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Title

Assessment of the plant growth promoting potential of some bacterial and fungal isolates under saline stress

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Abstract

Salt stress is a major abiotic stress influencing plant growth, development, and crop yield.

This study aims to characterize the beneficial effects of some endophytic microorganisms on plant growth.

Five bacteria *Bacillus subtilis, Aneurinibacillus migulanus, Baillus parenthracis, Brevibacillus invocatus, and Zhiengliuella alba* and one fungus, *Cladsporium halotoleans* were evaluated in vitro for their tolerance to salt under different concentrations (0, 300, 600, 900 mM NaCl). In addition, their potential to produce indole-3-acetic acid and siderophores was also measured. In vivo tests consisted in the determination of the capacity of the tested microbial strains to promote germination and growth of maize seeds under salt stress.

Results demonstrated that all the microbial strains tested were tolerant to salinity; however, *Bacillus subtilis* demonstrated a higher tolerance to salt (900 mM NaCl) compared to the other isolates.

Furthermore, all microbial strains can produce indole-3-acetic acid (IAA) under different salt concentrations, with *Bacillus subtilis and Bacillus parenthracis* showing higher concentrations of IAA. In addition, all the tested isolates produce siderophores at all the tested NaCl concentrations with *Zhiengliuella alba and Brevibacillus invocatus* demonstrating the higher percentages of production.

Regarding the *in vivo* test, we observed that the microbial strains *Bacillus subtilis, Brevibacillus invocatus, Cladosporium halotolerant, and Aneurinibacillus migulanus* have better effect on the growth parameters of maize seeds, such as higher root length and shoot length.

It is very important to understand the dialog and sensing that occur between microorganisms and plants in order to manage very well the use of Plant Growth Promoting Microorganisms for salt soils rehabilitation. Additional studies regarding the determination of the synthesis of other plant hormones and the ability to fixe and solubilize nutrients are also needed.

Keywords: Salt stress, plant growth promoting microorganisms, indole-3-acetic acid, siderophore, maize seeds.

Résumé

Le stress salin est un stress abiotique majeur qui influence la croissance, le développement et le rendement des plantes.

Cette étude vise à caractériser les effets bénéfiques de certains micro-organismes endophytes sur la croissance des plantes.

Cinq bactéries, *Bacillus subtilis, Aneurinibacillus migulanus, Baillus parenthracis, Brevibacillus invocatus et Zhiengliuella alba*, ainsi qu'un champignon, *Cladsporium halotoleans*, ont été évalués in vitro pour leur tolérance au sel à différentes concentrations (0, 300, 600, 900 mM de NaCl). De plus, leur potentiel à produire de l'acide indole-3-acétique et des sidérophores a également été mesuré. Les essais in vivo ont consisté à déterminer la capacité des souches microbiennes testées à favoriser la germination et la croissance des graines de maïs en stress salin.

Les résultats ont démontré que toutes les souches microbiennes testées étaient tolérantes à la salinité ; cependant, *Bacillus subtilis* a démontré une tolérance au sel plus élevée (900 mM de NaCl) par rapport aux autres isolats.

Toutes les souches microbiennes peuvent produire de l'acide indole-3-acétique (AIA) sous différentes concentrations de sel, avec *Bacillus subtilis* et *Bacillus parenthracis* présentant des concentrations plus élevées d'AIA. De plus, tous les isolats testés produisent des sidérophores à toutes les concentrations de NaCl testées, *Zhiengliuella alba* et *Brevibacillus invocatus* démontrant les pourcentages de production plus élevés.

En ce qui concerne l'essai in vivo, nous avons observé que les souches microbiennes *Bacillus subtilis*, *Brevibacillus invocatus*, *Cladosporium halotolerant* et *Aneurinibacillus migulanus* ont un meilleur effet sur les paramètres de croissance des graines de maïs, tels que la longueur des racines et des pousses plus longues.

Il est très important de comprendre le dialogue et la détection qui se produisent entre les micro-organismes et les plantes afin de bien gérer l'utilisation des PGPM pour la réhabilitation des sols salés.

Des études supplémentaires concernant la détermination de la synthèse d'autres hormones végétales et la capacité de fixer et de solubiliser les nutriments sont également nécessaires.

Mots-clés : Stress salin, micro-organismes favorisant la croissance des plantes, acide indole-3-acétique, sidérophore, graines de maïs.

ملخص

.الإجهاد الملحي هو إجهاد لاأحيائي رئيسي يؤثر على نمو النبات وتطوره وإنتاجية المحاصيل

تهدف هذه الدراسة إلى تحديد الآثار المفيدة لبعض الكائنات الحية الدقيقة الداخلية على نمو النبات.

تم تقييم خمسة بكتيريا، *Bacillus subtilis*، *Aneurinibacillus migulanus*، *Baillus parenthracis*، *Brevibacillus invocatus* و *Zhiengliuella alba* ، بالإضافة إلى فطر واحد ، *Cladsporium halotoleans* ، في المختبر لتحمل الملح بتركيزات مختلفة (0 ، 300 ، 600 ، 900 mM NaCl). بالإضافة إلى ذلك ، تم أيضا قياس قدرتها على إنتاج حمض الإندول -3 أسيتيك ، و حامل الحديد.

ثم تمت دراسة تعزيز نمو النبات باستخدام بذور الذرة لتحديد قدرة السلالات الميكروبية المختبرة على تعزيز إنبات ونمو بذور الذرة تحت ضغط الملح.

أظهرت النتائج أن جميع السلالات الميكروبية التي تم اختبارها كانت متحملة للملوحة ولكن اكثرها هي *Bacillus subtilis* عند التركيز النهائي (900 ملم).

