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Research Article

DROUGHT TOLERANT AZOTOBACTER STRAIN AZT-7 EFFECT ON GROWTH AND DEVELOPMENT OF OKRA SEEDLINGS UNDER DROUGHT STRESS

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A B S T R A C T

The present research was designed to study the effect of *Azotobacter* strain Azt-7 inoculation on growth and development of okra seedlings under drought stress condition. A total of 29 *Azotobacter* spp. were isolated from the rhizosphere soil of different crops covering arid and semi-arid regions. Among 29 strains, Azt-7 exhibited PGP traits such as IAA, ammonia production, phosphate solubilisation and tolerance to drought stress. The strain was identified as *Azotobacter chroococcum* by 16S rRNA sequence analysis and the gene sequence was submitted to GenBank under the accession number KT374218.1. Strain Azt-7 was evaluated for growth promotion of okra under non-stress and drought stress condition. Seeds inoculated with Azt-7 showed better growth in terms of shoot, root length and dry biomass under both the conditions whereas, uninoculated plants showed stunted growth, rolling and wilting of leaves under drought stress. Inoculation improved the accumulation of soluble sugars, amino acids, proline, chlorophyll, and protein content under non-stress and drought stress conditions. The present study suggests the possible role of microorganisms inmitigating adverse effects of climate changes on crop growth and may lead to the development of microbial products to mitigate such effects.

INTRODUCTION

Drought is one of the major abiotic stress affecting the agricultural productivity in arid and semi-arid regions of the world (Ali *et al*., 2014). It affects the plant water relations at cellular as well as whole plant level causing economic losses in agriculture (Sandhya *et al*., 2009). Drought is a major factor that limits the growth of okra. It affects the seedling stage by reducing the root and shoot growth. Drought also lowers photosynthetic activity and nutrient uptake by roots. Research has done to develop drought tolerant varieties through biotechnological approaches, but these methods are time consuming and cost intensive. Plant growth promoting rhizobacteria (PGPR) have tremendous potential to facilitate plant growth and productivity, in a number of ways and another remarkable eminence on the credit of these marvellous microorganisms is their ability to support plants under stressed environments (Qudsia Bano *et al*., 2013). Recent reports suggested the role of microbial mediated tolerance in plants to abiotic stress, such as chilling injury (Ait Barka *et al*., 2006), metal toxicity (Dell Amico *et al*., 2008), drought (Sandhya *et al*., 2009), elevated temperature (Ali *et al*., 2009) and salinity (Chakraborthy *et al*., 2011).

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Recently, umpteen reports have come on *Azotobacter* mediated tolerance in plants to abiotic stresses, such as drought. Treating wheat plants with *Azotobacter chrocoocum*(E1) and *Pseudomonas* sp. (E2) significantly enhanced the root anatomical characters and improved water bio-productivity against water deficit (El-Afry *et al*., 2012). Dual inoculation with *Azotobacter* and *Azospirillum* enhanced maize growth under drought stress compared to uninoculated control (Naseri *et al*., 2013). Further, *Azotobacter* inoculation, enhanced growth of ficus seedlings (Amira and Qados, 2015) and tomato plants under drought stress condition (Viscardi *et al*., 2016). Therefore, in the present study, an attempt was made to isolate drought tolerant *Azotobacter* spp., their molecular characterization and effects on growth and development of okra seedlings under drought stress.

MATERIALS AND METHODS

Isolation of *Azotobacter* spp.

Azotobacter spp. were isolated from rhizosphere soil samples collected from different crop production systems growing under arid and semi-arid regions in India. The plants were uprooted with attached soil, brought to the lab under refrigerated conditions, and immediately processed. Excessive soil from the roots was removed by gentle shaking and root-adhering soil (RAS) was carefully collected and used for isolation of *Azotobacter* spp. by serial dilution spread plate method using Jenson's agar (Hi-media, India) and incubated at

28 ± 2°C for 48-72 h. The pure cultures were maintained on respective agar media slants under refrigerated conditions. Fresh broth cultures of each strain were prepared for further experiments.

