INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 2, p. 418-428, 2019

RESEARCH PAPER OPEN ACCESS

Evaluation of PGPR strains having ACC deaminase activity for their efficacy to induce water stress tolerance in sunflower under varied moisture regimes

Muhammad Shahid Siddique[1*], Ghulam Qadir[1], Shahid Maqsood Gill[2], Tariq Sultan[2], Riffat Hayyat[3]

[1] *Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*

[2] *Land Resources Research Institute, National Agricultural Research Centre, Islamabad-45500, Pakistan*

[3] *Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan*

Key words: PGPR strains, ACC deaminase activity, Drought stress tolerance, Sunflower.

http://dx.doi.org/10.12692/ijb/14.2.418-428 Article published on February 27, 2019

Abstract

Sunflower is an important oil seed crop in Pakistan but incidence of drought stress considerably hampers its growth and productivity. The present pot study was performed to evaluate the potential of PGPR strains having ACC deaminase activity for their efficacy to induce water stress tolerance in sunflower. Ten PGPR strains (KS7, KS8, KS10, KS15, KS17, KS25, KS28, KS41, KS42 and KS44) already isolated and identified (Kiani *et al.*, 2015) along with uninoculated control were tested at three soil moisture levels (25, 20 and 15% volumetric water contents representing no, medium and high soil water stress, respectively) in a two factor Completely Randomized Design with three replications. The volumetric water contents were determined daily with Time Domain Reflectometer (TDR) and different soil moisture regimes were maintained accordingly. Four plants were maintained in each pot. Two plants each after 30 and 60 days of germination were harvested for data recorded. The results revealed that at high water stressed condition (15%VWC),KS42 and KS17 caused maximum increase of upto 35% and 31%, respectively in chlorophyll SPAD readings over uninoculated control after 30 and 60 DAG. While in terms of growth traits PGPR strains KS42 and KS7 outperformed and gave maximum increment of upto 151% &104%, 255% & 205% and 139% & 100% in fresh & dry shoot weight, fresh & dry root weight and total root length, respectively over uninoculated control. It was concluded that PGPR strains KS42 and KS7 exhibited greater potential for mitigation of negative effects of drought stress in sunflower.

* **Corresponding Author:** Muhammad Shahid Siddique ✉ shahid.mub@hotmail.com

Introduction

Pakistan is facing acute shortage in production of edible oil, about 84% of the local edible oil demand is met through import. The total edible oil requirement during the year 2014-15 was 3.523 million tonnes. The domestic production share in total edible oil demand was 0.556 million tonnes, while the remaining 2.967 million tonnes were imported on spent of huge bill, ranking Pakistan as third largest importer of edible oil after China and India (Siddiqui, 2010; GOP, 2015).Sunflower (*Helianthus annus* L.) as an important oilseed crop promises to fill the gap between demand and supply because of its wide adaptability to agro-climatic conditions of Pakistan. It is one among the four oleaginous plants that are widely cultivated as oilseed crops around the globe. Its economic value is attributed to its premium quality oil that contains high percentage of poly unsaturated fatty acids that is highly preferred in diet (Rathore *et al*., 2001; Skoric *et al*., 2008).

The prevailing cropping systems, where sunflower is mainly grown offer narrow window for increase of area under this crop due to some non-adjustment reasons with major crops (Badar *et al*., 2002). Sunflower can be cultivated on marginal lands and semi-arid regions, as it can cope with abiotic stress conditions efficiently than any other crop due to its structural stability (Kiani *et al*., 2007; Skoric, 2009). It is also categorized as low to medium drought sensitivite crop, and its productivity is greatly influenced by water availability. Water stress during vegetative and reproductive growth stages may result in yield reduction of 40 to 61% in sunflower, respectively (Iqbal, 2004).

Drought is one of the key factor among various abiotic stresses that adversely hampers crop growth and productivity around the globe, predominantly on drylands (Debaeke and Abdellah, 2004; Lauteri*et al*., 2014). About 40% of the global land area is occupied by drylandsthat is mainly characterized by low mean annual precipitation, that also support two billion people mainly in developing countries (Millennium Ecosystem Assessment, 2005). The extent of annual yield losses caused by drought averaged about 15% of potential yield (Edmeades, 2008). In Pakistan about 5.1 million hectares (22%) of the total cropped area is rainfed. The contribution of rainfed area in the total production is one third, whereas rainfed area has two times less productivity than irrigated area (Khalil and Jan, 2002).

Climate change and rapid increase in population growth also advocate shift of major crops production to marginal lands, while irregular and erratic rainfalls limiting fresh water availability to critical levels on these lands (Edmeades, 2008; Chen *et al*., 2013). Latest studies elucidated that naturally inhabiting microbial communities could probably assist crops to cope with abiotic stresses more effectively by deploying range of activities (Kavamura *et al*., 2013).The rhizobacterial strains having 1-aminocyclopropane-1-carboxylate (ACC) activity possess great potential of ameliorating plant physiological status under water deprived conditions (Marasco *et al*., 2012). PGPRs are also well adapted to hostile environments and may strengthen plants from damages of drought stress, thus improving crop growth and yield under arid or semiarid conditions (Kasim *et al*., 2013). Ethylene production in plants up to a certain level regulates various processes, while its biosynthesis is influenced by various biotic and abiotic stresses (Hardoim *et al*., 2008b). Ethylene acts as growth inhibitor in plants when its concentration goes beyond a certain limit than required level (Huang *et al*., 2003).Under drought stressed condition, plant homoeostasis is regulated by endogenous production of ethylene that result in reduced root and shoot growth. The rhizosphere naturally populated with ACC deaminase containing rhizobacteria have ability to cleave ACC (immediate precursor of ethylene) released through root exudates under stressed conditions, thereby lowering of ethylene alleviate negative effects of drought stress and also ameliorate plant growth (Glick *et al*., 2007; Nadeem *et al*., 2013).