من ناحية أخرى، تنتج هذه الكائنات الحية الدقيقة حمض الإندول 3-أسيتيك (IAA) بحيث Bacillus subtilis، و *Bacillus parenthacis* كشفت عن تركيز أعلى لهذا الهرمون النباتي. زيادة عن ذلك، كانت جميع الميكروبات لديها القدرة على إنتاج حاملات الحديد عند التركيز النهائي (900 ملم)، ولكن *Zhiengliuella alba* و*Brevibacillus invocatus* أظهر إنتاجًا أعلى.

بالنسبة للتجربة على النبات، لاحظنا أن السلالات الميكروبية التي أثرت على نمو بذور الذرة (طول الجذر والبراعم) هي *Bacillus subtilis*، *Brevibacillus invocatus*، *Cladosporium halotolerant*، *Aneurinibacillus migulanus*.

من المهم جدا فهم الحوار والاستشعار الذي يحدث بين الكائنات الحية الدقيقة والنباتات من أجل إدارة استخدام الكائنات الحية الدقيقة المعززة لنمو النبات بشكل جيد للغاية لإعادة تأهيل التربة المالحة. هناك حاجة أيضا إلى دراسات إضافية بشأن تحديد تخليق الهرمونات النباتية الأخرى والقدرة على إصلاح العناصر الغذائية وإذابتها.

الكلمات المفتاحية: الإجهاد الملحي ، الكائنات الحية الدقيقة المعززة لنمو النبات ، حمض الإندول3 -أسيتيك ، حامل الحديد ، بذور الذرة.

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List of abreviations

IAA: Indole acetic acid.

mM: milimolaire.

NaCl: sodium chloride.

PGPF: Plant growth promoting fungi

PGPM : plant growth promoting microorganisms.

PGPR : plant growth promoting rhizobacteria.

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Introduction

Introduction

Abiotic stressors, or environmental stressors, are events that put plants, soil, and microorganisms under stress. Natural systems depend on a delicate balance, and when either of these factors is out of the ordinary, the ecosystem is stressed, endangering the health of all living creatures (Enebe and Babalola 2018). Some of the main abiotic elements that affect plants, microorganisms, and soil are water, nutrients, salts, temperature, and pH (Trinayana et al. 2024).

Salinity is considered one of the most devastating environmental stresses that drastically curtails the productivity and quality of crops across the world. More than 20% of the world's cultivable lands are dealing with the adversity of salt stress, and these salt prone areas are continuously increasing due to both natural and anthropogenic activities (Mirza and Masayuki 2020). However, this adversity has become much more severe in arid and semi-arid regions (Stavropoulou and Archontia2011).

In Algeria, about 3.2 million hectares of agricultural land are salt-affected (Bioud et al. 2023). In the Algerian Sahara, the problems of soil salinization have particular importance. It is estimated that the electrical conductivity of the soils is excessively high, up to 5.000 mS/m^2 in the surface horizons in the summer season (Hadjadj et al. 2022).

Salt stress adversely impacts plants by hindering seed germination, growth, and development, as well as flowering and fruiting. It leads to various physiological and molecular changes and impedes plant growth by inhibiting photosynthesis, thus reducing the available resources and repressing cell division and expansion (Shuangshuang et al. 2021).

Saline soils also lead to nutrient imbalance, ion toxicity, disruption of soil structure, and also osmotic stress among many deleterious effects on plants (Munnset al. 2002). Plant cell death due to osmotic stress may be caused by an increase in sodium ions in the cell walls. Crop development processes such as microsporogenesis, stamen elongation, ovule development, and embryogenesis can be impacted by the soil's salinity, which also promotes programmed cell death (Trinayana et al. 2024).

Soil microorganisms constitute less than 0.5% (w/w) of the soil mass, but they play a key role in soil properties and processes. Salinity affects plants and microbes via two primary mechanisms; osmotic effect and specific ion effects. Another factor influencing plants and microbes is soil water content; soil water potential which relates to the energy level by which the water is held in the soil also closely related to soil salinity, is influenced by osmotic potential in the soil solution (Nan et al. 2021).

Higher NaCl concentrations encourage the expression of a certain gene in bacteria, which is known as a stress-induced increase in the production of a group of proteins.

New ways of biological stress tolerance enhancement are found by the use of beneficial microorganisms that could help plant growth and tolerance to stresses; these are called plant growth promoting microorganisms PGPM (Stavropoulou and Archontia 2011).

In this context, this study aims to characterize beneficial effects of some endophytic strains isolated previously from a plant growing in the saline soils of the Sebkha of Sekhouna region (North West of Algeria) and microorganisms isolated from soil polluted by petroleum hydrocarbons from different regions in Algeria. This will be performed under saline stress both *in vitro* and *in vivo* using Maize seeds.

Literature review

Literature review

Salt stress is considered an alarming condition as it decreases the agricultural productivity of soil and results in reduced crop yields (Mahmood et al. 2019). It affects molecular, morphological, physiological, and biochemical processes in plants, resulting in growth suppression and cell death (Kekeletso et al. 2021).

1. Plant growth promoting microorganisms (PGPM)

Plant growth promoting microbes, or PGPM, are a class of microorganisms that positively impact plant development and health (Chennappa et al. 2019), they are characterized by three intrinsic traits, they must be able to colonize the root rhizosphere, endure and proliferate in root associated microhabitats, competing with other microbiota for the duration required to express their protection and plant promoting activities, and stimulate plant growth (Kumar and Vankayalapati 2016). Numerous actinomycetes, fungus, bacteria, and other eukaryotic microorganisms that may be cultivated in controlled environments can be found in these microbiomes. Rhizobacteria that support plant growth are all the bacteria that live in the rhizosphere and work together to increase crop output and plant growth. Plant growth can be mediated by rhizosphere dwelling microbes through many direct and indirect methods. Certain rhizosphere fungi, functionally known as "plant-growth-promoting-fungi (PGPF), enhance plant development after root colonization, much as PGPRs (table 1) (El-Maraghy et al. 2021). The potential of these PGPM to produce a variety of substances, including siderophores, organic acids, phytohormones, atmospheric nitrogen fixation, phosphate solubilization, and antibiotics, as well as to promote systemic resistance to control plant diseases, has been widely credited to their beneficial benefits (Chennappa et al. 2019).