Plant growth promoting traits

All the *Azotobacter* strains were tested *in vitro* for PGP traits. For testing ammonia production, culture was raised in 10 ml of peptone water at 28 °C for 2 days, and 1 ml of Nessler's reagent was added. Development of yellow to brown colour indicated production of ammonia (Dey *et al*., 2004). For hydrogen cyanide (HCN) production, the culture was streaked on Jenson's agar and Whatman number 1 filter paper disc soaked in 0.5% picric acid (in 2% sodium carbonate) was placed in the lid of Petri plate. The plates were sealed with Parafilm and incubated at 28 °C for 4 days for the development of deep orange colour (Bakker and Schipper, 1987). For siderophore production, 10 µl of overnight raised culture in Luria broth (LB) (10^8 cfu/104 ml) was spotted on ChromeAzurol S (CAS) agar plates and incubated at 28 °C for 48 – 72 h. Plates were observed for the appearance of an orange halo around the bacterial colony (Schwyn and Neilands, 1987). The method of Gordon and Weber, (1951) was followed for the estimation of IAA. 1 ml of the broth culture, raised in LB (amended with 5 mM tryptophan), was centrifuged and supernatant was carefully decanted in a separate test tube; 4 ml of Salkowsky reagent was added to 1 ml of supernatant and then the mixture was incubated for 1 h at room temperature for the development of pink colour under dark condition. After incubation, the absorbance was read at 530nm. For studying phosphate solubilisation, 5 µl of overnight raised culture (10^8 cfu/ml) was spotted on Pikovskaya's agar plates containing 2% tricalcium phosphate. The plates were incubated at 28 °C for 48 –72 h and observed for the appearance of the solubilisation zone around the bacterial colonies.

Screening for drought stress

In order, to screen the strains for drought stress tolerance, LB broth with differentwater potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa) was prepared by adding appropriate concentrations of Polyethylene glycol 6000 (PEG 6000) (Michel and Kaufmann, 1973; Sandhya *et al*., 2009) and inoculated with overnight grown cultures of the respective bacterium with a cell population of 10^8 cfu/ml (0.1% v/v). Six replicates of each strain and each concentration were prepared. After incubation at 28 °C under shaking conditions (120rpm) for 24 h, growth was estimated by plate counts on LB agar medium. The growth of strains at various water potential levels was recorded.

Molecular characterization

For molecular characterization, bacterial genomic DNA was isolated (Chen and Kuo,1993) and subjected to Polymerase Chain Reaction (PCR) for amplification of 16S rRNAgene using universal forward 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse1492R (5′CGGTTACCTTGTTACGACTT-3′) (Meier *et al*., 2012) primers under standard conditions (initial denaturation at 94 °C for 129 5min, 30 cycles of denaturation at 94 °C for 1min, annealing at 50 °C for 40 s, extension at 72 °C for 90 s, and final extension at 72 °C for7 min). The PCR (approximately 1.5 kb) product was purified and sequenced (Xcelris LabsLtd, India). The 16S rRNA sequence obtained was compared with the existing database and submitted to NCBI GenBank.

Phylogenetic analysis

Sequence analysis of selected strain was performed using Basic Local Alignment Search Tool (BLAST) search tool available on the NCBI homepage (http://www.ncbi.nlm.nih.gov). Homologous gene sequences were collected from the NCBI database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence data were aligned with Clustal W 1.6 and evolutionary tree construction was done by using Molecular Evolutionary Genetics Analysis 6.0 (MEGA6) software (Tamura *et al*., 2013; Rana *et al*., 2014). Tree branches were evaluated using the bootstrap method (Felsenstein, 1985; Islam *et al*., 2015).

Plant growth studies

Red soil (field soil) was used to evaluate the potential of drought-tolerant strain Azt-7 on okra seedlings. Seeds were surface sterilized with 0.1% HgCl2, 70% ethanol and bacterized with overnight grown cultures (10^8cfu/ml) of bacterial strains, shade dried and sown in plastic pots filled with 4 kg of sterile soil. Both inoculated and uninoculated treatments were replicated six times, maintaining three plants per pot. Soil moisture was maintained constantly with sterile distilled water. After 21 days of germination, drought stress was induced in three out of six replicates by discontinuing water. Drought-stressed seedlings and their corresponding non-stressed controls were harvested on the sixth day of exposure to drought.

