

# The role of biofertilizers in reducing the adverse effects of drought stress and adjusting water deficit conditions in maize (*Zea maize* L.) SC704

Asma Najafi<sup>1</sup>

<sup>1</sup>Master student of Crop Physiology, Ferdowsi University of Mashhad, Iran. Mohammad Javad Ahmadi-Lahijani<sup>2</sup>

<sup>2</sup>Assistant Professor, Department of Agrotechnology, Ferdowsi University of Mashhad, Iran.

Farnoosh Fallahpoor<sup>2</sup> <sup>2</sup>Assistant Professor, Department of Agrotechnology, Ferdowsi University of Mashhad, Iran. Armin Oskoueian<sup>3</sup> <sup>3</sup>PhD of Crop Physiology, Department of Agrotechnology, Ferdowsi University of Mashhad, Iran.

# Abstract

Drought stress, as one of the most important environmental stresses, has a negative effect on plant performance and reduces plant productivity. Biofertilizers can reduce the effects of drought on plants in water deficit conditions. This research was carried out to investigate the effects of biological fertilizers on the biochemical characteristics and growth of maize SC 704 under drought stress in 2022. Five fertilizer treatments including *Control, Nitrobacter, Phosphopowerbacter, Potashpowerbacter, and the combination of all three fertilizers* were applied under two levels of irrigation (normal irrigation and drought stress) in 6 replications. The results showed that drought stress affected the proline and leaf chlorophyll content and photosynthetic parameters. At the 8-leaf stage, the application of Nitrobacter increased photosynthesis and transpiration content by 37% and 27%, respectively. Also, the combined application of biofertilizers at the tasseling stage resulted in a 38% increase in intercellular CO<sub>2</sub> concentration. At the tasseling stage, the combined biofertilizers increased leaf chlorophyll a and proline content by 64% and 110%, respectively, under drought stress conditions compared to the control. In general, the results showed that the use of Nitrobacter in normal irrigation and the combined application of biological fertilizers under drought stress conditions compared to the control. In general, the results showed that the

Keywords: Drought stress, chlorophyll, proline, photosynthesis, PGPR.

# Introduction

Drought is a condition in which the plant faces a lack of moisture in the root environment resulting in a decrease in plant yield. Depending on the type of plant and variety, the water holding capacity of the soil, and the atmospheric conditions the level of damage is different. Iran is one of the countries that prone to stressful factors such as drought stress in the production of crops, which has resulted in reduced yield, soil fertility, and the impossibility of continuing agriculture [1].

Maize is one of the most important food grains and strategic agricultural products, which is very important in livestock, poultry nutrition, and food security, especially in developing countries. In recent years, maize cultivation has gradually expanded in arid and semi-arid regions. This change has helped to solve food security issues in countries with limited water resources [2,3].

It is important to supply the water required for plants in the vegetative and reproductive growth stages. Depending on the developmental stage and genotype, the growth and development of plants will be affected by the negative effects of drought stress [4]. Among the growth stages of maize plants, seedling growth, pollination, and seed filling stages are the most sensitive to drought stress and reduction of soil water potential. Water deficiency affects the accumulation of proline, photosynthetic pigments, and the rate of photosynthesis. There is a relationship between the chlorophyll content and the rate of leaf photosynthesis. Low chlorophyll content under drought stress conditions is due to oxidative stress, which causes the photo-oxidation of pigments and the destruction of chlorophyll pigments in leaves [5].

Osmotic adjustment is one of the most common adaptation responses to water deficit with the accumulation of different compatible solutes such as proline inside the cells [5,6].

The production of such osmolytes in excess amounts helps plants cope with drought conditions by maintaining the osmotic balance of the cell and stabilizing the membrane and protein structures. The use of useful microorganisms in the form of biological inoculation is proposed as a biotechnological tool for sustainable and environmentally friendly agricultural measures. Since this approach is compatible with the environment, it has environmental benefits and helps sustainable agricultural measures in water shortage conditions [7].