Table 1. Different microorganisms reported as plant growth promoting PGPM.

PGPM	**Microorganisms**	**References**
PGPR	- *Bacillus subtilis* - *Brevibacillus* spp.	Munees and Mulugeta 2013.
	- *Bacillus megaterium*	Srividhya et al. 2020.
	- *Zhihengliuella* sp.	Sagar et al. 2021.
	- *Zhihengliuella alba*	Ajar Nath et al. 2020.
	- *Pseudomonas*	Habtamu and Mulugeta 2021.
	- *Pseudomonas aeruginosa*	Ajar Nath et al. 2020.
	- *Mesorhizobium* spp.	Munees and Mohammad 2012.

PGPF	- *Aspergillus sp.* - *As. fumigatus* - *As. niger* - *As. terreus*	Motaher and Farjana 2020.
	- *Penicillium simplicissimum*	Hossain et al. 2007.
	- *Penicillium chrysogenum*	Rodrigo et al. 2023.
	- *Trichoderma harzianum*	Iriset al. 2001.
	- *Fusarium* spp.	Shaikhul et al. 2014.
	- *Cladosporium* sp.	Muhammad et al. 2010.

1.1. Plant growth promoting rhizobacteria (PGPR)

Kloepper and Schroth were the first to define PGPR in 1980. A class of bacteria known as the PGPR actively colonize the roots of plants in order to stimulate plant growth and raise agricultural yield. Rhizobacterial strains are known as PGPR because it has been observed that they boost plant development following seed inoculation (Chennappa et al. 2019), they can significantly contribute to the establishment and growth of plants in nutrient deficient environments (Rifat et al. 2012). PGPR’s are the potential tools for sustainable agriculture and trend for the future (Jeyanthi and Kanimozhi 2018). In order to demonstrate their plant growth promotion/protection capabilities, they must be skilled at colonizing the root surface, live, multiply, and compete with other microbiota and support plant development (Munees and Mulugeta 2013). PGPR has been shown to have positive effects on several physiological processes, including as the intake of water and nutrients, photosynthesis, and source-sink connections that facilitate growth and development (Jeyanthi and Kanimozhi 2018). Plant development can be directly or indirectly enhanced by PGPR in a variety of ways. A number of processes, including phosphate solubilization, nitrogen fixation, siderophore synthesis, HCN, ammonia, vitamins, and phytohormones (including auxin, cytokinin, and gibberellins), are involved in direct mechanisms. The synthesis of antibiotics, hydrolytic enzymes, ACC deaminase activity, and induced systemic resistance (ISR) of phytopathogens are examples of indirect mechanisms (Habtamu and Mulugeta 2021). PGPR are currently divided into four categories, "biofertilizers" due to their capacity to fix atmospheric nitrogen and solubilize mineral phosphates, "phytostimulators" due to their ability to produce hormones in plants, "rhizoremediators" due to their ability to break down organic pollutants, and finally "biopesticides" due to their ability to produce siderophores and to synthesize antibiotics, enzymes, and or fungicidal compounds (Nadège et al. 2016).

1.2. Plant growth promoting fungi (PGPF)

PGPF is the term for the class of rhizosphere fungi that colonize plant roots and promote plant growth (Motaher and Farjana 2020). Plant growth-promoting fungi (PGPF) are a diverse group of nonpathogenic fungi that are connected to plants and boost their health and growth (Motaher et al. 2017). PGPF are non-pathogenic saprophytes that live in the soil. They are said to be helpful to a variety of crop plants, both by fostering plant growth and by preventing disease (El-Maraghy et al. 2021). The majority of true fungi classified as PGPF are primarily found in the phylum Ascomycota, which includes the following genera: *Aspergillus, Aureobasidium, Chaetomium, Cladosporium, Colletotrichum, Exophiala, Penicillium, Trichoderma, Fusarium, Gliocladium, Phoma, Phomopsis, Purpureocillium,* and *Talaromyces*. A smaller number of them are found in the Basidiomycota, which includes *Limonomyces, Rhodotorula, Rhizoctonia,* and soil-forming fungi, as well as Zygomycota (Motaher et al. 2017). The several mechanisms by which PGPF enhances plant growth in the rhizosphere can be summed up as follows: siderophores biosynthesis, supplying plants with phytohormones like cytokinins, gibberellic acids, and 3-indole acetic acid, increasing insoluble minerals bioavailability as a result of mobilization, reduction of the negative effects of stress on plants and enhancement of their resistance to various stressors, such as salinity, temperature, and drought (El-Maraghy et al. 2021).

1.3. Mechanisms of action of PGPM

Through their interactions with plants, microbes use biochemical and molecular pathways to help mitigate the detrimental effects of abiotic stressors on plant growth (Koza et al. 2022). The diverse methods exhibited by the bacteria contribute to the growth and development of crop plants, either directly or indirectly, ultimately leading to an improvement in crop productivity. Direct mechanisms are those that aid in the microbial production of chemicals or aid in the uptake of environmental nutrients. These include nitrogen fixation, phosphate solubilization, siderophore synthesis, HCN, ammonia, vitamins, and phytohormones (including gibberellins, cytokinins, and auxin). While indirect mechanisms, such as the synthesis of antibiotics and enzymes that damage cell walls, do not directly contribute to the increase of growth, they do generate a variety of inorganic and organic substances through different pathways (Fig. 1) (Olanrewaju, et al. 2017).

