

Original Research Article

Influence of endophytic bacterial consortia on plant biochemical parameters of maize under moisture stress conditions in vitro

ABSTRACT:

Mitigation strategies based on plant–microbe interactions to increase the performance of plants under water-deficit conditions are well documented. However, little is known about a suitable consortium of bacterial inoculants and underlying physiological and enzymatic events to improve drought tolerance in maize. This study aimed to investigate the synergistic interactions among plant growth-promoting bacteria to alleviate drought-induced damages in maize. In pot culture experiment endophytic bacterial consortium inoculated treatments imposed with moisture stress (75% water holding capacity) with full dose (T9) and 75% recommended dose of fertilizers (T8) were found to excel in many plant biochemical properties when compared to un-inoculated control. For instance chlorophyll stability index T9 (138.67%) T8 (133.33%), relative water content T9 (95.20%), The N, P and K uptake was found significantly higher in T9 with 13.98 g plant⁻¹, 3.38 g plant⁻¹ and 17.29 g plant⁻¹ respectively. Thus, the current research advocates the use of endophytic microbial consortium to mitigate moisture stress and to improve plant biochemical properties which ultimately enhances the plant health and yield.

Key words: Endophytic bacteria, Biochemical properties, WHC, NPK Uptake, Moisture stress

Comment [SL1]: Good and Novel approach
However statistical evidence may be added in the abstract

1. INTRODUCTION:

The climate change has become main source of creating stress for foliar growth and emerged as an alarming threat to natural ecosystems. The environmental stress is simply classified as abiotic and biotic stress based on their nature and source of effect. Sometimes abiotic stress may influence the biotic stress and reduces crop productivity [1]. Among the environmental stresses, moisture stress has major impact on crop growth and productivity throughout the world. By 2050 more than 50% of arable lands are expected to have negative impact on crop growth because of drought [2]. Drought stress has been reported to cause yield reductions of up to 21% in wheat and 40% in maize around the world [3].

A protection system is possessed by plants naturally that can tackle adverse stress conditions, even so, plants also interact with a many microorganisms that can alleviate the stress and protects the plant [4]. Plants are more contingent on microorganisms which are capable to enhance their metabolic activity to combat stress [5]. Upon exposure of plants to hostile conditions microbes can pre sensitize the plant cell metabolism, and so microbial treated plants will respond more quickly than untreated plants [6]. Thus, use of beneficial microbial inoculants as a stress protecting agent for plants may reassure for sustainable and chemical free eco-friendly agriculture.

Plants harbour several microbes, which reside within the cells in intercellular spaces or in vascular system, without affecting the plants are known as endophytes [7]. Bacterial endophytes are beneficial over rhizospheric bacteria as they reside within plant tissue with more intimate contact and they have no competition with rhizosphere microorganisms [8]. Their ability for plant growth and to alleviate the biotic and abiotic stresses is well studied [9,10, 7]. The interaction of plant with endophytic bacteria alleviates the tolerance towards drought stress [11].

Thus an effective endophytic microbial consortia was developed using four compatible endophytic bacterium and their efficiency was investigated in the present study by providing

maize plants with moisture stress under invitro conditions in pot culture and evaluated the influence of inoculated bacterial consortia on different plant biochemical parameters.

2. MATERIALS AND METHODS:

2.1 Location of the experiment: The pot culture experiment was carried out at Regional Agricultural Research Station, Anakapalle, Andhra Pradesh.

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2.2 Soil Preparation: The soil used in this experiment was loamy field soil which was sieved to remove stones and any stubbles to get fine soil. Soil was sterilized at 121°C for 15 minutes at 15 lb pressure and about 12 kg of sterilized soil was filled into each pot.

2.3 Sowing and treatment details: Maize hybrid PIONEER 3396 was selected and sown at the rate of 4 seeds/pot then thinning was done and only 2 plants per pot were maintained

2.4 Plant Biochemical Analysis

The total chlorophyll content was estimated and calculated by the method of Arnon [12] and chlorophyll stability index (CSI) was estimated by using the method of Murthy and Majumdar [13].

$$CSI = \frac{\text{Total chlorophyll content of the treated leaves}}{\text{Total chlorophyll content of untreated leaves}} \times 100$$

Leaf RWC was measured in control and stressed seedlings. Fully expanded leaves were excised and fresh weight (FW) was immediately recorded from control and stressed plants, then the leaves were soaked for four hours in distilled water at room temperature under constant light and turgid weight (TW) was recorded. After drying for 24 hours at 80 °C total dry weight (DW) was recorded. RWC was calculated according to the formula:

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$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

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Estimation of H₂O₂ was done by the standard method [14]. Proline content was determined by the method of Bates *et al.* [15]. Peroxidase was estimated as per the method given by Harde *et al.* [16].

