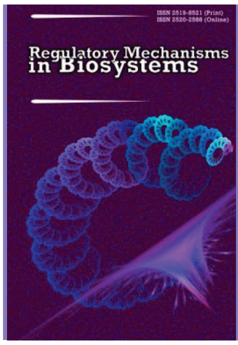

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Effects of bacterization on the development of *Zea mays* during droughts in the conditions of a vegetation experiment

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Sustainable agriculture is a concept developed in the late 20th century to promote the production of crops using the biological capabilities of cultivated organisms while minimizing the human impact on agrocenoses. In recent years, the discovery of new types of bacteria that positively impact plant growth has opened up opportunities for the development of effective microbiological preparations and they offer promising prospects for adaptive crop production, enabling farmers to adjust to changing environmental conditions while reducing reliance on anthropogenic inputs. Corn, as the third most important grain crop after wheat and rice, is sensitive to a lack of moisture. Therefore, understanding the mechanisms of adaptation and resistance of this plant to drought remains an urgent task. The aim of the work was to determine the effect of bacterization of *Zea mays* ("Early gourmet 121") seeds by strains of rhizobacteria on the development of corn under conditions of artificial drought in a vegetation experiment. Strains of bacteria with known properties useful for plant growth and development were used – *Bacillus cereus*, *Pseudomonas putida*, *Azospirillum brasilense* and *Azotobacter chroococcum*. The study investigated the indicators of seedlings and the content of mono-, oligo-, and soluble sugars in the leaves and the activity of polyphenol oxidase in the roots. The results showed that bacterization of seeds with suspensions of *P. putida* led to an increase in the length of roots in plants grown under moisture deficit conditions and significantly affected the above-ground parts. Positive effects on the mass of the above-ground parts were observed when seeds were bacterized with suspensions of *P. putida*, *A. chroococcum* and a mixture of bacterial suspensions and grown under sufficient irrigation. A similar positive effect occurred when seeds were bacterized only with a mixture of suspensions of the tested bacteria under drought conditions. Bacterization of corn seeds with *P. putida* or *A. brasilense* resulted in increased root mass and sugar accumulation in leaves when growing seedlings under conditions of moisture deficit. Additionally, inoculation of *P. putida* or *A. chroococcum* seeds increased the activity of polyphenol oxidase enzyme in seedling roots. While the bacteria showed a positive impact on corn development under some conditions, this effect was not observed when there was sufficient moisture. Therefore, we believe that a more promising approach for developing biological preparations based on beneficial microorganisms is to explore combinations of multiple soil bacteria strains. By identifying a mix of bacteria that can effectively stimulate plant growth in both stressed and favourable conditions, we can maximize the potential benefits for crop production. Such combinations of bacteria may enhance plant growth and yield not only during drought stress but also under optimal conditions, providing a more robust and reliable solution for crop management.

Keywords: water scarcity; soil microbiome; plant growth and development; soluble carbohydrates; polyphenol oxidase.

Introduction

Drought is one of the main environmental problems facing most countries today. Drought is a concern because it reduces the productivity of crops (Chukwuneme et al., 2020). Therefore, it is important to find ways to reduce the negative effects of drought in order to maintain food security. As drought is one of the main causes of declining crop yields in the world, the search for effective strategies to improve plant growth and yield under stress is relevant. Water scarcity seriously threatens global food security and limits crop production. Therefore, increasing the resistance of crops to water stress is a critically important way of ensuring agricultural production (Talaat et al., 2022).

An important issue in modern plant physiology is the clarification of the mechanisms of plant resistance to adverse environmental conditions. This is due to the growing anthropogenic pressure on the biosphere and global climate change that humanity has seen in recent decades (Tkalich et al., 2018, 2020). Every year there is a growing need for the production of safe food, which, in turn, requires the use of environmentally friendly methods of crop treatment, limiting the use of fertilizers, chemical pesticides, and the use of inefficient or expensive mechanical agricultural methods (Eisenstein, 2013; Chiaranunt & White, 2023). For more than half a century, the theory of biological or alternative agriculture has been developed and implemented in agricultural practice in many countries around the world. The need to develop alternative farming methods is primarily due to the accumulation of data on the negative effects of agrochemicals on human health (Wołejko et al., 2020; Bertola et al., 2021; Shah et al., 2021). An alternative to chemical fertilizers and pesticides is the use of biological preparations based on active strains of microorganisms isolated from different types of soil (Hanaka et al., 2021).

Also, more attention is drawn to the structural and ecological aspects of the interaction of plants with non-pathogenic soil microflora, which affects yields, stimulates growth, reduces sensitivity to phytopathogens, increases the resistance of agricultural plants to biotic and abiotic stressors (Armada et al., 2018; Shukla, 2019). It is known that under the influence of environmental factors a certain number and species composition of the microflora form on the roots of plants, this is what provides a variety of processes of transformation of substances in the root zone. In addition, the use of microorganisms of complex biological action, which are in mutualistic relationships with agricultural plants, is one of the ways of ecologically oriented management of agroecosystems (Paul & Lade, 2014; Vejan et al., 2016; Backer et al., 2018). Such microorganisms include nodule bacteria, mycorrhizal fungi, rhizosphere PGPR bacteria, and endophytic bacteria, which often act as elicitors. All these representatives of the soil microbiome are with the plant, mainly in mutually beneficial relations (Cordero et al., 2018; Guerrieri et al., 2020; Alemneh et al., 2022; Chiaranunt & White, 2023). Representatives of these microorganisms are considered as a potential basis for biological products for crop production.

The study of the interaction between plants and microorganisms at the biochemical, physiological and molecular levels shows that microbial associations can control or regulate plant responses to stress. The rhizosphere microbiome, being in particularly close contact with the plant organism, is particularly involved in the adaptation of plants to adverse factors. For example, the lack of water in the soil disrupts the water regime of plants and reduces the water content in all organs and tissues. Water deficiency can cause morphological, biochemical and physiological damage to plants, affecting various important cellular processes. Among the most detrimental effects are damage to the photosynthetic apparatus, oxidative damage to proteins, membrane lipids and other cellular components. In addition, water shortages can reduce the size of crop organs, delay flowering, fertilization and ultimately reduce grain yield and quality. These adverse effects are often associated with reduced soil microbial activity and limited availability of macro- and micronutrients to the plant (Pereira et al., 2020).

Vegetation experiments on different crops have shown that in conditions of soil drought, plant growth is primarily impaired (Saikia et al., 2018; Barnawal et al., 2019; Goswami & Deka, 2020). It has been established that inoculation of seeds with bacterial preparations reduces inhibition of growth processes. The use of rhizobacteria in drought had a positive effect on the preservation of the leaf surface, delayed the death of the lower leaves in treated plants, which led to a longer functioning of the leaf apparatus of plants. The treatment of seeds with rhizobacteria improved the development of the growth tube of seeds and root hairs, there was a thickening of the roots, which is probably due to the positive effect of bacterial metabolites. Thus, inoculation of plants with growth-promoting microorganisms can improve water retention strategies and drought resistance of plants grown in arid conditions. These beneficial microorganisms inhabit the rhizosphere, including endogenous plant microbiome – almost all of which, using direct or indirect mechanisms, promote plant growth (Khan et al., 2020). A large number of biological products based on active strains of microorganisms have appeared on the market, but not all manufacturers can support the quality of products and in many cases such products are ineffective. In addition, there is no unambiguous opinion in the literature on the possibility of using the same strains of effective microorganisms for different crops and different types of soils.