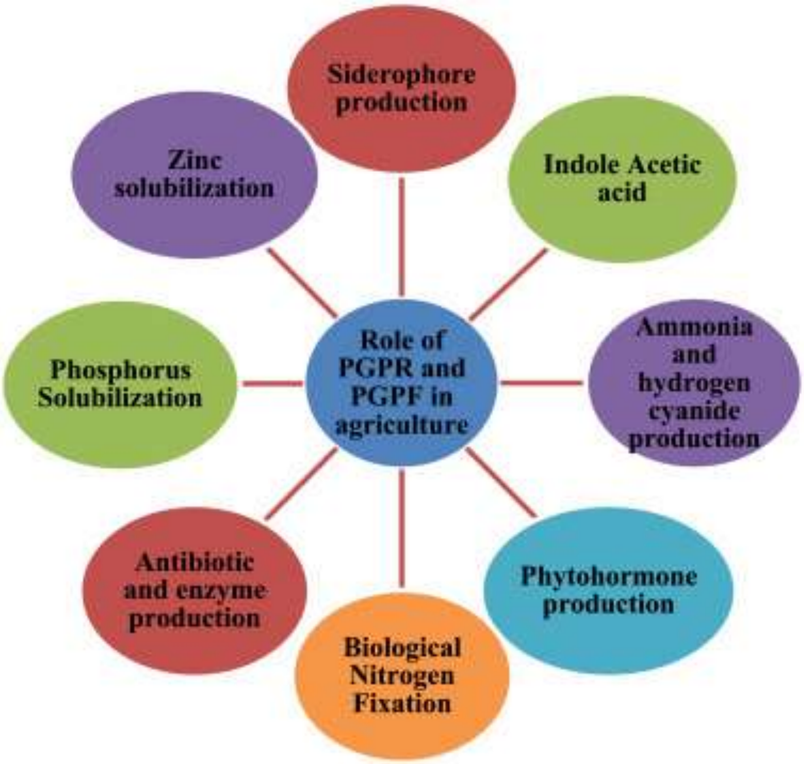


Figure 1. Important mechanisms of microbes to enhance growth and productivity of crops (Kumar et al. 2022).

1.3.1. Siderophores production

Numerous bacteria and fungi make and use siderophores, which are agents that chelate iron. The soil rhizosphere produces these low molecular weight chemicals under neutral to alkaline pH conditions. (Chennappa, et al. 2024). For several physiological functions, such as photosynthesis, electron transport, transpiration, and enzyme cofactor activity, plants require iron (Nandni et al. 2024). By producing siderophores, the microorganisms use iron, and this process is crucial for deciding whether or not the bacteria can colonize plant roots and for competing with other microbes for iron (Lorenzo et al. 2021).

1.3.2. Phytohormones

Through a variety of physiological and metabolic processes, including cell division, stem elongation, inhibition, root growth, activation of bud and branch development, promotion or delay of leaf senescence, and chlorophyll production, phytohormones are the primary regulators of plant growth and development in plants. etc (Kumar et al. 2022) as well as the mediators of environmental stress responses such as indole acetic acid (IAA) gibberellin and cytokinins (Muhammad et al. 2010; Bi-Xian et al. 2021).

1.3.3. Indole acetic acid (IAA)

The most common natural auxin, indole acetic acid (IAA), promotes root development (Sharma et al. 2024). Many of the microorganisms that colonize the seed coat or root surface have been proposed to work in concert with endogenous IAA to facilitate cell development and the uptake of minerals and nutrients from the soil (Rehman et al. 2016). IAA affects plant cell division and elongation extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity, and fluorescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stress (Kumar and Vankayalapati 2016; Bi-Xian et al. 2021).

Methodology

Methodology

1. Aim of the study

This study aimed to test the plant growth promoting potential of some microbial species under saline stress in vitro and in vivo with maize seeds.

2. Material and methods

2.1. Material

2.1.1. Microbial strains

In this study 5 bacteria and 1 fungus isolated in previous works have been tested for their potential to promote plant growth. These isolates have been identified as bacteria; *Bacillus subtilis*, *Bacillus parenthracis*, *Aneurinibacillus migulanus* (islolated from soil polluted by petroleum hydrocarbons), *Brevibacillus invocatus*, *Zhihengliuella alba* and a fungus *Cladosporium halotoleran*s that were isolated from Essebkha in Skhouna region (North West of Algeria).

2.1.2. Plant material

Maize seeds were used in this study to determine the effect of the microbial isolates in promoting its growth.

2.2. Methods

2.2.1. In vitro tests

2.2.1.1. Evaluation of the salt tolerance of the microbial strains

The tolerance to salinity of the bacterial isolates and the fungi was tested with increasing NaCl concentrations (0, 300, 600, 900 mM) using nutrient broth medium (NB), Escherichia coli was used as a reference for non-tolerant species. For each NaCl concentration, 100 µl of the bacterial and fungal strains were inoculated with an initial (OD600= 0,1) and incubated under shaking (150 rpm) at 30°C and 24 h for the bacteria and at room temperature for the fungus for 7 days. Then, microbial growth was measured as cell density determined using spectrophotometric readings at 600 nm. Each NaCl concentration was tested in triplicate (Sandeep et al. 2016).

2.2.1.2. Production of indole-3-acetic acid

The Production of indole-3-acetic acid (IAA) was tested utilizing the following steps. The bacterial strains were grown for 24 h and the fungus for 7 days until reaching. After that, 100 µl of freshly grown bacterial and fungal culture were taken separately at a density of approximately 1.5 x108 CFU/ml, and inoculated in nutrient-broth (NB) supplemented with 1% of L-tryptophan. The bacterial and the fungi strains were incubated on a rotary shaker at 180 rpm for 4 days at 30 ± 2°C for bacteria and for 7 days at room temperature for the fungus. After centrifugation for 10 min at 10,000 g, 1 ml of supernatants were collected and mixed with 4 ml Salkowski‘s reagent. The mixtures were incubated for 30 min in dark at 25±2°C then the absorbance was read using a spectrophotometer at 530 nm. The IAA quantification was performed based on standard curves prepared with pure IAA (Lebrazi et al., 2020). All experiments were performed in triplicate. Furthermore, we tested the effect of NaCl on IAA production by adding increasing NaCl concentrations (0, 300, 600 and 900 mM) to the media. After inoculation and incubation, in the same conditions as previously described, the amount of IAA produced was estimated spectrophotometrically at 530 nm.