Nitrogen content (%) in leaf was estimated by the micro kjeldhal method [17]. The nitrogen content in sample was estimated by using the formula

$$\% N = \frac{TV \times 0.00028 \times 100}{W}$$

Where,

TV= Titration value (Sample titration value-Blank titration value)

W= Weight of sample

The leaf phosphorus content in the triacid digest was determined by vanadomolybdo phosphoric acid yellow color method [17] and percent of phosphorus (% P) in plant sample was calculated by using following formula

$$\% \text{ Phosphorus} = P \times \frac{\text{Final Volume (50 mL)} \times 100 \times 100}{\text{Weight of sample} \times \text{Volume of aliquot} \times 1000000}$$

Where, P= Phosphorus concentration (ppm) obtained from standard curve

Weight of sample= 0.5g Volume of aliquot= 5 mL

The potassium content in the triacid digest was determined by using ELICO Flame photometer [17]. Percent of potassium (% K) in plant sample was calculated by using following formula

$$\% \text{ Potassium} = R \times \frac{100 \times 100}{\text{Weight of sample} \times 1000000}$$

Where, R= Concentration of potassium (K) in ppm

Weight of the sample = 0.5g

The nutrient uptake was calculated using the following formula and expressed in g plant⁻¹.

$$\text{Nutrient uptake} \left(\frac{\text{g}}{\text{plant}} \right) = \frac{\text{Nutrient content (\%)} \times \text{Dry matter production (g/plant)}}{100}$$

2.5 Statistical analysis:

The data obtained in different experiments was statistically analyzed using Completely Randomized Design (CRD) as per the procedures given by Snedecor and Cochran [18]. Statistical significance was tested by F-value at 0.05 level of probability and critical difference was worked out where ever the effects were significant.

3. RESULTS:

3.1 Chlorophyll Stability Index (CSI %)

Chlorophyll stability index is the most promising character to screen the plants under drought stress [19]. At vegetative and harvesting stage significantly higher CSI was found in T9:75 % WHC+ 100 % RDF + MC (128.33%, 90%) and T8:75 % WHC+ 75 % RDF + MC (116.33%, 89%). At flowering stage four treatments were found to have CSI significantly more than the control in the following order T9:75 % WHC+ 100 % RDF + MC (138.67%), T8:75 % WHC+ 75 % RDF + MC (133.33%), T6: 50% WHC + 100 % RDF + MC (129.67%) and T5: 50% WHC + 75 % RDF + MC (128.00%) (Table 1).

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The CSI was observed to increase at flowering stage than the vegetative stage and was gradually reduced at harvesting stage. And the treatments with water deficit conditions have shown less CSI than treatments given with full irrigations because drought impairs the photosynthesis by reversible inhibition of chlorophyll activity. However treatments T9, T8, T6 and T5 were found to have higher CSI though given lesser irrigations than the control which might be due to the application of osmotolerant and plant growth promoting microbial consortium in particular nitrogen fixing ability increases N content in plants enhancing the synthesis of chlorophyll than in control.

Sehgal *et al.* [20] reported that the drought inversely affects the chlorophyll content, with increased drought intensity chlorophyll content decreases results in inhibition of photosynthesis and accelerating the senescence process in plants. But, studies have proved that inoculation of microbes have improved chlorophyll content even under drought conditions. In maize inoculated with *Azospirillum* sp. and *Herbaspirillum* sp. under drought stress increased total chlorophyll content was observed suggesting that the available nitrogen in could have been used for chlorophyll synthesis [21]. Inoculation of *Pseudomonas syringae*, *Enterobacter aerogenes* and *P. fluorescens* to maize increased the chlorophyll content under drought stress [22].

Table 1. Influence of endophytic microbial consortia on plant biochemical parameters of maize in pot culture