Research has shown that inoculating plants with plant growth-promoting bacteria (PGPR) as an effective tool in reducing the negative effects of various environmental stresses, including drought stress, has a positive effect on maize plants [7]. Growth-promoting bacteria can directly affect plant growth through nitrogen fixation, soil insoluble phosphorus solubilization, hydrogen cyanide production, production of phytohormones such as auxin, cytotoxin, gibberellin, and reduction of ethylene production. Part of the increase in yield due to the use of PGPR in both full irrigation and water limitation conditions can be attributed to the increase in chlorophyll content and the improvement of stomatal conductance [8]. Various studies have shown that maize is able to maintain N2-fixing microorganisms in its rhizosphere [6].

One of the possible mechanisms of drought tolerance caused by PGPR in plants is the production of plant hormones such as abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA), as well as ACC deaminase to reduce the level of ethylene in the roots [9].

This research was carried out to investigate the effect of biofertilizers on reducing the adverse effects of drought stress and the effect of nitrogen-fixing, phosphorus- and potassium-fixing biofertilizers on the photosynthetic parameters of maize plants.

### Materials and methods

This research was carried out at the research greenhouse of the Ferdowsi University of Mashhad in 2022. The experiment was conducted as a two-factorial arrangement in a randomized complete block design with six replications. Maize plants (cv. SC704) under different fertilizer conditions at 5 levels (1- Nitrobacter *including Azospirillum, Azotobacter, and Bacillus strains,* 2- Phosphopowerbacter and 3- Potashpowerbacter *including a collection of Pseudomonas and Bacillus strains,* 4- mixing treatments of Nitro Bacter, Phospho Power Bacter and Potas Power Bacter, and 5- control without adding fertilizer) and two irrigation levels (90% as control and 60% of the field capacity, FC) were investigated.

Biofertilizers were applied to the soil after the seedling was fully established and at the 4-leaf stage of the plant (BBCH 14) and thinning of the weaker plants was done a week later. The amount of fertilizers used for each pot was 20 ml along with 100 ml of water, which were mixed in separate containers, and after adding to the soil for the penetration of microorganisms, irrigation was done again. Fertilizers used in this experiment were obtained from Kusheprovan Zistfanavar Biotechnology Company and maize seeds were obtained from Yekan Seed Company. For planting, 12 kg pots with a diameter of 25 cm and a height of 30 cm were used, which were filled with a ratio of 1:3 of sieved soil with loam texture and sand. The day and night temperatures of the greenhouse were 30 and  $20\pm5$  °C, respectively. The seeds were planted at a depth of 4 cm in the soil and 4 seeds were planted in each pot with proper distance, and after establishment in the 4-leaf stage, they were thinned to two plants [10]. In two growth stages



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(BBCH 18 and 53; 8-leaf stage and tasseling stages, respectively), three uniform plants were selected for further measurements [11].

Irrigation treatments were applied by weighing and watering the pots and then based on the day [12]. The pots were watered every four days at 90% FC and every eight days at 60% FC [13]. The first irrigation was done immediately after planting and once every four days until the establishment of the plant, and irrigation treatments were applied to all the pots after fertilizer treatments were applied.

Gas exchange variables were measured using a portable photosynthesis system (LCA-4) on the youngest and fully developed leaves of the plant at the two stages of 8 leaves and tasseling. The total content of leaf pigments was determined using the method of Lichten Thaler and Welburn (1983) using a spectrophotometer (SP2000, Amrican). The leaf proline content was measured according to the method of Bates et al. (1973). It was used to measure the proline content using a spectrophotometer at a wavelength of 520 nm (SP2000, Amrican) [12].

Statistical analysis and drawing graphs were performed using SAS v. 9.4 and Excel. The mean comparison was done using the LSD test with a probability level of 5%.

#### **Results Discussion**

According to the results, the effect of biofertilizers on photosynthetic parameters, including net photosynthesis and transpiration, was significant only at the 8-leaf stage and on the intercellular  $CO_2$  concentration (Ci) concentration parameter at the tasseling stage (Table 1). The results showed that Nitrobacter treatment increased photosynthesis and transpiration at the 8-leaf stage by 37% and 27%, respectively. Also, a 38% increase in the intercellular  $CO_2$  concentration was observed with the application of biofertilizers in combination at the tasseling stage (Table 2). The effect of drought stress was also significant on the net photosynthesis, Intercellular  $CO_2$  Concentration, and transpiration rate in the tasseling stage (Table 1). In general, during both growth stages, under drought stress conditions, these parameters, as well as leaf stomatal conductance, decreased significantly (Table 3).