2.3.3. Production of siderophores

Quantitative estimation of siderophore was done by taking supernatant of bacterial cultures grown in LB broth medium. For this, 1 ml broth was taken in 1.5 ml centrifuge tube (one for each bacterial culture) and after sterilization inoculated with 100 µl of freshly grown bacterial culture (10^8 CFU/ml). Three replicates (tubes) were taken for each strain. Apart from this, control tube (uninoculated broth) was also maintained. After incubation at 28°C for 48 h, bacterial cultures were centrifuged at 10,000 rpm for 10 min, cell pellets were discarded, and supernatant was used to estimate siderophore. Supernatant (0.5 ml) of each bacterial culture was mixed with 0.5 ml Chrome Azurol S (CAS) reagent and after 20 min optical density was measured at 630 nm.

We tested the effect of NaCl on siderophore production by preparing LB media (as previously described) with increasing NaCl concentrations (0, 300, 600 and 900 mM). After inoculation and incubation (in the same conditions as previously described), the amount of siderophore produced was estimated spectrophotometrically at 630 nm. Siderophore produced by strains was measured in percent siderophore unit (psu) which was calculated according to the following formula (Arora & Verma, 2017):

Siderophore production (psu) = $[(Ar-As)/Ar]\times 100$

where Ar = absorbance of reference (CAS solution and un-inoculated broth), and

As = absorbance of sample (CAS solution and cell-free supernatant of sample)

2.2.2. *In vivo* Tests

2.2.2.1. Plant growth-promoting potential of the isolates

Maize (*Zea mays*) seeds were surface sterilized with bleach for 5 min. The seeds were washed two times with sterilized distilled water for 5 min. To prepare the inoculum (10^8 CFU/ ml), the microorganisms were previously grown in Petri dishes containing nutrient agar medium for 24 h at 35°C for the bacteria and at 30 ± 2°C during 7 days for the fungi.

For seed treatment, 10 ml of the microbial suspension was added to 120 seeds and homogenized to contact the seeds with the microorganisms. 10 maize seeds were sown in Petri dish containing filter paper. The experiment was designed to have three treatments and three repetitions: (i) Control without microorganisms and normal irrigation; (ii) inoculated with microorganisms and without salt stress; (iii) irrigated with 50, 300, 600, 900 mM NaCl and inoculated with microorganisms. The pots were maintained at 25 °C ± 2. After three days, thinning was performed, keeping one per Petri dish. After that we seed and we put them in sterilized plastic pots. To simulate normal irrigation with tap water, after every 24 hours, the plants were collected to measure roots and stems lengths.

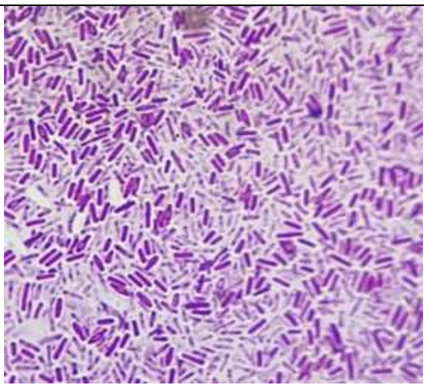
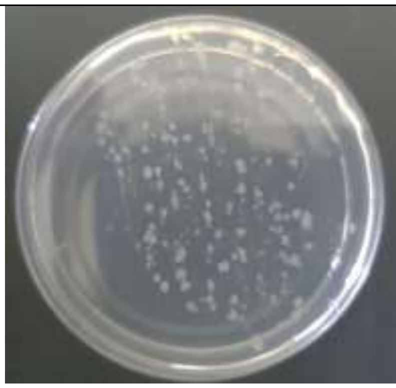
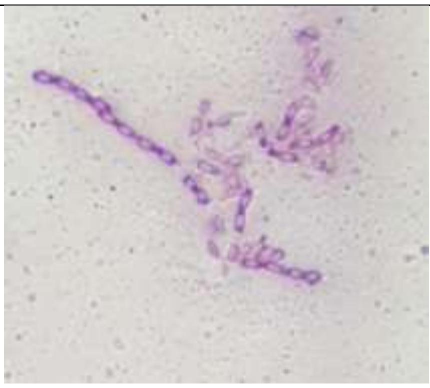
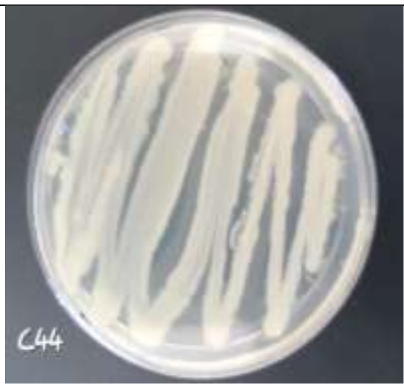
Results