Treatments	Chlorophyll Stability Index (CSI %)			Relative Water Content (RWC %)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T₁ : 25 % WHC + 50 % RDF + MC	67.67	92.00	58.67	57.20	65.60	57.33
T₂ : 25 % WHC + 75 % RDF + MC	72.67	96.00	56.67	54.37	65.03	63.63
T₃ : 25 % WHC+ 100 % RDF + MC	86.33	100.00	65.33	53.40	71.23	60.07
T₄ : 50 % WHC+ 50 % RDF + MC	79.67	92.00	61.33	63.57	89.53	72.97
T₅ : 50 % WHC+ 75 % RDF + MC	96.33	128.00	71.33	63.90	91.00	79.13
T₆ : 50 % WHC+ 100 % RDF + MC	99.00	129.67	75.00	63.03	89.80	80.77
T₇ : 75 % WHC+ 50 % RDF + MC	88.67	99.67	73.67	68.97	91.33	82.80
T₈ : 75 % WHC+ 75 % RDF + MC	116.33	133.33	89.00	62.07	91.50	83.37
T₉ : 75 % WHC+ 100 % RDF + MC	128.33	138.67	90.00	66.27	95.20	87.87
T₁₀ : 100% WHC+ 100 % RDF (Control)	100.00	100.00	100.00	59.63	92.96	82.70
CD(P=0.05)	5.93	4.28	5.31	5.35	4.30	5.38
S.Em. ±	1.99	1.44	1.78	1.80	1.45	1.81
S.Ed	2.82	2.03	2.53	2.55	2.05	2.56

3.2 Relative Water Content (RWC %)

At vegetative stage maximum and significantly higher RWC was observed in T7:75 % WHC+ 50 % RDF + MC (68.97%) followed by T9 :75 % WHC+ 100 % RDF + MC (66.27%) while T8 :75 % WHC+ 75 % RDF + MC (62.07%) and all the three treatments with 50% WHC T6 (63.03%), T5 (63.90%) and T4 (63.57%) were recorded to have RWC on par with the control T10:100% WHC+ 100 % RDF (59.63%). At flowering stage there was no significant difference among the treatments given with 50% WHC and 75% WHC and they are on par with the control given with 100% WHC and no microbial inoculums (Table 1).

Relative water content (RWC) of leaf also known as relative water turgidity is a measurement of actual water content relative to its extreme water holding capacity. It shows the degree of drought and heat stress, and provides one of the best criteria for measuring water deficit in leaves [23]. Higher relative water content of a species shows that the species are better adapted to environmental stress, i.e., drought stress. Hence, a higher RWC is considered as an important strategy for higher drought tolerance compared to a lower RWC [24]. To check the potential of endophytic PGP bacteria as a drought stress–alleviating agent, RWC of plant leaves could be used as a parameter [25]. In this regard, developed endophytic bacterial consortia have been investigated that alleviate drought stress in plants by increasing RWC in inoculated plants under given stress conditions.

In the present investigation RWC of all the treatments were observed to increase at flowering stage than vegetative stage and found to decrease at harvesting stage. The higher RWC may protect plant from osmotic and oxidative stress triggered by water deficit conditions. The increased RWC under water deficit conditions is due to altered physiological processes in plants by microbes producing phytohormones like Abscisic acid which induces closure of stomata thereby reducing transpirational losses.

Dodd *et al.* [26] reported that the increased RWC in microbial treated plants could be due to altered physiological processes such as stomatal closure and Grover *et al.* [27] has demonstrated that 24% increase RWC was observed in sorghum plants treated with *Bacillus* sp. strain KB 129 under drought stress. Ma *et al.* [28] isolated an endophytic bacteria, *Pseudomonas azotoformans* from the leaves of *Alyssum serpyllifolium* and tested for enhanced drought tolerance. Inoculation of *Trifolium arvense* with *Pseudomonas azotoformans* increased leaf RWC in the absence as well as presence of drought stress.

3.3 H₂O₂ (μmol g⁻¹) content

At vegetative stage the H₂O₂ content in control i.e., T10:100% WHC+ 100 % RDF given no moisture stress and microbial treatment was found to be 28.50 μmol g⁻¹ and the treatments which were recorded lower H₂O₂ than the control even though provided with moisture stress are found to be T9

(23.97 $\mu\text{mol g}^{-1}$), T8 (25.20 $\mu\text{mol g}^{-1}$) and T7 (28.13 $\mu\text{mol g}^{-1}$)(Table 2).At flowering stage minimal H_2O_2 content were found in treatments given with moisture stress are T9 (37.97 $\mu\text{mol g}^{-1}$), T8 (37.77 $\mu\text{mol g}^{-1}$) and T7 (36.97 $\mu\text{mol g}^{-1}$). At harvesting stage lower content of H_2O_2 were found in treatments given with moisture stress are T9 (30.43 $\mu\text{mol g}^{-1}$), T8 (31.87 $\mu\text{mol g}^{-1}$), T7 (32.80 $\mu\text{mol g}^{-1}$). Maximum H_2O_2 content was observed in treatments given with moisture stress are T1 (56.07 $\mu\text{mol g}^{-1}$) and T2 (54.70 $\mu\text{mol g}^{-1}$).

