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Full Length Article

Bio-Invigoration of Rhizobacteria Supplemented with Exogenous Salicylic Acid and Glycine Betaine Enhanced Drought Tolerance in Sunflower

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Abstract

Water deficit drastically hamper productivity of sunflower. In this study, the role of bio-invigoration of ACC deaminase rhizobacteria supplemented with chemicals *i.e*., glycine betaine and salicylic acid in alleviating negative effect of drought stress in sunflower was investigated. The inoculants of rhizobacteria *i.e*., KS7 and KS42 were applied as seed coating. While plants were foliar sprayed with 0.724 m*M* salicylic acid and 100 m*M* glycine betaine solutions at vegetative and flowering stage. Soil moisture was monitored with Time Domain Reflectometer (TDR) on weekly basis. Data on plant water relations, leaf free proline, leaf glycine betaine and total soluble sugars were recorded at flowering stage, while yield was recorded at maturity stage. Maximum improvement in selected plant traits and water use efficiency was caused by treatments where seed inoculation of KS42 and KS7 was supplemented with foliar spray GB or KS42 was combined with SA or sole inoculation of KS42 was performed. This novel approach of using biological and chemical means together may be recommended for field areas, where water deficit conditions limit sunflower production. © 2020 Friends Science Publishers

Keywords: PGPR strains; Glycine betaine and salicylic acid; Drought stress tolerance; Sunflower

Introduction

Water deficit induced by lack of surface water availability drastically hampers crop productivity including of sunflower on irrigated and rainfed fields. Drought is considered one of the major threats predicted to cause problems for crop production on 50% of the arable lands across the globe by 2050 (Jensen and Mogenson 1984; Vinocur and Altman 2005; Cai *et al*. 2013; Lesk *et al*. 2016). There are assessments showing that 30–60% water loss from the total irrigation water applied to soil due to evaporation in the arid and semi-arid areas (Ashraf 2010). The severity of water stress is further harnessed by depletion of water from root zone and escalated deficit in atmospheric vapor pressure may cause reduction in productivity of the crops ranging from 50 to 73% under water-limited conditions (Davenport *et al*. 2003; Berry *et al*. 2013; Ahanger *et al*. 2014).

Sunflower is one of the most important oilseed crop, attributed to its high yield potential, wide ranging adaptability with short growing period. In early seventies, this oil seed crop was introduced in Pakistan, now it ranks at second position among cash crops. However, its history of four decades reveals that area and production under sunflower is declining (Arshad *et al*. 2010). The area under this crop has been declined to 2, 16,000 hectares with the total production 109 and 41, 000 tones for seed and oil, respectively (GOP 2016–2017). The country has an average yield of sunflower 1,060 kg/ha which is far below than its potential yield of 4,000 kg/ha. Whereas, the history of last three decades for edible oil reveals that amount of locally produced edible oil is growing at the rate of 2.56% annually against domestic consumption which is increasing about 8% annually. Thus, the local production of edible oil from all available resources could not meet the demand of ever-increasing population. Rapid expansion in area and increase in domestic oilseed production has become major challenge for policy makers due to escalating import bill of edible oil.

Sunflower as low to medium drought sensitive crop is very responsive to environmental conditions such as soil moisture availability, which adversely affect crop production especially in semi-arid regions across the globe. It has been investigated that both magnitude and supply of water could adversely affect achene and oil yield of

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sunflower (Krizmanic *et al*. 2003; Reddy *et al*. 2003; Iqbal *et al*. 2005). Sunflower subjected to water stress at vegetative and reproductive stage may result in yield reduction of 40 to 61%, respectively (Iqbal *et al.* 2004).

The prevailing cropping systems in rice and cotton zones offer narrow window for sunflower adjustment, although it is agronomically adapted to agroecological conditions of Pakistan (Badar *et al*. 2002). The marginal lands act as necessary and candidate resource for food production. Use of marginal lands for cultivation of crops is unavoidable due to reduction in productive cropped areas mainly in overpopulated areas, on the other hand it is immensely required to meet increasing demand of food in developing countries (Laird 1951; Nelson *et al*. 1997; FAO 2008). Pakistan has total cropped area of 23.76 million hectares. Out of this 79% (18.77 million ha) is irrigated and remaining 21% (4.99 million ha) 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 (Baig *et al.* 2013).

In Pakistan, shortage of water is one of the limiting factor that leads toward low agricultural productivity. The availability of water is continuously declining since the time of independence. Historically, Pakistan was ranked as water surplus country owning to Indus River system, but now it is included in the list of water deficit countries. In 1947, availability of water per capita was about 5000 cubic meters; predicted to decrease up to 1200 cubic meter per capita by 2025 (Bhatti 1999). In Pakistan, the demand for water is predicted to grow by a factor of 2.2 by 2050 (Bates *et al.* 1973). It is obvious that the country is facing acute shortage of water for use in agriculture. Hence, strategic planning with concrete measures to properly manage irrigation water has becomes indispensable (Samdani 2004).

Therefore, one of the emerging interest is to find out the solutions of water-related problems like drought and its impacts on food security (Alexanratos and Bruinsma 2012). Especially, it is required to find out solutions to induce drought tolerance in plants with amelioration of crop growth to satisfy food demands under limited availability of water resource (Editorial 2010; Mancosu *et al*. 2015). Recent studies have elucidated that soil microbes can help crops to withstand abiotic stresses more effectively. Plant growth-promoting rhizobacteria (PGPR) have great potential to ameliorate nutritional, biochemical, physiological and morphological responses of many plants and, thus confer resistance in plants to alleviate the negative impact of biotic and abiotic stresses (Marasco *et al*. 2013). Further, PGPRs are well adapted to hostile environments and may help plants against damages caused by drought stress, thus ameliorate crop growth and yield in arid or semiarid regions (Marulanda *et al*. 2007; Kavamura *et al*. 2013; Kasim *et al*. 2013). Drought like other abiotic stresses induces accelerated ethylene production in plant tissues which causes abnormal growth in plants (Saleem *et al.* 2007; Bresson *et al*. 2013). Inoculation with PGPR having ACC deaminase activity may ameliorate plant growth by alleviating deleterious effects of ethylene. The rhizosphere naturally inhabiting the specific PGPR having 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme have the ability to break ethylene precursor ACC, thus reduce the ethylene level in plants under water stressed conditions (Glick 2004; Nadeem *et al.* 2013).

Besides PGPRs some chemicals like glycine betaine, kinetin and salicylic acid are being reported that may increase yield of different crops by ameliorating the stress induced inhibition of plant growth (Khan *et al*. 2003). Plants exhibit a range of defense mechanisms upon experiencing environmental stresses that may also be modified artificially or improved by the exogenous application of chemicals (Raskin 1995; Rajasekaran and Blake 1999). Many commercially available chemical compounds like salicylic acid, proline, amino acids and glycine betaine could be applied as promotors to modify status of plant secondary metabolites and consequently the bioactivity in drought affected plants.

Salicylic acid act as signal molecule and plays a vital role in modifying the plant responses to environmental stresses (Baghizadeh and Rezaei 2011). SA could modulate plant responses against numerous abiotic stress factors such as drought (Larkindale and Knight 2002). Several reports revealed that glycine betaine plays an important role in enhancing of plant tolerance under wide range of abiotic stresses including of drought (Quan *et al*. 2004). Accumulation of organic solutes like proline and glycine betaine help plants for turgor maintenance, strengthening of proteins and membranes to alleviate negative impact of abiotic stresses including salinity, drought and temperature extremes that confer cellular water depletion (Farooq *et al.* 2008a, b). Hence, exogenous application of such chemicals offers an alternative/additional way to genetic engineering for enhancing of crop yield under abiotic stresses (Heuer 2003).