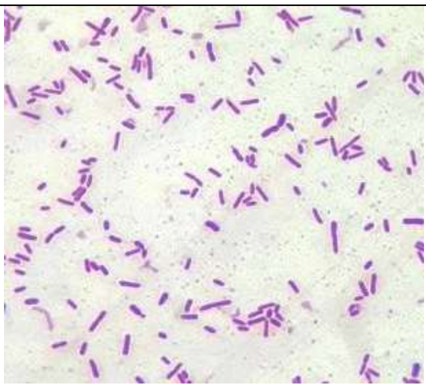
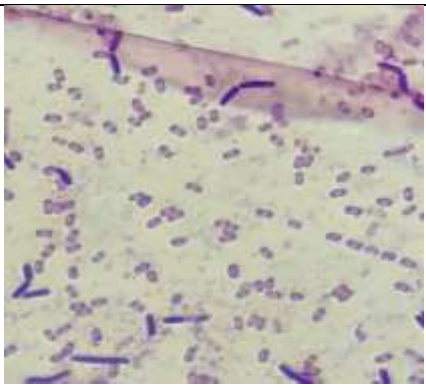
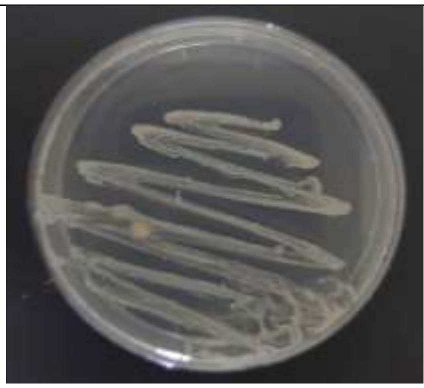
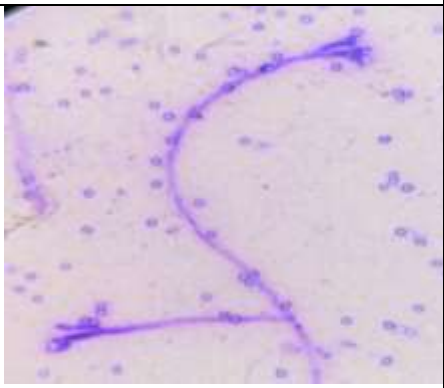
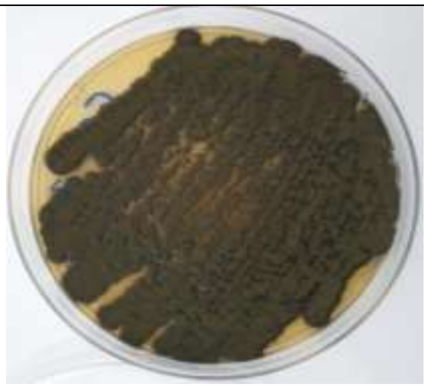
Results

1. Macroscopic and microscopic observations of the tested microbial isolates

The macroscopic and microscopic observations of the tested microorganisms are represented in table 2.

Table 2. Macroscopic and microscopic observations of the tested microorganisms

Aneurinibacillus migulanus (k20)		
Bacillus parenthracis (C44)		
Zhihengliuella alba (B12)		

Brevibacillus invocatus B11(1)		
Bacillus subtilis (k1)		
Cladosporium halotolerans (Ca)		

2. *In vitro* tests

2.1. Evaluation of the salt tolerance of the microbial strains

We observed that all microbial strains showed growth in the different concentrations of NaCl tested. However, *Bacillus subtilis* demonstrated the higher growth rate at the higher concentration of NaCl (900 mM) in addition to the formation of a biofilm at the surface of the medium. Moreover, all the tested microbial species showed better growth at 600 mM NaCl except *Z. alba* where growth was seen only at 300 mM of NaCl (Fig. 2).

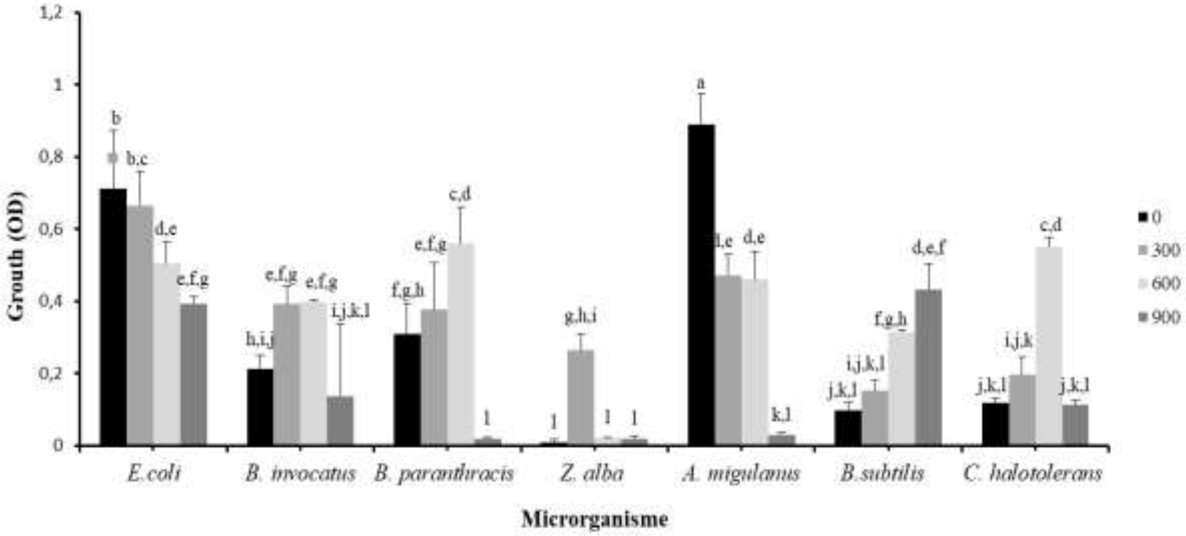


Figure 2. Halotolerance of the tested microbial isolates.

2.2. Production of indole-3-acetic acid (IAA)

All microbial strains tested have ability to produce indole acetic acid (IAA) but there remains a difference in high concentration of NaCl. *Bacillus subtillis* showed ability to produce IAA with high percentage in highest concentration of salt followed by *Bacillus paranthracis* (Fig. 3).