Exposure of plants to abiotic stress leads to increased production of reactive oxygen species (ROS) by partial reduction of atmospheric O_2 leading to production of hydrogen peroxide (H_2O_2), hydroxyl radical (HO^\bullet) and superoxide anion radical (O_2^-) [29] causing oxidative damage to lipids, proteins, and other macro molecules [30]. However, the production ROS can be minimized in the plants by the application of endophytic bacteria under drought stress as reported [31, 32]. Hence, in present investigation H_2O_2 content was taken as parameter to evaluate the function of developed endophytic microbial consortia in decreasing ROS under water deficit stress conditions.

In a case study, 7 endophytic bacteria, i.e., *Acinetobacter calcoaceticus*, *Burkholderia vietnamiensis*, *Enterobacter asburiae*, *Rhizobium tropici*, *Sphingomonas yanokuyae*, *Curtobacterium* sp., *Rhanella* sp., and *Pseudomonas* sp., were isolated from stem of poplar and willow plants. Poplar plants were inoculated with these bacteria and evaluated for their drought tolerance as a result plants showed decreased ROS activity, in addition to other drought tolerance-enhancing parameters [32].

Table 2. Influence of endophytic microbial consortia on antioxidant enzymes and proline content in maize in pot culture

Treatments	H ₂ O ₂ (μmol g ⁻¹)			Peroxidase (units min ⁻¹ g ⁻¹)			Proline (μg g ⁻¹)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T₁: 25 % WHC + 50 % RDF + MC	53.96	71.37	56.07	35.83	76.73	44.57	65.27	67.63	69.97
T₂: 25 % WHC + 75 % RDF + MC	44.16	69.10	54.70	35.27	75.23	43.33	66.77	65.03	73.17
T₃: 25 % WHC+ 100 % RDF + MC	43.00	66.43	52.70	34.63	73.43	45.80	63.87	68.53	68.80
T₄: 50 % WHC+ 50 % RDF + MC	34.26	46.13	45.50	30.03	68.77	37.57	35.60	34.43	40.20
T₅: 50 % WHC+ 75 % RDF + MC	33.96	46.53	43.03	30.03	70.03	35.17	34.73	33.63	39.60
T₆: 50 % WHC+ 100 % RDF + MC	32.00	43.83	40.97	30.37	66.57	34.17	33.80	35.13	37.77
T₇: 75 % WHC+ 50 % RDF + MC	28.13	36.97	32.80	26.47	54.27	30.50	28.73	26.43	33.60
T₈: 75 % WHC+ 75 % RDF + MC	25.20	37.77	31.87	26.97	56.47	29.63	30.33	29.40	31.97
T₉: 75 % WHC+ 100 % RDF + MC	23.96	37.97	30.43	24.83	54.43	29.47	29.47	28.80	31.40
T₁₀: 100% WHC+ 100 % RDF (Control)	28.50	42.70	33.40	24.80	55.43	30.43	25.17	25.50	26.77
CD(P=0.05)	2.56	3.97	3.63	3.47	4.44	2.27	2.84	3.78	4.79
S.Em. ±	0.86	1.34	1.22	1.16	1.49	0.76	0.95	1.27	1.61
S.Ed	1.22	1.89	1.74	1.64	2.11	1.08	1.35	1.79	2.28
CV (%)	4.30	4.64	5.04	6.72	3.97	3.67	3.99	5.32	6.17

3.4 Peroxidase (units min⁻¹ g⁻¹)

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At vegetative stage highest peroxidase content was produced by T1 (35.83 $\mu\text{mol g}^{-1}$), T2 (35.27 $\mu\text{mol g}^{-1}$), T3 (34.63 $\mu\text{mol g}^{-1}$) while lower content of peroxidase was observed in T7 (26.47 $\mu\text{mol g}^{-1}$), T8 (26.97 $\mu\text{mol g}^{-1}$), T9 (24.83 $\mu\text{mol g}^{-1}$) which were found on par with the control given with no water stress T10 (24.80 $\mu\text{mol g}^{-1}$) and similar trend was observed in flowering and harvesting stage (Table 2).

At all the growth stages peroxidase content is higher in treatments provided with 75% and 100% RDF under water deficit stress conditions. The results suggested that at optimum levels of fertilizer application could regulate increased tolerance to drought stress by improving levels of oxidative enzymes. As similar results were reported by Qu *et al.* [33] that phosphorus in maize leaves efficiently kept higher RWC and also increased superoxide dismutase (SOD) and peroxidase (POD) enzyme activities. Sun *et al.* [34] has observed increased activities of antioxidant enzymes SOD, POD, and CAT with a suitable nitrogen level under a water deficit stress.

