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EFFECT OF *AZOSPIRILLUM* INOCULATION ON MAIZE (*ZEA MAYS* L.) UNDER DROUGHT STRESS

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Abstract

Azospirillum strains isolated from water stressed conditions can mitigate drought effects when used as inoculants. In this context, the research was designed to study the effects of *Azospirillum lipoferum* strain (Accession no. GQ255950) inoculation on biochemical attributes and growth of maize plant under drought stress. Effect of seed inoculation and rhizosphere inoculation were studied in two varieties of maize, which were subjected to drought stress at vegetative stage. Water deficiency affected accumulation of free amino acids, soluble sugars, proline and soluble protein contents. However, seed inoculated plants had an increased accumulation of 54.54 percent and 63.15 percent free amino acids and soluble sugars respectively, while rhizosphere inoculated plants showed 45.45 percent increase in free amino acids and 31.57 percent increase in soluble sugars as compared to control. The concentrations of soluble proteins on the contrary decreased in the similar order. The plants growth aspect i.e. shoot and root fresh weight, shoot and root dry weight, shoot length and root length, also showed results in consistence with the biochemical attributes. Thus *Azospirillum* strain showed promising effects and can be a potent inoculant for maize that can help the crop to endure limited water availability.

Introduction

Beneficial rhizobacteria have tremendous potential to facilitate plant growth and productivity, in a number of ways. Another remarkable eminence on the credit of these marvelous creatures is their capability to support plants under stressed environments. When established in soils exposed to abiotic stresses, the populations of rhizobacteria become adapted to such stressed conditions thereby developing tolerance and further they can be isolated to be used as inoculum to support crops grown in correspondingly stressed environments (Sandhya *et al*., 2010, Khan *et al*., 2012). They can protect plants against deleterious effects of different environmental stresses to which crop plants are intermittently exposed, like heavy metals, flooding, salt and drought (Mayak *et al*., 2004). Among such abiotic stresses, drought is becoming more prevalent especially in arid and semi-arid regions of the world, where it sternly influences the crop yields (Sandhya *et al*., 2010, Hamayun *et al*., 2010). Soil water deficit is normally the environmental factor in lots of natural settings that compels the furthermost hold back on plant growth (Wahbi & Sinclair, 2007), one of the key environmental features restricting crop yields. It sways more or less all aspects of plant physiology, biochemistry and growth metabolism (Turner & Kramer, 1983), thereby reducing yield (Li, 2007) most decisively, as sufficient availability of water is very critical to growth and development of plants (Shao *et al*., 2008). Crucial changes in water homeostasis escort to osmotic stress, and are amid primary effects of drought stress. Osmotic adjustment is one among the most frequent acclimatization responses to water deficit that refers to the decreased osmotic potential of the plants by active accumulation of various compatible solutes (Yordanov *et al*., 2000), like amino acids such as proline, betains and sugars (Mohammadkhani & Heidari, 2008) within the cells in higher concentrations without smashing up the normal metabolism (Choluj *et al*., 2008). Production of such osmolytes in surplus quantities helps plants to cope up with drought by maintaining osmotic balance of the cell, thereby protecting them against dehydration by stabilization of membrane and protein structures (Hoekstra *et al*., 2001). Plant microbe interactions intercede to the plant fitness in a variety of ways (Mascher, 2007). Beneficial, symbiotic interactions of plants with microbes can shield plants from biotic and abiotic stresses (Mascher, 2007). Microorganisms have the potential to alter the plant health status and productivity and can elevate crop yield to a remarkable level. The soil microbial communities have definite interactions with plants and can play remarkably important roles in plant growth and development. Microbial strains, isolated from arid or semi arid soils have not been only well adapted to such environments, but also can abet plant mitigate the effects of restricted water availability by improving the plant water status through amplified osmolytes production, when used as inoculants. *Azospirillum* is one such competent genus of rhizobacteria that can bring about incredible outcomes in context of plant growth promotion and augmenting the drought stress tolerance, when segregated from soils with low water content. The genus consists of free-living plant growth-promoting bacteria (PGPR), capable of affecting growth and yield of copious plant species, many of agronomic and ecological significance (Bashan, *et al.*, 2004). Various studies have accounted that maize is able to hold up free living N_2 fixers in its rhizosphere (Estrada de Los Santos *et al.*, 2001; Ding *et al.*, 2005; Naureen *et al.*, 2005; Perin *et al.*, 2006; Mehnaz *et al.*, 2007). Maize being an important cereal crop, ranked third after wheat and rice globally is also facing water scarcity. Syndhya *et al*., (2010) wrap up their study by the maxim that improved biomass and average weight; better water relations and reduced water loss are scrutinized due to seeds being inoculated with PGPR, in contrast to the un-inoculated ones under withheld irrigation. Nitrogen fixed by PGPR in rhizosphere, either symbiotically or asymbiotically (Kang *et al*., 2012), picks