Materials and Methods

Experimental material

Experiment was conducted at the research area of Oil Seed Research Programme, National Agriculture Research Centre, (NARC) Islamabad (33.69°N, 73.03°E and 470 m. a. s. l.), Pakistanduring February–June of the years 2016 and 2017 by reproducing of same layout each year. The type of experimental soil was sandy clay loam, with pH 7.9, EC 0.35 dS/m, 0.82% organic matter. Seed of sunflower hybrid NK-S-278 was obtained from Oil Seed Research Programme, NARC, Islamabad. The inoculants of ACC deaminase rhizobacteria *i.e*., KS7 and KS42 were collected from Soil Microbiology Programme. NARC, Islamabad. While plants were supplemented with foliar spray of salicylic acid and glycine betaine solutions at bud initiation (VS) and flower initiation (FS) stages.

Treatments

The present experiment was laid out in randomized complete block design with split plot arrangement and was replicated three times by maintaining a net plot size of 7 m x 10 m. Two levels of moisture regimes and various combinations of ACC deaminase rhizobacteria with SA and GB were the experimental treatments randomized in the main and sub plots respectively.

Experimental procedure

Prior to seed bed preparation for planting, field was well presoaked by applying 10 cm irrigation when soil reached to optimum workable moisture level. Seed bed was prepared by ploughing with cultivators 2–3 times followed by planking after each cultivation. The planting was performed with the help of dibbler by putting two seeds per hill at the rate of 8 kg ha^{-1}. The row to row distance of 75 cm and plant to plant distance of 25 cm was maintained. After complete emergence at four leaf stage thinning was performed and one plant per hill was maintained. Two moisture regimes *i.e*., M0 = irrigated regime (no water stress) and M1= rainfed regime (water stress) were maintained. Soil moisture was monitored using Time Domain Reflectometry (TDR) on weekly basis in both moisture regimes. The water was applied to irrigation regime in a measured amount with the help of cut-throat flume by using of formula prescribed by Buland *et al*. (1994):

$$QT = AD$$

In equation, Q represents discharge rate from flume, T for time, A for area to be irrigated and D indicates depth of irrigation water applied.

Four irrigations were applied according to crop requirements in irrigated regime and 300 mm (1=75 mm), while rainfed regime was not irrigated to maintain two different soil moisture regimes in the field. The inoculants of ACC deaminase rhizobacteria were used as seed coating. Plants were supplemented with foliar spray of 0.724 m*M* salicylic acid and 100 m*M* glycine betaine solutions at bud initiation (VS) and flower initiation (FS) stages, respectively while control treatments received distilled water only. Measured quantity of salicylic acid was added in beaker containing 200 mL water and dissolved on magnetic stirrer hot plate at 160^{o}C for 1 h. The solution was transferred to volumetric flask and 1L volume was made with distilled water. For glycine betaine, weighted amount of glycine betaine was added to graduated cylinder and final volume of 1L was prepared in volumetric flask with distilled water. Recommended doses of fertilizers *i.e.,* 150 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 50 kg K ha^{-1} were applied. Nitrogen was applied in the form of urea and DAP, phosphorus in the form of DAP while potassium in the form of K_2SO_4. Half of the nitrogen, whole phosphorus and potassium were applied at sowing, while remaining half dose of nitrogen was applied with first irrigation. Weeds were kept under control by hoeing throughout the life cycle of crop. Plant protection measures were applied as and when required to keep crop free from insects and diseases. Chlorpyrefos and Radomil Gold were sprayed to control whitefly and head rot respectively. The meteorological data for the growth period of crop during two years 2016 and 2017 was collected from the National Agro Met Observatory of NARC located near the experimental site. During 2016, low rainfall of (36.72 mm) and in traces (3.79 mm) was recorded at bud initiation (VS) and flower initiation stage, respectively. However, in year 2017 low rainfall of (8.35 mm) was recorded at flower initiation (FS).

Plant measurements and statistical analysis

Data regarding plant water relations, compatible solutes were recorded after 85 days of sowing. The third leaf from top of the two randomly selected plants from each treatment was used to determine the leaf water potential (ψ) with the help of Scholander pressure chamber by using technique suggested by Scholander *et al*. (1965). For determination of osmotic potential, the same leaves were frozen in a freezer at temperature below -20^{o}C for seven days. After that freezing process leaf material was thawed and to collect cell sap disposable syringe was used. The cell sap extracted was used for determination of osmotic potential with the help of an osmometer (Wescor 5500). Turgor potential was calculated with the help of following formula by taking the difference of osmotic potential (Ψs) and water potential (Ψw) values.

$$(\Psi p) = (\Psi w) - (\Psi s)$$

The leaves were soaked for 16–18 h to determine turgid weight. Then the same leaves were kept in oven for 72 h at 65^{o}C until constant dry weight (DW) was obtained. Relative leaf water content (RLWC) was computed with the help of following formula proposed by (Schonfeld *et al*. 1988) and then averaged.

$$RLWC\ (\%) = (FW\text{-}DW)/\ (TW\text{-}DW)\ x100$$

The ratio between achene yield and water applied was taken as water use efficiency (WUE).

The leaf free proline from fresh leaf sample was determined by using protocol mentioned by Bates *et al*. (1973). The glycine betaine from dry leaf sample was estimated by using procedure given by Grieve and Grattan (1983). From the dried leaf samples, total soluble sugars were extracted and determined by anthrone method of (Riazi *et al*. 1985) as modified by Ibrahim (1999). Plants were harvested on June 23, 2016 and June 25, 2017 at harvesting maturity, respectively to record achene yields. The adjustment in achene yield data was made by considering of moisture content up to 10% and expressed in kg ha^{-1}.

The data regarding selected traits were subjected to analysis of variance using software Statistix version 8.1 and

means were compared by Least Significant Differences (LSD) Test at α=0.05.

Results

Leaf relative water content

Drought stress had negative effects on plant water relations and water use efficiency, but these parameters were considerably ameliorated when crop was grown by seed invigoration of ACC deaminase rhizobacteria and receive exogenous application of SA and GB at vegetative (VS) and flowering stage (FS) during consecutive years *i.e*., 2016 and 2017 (Table 1). The results indicated that in case of irrigated regime (M0), more leaf relative water contents (LRWC) were recorded as compared to rainfed regime (M1) during both years *i.e*., 2016 and 2017 (Table 1). Seed inoculation of rhizobacteria KS7 and KS42 as alone or integrated with salicylic acid and glycine betaine caused significant difference in LRWC over control. Maximum LRWC was recorded from treatment C2P2 followed by C2P1, C1P2 and C0P2, which gave an improvement upto 12, 10, 8, and 7%, respectively over C0P0 (control) having minimum LRWC during 2017 (Table 1). The interaction between moisture regimes (M) and various combinations of rhizobacteria *i.e*. KS7 and KS42 with chemical agents *i.e*. SA and GB (CP), M x CP, had significant effect on LRWC during both years *i.e*. 2016 and 2017 of study (Table 2). During 2016, the maximum LRWC were recorded from M0C2P2 (irrigated regime; seed inoculation with KS42 and foliar spray of chemicals *i.e*., GB at VS and FS stages) followed by M0C2P1, M0C1P2 and M0C0P2 against the minimum in M1C0P0 (rainfed regime; un-inoculated without foliar spray of chemicals *i.e*., SA and GB). However, remaining treatments also caused considerable improvement in LRWC under varied moisture regimes, but it was statistically at par with M0C2P0, M0C1P1 and M0C0P1. While, the effect of treatments M0C1P0 & M1C2P2 and M0C0P0 & M1C2P1 was found similar and statistically non-significant. In year 2017, maximum LRWC was observed in M0C2P2 followed by M0C2P1 and M0C1P2, but both produced similar and statistically non-significant effect. The rest of the treatments also caused improvement in LRWC against M1C0P0 which gave minimum RLWC, but was at par with M1C1P0.