 Table 1. Analysis of variance results of photosynthesis, photosynthetic pigments, and leaf proline content during two

 harvest stages under drought stress conditions and application of biofertilizers in maize SC704

S.O.V	df -	А		Ci		Е		gs	
		8-leaf	Tasseling	8-leaf	Tasseling	8-leaf	Tasseling	8-leaf	Tasseling
Block	2	0.327 <sup>ns</sup>	0.584 <sup>ns</sup>	997.1 <sup>*</sup>	14520 <sup>ns</sup>	0.022 <sup>ns</sup>	0.001 <sup>ns</sup>	0.0009 <sup>ns</sup>	0.000004 <sup>ns</sup>
<b>Biofertilizer (B)</b>	4	$0.444^{*}$	0.187 <sup>ns</sup>	275.5 <sup>ns</sup>	$22022^{*}$	$0.026^{*}$	$0.025^{**}$	0.001 <sup>ns</sup>	0.001 <sup>ns</sup>
Drought stress (S)	1	0.124 <sup>ns</sup>	$1.158^{*}$	158.9 <sup>ns</sup>	57460**	0.025 <sup>ns</sup>	$0.023^{*}$	$0.0007^{ns}$	0.001 <sup>ns</sup>
B×S	4	0.035 <sup>ns</sup>	0.145 <sup>ns</sup>	230.6 <sup>ns</sup>	9301 <sup>ns</sup>	0.004 <sup>ns</sup>	$0.005^{ns}$	$0.0003^{*}$	$0.006^{**}$
Error	18	0.115	0.183	162.7	5282	0.006	0.003	0.0002	0.001
CV (%)		12.6	25.1	3.8	19.0	15.4	16.2	1.4	2.8

			Continuation	of Table 1				
S.O.V	df	Chlor	ophyll a	Chlor	ophyll b	Proline		
5.U.V	ai	8-leaf	Tasseling	8-leaf	Tasseling	8-leaf	Tasseling	
Block	2	0.010 <sup>ns</sup>	0.032 <sup>ns</sup>	0.002 <sup>ns</sup>	0.0001 <sup>ns</sup>	2.61 <sup>ns</sup>	0.078 <sup>ns</sup>	
<b>Biofertilizer</b> (B)	4	0.049 <sup>ns</sup>	0.011 <sup>ns</sup>	$0.004^{ns}$	0.002 <sup>ns</sup>	0.916 <sup>ns</sup>	0.429 <sup>ns</sup>	
Drought stress (S)	1	0.018 <sup>ns</sup>	0.020 <sup>ns</sup>	0.004 <sup>ns</sup>	$0.000004^{ns}$	6.42 <sup>ns</sup>	$0.968^{*}$	
B×S	4	$0.065^{*}$	$0.046^*$	0.003 <sup>ns</sup>	0.003 <sup>ns</sup>	7.77**	$0.884^{**}$	
Error	18	0.018	0.010	0.001	0.002	1.595	0.155	
CV (%)		8.1	7.0	3.5	4.2	21.1	16.5	

ns, \* and \*\*; represent non-significant, significant at 1% and significant at 5% respectively. A (Photosynthetic Rate), C<sub>i</sub> (Intercellular CO<sub>2</sub> concentration), E (Evapotranspiration) and g<sub>s</sub> (stomatal conductance)

Table 2. Mean comparison of the effects of biofertilizers on leaf photosynthesis and the photosynthetic pigments content
during two harvest stages in maize SC704