Plant biochemical parameters

Plant experiments were repeated three times to study the mechanism of protection of seedlings exposed to drought stress. 27-day old seedlings (6 days after exposure to drought) were harvested for analysis. Shoot and root length were measured according to the manual method and dry biomass was recorded after drying the samples at 60 ^oC. Relative water content (RWC) of leaves was determined by recording fresh weight, saturated weight, and dry weight of leaves (Teulat *et al*., 2003). Leaf water loss was determined by recording fresh weight, and the leaves were left to evaporate under room temperature for 2 h and reweighed (Xing *et al*., 2004). Chlorophyll content was estimated by immersing leaf samples in dimethylsulphoxide (DMSO) and incubating them at 70 ^oC for 4 h. The absorbance of the solution was read at 645, 663 and 480 nm (Barnes *et al*., 1992). Free proline content was determined by the method of Bates *et al*. (1973). Leaf samples were homogenized in 3% (w/v) sulphosalicylic acid, centrifuged at 10,000 rpm for 15 min at 4^oC and the supernatant was heated at 100 ^oC after the addition of acidic ninhydrin. The samples were extracted with toluene and the chromophore-containing toluene was aspirated and cooled to room temperature and absorbance was read at 520 nm. The contents of sugars and amino acidswere determined by incubating 1 g of leaf sample with methanol: chloroform: water (60:25:15 v/v) mixture at 60 ^oC for 2 h. The samples were centrifuged at 10,000 rpm for 15min at 4 ^oC and the content of total sugars of the supernatant was estimated by phenolsulfuric acid method (Dubois *et al*., 1956). Total amino acids content was determined by heating 1 ml of the supernatant with 1 ml of 0.1 M acetate buffer and 1 ml of ninhydrin (5%w/v) at 95 ^oC for 5 min. The samples were

cooled and absorbance was read at 570 nm (Chen *et al*., 2007). The protein content was determined by grinding 0.1 g of leaf tissue in phosphate buffer (pH 7.0) and estimated by Bradford method (Bradford, 1976).

Statistical analysis

Each experiment was analyzed with six replicates and the data obtained was analyzed by analysis of variance (ANOVA) and expressed in mean ± SD of six replicates.

RESULTS

Isolation and screening for PGP traits

A total of 29 *Azotobacter* spp. were isolated from ten rhizosphere soil samples collected from different crop production systems grown under arid and semi-arid regions of India. The colonies showing slimy, glistering, watery like appearance were purified and preserved in nutrient agar slants under refrigerated conditions. *In vitro* screening of the strains revealed variations in the production of PGP traits (Table 1).

Screening for drought stress tolerance

All the four strains (Azt-7, Azt-9, Azt-14 and Azt-20) were screened for drought tolerance using LB broth at varying water potential using PEG 6000. Strains Azt-9, Azt-14 and Azt-20 could grow at a water potential of −0.49 MPa with cell population of 2.5 x 10^3, 3.8 x 10^2 and 4.1 x 10^3cfu/ml respectively whereas, strain Azt-7 could grow at a water potential of −0.73 MPa and showed highest cell population of 4.8 x 10^5cfu/ml

Molecular characterization

A BLASTN search was performed for nucleotide sequence of partial length of strain Azt-7; the sequence showed a 100% homology with the 16S rRNA sequence of *Azotobacterchroococcum* strain IAM 12666 (NR 041035.1). The sequence was submitted to GenBank under the accession no. KT374218.1. Dendogram was constructed from the 16S rDNA sequence data (Fig. 1) using neighbour joining method to investigate the relationship of *Azotobacter* strain Azt 7 with NCBI GenBank data.

Table 1 Plant growth promoting traits of *Azotobacter* spp.