The significant reduction of antioxidant enzyme activity was observed in the plants treated with the consortium T3 and T4 respectively, as compared to single bacterial strain inoculation (T1 and T2) under drought stress conditions [35]. A drought-resistant endophytic bacteria *Pseudomonas azotoformans* ASS1 isolated from leaves of *Alyssum serpyllifolium* enhanced plant chlorophyll content, peroxidase, catalase, superoxide dismutase, and proline content under drought stress [31].

3.5 Proline ($\mu\text{g g}^{-1}$)

Drought stress is associated with an increase in a number of compatible solutes, specifically proline [36]. The accumulation of compatible solutes namely sugars, organic acids, inorganic ions and proline under water deficit stress conditions is termed as osmotic adjustment. Thus, influence of applied endophytic microbial consortium on accumulation of proline content under water deficit stress conditions was evaluated in the present study (Table 2).

At vegetative stage maximum proline content was found in T2 (66.77 $\mu\text{g g}^{-1}$) followed by T1 (65.27 $\mu\text{g g}^{-1}$) and minimum production of proline was observed in T7 (28.73 $\mu\text{g g}^{-1}$). At flowering stage highest proline content was found in by T1 (67.63 $\mu\text{g g}^{-1}$) followed by T3 (68.53 $\mu\text{g g}^{-1}$) and lowest production of proline was observed in T7 (26.43 $\mu\text{g g}^{-1}$), T8 (29.40 $\mu\text{g g}^{-1}$) and T9 (28.80 $\mu\text{g g}^{-1}$) which were found on par with the control given with no water stress T10 (26.60 $\mu\text{g g}^{-1}$). At harvesting stage highest proline content was found in by T2 (73.17 $\mu\text{g g}^{-1}$) followed by T3 (73.00 $\mu\text{g g}^{-1}$) and lowest production of proline was observed in T7 (31.30 $\mu\text{g g}^{-1}$), T8 (31.97 $\mu\text{g g}^{-1}$) and T9 (29.33 $\mu\text{g g}^{-1}$) which were found on par with the control given with no water stress T10 (32.23 $\mu\text{g g}^{-1}$).

At all the growth stages proline content was found highest in treatments (T1, T2 and T3) given with 25% WHC while the treatments provided with 75% WHC (T7, T8 and T9) were found to have lesser proline content than the control T10 provided with no water stress and no microbial inoculums, indicating that applied microbial consortia might have improved osmotic tolerance by reducing the compatible solutes like proline. In study of Marulanda *et al.* [37], *P. putida* and *B. megaterium* exhibited the highest osmotic tolerance, and both strains also showed increased proline content, involved in osmotic cellular adaptation.

3.6 Uptake of nitrogen (g plant⁻¹)

At vegetative stage significantly higher uptake of nitrogen by maize was found (Table 3) in treatments T9:75 % WHC+ 100 % RDF + MC (5.33g plant⁻¹), T6:50 % WHC+ 100 % RDF + MC (4.73 g plant⁻¹) and T8:75 % WHC+ 75 % RDF + MC (4.68 g plant⁻¹) while the control T10:100% WHC+ 100 % RDF has shown 4.01 g plant⁻¹ of nitrogen uptake. At flowering stage significantly higher amounts of nitrogen uptake was found in treatments T9 (13.98 g plant⁻¹), T8 (12.26 g plant⁻¹) and T6 (10.24 g plant⁻¹) while the control T10 has shown 9.96 g plant⁻¹ of nitrogen uptake. At harvesting stage significantly higher nitrogen uptake by plants was found in treatments T9 (10.56 g plant⁻¹), T8 (10.28 g plant⁻¹) and T6 (10.08) when compared to control T10 (8.45 g plant⁻¹)

Table 3. Influence of endophytic microbial consortia on uptake of macro nutrients by maize in pot culture