The effect of beneficial microbes on several crops has been explored previously. However, the role of

rhizobacterial strains having ACC deaminase activity in enhancement of drought stress tolerance, that subsequently also promote plant growth traits has been earlier reported by some researchers (Tank and Saraf, 2010). But, very few studies have been conducted to elucidate the effects of microbial diversity associated with sunflower, especially under water stressed conditions. The present pot experiment was thus performed to test the efficacy of indigenous rhizobacterial strains containing ACC deaminase in enhancement of water stress tolerance in sunflower.

Materials and methods

Experimental procedure

A pot experiment in glasshouse of Plant Genetic Resources Institute, National Agricultural Research Centre (NARC), Islamabad, Pakistan was conducted during May-July, 2015.The efficacy of ten PGPR strains (KS7, KS8, KS10, KS15, KS17, KS25, KS28, KS41, KS42 and KS44) having ACC deaminase activity and an uninoculated control without PGPR was tested at three water stress levels (No, moderate and high) on sunflower (*Helianthus annuus* L. hybrid NKS-278) in a two factor Completely Randomized Design with thee replications making a total of 99 pots. Sunflower seed was obtained from Oilseed Research Program, NARC. Field soil was collected from 0-30cm depth of an uncultivated land having sandy clay loam texture, pH of 8.27, $EC_{1:1}$of 0.40dSm^{-1} and NO_3-N, available P_2O_5, extractable K_2O, Zn, Fe, Cu and Mn contents of 0.65, 0.71, 76.0, 2.57, 10.31, 1.92 and 2.28 mg kg^{-1} soil, respectively. The soil was ground, sieved, autoclaved at 121°C for 25min and filled in PVC pots (25cm diameter and 30cm height) sterilized with 2% sodium hypochlorite solution. The pots were filled with 17kg soil in each pot and tapped on the floor to bring to the same height for attaining uniform soil bulk density, consequently uniform total soil porosity (TSP).Half of the fertilizer dose of N and full doses of P and K corresponding to the recommended rates of 100, 60 and 50 kg ha^{-1}, respectively, were thoroughly incorporated in all the pots. Eight inoculated seeds of sunflower were planted at a depth of 2cm in each pot, which were thinned to four plants at 4-leaf stage. The second half of the N fertilizer was applied after three weeks of sowing. The pots were kept under natural light in the greenhouse with average temperature and relative humidity of 31.6±3.8°C and 69.4±4.9%, respectively, during the growing period. Weeds were hand-pulled whenever necessary. Chlorpyrefos pesticide was used thrice during the growing period to control the attack of army worm.

Inocula preparation and application

Indigenous strains of PGPR, isolated from saline rhizosphere of sunflower having ACC deaminase activity were collected from Soil Biology Program, Land Resources Research Institute, NARC. The liquid cultures of respective strains were multiplied in Luria-Bertini (LB) broth media (Maniatis *et al*., 1982) and kept in orbital shaking incubator for an incubation period of 48 hours at 28±1°C at continuous shaking of 100rpm. The inocula of respective strains when reached to (10^8-10^9 cfu mL^{-1}) were prepared by injecting of suspension into autoclaved black soil carrier material packed in cellophane bags (100 ml kg^{-1}).Sunflower seeds of uniform size and weight were used. Before sowing of seeds were surface sterilized by dipping in 95% ethanol solution for 5minutes then treated with 0.2% $HgCl_2$ solution for 3 minutes and rinsed thoroughly with distilled water (Khalid *et al*., 2004).Culture bags containing different PGPR strains were mixed with 10% sugar solution (100gL^{-1}) to make a slurry (Arshad *et al*., 2008). Seed dressing was done by mixing of seeds with the slurry (1:1) containing different PGPR strains, while for uninoculated control treatments seeds were treated with slurry mixture of LB broth and sugar solution containing no PGPR strain. Treated seeds were spread and air dried under shade condition for 6 to 8hours before sowing.

Development of water stress levels

Three water stress levels were developed based on volumetric soil water (VSW) contents 25% VSW, representing soil water content at field capacity (No water stress), 20% VSW (Moderate water stress), and 15% VSW, representing soil water content slightly

higher than permanent wilting point (High water stress).The pots were first irrigated with a uniform volume of tap water (equivalent to TSP) to saturate the soil and left for drying. VSW was monitored daily from each pot with Time Domain Reflectometer (TDR) model TRIME-FM (IMKO Micromodultechnik GmbH, Germany). Sunflower seeds were planted at field capacity water contents (25% VSW) and the pots were maintained at this water level. After three weeks of planting, the pots subject to moderate and high water stress were stopped irrigation until the desired water stress level was attained. Afterwards, water required to maintain different stress levels were applied daily to the respective pots.

