



A COMPARISON OF THE EFFECTS OF *RHIZOPHAGUS INTRARADICES*, *SERENDIPITA INDICA*, AND *PSEUDOMONAS FLUORESCENS* ON SOIL AND *ZEA MAIZE L.* PROPERTIES UNDER DROUGHT STRESS CONDITION

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ABSTRACT

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Drought is one of the most critical environmental stresses that reduce agricultural production. This study aimed to examine the effects of individual and simultaneous inoculation of *Rhizophagus intraradices*, *Serendipita indica*, and *Pseudomonas fluorescens* on the physical properties of soil and the growth parameters of single cross 704 maize under three levels of drought stress (80%, 50%, and 25% available water). It was found that *Rhizophagus intraradices* significantly increased soil hydrophobicity at all levels of drought stress, as did *Serendipita indica* at the second and third levels. *Pseudomonas fluorescens*, on the other hand, decreased soil hydrophobicity at all drought levels. At the optimum moisture level, individual inoculations of the investigated microorganisms did not significantly affect mean weight diameter, but all studied microorganisms increased mean weight diameter as drought stress increased. Additionally, inoculating plants with *Rhizophagus intraradices* at all levels of drought stress significantly increased the dry and fresh weight of shoots. Nevertheless, inoculating plants with *Rhizophagus intraradices* and *Pseudomonas fluorescens* at all levels of drought stress led to a significant increase in plant shoot height. Plant shoot potassium concentrations were significantly reduced by individual inoculation of *Pseudomonas fluorescens* and *Serendipita indica* under drought stress at the first and third levels. However, at all drought stress levels, inoculating plants with *Rhizophagus intraradices* significantly increased phosphorus concentrations in the shoots. Based on the results of this study, simultaneous insemination of maize with *Rhizophagus intraradices* and *Serendipita indica* was the most effective microorganism treatment for reducing the harmful effects of drought stress and improving soil properties.

Contribution/Originality: An attempt has been made to compare the effects of mycorrhiza, endophyte, and bacteria on plant and soil properties under drought stress conditions. So, this study can take a new step by investigating the effect of different types of microorganisms in drought conditions and identifying which type has the greatest effect on reducing drought stress on maize.

1. INTRODUCTION

Maize (*Zea mays L.*) is one of the oldest crops used by humans, livestock, and especially poultry (Etemadi, Hashemi, Zandvakili, Dolatabadian, & Sadeghpour, 2018). Globally, this plant is ranked first in yield and third in

cultivation (after wheat and rice) (Dowswell, Paliwal, & Cantrell, 2019; Soare & Dobre, 2016). Due to ease of cultivation, C4 photosynthesis pathway, high storage capacity, and high yield, maize has the highest genetic diversity among all crops (Azad, Biswas, Alam, & Alam, 2012; Drewry et al., 2010).

Plant growth, structure, and organ function are negatively affected by drought stress (Farooq, Hussain, Wahid, & Siddique, 2012), one of the most harmful environmental stresses, which ultimately reduces crop production efficiency (Fathi & Tari, 2016). As water scarcity creates stress for plants in semi-arid and arid regions, it is essential to increase a plant's water storage efficiency and use or drought resistance (Shirinbayan, Khosravi, & Malakouti, 2019). The effects of drought stress include reduced plant growth, shrinkage of leaves caused by reduced photosynthesis and inflammation of the cells, disorders of protein metabolism, and amino acid synthesis, leading to decreased cell division and enzymatic activity in plants (Fathi & Tari, 2016). Stresses such as salinity often directly and indirectly affect plants by causing drought stress. A plant that is tolerant of drought stress will be relatively tolerant of other stresses as well (Mahajan & Tuteja, 2005).

Inoculating plants with beneficial soil microorganisms such as plant growth-promoting rhizobacteria (PGPR), mycorrhiza, and endophytes assist plants in coping with environmental stresses (Lata, Chowdhury, Gond, & White Jr, 2018). PGPRs are a group of bacteria in the rhizosphere that directly (through reducing stress ethylene, fixing nitrogen, dissolving insoluble phosphates, supplying iron via the production of siderophores, and making plant hormones) or indirectly (through reducing and preventing the harmful effects of plant pathogens) increase plant growth (Nadeem, Ahmad, Zahir, Javaid, & Ashraf, 2014; Prasad, Kumar, & Varma, 2015). In soil under biotic and abiotic stress, microbial endophytes cause physiological and ecological changes in plants, increase plant yield per unit area, and develop plant cultivation (Hardoim et al., 2015; Hereme et al., 2020).

Serendipita indica (*S. indica*), formerly known as *Piriformospora indica*, is an endophytic fungus that can coexist with most crops and also reproduce in artificial culture media (Verma et al., 1998). In 1998, Verma et al. (1998) isolated this fungus from the rhizosphere of *Zizyphus nummularia* W. and *Prosopis juliflora* DC. in the Thar desert of India (Verma et al., 1998). Studies showed that inoculating plants with mycorrhizal fungus increased their tolerance to drought, salinity, pathogens, and improper soil temperature and pH (Meena, Vijayakumar, Yadav, & Mitran, 2018). Mycorrhizae increase plant growth and stress tolerance by improving water and nutrient uptake, repairing the soil structure, and producing plant hormones (Nadeem et al., 2014).

The stability of soil aggregates is a crucial component of plant growth in arid and semi-arid climates (Bird, Herrick, Wander, & Murray, 2007). Soil structure significantly affects nutrient uptake, water retention, ventilation, hydraulic conductivity, thermal conductivity, soil resistance to erosion, and plant growth and yield (Dexter, 2004). Different biological and physical factors, as well as soil composition, affect aggregate formation (Mbagwu, 2004).

Drought stress conditions reduce crop growth and yield, so it is necessary to provide any appropriate proposal to deal with this problem. Due to the effects of soil hydrophobicity and mechanical dispersion of clay on water infiltration, soil erosion, soil structure stability, and plant growth and yield, controlling these factors is essential to improve soil protection. Improvement of some growth indices of maize under drought stress by inoculating *Rhizophagus intraradices* (*R. intraradices*), *S. indica*, and *Pseudomonas fluorescens* (*P. fluorescens*) individually and simultaneously 2). Individual and simultaneous application of three microorganisms improve some physical characteristics of soil, including mechanical dispersion of clay, soil hydrophobicity, and mean weight diameter in soils under drought stress.