Leaf water and osmotic potential

The results of leaf water and osmotic potential showed that more leaf water potential and less negative leaf osmotic potential values were recorded from irrigated regime, whereas more negative values of leaf osmotic potential and low leaf water potential values were found in case of rainfed regime (Table 1). Seed invigoration of ACC deaminase rhizobacteria alone or in supplementation with exogenous application of chemicals *i.e*. SA and GB significantly ameliorated leaf water potential and osmotic potential during both years *i.e*., 2016 and 2017 (Table 1). In year

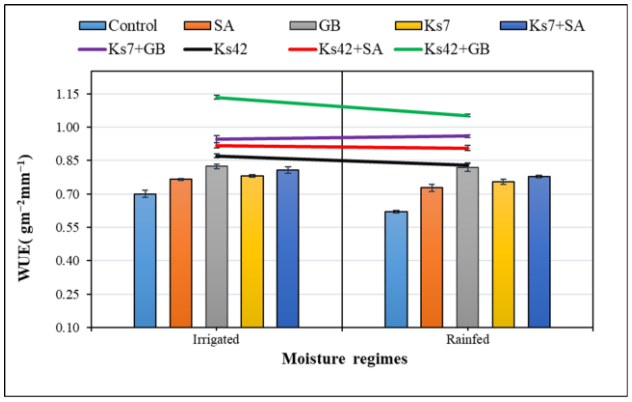

Fig. 1: Effect of seed bio-invigoration of rhizobacteria combined with exogenous SA and GB application on water use efficiency (WUE) of sunflower. Different color bars/lines indicating the effect of various treatments on (WUE)

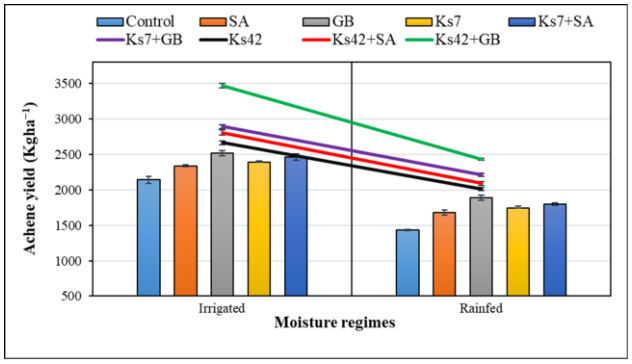

Fig. 2: Effect of seed bio-invigoration of rhizobacteria combined with exogenous SA and GB application on achene yield of sunflower. Different color bars/lines indicating the effect of various treatments on achene yield

2016, maximum improvement in leaf water potential was observed in C2P2 (where seed inoculation of KS42 with exogenous application of GB was practiced at bud and flower initiation stage) followed by C2P1, C1P2 and C0P2, against control C0P0 (un-inoculated and did not receive foliar spray of chemicals. In 2017, same trend of improvement in leaf water potential was caused by various treatment combinations as it was observed produced during 2016. The maximum improvement in leaf water potential and in leaf osmotic potential observed in C2P2 against minimum in case of control C0P0 (un-inoculated and did not receive foliar spray of chemicals *i.e*., SA and GB at bud and flower initiation stage). The interaction between two factors, M × CP, caused pronounced effect on leaf water potential during both years *i.e*., 2016 and 2017 (Table 2). In year 2016, maximum leaf water potential was recorded from M0C2P2 (irrigated regime; seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at bud and flower initiation stages) followed by M0C2P1, M0C1P2, M0C0P2 and M0C2P0, which gave statistically at par effect on

Table 1: Effect of seed bio-invigoration of rhizobacteria combined with exogenously applied SA and GB on plant water relations and water use efficiency (WUE) of sunflower under varied moisture regimes

	RWC (%)		Water Potential (−MPa)		Osmotic Potential (−MPa)		Turgor Pressure (MPa)		WUE (kg m^{-3})	
Treatments	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
M0	82.17 a	81.04 a	0.859 b	0.916 b	1.319 b	1.36 b	0.46 a	0.444 a	0.88 a	0.84 a
M1	74.03 b	72.85 b	1.054 a	1.107 a	1.447 a	1.471 a	0.393 b	0.364 b	0.87 b	0.79 b
LSD (0.05)	**1.091**	**0.888**	**0.052**	**0.064**	**0.049**	**0.044**	**0.028**	**0.022**	**0.011**	**0.027**
C0P0	74.17 i	73.37 g	1.055 a	1.08 a	1.328 h	1.342 g	0.273 i	0.262 g	0.68 h	0.64 i
C1P0	75.40 h	73.38 g	1.01 b	1.062 ab	1.362 f	1.393 f	0.352 h	0.332 f	0.78 g	0.71 h
C2P0	77.93 e	76.62 e	0.94 d	1.012 c	1.372 e	1.417 d	0.432 e	0.405 e	0.85 d	0.79 e
C0P1	76.28 g	74.98 f	0.982 c	1.055 b	1.35 g	1.395 ef	0.368 g	0.34 f	0.80 f	0.73 g
C1P1	77.30 f	76.00 e	0.992 c	1.012 c	1.383 d	1.405 e	0.392 f	0.393 e	0.83 e	0.76 f
C2P1	81.20 b	80.88 b	0.89 f	0.957 e	1.415 b	1.44 b	0.525 b	0.483 b	0.98 b	0.92 b
C0P2	79.02 d	77.68 d	0.928 de	0.995 cd	1.39 cd	1.425 cd	0.462 d	0.43 d	0.86 d	0.84 d
C1P2	80.03 c	79.27 c	0.913 e	0.985 d	1.397 c	1.433 bc	0.483 c	0.448 c	0.94 c	0.88 c
C2P2	82.95 a	81.82 a	0.877 f	0.923 f	1.427 a	1.47 a	0.55 a	0.547 a	1.11 a	1.07 a
LSD (0.05)	**0.535**	**0.876**	**0.017**	**0.018**	**0.014**	**0.010**	**0.013**	**0.012**	**0.016**	**0.014**

M0 = Irrigated (no water stress), M1 = Rainfed (water stress), C0P0 = Control (un-inoculated and did not receive foliar spray of chemicals *i.e*., SA and GB), C1P0 = Foliar spray of 0.724 m*M* SA at bud initiation (VS) and flowering initiation (FS) stage, C2P0 = Foliar spray of 100 m*M* GB at VS and FS stage, C0P1 = Seed inoculation with KS7, C1P1 = Seed inoculation of KS7 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P1 = Seed inoculation of KS7 with foliar spray of 100 m*M* GB at VS and FS stage, C0P2 = Seed inoculation with KS42, C1P2 = Seed inoculation of KS42 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P2 = Seed inoculation of KS42 with foliar spray of 100 m*M* GB at VS and FS stage, WUE = Water use efficiency, LSD = Least significant difference. Values sharing same letters in columns are statically non-significant at $P = 0.05$

Table 2: Interactive Effect of seed bio-invigoration of rhizobacteria combined with exogenously applied SA and GB on plant water relations and WUE of sunflower under varied moisture regimes