Treatments	Nitrogen uptake (g plant ⁻¹)			Phosphorus uptake (g plant ⁻¹)			Potassium uptake (g plant ⁻¹)		
	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage	Vegetative Stage	Flowering Stage	Harvest Stage
T₁: 25 % WHC + 50 % RDF + MC	2.55	4.88	5.08	0.23	0.77	0.72	3.13	7.02	10.18
T₂ : 25 % WHC + 75 % RDF + MC	2.92	6.03	5.90	0.33	0.91	0.84	3.55	8.98	11.09
T₃ : 25 % WHC+ 100 % RDF + MC	3.11	6.33	6.59	0.36	1.03	1.03	3.65	9.68	14.03
T₄ : 50 % WHC+ 50 % RDF + MC	2.42	5.57	4.57	0.31	0.81	0.74	2.83	6.69	10.27
T₅ : 50 % WHC+ 75 % RDF + MC	3.49	7.27	7.89	0.46	1.15	1.38	4.27	9.65	15.38
T₆ : 50 % WHC+ 100 % RDF + MC	4.73	10.24	10.08	0.59	1.71	1.78	5.40	12.75	18.19
T₇ : 75 % WHC+ 50 % RDF + MC	3.49	7.18	7.14	0.39	1.23	1.39	3.89	9.15	12.16
T₈ : 75 % WHC+ 75 % RDF + MC	4.68	12.26	10.28	0.62	2.69	2.06	5.44	16.36	19.23
T₉ : 75 % WHC+ 100 % RDF + MC	5.33	13.98	10.56	0.65	3.38	2.19	6.36	16.33	20.14
T₁₀: 100% WHC+ 100 % RDF (Control)	4.01	9.93	8.45	0.52	1.79	1.69	4.99	12.80	16.07
CD(P=0.05)	0.38	0.88	0.57	0.03	0.19	0.14	1.02	1.08	0.93
S.Em. ±	0.13	0.30	0.19	0.01	0.06	0.05	0.34	0.36	0.31
S.Ed	0.18	0.42	0.27	0.01	0.09	0.07	0.48	0.51	0.44
CV (%)	6.01	6.11	4.31	3.54	7.00	6.08	13.60	5.75	3.68

3.7 Uptake of phosphorus (g plant⁻¹)

At vegetative stage maximum uptake of phosphorus by plants was found (Table 3) in treatments T9:75 % WHC+ 100 % RDF + MC (0.65 g plant⁻¹), T8:75 % WHC+ 75 % RDF + MC (0.62 g plant⁻¹) and T6:50 % WHC+ 100 % RDF + MC (0.59 g plant⁻¹) while the control T10:100% WHC+ 100 % RDF has shown 0.52 g plant⁻¹ of P uptake by plants. At flowering stage maximum P uptake by plants was recorded in T9 (3.38 g plant⁻¹) followed by T8 (2.69 g plant⁻¹) while the control T10 has shown 1.79 g plant⁻¹ of P uptake by plants. At harvesting stage higher uptake of P by plants was found in T9 (2.19 g plant⁻¹) followed by T8 (2.06 g plant⁻¹) and T6 (1.78 g plant⁻¹) while lowest the control T10 has shown 1.69 g plant⁻¹ of P uptake.

3,8 Uptake of potassium (g plant⁻¹)

At vegetative stage significantly high quantity of potassium uptake by plants was found (Table 3) in T9:75 % WHC+ 100 % RDF + MC (6.36 g plant⁻¹) while T8:75 % WHC+ 75 % RDF + MC (5.44 g plant⁻¹), and T6:50 % WHC+ 100 % RDF + MC (5.40 g plant⁻¹) were found to have K uptake on par with the control T10:100% WHC+ 100 % RDF (4.99 g plant⁻¹). At flowering stage in plants significantly more K uptake was found in the treatments T9 (16.33 g plant⁻¹) and T8 (16.36 g plant⁻¹) when compared to control T10 (12.80 g plant⁻¹). At harvesting stage significantly higher uptake of K by plants was observed in T9 (20.14 g plant⁻¹) followed by T8 (19.23 g plant⁻¹) and T6 (18.19 g plant⁻¹) while the control T10 has shown 16.07 g plant⁻¹ of K uptake by plants.

Application of microbial consortium to maize has improved uptake of N, P and K even under drought stress which might be due to the ability of endophytic bacterial consortium to produce IAA efficiently and ACC deaminase activity that reduces ethylene levels all together enhancing the root growth and root hair production that helps the plants to uptake relatively more water and minerals available thereby increasing concentration of N, P and K in plant. Endophytic bacterial strains *Bacillus* and *Brevibacillus* isolated from the leaves two medicinal plants *Fagoniamollis* and *Achilleafragrantissima* had significant positive effects on plant growth and shoot nitrogen (N) and phosphorus (P) contents [38].

By inoculating plants with endophytic bacteria, an increase in the lateral roots and root hairs were observed which lead to the increased surface area and consequently enhanced moisture and mineral uptake from the soil [39]. In the study of Danish *et al.* [40] a significant improvement in shoot N, P and K, was recorded which might be due to increased root elongation. According to Zahir *et al.*[41] the improved root elongation helps the plants to uptake relatively more water thus increasing water use efficiency under drought stress. Better uptake of nutrients was also observed in pea plants treated with ACC deaminase containing PGPR *P. brassicacearum* and *P. marginalis*. The similar results were observed by Raúl *et al.*[42] and Krishnaveni [43] that the application of microbial inoculants increased the uptake of both

macro- and micronutrients. It was observed that microbial inoculants application had enhanced the uptake of all plant nutrients especially phosphorus content in the plant tissue.

