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Role of exopolysacharide bacteria in improving the tolerance of *Zea mays* (L.) Seedlings to drought stress

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Abstract

In this study, 20 *Bacillus* and 20 *Pseudomonas* bacteria were isolated from arid and semiarid soil samples. Bacillus and Pseudomonas bacteria are soil-dwelling bacteria that can promote plant growth. They are known to produce exopolysaccharides (EPS), which can help plants retain water and tolerate drought stress. The isolates were morphologically and microscopically characterized and tested for their ability to produce exopolysaccharides. The isolates that were most capable of producing exopolysaccharides were used as biofertilizer to improve drought tolerance of maize seedlings. The seedlings were irrigated every 24, 48, or 72 h. The results showed that biofertilizers containing the most EPS-producing Bacillus and Pseudomonas isolates, B. subtillus, B. brevibacillus, P. putida, P. fluorescens, significantly improved the transpiration rate, stress tolerance index, drought tolerance, chlorophyll stability and membrane damage index of maize seedlings.

Keywords: drought stress, bacillus, pseudomonas, Exopolysaccharide.

Introduction

Water scarcity is a major issue in the face of climate change and water scarcity. This has forced researchers and farmers to find alternatives and strategies to achieve food security for the growing population, which is considered one of the major current risks [1]. Thousands of hectares of land are left fallow every year worldwide due to the effects of drought. In Iraq, drought is one of the most important factors affecting crop production due to poor water management and drought conditions. One of the strategies used to improve plant growth and tolerance to drought stress is the use of plant growth-promoting bacteria (PGPB). These bacteria efficiently colonize plant roots and are present in the soil and improve their growth and production through several direct and indirect mechanisms [2]. The first is a symbiotic relationship with the plant, and the other is free-living [3] which stimulates plant growth-promoting compounds such as hormones (auxins, gibberellins and cytokinins) which help improve the ability of plants to tolerate stresses such as drought stress [4]. *Bacillus* and *Pseudomonas* are bacterial genera present in the soil. Some species of this genus have the can produce exopolysaccharide (EPS). Bacteria

secrete exopolysaccharide (EPS) to survive and live under stress conditions [5]. It allows them to maintain a higher water content, which helps them survive and live under conditions of low water content in the soil. The use of this type of bacteria as a bacterial inoculant or biofertilizer to reduce the use of chemicals, which are among the most important environmental pollutants of the ecosystem [6,7,8] . Therefore, this study aims to improve the tolerance of maize seedlings to drought, which is one of the important crops in Iraq, using a relatively easy and inexpensive method, which is inoculating seeds with two bacterial strains of Bacillus and two strains of Pseudomonas to determine the ability of the isolates to alleviate drought stress and the efficiency of the bacteria to improve plant growth for some indicators.

Materials and Methods

Collection and preparation of soil Sample

40 strains of bacteria were isolated from a soil sample from the root zone of cultivated lands in semi-arid areas. The soil characteristics are presented in Table 1. The samples were collected according to the method described by (9). where a small shovel was used to clean the soil. The soil was dug to a depth of 15 cm from the ground surface, and the samples were placed in clean, sterile tubes and transported to the laboratory as described by [10].

Table (1) : Soil characteristics

Test	EC ds m^{-1}	T.D.S ppm	pH	O.M %	NO_3^- ppm	NH_4^+ ppm	Cl^- ppm	K^+ ppm	Na^+ ppm	Mg^{++} ppm	Ca^{++} ppm
Sample	3.1	1985	8.1	1.8	20.1	40	2130	176	414.2	70.5	360

Isolation and diagnosis of bacteria from soil

strains ware isolated from soil samples according to the method of Becking (1981), where Nutrient agar and King-B medium ware used, and the bacteria were purified using the method of [11]. The appearance of colonies, pigmentation, Gram stain, cell morphology, and cyst formation were evaluated. [12]