Plant measurements and statistical analysis

Two of the four plants maintained in each pot were harvested 30 days after germination (DAG) and data regarding chlorophyll contents, plant height and fresh & dry shoot weights were recorded. Rest of the two plants were allowed to grow and harvested 60 DAG and parameters like chlorophyll contents, plant height, fresh & dry shoot weights, and fresh &dry root weights and total root length were recorded. The data collected on different plant parameters were subject to analysis of variance using software Statistix version 8.1 and means were compared by Tukey's Honest Significant Difference Test at α=0.05.

Results

Chlorophyll contents

The data of chlorophyll contents in response to inoculation of PGPR under various moisture levels is represented in DAG (Fig. 1). The results revealed that generally higher chlorophyll SPAD readings were found after 30 DAG than was recorded after 60.

As the over all, maximum chlorophyll SPAD readings were found at moderate water stress level as compared to no and high water stress levels at both growth stages. High water stress level resulted in significant decrease in chlorophyll contents, while inoculation of PGPR strains not only countered the negative effects of water stress even caused an increase in chlorophyll contents compared to uninoculated control.

Table 1. Effect of PGPR having ACC deaminase activity on shoot fresh weight (g plant^{-1}) in sunflower under different water stress levels after 30 and 60 days of germination.

PGPR Strains	30 Days After Sowing				60 Days After Sowing			
	Water Stress Levels				Water Stress Levels			
	No	Medium	High	Mean	No	Medium	High	Mean
No PGPR	10.4mno	10.00p	8.4p	9.6E	47.0pq	53.7op	27.8u	42.8G
KS7	14.2b-f	13.7c-i	12.4g-l	13.5BC	78.7efg	77.8fgh	56.8no	71.1C
KS8	14.7bcd	14.1c-g	11.9j-n	13.6BC	72.6g-j	89.8cd	39.1rs	67.2D
KS10	12.9d-k	12.2h-l	11.1l-o	12.1D	65.9jkl	77.2fgh	30.8tu	58.0E
KS15	15.5abc	14.6bcd	12.6e-l	14.2B	75.7f-i	99.2b	39.7rs	71.5C
KS17	16.5a	16.0ab	14.4b-e	15.6A	82.8def	112.1a	41.2qrs	78.7B
KS25	13.2d-j	12.6f-l	10.9l-o	12.2D	73.2g-j	52.9op	35.8st	54.0F
KS28	12.1i-m	12.0i-n	10.2no	11.4D	58.1mno	72.2g-j	30.2tu	53.5F
KS41	13.9c-h	13.6d-j	12.3h-l	13.3C	70.5hk	62.5lmn	44.2qr	59.1E
KS42	14.6bcd	14.6bcd	13.5d-j	14.2B	85.5cde	92.0bc	69.7i-l	82.4A
KS44	12.6f-l	11.3k-o	10.8l-o	11.6D	66.8jk	64.7klm	28.4u	53.3F
Mean	13.7A	13.2B	11.7C		70.6B	77.7A	40.3C	

Means sharing the same letters within a column differ non significantly (P>0.05).

At high and moderate stress level, individual inoculation of KS42 and KS17 caused maximum increment of upto (35%& 31%) and (32%& 24%) in chlorophyll contents, respectively over uninoculated control at 30 and 60 DAG. Under various water stress levels, seed inoculation of KS42, KS17, KS15 and KS7 gave significantly low extent of decrease in chlorophyll contents compared to uninoculated control and rest of the strains.

Shoot fresh and dry weight

The data of fresh and dry shoot weights recorded in response to seed inoculation of PGPR at various water stress levels is represented in (Table 1& 2).

Table 2. Effect of PGPR having ACC deaminase activity on shoot dry weight (g plant^{-1}) in sunflower under different water stress levels after 30 and 60 days of germination.

PGPR Strains	30 Days After Sowing				60 Days After Sowing			
	Water Stress Levels				Water Stress Levels			
	No	Medium	High	Mean	No	Medium	High	Mean
No PGPR	3.48opq	3.28qr	2.78r	3.18G	14.8mn	17.6klm	9.4q	13.9G
KS7	4.67b-h	4.64c-h	4.18g-n	4.50CD	25.6cde	25.0c-f	18.5jkl	23.0BC
KS8	4.98bcd	4.58d-i	3.93j-p	4.50CD	19.6i-l	27.7bcd	12.5n-q	19.9D
KS10	4.33e-k	3.99i-o	3.68m-q	4.00EF	19.0jkl	23.7e-h	11.0opq	17.9EF
KS15	5.25ab	4.69b-g	4.24f-m	4.73BC	21.6f-j	30.3b	13.1nop	21.7C
KS17	5.61a	5.19abc	4.78b-f	5.19A	24.3d-g	33.8a	14.2mno	24.1B
KS25	4.29f-l	4.13g-n	3.70l-q	4.04E	24.0efg	17.3klm	11.7n-q	17.7F
KS28	3.98i-o	3.89k-p	3.35pqr	3.74F	16.7lm	22.6e-i	10.1pq	16.5F
KS41	4.62c-h	4.48d-j	4.08h-n	4.39D	23.3e-h	20.4h-k	14.7mn	19.4DE
KS42	4.95bcd	4.89b-e	4.56d-i	4.80B	28.4bc	30.3ab	22.8e-i	27.2A
KS44	4.20f-n	3.86k-q	3.62n-q	3.89EF	22.0f-j	21.2h-k	9.7mn	17.6F
Mean	4.58A	4.33B	3.90C		21.7B	24.5A	13.4C	

Means sharing the same letters within a column differ non significantly (P>0.05).