	RWC (%)		Water Potential (−MPa)		Osmotic Potential (−MPa)		Turgor Potential (MPa)		WUE (kg m^{-3})	
Treatments	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
M0C0P0	78.00 hi	77.03 g	0.967 e	0.987 e	1.283 h	1.283 h	0.307 k	0.297 i	0.71 l	0.68 kl
M0C1P0	79.30 g	77.13 g	0.907 f	0.953 f	1.333 g	1.333 g	0.38 ghi	0.38 f	0.79 jk	0.74 ij
M0C2P0	82.47 e	81.53de	0.82 g	0.91 g	1.367 e	1.367 e	0.493 d	0.457 d	0.84 gh	0.80 gh
M0C0P1	80.77 f	79.80 f	0.887 fg	0.947 f	1.327 g	1.327 g	0.39 gh	0.38 f	0.80 jk	0.76 i
M0C1P1	81.83 e	80.90ef	0.883 fg	0.89 gh	1.35 f	1.35 f	0.43 ef	0.46 d	0.83 hi	0.79 h
M0C2P1	85.20 b	84.20 b	0.807 g	0.867 hi	1.383 e	1.383 e	0.543 a	0.517 b	0.97 d	0.93 c
M0C0P2	83.57 d	82.57cd	0.807 g	0.897 g	1.367 e	1.367 e	0.513 bc	0.47 d	0.89 f	0.85 ef
M0C1P2	84.33 c	83.33bc	0.813 g	0.89 gh	1.38 e	1.38 e	0.523 bc	0.49 c	0.94 e	0.90 d
M0C2P2	86.83 a	85.87 a	0.797 g	0.857 i	1.407 d	1.407 d	0.56 a	0.55 a	1.14 a	1.13 a
M1C0P0	70.33 n	69.70 l	1.143 a	1.173 a	1.383 f	1.4 de	0.24 l	0.227 i	0.65 m	0.60 m
M1C1P0	71.50 m	69.63 l	1.113 b	1.17 a	1.437 d	1.453 c	0.323 k	0.283 i	0.78 k	0.67 l
M1C2P0	73.40 l	71.70 ij	1.06 c	1.113 b	1.43 de	1.467 c	0.37 hi	0.353 g	0.87 fg	0.77 hi
M1C0P1	71.80 m	70.17 kl	1.077 b	1.163 ab	1.423 e	1.463 c	0.347 j	0.3 i	0.81 ij	0.70 k
M1C1P1	72.77 l	71.10 jk	1.1 b	1.133 b	1.453 c	1.46 c	0.353 ij	0.327 h	0.83 hi	0.73 j
M1C2P1	77.20 i	77.57 g	0.973 e	1.047 d	1.48 b	1.497 b	0.507 cd	0.45 d	1.00 c	0.91 cd
M1C0P2	74.47 k	72.80 i	1.05 c	1.093 c	1.46 c	1.483 b	0.41 fg	0.39 ef	0.83 hi	0.83 fg
M1C1P2	75.73 j	75.20 h	1.013 d	1.08 c	1.457 c	1.487 b	0.443 e	0.407 e	0.95 de	0.87 e
M1C2P2	79.07 gh	77.77 g	0.957 e	0.99 e	1.497 a	1.533 a	0.54 ab	0.543 a	1.09 b	1.01 b
LSD (0.05)	**0.756**	**1.238**	**0.024**	**0.026**	**0.012**	**0.015**	**0.019**	**0.018**	**0.023**	**0.021**

M0 = Irrigated (no water stress), M1 = Rainfed (water stress), C0P0 = Control (un-inoculated and did not receive foliar spray of chemicals i.e. SA and GB), C1P0 = Foliar spray of 0.724 m*M* SA at bud initiation (VS) and flowering initiation (FS) stage, C2P0 = Foliar spray of 100 m*M* GB at VS and FS stage, C0P1 = Seed inoculation with KS7, C1P1 = Seed inoculation of KS7 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P1 = Seed inoculation of KS7 with foliar spray of 100 m*M* GB at VS and FS stage, C0P2 = Seed inoculation with KS42, C1P2 = Seed inoculation of KS42 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P2 = Seed inoculation of KS42 with foliar spray of 100 m*M* GB at VS and FS stage, WUE = Water use efficiency, LSD = Least significant difference. Values sharing same letters in columns are statically non-significant at $P = 0.05$

LRWC. Amongst, the rest of treatments M0C0P1 and M0C1P1 gave statistically at par results, but it was similar and statistically non-significant to M0C1P0 under irrigated regime. The leaf water potential observed from M0C0P0 under irrigated regime was found statistically at par with M1C2P1 and M1C2P2 under rainfed regime.

A minimum leaf potential was recorded from control M1C0P0 (rainfed regime; un-inoculated and without exogenous application of chemicals *i.e*., SA and GB at bud and flowering initiation stage). In 2017, maximum leaf water potential was recorded from M0C2P2 (irrigated regime; seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at bud and flower initiation stage) followed by M0C2P1, but the effect of both was found statistically non-significant. The rest of the treatments (M0C1P2 & M0C1P1; M0C0P2 &M0C2P0 and M0C0P1 & M0C1P0) gave statistically at par results. While Leaf water potential recorded from M0C0P0 (irrigated regime; un-inoculated and not receive exogenous application of chemicals *i.e*., SA and GB) and M1C2P2 (rainfed regime; where seed was inoculated with KS42 and also supplemented with foliar spray of chemicals *i.e*., GB) were found statistically at par. The other treatments also produced statistically significant effect against M1C0P0, which gave minimum leaf potential, but it was found statistically at par to M1C1P0. The interaction between two factors, M x CP, gave pronounced effect on leaf osmotic potential during both years *i.e*., 2016 and 2017 (Table 2). The results of

osmotic potential in response to seed invigoration of ACC deaminase rhizobacteria with SA and GB foliar spray at VS and FS stage illustrated that more negative leaf osmotic potential was recorded from rainfed regime as compared to irrigated regime. In year 2016, the maximum negative leaf osmotic potential was recorded from M1C2P2 (rainfed regime, seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at bud and flower initiation stage) followed by M1C2P1, whereas other treatment combinations M1C0P2, M1C2P2 and M1C1P1 caused produced statistically at par results of leaf osmotic potential. The effect of M1C1P0 (rainfed regime; un-inoculated and receive foliar spray of SA) and M0C2P2 (irrigated regime; seed inoculation of KS42 with foliar spray of GB) was found statistically at par. Among rest of the treatments M1C0P1, M0C1P2, M0C0P2, M0C2P1 and M0C2P0 gave statistically at par results. The effect of M1C0P0 and M0C1P1 on leaf osmotic potential was found also statistically at par. The less negative leaf osmotic potential was recorded from M0C0P0 (irrigated regime; un-inoculated and did not receive foliar spray of chemicals *i.e*. SA and GB. In 2017, maximum negative leaf osmotic potential was caused by M1C2P2 (rainfed regime, seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at bud and flower initiation stages) followed by M1C2P1, M1C1P2 and M1C0P2, while their effect was found statistically at par. Amongst, the other treatments M1C1P1, M1C0P1, M1C2P0 and M1C1P0 produced statistically at par results of leaf osmotic potential. The rest of treatments M0C1P2, M0C0P2, M0C2P1 and M0C2P0 produced statistically at par results against M0C0P1 and M0C1P1 and M0C0P0 produced minimum negative leaf osmotic potential.