Maize is one of the most common cereal crops in many regions around the world. Drought is becoming the biggest problem for growing this crop. It is estimated that up to 15% of the potential corn yield is lost annually due to soil moisture deficiency. Corn belongs to those crops that require a significant amount of water to obtain high yields (Talaat et al., 2022). The realization of the potential harvest depends on many factors – the sensitivity of the variety to water stress, other environmental factors (the composition and structure of the soil, the diversity and functioning of the soil microbiome). However, crop formation begins at the stage of seed germination. It is at the beginning of the growth of seedlings that it is most important to create acceptable conditions for their further development. The resistance to major biotic and abiotic stresses is one of crucial characteristics required for modern varieties of crops, as well as for technologies of their growing. Therefore, studies focused on the role of rhizosphere microorganisms in maintaining plants' ecological sustainability are highly relevant and have practical value. When searching for strains of microorganisms with beneficial properties, the focus is often on those that have a general stimulating effect or help plants cope with stress. However, plants do not always experience stress in their natural environment. Therefore, it is important to search for strains or develop complex biopreparations that are equally effective in both stressful and favourable conditions. By prioritizing the creation of such preparations, we can ensure that plants receive consistent support throughout their development, ultimately leading to a more resilient and productive crop. In view of the above, the aim of our research was to determine the effect of bacterization of *Zea mays* seeds by rhizobacterial strains on the development of maize under artificial drought in a vegetation experiment.

Materials and methods

The object of the study was the seeds of sweet corn (*Zea mays* L.) cultivar "Early gourmet 121" (FAO 120). This cultivar is considered capricious to environmental conditions especially at the beginning of growth and development. Plants were grown in a vegetation chamber, in soil culture, in one liter pots. Prior to the commencement of the experiment, the soil was sterilized for 30 minutes by autoclaving at a temperature of 120 ºC (Medical Equipment, 2017) in paper bags of 200 g. Initially, in all versions of the experiment, the soil was moistened to 60% of the total moisture content. Containers with sown seeds were placed in the growing chamber. The plants were grown under a photoperiod of 16 hours of light and 8 hours of darkness, with a temperature range of 20–24 °C during the day and 17–20 °C at night. The plants were illuminated by white fluorescent lamps with a light intensity of 300 μmol photons/m^2/s, measured just above the plant canopy. Each of three pots contained 10 plants. Half of the plants were grown on a substrate whose humidity was maintained at 60% of the total moisture content (optimal water supply conditions), for the other half of the plants in each version of the experiment and control, we created a model drought by reducing watering (up to 30% of total moisture content). The experiment lasted 30 days. All experiments were performed in triplicate.

The inoculations were performed using strains of *Bacillus cereus*, *Pseudomonas putida*, *Azospirillum brasilense* and *Azotobacter chroococcum* from the microorganism collection of the Department of Physiology and Biochemistry of Plants and Microorganisms at V. N. Karazin Kharkiv National University. *Bacillus cereus* strain and *P. putida* were cultured on MPA medium (Ukraine, 2019) in a thermostat (Medaparat, Ukraine, 2018) at 33 °C. *Azospirillum brasilense* was cultured on potato agar, g/L: malic acid 2.5 (China, 2017); $C_6H_{12}O_6$ – 2.5 (Ukraine, 2017); agar – 20.0 (Spain, 2017); potato broth (200 g of raw potatoes boiled in 1 liter of water); pH 6.5 (MT, Russia, 2016) at a temperature of 28 °C using a thermostat. *Azotobacter chroococcum* was cultured in a thermostat (Medaparat, Ukraine, 2018) at a temperature of 28 °C on Ashby medium, g/L: $C_6H_{12}O_6$ – 20.0 (Ukraine, 2017); K_2HPO_4 – 0.2 (China, 2015); NaCl – 0.2 (Ukraine, 2017); $MgSO_4 \times 7H_2O$ – 0.4 (China, 2015); K_2SO_4 – 0.1 (China, 2015); $CaCO_3$ – 5.0 (Ukraine, 2014); agar – 20.0 (Spain, 2017); distilled water; pH 6.8 – 7.0 (MT, Russia, 2016).

One-day cultures were used for bacterization by *B. cereus* strain, *P. putida*, *A. chroococcum* and three-day cultures for *A. brasilense*. Under conditions of sterility of the laminar box (Technogen, Ukraine, 2019), bacterial suspensions with a cell number of 10^6 cells/mL were prepared (according to the turbidity standard No. 5, Ukraine, 2014). Vortex was used to obtain a homogeneous bacterial suspension (Biosan, Latvia, 2016). The research scheme is illustrated in Figure 1.

Variants of the experiment: the non-inoculated group, in which sweet corn seeds were sterilized with 70% ethanol for 15 minutes and washed with sterile distilled water before sowing, the inoculated group, in which sweet corn seeds were sterilized with 70% ethanol for 15 minutes and washed under sterile distilled water and after that inoculated by aqueous suspensions of bacteria *B. cereus* strain, *P. putida*, *A. brasilense*, *A. chroococcum* with a titre of 10^6 cells/mL (Turbidity Standard No. 5, Ukraine, 2014), co-inoculated group – sweet corn seeds before sowing were sterilized with 70% ethanol for 15 minutes washed under sterile distilled water and co-inoculation by a mixture of all of these bacteria *B. cereus* strain + *P. putida* + *A. brasilense* + *A. chroococcum* with a titter of 10^6 cells/mL (Turbidity Standard No. 5, Ukraine, 2014).

Sweet corn plants were harvested from pots after 30 days of germination. The measurement of root length (cm), shoots length (cm), root dry weight (g), shoot dry weight (g) was measured. The plants were gently extracted from their respective pots, the aboveground part was separated, and the root system was thoroughly washed with tap water, dried with filter paper and measured. After that, the aboveground and underground parts of the plants were dried in a dry oven (Medical Equipment, Russia, 2006) at a temperature of 60 ºC to constant weight and measured using laboratory scales. The carbohydrate content was determined in dried

(120 ºC for 30 min) leaves of 10 plants for each of the repeats. The sum of sugars and monosaccharides was determined by the Shvetsov and Lukyanenko micromethod (Yermakov et al., 1987), measuring the optical density of the extract at a wavelength of 670 nm (Spectrophotometer Ulab 102UV, China, 2019). The oligosaccharide content was determined by the difference between the sum of sugars and the content of monosaccharides.

Determination of polyphenol oxidase (PPO, EC 1.14.18.1) activity was carried out by the method of Boyarkin (Yermakov et al., 1987) in washed raw roots from 10 plants. Polyphenol oxidase activity was determined spectrophotometrically (Spectrophotometer Ulab 102UV, China, 2019), in kinetic mode at a wavelength of 420 nm, recording the value of the oxidation reaction of pyrocatechin for 120 seconds.