2. MATERIALS AND METHODS

2.1. Planting Conditions

The experiment was conducted in the research greenhouse of the Isfahan University of Technology (N 32° 43.2021' and E 51° 31.9807') in a completely randomized factorial design with three replications. The factors of the present experiment included two levels of *S. indica* (inoculation and non-inoculation), two levels of arbuscular

mycorrhizal fungus (*R. intraradices*) (inoculation and non-inoculation), two levels of *P. fluorescens* (inoculation and non-inoculation), and three levels of drought stress (80 %, 50%, and 25% available water (AW)). In this experiment, the control check sample (CK) represented maize not inoculated with microorganisms at all drought levels. The purpose of this study is to investigate the interaction between the treatments on the growth indices of maize and the physical properties of the soil. Several parameters were determined, including shoot fresh weight, shoot dry weight, shoot height, shoot potassium content, shoot phosphorus content, soil hydrophobicity, mean weight diameter, and clay's mechanical dispersion.

For disinfection, healthy and uniform maize seeds (Single Cross 704) were soaked for 30 seconds in 97% ethanol alcohol, then for 5 minutes in sodium hypochlorite solution, and then they were washed with distilled water. For germination, seeds were placed in Petri dishes with distilled water and incubated at 24–26 °C in an incubator. As soon as the roots reached 1–1.5 cm in length, the seedlings were transplanted into pots (3 L, 25 cm diameter).

The pots were filled with soil (Table 1 presents the Physical and chemical properties of soil), and then arbuscular mycorrhizal inoculum (sand and infected root fragments) was added to the soil. The top layer of soil was then returned to the pots. For the *S. indica* treatment, germinated maize seeds were inoculated with 50 ml of a spore suspension containing 5×10^7 spores per ml of culture medium. The soil was treated with *P. fluorescens* (bacterial treatment) inoculum suspension containing 5×10^7 bacterial cells per ml of culture medium two days after seedlings were planted.

Table 1. Physical and chemical properties of soil used in research.

Soil Classification	PWP	FC	pH	EC (dS/m)	OM%	TN%	P (mg/kg)	b ρ (g/cm ³)	Lime %
Clay	16.2%	28.18%	8.05	0.3%	0.8	0.07	28.4	1.23	31%

To prevent the growth of other microorganisms, the soil was sterilized for 60 minutes at 121 °C in an autoclave. As soon as the pots were ready, they were transferred to a greenhouse with 16 hours of daily light, a maximum daily temperature of 22–24 °C, a night temperature of 18 °C, and a light intensity of 10,000 lux. The seedlings were harvested after three months at the end of the vegetative stage but before the reproductive stage (clustering).

2.2. Apply Drought Stress

The field capacity (FC) and permanent wilting point (PWP) of maize were determined in a laboratory using pressure plate extractors, 28.18% and 16.2% by weight, respectively. Taking into account the weight of empty pots and dry soil (the amount was constant in all pots), the amount of water required for each stress level was calculated (80%, 50%, and 25% of available water) (Motesharezadeh & Asgari Lajayer, 2014). The pots were kept at FC level for 21 days (up to the four or five leaves stage), and drought stress treatments were started 21 days after planting. A daily weighing and adjustment of the pots' weight were made to apply drought stress.

2.3. Propagation and Inoculation of *P. indica*

According to Table 2, a complex culture medium was used to propagate *S. indica* (Varma et al., 2001), and fungal isolates were cultured at 24 °C for four weeks in an incubator to ensure sufficient spore production and fungal growth. To collect fungal spores from the culture medium, sterile rubber and water-twin (20 L) were used under the laminar hood. After passing liquid containing fungal spores through filter paper, the solution was poured into several 50 ml falcons and centrifuged for seven minutes at 7 °C at 500 rotations per minute. Following centrifugation, the supernatant was discarded, water-twin was added to the residue, and then sonicid was applied. The above steps were repeated three times. A Neobar lam was used to count the spores in the fungal inoculum, which was adjusted to 107×5 spores per milliliter containing water-twin 20%.

Table 2. The formula of *S. indica* culture medium (one liter).

Combination	Amount
Glucose	20 g
Fungi Yeast	1 g
Casamino Acid	1 g
Salt solution	50 ml
Peptone	2 g
Microelement	1 ml
Agar	15 g

2.4. Inoculation of Plant Growth-Promoting Rhizobacteria (PGPR)

Growth-inducing bacteria of *Pseudomonas fluorescens* were obtained from the microbial collection of the Isfahan University of Technology. To prepare the bacterial inoculum, a loop of bacteria was transferred from a solid culture medium to 250 ml nutrient broth (NB) under completely sterile conditions and aerated on a shaker at 120 rotations per minute. A colony counting method was used to adjust the bacteria population in a nutrient broth culture medium, which yielded approximately 5×10^7 bacterial cells per ml (Maleki, Mostafaee, Mokhtarnejad, & Farzaneh, 2010).

2.5. Preparation of Arbuscular Mycorrhizal Fungi

Simultaneously with seed sowing, the trap method was used for the propagation of arbuscular mycorrhizal fungi (*Rhizophagus intraradices*). One part of the soil was mixed with four parts of sterile sand in the pots, the topsoils were removed, and 100 g of inoculum of the desired species of fungus was added to the substrate. Then the removed litter was returned to the pots, and five maize seeds were planted in each pot. The seeds were reduced to three after germination, then kept in a greenhouse for five months so the maize roots would trap and propagate fungi (Leal, Stürmer, & Siqueira, 2009). Following this period, the aerial part was cut off, and the roots were mixed with the substrate contents. However, two grams of roots were collected, washed thoroughly, and then stored in FAA solution (formaldehyde-acetic acid-50 percent alcohol in a volume ratio of 5-5-90). The inoculation potential of each fungal treatment in the culture was calculated by determining the number of spores and the percentage of roots colonized in the culture. The Kormanik and McGraw (1982) method was used to determine root colonization.