Estimation of exopolysaccharide

The method described by [13] was followed in extracting exopolysaccharides. After the end of the incubation period (48 hours), the resulting medium was placed in a water bath at 91 degrees Celsius for 11 minutes, and the cells were separated from the fermentation medium using a centrifuge at 8000 speed. RPM for 11 minutes. The cells were dried for the purpose of calculating the dry weight of the biomass, while the filtrate was used. Trichloroacetic acid (TCA) was added to it at a concentration of 8% (volume/volume) and left for 3 hours at 4°C. Then it was quickly centrifuged. 8000 rpm for 11 minutes in to precipitate the protein present in the medium. After that, the sediment was discarded and the filtrate was taken. Two volumes of refrigerated ethanol at a

concentration of 95% were added to it and left at 4°C for 24 hours. Then the EPS was separated by centrifugation at a speed of 12000 rpm for 12 minutes. The filtrate was discarded and the sediment was dried at 41°C for 24 hours for the purpose to calculate calculating the dry weight. [14]

sterase and oxidase into a single enzymatic reagent for the detection of total cholesterol; by using the spectrophotometric method [15].

Preparing the bacterial inoculated

The bacterial isolates preserved on Slant were activated by growing them on a suspension of bacterial inoculum prepared for the isolates for seed treatment. They were activated by mixing one colony of each bacterial strain in nutrient Broth medium and incubating the culture for 24 hours at 37°C. [16].

Inoculation of maize (Zea mays L.) with EPS producing bacteria

The bacterial isolates found on Slant, preserved, isolated and previously characterized, whose exopolysaccharides and their ability to tolerate drought were previously determined, were activated. They were activated on N.B. After 24 hours, the isolates were taken and inoculated with lurian bacteria (L.B) and then placed in a vibrating incubator at a temperature of 30 for 72 hours. Then the isolates were centrifuged at (300 revolutions/minute for 10 minutes). After that, the filtrate is discarded and the sediment (pellet) is taken. Then we add small amounts of water to the sediment in to obtain a reading (OD = 1) at 660 nano, which is equivalent to (10-10) CFU ml-1 (colony forming unit) . The sterilized seeds were then soaked in this bacterial inoculum for 2-3 hours and planted in soil. [17,18,19]

Transpiration rate

The transpiration rate was measured by measuring water loss. The experiment was carried out by the weighing method, the seedlings were placed in glass beakers containing 20 ml of distilled water. Drops of oil were added to the surface of the water to prevent water loss due to evaporation, and then the initial weight was calculated. Then the transpiration rates were estimated for 6 days. On the sixth day, the weight of each (plants + beaker + water + oil drops) was recorded to determine the amount of water lost from each treatment.

Low weight indicates the loss of water by the plant (due to transpiration). The transpiration rate is measured as the amount of water lost expressed in units of grams/day/plant. [20]

Stress tolerance index

The Stress Tolerance Index (STI) was calculated for both seedlings and cuttings according to the following equation: [21]

$$STI = (Ypi \times Ysi) / Ypi^{2}$$

- Ysi = Dry weight of cuttings.
- Ypi = Dry weight of cuttings treated.

Stress intensity

The severity of stress was measured according to the method of Fischer & Murrer, (1978) and based on the dry weight of plant samples [22] and was calculated from the following equation:

$$\text{Stress intensity} = 1 - (\text{Ysi} / \text{Ypi})$$

Cell membrane stability

The cell membrane stability index was determined based on the number of ions contained in Double distilled water (ddH_2O) (according to Guo et al. (2007) [23] and Singh et al. (2017)[24]. Two hundred mg of leaf were cleaned and cut with a length of 5mm and put in a tube containing 20 ml ddH_2O. The tubes were incubated for 12 hours at room temperature with constant lighting The value of the conductivity of the solution is measured with an Electro-conductivity meter (EC-meter CM-21P, TOA Corp, Japan) as the initial conductivity (EC1), then the solution is boiled to 100ºC for 15 minutes and cooled to 25 ºC then the EC is measured as EC2 and ISM determined by the following formula : [25,26]

$$\text{ISM (\%)} = \left[1 - \frac{\text{EC1}}{\text{EC2}}\right] \times 100\%$$

Extraction of chlorophyll

Chlorophyll was estimated by Hiscox and Israelstam method (1979), which involves the estimation of plant chlorophyll without maceration. 100 mg of leaves were washed with DW and chopped. These chopped leaves were taken in test tubes in triplicates and 10 ml DMSO was added to each test tube. Test tubes were incubated in a water bath at 60 0 C for 15 min. The absorbance of the solutions was recorded at 663, 645 nm on UV-visible double - beam spectrophotometer. (Model. ELIT, BioEra Ltd.)