up the plants mechanism for drought resistance, thereby contributing to enhanced growth and development under limited water supply (Zhang *et al*., 2011). Besides nitrogen fixation, *Azospirillum* produces plant growth regulators, the compounds that impetus the early developmental stages of maize, as being the first contact between the microbe and the seeds (Cassan *et al*., 2009). Healthier maize seedlings are brought into being when exposed to osmotic stress following inoculation with live cultures of *Azospirillum* in contrast to control. Further the seedlings demonstrated an improved water status (Casanovas *et al*., 2002). Plant adaptability to drought stress in corn can be looked up by PGPR's such as *Azospirillum*, inoculation that causes raised leaf proline contents (Kandowangko *et al*., 2009), free amino acids and sugars (Sandhya *et al*., 2010). Microbial strains isolated from soils with moisture stressed conditions have even more potential to induce tolerance to host plant, when inoculated (Ilyas *et al*., 2012).

Thus in view of this scenario, the present study was designed to cram what positive effects on plant growth and yield of maize can be observed via osmotic adjustment by means of enhanced osmolyte production through *Azospirillum lipoferum* strain (Accession no. GQ255950) inoculation, a novel strain isolated from arid zone of Punjab.

Materials and Methods

Inoculum preparation: *Azospirillum lipoferum* strain (Accession no. GQ255950) was grown in LB broth medium placed in incubator shaker at 35^{o}C till the desired concentration i.e., 10^7 CFU was achieved (i.e. for five days). The bacterial concentration for inoculums was determined spectrophotometerically to find the CFU. One of the flasks containing inoculums was autoclaved in order to achieve heat killed inoculum. Seeds of two different maize varieties i.e., Islamabad Gold and R.C.P. selected at random were surface sterilized in 0.1% mercuric chloride solution followed by several and thorough washings with distilled water. The sterilized seeds were soaked in the inoculum and heat killed inoculum for 4 hours to inoculate them with *Azospirillum* strain. Rhizosphere inoculation was also carried out during the course of work by the application of inoculum in the close vicinity of roots so as to make possible the direct access of bacteria to the roots to be inoculated, instead of seeds.

Greenhouse experiment: Three to five inoculated seeds were sown into plastic pots having diameter 45 cm and containing 12 kg sterile soil compost. The pots were pre-irrigated to the field capacity before sowing. The Soil compost used has a composition of soil: sand in a ratio of 2:1. For rhizosphere inoculation the inoculum and heat killed inoculum were prepared in the same way as described earlier. Rhizosphere inoculation was carried out by adding 1 ml of inoculum or heat killed inoculum as per requirement of the treatment in the close vicinity of the roots at 15 DAS. Drought was imposed at vegetative stage by holding water so as to maintain the soil moisture content at 15± 1% (i.e. 65 ± 5% by weight of field capacity), whereas the moisture content of well watered plants was maintained at 19±1% (i.e. 85 ± 5% by weight of field capacity). Vegetative stage was selected in particular, to induce drought, because despite of being imperative to the crop, this stage is also critical in terms of successful colonization and establishing interaction between *Azospirillum* strains and maize roots, which, if under the influence of drought fails to prevail can reduce the inoculums efficiency. There were 10 treatments in all. Where, T_0= well watered and un-inoculated, T_1= stressed and un inoculated, T_2= seed inoculated and well watered, T_3= Seeds inoculated with heat killed inoculum & well watered, T_4= Rhizosphere inoculated with *Azospirillum* & well watered, T_5= Rhizosphere inoculated with heat killed inoculum & well watered, T_6= Seeds inoculated with *Azospirillum* & drought exposed, T_7= Seeds inoculated with heat killed inoculum & droght exposed, T_8= Rhizosphere inoculated with *Azospirillum* & drought exposed, T_9= Rhizosphere inoculated with heat killed inoculum & drought exposed.