Strains	Phosphate solubilisation (µg/ml)	IAA (µg/mg protein)	Siderophore	HCN	Ammonia
Azt-1	12.33 ± 1.15	2.93 ± 0.14	-	-	+++
Azt-2	10.93 ± 0.05	5.55 ± 0.63	-	-	++
Azt-3	10.11 ± 0.12	6.00 ± 0.09	-	-	++
Azt-4	10.76 ± 0.12	1.84 ± 0.17	-	-	++
Azt-5	12.33 ± 0.03	4.11 ± 1.51	-	-	++
Azt-6	12.29 ± 0.67	6.22 ± 0.59	-	-	++
Azt-7	14.57 ± 0.12	8.91 ± 0.42	-	-	+++
Azt-8	11.81 ± 1.81	6.95 ± 0.71	-	-	++
Azt-9	14.01 ± 0.14	7.73 ± 0.41	+	-	++
Azt-10	12.53 ± 0.17	6.25 ± 0.47	-	-	++
Azt-11	10.30 ± 0.22	1.24 ± 0.64	-	-	+
Azt-12	12.99 ± 0.59	5.84 ± 0.24	-	-	++
Azt-13	12.21 ± 0.94	6.08 ± 0.19	-	-	++
Azt-14	13.27 ± 0.63	7.97 ± 0.36	-	-	++
Azt-15	10.08 ± 0.04	5.97 ± 0.09	-	-	+
Azt-16	11.37 ± 0.34	6.31 ± 0.31	-	-	++
Azt-17	10.12 ± 0.23	5.64 ± 0.72	-	-	+
Azt-18	11.94 ± 0.73	5.51 ± 0.22	-	-	++
Azt-19	10.32 ± 0.11	2.68 ± 0.13	-	-	+
Azt-20	13.25 ± 1.45	7.55 ± 0.45	-	-	++
Azt-21	10.43 ± 0.27	3.37 ± 0.39	-	-	+
Azt-22	10.54 ± 0.22	2.44 ± 0.37	-	-	+
Azt-23	12.15 ± 0.92	6.26 ± 0.21	-	-	++
Azt-24	11.85 ± 0.64	6.40 ± 0.71	-	-	+
Azt-25	12.85 ± 0.97	5.55 ± 0.16	-	-	++
Azt-26	11.07 ± 0.06	6.35 ± 0.32	-	-	++
Azt-27	12.77 ± 1.32	5.28 ± 0.63	-	-	++
Azt-28	11.43 ± 1.77	5.33 ± 0.12	-	-	++
Azt-29	12.86 ± 1.78	4.24 ± 0.53	-	-	+

-, absent; +, presence; +, fair; ++, good; +++, excellent. Values are the means of three replicates with ± SD value.

Strain Azt-7 solubilized maximum amount of phosphate (14.57 ± 0.12 µg/ml) followed by Azt-9 (14.01 ± 0.14 µg/ml)Azt-14 (13.27 ± 0.63 µg/ml) and Azt-20 (13.25 ± 1.45 µg/ml). All the strains produced IAAbut, variation was observed among the strains. Strain Azt-7 was the best producer of IAA(8.91 ± 0.42 µg/mg) followed by Azt-14 (7.97 ±0.36 µg/mg), Azt-9 (7.73 ± 0.41 µg/mg) andAzt-20 (7.55 ± 0.45 µg/mg). Siderophore production was observed only in strain Azt-9. Allthe 29 strains were negative for HCN whereas, positive for ammonia production (Table 1).Strains Azt-7, Azt-9, Azt-14 and Azt-20 were selected for further studies.

Azotobacter strain Azt-7 showed close relationship (bootstrap value of 76%) with reference strain *A. chroococcum* strain IAM 1266(NR041035.1) from NCBI database followed by other species. *Pseudomonas putida* strain FBKV2 was used as the out group (Fig. 1) for dendogram construction.

Evaluation of strains for plant growth promotion

The effect of seed inoculation of drought-tolerant strain Azt-7 on the growth of okra seedlings was studied under drought stress and non-stress conditions. Under drought stress condition uninoculated plants started wilting from the sixth day and completely died at the end of eight day.

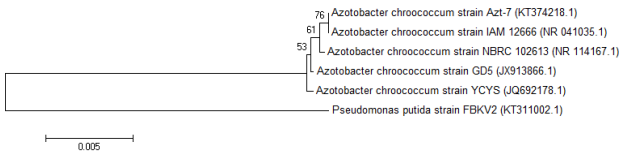

Fig 1 Phylogenetic analysis of Azotobacter spp. based on 16S rRNA gene sequences available from the NCBI Genbank database. Distances and clustering analysis with the neighbour joining method was performed by using the software packages Mega ver. 6.0. Bootstrap values (n=500) are listed as percentages at the branching points.

Strain Azt-7 inoculated plants started wilting from eight day and survivedup to ten days after drought stress. Strain Azt-7 inoculated plants and uninoculated plants showed significance difference in plant growth parameters like root length, shoot length and dry biomass. Inoculated plants showed highest root length (23% higher under non-stress 17.82% higher under drought stress conditions) and shoot length (17.49% higher under nonstress, 19.29% higher under drought stress conditions) (Table 2) than uninoculated plants. Bacterial inoculation resulted in increased total dry biomass (20.94%) under drought stress (26.67%) and non-stressed conditions (Table 2).

Table 2 Root, shoot length and dry mass of okra seedlings inoculated with Azotobacter strain Azt-7 exposed to drought stress and non-stress conditions.