For evaluation of chlorophyll stability index, chlorophyll extract was prepared from 100 mg fresh plant material as well as 100 mg fresh plant material kept in an oven at 600 C for 1 hour using DMSO as a solvent.

It was also calculated periodically using the following formula: [27,28]

$$\text{Chlorophyll Stability Index} = \frac{Total\ chl.content\ of\ heated\ plant\ material}{Total\ chl.content\ of\ fresh\ plant\ material} \times 100$$

The Statistical Analysis

Acompletely Randomized Design was used, data were analyzed statistically using a computer, and L.S.D values were used to compare the means of the treatments at a probability level of (0.05) in all experiments [29].

Results and Discussion

Bacterial isolation

40 bacterial isolates were obtained from 40 soil samples suffering from drought-affected desert lands west of Karbala.a Governorate, through the process of isolating and purifying these isolates and cultivating them on selective culture media in the laboratories of the College of Science at the University of Kerbala.

Microscopic and biochemical diagnosis

Bacillus spp., which were grown on Nutrient agar, showed dry, white, Gram-positive, irregular-edged, rod-shaped, spore-forming colonies, and some of them were anaerobic.

When conducting biochemical tests on the isolates of Bacillus Spp. bacteria obtained in this study, it turned out that all of the isolates were positive for the catalase, oxidase, and indole tests. It can grow in 1% of both NaCl and Glycerol. While it was not able to grow in 0.1% phenol.

Pseudomonas spp. bacteria on the King's B medium showed colonies with a greenish-yellow halo around the colony, negative for Gram stain, viable at 4-42 °C, and rod-shaped bacteria. The appearance of a greenish-yellow halo around the colonies in the dish under normal light is evidence that the test is positive for fluorescein stain.,who indicated that after incubating the bacteria for 48 hours at 28 °C, small colonies with smooth edges and a convex surface were purified and showed clear fluorescence under ultraviolet light on King's B medium.

When conducting biochemical tests on the isolates of *Pseudomonas spp* bacteria obtained in this study, it turned out that all of the isolates were positive for the catalase, oxidase, and indole tests. It has the ability to grow in 1% of both NaCl and Glycerol. While it was not able to grow in 0.1% phenol.

Estimated of exopolysaccharide

All bacterial isolates were examined. *Bacillus Spp.* and *Pseudomonas Spp.* to test their ability and determine the most efficient among all 40 isolates to produce EPS by calculating the dry weight of EPS as shown in Table No. (1)

The results of the dry weight of EPS mg/L showed variation in its ability to produce exopolysaccharides, as production ranged for *bacillus* from (1.02) mg/L for isolate B6 to (2.22) mg/L for isolate B19. and production ranged for *Pseudomona*s from (1.16) mg/L for isolate Ps9 to (3.04) mg/L for isolate Ps18

Table (2): Screening of bacterial isolates *Bacillus spp.* to produce extracellular polysaccharides

Symbol of isolation	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
Dry weight of EPS mg/L	950	678	720	816	633	700	912	830	650	733	600	618	690	708	906	588	650	733	600	618
Dry weight of cells mg/L	500	350	410	420	402	680	500	493	424	406	320	461	483	486	700	340	330	415	350	590
Dry weight of EPS/dry weight of cells	1.9	1.93	1.75	1,94	1.57	1.02	1.82	1.68	1.53	1.8	1.87	1.34	1.42	1.45	1.29	1.72	1.51	1.91	2.22	1.35

Table (3): Screening of bacterial isolates *Pseudomonas* spp. to produce extracellular polysaccharides

Symbol of isolation	Ps1	Ps2	Ps3	Ps4	Ps5	Ps6	Ps7	Ps8	Ps9	Ps10	Ps11	Ps12	Ps13	Ps14	Ps15	Ps16	Ps17	Ps18	Ps19	Ps20
Dry weight of EPS mg/L	700	380	590	160	660	490	520	560	620	580	492	556	540	530	751	460	570	670	573	516
Dry weight of cells mg/L	360	140	240	110	220	245	310	210	530	240	252	341	330	390	412	240	311	220	233	342
Dry weight of EPS/dry weight of cells	1.9	2.71	2.45	1.5	3	2	1.67	2.6	1.16	2.4	1.95	1.63	1.6	1.35	1.82	1.91	1.83	3.04	1.72	1.5