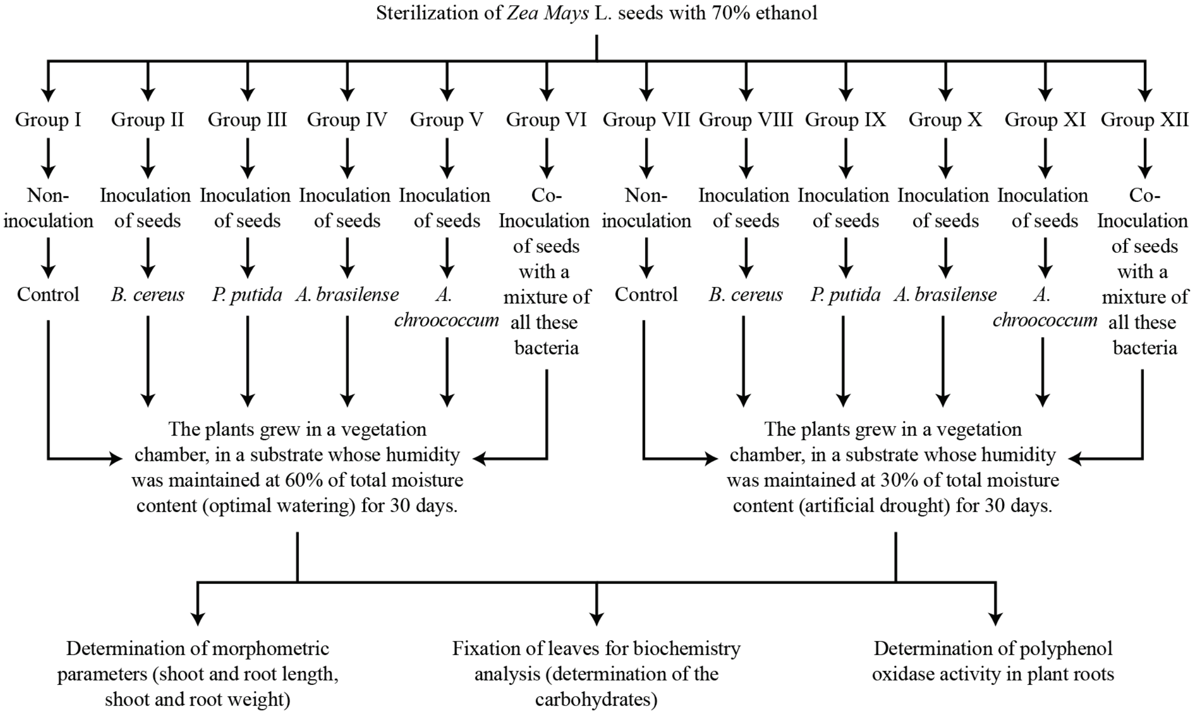

Fig. 1. The scheme of the research

The values of the parameters measured in three replicates of each experiment were pooled together and their mean was calculated. Means from three repeated experiments were averaged, and the respective standard error (SE) for the overall mean was estimated. Means were compared between experimental series using Tukey's test, considering the difference significant at ($P < 0.05$, $n = 3$).

Results

Figure 2 presents the results of the study, which indicate that seed inoculation did not significantly stimulate shoot length elongation compared to the control group under both optimal water supply conditions and artificial drought. Co-inoculation with a mixture of all of these bacteria significantly increased length of shoots under optimal water supply conditions by an average of 23.0% compared to the control variant of the experiment.

Artificial drought significantly reduced the length of shoots compared to all variant of the experiment with inoculation and co-inoculation under optimal water supply conditions (Fig. 2).

Under drought conditions the root length was significantly enhanced of non-inoculated sweet corn plants (on average by 18.1%), plants inoculated with *P. putida* (on average by 20.9%) and inoculated with *A. brasilense* (on average by 22.5%) compared to plants that were not affected by drought (Fig. 3).

In other variants of the experiment, the length of the roots of seedlings grown in drought conditions was significantly lower compared to plants of similar variants, but which were grown under optimal water supply conditions (Fig. 3). The root length of plants inoculated with *A. brasilense* or *A. chroococcum* significantly decreased (by 38.3% to 24.5%) under optimal water supply conditions and under drought conditions by 34.2–54.1% compared with the control variant of the experiment (Fig. 3).

Figure 4 shows that the drought significantly reduced the mass of the shoots in the control and all variants of the experiment compared to the plants of similar variants grown in sufficient moisture. Inoculation with *B. cereus* significantly decreased the mass of shoots under optimal water supply conditions by an average of 18.2% and under drought conditions by 25.0% compared to the control variant of the experiment (Fig. 4). Inoculation with *A. chroococcum* significantly increased the mass of shoots under optimal water supply conditions by an average of 18.2% compared to the control variant of the experiment. In the variants of the experiment under drought conditions there was no positive effect of bacterization on the mass of the shoots. An exception was the option with bacterization of seeds with a mixture of bacterial suspensions – there was a slight increase in the mass of the shoots, compared with the control (Fig. 4).

Figure 5 shows that the drought adversely affected the mass of the underground part of the seedlings in the control and most variants of the experiment. Exceptions were variants with bacterization of *P. putida* and *A. brasilense* – the mass of roots of sweet corn plants grown in drought conditions was significantly higher by 50.0% and 33.3%, respectively than that of plants of the corresponding variants grown in sufficient moisture (Fig. 5).

Data regarding the enzyme showed that rhizobacteria treatments improved the polyphenol oxidase (PPO) activity under normal conditions in variants with single inoculation with *B. cereus* (on average by 314.3%), with *P. putida* (on average by 100.0%) and *A. chroococcum* (on average by 71.4%) compared with the control. Under drought conditions PPO activity was significantly enhanced in variants single inoculation with *P. putida* (on average by 50.0%) and *A. chroococcum* (on average by 66.7%) compared with the control (Fig. 6). In general, the activity of the enzyme in the roots of seedlings exposed to drought was lower compared to the option of growing in sufficient moisture (Fig. 6).

The content of water soluble carbohydrate in the leaves of sweet corn plants in the variant co-inoculation with *B. cereus* + *P. putida* + *A. brasilense* + *A. chroococcum* increased by 14.3% under normal conditions and significantly increased by 50.0% under drought conditions, compared with the control (Fig. 7). Treatment of seeds with a suspension of *A. brasilense* or *A. chroococcum* had a positive effect on the water soluble carbohydrate content in the leaves of both plants grown under normal and drought conditions. In other variants of the experiment, bacterization with rhizobacteria did not significantly affect the water soluble carbohydrate content in corn leaves (Fig. 7).

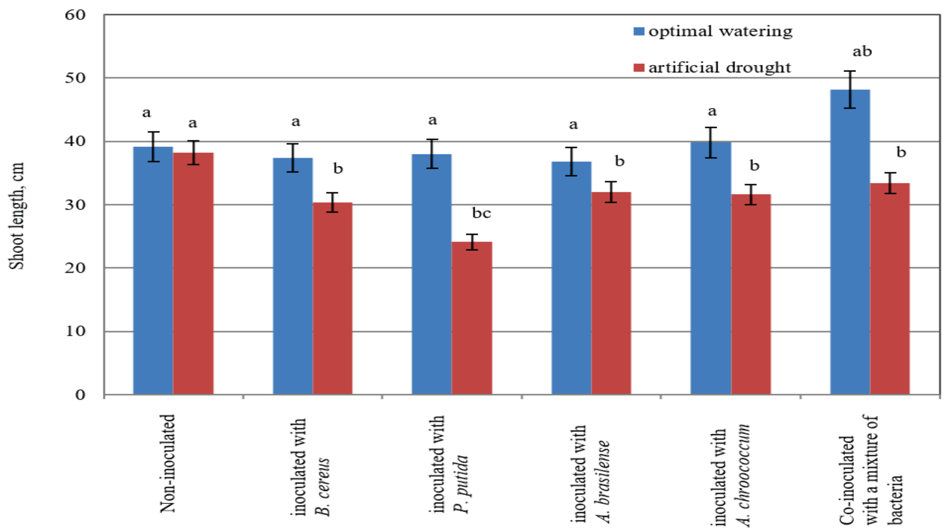

Fig. 2. Effect of treatment of seeds with different rhizobacteria on shoot length under normal and drought conditions after 30 days of growth (cm): control group – non-inoculated, inoculated – single inoculation with *B. cereus*, inoculated – single inoculation with *P. putida*, inoculated – single inoculation with *A. brasilense*, inoculated – single inoculation with *A. chroococcum*, co-inoculated – co-inoculation with *B. cereus* strain + *P. putida* + *A. brasilense* + *A. chroococcum*, comparisons were made within sweet corn (*Zea mays* L.), means with the same letter are not significantly different from each other on the results of comparison using the Tukey test ($P < 0.05$) with Bonferroni correction ($n = 3$, $x \pm SE$)