2.6. Soil Hydrophobicity

A micro-penetration machine and intrinsic sorptivity method were used to measure soil hydrophobicity. The soil samples were dried in an oven at 65 °C. Then water sorptivity of samples was measured, the samples again were dried, and the ethanol sorptivity was measured. During the initial infiltration process (0 to 180 seconds), the effect of water and ethanol weight loss (soil adsorption) was recorded every five seconds. Most of the flow occurs under the effect of the matrix gradient, and the effect of gravity is of little importance. The effective parameter in the initial infiltration value is soil sorptivity, which was calculated through the steady flow of liquid (water or ethanol) in a short infiltration time using the following equation (Hallett, Baumgartl, & Young, 2001).

$$S = \sqrt{\frac{QF}{4br}}$$

Which Q is a steady flow of liquid, b is a parameter dependent on the soil water diffusivity function, which was considered equal to 0.55, r is the radius of the end of the infiltration tube in contact with the soil (cm), and f is the porosity filled with soil air (total porosity in dry conditions). In general, coarse soils have further hydraulic conductivity and more negligible soil sorptivity than fine soils (Hallett & Gaskin, 2007).

Tillman, Scotter, Wallis, and Clothier (1989) proposed the R index to assess soil hydrophobicity .This index was calculated by measuring the sorptivity of water (S_w) and ethanol (S_e) using the following equation:

$$R = 1.95 \left(\frac{S_E}{S_W} \right)$$

A constant of 1.95 is considered due to the difference in surface tension and viscosity between water and ethanol. Ethanol penetrates all soils due to its low surface tension and non-polarity, independent of soil hydrophobicity. Therefore, ethanol uptake is affected by soil pores' porosity and particle size distribution (Hallett et al., 2001). In completely hydrophilic soils, R is equal to one, and with increasing soil hydrophobicity, the hydrophobicity index increases due to the decrease of S_w . The angle of contact of water with soil can also be calculated, and as the water-soil contact angle increases, soil hydrophobicity also increases (Letey, Carrillo, & Pang, 2000).

2.7. Mean Weight Diameter (MWD)

The Kemper and Rosenau (1986) method determined the soil aggregates' mean weight diameter. In this method, 50 grams of soil aggregate with less than a 4 mm diameter were weighed. The series of sieves used were 2, 1, 0.5, 0.25, and 0.05 mm, and the sieve set was moved in water at a 1.5 inches vertical oscillation at a speed of 30 rpm for five minutes. Then the remaining amount on each sieve was weighed after drying in the oven (at 105 °C), and finally, the mean weight diameter of the soil aggregates was obtained using the following equation:

$$MWD = \sum_{i=1}^n Wi \times \bar{Xi}$$

Wi is the weight ratio of the soil aggregates to total soil (excluding sand and gravel particles) and \bar{Xi} is the average soil aggregates on the sieve.

2.8. Mechanical Dispersion of Clay (MDC)

The Rengasamy, Greene, Ford, and Mehanni (1984) method was used to measure the mechanical dispersion of clay. First, a 1:5 suspension was prepared by adding without pounding soil (particles smaller than 2 mm) into the water. Then the samples (at a speed of 100 rpm and an orbit of 2 cm) were shaken by a reciprocating shaker for 15 minutes. Then the samples were transferred to one-liter sedimentation containers, and after 12 hours, the supernatant of the suspension was stirred slowly and evenly. After four hours, it was sampled (according to Stokes' law) from a depth of 5 cm above the suspension by pipette, and then the mechanical dispersion of clay was measured by the weighting method.

2.9. Shoot Height, Shoots Fresh, and Dry Weight

Pre-harvest plant shoot height was measured with a ruler. Immediately after harvesting, plant samples were also weighed (fresh weight of shoots). Afterward, they were placed in an oven at 70 °C for two days and weighed by an electric scale (dry weight of shoots) after cooling (Barrs & Weatherley, 1962).

2.10. Measurement of Shoot Phosphorus (P) and Potassium (K) Concentrations

The dried shoot specimens were ground first, and then 0.5g of each specimen was placed in porcelain crucibles and heated at 550 °C for four hours to determine the P and K concentrations. As the samples cooled, a standard solution of hydrochloric acid (12.1 M) was added to dissolve the elements in the ash. As a means of better dissolving the ash elements in acid, the samples were gently heated with an electric heater, and the final extract was passed through filter paper (Whatman 42). By adding distilled water, the extract volume increased to 50 ml (Tadesse, Haque, & Aduayi, 1991). The P concentration in the extracts of the samples was determined by an autoanalyzer (QuikChem

Series 8000, Lachat Instruments Inc., USA) and the K concentration by an atomic absorption spectrophotometer (Perkin-Elmer 5100 PC).

2.11. Statistical Analyses

Statistical analysis of the total determination was conducted using one-way ANOVA and pairwise least significant difference (LSD) tests ($p \leq 0.05$). The analysis was conducted using the Statistical Package for Social Sciences (SPSS) 26.0.

3. RESULTS

3.1. Soil

3.1.1. Soil Hydrophobicity

The inoculation of *P. fluorescens* reduced soil hydrophobicity at all levels of drought stress, though it was not significant at the third level of stress (25% AW). As a result of individual inoculations of *S. indica*, soil hydrophobicity increased significantly compared to the control at the second and third levels of drought stress (50% AW and 25% AW) but was not significant at the first level (Table 4). The simultaneous inoculation of mycorrhizal fungi with *S. indica* significantly increased soil hydrophobicity compared to the control at all three moisture levels. It showed the highest soil hydrophobicity across all treatments at the first and second levels of drought stress (80% AW and 50% AW). The simultaneous inoculation of *S. indica* and *P. fluorescens* significantly increased soil hydrophobicity compared to control at the second and third levels of drought stress but decreased at the optimal level of moisture (80% AW). In all three levels of drought stress, simultaneous inoculation of mycorrhizal fungi and *P. fluorescens* did not significantly change soil hydrophobicity. Nevertheless, triple inoculation of microorganisms significantly increased it at all levels of stress (Table 4). Based on the results, it was found that the soil hydrophobicity increased with decreasing water and ethanol sorptivity levels. The presence of mycorrhizal fungi (at all moisture levels) and *S. indica* (at the second and third stress levels) significantly reduced water and ethanol sorptivity when compared to CK. Additionally, *P. fluorescens* significantly increased the sorptivity of water and ethanol at 80% AW and 50% AW at the same time (Table 4, Figure 1).