Effect of bacterial inoculum on maize sedling

After obtaining the results of EPS production tests and for the 20 isolates of *Bacillus Spp* and 20 isolates of *Pseudomonas spp*, the two isolates of Bacillus B4 and B18 were selected,and two isolates of *Pseudomonas* Ps18 and Ps5 were selected as these isolates were the highest in EPS production, to determine the effects of these isolates on the following criteria:

The effect of isolates inoculum on the rate of transpiration

The results of this study also showed the measurement of transpiration rates gm/hour/plant for maize plants after treating the seeds with different types of biological bacterial fertilizers. The results showed that all types of bacteria contributed to a decrease in the transpiration rate when the plant was exposed to drought conditions compared to the plant under control.

The effect of 24 hours of irrigation on transpiration rates was greater than the effect of irrigation for 48, 72 hours for each of the bacteria *Bacillus, pseudomonas*.

However But when the irrigation rates increased to 72 hours, a sharp and variable decrease in the transpiration rate appeared in the presence of bacterial fertilizers.

The transpiration rate after 72 hours of irrigation was the lowest when treating maize plants with bacteria of the type P. *fluorescens* & *B. subtillus*.

The highest value of transpiration in the same irrigation period was in the presence of bacteria of the type *P. putida* & B. *Brevibacillus* .

Table (4) Transpiration rate g/hour/plant of maize plants after treating the seeds with isolates of *Bacillus spp.* bacteria

Treatment	Control Un inoculated	*B. subtillus*	*B. Brevibacillus*
watering 24 hours	0.18	0.26	0.25
watering 48 hours	0.14	0.22	0.14
watering 72 hours	0.09	0.18	0.19

Table (5) : Transpiration rate g/hour/plant of maize plants after treating the seeds with isolates of *Pseudomonas spp.* bacteria

treatment	Control uninoculated	*P. fluo-rescens*	*P. putida*
watering 24 hours	0.18	0.29	0.25
watering 48 hours	0.14	0.259	0.22
watering 72 hours	0.09	0.16	0.21

The effect of isolates inoculum on stress intensity

Table 6 shows that all maize plants after treatment with biofertilizers suffered less stress when exposed to drought conditions than after irrigation, as treating the seeds with biofertilizers led to a reduction in the stress severity in maize plants under drought conditions.

As for irrigation for different periods, the greatest effect was at 72 hours of irrigation, followed by 48 hours and then 24 hours of irrigation. When comparing the stress rate of maize plants under control conditions and without treatment and drought.

P. putida & B. Brevibacillus were the most effective bacteria in reducing stress severity when treated for 72 hours of drought.

These results indicate that treating seeds with biofertilizers is effective in reducing stress severity in maize plants under drought conditions. This effect may be due to the ability of bacteria to improve water and nutrient uptake by plants, as well as stimulate the production of antioxidants that help protect plants from stress. The duration of irrigation of maize plants after seed treatment may also play a role in reducing stress severity. The effectiveness of different types of bacteria in reducing stress severity may vary.[30].

Table (6) : The stress intensity of maize plants after treating the seeds with two isolates of *Bacillus* spp.

treatment	Control uninoculated	*B. subtillus*	*B. Brevibacillus*
watering 24 hours	0.17	0.19	0.21
watering 48 hours	0.16	0.18	0.18
watering 72 hours	0.08	0.17	0.15

Table (7) : The stress intensity of maize plants after treating the seeds with two isolates of *Pseudomonas* spp.

treatment	Control Un inoculated	*P. fluorescens*	*P. putida*
watering 24 hours	0.17	0.19	0.19
watering 48 hours	0.16	0.14	0.17
watering 72 hours	0.08	0.13	0.11

The effect of isolates inoculum on stress tolerance index

Table (8) shows the difference in the effectiveness of biofertilizers and their effect on plant tolerance to stress, as the stress tolerance of all maize plants increased when exposed to drought conditions. Treating seeds with biofertilizers also led to an increase in the stress tolerance coefficient under drought conditions. The results also indicated that the greatest effect came after 72 hours of irrigation, then 48 hours of irrigation, then 24 hours of irrigation. The isolates of B. Brevibacillus and P. putida showed the most effectiveness in increasing the stress tolerance coefficient during the 72-hour irrigation period, while the effect of both B. Brevibacillus and P. putida was the least effective in increasing the tolerance coefficient when irrigated for 24 hours.