Relative water content: Pre-weighed fresh leaves were soaked in water and weighed at regular intervals to record the maximum fresh (turgid) weight. Same leaf samples were then oven dried at 65^{0}C for one week to get the dry weight. The ratio of fresh weight and maximum fresh weight was taken as a measure of relative water content, after subtracting dry weight of upper fully developed leaf (Unyayer *et al*., 2005).

Leaf osmotic potential: Leaf osmotic potential was determined, following the method of Garnier and Berger (1985). For the estimation, 0.5-1 g fully expanded young leaves were detached from each plant and frozen in polypropylene tubes for two weeks. Then frozen samples were thawed and the sap was extracted by crushing with a metal rod. The sap was centrifuged at 8000 x g for four min to be used for the estimation of osmotic potential by a vapor pressure osmometer using Vapro 5500.

Soluble proteins: Soluble proteins were determined spectrophotometrically (Bradford, 1976). 0.5ml of the sample extract was homogenized with 0.5ml distilled water and 3ml of coomassie bio red dye. Absorbance was read at 595nm after five minutes. Soluble protein was calculated as follows:

$$\text{Total protein} = \frac{\text{Absorbance of sample x K value x Dilution factor}}{\text{Weight of fresh tissue}}$$

Free amino acids: Nninhydrin method was used for determination of free amino acids spectrophotometerically (Hamilton & Van Slyke, 1943). The leaf extract was allowed to react with 10% pyridine and 2% Ninhydrin solution, followed by subsequent boiling in water bath for 30 minutes. The reaction mixture was diluted and absorbance recorded at 570nm using spectrophotometer. Free amino acids were calculated using following formula:

$$\text{Total free amino acids} = \frac{\text{Absorbance of sample x Sample volume x Dilution factor}}{\text{Weight of fresh tissue x 1000}}$$

Soluble sugars: Soluble sugars were estimated after the method of Dubois (1951). 0.5g fresh leaf material was added, 80% ethanol and heated at 80°C for one hour in water bath. 0.5 ml of the aliquot was mixed with 18% phenol and left for incubation for one hour at room temperature with a subsequent addition of sulphuric acid. The reaction mixture was finally shaken and absorbance recorded at 490nm. Where,

$$\text{Soluble sugars} = \frac{\text{Absorbance of sample x K value x Dilution factor}}{\text{Weight of fresh tissue}}$$

Proline: For the spectrophotometric determination of proline, the protocols of Bates *et al*., were followed (Bates *et al*., 1973). 0.5g of fresh plant leaf was homogenized in 3% sulfosalicylic acids. The filtrate was treated with Glacial acetic acid and ninhydrin reagent; and boiled for one hour in water bath. The reaction was finally stopped in ice followed by addition of toluene. The absorbance of upper layer was recorded at 520 nm. Total proline was calculated as:

$$\text{Total proline} = \frac{\text{Absorbance of sample x K value x Dilution factor}}{\text{Weight of fresh tissue}}$$