3.1.2. Mean Weight Diameter (MWD)

At the second and third moisture levels (50% AW and 25% AW), inoculating individual mycorrhizal fungi significantly increased MWD. However, at the first moisture level, this increase was insignificant. Individual *S. indica* inoculations showed similar results. It was observed that MWD increased significantly after individual inoculations of *P. fluorescens* only in the second level of drought stress but not in the optimal (80% AW) or severe levels of moisture (25% AW). According to the results, individual *S. indica* inoculation in the second and third levels of drought stress was the most effective treatment for increasing MWD (Tables 3 and 4). In all levels of drought stress, simultaneous inoculation of mycorrhizal fungi and *P. fluorescens* significantly increased MWD compared to CK ($p \leq 0.05$). However, the simultaneous inoculation of *S. indica* and *P. fluorescens* increased MWD only at the second and third levels of drought. There was a significant increase in MWD at the first and second levels of drought stress (80% AW and 50% AW) after triple inoculation of microorganisms, but not at the third level (Tables 3 and 4).

3.1.3. Mechanical Dispersion of Clay (MDC)

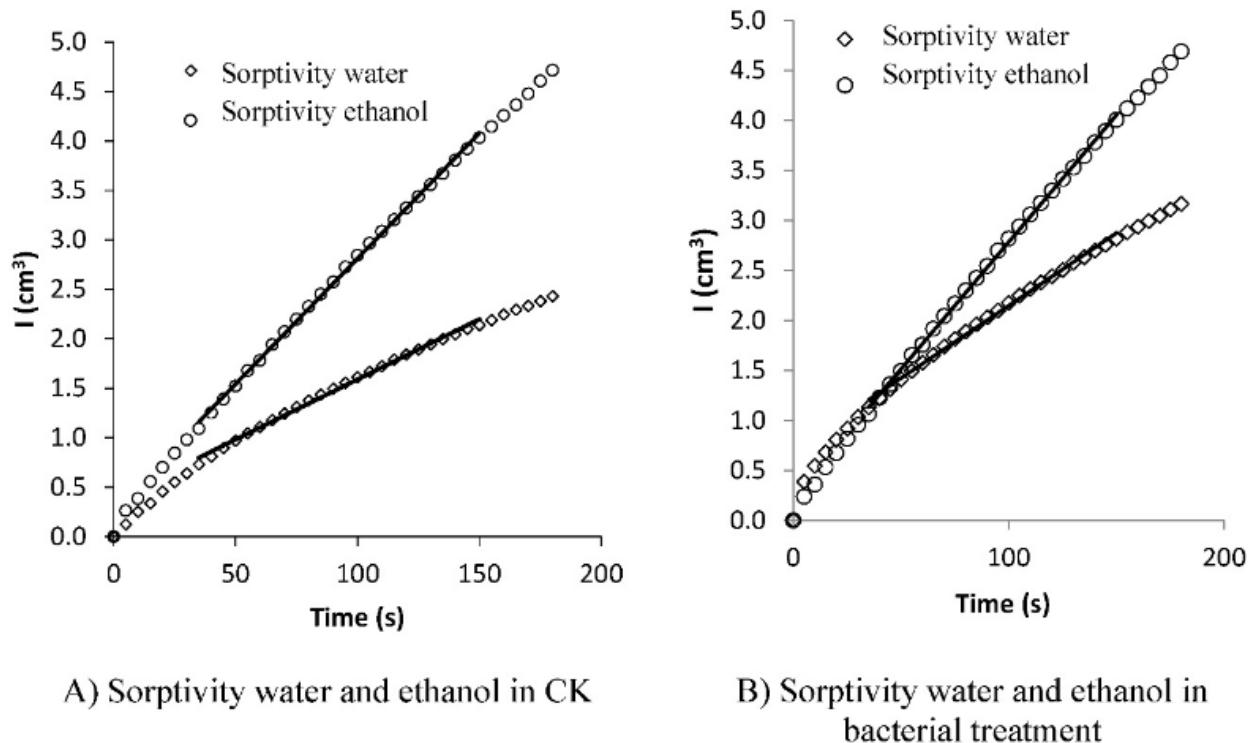
According to the results, MDC was affected differently by mycorrhizal fungi, *S. indica*, and *P. fluorescens* at different levels of moisture. The inoculation of individual mycorrhizal fungi significantly reduced MDC at the optimum moisture level (80% AW), but with decreasing soil moisture, there was no significant change in MDC. In all levels of drought stress, individual inoculation with *S. indica* and *P. fluorescens* significantly reduced MDC compared to the control ($p \leq 0.05$). Nevertheless, the simultaneous inoculation of *S. indica* and *P. fluorescens*, as well

as the simultaneous inoculation of *S. indica* and mycorrhizal fungi, significantly reduced MDC in comparison with the control group. In contrast, the simultaneous inoculation of mycorrhizal fungi and *P. fluorescens* reduced MDC at optimal and moderate moisture levels (80% AW and 50% AW) but not at severe drought stress (25% AW). At all three levels of drought stress, MDC was significantly reduced when all three microorganisms were inoculated simultaneously. This experiment showed that mycorrhizal fungi inoculation at the optimal moisture level reduced MDC most effectively, but *S. indica* inoculation performed better in the second and third stress levels (Tables 3 and 4).

Table 3. Results of ANOVA on main and interaction effects of *R. intraradices* (mycorrhizal fungi), *S. indica*, *P. Fluorescens* (Bacteria), and drought stress on the measured characteristics in shoots and soil under cultivation of maize.

Source of Variation	df	Shoot dry weight	Shoot height	P	MWD	MDC
Bacteria	1	0.67*	145.18**	0.0038ns	0.001*	19.03**
Mycorrhiza	1	122.19**	712.27**	2.14**	0.02**	0.63ns
<i>S. indica</i>	1	0.16 ^{ns}	44.05**	0.16**	0.008**	3.26**
Drought	2	218.19**	3893.81**	0.54**	0.03**	4.34**
Bacteria × Mycorrhiza	1	1.99**	69.97**	0.07**	0.004**	1.98*
Bacteria × <i>S. indica</i>	1	1.41**	15.79 ^{ns}	0.04**	0.006**	4.89**
Bacteria × drought	2	0.41 ^{ns}	48.006**	0.02**	0.003**	2.54**
Mycorrhiza × <i>S. indica</i>	1	0.80*	18.74*	0.002 ^{ns}	0.001 ^{ns}	8.55**
Mycorrhiza × drought	2	32.80**	66.85**	0.01**	0.02**	1.03*
<i>S. indica</i> × Drought	2	0.07 ^{ns}	37.07**	0.009**	0.0005 ^{ns}	5.79**
Bacteria × Mycorrhiza × <i>S. indica</i>	1	0.01 ^{ns}	8.61 ^{ns}	0.007**	0.0006 ^{ns}	7.40**
Bacteria × <i>S. indica</i> × Drought	2	0.30 ^{ns}	42.67**	0.02**	0.001*	1.95**
Mycorrhiza × <i>S. indica</i> × Drought	2	0.25 ^{ns}	3.15 ^{ns}	0.02**	0.01**	5.72**
Bacteria × Mycorrhiza × Drought	2	0.97**	53.66**	0.01**	0.001*	16.96**
Quadruple interaction	2	0.27**	41.99**	0.01**	0.002**	0.31 ^{ns}
Error	24	0.4069	1.981	0.0365	0.0190	0.5528
Coefficient of variation		8.87	3.816	2.51	3.04	17.52

Note: ** Significant at the level of 1%, * Significant at the level of 5%, ns nonsignificant.