Leaf turgor potential

Results of leaf turgor potential revealed that varied moisture regimes caused pronounced effect on turgor potential (Table 1). Overall, maximum values of leaf turgor potential were resulted from irrigated regime compared with rainfed regime, which appreciably reduced turgor pressure. Various treatments of rhizobacteria as alone or integrated with salicylic acid and glycine betaine caused significant difference in leaf turgor potential over control (Table 1). In year 2016, the highest value of leaf turgor potential was caused by C2P2 (seed inoculated with KS42 and foliar spray of chemicals *i.e*., GB at VS and FS) followed by C2P1, C1P2 and C0P2 gave an increment of 101, 92, 77 and 69%, respectively. While minimum leaf turgor potential was recorded from C0P0 (when crop was grown without inoculation of rhizobacteria *i.e*., KS7 or KS42 and did not receive exogenous of chemical agents *i.e*., GB and SA at VS and FS stage. In 2017, maximum leaf turgor potential was recorded from C2P2 when seed inoculated with KS42 and foliar spray of chemicals *i.e*., GB at VS and FS followed by C2P1, C1P2 and C0P2 gave an increment of 109, 84, 71 and 64%, respectively over control. Minimum leaf turgor potential was caused by C0P0 (when crop was grown without rhizobacteria inoculation *i.e*., KS7 or KS42 and did not receive any foliar spray of chemicals *i.e*., SA and GB at VS and FS stage). The interaction between moisture regimes (M) and various combinations of rhizobacteria *i.e*., KS7 and KS42 with chemical agents *i.e*. SA and GB (CP), M x CP, appreciably affected leaf turgor potential during consecutive years *i.e*., 2016 and 2017 (Table 2). In year 2106, the highest value of turgor potential was recorded from M0C2P2 (irrigated regime; seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at VS and FS) followed by M0C2P1 which caused statistically at par results, but it was similar and non-significant to M1C2P2 (rainfed regime; seed inoculation of KS42 with exogenous spay of SA and GB at VS and FS). However, M0C1P2 and M0C0P2 gave statistically at par results of leaf turgor potential. The rest of treatments M1C2P1& M0C2P0; M1C1P2 & M0C1P1 also caused significant effect which were similar and statistically non-significant followed by M1C2P0 and M1C0P2, while M0C1P0 and M0C0P1 produced statistically non-significant results followed by M1C1P1 which was similar and non-significant to M1×C0P1. The leaf turgor potential with M1C1P0 and M0C0P0 was found statistically at par. Minimum leaf turgor potential was recorded from M1C0P0 (rainfed regime; without rhizobacteria inoculation and not receive foliar spray of chemicals *i.e*., chemicals *i.e*., SA and GB). In 2017, maximum turgor pressure was recorded from M0×C2P2 (Irrigated regime; seed inoculation of KS42 with exogenous application of GB) followed by M1C2P2 (rainfed regime; seed inoculation of KS42 with foliar spray of chemicals *i.e*., GB at VS and FS) which were found statistically at par. Amongst, other treatments M0C2P1 gave significant effect on leaf turgor potential followed by M0C1P2 which was statistically at par with M0C0P2, M1C2P1and M0C1P1, but it produced similar results to M1C1P2 which statistically non-significant to M1C0P2.The treatment combination M0C0P1 and M0C1P0 also produced statistically at par results. The less increment in leaf turgor potential was recorded from M1C1P0 and M1C0P1, but both were statistically at par to M1C0P0 (rainfed regime; without rhizobacteria inoculation and not receive foliar spray of chemicals *i.e*., Chemicals *i.e*., SA and GB at VS and FS which gave minimum leaf turgor potential.

Water use efficiency

The results of water use efficiency (WUE) illustrated that maximum water use efficiency was recorded from irrigated regime (M0) as compared with rainfed regime (M1), which caused a considerable reduction in water use efficiency during consecutive years *i.e*., 2016 and 2017 (Table 1). Seed inoculation of rhizobacteria in combination with foliar spray caused significant differences in WUE during consecutive years *i.e*., 2016 and 2017 (Table 1). During 2016, maximum

WUE was caused by C2P2 (66%) when crop was grown with seed inoculated KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS followed by C2P1 (44%), C1P2 (38%) and C0P2 (29%) which gave statistically at par effect with C2P0. Minimum WUE was recorded from C0P0 when crop was grown without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS stage (Fig. 1). In 2017, almost the same trend was found, where maximum WUE resulted from C2P2 (67%) followed by C2P1 (44%), C1P2 (38%) and C0P2 (31%), while minimum WUE resulted from C0P0 when crop was grown without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS stage. The interactive effect between moisture regimes (M) and various combinations of rhizobacteria *i.e.*, KS7 and KS42 with foliar spray of chemicals *i.e*., SA and GB (CP), M x CP, on WUE was found significant during consecutive years *i.e*., 2016 and 2017 (Table 2). During 2016, Maximum WUE was produced by M0C2P2 (irrigated regime; combination of KS42 with foliar spray of chemicals *i.e*., GB) followed by M1C2P2 and M1C2P1 (rainfed regime; combination of KS42 and KS7 with foliar spray of chemicals *i.e*., GB). Minimum WUE was caused by M0COP0 (irrigated regime: without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS). Amongst, other treatments M0C2P1& M1C1P2 and M0C0P2, M1C2P0 produced statistically non-significant results, while M0C1P1, M1C1P1 and M1C0P2 caused statistically at par results. In 2017, the same trend was resulted from various combinations of rhizobacteria and chemicals as was observed during 2016. Maximum WUE was produced by M0C2P2 (irrigated regime; combination of KS42 with foliar spray of chemicals *i.e.,* GB), whereas the minimum WUE was caused by M0C0P0 (without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS).

Leaf free proline

The data showed that leaf free proline contents were significantly affected under varied moisture regimes. Maximum free proline content were recorded from rainfed regime as compared to irrigated regime during consecutive years *i.e*., 2016 and 2017 (Table 3). Seed inoculation of rhizobacteria in combination with foliar spray of chemicals *i.e*., SA and GB at VS and FS stage caused significant effect on free proline content during consecutive years *i.e*., 2016 and 2017 (Table 3). During 2016, maximum free proline content were resulted from C2P2 (57%) when crop was grown with seed inoculated KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS followed by C2P1 (50%), C1P2 (49%) and C0P2 (40%), respectively over control. Minimum leaf free proline content were recorded from C0P0 when crop was grown without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS. During 2017, almost the same trend was found, where maximum free proline content resulted from C2P2 (55%) followed by C2P1 (48%), C1P2 (39%) and C0P2 (38%). While, minimum free proline content were recorded from C0P0 when crop when crop was grown without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS stage. The interactive effect of moisture regimes (M) and various combinations of rhizobacteria *i.e*., KS7 and KS42 with foliar spray of chemicals *i.e*., SA and GB (CP), M x CP, on free proline content was found significant during consecutive years *i.e*., 2016 and 2017 (Table 4). During year 2016, Maximum free proline content were produced by M1C2P2 and M1C2P1 (rainfed regime; combination of KS42 and KS7 with foliar spray of chemicals *i.e*., GB and) followed by M1C0P2 and M1C1P2. Contrarily, the minimum free proline content was caused by M0C0P0 (without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*., SA and GB at VS and FS). The rest of the treatments significantly affected leaf free proline contents, but M0C2P1 and M1C2P0 produced significant results of free proline content which were statistically at par. In 2017, the same trend was found, Maximum free proline content were produced by M1C2P2 and M1C2P1 (rainfed regime; combination of KS42 and KS7 with foliar spray of chemicals *i.e*., GB and), whereas the minimum free proline contents were caused by M0C0P0 (without seed inoculation of rhizobacteria and did not receive foliar spray of chemicals *i.e*. SA and GB at VS and FS).