Results and Discussion

Inoculation effects on growth attributes: The effect of plant's inoculation with culture suspension of *A. lipoferum* on growth attributes (Table. 1) had shown to be significant ($p>0.05$) compared to the control plants with both seed as well as seedling inoculation. Results revealed that *A. lipoferum* inoculation resulted in 53.94% and 43.89% increase in shoot length under well watered conditions with seed and rhizosphere inoculation respectively; and 9.7% and 2.69% increase in the plant height was observed when seeds and rhizosphere inoculated with heat killed inoculums. A similar trend was observed in the drought exposed plants, with 43.89% and 35.33% rise in the height with seed and rhizosphere inoculation correspondingly with *A. lipoferum.* The percentage of increase in length remained 8.1%, and 3.2% respectively with heat killed inoculums with the two mode of inoculation. Other growth aspects i.e. root length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight also exhibited the analogous tendency with different mode of inoculation with *A. lipoferum.* Both the maize cultivar responded in a comparable fashion to the bacterial inoculation, its mode of application and imposed drought. The improved growth response of the seedlings receiving *Azospirillum* inoculation treatments signifies enhanced drought tolerance in the host plants (Ilyas *et al*., 2012). There is a wide array of plants with economic importance, that have displayed improved morphology under different abiotic stresses, when treated with rhizobacteria with the capability to enhance plant growth (PGPR). In this context, different genera of *Azospirillum* have been catching researcher's interest on account of remarkable ability to improve plant growth and productivity under various environmental stresses. Inoculation with diverse *A. lipoferum* strains alleviated the plant drought stress by increasing wheat growth and yield (Arzanesh *et al*., 2011). In the present study, however, a novel *Azospirillum* strain, isolated from arid soils was used for inoculation.

Effects on relative water content and leaf osmotic potential: Leaf relative water content (RWC) is very frequently taken as substitute to the measure of plant water status, thereby serving as measure of the metabolic activity level within the tissues (Taiz & Zeiger 2002; Seghatoleslami *et al*., 2008). Relative water content (Fig. 1) of the plants growing under water stress was significantly low in comparison to the well watered plants. A considerable increase in the relative water content was observed with the seed inoculation of bacterial inoculums under both regularly watered as well as stressful plants. Plants inoculated with heat killed inoculum either before sowing or at vegetative stage, followed the same fashion. The two varieties divulged a comparable inclination. The most prolific effects were noticed with seed inoculation. The overall results were more pronounced under well watered conditions. Atteya (2003) reported similar upshots with significantly altered internal water status by decrease in water potential and RWC of corn under drought stress; that consequently lowered down the photosynthetic rate and reduced the final crop yield. Siddique *et al*., (2000) also noticed the same.

Analysis of the datum corresponding leaf osmotic potential under water deficit (Fig. 2), depicted a huge decrease as compared to normally irrigated plants, in both the varieties. The results of *Azospirillum* application were most enhanced in customarily irrigated plants, treated before sowing, over those of plants with imposition of deficit. In general, seed inoculation with live culture proved to be more efficient than rhizosphere being inoculated; and inoculation with autoclaved inoculum. Plants facing deficiency of irrigation water also pursued an almost identical pattern. The two varieties did not show significant differences in terms of alterations in leaf osmotic potential. Decreased water potential in different crops on account of restricted water supply has been reported in different crops (Meek *et al*., 2003).

Effects on compatible solutes

Soluble proteins: Among the incredible responses of plants to abiotic stresses like drought and salinity, is an enhanced production and accumulation of compatible solutes to osmotically adjust themselves (Serraj & Sinclair, 2002). Amid such compatible solutes the impact of soluble proteins is quite patent. Inoculating the maize cultivars with *A. lipoferum* (Fig. 3), demonstrated a decline in the concentration of soluble proteins with increasing water dwindles. There was a significant effect ($p>0.05$) of inoculation with inoculums containing *A. lipoferum* culture on soluble protein contents of 2 maize varieties, in comparison with control plants. Maximum concentration of soluble proteins was observed in the control plants; that were well watered and allowed to establish without inoculation. Results illustrated a