A) Sorptivity water and ethanol in CK

B) Sorptivity water and ethanol in bacterial treatment

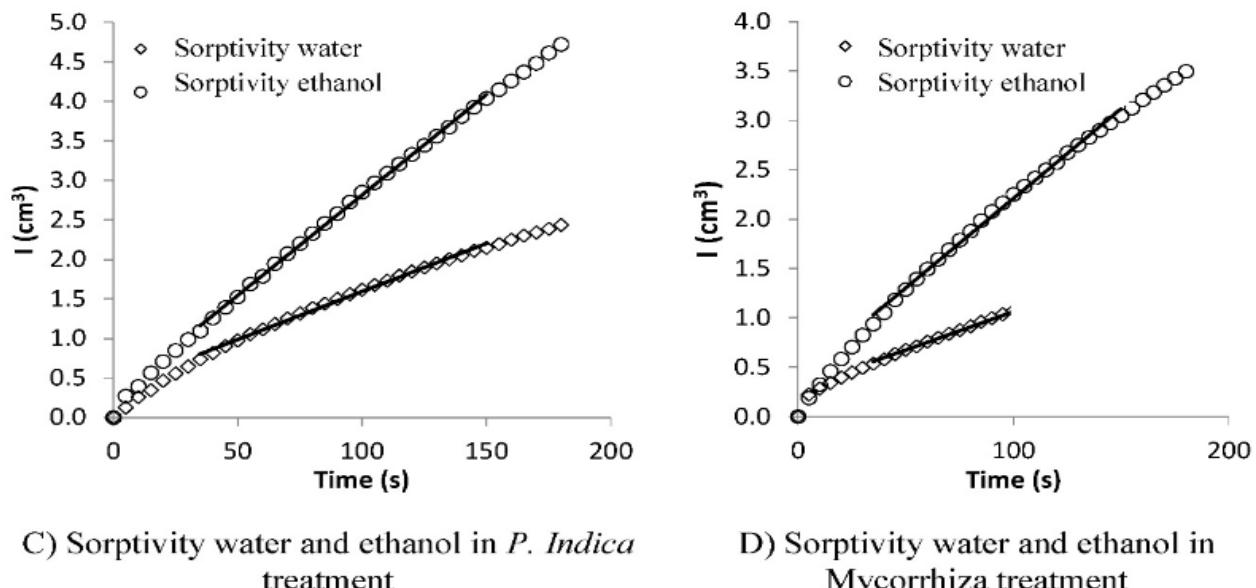


Figure 1. An example of the sorptivity of water and ethanol curves at the optimum moisture level (80% AW) in different treatments.

Table 4. Compare means interactions of *R. intraradices* (mycorrhizal fungi), *S. indica*, *P. Fluorescens* (Bacteria), and drought stress on measured physical characteristics of the soil.

Bacteria	Mycorrhiza	<i>S. indica</i>	Drought treatment (Available water)	Soil hydrophobicity	Soil-water contact angle (degree)	Water Sorptivity (cm/s ^{0.5})	Sorptivity ethanol (cm/s ^{0.5})	M.W.D. (mm)	MDC (%)
I	I	I	80%	2.92 bc	70.01 bc	0.124 e	0.186 abc	0.70 ab	4.35 bcd
			50%	2.90 bc	69.93 bc	0.099 j	0.148 i	0.62 ghi	2.19 ghi
			25%	2.77 cde	68.93 cdef	0.106 g	0.155 hi	0.65 defg	1.80 hi
	NI	NI	80%	2.84 cd	69.42 bcde	0.126 e	0.184 abcd	0.66 cde	2.48 gh
			50%	2.44 fg	65.81 hi	0.137 b	0.172 fg	0.63 fgh	1.90 hi
			25%	2.22 h	63.33 j	0.151 a	0.172 fg	0.67 bcd	3.52 def
	NI	I	80%	2.59 ef	67.29 fgh	0.137 b	0.182 bcd	0.53 j	1.87 hi
			50%	2.84 c	69.45 bcde	0.130 d	0.189 ab	0.59 j	3.81 cde
			25%	2.91 bc	69.90 bc	0.116 f	0.173 efg	0.68 abc	2.08 ghi
	NI	NI	80%	2.59 ef	67.25 fgh	0.137 b	0.182 bcd	0.54 j	1.98 hi
			50%	2.31 gh	64.33 d	0.150 a	0.178 def	0.62 ghi	2.98 efg
			25%	2.18 h	62.66 j	0.151 a	0.169 g	0.63 fgh	2.66 fgh
NI	I	I	80%	3.56 a	73.73 a	0.067 j	0.122 k	0.67 bcd	4.24 bcd
			50%	3.50 a	73.47 a	0.074 i	0.133 j	0.60 hi	4.32 bcd
			25%	2.85 c	69.44 bcde	0.106 g	0.155 hi	0.66 cdef	2.23 ghi
	I	NI	80%	3.08 b	71.10 b	0.100 h	0.158 h	0.56 j	1.36 i
			50%	3.53 a	73.60 a	0.076 i	0.138 j	0.61 hi	4.78 b
			25%	2.84 cd	69.41 bcde	0.131 d	0.191 a	0.68 abc	3.49 def
	NI	I	80%	2.85 c	69.50 bcd	0.126 e	0.184 abcd	0.54 j	4.51 bc
			50%	2.64 de	67.68 efg	0.134 c	0.182 bcd	0.65 cdef	1.90 hi
			25%	2.63 ef	67.73 defg	0.134 c	0.181 cde	0.71 a	1.94 hi
	NI	NI	80%	2.86 c	69.57 bc	0.125 e	0.183 abcd	0.54 j	6.59 a
			50%	2.55 f	66.94 gh	0.137 b	0.179 cdef	0.56 j	5.04 b
			25%	2.29 gh	64.23 ij	0.151 a	0.177 def	0.64 efg	3.56 def

Note: I (Inoculated) and NI (non-inoculation). $s^{0.5}/1/2$ second.