Leaf glycine betaine

The results of leaf glycine betaine exhibited that more leaf glycine betaine contents were recorded from rainfed regime as compared with irrigated regime during consecutive years *i.e*., 2016 and 2017 (Table 3). The leaf glycine betaine was significantly affected when crop was grown with inoculation of rhizobacteria and foliar spray of chemicals *i.e*., SA and GB at VS and FS during consecutive years *i.e.,* 2016 and 2017 (Table 3). During 2016, maximum leaf glycine betaine was caused by C2P2 (43%) when seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS, this increase in glycine betaine was more pronounced with C2P1 (35%), C1P2 (25%) and C0P2 (23%) as compared to C0P0 (without seed inoculation of rhizobacteria *i.e.*, KS7 and KS42 and foliar spray of chemicals *i.e*., SA and GB at VS and FS which produced minimum leaf glycine betaine, although leaf glycine betaine with C0P1 and C1P1 was found statistically at par. In 2017, the same trend of leaf glycine betaine was caused by various treatment combinations, maximum leaf glycine betaine was caused by C2P2 (44%) where seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS) followed by C2P1 (36%), C1P2 (27%) and C0P2 (25%).

Table 3: Effect of seed bio-invigoration of rhizobacteria combined with exogenously applied SA and GB on compatible solutes and achene yield of sunflower under varied moisture regimes

	Leaf proline content (μmol g^{-1} f. wt.)		Leaf glycine betaine (μmol g^{-1} d. wt.)		Total soluble sugar (mg g^{-1} d. wt.)		Achene yield (kg ha^{-1})	
Treatments	2016	2017	2016	2017	2016	2017	2016	2017
M0	4.33 b	4.46 a	11.34 b	11.47 b	78.40 b	78.80 b	2664 a	2557 a
M1	4.99 a	5.13 b	13.24 a	13.60 a	84.96 a	85.61a	2023 b	1818 b
LSD (0.05)	**0.226**	**0.130**	**0.314**	**0.364**	**1.360**	**1.486**	**48**	**33**
C0P0	3.71 i	3.69 i	10.18 h	10.18 h	77.38 i	77.88 i	1837 h	1734 h
C1P0	4.01 h	4.02 h	11.22 g	11.53 g	79.48 h	80.03 h	2103 g	1912 g
C2P0	4.56 e	4.70 e	12.13 e	12.43 e	81.50 e	82.02 e	2288 e	2120 e
C0P1	4.08 g	4.14 g	11.67 f	11.98 f	80.10 g	80.60 g	2156 fg	1975 fg
C1P1	4.34 f	4.33 f	11.73 f	12.05 f	80.83 f	81.35 f	2220 ef	2041 f
C2P1	5.50 b	5.67 b	13.72 b	13.87 b	83.98 b	84.52 b	2634 b	2471 b
C0P2	5.14 d	5.23 d	12.42 d	12.72 d	82.12 d	82.72 d	2415 d	2261 d
C1P2	5.17 c	5.36 c	12.62 c	12.90 c	82.92 c	83.47 c	2525 c	2372 c
C2P2	5.76 a	5.85 a	14.55 a	14.67 a	85.53 a	86.07 a	2999 a	2897 a
LSD (0.05)	**0.082**	**0.058**	**0.071**	**0.089**	**0.173**	**0.257**	**73**	**67**

M0 = Irrigated (no water stress), M1 = Rainfed (water stress), C0P0 = Control (un-inoculated and did not receive foliar spray of chemicals *i.e.*, SA and GB), C1P0 = Foliar spray of 0.724 m*M* SA at bud initiation (VS) and flowering initiation (FS) stage, C2P0 = Foliar spray of 100 m*M* GB at VS and FS stage, C0P1 = Seed inoculation with KS7, C1P1 = Seed inoculation of KS7 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P1 = Seed inoculation of KS7 with foliar spray of 100 m*M* GB at VS and FS stage, C0P2 = Seed inoculation with KS42, C1P2 = Seed inoculation of KS42 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P2 = Seed inoculation of KS42 with foliar spray of 100 m*M* GB at VS and FS stage, WUE = Water use efficiency, LSD = Least significant difference. Values sharing same letters in columns are statically non-significant at $P = 0.05$

Table 4: Interactive Effect of seed bio-invigoration of rhizobacteria combined with exogenously applied SA and GB on compatible solutes and achene yield of sunflower under varied moisture regimes

	Leaf proline content (μmol g^{-1} f. wt.)		Leaf glycine betaine (μmol g^{-1} d. wt.)		Total soluble sugar (mg g^{-1} d. wt.)		Achene yield (Kg ha^{-1})	
Treatments	2016	2017	2016	2017	2016	2017	2016	2017
M0COP0	3.50 n	3.47 m	9.90 o	9.83 o	73.33 q	73.70 q	2183 g	2095 gh
M0C1P0	3.69 lm	3.71 m	10.33 n	10.47 n	76.37 p	76.80 p	2403 f	2274 f
M0C2P0	3.99 jk	4.23 j	11.17 k	11.27 k	78.33 m	78.80 m	2578 d	2456 d
M0C0P1	3.86 m	3.88 l	10.53 m	10.70 m	76.83 o	77.23 o	2446 ef	2332 ef
M0C1P1	3.93 k	3.94 k	10.67 l	10.83 l	77.77 n	78.17 n	2527 de	2405 de
M0C2P1	5.00 f	5.20 f	12.23 g	12.40 h	80.17 j	80.60 j	2952 b	2831 b
M0C0P2	4.67 hi	4.86 h	11.53 j	11.67 j	79.13 l	79.57 l	2713 c	2612 c
M0C1P2	4.80 g	5.01 g	11.73 i	11.83 i	79.67 k	80.07 k	28657 b	2747 b
M0C2P2	5.21 e	5.42 e	13.17 e	13.30 f	81.47 i	81.87 i	3478 a	3456 a
M1COP0	3.92 kl	3.90 kl	10.47 m	10.53 n	81.43 i	82.07 i	1490 l	1374 m
M1C1P0	4.21 j	4.33 j	12.10 h	12.60 g	82.60 h	83.27 h	1804 jk	1550 l
M1C2P0	5.13 f	5.16 fg	13.10 e	13.60 e	84.67 e	85.23 e	1997 h	1783 j
M1C0P1	4.52 i	4.53 i	12.80 f	13.27 f	83.37 g	83.97 g	1865 j	1618 kl
M1C1P1	4.55 gh	4.72 h	12.80 f	13.27 f	83.90 f	84.53 f	1913 hi	1677 jk
M1C2P1	5.78 b	5.98 b	15.20 b	15.33 b	87.80 b	88.43 b	2316 fg	2111 g
M1C0P2	5.38 d	5.60 d	13.30 d	13.77 d	85.10 d	85.87 d	2118 g	1911 i
M1C1P2	5.28 c	5.71 c	13.50 c	13.97 c	86.17 c	86.87 c	2184 g	1997 hi
M1C2P2	6.30 a	6.28 a	15.93 a	16.03 a	89.60 a	90.27 a	2520 def	2336 def
LSD (0.05)	**0.116**	**0.082**	**0.101**	**0.126**	**0.244**	**0.363**	**117**	**107**