The results indicated that inoculation of PGPR significantly improved fresh and dry shoot weights of sunflower plants under various water stress levels when compared with uninoculated control. The results indicated that high water stress level resulted in a significant decrease of up to (15% & 48%) and (17% & 82%) in shoot fresh and dry weight, respectively, over no and moderate water stress condition after 30 and 60 DAG. While seed inoculation of PGPRs at various water stress levels significantly improved fresh and dry shoot weight over uninoculated control. At high water stress level, maximum increase of upto (151% &104%) and (144% & 97%) in fresh and dry shoot weights, respectively, was caused by KS42 and KS7 over uninoculated control. While at moderate water stress level inoculation with (KS17 & KS42) and (KS17 & KS15) gave significant increment of up to (109% &85%) and (93% & 73%) in shoot fresh and dry weight, respectively, over uninoculated control.

Table 3. Effect of PGPR having ACC deaminase activity on root fresh and dry weight (g plant^{-1}) in sunflower under different water stress levels 60 after days of germination.

PGPR Strains	Root Fresh Weight				Root Dry Weight			
	Water Stress Levels				Water Stress Levels			
	No	Medium	High	Mean	No	Medium	High	Mean
No PGPR	2.73l-o	4.89f-i	1.87o	3.16G	2.71pqr	4.29i-o	1.97r	2.99F
KS7	6.50de	6.66d	5.68def	6.28C	6.13d-g	6.43c-f	5.41f-i	5.99BC
KS8	4.81f-j	8.37c	2.91k-o	5.36D	4.47i-n	7.35cde	2.76pqr	4.86D
KS10	4.19g-k	6.73d	2.44mno	4.45E	3.86j-p	6.13d-g	2.19qr	4.06E
KS15	5.89def	9.83ab	3.14k-o	6.29C	5.10f-k	9.02ab	2.97n-r	5.70C
KS17	6.71d	10.60a	3.94h-l	7.08B	6.00e-h	10.05a	3.53l-q	6.53B
KS25	5.88bc	3.99c	3.50de	4.46E	5.80f-i	3.62k-q	3.29m-r	4.23DE
KS28	3.35k-n	6.60de	2.19no	4.05EF	2.91o-r	5.65f-i	2.12qr	3.56EF
KS41	4.99f-i	5.47d-g	5.27e-h	5.24D	4.53h-n	5.29f-j	4.84g-l	4.89D
KS42	8.59bc	8.36c	6.62de	7.86A	7.86bc	7.53bcd	6.45c-f	7.28A
KS44	4.21g-k	3.65i-m	3.47j-n	3.78FG	3.82j-p	3.45l-r	3.30m-r	3.52EF
Mean	5.26B	6.83A	3.73C		4.83B	6.25A	3.53C	

Means sharing the same letters within a column differ non significantly (P>0.05).

The most substantial increase in fresh and dry shoot weight at high water stressed conditions were recorded with KS42 and KS7 over respective uninoculated control. While PGPR strain KS25 exhibited low potential of improving fresh and dry shoot weight at water stressed conditions compared to uninoculated control and rest of the strains after 30 and 60 DAG.

Table 4. Effect of PGPR having ACC deaminase activity on total root length (cm) and root:shoot ratio in sunflower under different water stress levels after 60 days of germination.

PGPR Strains	Total Root Length				Root:Shoot Ratio			
	Water Stress Levels				Water Stress Levels			
	No	Medium	High	Mean	No	Medium	High	Mean
No PGPR	109ij	135e-j	104j	116C	0.183g-i	0.245c-h	0.211d-i	0.213D
KS7	204a-f	206a-e	207a-e	206AB	0.240c-i	0.256b-g	0.293abc	0.263AB
KS8	114hij	173b-j	137e-j	142C	0.228c-i	0.267a-f	0.220c-i	0.238A-D
KS10	112ij	151e-j	125a-i	129C	0.203e-i	0.259b-g	0.198d-i	0.220CD
KS15	158d-j	226a-d	183a-i	189B	0.236c-i	0.297abc	0.226c-i	0.253ABC
KS17	188a-h	240ab	191a-g	206AB	0.249c-h	0.297abc	0.248c-h	0.265AB
KS25	105j	133e-j	141e-j	126C	0.242c-i	0.209d-i	0.280a-d	0.244A-D
KS28	108j	145g-j	118e-j	123C	0.174hi	0.250c-h	0.210d-i	0.212D
KS41	139e-j	158c-j	141e-j	146C	0.195f-i	0.259b-g	0.333ab	0.262AB
KS42	232abc	240ab	248a	240A	0.277a-e	0.249c-h	0.282a-d	0.269A
KS44	131f-j	136e-j	118g-j	129C	0.174hi	0.162i	0.341a	0.226BCD
Mean	145 B	177 A	156 B		0.109 B	0.125 A	0.129 A	

Means sharing the same letters within a column differ non significantly (P>0.05).

