e	3271 C
90-75-0	3550 d	3683 c	3616 B
120-100-0	4042 b	4208 a	4125.0 A
Means	3586 B	3755 A	
LSD (Fertilizer) 168.23			
	Grain Yield (kg ha^{-1}) ARF, Vehari		
60-50-0	2926^{NS}	2953	2939 B*
90-75-0	3562	3575	3568 A
120-100-0	3555	3631	3593 A
Means	3348 B	3387 A	
LSD (Fertilizer) 93.65			

NS: Non-significant

*Mean sharing similar letter (s) in a column or row do not differ significantly at p=0.05

**Inocula were prepared by a consortium of SBW17 and SBW27 in 1:1 and one irrigation was skipped at flowering stage

levels but differences were non-significant at Vehari but significant at Khanewal.

Diverse biological heterogeneity in soils under natural conditions has demonstrated inconsistent yield responses to microbial inoculations (Dobbelaere *et al*., 2003). In our studies, wheat responded positively to ACC-deaminase containing PGPR. Under field condition, wheat yield was enhanced from 4-14% in different trials. Glick *et al.* (2007), Arshad *et al.* (2008) and Zahir *et al*. (2008) also reported that ACC-deaminase containing PGPR produced higher crop yields and provided stress relief especially under drought conditions.

Yang *et al.* (2008) proposed the term induced systemic tolerance (IST) for PGPR induced physical and chemical changes in plants that result in enhanced tolerance to abiotic stress. Results of our study also complement the proposal by demonstrating optimum yields obtained under water stress, but inoculated with ACC-deaminase containing bacteria.

Though PGPR is an emerging area of science but had proved its significance in plant growth promotion through enhanced nutrient acquisition, phytohormone production, and biological control (Nakkeeran *et al*., 2006). Phytoremediation and induced systemic resistance (ISR) are also well-thrashed (van Loon, 2007). Few reports have been published on stress tolerance by PGPR, so, a better understanding and exploitation of PGPR-induced systemic tolerance can make a breakthrough in raising crops under environmentally unfavorable conditions (Yang *et al*., 2008). The findings could be very useful in the arid regions, or the developing world relying mainly on low input agriculture.

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