M0 = Irrigated (no water stress), M1 = Rainfed (water stress), C0P0 = Control (un-inoculated and did not receive foliar spray of chemicals *i.e.*, SA and GB), C1P0 = Foliar spray of 0.724 m*M* SA at bud initiation (VS) and flowering initiation (FS) stage, C2P0 = Foliar spray of 100 m*M* GB at VS and FS stage, C0P1 = Seed inoculation with KS7, C1P1 = Seed inoculation of KS7 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P1 = Seed inoculation of KS7 with foliar spray of 100 m*M* GB at VS and FS stage, C0P2 = Seed inoculation with KS42, C1P2 = Seed inoculation of KS42 with foliar spray of 0.724 m*M* SA at VS and FS stage, C2P2 = Seed inoculation of KS42 with foliar spray of 100 m*M* GB at VS and FS stage, WUE = Water use efficiency, LSD = Least significant difference. Values sharing same letters in columns are statically non-significant at $P = 0.05$

However, minimum leaf glycine betaine was recorded from C0P0 (without seed inoculation of rhizobacteria i.e., KS7 and KS42 and foliar spray of chemicals i.e. SA and GB at VS and FS). The interaction between varied moisture regimes (M) and various combinations of rhizobacteria with chemical agents (CP), M x CP, had significant effect on leaf glycine betaine during consecutive years i.e., 2016 and 2017 (Table 4). During 2016, the results elucidated that maximum leaf glycine betaine was recoded from M1C2P2 (rainfed regime; seed inoculation of KS42 and foliar spray of chemicals i.e., GB at VS and FS) followed by M1C2P1, M1C1P2 and M1C0P2. The minimum leaf glycine betaine was recorded from M0COP0 (irrigated regime; un-inoculated and did not receive application of SA and GB at VS and FS). The rest of the treatments significantly enhanced leaf glycine betaine as compared to control, but it produced statistically at par results with M1C0P1 and M1C1P1; M0C0P1 and M1C0P0. In 2017, the same trend of leaf glycine betaine was found with various treatment combinations of rhizobacteria and chemical agents, maximum leaf glycine betaine was recoded from M1C2P2 (rainfed regime; seed inoculation of KS42 and foliar spray of chemicals *i.e.*, GB at VS and FS). Whereas, minimum leaf glycine betaine was recorded from M0C0P0 (irrigated regime; un-inoculated and did not receive application of SA and GB at VS and FS).

Leaf total soluble sugar

The results of total soluble sugar in response to varied moisture regimes illustrated that higher total soluble sugar was recorded from rainfed regime as compared with irrigated regime during consecutive years *i.e.* 2016 and 2017 (Table 3). The total soluble sugar was considerably affected when crop was grown with inoculation of rhizobacteria and foliar spray of chemicals *i.e*., GB and SA at VS and FS during consecutive years *i.e*., 2016 and 2017 (Table 3). During 2016, maximum total soluble sugar was resulted from C2P2 (11%) when seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS), this increase in total soluble sugar was more prominent with C2P1 (8%), C1P2 (7%) and C0P2 (6%) as compared to C0P0 (without seed inoculation of rhizobacteria *i.e*., KS7 and KS42 and foliar spray of chemicals *i.e*., GB and SA at VS and FS) which produced minimum total soluble sugar. In 2017, the same trend of total soluble sugar was caused by various treatment combinations, maximum total soluble sugar was caused by C2P2 (11%) when seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS) followed by C2P1 (9%), C1P2 (7%) and C0P2 (6%), whereas, minimum total soluble sugar was recorded from C0P0 (without seed inoculation of rhizobacteria *i.e*., KS7 and KS42 and foliar spray of chemicals *i.e.*, SA and GB at VS and FS). The interaction between varied moisture regimes (M) and various combinations of rhizobacteria with chemical agents (CP), M x CP, had significant effect on total soluble sugar during consecutive years *i.e*., 2016 and 2017 (Table 4). During 2016, the results indicated that maximum total soluble sugar was recoded from M1C2P2 (rainfed regime; seed inoculation of KS42 and foliar spray of chemicals *i.e*., GB at VS and FS) followed by M1C2P1, M1C1P2 and M1C0P2. The minimum total soluble sugar was recorded from M0COP0 (irrigated regime; un-inoculated and did not receive application of SA and GB at VS and FS). The rest of the treatments significantly enhanced total soluble sugar as compared to control, but it produced statistically at par results with M0C2P2 and M1C0P0. In 2017, the same trend of total soluble sugar was found with various treatment combinations of rhizobacteria and chemical agents, maximum total soluble sugar was recoded from M1C2P2 (rainfed regime; seed inoculation of KS42 and foliar spray of chemicals *i.e*., GB at VS and FS). Whereas, minimum total soluble sugar was recorded from M0C0P0 (irrigated regime; un-inoculated and did not receive application of SA and GB at VS and FS).

Achene yield

The data of achene yield in response to varied moisture regimes are represented in (Table 3). The results revealed that high achene yield was recorded from irrigated regime as compared with rainfed regime which caused a significant reduction in grain yield during consecutive years *i.e*., 2016 and 2017. Various combinations of rhizobacteria with chemical agents caused significant differences in achene yield as indicated in (Table 3). The achene yield was appreciably affected with inoculation of rhizobacteria and foliar spray of chemicals *i.e*., SA and GB at VS and FS during consecutive years *i.e.*, 2016 and 2017. During 2016, maximum achene yield was recorded from C2P2 (65%) seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS), the increase in achene yield was also prominent with other treatments C2P1 (43%), C1P2 (37%) and C0P2 (31%) as compared to C0P0 (without seed inoculation of rhizobacteria *i.e*., KS7 and KS42 and foliar spray of chemicals *i.e*., SA and GB at VS and FS), which caused minimum achene yield (Fig. 2). The effect of C1P0 and C0P1; C2P0 and C1P1 on achene yield was statistically similar and non-significant. In 2017, the same trend of achene yield caused by various treatment combinations, maximum achene yield was caused by C2P2 (67%) when seed inoculated with KS42 and receive foliar spray of chemicals *i.e*., GB at VS and FS) followed by C2P1 (42%), C1P2 (37%) and C0P2 (30%). Conversely, a minimum achene yield was recorded from C0P0 (without seed inoculation of rhizobacteria *i.e*., KS7 and KS42 and foliar spray of chemicals *i.e*., SA and GB at VS and FS). The interactive effect between varied moisture regimes (M) and various combinations of rhizobacteria with chemical agents (CP), M x CP, was found significant on achene yield during consecutive years *i.e.* 2016 and 2017 (Table 4). During 2016, the results illustrated that maximum achene yield was recoded from M0C2P2 (irrigated regime; seed inoculation of KS42 and foliar spray of chemicals *i.e.*, GB at VS and FS) followed by M0C2P1 which produced statistically at par results with M0C1P2. Whereas, minimum achene yield was recorded from M1COP0 (rainfed regime; un-inoculated and did not receive application of SA and GB at VS and FS). The rest of the treatments significantly enhanced achene yield when compared with control, but found statistically at par results with M0C0P0, M1C1P2 and M1C1P2. In 2017, the same trend of total soluble sugar was found with various treatment combinations of rhizobacteria and chemical agents, maximum achene yield was recoded from M0C2P2 (irrigated regime; seed inoculation of KS42 and foliar spray of chemicals *i.e*., GB at VS and FS). Whereas, minimum achene yield was recorded from M1C0P0 (rainfed regime; un-inoculated and did not receive application of SA and GB at VS and FS).