Root fresh and dry weight

The data regarding the effect of PGPR inoculation on fresh and dry root weights of sunflower under various water stress levels are summarized in (Table 3). The results showed that maximum fresh and dry root weights were recorded at moderate moisture level. While when sunflower plants were subjected to high moisture stress level both fresh and dry root weight were significantly decreased as compared to non-water stressed condition. Under high water stress level a significant decrease of (29% and 45%) and (28% &44%) in root fresh and dry weight was recorded over no and moderate stress conditions, respectively, after 60 DAG. It is evident from the results that PGPR inoculation significantly improved root fresh and dry weight under various water stress levels over uninoculated control. At high water stress level, PGPR strains KS42 and KS7 caused maximum increase of up to (255 &205%) and (227 & 191%) in root fresh weight and in dry weight, respectively over uninoculated control. At moderate water stress level, an increment of (116%& 70%) and (134% & 75%) in root fresh and dry weight was caused by KS17 and 15, respectively, over uninoculated control.

Total root length and root to shoot ratio

The data on total root length and root to shoot ratio of sunflower plants in response to seed inoculation of ACC deaminase containing PGPR strains at various water stress levels are summarized in (Table 4).The results revealed moderate and high water stress levels resulted in an increase 21% and 7%, respectively in total root length over non-stressed condition. The seed inoculation of PGPRs also extensively improved root length under various water stress levels. At high water stress level, individual inoculation with KS42 and KS7 caused maximum increment of 139% and 100% in root length, respectively over uninoculated control. Under moderate and non-stressed condition, KS42, KS17 and KS7 outperformed significantly ameliorated root length over uninoculated control. While PGPR strains (KS25 & KS28) and KS25 under no and moderate stressed conditions, respectively showed low potential of increase in total root length

of sunflower plants. In general, KS42 and KS7 outperformed and extensively increased total root length under water stressed conditions over uninoculated control and rest of the strains. The data on root to shoot ratio in response to seed inoculation of PGPRs under various water stress levels is represented in (Table 4).The results indicated that high and moderate water stressed conditions resulted in an increase of up to 18 and 15%in root to shoot ratio, respectively over non water stressed condition (Table 4). At high water stress level maximum increase of up to 62% in shoot to root ratio was recorded with KS44 followed by KS 7 and KS 42 that caused an increase of 47% and 34%, respectively over uninoculated control.

Discussion

Chlorophyll contents

In present study, moderate water stress level resulted in an increase of chlorophyll SPAD readings as compared to non-stressed condition, but more reduction in water content at high water stress level caused reduction in SPAD readings. Our results are in accordance with (Manivannan *et al.*, 2007b; Kiani *et al.*, 2008), reported a substantial decrease in chlorophyll contents of sunflower plants underwater stressed conditions. In contrast (Ommen *et al.*, 1999; Nezami *et al.*, 2008) during investigations observed that water deficit conditions resulted in a significant increase in chlorophyll contents and SPAD index of wheat and sunflower. While inoculation of PGPRs ameliorated chlorophyll contents in sunflower plants subjected to various water stress levels. Our results are in agreement with (Stefan *et al.*, 2013) found that seed inoculation of PGPR strains under moisture stress condition caused a significant increase in chlorophyll and carotenoid contents of runner bean.