	Α μmol m <sup>-2</sup> s <sup>-1</sup>		C <sub>i</sub> vpm		$E mmol m^{-2} s^{-1}$		g <sub>s</sub> mmol m <sup>-2</sup> s <sup>-1</sup>		Chlorophyll b mg/gFW	
Biofertilizer treatments										
	8-leaf	Tassel ing	8-leaf	Tasseli ng	8-leaf	Tasseli ng	8-leaf	Tassel ing	8-leaf	Tassel ing
Control	6.29 <sup>ab</sup>	1.88 <sup>a</sup>	319.2ª	337.8 <sup>b</sup>	0.471 <sup>b</sup>	0.451ª	0.135 <sup>ab</sup>	0.255 <sup>a</sup>	0.13 <sup>a</sup>	0.15 <sup>a</sup>
Nitrobacter	8.62 <sup>a</sup>	1.27 <sup>a</sup>	333.3ª	416.3 <sup>ab</sup>	0.601 <sup>a</sup>	0.345 <sup>bc</sup>	0.186 <sup>a</sup>	0.330 <sup>a</sup>	0.21 <sup>a</sup>	0.17 <sup>a</sup>
Phosphopowerbacter	4.29 <sup>b</sup>	2.90 <sup>a</sup>	333.1ª	327.4 <sup>b</sup>	0.432 <sup>b</sup>	0.292 <sup>c</sup>	0.108 <sup>b</sup>	0.292 <sup>a</sup>	0.24 <sup>a</sup>	0.19 <sup>a</sup>
Potashpowerbacter	6.44 <sup>ab</sup>	2.51ª	334.0 <sup>a</sup>	362.3 <sup>b</sup>	0.543 <sup>ab</sup>	0.391 <sup>ab</sup>	0.156 <sup>ab</sup>	0.350 <sup>a</sup>	0.26 <sup>a</sup>	0.25 <sup>a</sup>
mixing treatments	6.15 <sup>ab</sup>	2.10 <sup>a</sup>	322.7ª	468.6 <sup>a</sup>	0.468 <sup>b</sup>	0.301 <sup>bc</sup>	0.128 <sup>b</sup>	0.301 <sup>a</sup>	0.16 <sup>a</sup>	0.17 <sup>a</sup>
LSD	2.93	2.76	21.2	120.7	0.12	0.09	0.05	0.12	0.14	0.17

The same letters are not significantly different. LSD 5%. A (Photosynthetic Rate), C<sub>i</sub> (Intercellular CO<sub>2</sub> concentration), E (Evapotranspiration), and g<sub>s</sub> (stomatal conductance)



photosynthetic pigments during two harvest stages in maize SC704											
Levels of irrigation	А		Ci		Е		gs		Chlorophyll b		
	$\mu mol m^{-2} s^{-1}$		vpm		$mmol m^{-2} s^{-1}$		$mmol m^{-2} s^{-1}$		mg/gFW		
Levels of it figation	8-leaf	Tassel ing	8-leaf	Tasseli	8-leaf	Tasseli	8-leaf	Tasselin	8-	Tasselin	
				ng		ng		g	leaf	g	
Normal irrigation	6.59 <sup>a</sup>	2.85 <sup>a</sup>	330.9 <sup>a</sup>	420.0 <sup>a</sup>	0.527 <sup>a</sup>	0.379 <sup>a</sup>	0.151 <sup>a</sup>	0.325 <sup>a</sup>	0.22 <sup>a</sup>	0.19 <sup>a</sup>	
Drought stress	6.09 <sup>a</sup>	1.50 <sup>b</sup>	325.7ª	339.6 <sup>b</sup>	0.476 <sup>a</sup>	0.330 <sup>b</sup>	0.133ª	0.289ª	0.18 <sup>a</sup>	0.18 <sup>a</sup>	
LSD	1.86	1.75	13.4	76.5	0.08	0.06	0.03	0.07	0.09	0.10	

 Table 3. Mean comparison of the average effect of Irrigation levels on leaf photosynthesis and the content of photosynthetic pigments during two harvest stages in maize SC704

The same letters are not significantly different. LSD 5%. A (Photosynthetic Rate), C<sub>i</sub> (Intercellular CO<sub>2</sub> concentration), E (Evapotranspiration), and g<sub>s</sub> (stomatal conductance)

Stomata play a significant role in preventing water loss and facilitating the absorption of carbon dioxide during the process of photosynthesis in plants. With drought stress in the plant, the stomata tend to close and this reaction is induced in the early stages by abscisic acid (ABA) of the root to reduce transpiration from the leaf surface. The closing of the stomata under drought stress conditions ultimately leads to the reduction of leaf stomatal conductance and the discharge of Intercellular  $CO_2$  concentration (Ci). Also, reduced transpiration and increased leaf temperature were mentioned as effects of enhanced stomatal resistance. Therefore, the decrease in photosynthesis under drought stress conditions may be attributed to the closure of stomata and the reduction in stomatal conductance [1].

In a study, it was shown that rice plants under drought stress conditions and lack of inoculation with growthpromoting bacteria led to a decrease in water status, the amount of chlorophyll a and b, as well as a decrease in stomatal conductance, which indicates the negative effects of drought stress on rice. This reduction in growth parameters is due to the reduction of cell divisions caused by drought [14].