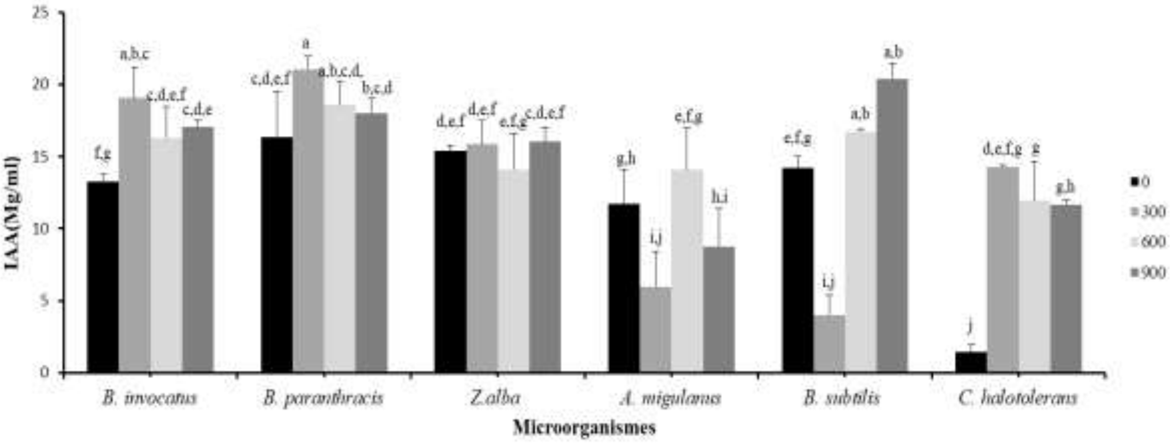


Figure 3. Production of IAA by the tested microbial isolates.

2.3 Siderophores production

This assay confirmed the production of siderophore by the different microbial strains in different concentration of NaCl (300, 600, 900 mM). All microbial strains showed ability to produce siderophore but we observed the capacity of *Zhihengliuella alba* to produce siderophores with highest percentages (93.10%) until the final concentration (900 mM) (Fig. 4)

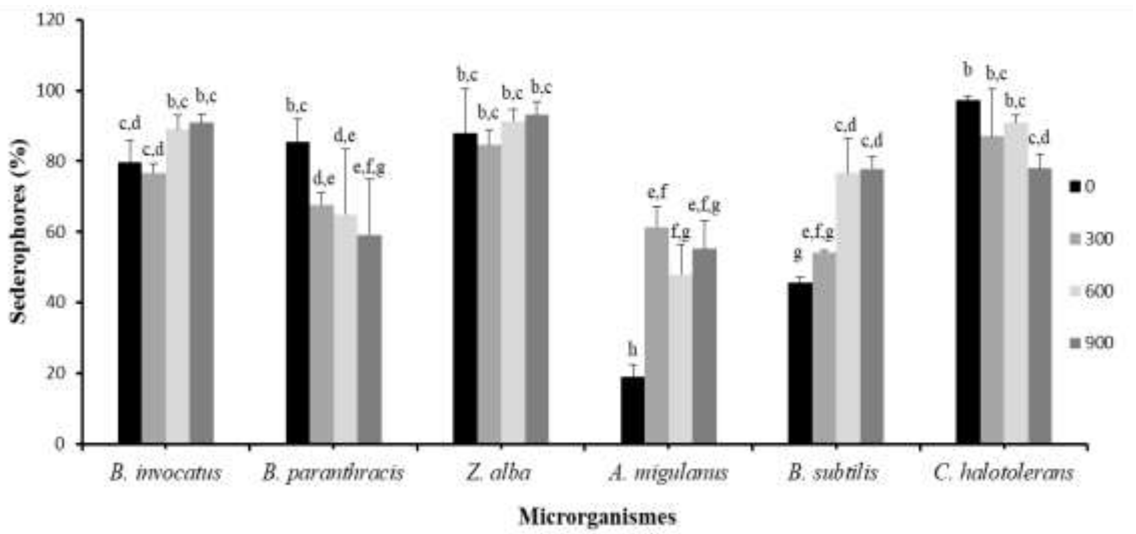


Figure 4. Production of siderophores in the tested microbial isolates

3. *In vivo* tests

It should be noted that the seed of Maize that were tested at concentration of 300, 600, and 900 mM of NaCl did not germinate and even got altered. However, the seed treated with 50 mM NaCl showed different responses depending on the microbial isolate being treated with (Fig. 5).

Figure 5. Maize (*Zea mays*) growth after inoculation with the microbial strains tested.

3.1. Root growth

After inoculation of maize seeds with the different microbial species, we noticed growth of the roots that was measured in terms of length and width. However, the results differed when salt was added. In maize seeds inoculated with *Bacillus invocatus* we noticed that root length increases in salt stress compared with control. While, no growth was seen in seeds inoculated with *Z. alba* under salt stress (Fig. 6).