CONCLUSION:

Based on the obtained results, improved or on par levels of plant biochemical components which are involved in plant osmotic adjustment under stress conditions viz., chlorophyll stability index, relative water content, H_2O_2 ($\mu\text{mol g}^{-1}$), peroxidase and proline content was observed. Plant uptake of NPK was also proved to be increased under application of consortia. Thus, it is evident that moisture deprived stress conditions in maize crop can be mitigated with the application of plant growth promoting endophytic microbial consortia.

REFERENCES:

1. Kumar A, Verma JP. Does plant—microbe interaction confer stress tolerance in plants: a review? *Microbiological Research*.2018; 207: 41–52.
2. Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current opinion in biotechnology*. 2005;16(2): 123-132.
3. Daryanto S, Wang L, JacinthePA. Global synthesis of drought effects on maize and wheat production. *PLoS One*. 2016; 11(5):e0156362.
4. Marulanda A, Barea JM, Azcon R. An indigenous drought tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retamasphaerocarpa*. *Microbial Ecology*. 2006; 52:670–678.
5. Kavamura VN, Santos SN, da Silva JL, Parma MM,Ávila LA,ViscontiA, Zucchi TD, Taketani RG, Andreote FD, de Melo IS. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiological Research*.2013; 168: 183–191.
6. Compant S, Duffy B, Nowak J, Clément C, Barka EA. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Applied Environmental Microbiology*.2005; 71(9): 4951-4959.
7. Sandhya V, Shrivastava M, Ali SZ, Prasad SSKV. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russian Agricultural Sciences*.2017; 43(1): 22–34.

8. Naveed MB, Mitter S, Yousaf M, Pastar M, Afzal, A, Sessitsch. The endophyte *Enterobacter* sp. FD17: a maize growth enhancer selected based on rigorous testing of plant beneficial traits and colonization characteristics. *Biology and Fertility of Soils*. 2014; 50: 249-262.
9. Chandran V, Shaji H, and Mathew L. Endophytic microbial influence on plant stress responses. *Microbial endophytes*. 2020; 7: 161-193.
10. Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA, Ihsan M, Laiq M, Ullah S, Fahad S, Khan A. Drought tolerance improvement in plants: an endophytic bacterial approach. *Applied microbiology and biotechnology*. 2019; 103(18): 7385-7397.
11. Paul D, Lade H. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agronomy for Sustainable Development*. 2014; 34:737-752.
12. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris*. *Plant Physiology*. 1949; 24: 1-15
13. Murthy KS, Majumder SK. Modifications of the technique for determination of chlorophyll stability index in relation to studies of drought resistance in rice. *Current Science*. 1962; 31: 470-471.
14. Patterson BD, Macrae EA, Ferguson IB. Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Analytical Biochemistry*. 1984; 139: 487-492.
15. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. *Plant and Soil*. 1973; 39: 205-207.
16. Harde AL, Perane RR, Shinde VS, Sakore GD. Biochemical defense mechanism in groundnut genotypes against rust caused by *Puccinia arachidis* Speg. *International Journal of Chemical Studies*. 2019; 7 (3): 834-841.
17. Piper CS. *Soil and Plant Analysis*. Academic press, New York. 1960; 357-367.
18. Snedecor GW, Cochran WG. *Statistical methods*. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 1967; 593-598.
19. Arunyanark A, Jogloy S, Akkasaeng C, Vorasoot N, Kesmala T, Nageswara Rao RC, Wright GC, Patanothai A. Chlorophyll stability is an indicator of drought tolerance in peanut. *Journal of Agronomy and Crop Science*. 2008; 194(2): 113-125.
20. Sehgal A, Sita K, Siddique KH, Kumar R, Bhogireddy S, Varshney RK, Hanumantharao B, Nair RM, Prasad PV, Nayyar H. Drought or/and heat-stress effects on seed filling in food crops:

impacts on functional biochemistry, seed yields, and nutritional quality. *Frontiers in Plant Science*. 2018; 9: 1-19.