Discussion

In present study, it is obvious from the results that seed inoculation of rhizobacteria combined with exogenous application of chemicals under varied moisture regimes may assist sunflower plants in alleviating adverse effects of drought stress. Leaf relative water contents were significantly reduced under rainfed regime (water stressed) compared to irrigated regime (well-watered). This decrease

in LRWC correspond to the earlier reports that water relations disturbed under water deficient condition elucidated a considerable reduction in RWC under water stressed conditions. The declined leaf water status implies loss of turgor that restrict cell expansion and growth of plants (Farooq *et al.* 2009; Castillo *et al.* 2013), but it was noticeably improved when crop was grown with seed inoculation ACC deaminase rhizobacteria *i.e*., KS7 and KS42 and receive foliar spray of chemicals *i.e*., GB at bud and flower initiation stage. These results are in conformity with earlier illustrated report that inoculation of PGPR and exogenous application of SA and GB acid improve RWC under drought stress, the increase in LRWC under water deficit conditions may be associated to modifications in sensitivity of physiological processes including of stomatal closure, proliferated lateral roots with high density and longer root hairs, which result in increased exchange surface area with soil, and higher water flux from whole root system up to the leaves through amelioration of tissue water status, principally due to enhanced osmotic adjustment in response to accumulation osmolytes (Dodd *et al*. 2010; Kechid *et al*. 2013; Grover *et al*. 2014; Zhang *et al*. 2014; Gontia-Mishra *et al*. 2016; Latif *et al.* 2016; Liu *et al.* 2017).

Crop water relations were adversely affected because of decline in moisture under rainfed regime. Plants promptly respond when exposed to drought stress by lowering of osmotic potential as an adaption strategy to combat water deficit conditions (Subbarao *et al*. 2000) which is attributed to accumulation of solutes in cells for osmotic adjustment (Bray 1997). It is obvious from the results of present study that seed inoculation of rhizobacteria cum exogenous application of SA and GB caused a significant amelioration in leaf osmotic potential, which was more pronounced under rainfed regime. Improvement in turgor potential in response to seed inoculation of rhizobacteria *i.e.*, KS7 and 42 with exogenous application SA and GB might be directly related to enhanced leaf water potential and high negative leaf osmotic potential which helped plants to withstand water deficient conditions. Lowering of leaf osmotic potential by seed inoculation of rhizobacteria with exogenous application of SA and GB might be attributed to accumulation organic solutes like, Proline, GB and total soluble sugars etc., which then ameliorated the osmoregulation ability of crop under water deficit conditions (Farooq *et al.* 2009, 2010; Sandhya *et al*. 2011). Osmotic adjustment is considered as an effective component of drought resistance that assist crop plants under water limited conditions. Osmotic adjustment involves the net accumulation of solutes in a cell in response to a fall in the water potential of the cell's environment, as a consequence, the osmotic potential of the cell is lowered, which gradient for water influx into the cell and tends to maintain turgor pressure. Improved tissue water status may be achieved through osmotic adjustment or changes in cell wall elasticity. This is essential for maintaining physiological activity for extended period of drought. Changes in tissue elasticity in response to drought, which modify the relationship between turgor pressure and cell volume, might contribute to drought tolerance, as observed in sunflower (Kramer and Boyer 1995; Maury *et al*. 2000) and common bean (Zlatev 2005).

Water use efficiency was appreciably reduced under rainfed condition when compared with irrigated regime. Our results are in accordance with the Reza *et al.* (2014) reported that a decline in water use efficiency of sunflower under water deficit conditions. The improvement in WUE in response to seed inoculation of rhizobacteria and foliar spray of chemicals *i.e*., GB might be attributed to increase in yield as same amount of water utilized by all treatments including of control when seed was not inoculated and crop did not receive any foliar spray of chemicals *i.e*., SA and GB. This improvement in WUE by seed inoculation of rhizobacteria and foliar spray of chemicals *i.e*., SA and GB was also previously described by (Belimov *et al*. 2009; Shahbaz *et al.* 2011; Zoppellari *et al*. 2014)

The present study results revealed that leaf free proline, glycine betaine and total soluble sugars were increased when moisture contents declined under rainfed regime. These results comply with (Manivannan *et al*. 2007) reported that a significant increment in free proline, glycine betaine and total soluble sugars in sunflower plants under water stressed conditions. The accumulation of compatible solutes in plants when exposed to water stressed conditions is one of the universal responses that plants exhibit and its role in acclimation of plants is well accredited (Agboma *et al*. 1997; Raymond and Smirnoff 2002). Seed inoculation of rhizobacteria cum exogenous application of chemicals *i.e.* SA and GB enhanced free proline, glycine betaine and total soluble sugars under varied moisture regimes. Our results comply with earlier described reports that rhizobacteria and chemicals *i.e*., SA and GB improved free proline, glycine betaine and sugars in plants when exposed to drought stress (Heidari *et al*. 2012; Naeem *et al*. 2011; Sandhya *et al*. 2011; Dawood and Sadak 2014; Ma *et al*. 2014; Jalaludin *et al*. 2015; Zaidi *et al*. 2015; Gontia-Mishra *et al*. 2016; Tiwari *et al*. 2016; Vurukonda *et al*. 2016). The role of osmolytes accumulated under water stressed conditions might be related to improvement in osmoregulation that mainly assist plants to withstand water deficit conditions through its protective role as a compatible solute and the stabilization of macromolecules which allows root growth and photosynthesis during drought stress (Delauney and Verma 1993; Verbruggen and Hermans 2008; Blum 2011). Amongst, various modifications that plants adapt to withstand drought stress, osmotic adjustment (OA) is considered as basic stress tolerance mechanism which is accomplished through production of various organic solutes (Serraj and Sinclair 2002).

Water deficit caused a significant reduction in achene yield when moisture contents declined under rainfed regime as compared with irrigated regime. Our results are in conformity with Buriro *et al.* (2015) reported that water

stress had severe negative effect on seed yield of sunflower. The limited water supply caused significant decline in yield trait of crops which might be related to impaired gas exchange properties of leaf which not only reduce the size of source and sink tissues but had negative effect on phloem loading, assimilate translocation and dry matter portioning (Farooq *et al*. 2009). However, the seed inoculation of rhizobacteria and chemical agents *i.e.* SA and GB improved achene yield under varied moisture regimes. The improvement in achene yield was more pronounced at rainfed regime as compared with irrigated regime. The results of achene yield obtained in our investigation are in accordance with the earlier illustrated report by (Dey *et al*. 2004; Arshad *et al*. 2008; Arzanesh *et al*. 2011; Ahmad *et al*. 2014; Osman 2015; Noreen *et al*. 2017). This increase in achene yield might be correlated to accumulation of compatible solutes in response to seed inoculation of rhizobacteria and chemical agent's *i.e.*, SA and GB which caused improvement in osmotic adjustment under water deficit condition. Plants accumulate compatible solutes in the cell to lower down osmotic potential which improve water influx into the cell to maintain turgor potential. This osmotic adjustment might have helped plants in bringing about different cell organelles and cytoplasmic activities at normal rate which ultimately improved growth, photosynthesis and assimilate partitioning to grain filling (Ludlow and Muchow 1990; Subbarao *et al*. 2000; Compant *et al*. 2010).

Conclusion

Moisture conditions during rainfed regime caused a significant reduction in plant water relations, WUE and achene yield of sunflower. Nevertheless, various combinations of seed inoculation of ACC deaminase rhizobacteria *i.e*. KS7 and KS42 with exogenous application of chemical agents *i.e*. salicylic acid and glycine betaine appreciably ameliorated the negatively impaired traits under normal and water stressed conditions. While the extent of increment caused over control was more pronounced with treatment combinations KS42+GB, KS7+GB, KS42+SA and KS42. The role of PGPR with chemical agents might be further explored by investigation of key enzymes and gene expression involved in metabolism and their relationship to drought tolerance in plants inoculated with ACC deaminase rhizobacteria KS7 and KS42 and also receive foliar spray of chemicals *i.e*. SA and GB could provide key insights for induction of drought tolerance in sunflower.

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