According to the results, the interaction of biofertilizers and drought stress on stomatal conductance was significant in both the 8-leaf stage and the tasseling stage (Table 1).

At the 8-leaf stage, under normal irrigation conditions, Nitrobacter and Potashpowerbacter treatments had the greatest effect on increasing stomatal conductance, and there was no significant difference between them. Under drought stress conditions, all biofertilizer treatments showed better performance than the control treatment, but all treatments except Nitrobacter treatment decreased stomatal conductance under drought stress conditions, and Nitrobacter treatment was better able to moderate water deficit conditions, and leaf stomatal conductance did not change much (Figure 1-A)

In the stage of tasseling, under normal irrigation conditions, Nitrobacter treatment showed the greatest effect on increasing stomatal conductance, and there was no significant difference between treatments under drought stress conditions. Therefore, it can be said that Nitrobacter treatment was more effective during plant growth and at different irrigation levels. In the investigation carried out during the growth process of the plant under normal irrigation conditions and without the use of biofertilizer, the stomatal conductance decreased but showed a significant increase under drought stress conditions (Figure 1-A).

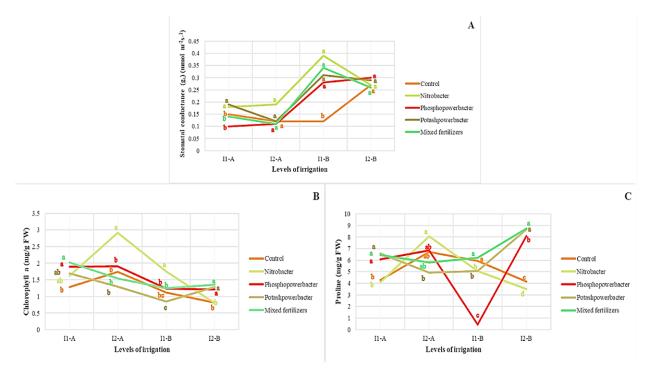




Figure 1. The effects of different levels of irrigation and application of biofertilizers (no application of biofertilizers, Nitrobacter, Phosphopowerbacter, Potashpowerbacter, and mixed fertilizers) on the amount of stomatal conductance (A), chlorophyll a (B) and proline (C) in leaves at different levels of irrigation in the 8-leaf stage (I1-A: normal irrigation and I2-A: drought stress) and the tasseling (I1-B: normal irrigation and I2-B: drought stress) in maize SC704

In a similar study, it was shown that leaf stomatal conductance during the growth period of the plant has a decreasing trend, and the highest stomatal conductance was obtained under conditions of full irrigation and inoculation with *Azospirillum*. Jalili et al. (2012) reported that the inoculation of canola seeds with growth-promoting bacteria increased the stomatal conductance. Mia et al. (2010) stated that growth-promoting bacteria increase the stomatal conductance of plants, and the improvement in the growth response of seedlings receiving *Azospirillum* inoculation treatments indicates an increase in drought tolerance in host plants [8].

According to the results of the analysis of variance, the interaction effect of bacteria and irrigation levels on chlorophyll a and proline content of leaves in both growth stages was significant at 5% and 1% levels, respectively (Table 2). In drought stress conditions, in the 8-leaf stage of corn plants, on average, Nitrobacter treatment and in tasseling stage, mixed fertilizer treatment had the greatest effect on the increase of proline and chlorophyll a (Figure 1-B&C).

In the first stage, Nitrobacter treatment under drought stress conditions caused a 67% increase in the content of chlorophyll a and a 19% increase in the proline content of leaves compared to the control treatment, and in the second stage of harvest, the treatment with a mixture of fertilizers caused a 64% increase in the content of chlorophyll a and a 110% increase in the proline content of leaves compared to the treatment (Figure 1-B&C).

The comparison of means showed that under drought stress conditions at the tasseling stage, most treatments exhibited an increase in leaf proline content, while there was a decrease in chlorophyll a content. Additionally, the application of biofertilizers under drought stress conditions, particularly during the early stages of plant growth, had a significantly positive effect compared to its application under normal irrigation conditions (Figure 1-B&C).