These letter shows the significant differences between factors and treatments.

3.2. Plant

3.2.1. Shoot Dry Weight

Comparing means revealed that different levels of drought stress affected the dry weight of shoots differently depending on *R. intraradices*, *S. indica*, and *P. Fluorescens*. Plants inoculated with *R. intraradices* gained significantly more dry weight in all three stress levels than CK. In addition, the results show that the dry weight of shoots in

plants inoculated with mycorrhizal fungi was significantly higher than that of plants inoculated with *S. indica* or *P. Fluorescens* at all stress levels (Tables 3 and 5). At the first and third levels of stress (80% AW and 25% AW), the shoot dry weight was not significantly affected by *S. indica*. However, shoot dry weight increased significantly under the second level of stress. Plants inoculated with *P. Fluorescens* produced significantly higher shoot dry weights at the first and second levels of drought stress than CK. However, there was no significant effect at the third stress level (25% AW) on shoot dry weight. At all drought levels, simultaneous inoculation of *R. intraradices* and *S. indica* increased the dry weight of maize shoots compared to CK ($p \leq 0.05$). Under optimal moisture conditions, the maximum shoot dry weight was observed (Tables 3 and 5). At the first and second stress levels, simultaneous inoculation of *S. indica* and *P. Fluorescens* significantly increased shoot dry weight compared to the control ($p \leq 0.05$). However, the third level of stress had no significant effect on the dry weight of the shoots. Compared to the control and individual treatment of *P. fluorescens*, simultaneous inoculation of mycorrhizal fungi and *P. fluorescens* increased the dry weight of shoots at all stress levels (Tables 3 and 5). In addition, triple inoculation of mycorrhizal fungi, *S. indica*, and *P. fluorescens* significantly increased maize shoot dry weight at all levels of drought stress.

3.2.2. Shoot Fresh Weight

By reducing soil moisture from 80% AW to 25% AW, the fresh weight of shoots in all treatments decreased following the dry weight of shoots (Table 5). Fresh weights of maize shoots treated with mycorrhizal fungi were significantly higher than those treated with *S. indica* or *P. fluorescens* individually at all three levels of drought stress. Both *S. indica* and *P. fluorescens* individual inoculations increased the fresh weight of shoots compared to CK ($p \leq 0.05$) at the first and second levels of drought stress (Table 5).

When mycorrhizal fungi and *S. indica* were inoculated simultaneously, the fresh weight of shoots was increased compared to all other treatments, regardless of stress level. However, it was not significant for the mycorrhizal inoculation alone or the simultaneous inoculation with mycorrhizal fungi and *P. fluorescens* at the second level of drought stress. The simultaneous inoculation with *S. indica* and *P. fluorescens* significantly increased shoot fresh weight at the first level of drought stress compared to the control ($p \leq 0.05$). At all levels of drought stress, triple inoculation significantly increased the fresh weight of shoots compared to the control (Table 5).

3.2.3. Shoot Height

Increasing drought stress from 80% AW to 25% AW reduced shoot height in all treatments, similar to the results of dry and fresh shoot weight (Tables 3 and 5). Individual inoculation of mycorrhizal fungi and *S. indica* resulted in significant increases in shoot height compared to control ($p \leq 0.05$) at all three drought stress levels. However, individual *S. indica* inoculation at 80% AW and 25% AW moisture levels significantly increased plant shoot height compared to CK. At all levels of drought stress, simultaneous inoculation of mycorrhizal fungi and *S. indica* significantly increased maize shoot height compared to the control ($p \leq 0.05$), and maximum shoot height was obtained under optimal moisture conditions. As a result of simultaneous inoculation of *S. indica* and *P. fluorescens*, plant height increased significantly in comparison to CK in both the first and second drought stress levels (Table 5). A significant increase in shoot height was observed at all levels of drought stress after inoculations of all microorganisms. As can be seen, there was no significant difference in shoot height between the triple treatment and the treatment with individual mycorrhizal fungi and simultaneous inoculation with mycorrhizal fungi and *P. fluorescens* (Table 5).

3.2.4. Shoot Potassium (K)

Based on the analysis of variance, all treatments (except *S. indica*) affected shoot potassium significantly. It was found that the interaction between microbial treatments (except *P. fluorescence* and *S. indica*) on shoot potassium was significant. However, the dual interaction of microbial treatments with increasing drought stress did not show a significant increase in shoot potassium (Table 5).

Table 5. Compare mean interactions of *R. intraradices* (mycorrhizal fungi), *P. indica*, *P. Fluorescens* (Bacteria), and drought stress on the measured traits of maize.

Bacteria	Mycorrhiza	<i>S. indica</i>	Drought treatment (Available water)	Shoot dry weight (g)	Shoot fresh weight (g)	Shoot height (cm)	K (mg/g)	P (mg/g)
I	I	I	80%	11 b	69.1 c	70.5 b	12.2 jkl	1.71 b
			50%	5.2 e	36.6 fg	53.9 e	14.1 h	1.67 bc
			25%	3 g	20.7 i	42.3 ijkl	15.8 bcd	1.42 e
	NI	NI	80%	10.9 b	67 c	70.7 b	12.09 kl	1.65 c
			50%	5 e	38.5 ef	50.7 ef	14.7 fgh	1.63 c
			25%	3.1 g	20.6 i	44.5 ijkl	15.1 def	1.40 e
	NI	I	80%	6.1 d	45.5 d	69.9 b	13.1 i	1.55 d
			50%	3.3 fg	20.6 i	48.9 fg	14.8 fgh	1.39 ef
			25%	2.2 h	12 k	36.1 m	16.9 a	1.04 i
	NI	NI	80%	6.9 c	44.6 d	62 c	12.9 ij	1.43 e
			50%	3.8 f	22 hi	48.5 fgh	15.3 cdef	1.30 g
			25%	2.2 h	12.2 k	41.4 l	16.4 ab	1.09 i
NI	I	I	80%	11.8 a	78.6 a	74.3 a	12.2 jkl	1.80 a
			50%	5.2 e	39.2 e	45.7 ghi	14.4 gh	1.77 b
			25%	3.4 fg	23.4 h	44.9 ijkl	15.3 cdef	1.49 d
	I	NI	80%	10.8 b	72.6 b	68.7 b	12.5 ijkl	1.66 bc
			50%	4.8 e	38.4 ef	50.7 ef	14.3 gh	1.65 bc
			25%	3 g	17.4 j	43.2 ijkl	15.5 cde	1.41 e
	NI	I	80%	5.2 e	36.7 fg	58.1 d	12.7 ijk	1.30 g
			50%	3.7 f	20.5 i	45.3 hij	15.4 cdef	1.51 d
			25%	2 h	12 k	41.9 kl	15.9 bc	1.10 i
	NI	NI	80%	5.2 e	34.7 g	53.8 e	12 l	1.34 fg
			50%	2.9 g	17.6 j	42.9 ijkl	14.8 efg	1.20 h
			25%	2.2 h	12.1 k	36 m	15 efg	1.05 i