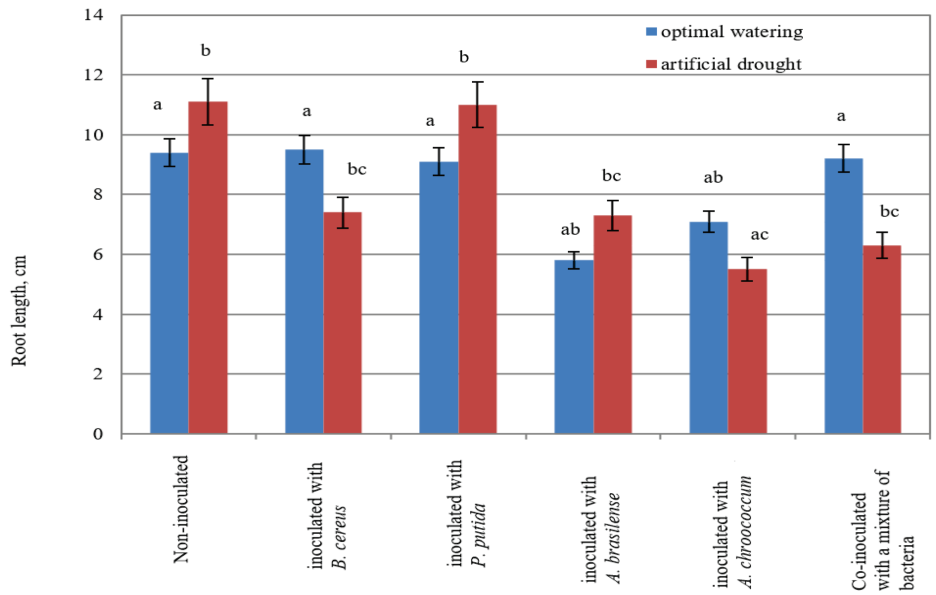

Fig. 3. Effect of treatment of seeds with different rhizobacteria on root length under normal and drought conditions after 30 days of growth (cm): the legend is the same as described for Figure 2

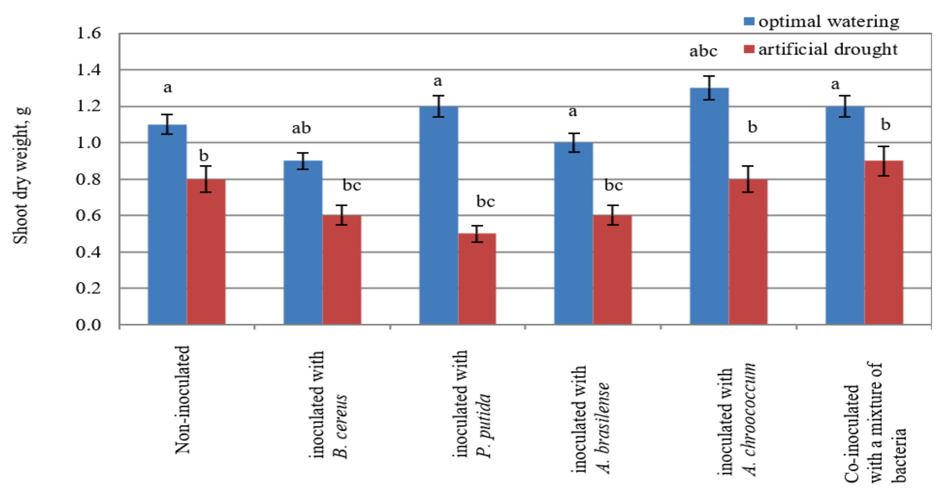

Fig. 4. Effect of treatment of seeds with different rhizobacteria on shoot dry weight under normal and drought conditions after 30 days of growth (g): the legend is the same as described for Figure 2

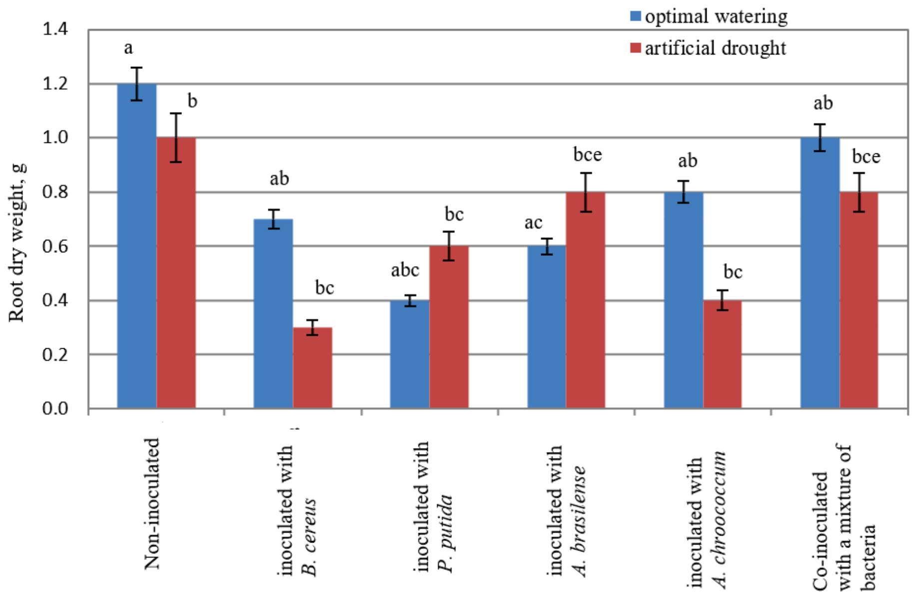

Fig. 5. Effect of treatment of seeds with different rhizobacteria on root dry weight under normal and drought conditions after 30 days of growth (g): the legend is the same as described for Figure 2

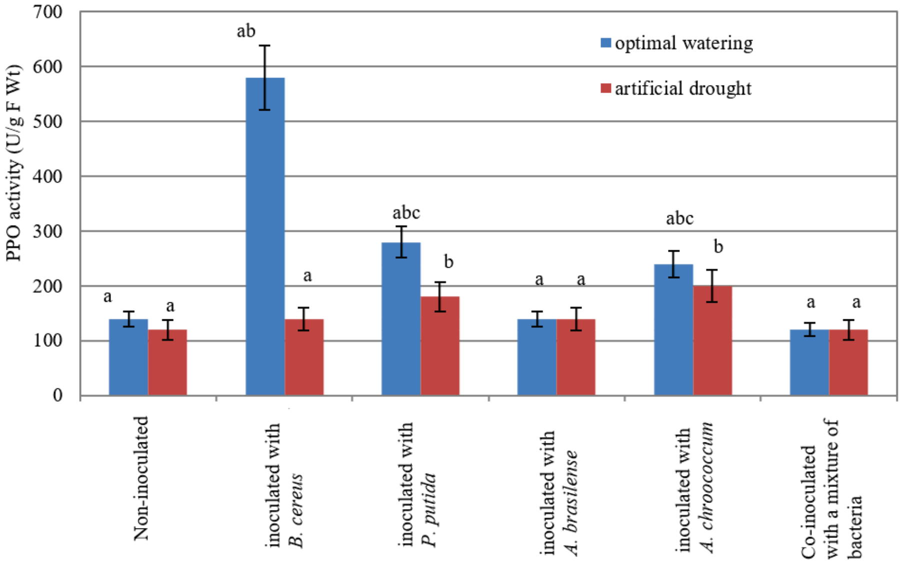

Fig. 6. Effect of treatment of seeds with different rhizobacteria after 30 days of growth on enzyme polyphenol oxidase (PPO) activity under normal and drought conditions after 30 days of growth (U/g F Wt): the legend is the same as described for Figure 2 above