Table (8): Stress tolerance index of maize plants after seed treatment with two isolates of *Bacillus* spp.

Treatment	Control uninoculated	*B. subtillus*	*B. Brevibacillus*
watering 24 hours	0.84	0.79	0.55
watering 48 hours	0.92	0.75	0.71
watering 72 hours	1.12	0.91	0.92

Table (9) : Stress tolerance index of maize plants after seed treatment with two isolates of *Pseudomonas* spp.

treatment	Control uninoculated	*P. fluo-rescens*	*P. putida*
watering 24 hours	0.84	0.67	0.42
watering 48 hours	0.92	0.71	0.61
watering 72 hours	1.12	0.95	0.97

The effect of isolates inoculum on cell membrane stability

Table (10) shows that all maize plants suffered from a decrease in plasma membrane stability when exposed to drought conditions. Compared to untreated seeds and without drought, treating the seeds with bacteria led to a decrease in plasma membrane stability depending on the type of bacteria. The rate of effect of bacteria on plasma membrane stability was greatest at 72 hours of irrigation when treated with the bacterial isolate P. putida. The bacterial isolate B. subtillus was the best in raising membrane stability to the highest degree and improving plasma membrane stability at a 24-hour irrigation period. These results indicate that treating seeds with biofertilizers is effective in improving plasma membrane stability in maize plants under drought conditions. This effect is due to the ability of bacteria to improve the absorption of water and nutrients by plants, as well as stimulating the production of antioxidants that help protect the plasma membrane from damage.[31].

Table (10): Stability of the plasma membrane of maize plants after treating the seeds with two isolates of *Bacillus* spp.

Treatment	Control uninoculated	*B. subtillus*	*B. Brevibacillus*
watering 24 hours	30.63	62.73	32.77
watering 48 hours	34.17	39	39.63
watering 72 hours	52.13	40	55.43

Table (11): Stability of the plasma membrane of maize plants after treating the seeds with two isolates of *Pseudomonas* spp.

Treatment	Control uninoculated	*P. fluorescens*	*P. putida*
watering 24 hours	30.63	36.03	45.14
watering 48 hours	34.17	40.3	52.18
watering 72 hours	52.13	57.58	71.33

The effect of isolates inoculum on chlorophyll stability

The results of the current study showed that chlorophyll stability increased with the two bacterial isolates depending on the irrigation rate (either 24 hours, 48 hours or 72 hours) compared to untreated seeds and without irrigation. (Table 12) The increase in growth was greatest at 24 hours of irrigation, then 48 hours of irrigation, then 72 hours of irrigation. The results indicate that the bacterial isolate B. Brevibacillus contributed to a significant increase in chlorophyll stability, especially after irrigation for24 hours. The bacterial isolate P. putida was the best in raising the stability of chlorphyll to the highest degree and improving it with an irrigation period of 24 hours.

Table (12): Chlorophyll stability in maize plants after treating the seeds with two isolates of *Bacillus* spp.

treatment	Control uninoculated	*B. subtillus*	*B. Brevibacillus*
watering 24 hours	66.03	80.69	84.02
watering 48 hours	60.11	79.71	71.83
watering 72 hours	53.21	75.65	69.95

Table (13): Chlorophyll stability in maize plants after treating the seeds with two isolates of *Pseudomonas* spp.