Note: In each column, the different letters significantly showed differences ($p \leq 0.05$) between treatments. I (Inoculated) and NI. (non-inoculation).

Compared to CK ($p, 0.05$) in any drought stress level, the potassium of shoots inoculated with mycorrhizal fungi and simultaneously inoculated with mycorrhizal fungi+*S. indica* and mycorrhizal fungi+*P. fluorescence* did not show a significant difference ($p \leq 0.05$). *S. indica* and *P. fluorescens* individually or simultaneously inoculated resulted in significant increases in shoot potassium compared to control during the first and third levels of drought stress. When plants were inoculated with all three microorganisms, shoot potassium increased significantly compared to control at the third level of drought stress (25% AW) but not at the first or second levels (Table 5).

3.2.5. Shoot Phosphorus (P)

There was a significant effect of main treatments except for *P. fluorescens* on shoot phosphorus, according to the analysis of variance. On the other hand, the interaction of treatments with each other on shoot phosphorus was significant in all cases except for the interaction between mycorrhizal fungi and *S. indica* (Table 3).

In all three levels of drought stress, the phosphorus content of shoots in plants treated individually with mycorrhizal fungi increased significantly compared to CK ($p \leq 0.05$). Only inoculation of *S. indica* at the second level caused a significant increase. Simultaneous inoculation of *S. indica* and *P. fluorescens* induced an increase in shoot phosphorus compared with the control ($p \leq 0.05$), and it also increased shoot phosphorus compared with individual inoculation of *P. fluorescens* at the first and second stress levels.

Compared to CK at all levels of drought stress, simultaneous inoculation of all three microorganisms increased shoot phosphorus significantly. Maize shoot phosphorus changed in the same manner as the effect of microbial treatments on shoot dry weight, so with the increase in plant biomass, shoot phosphorus content increased (Tables 3 and 5).

4. DISCUSSION

Increasing drought stress reduced the dry weight, fresh weight, and shoot height due to reduced photosynthesis. However, drought stress disrupts cell inflammation, cell division, plant enzymatic activity, uptake and transport of water and nutrients, protein metabolism, and amino acid synthesis (Fathi & Tari, 2016; Men, Wang, Li, Su, & Chen, 2018). Similarly, inoculation of maize with mycorrhizal fungi increased dry and fresh weight, height, leaf water potential, carbon dioxide concentration, plant nutrition, and water uptake rate under drought conditions (Sarah et al., 2019; Sun et al., 2021; Zhu, Song, & Xu, 2010). Mycorrhizal fungi promote better water and nutrient uptake, leading to more plant growth, which ultimately makes plants more resistant to biotic and abiotic stresses (Fernández-Lizarazo & Moreno-Fonseca, 2016; Meddad-Hamza et al., 2010).

In the present experiment, inoculating maize with *S. indica* improved plant physiological factors and increased resistance to drought stress. This is because *S. indica* helps plants resist oxidative stress by increasing proteins such as vacuolar proton-ATPase, methionine sulfoxide, and drought-induced enzymes that destroy reactive oxygen species (Wiszniewska, 2021). As the natural location of *S. indica* is mostly in the desert and arid regions, reducing soil moisture to 50% AW enhanced *S. indica*'s effects on inoculated plants. This result can be interpreted as the fungus can better exert its effects on plants under drought stress (Hosseini, Mosaddeghi, Dexter, & Sepehri, 2018; Xu, Wang, Wang, Wei, & Zhang, 2017).

The results indicated that the inoculation of maize with *P. fluorescens* had beneficial effects on the physiological factors of plants under drought stress conditions. This is because *P. fluorescens* increases the ability to dissolve P, the production of auxin, chitinase, and 1-aminocyclopropane-1-carboxylic acid (ACC-deaminase), and metabolites such as siderophore and hydrogen cyanide, which ultimately improve plant growth (Bano & Fatima, 2009; Sarma & Saikia, 2014; Shahroona, Jamro, Zahir, Arshad, & Memon, 2007). Researchers found that a combined inoculation of *S. indica* and *P. fluorescens* could be an effective ecological system for providing plants with water, macronutrients, and micronutrients (Abadi, Sepehri, Khatabi, & Rezaei, 2021; Baghaie, 2020; Mensah et al., 2020).

According to the results, shoot K content increased with increasing drought stress, which was consistent with similar studies (Aslam et al., 2013; Cui et al., 2019). In drought-stressed crops, K is thought to increase because it regulates osmotic pressure and stomata opening and closing (Bregante, Carpaneto, Pastorino, & Gambale, 1997). A study by Haghigatnia, Nadian, and Rejali (2011) found that mycorrhizal fungi did not significantly affect leaf K concentration under optimal moisture and medium drought stress conditions. When drought stress was severe, K concentration decreased (Haghigatnia et al., 2011). Nautiyal et al. (2010) also stated that co-inoculation of chickpea with *S. indica* and *P. fluorescens* increased shoot potassium and phosphorus compared to CK, whereas the dual treatment caused a decrease in shoot potassium compared to individual inoculation of *S. indica* or *P. fluorescens* (Nautiyal et al., 2010).

For all stress levels, individual inoculation of *P. fluorescens* showed the most significant reduction in soil hydrophobicity, and simultaneous inoculation of *R. intraradices* and *S. indica* showed the most significant increase. Plant growth was significantly increased by simultaneous inoculation of these two fungi compared to other treatments, suggesting that organic matter production, hydrophobic root secretions, and more root volume were responsible for this increase in soil hydrophobicity (Hosseini et al., 2018; Xu et al., 2017; Zhang et al., 2021).