21. Curá JA, Franz DR, Filosofía JE, Balestrasse KB, Burgueño LE. Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of maize to drought stress. *Microorganisms*. 2017; 5(3): 41.
22. Nadeem SM, Zahir Z, Naveed M, Arshad M, Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. *Canadian Journal of Microbiology*. 2007; 53 (10): 1141-1149.
23. Mullan D, Pietragalla J. Leaf relative water content physiological breeding II: a field guide to wheat phenotyping CIMMYT, Mexico. 2012; 25-27.
24. Nounjan N, Chansongkrow P, Charoensawan V, Siangliw JL, Toojinda T, Chadchawan S, Theerakulpisut P. High performance of photosynthesis and osmotic adjustment are associated with salt tolerance ability in rice carrying drought tolerance QTL: physiological and co-expression network analysis. 2018; *Frontiers in Plant Sciences*. 9:1135
25. Ngumbi E, Kloepper J. Bacterial-mediated drought tolerance: current and future prospects. *Applied Soil Ecology*. 2016; 105:109–125.
26. Dodd IC, Zinovkina NY, Safronova VI, Belimov AA. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology*. 2010; 157(3): 361-379.
27. Grover M, Madhubala R, Ali SZ, Yadav SK, Venkateswarlu B. Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *Journal of Basic Microbiology*. 2014; 54(9): 951-961.
28. Ma Y, Rajkumar M, Moreno A, Zhang C, Freitas H. Serpentine endophytic bacterium *Pseudomonas azotoformans* ASS1 accelerates phytoremediation of soil metals under drought stress. *Chemosphere*. 2017; 185: 75-85.
29. Ullah A, Sun H, Yang X, Zhang X. Drought coping strategies in cotton: increased crop per drop. *Plant Biotechnology Journal*. 2017; 15:271–284.
30. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Sciences*. 2002; 7: 405-410.

31. Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al Khiziri S, Ullah I, Ali L, Jung HY, Lee IJ. Bacterial endophyte *Sphingomonas* LK11 produces gibberellins and IAA and promotes tomato plant growth. *Journal of Microbiology*.2014; 52:689–695.
32. Khan Z, Rho H, Firrincieli A, Hung SH, Luna V, Masciarelli O, Kim SH, Doty SL. Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Current Plant Biology*. 2016; 6: 38-47.
33. Qu D, Wang BL, Shan L, Wang PH, Su P. Effects of phosphorous on SOD and POD activities of maize under water stress and highly varied water conditions. *Acta University AgriculturaeBoreali-Occidentalis* (in Chinese).1996; 24: 48-52.
34. Sun Q, Liang ZS, Wang WL, Li XJ,Zhang PS. Effect of membrane lipid peroxidation and photosynthesis rate on water stress under different nitrogen level in maize. *ActaAgriculturaeBoreali-OccidentalisSinica* (in Chinese). 2001; 10: 7-10.
35. Joshi B, Chaudhary A, Singh H, Kumar PA. Prospective evaluation of individual and consortia plant growth promoting rhizobacteria for drought stress amelioration in rice (*Oryza sativa* L.). *Plant and Soil*. 2020; 457(1): 225-240.
36. Kiani SP, Talia P, Maury P, Grieu P, Heinz R, Perrault A, Sarrafi A. 2007. Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. *Plant Science*.2007; 172:773–787.
37. Marulanda A, Barea JM, and Azcón R. 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM Fungi and Bacteria) from dry environments: mechanisms related to bacterial effectiveness. *Journal of Plant Growth Regulators*.2009; 28:115–124.
38. ALKahtani MD, Fouda A, Attia KA, Al-Otaibi F, Eid AM, Ewais EED, Hijri M, St-Arnaud M, Hassan SED, Khan N, Hafez YM. Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. *Agronomy*. 2020; 10(9): 1325.
39. Spaepen, S. and Vanderleyden, J. 2011. Auxin and plant-microbe interactions. *Cold Spring Harbor perspectives in biology*. 3(4): 001438.
40. Danish S, Zafar-ul-Hye M, Hussain M, Riaz M, Qayyum MF. Mitigation of drought stress in maize through inoculation with drought tolerant ACC deaminase containing PGPR under axenic conditions. *Pakistan Journal of Botany*. 2020; 52(1): 49-60.

41. Zahir ZA, Munir A, Asghar HN, Shaharoon B, Arshad M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *Journal of Microbiology and Biotechnology*. 2008; 18(5): 958-963.
42. Raúl OP, Carlos HB, Silvia CB, Paulo MFBS, Kátia RST. 2009. *Azospirillum* inoculation and nitrogen fertilization effect on grain yield and on the diversity of endophytic bacteria in the phyllosphere of rice rainfed crop. *European Journal of Soil Biology*. 2009; 45(1): 36-43.
43. Krishnaveni MS. Studies on phosphate solubilizing bacteria (PSB) in rhizosphere and non-rhizosphere soils in different varieties of foxtail millet (*Setaria italica*). *International Journal of Agriculture and Food Science Technology*. 2010; 1:23-29.

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