Treatment	Root length (cm)	Shoot length (cm)	Dry biomass (g/plant)
CNS	25.28 ± 1.21	32.14 ± 1.25	0.55 ± 0.079
INS	32.83 ± 1.53	38.91 ± 1.02	0.75 ± 0.065
CDS	21.68 ± 1.69	27.75 ± 1.33	0.34 ± 0.083
IDS	26.38 ± 1.08	34.38 ± 1.29	0.43 ± 0.091

CNS control non-stress, CDS control drought stress, INS inoculated non-stress, IDS inoculated drought stress.
Numerical values are mean ± SD of three replicates with three independent experiments.

Inoculation of okra seedlings with strain Azt-7 significantly enhanced RWC as compared to un-inoculated seedlings under both the conditions. Furthermore, inoculation decreased the leaf water loss under drought stress and non-stress conditions respectively (Table 3). The effect of inoculation on biochemical status of plants was studied under drought stress andnon-stress conditions. Inoculation with strain Azt-7 enhanced biochemical parameters of okra seedlings compared to uninoculated seedlings under both the conditions. Inoculation significantly enhanced chlorophyll, proline, total protein, amino acids and soluble sugars (Table 3). Treatment with strain Azt-7 improved proline content under non-stress and droughtstress (3.92 µ mol/g and 53.60 µ mol/g) conditions compared to uninoculated treatment (2.84µ mol/g and 13.60 µ mol/g). Similarly, inoculation significantly enhanced amino acids content 48.55 µ mol/g (non-stress) and 151.85 µ mol/g (drought stress) over uninoculated seedlings (43.15 µ mol/g (non-stress) and 100.65 µ mol/g (drought stress) respectively. A positive effect of inoculation was also observed on total soluble sugar content as compared to uninoculated control under both the conditions. Inoculation enhanced soluble sugar content under non-stress (32.24 µ mol/g) and drought stress (132.20 µ mol/g) than uninoculated seedlings (Table 3). The positive influence of Azt-7 inoculation was also observed on shootchlorophyll content as compared to uninoculated control under drought stress and non-stressconditions. Bacterial inoculation significantly increased the chlorophyll content (472.69 mg/g drought stress and 485.25 mg/g non-stress) (Table 3). A similar response was also observed in total soluble protein content in Azt-7 inoculated seedlings compared to uninoculated seedlings under both the conditions (Table 3).

Table 3 Effect of Azt-7 inoculation on physiological and biochemical parameters of okra seedling under non-stressed and drought stressed condition.

Treatment	Control		Azt-7 strain	
	NS	DS	NS	DS
Relative water content (%)	14.40 ± 1.41	11.77 ± 0.66	22.45 ± 1.34	15.50 ± 0.71
Leaf water loss (%)	22.60 ± 1.98	13.65 ± 2.33	18.32 ± 1.41	8.31 ± 1.43
Chlorophyll content (mg/g)	326.87 ± 2.28	313.84 ± 3.51	485.25 ± 1.31	472.69 ± 2.22
Proline (µ mol/g)	2.84 ± 0.21	13.60 ± 0.91	3.92 ± 0.15	53.60 ± 1.09
Soluble sugars (µ mol/g)	23.94 ± 1.96	80.50 ± 2.55	32.24 ± 1.53	132.20 ± 2.26
Amino acids (µ mol/g)	43.15 ± 1.20	100.65 ± 2.05	48.55 ± 1.77	151.85 ± 0.35
Protein (mg/g^{-1})	622.61± 1.92	603.53 ± 1.93	630.78 ± 1.93	608.98 ± 1.93

Numerical values are mean ± SD of three replicates with three independent experiments; NS, non-stress; DS, drought stress.