Treatment	Control uninoculated	*P. fluorescens*	*P. putida*
watering 24 hours	66.03	80.69	83.64
watering 48 hours	60.11	74.71	81.62
watering 72 hours	53.21	71.61	72.55

Drought is a prominent abiotic stress that affects global food production, leading to economic losses in crops, especially cereals such as wheat, rice, and maize. This study investigated the abilities of strains of bacteria to mitigate the effects of drought stress on maize varieties to enhance their growth. The presence of bacteria in the root area leads to a beneficial exchange between the plant and the bacteria and leads to enhanced plant growth. Due to the richness of secretions released by plants, the rhizosphere constitutes a suitable place for abundant microbial activities. The use of beneficial microbes, especially rhizobacteria within the rhizosphere of plants that possess multifunctional growth-promoting factors and the ability to withstand abiotic stresses, is a cheap and alternative way to increase plant growth under abiotic stresses. Previous researchers indicated the application of PGPR reduces drought stress in plants. [32,33,34,35]

Maize was inoculated with two types of sugar-producing bacteria. The strains improved some plant growth parameters such as biomass. Inoculation with the isolated bacteria also improved the water use efficiency of the plant. [36,37]

The bacterial isolates enhanced plant growth under drought stress conditions by increasing soil moisture content. This was made possible by the ability of the rhizobacterial strains to produce exopolysaccharides that conserve soil moisture by increasing its water holding capacity and thus protecting bacteria and plant roots from desiccation [38]. Thus, the production of EPS by these microbes increased the ability of the soil to balance its water potential and maintain soil aggregation which enhanced nutrient uptake with the resulting growth of maize plants and protection from drought [39]. In terms of mitigating abiotic stresses such as drought, EPS-producing microbes are indispensable because they increase the water-holding capacity of the soil, thus alleviating stress on plants [39].

It is known that under drought conditions, plants adopt a strategy of increasing the length of the root system in to obtain moisture more efficiently, and thus the plant suffers from the consumption of mineral elements, nutrients and stored water surrounding the root. In the case of using rhizobacteria, a balance must be achieved between the amount of growth promoters they release and the need for external bioactive substances for the plants themselves. Bacteria maintain the moisture content of the plant to promote plant growth by balancing drought stress, transpiration rates, stress tolerance indicators, chlorophyll stability and plasma membrane, thus leading to an increase in plant root mass and improved growth, which is a more beneficial strategy for plant growth under drought stress. [40,41].

Exopolysaccharide-producing isolates

EPS production can be influenced by environmental factors such as nitrogen limitation, excess carbohydrates, and temperature. For example, Pseudomonas ncibii264 produces EPS to its maximum under nitrogen limitation with excess carbohydrates [42]. While genetic differences in *Pseudomonas spp*. are important factors affecting EPS production, different strains of *Pseudomonas spp*. can produce different types and amounts of EPS. For example, some strains may produce only one or two types of EPS, such as Pel, Psl, and alginate, while other strains may produce all three types [43]. Some mucoid strains of *Pseudomonas spp*. can produce excess amounts of alginate, which is beneficial in harsh environments. However, non-mucoid strains do not need to express alginate biosynthesis genes to form biofilms, using either Pel or Psl as the primary structural polysaccharide of the matrix. [44] Some strains of Pseudomonas spp. can tolerate and produce EPS in response to various abiotic stresses such as drought, temperature, and salt, with EPS composition and polysaccharide ratios increasing under stress conditions, which may lead to osmotic and thermotolerance in the bacteria. The choice of carbon source can also influence EPS production. For example, glycerol was found to be the best carbon source for EPS production in Pseudomonas putid under both stress and non-stress conditions. [45] EPS production can be affected by environmental factors such as the presence of heavy metals. For example, the presence of mercury can suppress EPS production in some isolates but stimulate it in others. Different strains of Bacillus spp ,Pseudomonas spp can produce different types and amounts of EPS. For example, some strains may produce more EPS in response to certain carbon sources or under specific growth conditions. The choice of carbon source can influence exopolysaccharide production. For example, Bacillus spp ,Pseudomonas spp grown on 4-hydroxybenzoic acid as the sole carbon source produced EPS, but the rate and extent of EPS production differed compared to growth on sugars. [46,47] The presence of heavy metals such as mercury can affect EPS production. Some isolates can grow and produce EPS in the presence of mercury, while others may not. These differences in EPS production are essential for understanding the roles of specific exopolysaccharides in biofilm formation, heavy metal tolerance, and plant growth promotion. [48] The difference in production may be because the antioxidant activity of EPS is a function of a combination of several factors since the antioxidant capacity of EPS mainly depends on its structural distinction and glycosidic linkage as reported [49]. It could also be due to other activities related to the presence of other antioxidant components in the crude EPS extract such as peptides, proteins, and trace elements [50].