51.83% decline in soluble proteins in drought exposed plants. *A. lipoferum* inoculation mitigated the trend with inoculated seeds resulting in 11.28% decrease in well watered and 15.78% decrease in stressed plants compared to respective controls. Inoculation of plant rhizosphere, consistently proved to be efficient, with 12.54% decreased protein contents in normal and 21.84% decrease in drought exposed plants. Results in consistent trend, were obtained with the application of heat killed inoculums in either mode of inoculation, though not as efficient as the inoculums containing *A. lipoferum*. The difference between the responses of the two varieties was not significant. Under limited water accessibility to the plants, decline in protein synthesis is an important biochemical manifestation, flanked by many others as well, to overcome the injury (Irigoyen *et al*., 1992). Parallel were the findings of Mohammadkhani & Heidari (2008), who establish the reduction of total soluble protein content in roots and leaves of 2 maize varieties, whereby the reduction was proportionate to the drought intensity and drought duration. Decrease in the concentration of soluble proteins is often discerned thrusty plants. A blatant cause seems to be a stern decline in photosynthesis that frequently occurs in drought stress plants. When plants are exposed to stress causing conditions the demonstrate inhibition of starch biosynthesis (Schellenbaum *et al*., 1998, Kidokoro *et al*., 2009). Despite of the report of stress protein production, as an immediate response of the stress, the protein counts gradually decrease as the stress lingers on, due to radical decrease in photosynthesis, moreover on account of unavailability of raw materials for protein synthesis, there occurs a striking decline or even complete termination of the process (Mohammadkhani and Heidari, 2008). Another doable reason behind reduced protein concentration may be protein degradation more rapidly than they are synthesized. The speedy degradation of proteins is a consequence of amplified activity of protease or other catabolic enzymes, that activate drought stress, or else, due to crumbling of proteins owing to toxic effects of reactive oxygen species consequently ending up in reduced protein content; lowered levels of protein are thus a characteristic symptom of oxidative stress and are been experienced times and again in plants facing drought stress (Seel *et al*., 1992; Moran *et al*., 1994). In present study, similar trend was observed, however application of the bacterial inoculum mitigated the effects by causing a considerable decrease in the proportions with which it decreased under stress without inoculating the plants.

Amino acids: In this study an increase in the amount of free amino was encountered with increase in the water shortage to the plants (Fig. 4). There was a significant increase in the amino acids amounts of plants with drought stress in comparison to the control plants. Inoculating the seeds with *A. lipoferum*, caused a significant increase in the level of free amino acids. There was not any considerable difference among the two modes of application and live cultures and heat killed inocula. A tremendous increase in amino acid levels was observed when treated with the bacterium under drought stress. The differences in the application mode and inoculum type under stress, was again not considerable. Likewise, a 2.4 and 2 fold increase in amino acid pool was observed under drought stress condition in two cotton genotypes (Parida *et al*., 2007). The contribution of amino acids for plants under stressful conditions due to restricted water supply; to adjust osmotically, their inner cellular environment is being point up repeatedly. The elevated amounts of amino acids under drought stress have been repoted in crops like sorghum, pepper and wheat (Yadav *et al*., 2005). Amplification of amino acid levels within the cytoplasm are taken as a measure of drought tolerance in plants. The decrease in total soluble proteins is correlated with the high accumulation of free amino acids (Iqbal *et al*., 2011). These larger pools of free amino acids are the outcome of hydrolysis of proteins, that crop up in response to alterations of osmotic adjustment; particularly the breakdown of structural proteins into the constituent amino acids (Iqbal *et al*., 2011). They also serve to mitigate the activity of ROS (reactive oxygen species), excessively produces under drought conditions (Sandhya *et al*., 2010).

Sugars: A colossal increase in soluble sugars accumulated in the plants with restricted water availability was observed in present study. The sets of plants inoculated with the microbial strain made evident a rise in these pre-elevated amounts of soluble sugars (Fig. 5). Differences among the nature of inoculums and inoculation was noticed under well watered conditions, along with a noteworthy bump up from the control. The results were well pronounced in case of droughted plants. Maximum concentrations were found to be displayed with seed inoculations judged against drought imposed plants without any inoculations. Rhizosphere of stressed plants, inoculated with heat killed inoculum illustrated, in contrast the minimal amount of soluble sugars evaluated against stressed plants without inoculation Proportions of soluble sugars mounted up in different plant parts, is elevated in consequence of several abiotic stresses (Prado *et al*., 2000). Two mechanism have been proposed in the literature regarding the protective mechanism behind the feat of soluble proteins in the stress exposed cells wiz during drought stress, the hydroxyl groups of sugars may surrogate for water so as to uphold hydrophilic interaction in membranes and proteins through hydrogen bonding, thus sustain the membrane integrity besides shunning the protein denaturation (Leopold *et al*., 1994). The other postulate is their role as a key factor in vitrification, a distinct biological phenomenon in the scorched cells (Leopold *et al*., 1994; Buitink *et al*., 1998). Soluble sugars are engaged in very intricate roles within the cell under normal as well as stressed conditions. These roles include serving as substrate in biosynthesis processes, energy production, being the products of hydrolytic metabolic pathways, they may also contribute as regulatory signal molecules for metabolic regulation (Sheen *et al*., 1999; Smeekens, 2000; Gibson, 2005,). Their role as osmoprotectant cannot be shorn of, where they stabilize cellular membranes (Hoekstra *et al*., 2001), contribute to cell turgor maintenance; and as regulators of the gene expression (Koch, 1996).