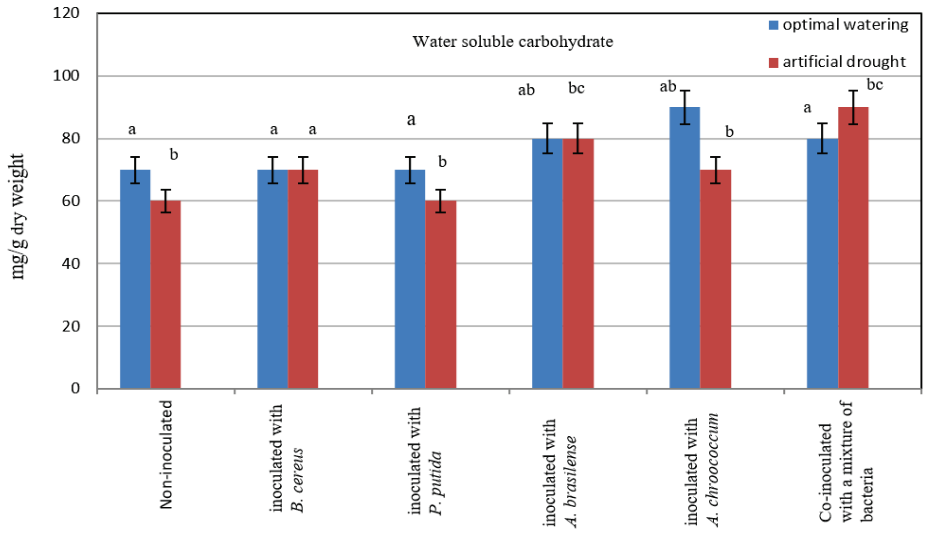

Fig. 7. Effect of treatment of seeds with different rhizobacteria on water soluble carbohydrate content in leaves under normal and drought conditions after 30 days of growth (mg/g dry weight): the legend is the same as described for Figure 2 above

The content of monosaccharides in the leaves tended to increase due to bacterization of *A. brasilense* seeds or a mixture of suspensions of rhizobacteria under drought conditions by an average of 20.0% compared to the control (Fig. 8). Treatment of seeds with a suspension of *A. chroococcum* had a positive effect on the monosaccharides' content under optimal water supply conditions. Inoculation with *P. putida* significantly decreased the content of monosaccharides in the leaves under optimal water supply conditions by an average of 20.0% compared to the control (Fig. 8).

Analysis of oligosaccharides' content in leaves under normal and drought conditions after 30 days of growth revealed significant change in content due to rhizobacterial inoculation compared to control (Fig. 9). Inoculation with *P. putida*, *A. brasilense*, *A. chroococcum* and co-inoculation with a mixture of all of these bacteria significantly increased the oligosaccharides' content under optimal water supply conditions by an average of 50.0% compared to the control variant of the experiment (Fig. 9). Under drought conditions the oligosaccharides' content significantly increased in leaves, inoculated with *B. cereus* (on average by 100.0%), *A. brasilense* (on average by 100.0%) and *A. chroococcum* (on average by 100.0%) and co-inoculation with a mixture of all of these bacteria (on average by 200.0%) compared to control (Fig. 9). Artificial drought significantly reduced the oligosaccharides' content in the following variants of the experiment: in control (on average by 100.0%), with inoculation *P. putida* (on average by 66.7%), *A. brasilense* (on average by 33.3%) and *A. chroococcum* (on average by 33.3%) compared to optimal water supply conditions (Fig. 9).

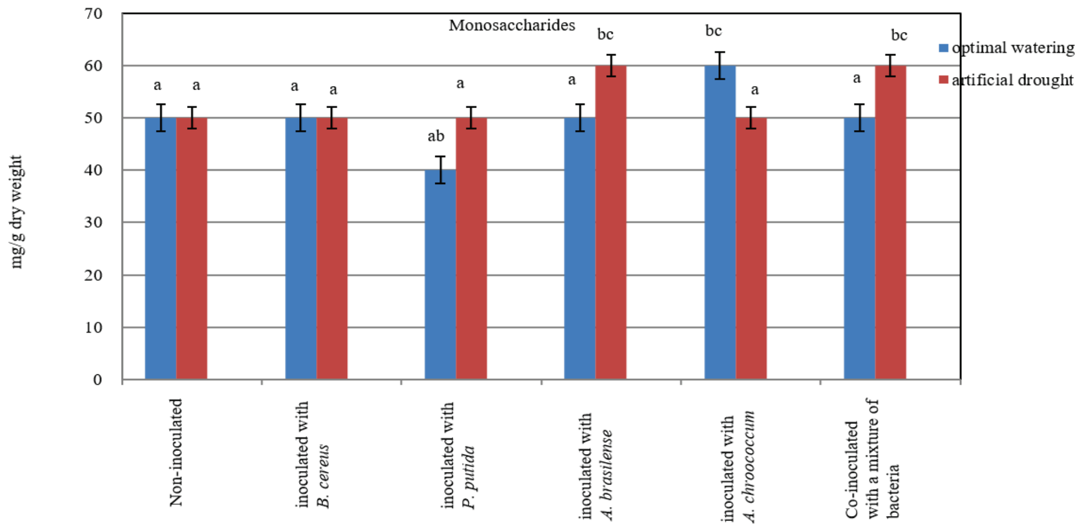

Fig. 8. Effect of treatment of seeds with different rhizobacteria on monosaccharide content in leaves under normal and drought conditions after 30 days of growth (mg/g dry weight): the legend is the same as described for Figure 2

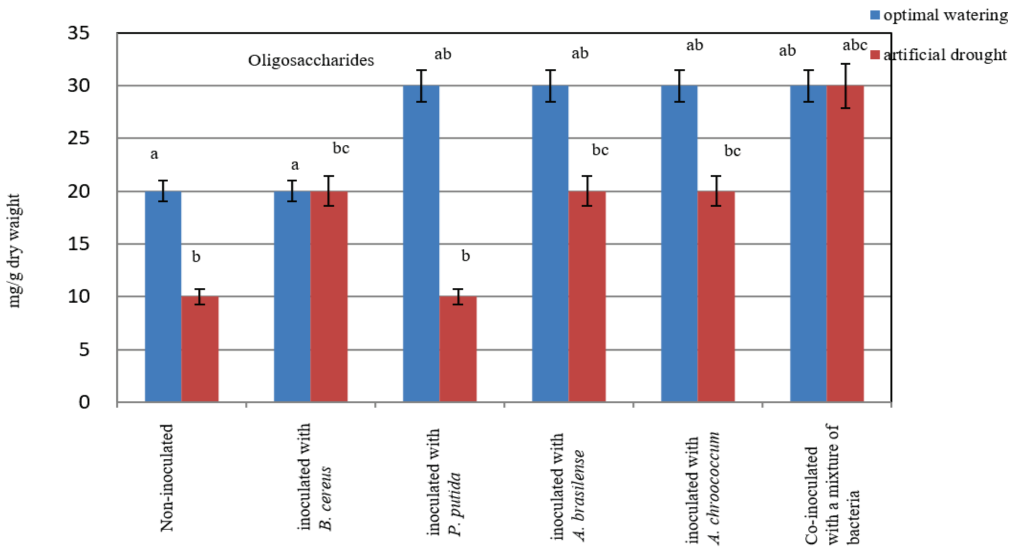

Fig. 9. Effect of treatment of seeds with different rhizobacteria on oligosaccharides' content in leaves under normal and drought conditions after 30 days of growth (mg/g dry weight): the legend is the same as described for Figure 2