Transpiration

Some studies suggest that some bacteria can indirectly reduce plant transpiration by enhancing soil moisture retention and nutrient uptake. For example, [51] reported that Bacillus subtilis inoculation reduced transpiration in wheat plants under drought stress.[52] Zlatev et al. (2016) found that drought stress significantly increased transpiration rates in sunflower plants. Plants under drought stress typically increase transpiration

rates in an attempt to absorb water from dry soil. However, this can lead to water loss and further stress. Drought leads to water stress maize plants, this is primarily due to limited water supply to the roots and increased transpiration rates [53].

Stress tolerance Index

It has been reported [54] that inoculation of maize plants with Pseudomonas putida under drought stress conditions improved fresh/dry weight ratio, dry matter content and grain yield compared to uninoculated plants. *Pseudomonas, putida* was able to solubilize large amounts of phosphate and improve seedling vigor under drought stress

Inoculation of maize plants with Bacillus spp. showed a decrease in oxidative stress markers such as malondialdehyde and hydrogen peroxide under drought conditions and thus reduced stress severity [55].

Stress Severity

B.bacillus bacteria stimulate plant growth and increase drought tolerance through direct and indirect means such as siderophore synthesis, increased plant hormones and improved nutrient motivation. These results are consistent with previous studies which reported that B.bacillus bacteria increase tolerance to water stress.[56]

Bacillus subtilis isolate proved superior in its ability to express antioxidant activity, leaf water potential, relative water content and drought-responsive gene expression. The bacterial isolates were good at secreting exopolysaccharides and showed biofilm formation, where all these factors increase the ability of inoculated seedlings to tolerate drought conditions [57]. Some studies have found that inoculation of *Arbidops.s* with B.bacillus bacteria improved tolerance to water drought stress, through increased transcription and regulation of drought-induced genes due to drought stress. Given these results, it can be used as a bioinoculation that effectively reduces damage to plants due to environmental stresses [58].

Plasma membrane

Increased membrane damage is associated with the accumulation of enzymatic and non-enzymatic antioxidants. Membrane damage caused by drought stress is due to oxidative stress damage. Ion leakage is less in seedlings inoculated with seeds compared to uninoculated seeds under stress conditions. This indicates that inoculation with bacterial isolates gave tolerance to plants or seedlings under drought stress. The permeability of leaf membranes of inoculated seedlings may be [59].

showed the least increase compared to drought-stressed seedlings and the damage rates increased by about (45%) in non-stressed seedlings and the positive correlation between drought stress and membrane damage was observed [60] where high leakage (high damage rate) was observed in drought-stressed maize plants compared to the control. The bacterial isolates in the current study stimulate the withdrawal of nutrients and elements from the soil, including potassium, as potassium is very important for drought tolerance in plants through its contribution to cell elongation, membrane stability, aquaporin activation, water absorption, stomata regulation and osmotic adjustment.

Chlorophyll

Drought stress reduces the biosynthesis of chlorophyll pigments (chlorophyll content), which results in a decrease in the level of photosynthesis rates [61] and the content of maize seedling leaves decreased under stress conditions in this study and the increase in photosynthetic pigments in maize seedlings inoculated with bacterial isolates may result from the activation or increase in the effectiveness of enzymes involved in the chlorophyll biosynthesis pathways and the limited production of free radicals (Reactive Oxygen species) or the increase in the solubility and availability of organic minerals such as Mg, N [62]

The results of the study are consistent with the results of previous studies that plant growth-promoting bacteria, including *Bacillus Spp*., achieve chlorophyll growth in maize plants, which is due to the increase in chlorophyll synthesis and nutrient balance. [63] In a previous study, inoculation with bacterial isolates led to an increase in chlorophyll, nitrogen and phosphorus content compared to uninoculated plants [33], which is also consistent with the current study.

Maize seedlings subjected to drought stress exhibited decreased tolerance, increased stress severity, and damage to plasma membranes and chlorophyll. The use of bacteria extract significantly alleviated these negative impacts, demonstrating its potential for enhancing drought resilience in maize.[64]

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