Hallett et al. demonstrated that organic compounds make soil hydrophobic, and the environment surrounding the roots of plants has higher soil hydrophobicity levels (Hallett, Gordon, & Bengough, 2003). Due to the lack of organic matter in the soils of dry areas, they have less water hydrophobicity than the soils of wet areas. Consequently, soil hydrophobicity decreases with decreasing soil moisture due to reduced plant growth and lower organic matter production (Doerr, Shakesby, & Walsh, 1998; Zhang, Chen, & Fan, 2020). In this way, repeated dry-wet cycles affect the development and durability of soil hydrophobicity (Cosentino, Chenu, & Le Bissonnais, 2006). Researchers report that adding microbes to soil increases soil hydrophobicity after a dry-wet cycle, but soil hydrophobicity decreases in subsequent dry-wet cycles (Cosentino et al., 2006). It should be noted that fungi increase soil hydrophobicity while

bacteria decrease it Hallett et al. (2001). Rillig, Mardatin, Leifheit, and Antunes (2010) observed that the hydrophobicity of soil is increased by arbuscular mycorrhizae (Rillig et al., 2010), while Roper showed that bacteria reduce soil hydrophobicity by degrading hydrophobic compounds and producing biosurfactants, and use organic compounds as carbon and energy sources (Roper, 2004) (Figure 2).

It is important to note that ethanol sorptivity is only affected by the geometry of soil pores, while water sorptivity is affected by the geometry of soil pores and the hydrophobic surface of the soil. Therefore, S_w and S_E indices are useful for determining whether soil pores or soil hydrophobicity has changed after treatment (Feeney et al., 2006).

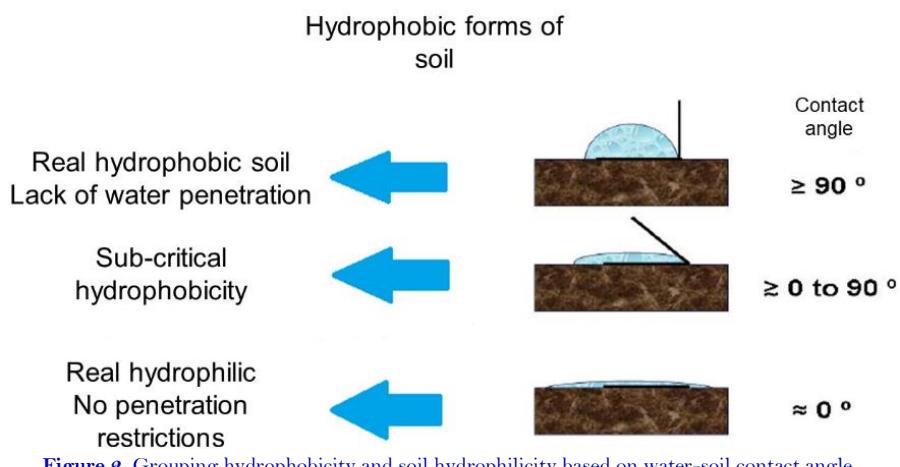


Figure 2. Grouping hydrophobicity and soil hydrophilicity based on water-soil contact angle.

According to the results, root diameter increased with decreasing soil moisture due to intensified wet-dry cycles, increased cohesion of dry soil, and further root development under stress (Cosentino et al., 2006). Mycorrhiza reversed this trend by improving MWD. Therefore, mycorrhiza has a more significant impact on soil aggregates, structure, and stability (reduction of MDC) at sufficient soil moisture. However, its effectiveness at 50% AW and 25% AW is more pronounced. Through their hyphae and deposition of organic compounds, mycorrhizal fungi play an important role in stabilizing aggregates (Wu, Cao, Zou, & He, 2014). Increasing the stability of aggregates due to *S. indica* inoculation can be indirectly related to the growth of roots, increasing root width and length, attracting soil microorganisms, and providing suitable pores for microorganism activity (Mosaddeghi, Hosseini, Hajabbasi, Sabzalian, & Sepehri, 2021; Varma, Chordia, Bakshi, & Oelmüller, 2013). It is also reported that *S. indica* increases aggregate stability directly through various secretions, including those from organic matter, hormones, enzymes, proteins, and the distribution of its mycelium around the roots of the host plant (Rai, Acharya, Singh, & Varma, 2001). Research has also shown *P. fluorescence* produces Levan (an exopolysaccharide) in soybean plants, which increases the water permeability and apparent density of soil under soybean cultivation under dry conditions (Budac, 2002).

In the results, microorganisms reduced clay mechanical dispersion. At the optimum moisture level, mycorrhizal fungi increase hydrophobic secretions, resulting in decreased mechanical dispersion of clay (Rillig et al., 2010). In support of this argument, other researchers have demonstrated that hydrophobic compounds secreted by the mycorrhizal fungus stabilize aggregates and improve soil structure (Cosentino et al., 2006; Rillig et al., 2010). *S. indica* reduces the mechanical dispersion of clay by producing organic compounds and hormones and distributing its mycelium around the host plant root (Rai et al., 2001). Furthermore, indirectly through the effect that *S. indica* has on the root area and its secreted compounds on the number of soil microorganisms by providing suitable habitat for them (Mosaddeghi et al., 2021; Varma et al., 2013).

5. CONCLUSIONS

This experiment showed that increasing drought stress resulted in decreased dry weight, fresh weight, height, and P levels in maize shoots. However, inoculation of maize with *R. intraradices*, *S. indica*, and *P. fluorescence* under

drought stress improved plant physiological parameters. Under drought stress conditions, inoculation of maize with different microorganisms also increased K content in the shoots. The simultaneous inoculation of *R. intraradices* and *S. indica* improved the physiological parameters of maize at the optimal moisture level. The individual inoculation of each microorganism at the optimal moisture level did not affect MWD, but with increasing drought, all three microorganisms increased MWD. Hydrophobicity of soil was increased significantly by *R. intraradices* and *S. indica*, whereas it was decreased by *P. Fluorescens*. However, *S. indica* was the most effective treatment for reducing MDC and increasing MWD under drought stress. Based on the obtained results, it is recommended to study the effects of these microorganisms on other stresses, such as cold, salinity, and heavy metals. In future studies, it is also recommended to perform advanced molecular experiments to identify and understand the molecular mechanisms of increasing resistance of plants inoculated with *S. Indica*, *P. Fluorescens*, and *R. intraradices*.

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