Discussion

Corn is one of the most important cereals in the world. However, this is a crop with high water consumption, which prevents the increase of corn acreage in modern climate change, when the availability of water resources is reduced. Drought directly affects the morphology, anatomy, physiology and biochemistry of plants. Changes at the transcriptomic and metabolomic levels can also provide evidence of its influence on plants (Bogati & Walczak, 2022). At the same time, plants modify their morphological and physiological features, which are necessary for adaptation to conditions of water deficiency in the environment (Goswami & Deka, 2020). Also, the negative effects of drought can be mitigated by rhizosphere microbial communities, especially bacteria that stimulate plant growth. These microorganisms adapt their structural and functional composition to water scarcity. They can accumulate moisture in their capsules and mucous covers, secrete phytohormones, siderophores and other active substances. Even in case of mass death, bacteria can become a source of nitrogen for the plant (Bogati & Walczak, 2022).

It is known that endogenous and exogenous growth regulators (including microbial origin) play an important role in plants' response to abiotic stress (Shukla, 2019). For example, field and vegetation studies using different cereals have shown positive effects on growth and grain yield of a combination of strains *Bacillus megaterium*, *Azotobacter chlorophenolicus* and *Enterobacter* sp. (Kumar et al., 2014; Majeed et al., 2015) and bacterization of seeds by monocultures of *Azotobacter* sp. (Shirinbayan et al., 2019; Song et al., 2020; Ali & Kamal, 2020). The positive effect of PGPR on the growth, morphology and architecture of maize roots has been described (Barnawal et al., 2019). According to the authors, seed treatment with PGPR strains led to an increase in biomass and root length of plants grown under drought.

The main difference between our research and that presented in most literature sources is our use of sterile soil for growing plants and the use of sterile tap water for irrigation. The positive effects of bacterization of seeds with a mixture of rhizobacteria on the length of the shoots of corn seedlings perhaps related to the production by bacteria of phytohormones (IAA, auxins), which stimulate plant growth processes. Moreover, it was more effective to use a mixture of bacteria and growing corn under conditions of sufficient moisture. The fact that rhizobacteria produce phytohormones and stimulate the growth processes of various agricultural plants (direct impact on the plant organism) has been demonstrated by various authors (Braccini et al., 2012; Kudoyarova et al., 2015; Shi et al., 2017; Balbinot et al., 2020).

According to our study, bacterization did not increase the mass of the shoot of the corn seedlings, exceptions was variant with bacterization of *A. chroococcum* – in this variant of bacterization under optimal water supply conditions there was a significant increase in the mass of the shoots. Stimulation of growth processes in corn and other grain crops by different strains of *A. chroococcum* was shown by other authors (Shirinbayan et al., 2019; Ali & Kamal, 2020; Song et al., 2020). It should be noted that according to Barnawal et al. (2019), inoculation with strains of *Bacillus* spp. caused an increase in the length of shoots and dry biomass of corn, which was grown in the conditions of vegetation experiment under drought conditions. However, the bacillus strain used in our study did not have a stimulating effect on seedling growth size. Thus, the decrease in growth rates of seedlings that were bacterized by rhizobacteria and grown in arid conditions may indicate the development of protective mechanisms against stressors, in our case – a drought. Similar assumptions have been discussed in the literature (Bechtold & Field, 2018). The root system plays a crucial role in plant development from seed germination to maturation. Necessary changes in the growth and development of roots are most important for the adaptation of plants under stress. Today, varieties of agricultural plants with a developed root system are a priority in the cereals market (Watt et al., 2013; Kudoyarova et al., 2019). According to the results of our research, in some variants of the experiment, an increase in the length and mass of the roots of seedlings was found. Namely, this occurred in variants with bacterization of *P. putida* and *A. brasilense* seeds, as well as in plants whose seeds were not inoculated with bacteria. Moreover, it engaged to a greater extent the length of the roots. It is a well-known fact that in drought conditions the plant implements a strategy to increase the length of the root system in order to more efficiently obtain moisture. However, in the case of the use of rhizobacteria, a balance must be struck between the amount of growth stimulants they release and the need for exogenous biologically active substances of the plants themselves. There is evidence, for example, that with increasing concentrations of exogenous auxins, there is an inhibition of growth of the root system of plants (Kudoyarova et al., 2019). In general, increasing the root mass of plants and optimizing the excretory function (to regulate the development of the microbiome in the rhizoplane and rhizosphere) is a more advantageous strategy for plant development under stress.

Therefore, according to many authors, bacterization by different species of rhizobacteria leads to an increase in the length and weight of the underground part of plants that developed in drought conditions (Czarnes et al., 2020; Khan et al., 2020; Lin et al., 2020). However, our studies have shown the opposite effect in variants with bacterization of *B. cereus*, *P. putida* and *A. brasilense*. Regarding the representative of *Azospirillum*, it is known that members of the genus have the ability to retain water around the root by developing mucous cysts around the root (Khan et al., 2020). It is possible that due to this property azospirals had a positive effect on the root system of experimental plants of corn. After analyzing the data obtained by us, we have reached the assumption that the stimulating effect on the root system of plants may manifest itself at later stages of their development. It is known (Anzuay et al., 2021) that the activity of the rhizosphere microbiome depends on the phenological stage of plant development. In studying the effects of various stressors on the plant organism, in addition to the length and mass of their underground part, an important component is information about the morphology of roots. According to Goswami & Deka (2020), plant roots change their morphology depending on the amount of moisture available in the soil. The presence of a large number of short and thin lateral roots in plants, in addition to other roots, leads to an increase in the root surface area and improves water absorption. Therefore, the area and system architecture of the root system of plants is crucial to increase the resistance of the plant organism to drought. The changes in the morphology of the root system of the maize seedlings recorded by us indicate that, despite the lack of significant effect on the length and weight of the underground part of the seedlings, bacterization had a positive effect on the morphology and architecture of the roots. Such a positive effect in the future may improve plant nutrition, which will eventually lead to increased plant productivity.

In recent years, the possibility of the participation of redox enzymes in regulatory processes has been actively discussed, namely in the transmission of signals that ensure the formation of the plant cell response to biotic and abiotic factors. Researchers note an increase in polyphenol oxidase activity in the roots and leaves of plants under conditions of anthropogenic pollution, phytopathogenic load and point to the important role of polyphenol oxidase in the regulation of oxidative processes under stress (Chen et al., 2000; Konappa et al., 2020). A slight increase in polyphenol oxidase activity in plant roots may indicate the interaction of the plant with microorganisms, including beneficial ones (Abd El-Daim et al., 2014). In our study, higher than control activity of the enzyme, under normal and drought conditions was found in variants with bacterization of *P. putida*, *A. chroococcum* and under normal conditions in the variant with bacterization of *B. cereus*. The obtained data may indicate the potential ability of these bacteria to induce systemic resistance, thus providing a positive effect of bacterization of plants on the adaptation of the root system to the lack of moisture in the soil.