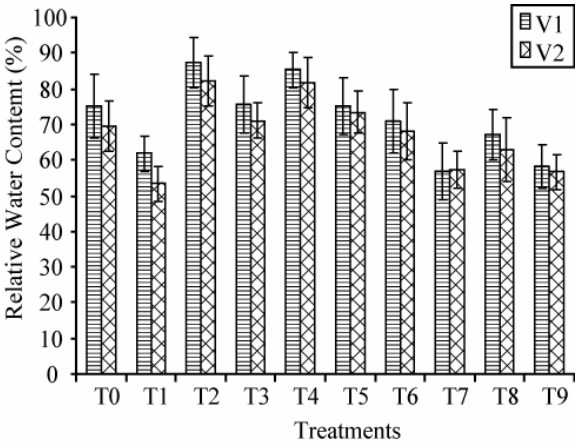

Fig. 1. Effect of *Azospirillum* inoculation on relative water content of two maize varieties under drought stress.

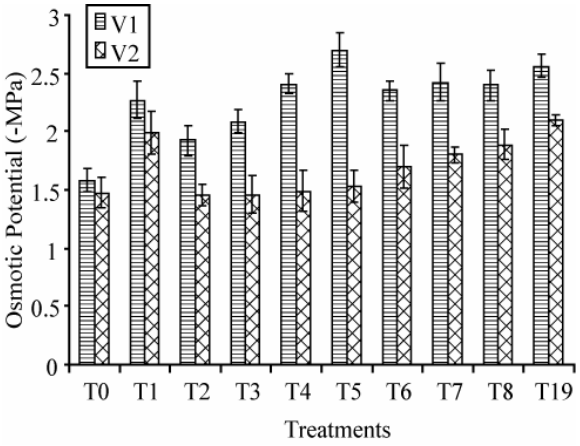

Fig. 2. Effect of *Azospirillum* inoculation on leaf osmotic potential of two maize varieties under drought stress.

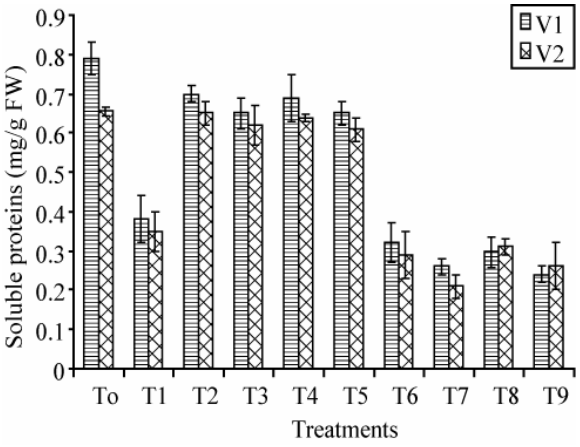

Fig. 3. Effect of *Azospirillum* inoculation on soluble proteins of two maize varieties under drought stress.

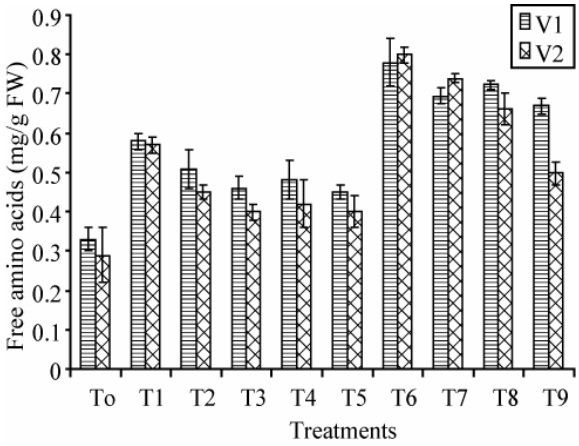

Fig. 4. Effect of *Azospirillum* inoculation on free amino acids of two maize varieties under drought stress.