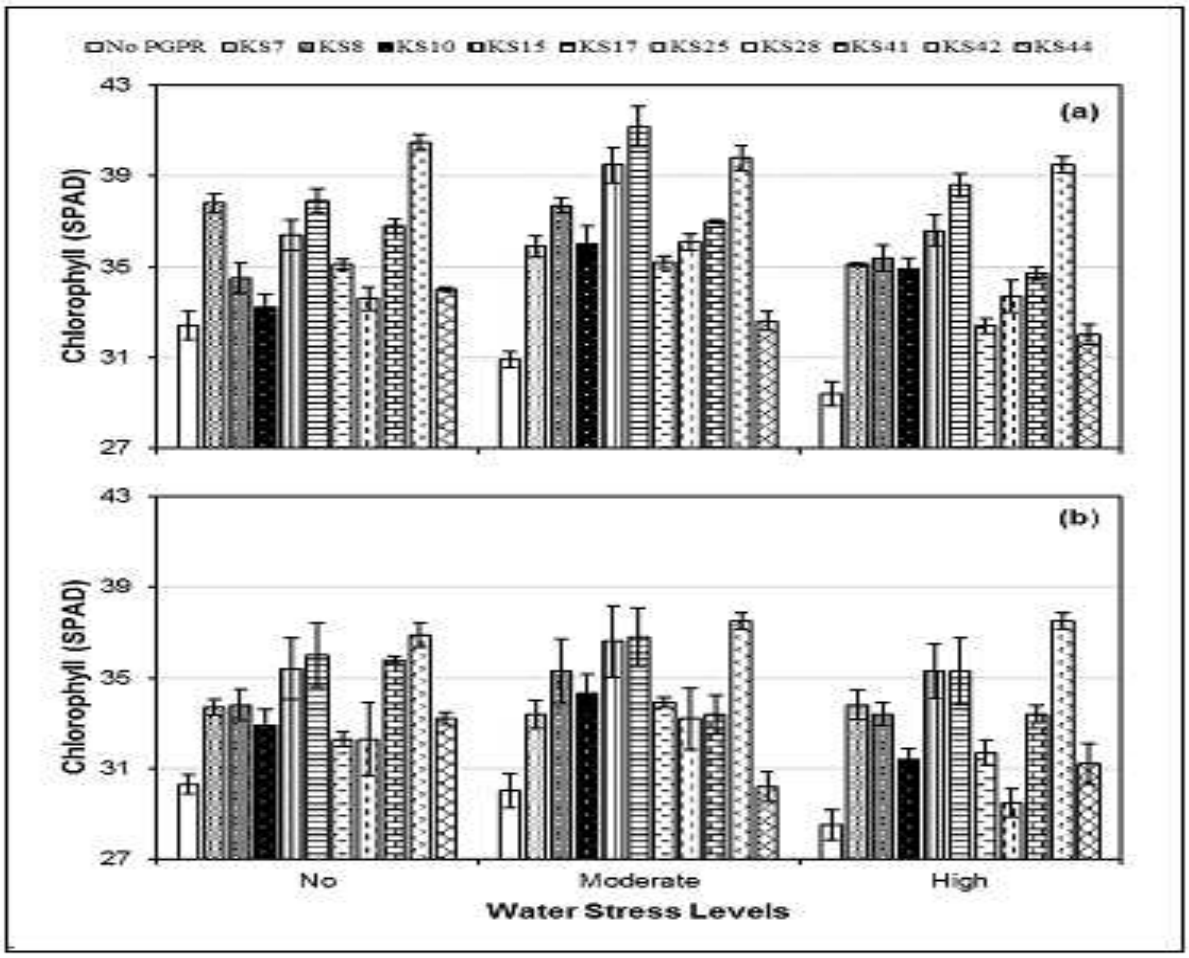

Fig. 1. Effect of PGPR having ACC deaminase activity on chlorophyll contents (SPAD) in sunflower under different water stress levels after 30 (a) and 60 (b) days of germination.

Shoot fresh and dry weight

A considerable reduction in fresh and dry shoot weights was recorded at high water stress level after 30 and 60 days of germination, while inoculation of PGPR caused a significant increment in both fresh and dry shoot weights of sunflower plants. Our results are in commitment with (Zhao *et al.*, 2006) reported that a common detrimental effect of water stress on crop plants is reduction in fresh and dry biomass. While inoculation of PGPRs alleviated the negative impact of moisture stress and subsequently ameliorated shoot weight of maize under water deficit conditions (Shaharoona *et al.*, 2006).Under water stressed conditions pea plant growth status was significantly improved by inoculation of PGPRs that was observed in terms of increased shoot and root biomasses (Munir *et al.*, 2008). Amongst, various PGPR strains used in this pot study KS42 and KS7 caused maximum increase of upto (151% & 104%) and (144% & 97%) in fresh and dry shoot weights, respectively over uninoculated control at high water stress level.

Root fresh and dry weight

It was evident that PGPR inoculation improved sunflower growth under drought stress and subsequent recovery, which was evident from increased fresh and dry root weights and proliferated roots. Seed inoculation with rhizobacterial strains caused significant increase in fresh &dry root weights and root length of sunflower and maize plants under drought stressed conditions (Nain *et al.*, 2010; Ambrosini and Beneduzi, 2012). Our results are in accordance with earlier reports showed the beneficial effect of inoculation with PGPRs for alleviation of drought stress symptoms in sunflower plants (Sandhya *et al.*, 2010).The previous studies indicated that PGPR strains caused a considerable increment in root dry weight and root length of wheat seedlings at various water stress levels (Bangash *et al.*, 2013). It is most likely to be ascribed that ACC deaminase containing rhizobacteria have ability to metabolize ACC, thus lowering of endogenous ethylene level in stressed plants ultimately ameliorated root and shoot growth (Kang *et al.*, 2010). The PGPR strains KS42 and KS7, prominently improved root growth and caused maximum increment of up to (255 &205%) and (227 & 191%) in root fresh and dry weight, respectively over uninoculated control at high water stress level.

Total root length and root to shoot ratio

In the present study, total root length showed a significant increase under drought stress which conformed to some earlier published report on mung bean (Ranawake *et al.*, 2011). It was found that root length of sunflower plants subjected to drought stress was increased over non stressed control on 50DAS, while a significant reduction in root length was recorded in drought stressed plants over non stressed control on 70DAS (Manivannan *et al.*, 2007). It was found that root length in pisum plants was prominently increased by inoculation of Pseudomonas spp., a rhizobacterial strain having ACC deaminase activity, which improved water absorption ability of drought stressed plants (Zahir *et al.,* 2008). Under water stressed conditions roots proliferate and increase in number, resulting in increased surface area for better uptake of water. While extensive lateral root growth increases surface area and subsequently the nutrient uptake efficiency of plants (Vasudevan *et al.*, 2002). The seed inoculation with KS42 and KS7caused a significant increase of up to 139% and 100%, respectively in total root length at high water stressed condition over uninoculated control. The results of pot study showed that root to shoot ratio of sunflower plants increased significantly under water stressed conditions. Our results are in agreement with (Fulda *et al.*, 2011) revealed that shoot growth was more restricted than root growth under water stressed conditions, resulting in extensive increase in root to shoot ratio of water stressed plants. The results also indicated that inoculation of PGPR caused a significant increase in root to shoot ratio of sunflower plants under induced water stressed conditions.