An important adaptive response to the effects of drought is the accumulation of inorganic and organic osmolytes by the plant organism, which include soluble sugars. Reducing sugars can also function as signaling molecules that control plant metabolism, growth, development, and stress responses. According to Vishnupradeep et al. (2022), the content of reducing sugars and sucrose increases in the leaves at the beginning of the drought. In our own study, we found that the monosaccharide content in the leaves of control plants remained constant, while oligosaccharide levels decreased. A slight difference was observed only when measuring the content of soluble sugars in the leaves of seedlings grown under different moisture conditions. Accumulation of monosaccharides in the leaves of plants exposed to drought occurred under the conditions of inoculation of seeds of *P. putida*, *A. brasilense* and a mixture of suspensions. It is possible that stimulation of the osmotic adjustment of the plant organism took place under conditions of moisture deficiency. After all, an increase in the concentration of soluble sugars is considered one of the characteristic physiological responses to drought. According to modern ideas, the accumulation of hexoses during water stress is one of the early responses to drought (Vishnupradeep et al., 2022). Rhizosphere bacteria can stimulate the plant's synthesis of osmolytes, including those of sugar nature (Chiaranunt & White, 2023).

In general, in the current study it was established that only inoculation with *P. putida* stimulated development of the root system of plants and the synthesized sugars were distributed predominantly to the root system growth. The decrease in the content of oligosaccharides in conditions of limited water availability is a consequence of reduced photosynthesis and insufficient absorption of nutrients, which in turn leads to a decrease in the mass of shoots and roots. Plants secrete fixed carbon by root exudation and feed the rhizosphere bacteria, which is why treatment of seeds with different rhizobacteria in our experiment led to reduction of the mass of roots under normal and drought conditions. According to the literature, plant-associated microorganisms also constitute a strong sink for plant carbon, thereby increasing concentration gradients of sugars and affecting root exudation (Canarini et al., 2019). Root exudation of carbohydrates is driven by facilitated diffusion but plants and microbes can control the root exudation process by modifying concentration gradients depending on their nutritional status (Canarini et al., 2019). Our data show that plant belowground carbon allocation and root exudation in response to environmental changes in comparison to fertilization are insufficiently studied and have a wide potential for use in the manipulation of plant carbon transport for improving agricultural productivity.

Therefore, the absence of a significant positive effect of the bacteria we used on the growth and development of maize seedlings in drought conditions may also be related to changes in the composition of root secretions. It is known (Bogati & Walczak, 2022) that the presence of stress signals in root exudates can negatively affect the development of the soil microbiome. The trends established by us regarding the positive effect of bacterization on certain physiological and biochemical reactions of plants that developed in drought conditions can be more significantly manifested during longer observations.

Conclusion

One of the most urgent problems of modern plant physiology is to find out the mechanisms of their resistance to adverse environmental conditions. A fairly promising approach to creating resistant varieties is the use of strains of microorganisms capable of protecting plants and exerting a stimulating effect on growth processes. When subjected to artificially induced drought, the length of the aerial parts of corn plants was negatively affected. The bacterial strains we used had different effects on the development of corn both under conditions of moisture deficit and when the soil was sufficiently moistened. Thus, *P. putida* stimulated the development of the root system of seedlings, and the bacterization of seeds with a mixture of bacterial suspensions had a positive effect on the development of the aerial part of seedlings grown in drought conditions. Significantly higher activity of polyphenol oxidase in the roots of seedlings, both in conditions of sufficient moisture and in conditions of drought, was noted during the bacterization of corn seeds by cells of *P. putida* and *A. chroococcum*. This may indicate the establishment of closer contact between the plant and microorganisms and in the case of *P. putida* bacterization, the activity of PFO correlates with the stimulation of the development of the root system of seedlings. The accumulation of mono- and oligosaccharides in the leaves of plants grown under drought conditions and under the influence of inoculation with *P. putida*, *A. brasilense* and a mixture of bacterial suspensions may indicate the stimulation of protective reactions of plants in response to stress. Therefore, of all the bacterial strains used, only *P. putida* had a positive effect on various physiological and biochemical properties of plants that developed under the effects of drought. However, the same bacteria did not always have a positive effect on the development of corn under conditions of sufficient moisture. In our opinion, a more promising direction for the creation of biological preparations based on useful strains of microorganisms is the search for a combination of several different types of soil bacteria that will maximally effectively stimulate the development of plants both under conditions of stress and in more or less suitable conditions.

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References

Abd El-Daim, I. A., Bejai, S., & Meijer, J. (2014). Improved heat stress tolerance of wheat seedlings by bacterial seed treatment. Plant and Soil, 379, 337–350.

Alemneh, A. A., Zhou, Y., Ryder, M. H., & Denton, M. D. (2022). Soil environment influences plant growth-promotion traits of isolated rhizobacteria. Pedobiologia, 90, 150785.

Ali, H. A., & Kamal, J. A. (2020). Effect infection of *Azotobacter chroococcum*, *Azospirillum brasilense*, poultry residues in ammonium concentration and (*Sorghum bicolor* L.). Al-Qadisiyah Journal For Agriculture Sciences, 10(1), 232–240.

Anzuay, M. S., Viso, N. P., Ludueña, L. M., Morla, F. D., Angelini, J. G., & Taurian, T. (2021). Plant beneficial rhizobacteria community structure changes through developmental stages of peanut and maize. Rhizosphere, 19, 100407.

Armada, E., Leite, M. F. A., Medina, A., Azcón, R., & Kuramae, E. E. (2018). Native bacteria promote plant growth under drought stress condition without impacting the rhizomicrobiome. FEMS Microbiology Ecology, 94(7), 1–13.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. Frontiers in Plant Science, 9, 1473.

Balbinot, W. G., Rodrigues, S., & Botelho, G. R. (2020). Isolates of *Bacillus* sp. from garlic: Effect on corn development and plant growth-promoting mechanisms. Revista Brasileira de Ciência do Solo, 44, e0200043.

Barnawal, D., Singh, R., & Singh, R. P. (2019). Role of plant growth promoting rhizobacteria in drought tolerance: Regulating growth hormones and osmolytes. In: PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, Sawston. Pp. 107–128.

Bechtold, U., & Field, B. (2018). Molecular mechanisms controlling plant growth during abiotic stress. Journal of Experimental Botany, 69(11), 2753–2758.

Bertola, M., Ferrarini, A., & Visioli, G. (2021). Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by – Omics approaches: A perspective for the environment, food quality and human safety. Microorganisms, 9, 1400.

Bogati, K., & Walczak, M. (2022). The impact of drought stress on soil microbial community, enzyme activities and plants. Agronomy, 12(1), 189–215.

Braccini, A. L., Dan, L. G. M., Piccinin, G. G., Albrecht, L. P., Barbosa, M. C., & Ortiz, A. H. (2012). Seed inoculation with *Azospirillum brasilense*, associated with the use of bioregulators in maize. Revista Caatinga, 25(2), 58–64.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. Frontiers in Plant Science, 10, 157.

Chen, C., Belanger, R. R., Benhamou, N., & Paulitz, T. C. (2000). Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology, 56(1), 13–23.