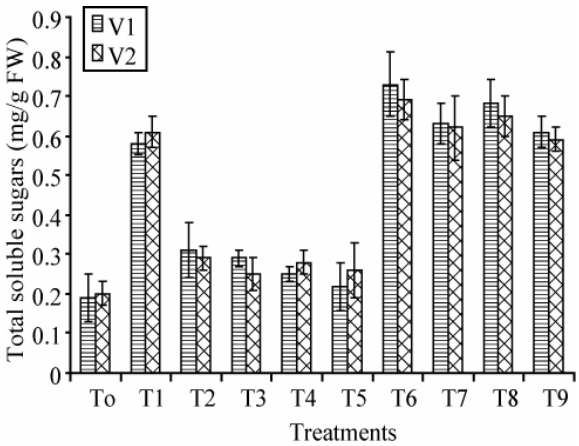

Fig. 5. Effect of *Azospirillum* inoculation on total soluble sugars of two maize varieties under drought stress.

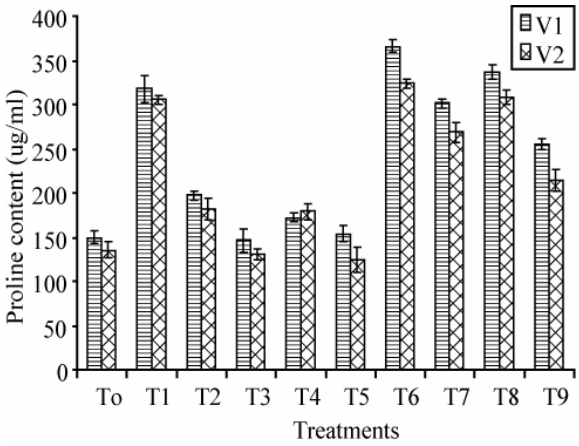

Fig. 6. Effect of *Azospirillum* inoculation on proline content of two maize varieties under drought stress.

Graphs showing the Effect of *Azospirillum* Inoculation on the two Varieties of Maize under Drought Stress.

Where, T_0= well watered and un-inoculated, T_1= stressed and un inoculated, T_2= seed inoculated and well watered, T_3= Seeds inoculated with heat killed inoculum & well watered, T_4= Rhizosphere inoculated with *Azospirillum* & well watered, T_5= Rhizosphere inoculated with heat killed inoculum & well watered, T_6= Seeds inoculated with *Azospirillum* & drought exposed, T_7= Seeds inoculated with heat killed inoculum & droght exposed, T_8= Rhizosphere inoculated with *Azospirillum* & drought exposed, T_9= Rhizosphere inoculated with heat killed inoculum & drought exposed. And V_1= Maize variety, Islamabad Gold; and V_2= maize variety, R.C.P.

Table. 1. Showing the effects of *Azospirillum* inoculation on growth attributes of two maize varieties under drought stress.

	Leaf Count		Shoot length (cm)		Root length (cm)		Shoot fresh wt. (gm)		Shoot dry wt. (gm)		Root fresh wt. (gm)		Root dry wt. (gm)	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
T_0	14 b	14 e	63 c	58.125 e	24 f	21.25 h	101.636 d	92.980 g	44.465 c	33.337 f	6.916 d	6.448 d	1.44 h	1.015 c
T_1	10.25 h	10 c	43 e	42.5 g	34.125 e	31.5 g	85.1917 fg	81.7444 cd	28.911 Fg	26.199 ab	7.024 c	6.816 a	1.5017 g	1.405 b
T_2	15 a	14.75 d	97 a	91.5 c	41.125 cd	38.75 ef	150.06 a	132.167 d	53.674 a	46.625 d	7.292 b	7.148 e	2.567 b	1.87 e
T_3	11.25 e	11.5 h	68 c	50 a	29.875 e	34.75 e	94.2752 ef	82.318 g	39.049 d	30.251 g	6.789 de	6.204 cd	2.033 cd	1.902 fg
T_4	13.5 c	13.75 f	86.75 b	57.5 d	38.25 de	33.75 fg	131.522 b	109.903 e	49.53 b	39.994 e	7.153 b	7.125 e	1.993 de	1.698 gh
T_5	11 g	10.5 b	64.75 d	39.75 g	30.25 e	29.75 g	99.228 d	98.084 g	47.916 c	32.161 f	6.118 f	6.022 f	1.873 fg	1.318 ab
T_6	13.25 d	12.75 g	61.875 c	59.5 e	94.125 a	91.5 c	118.069 c	96.234 f	33.85 de	31.45 gh	7.942 a	7.675 c	2.6523 a	2.35 d
T_7	10.75 g	11b b	46.5 de	48.25 g	62 b	65.25 d	94.595 de	89.722 bc	31.55 ef	29.158 de	7.43 b	7.049 e	2.161 ab	2.091 de
T_8	11.5 f	10.75 a	58.194 d	43 b	85.25 b	49.75 b	107.993 de	76.993 a	31.229 fg	24.56 bc	6.994 d	6.271 b	1.835 ef	1.62 a
T_9	10 h	10.5 c	44.375 e	41.11 h	50.25 c	39.625 a	83.331 g	69.681 b	30.12 g	20.636 g	6.563 e	6.113 b	1.746 g	1.294 B