Conclusion

The results of pot study demonstrated that the PGPR stains having ACC deaminase activity proficiently

alleviated negative effects of water stress in sunflower. According to the results, PGPR strains KS42 and KS7 have been recognized as the most promising strains, that outperformed and extensively ameliorated chlorophyll contents and plant growth traits of sunflower plants under induced water stressed conditions. These findings may imply that inoculation with rhizobacterial strains having ACC deaminase activity could be used as effective inoculants for improving growth of sunflower plants under drought abiotic stress condition. In future, there is need to perform such experiments under field conditions to explore the efficacy of PGPR strains having ACC deaminase activity in alleviation of drought stress negative effects and subsequent improvement of crop production under water deprived conditions.

References

Arshad M, Shaharoona B, Mahmood T. 2008. Inoculation with Pseudomonas spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.). Pedosphere **18(5),** 611-620. https://doi.org/10.1016/S1002-0160(08)60055-7

Ambrosini A, Beneduzi A, Stefanski T, Pinheiro FG, Vargas LK, Passaglia LMP. 2012. Screening of plant growth promoting Rhizobacteria isolated from sunflower (*Helianthus annuus* L.). Plant Soil **356(2),** 245–264. http://dx.doi.org/10.1007/s11104-011-1079-1

Badar H, Javed MS, Ali A, Batool Z. 2002. Production and Marketing Constraints Limiting Sunflower Production in Punjab (Pakistan). International journal of Agriculture and Biology **4(2),** 267-271.

Bangash N, Khalid A, Mahmood T, Siddique MT. 2013. Screening rhizobacteria containing ACC-deaminase for growth promotion of wheat under water stress. Pakistan Journal of Botany **45,** 91-96

Chen L, Dodd IC, Davies WJ, Wilkinson S. 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. Plant, Cell and Environment **36(10),** 1850–1859. https://doi.org/10.1111/pce.12094

Debaeke P, Abdellah A. 2004. Adaptation of crop management to water limited environments. European Journal of Agronomy **21(4),** 433–446. https://doi.org/10.1016/j.eja.2004.07.006

Edmeades GO. 2008. Drought tolerance in maize: an emerging reality. A feature. In: James, C. (Ed.), Global Status of Commercialized Biotech/GM Crops. ISAAA Brief **39**, 197-217.

Fulda S, Mikkat S, Stegmann H, Horn R. 2011. Physiology and proteomics of drought stress acclimation in sunflower (*Helianthus annuus* L.). Plant Biology **13(4),** 632-642. https://doi.10.1111/j.1438-8677.2010.00426.x.

Glick BR, Cheng Z, Czarny JC, Duan J. 2007. Promotion of plant growth by ACC-deaminase containing soil bacteria. European Journal of Plant Pathology **119,** 329-339. http://dx.doi.org/10.1007/sI0658-007-9162-4

GOP. "Economic Survey of Pakistan 2014-2015". Government of Pakistan, Ministry of Food, Agriculture and Livestock, Agriculture & Livestock Division (Economic Wing), Islamabad.

Huang Y, Hutchison LH, Laskey CE, Kieber JJ. 2003. Biochemical and functional analysis of CTR1, a protein kinase that negatively regulates ethylene signaling in Arabidopsis. The Plant Journal **33(2),** 221-233. https://doi.org/10.1046/j.1365-313X.2003.01620.x

Hardoim PR, Van Overbeek LS, Van Elsas JD. 2008. Properties of bacterial endophytes and their proposed role in plant growth. The Trends in Microbiology **16(10),** 463–471. https://doi.10.1016/j.tim.2008.07.008

Iqbal N. 2004. Influence of exogenous glycine betaine on drought tolerance of sunflower (*Helianthus annuus* L.). Ph.D. Thesis, Dept. of Bot, Univ. of Agri., Faisalabad.

Khalil IA, Jan A. 2002. Cropping technology, National Book Foundation, Islamabad. Pakistan, p 28.

Kasim WA, Osman ME, Omar MN, Abd El-Daim IA, Beja S, Meijer J. 2013. Control of drought stress in wheat using plant growth-promoting bacteria. Journal of Plant Growth Regulation **32(1),** 122–130.

Khalid A, Arshad M, Zahir ZA. 2004. Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology **96(3),** 473–480. https://doi.org/10.1046/j.1365-2672.2003.02161.x

Kiani SP, Grieu P, Maury P, Hewezit T, Gentzbittel A. Sarrafi S. 2007. Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower. Theory of Applied Genetics **114(2)**, 193-207.

Kiani SP, Maury P, Sarrafi S, Grieu P. 2008. QTL analysis of chlorophyll fluorescence parameters in sunflower (*Helianthus annuus* L.) under well-watered and water-stressed conditions. Plant Science **175(4),** 565-573.