Chiaranunt, P., & White, J. F. (2023). Plant beneficial bacteria and their potential applications in vertical farming systems. Plants, 12(2), 400–427.

Chukwuneme, C. F., Babalola, O. O., Kutu, F. R., & Ojuederie, O. B. (2020). Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. Journal of Plant Interactions, 15(1), 93–105.

Cordero, I., Balaguer, L., Rincon, A., & Pueyo, J. J. (2018). Inoculation of tomato plants with selected PGPR represents a feasible alternative to chemical fertilization under salt stress. Journal Plant Nutrition and Soil Science, 181(5), 694–703.

Czarnes, S., Mercier, P. E., Lemoine, D. G., Hamzaoui, J., & Legendre, L. (2020). Impact of soil water content on maize responses to the plant growth-promoting rhizobacterium *Azospirillum lipoferum* CRT1. Journal of Agronomy and Crop Science, 206(5), 505–516.

Eisenstein, M. (2013). Plant breeding: Discovery in a dry spell. Nature, 501, S7–S9.

Goswami, M., & Deka, S. (2020). Plant growth-promoting rhizobacteria – alleviators of abiotic stresses in soil: A review. Pedosphere, 30(1), 40–61.

Guerrieri, M. C., Fanfoni, E., Fiorini, A., Trevisan, M., & Puglisi, E. (2020). Isolation and screening of extracellular PGPR from the rhizosphere of tomato plants after long-term reduced tillage and cover crops. Plants, 9(5), 668.

Hanaka, A., Ozimek, E., Reszczyńska, E., Jaroszuk-Ściseł, J., & Stolarz, M. (2021). Plant tolerance to drought stress in the presence of supporting bacteria and fungi: An efficient strategy in horticulture. Horticulturae, 7(10), 390.

Khan, N., Ali, S., Tariq, H., Latif, S., Yasmin, H., Mehmood, A., & Shahid, M. A. (2020). Water conservation and plant survival strategies of rhizobacteria under drought stress. Agronomy, 10(11), 1683.

Khan, N., Martínez-Hidalgo, P., Humm, E. A., Maymon, M., Kaplan, D., & Hirsch, A. M. (2020). Inoculation with a microbe isolated from the Negev Desert enhances corn growth. Frontiers in Microbiology, 11, 1149.

Konappa, N., Krishnamurthy, S., Arakere, U. C., Chowdappa, S., & Ramachandrappa, N. S. (2020). Efficacy of indigenous plant growth-promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in a tomato. Egyptian Journal of Biological Pest Control, 30, 106.

Kudoyarova, G. R., Arkhipova, T. N., & Melentev, A. I. (2015). Role of bacterial phytohormones in plant growth regulation and their development. In: Bacterial metabolites in sustainable agroecosystem. In: Maheshwari, D. (Eds.). Sustainable development and biodiversity. Springer, Cham. Vol. 12. Pp. 69–86.

Kudoyarova, G., Arkhipova, T., Korshunova, T., Bakaeva, M., Loginov, O., & Dodd, I. C. (2019). Phytohormone mediation of interactions between plants and non-symbiotic growth promoting bacteria under edaphic stresses. Frontiers in Plant Science, 10, 1368.

Kumar, A., Maurya, B. R., & Raghuwanshi, R. (2014). Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). Biocatalysis and Agricultural Biotechnology, 3(4), 121–128.

Lin, Y., Watts, D. B., Kloepper, J. W., Feng, Y., & Torbert, H. A. (2020). Influence of plant growth-promoting rhizobacteria on corn growth under drought stress. Communications in Soil Science and Plant Analysis, 51(2), 250–264.

Majeed, A., Abbasi, M. K., Hameed, S., Imran, A., & Rahim, N. (2015). Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Frontiers in Microbiology, 6, 198.
Paul, D., & Lade, H. (2014). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: A review. Agronomy for Sustainable Development, 34, 737–752.
Pereira, S. I. A., Abreu, D., Moreira, H., Vega, A., & Castro, P. M. L. (2020). Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (*Zea mays* L.) under water deficit conditions. Heliyon, 6(10), 05106.
Saikia, J., Sarma, R. K., Dhandia, R., Yadav, A., Bharali, R., Gupta, V. K., & Saikia, R. (2018). Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Scientific reports, 8, 3560.
Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., Rabileh, M., Zajonc, J., & Smith, D. L. (2021). PGPR in agriculture: A sustainable approach to increasing climate change resilience. Frontiers in Sustainable Food Systems, 5, 667546.
Shi, T. Q., Peng, H., Zeng, S. Y., Ji, R. Y., Shi, K., Huang, H., & Ji, X. J. (2017). Microbial production of plant hormones: opportunities and challenges. Bioengineered, 8(2), 124–128.
Shirinbayan, S., Khosravi, H., & Malakouti, M. J. (2019). Alleviation of drought stress in maize (*Zea mays*) by inoculation with *Azotobacter* strains isolated from semi-arid regions. Applied Soil Ecology, 133, 138–145.
Shukla, A. K. (2019). Ecology and diversity of plant growth promoting *Rhizobacteria* in agricultural landscape. In: PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, Sawston. Pp. 1–15.
Song, Y., Liu, J., & Chen, F. (2020). *Azotobacter chroococcum* inoculation can improve plant growth and resistance of maize to armyworm, *Mythimna separata* even under reduced nitrogen fertilizer application. Pest Management Science, 76(12), 4131–4140.
Talaat, N. B., Ibrahim, A. S., & Shawky, B. T. (2022). Enhancement of the expression of ZmBZR1 and ZmBES1 regulatory genes and antioxidant defense genes triggers water stress mitigation in maize (*Zea mays* L.) plants treated with 24-epibrassinolide in combination with spermine. Agronomy, 12(10), 2517.
Tkalich, Y. I., Tsyliuryk, A. I., Masliiov, S. V., & Kozechko, V. I. (2018). Interactive effect of tank-mixed post emergent herbicides and plant growth regulators on corn yield. Ukrainian Journal of Ecology, 8(1), 961–965.
Tkalich, Y., Tsyliuryk, O., Kozechko, V., Rudakov, Y., Tkalich, O., & Bagorka, M. (2020). Weed chemical control in the winter wheat planting after non fallow predecessors in the Northern Steppe of Ukraine. Scientific Papers, Series A – Agronomy, 63(1), 567–573.
Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., & Boyce, A. N. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability – a review. Molecules, 21(5), 573.
Vishnupradeep, R., Bruno, L. B., Taj, Z., Karthik, C., Challabathula, D., Tripti, Kumar, A., Freitas, H., & Rajkumar, M. (2022). Plant growth promoting bacteria improve growth and phytostabilization potential of *Zea mays* under chromium and drought stress by altering photosynthetic and antioxidant responses. Environmental Technology and Innovation, 25, 102154.
Watt, M., Moosavi, S., Cunningham, S. C., Kirkegaard, J. A., Rebetzke, G. J., & Richards, R. A. (2013). A rapid, controlled-environment seedling root screen for wheat correlates well with rooting depths at vegetative, but not reproductive, stages at two field sites. Annals of Botany, 112(2), 447–455.
Wołejko, E., Jabłońska-Trypuć, A., Wydro, U., Butarewicz, A., & Łozowicka, B. (2020). Soil biological activity as an indicator of soil pollution with pesticides – a review. Applied Soil Ecology, 147, 103356.
Yermakov, A. I., Arasimovich, V. V., & Yarosh, N. P. (1987). Metody biokhimicheskogo issledovaniya rastenii [Methods of biochemical research of plants]. Agropromizdat, Leningrad (in Russian).