DISCUSSION

Drought stress is one of the major agricultural problems limiting crop productivity (Sandhya *et al*., 2009). Drought is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050 (Kasim *et al*., 2013; Vurukonda *et al*., 2016). Among different strategies to cope with drought stress, seed treatment with drought tolerant bacteria is an easy technique to overcome the drought problem. In the present study, a total of 29 *Azotobacter* spp. were isolated from the rhizosphere soil of different crop production systems grown under arid and semi-arid conditions. Out of 29 strains, four (Azt-7, Azt-9, Azt-14 andAzt-20) could show highest plant growth promoting traits like phosphate solubilisation, ammonia production, IAA and siderophore production. Bacterial plant growth promotion is achieved by more than one PGP trait by the associated bacterium and helps plants tolerate toabiotic stresses (Yang *et al*., 2009). Physiologically most active auxin in plant growth and development is IAA. Various plant species inoculated with IAA-producing bacteria increased root growth and/or enhanced formation of lateral roots and root hairs (Dimkpa *et al*., 2009) thus increasing water and nutrient uptake by plants to cope with drought stress (Egamberdieva and Kucharova, 2009). In our study, strain Azt-7 showed highest IAA production responsible for adventitious root development in okra seedlings, enhancing drought tolerance. The amount of phosphorus in the soil is generally high, most of this phosphorus is insoluble and therefore not available to support plant growth. Solubilization of phosphorus by phosphate-solubilizing bacteria is an important trait (Rodríguez and Fraga, 1999; Richardson, 2001). *Azotobacter* strain Azt-7 showed highest phosphate solubilisation helping plant in better nutrient uptake. Strain Azt-7 also screened for drought tolerance which could tolerate the maximum level of drought stress (-0.73 MPa) and protecting okra seedlings under drought stress. Molecular characterization of the strain was done on the basis of 16SrRNA gene sequence analysis and identified as *Azotobacter chroococcum* and the sequence was submitted to GenBank under the accession number KT374218. Dendogram of *Azotobacter* strain Azt7 showed close relationship with reference strain *A. chroococcum* strain IAM 1266 (NR041035.1) from NCBI database with a bootstrap value of 76%. Inoculation with drought tolerant *Azotobacter* strain Azt-

7 resulted in 23% higher root lengthunder non-stress and 17.82% under drought stress compared to uninoculated plants. In asimilar study, pepper plants inoculated with bacterial strains enhanced the root system up to 40% (Marasco *et al*., 2013). Enhancement in root length thus increases water and nutrientsuptake from soil helping plants to cope with water deficit (Egamberdieva and Kucharova, 2009). Similarly, inoculation also enhanced shoot length and dry biomass under both nonstress and drought stress condition, similar to the reports given by Sandhya *et al*. (2011). Relative water content and leaf water loss decreased under drought stress in both inoculated and uninoculated conditions. However, bacterial inoculation did help plants to maintain their relative water content and leaf water loss during drought periods, similar results found inmaize seedling inoculated with *Pseudomonas* spp. (Sandhya *et al*., 2010).

Many studies revealed that osmotic regulations in plants during abiotic stresses occurred through the accumulation of osmotically active compounds or osmoprotectants (Ranganayakulu *et al*., 2013; Shahbaz *et al*., 2013; Talat *et al*., 2013; Filippou *et al*., 2014;Singh *et al*., 2015). Osmoprotectants such as proline, amino acids, and soluble sugars canstabilize proteins and membranes, and reduce the osmotic potential of membranes to prevent dehydration inside the cell (Wani *et al*., 2013; Singh *et al*., 2015). In the present study under drought stress condition inoculated plants showed significantly higher proline level than uninoculated control. Proline is essential for primary metabolism in the plant. Under stress conditions proline is involved in the maintenance of cell turgor, promoting growth under drought stress condition (Mullet and Whitsitt, 1999). Inoculated plants showed 0.79 foldsmore amino acid content compared to uninoculated drought stressed plants. The accumulation may be due to hydrolysis of protein and may occur in response to changes inosmotic adjustment of their cellular content (Sandhya *et al*., 2010). Soluble sugars also showed the significant difference between uninoculated and inoculated seedlings. Soluble sugars function as osmoprotectants, stabilizing cellular membranes and maintaining turgor (Mohammadkhani and Heidari, 2008). Furthermore, inoculation significantly enhanced chlorophyll content under both non-stress and drought stress conditions respectively. Under drought stress condition protein content was decreased in inoculated and uninoculated plants. However, the decrease was much higher in uninoculated plants compared to inoculated plants, indicating that the protein synthesizing mechanism may have protected by thebacteria. Similar findings were reported in maize seedlings, inoculated with *Pseudomonas* spp. (Sandhya *et al*., 2010).

CONCLUSION

The present study demonstrates that, inoculation with drought tolerant *Azotobacter* strain Azt-7 influenced the biochemical and physiological parameters of okra seedlings and mitigated the drought stress. *Azotobacter* strain Azt-7 isolated in the existing study can be further tested under field conditions and may be developed as bio-inoculant for rain-fedcrops.

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