Where, T_0= well watered and un-inoculated, T_1= stressed and un inoculated, T_2= seed inoculated and well watered, T_3= Seeds inoculated with heat killed inoculum & well watered, T_4= Rhizosphere inoculated with *Azospirillum* & well watered, T_5= Rhizosphere inoculated with heat killed inoculum & well watered, T_6= Seeds inoculated with *Azospirillum* & drought exposed, T_7= Seeds inoculated with heat killed inoculum & droght exposed, T_8= Rhizosphere inoculated with *Azospirillum* & drought exposed, T_9= Rhizosphere inoculated with heat killed inoculum & drought exposed. And V_1= Maize variety, Islamabad Gold; and V_2= maize variety, R.C.P.

Proline: Proline when accumulated in plants serves as an osmoticum and helps plants to maintain their water potential under stress consequently prop up the plant to haul out water from soil (Hanson *et al*., 1979). Loads of material supporting the evidence of proline accumulation and behaving as an effective osmolyte is available. During the course of present study, we arrived at the parallel outcomes. Proline accumulates had a massive boost under stress conditions. The effects were improved with application of *A. lipoferum.* In normally watered plants the proline contents remained higher in inoculums treated plants than that in the non-treated ones (Fig. 6). *Azospirillum* strain facilitated the plants to maintain proline contents at an even higher level in comparison to the drought exposed and untreated plants, with a significant difference among the treatment mode and inoculum type. Such a high accumulation of proline within the cell (up to 80% of the amino acids pool under stress and 5% under normal conditions) may be attributed to increased synthesis and decreased degradation of proline under water and salt stress in various species (Szabados & Savourè, 2009). Under water stress, maize seedlings inoculated with *Azospirillum* accumulated much more proline than the un-inoculated ones (Casanova *et al*., 2002). The proline concentrations increased in stressed plants so as to maintain an osmotic power in plant cells to overcome the detrimental effects of drought (Valentovic *et al*., 2006). This generated an influx of water molecules available in the immediate vicinity of the plant roots. Inoculating the plants with plant growth promoting rhizobacteria adds up to the amounts of accumulating osmolyte. A sizeable increase in its quantity was observed when plants were inoculated with *P. mendocina* (Kohler *et al*., 2008). The role of proline in plants efficient survival under stressed conditions is complicated multifarious. It is most commonly regarded as compatible solute that has the potential to pull in higher amounts of water. Proline may also aid in stabilization of protein structures within the cell. Other benefits on its credits include, enhancing the activity of various enzymes, maintaining pH within the cell and antioxidant activity by scavenging reactive oxygen species (Verbruggen & Hermans, 2008).

Conclusion

The results of the study demonstrate that *Azospirillum lipoferum* strain (Accession no. GQ255950) is well adapted to restricted moisture supply. The selected strain is capable to mitigate the deleterious effects of drought on maize, thereby boosting it up in normal irrigation practices, even more effectively. This particular strain is a potent inoculum for better corn crop in normal as well as drought stress conditions.

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