The inoculation of PGPR (Plant Growth-Promoting Rhizobacteria) can stimulate plant growth through the production of plant growth regulators by bacteria in the rhizosphere [9]. Molecules such as amino acids like proline, certain phytohormones, and chlorophyll all share a common feature in their structure, which is the presence of the nitrogen element. If nitrogen is available to the plant in sufficient amounts, it can have a significant impact on increasing plant performance [15]. The application of nitrogen-fixing bacteria, including *Azospirillum*, which is one of the beneficial rhizobacteria commonly used in Nitrobacter biofertilizers, can play a significant role in enhancing plant growth and increasing drought stress tolerance. Therefore, the increase in proline and chlorophyll a during the early growth stages under drought stress conditions, as observed in Nitrobacter treatment (Figure 1-B&C), may involve hormonal interactions that regulate natural growth and responses to drought stress [16].

The application of biofertilizers and different irrigation levels did not have a significant impact on the chlorophyll b content. However, in both stages, there was a general reduction in its level under drought stress conditions. It was observed that the use of biofertilizers led to an increase in chlorophyll b content compared to the control treatment.

The negative effects of drought stress on the growth and productivity of maize include the reduction of leaf size, chlorophyll content, and nutrient content [7]. The use of PGPRs in plants has a positive effect on increasing yield and reducing drought stress through various mechanisms, such as reducing oxidative stress, increasing proline content, enhancing photosynthetic capacity, improving nutrient content, promoting vegetative growth, and increasing the production of growth regulators such as abscisic acid, auxin, gibberellin, and cytokinin [14].

In drought stress conditions, the breakdown of proteins accelerates, leading to an increase in the production of amino acids such as proline and amides. The accumulation of osmolytes, including proline, persists for several days, and its concentration may increase to up to 10% of the dry weight of the entire leaf. Since glutamate is a precursor for the synthesis of chlorophyll and proline, under drought stress conditions, this substance is converted into proline, leading to a decrease in the amount of chlorophyll. Steele et al. (1991) reported a positive correlation between the intercellular CO2 concentration and the concentration of chlorophyll b. In cultivars with higher amounts of chlorophyll b, carbon dioxide processing is more efficient [1].

In a study, it was demonstrated that an *Azospirillum* strain assists plants in maintaining a higher level of proline content when compared to drought-stressed plants and untreated plants. The high intracellular proline accumulation in *Azospirillum* inoculated maize plants may be attributed to increased synthesis and reduced proline degradation under drought stress in different species. Also, in another study, it was demonstrated that the inoculation of plants with a *Pseudomonas* bacterial strain significantly increases the accumulation of osmolytes [6].

Shinde and Khade (2019) found that the combination of *Azotobacter* and phosphate-solubilizing bacteria (PSB) had the most significant impact on the levels of chlorophyll a and total chlorophyll in maize plants [17].

The relative content of chlorophyll and photosynthetic pigments was strongly influenced by the interaction between PGPR and irrigation treatments. Abd El-Mageed et al. (2022) reported that the relative content of chlorophyll and photosynthetic pigments was significantly affected by irrigation, PGPR treatments, and their interaction. In the study, rice plants inoculated with PGPR showed an increase in the content of chlorophyll a by 10.5% and chlorophyll b by 14.3% compared to non-inoculated plants [14].

The results of a survey on cotton plants showed that the combination of nitrogen-fixing and phosphate-dissolving rhizobacteria, including strains from the genera *Azospirillum*, *Bacillus*, *Pseudomonas*, and *Azotobacter*, had the most



significant effect on the levels of chlorophyll a and b. Studies have shown that the inoculation of maize plants with strains of the genus *Pseudomonas* led to a significant improvement in plant chlorophyll content under drought stress, as well as an increase in proline content [8]. In another study involving maize and sorghum plants, the greatest increase in chlorophyll content was achieved with *Bacillus* and *Pseudomonas* strains [18].

#### Conclusions

In general, drought stress gradually decreased the photosynthetic parameters and the pigment levels throughout the growth stages. The decline in photosynthesis and chlorophyll content was consistently accompanied by an increase in proline. Although drought negatively impacts plant performance, the application of pesticides can mitigate its adverse effects. It appears that corn can establish a beneficial symbiosis with growth-promoting bacteria in the rhizosphere, and the use of a combination of different bacterial strains could be the most effective approach to enhance the physiological characteristics of the plant under drought stress conditions.



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