Kiani MZ, Ali A, Sultan T, Ahmed MM. 2015. Characterization of plant growth promoting rhizobacteria isolated from root system of sunflower. Pakistan Journal of Agricultural Research **28(2),** 136-142.

Kang BG, Kim WT, Yun HS, Chang SC. 2010**.** Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnology **4(3),** 179-183. http://dx.doi.org/10.10.1007/s11816-010-0136-1

Kavamura VN, Santos SN, da Silva JL, Parma MM, ÁvilaL A, Visconti A, Zucchi TD, Taketani RG, Andreote FD, de Melo IS. 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. Microbiological Research **168(4),** 183–191. https://doi.10.1016/j.micres.2012.12.002

Lauteri M, Haworth TD, Serrajn R, Monteverdi MC, Centritto M. 2014. Photosynthetic diffusional constraints affect yield in drought stressed rice cultivars during flowering. PLoS One **9(9),** e109054. http://dx.doi.org/10.10.1371/journal.pone.0109054;10.1371/journal.pone.0117631

Maniatis T, Frtch EF, Sambook. 1982. Molecular Coloning; A Laboratory Manual. ColdSpring Harber laboratory, New York, USA., 545.

Manivannan P, Jaleel CA, Sankar B, Kishorekumar A, Somasundaram R, Alagu LGM, Panneerselvam R. 2007b. Growth, biochemical modifications and proline metabolism in Helianthus annuus L. as induced by drought stress. Colloids Surfaces B: Biointerfaces **59(2),** 141-149. https://doi.10.1016/j.colsurfb.2007.05.002

Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S. 2012. A droughtresistance-promoting microbiome is selected by root system under desert farming. PLoS ONE **7,** e48-479. http://dx.doi.org/10.10.1371/journal.pone.0048479

Nezami A, Khazaei HR, Rezazadeh ZB, Hosseini A. 2008. Effects of drought stress and defoliation on sunflower (*Helianthus annuus* L.) in controlled conditions. Desert **12(2),** 99-104. http://jdesert.ut.ac.ir

Nain L, Rana A, Joshi M, Shrikrishna JD, Kumar D, Shivay YS, Paul S, Prasanna R. 2010. Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. Plant and Soil **331**, 17–230

Nadeem SM, Zahir ZA, Naveed M, Ashraf M. 2013. Microbial ACC-deaminase: prospects and applications inducing salt tolerance in plants. Critical Reviews in Plant Sciences **29(6),** 360-393.

Ommen OE, Donnelly A, Vanhoutvin S, Vanoijen M, Manderscheid R. 1999. Chlorophyll content of spring wheat flag leaves grown under elevated CO2 concentration and other environmental stress within `ESPACE-Wheat` project. European Journal of Agronomy **10(4),** 197-203 http://dx.doi.org/10.10.1016/S1161-0301(99)00011-8

Rathore VS, Gautam RC, Kaushik SH. 2001. Yield, quality and nutrient uptake by sunflower as influenced by weed and nutrient management. Journal Annals of Agricultural Research **22(3),** 443-444.

Ranawake AL, Dahanayaka N, Amarasingha UGS, Rodrigo WDRJ, Rodrigo UTD. 2011. Effect of water stress on growth and yield of mung bean (*Vigna radiata* L.). Tropical Agricultural Research and Extension **14(4),** 76-79. http://www.agri.ruh.ac.lk/tare/pdf/V

Shaharoona B, Arshad M, Zahir ZA. 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (Zea mays L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Letters in Applied Microbiology **42,** 155-159.

Skoric D, Jocic S, Lecic, N. 2008. Genetic possibilities for altering sunflower oil quality to obtain novel oils. Canadian Journal of Physiology and Pharmacology **86(4),** 215-221.

Skoric D. 2009. Sunflower breeding for resistance to abiotic stresses. Helia **32(50),** 1-16.

Siddiqui MH. 2010. Nutrient management for sunflower production. PhD diss., Tendo Jam univ. Sindh, (Pakistan).

Sandhya V, Ali SKZ, Grover M, Reddy G, Venkateswarlu B. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regulation **62(1),** 21-30. https://doi.10.1007/s10725-010-9479-4

Stefan M, Munteanu N, Staler N, Mihasan M. 2013. Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean. Romanian Biotechnological Letters **18(2),** 8132-8143.

Tank N, Saraf M. 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. Journal of Plant Interaction **5(1),** 51–58. https://doi.org/10.1080/17429140903125848

Vasudevan P, ReddyMS, Kavitha S, Velusamy P, Paul Raj RSD, Purushotta-man SM, Priyadarsini VB, Bharathkumar S, Kloepper JW, Gnanamanickam SI. 2002. Enhancement of rice seedling growth and grain yield. Current Science **83,** 1140–1143.

Zhao TJ, Sun S, Liu Y, Liu JM, Liu Q, Yan YB, Zhou HM. 2006. Regulating thedrought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and transinactive DRE binding factors in Brassica napus. Journal of Biological Chemistry **281(16),** 10752-10759.

Zahir ZA, Munir AHN, Shahroona B, Arshad M. 2008. Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum* L.) under drought conditions. Journal of Microbiolology & Biotechnology **18(5),** 958-963.