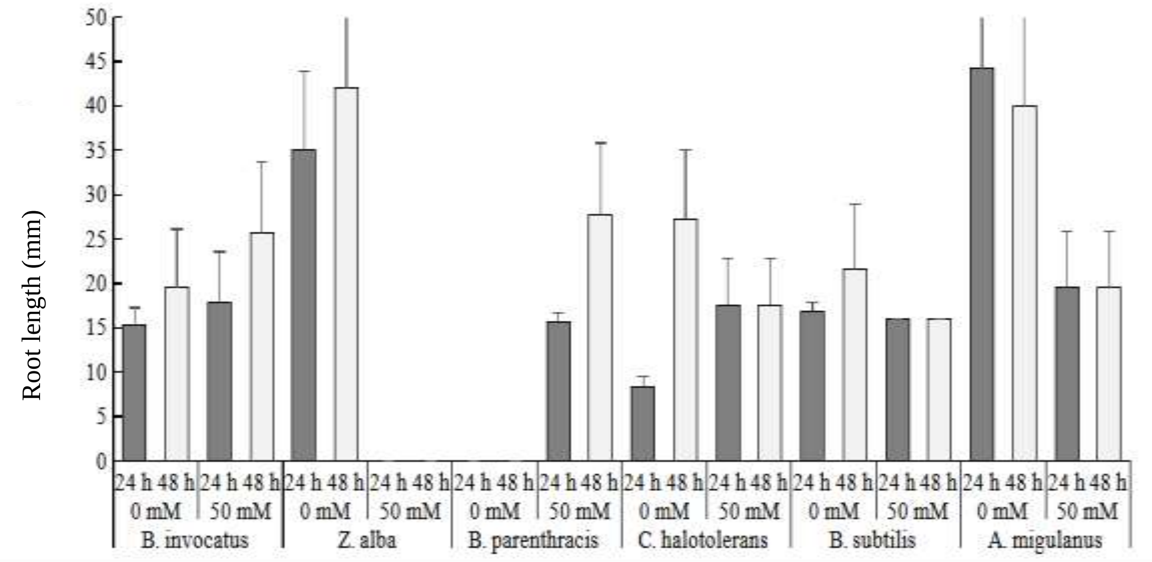


Figure 6. Root length of maize inoculated with microbial strains under normal and salt stress conditions.

3.2. Shoot growth

We observed that all seeds treated with the different microbial species had their shoots grow. However, under salt stress, seeds inoculated with *Z. alba* and *B. paranthracis* did not develop their shoots. Besides, seed inoculated with *Bacillus subtilis* showed an increase in the shoot length under salt stress while seeds inoculated with *B. invocatus* showed the highest shoot length that remained the same in normal and under salt stress conditions (Fig. 7).

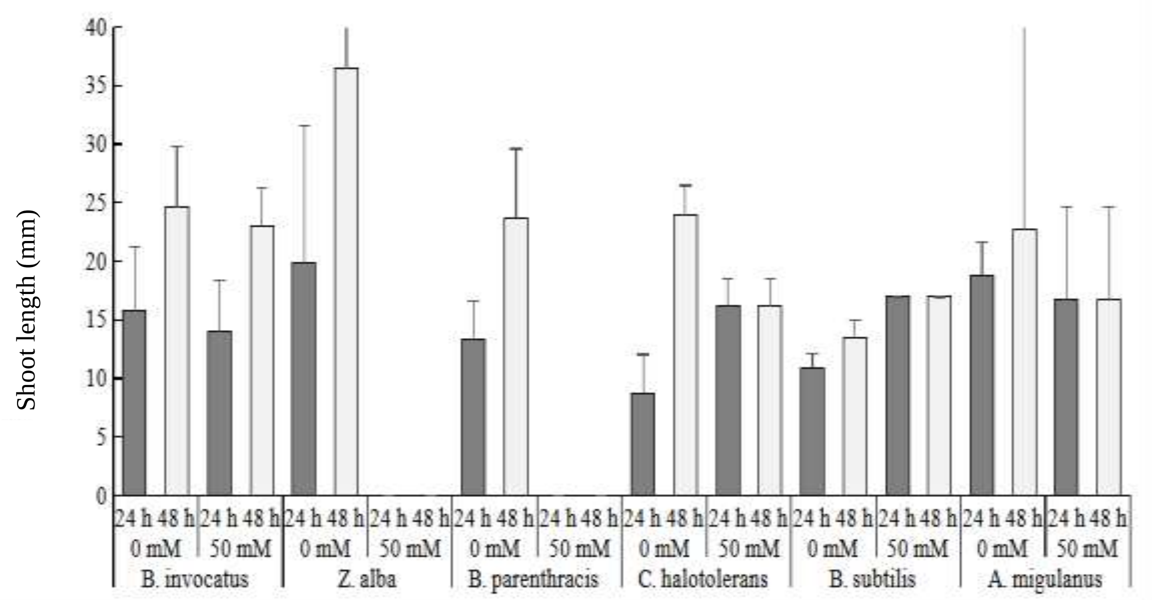


Figure 7. Shoot length of maize inoculated with microbial strains under normal and salt stress conditions.

Discussion

Discussion

Salinity, a harsh environmental factor, significantly impacts crop plant productivity, affecting land area on an increasing scale (Pooja and Rajesh 2015).

Through our investigation, previously isolated microorganisms were tested for their potential to promote plant growth under salt stress. Six microbial strains were tested in this study, 5 bacteria and one fungus.

All microbial strains tested in this study showed ability to grow at the different concentrations of NaCl tested (300, 600, 900 mM) at different rates. *Bacillus subtilis* and *Brevibacillus invocatus* demonstrated higher growth rates at the higher concentration of salt (900 mM), this is in agreement with research done by Saboor et al. (2023) who demonstrated also that *Bacillus subtilis* has ability to tolerate high concentration of salt. In addition, when exposed to environmental stressors, *B. subtilis* has the ability to generate very resistant dormant endospores (Earl et al. 2008). Also, we observed the formation of biofilm in the test tube during the experiment which was also observed in the study performed by Earl et al. (2008) that observed growing biofilms when *B. subtilis* was injected onto *Arabidopsis thaliana* roots.

It was shown that *B. subtilis* inoculation decreased the generation of reactive oxygen species (ROS) and the activities of certain enzymes. This finding is in line with other studies that demonstrated elevated antioxidant enzyme activities in wheat cultivars under salinity stress (Saboor et al. 2023).

Regarding the tested fungus, higher growth was observed under 600 mM NaCl, which is similar to the result of Ming et al. (2023) that demonstrated that *Cladosporium* has ability to grow in high concentration of NaCl. In fact, endophytic fungi counteract salt stress in plants by enhancing the amount of osmoprotectants, lowering salt-induced root respiration, altering the phytohormone profile, and stimulating the antioxidant system (Siddiqui et al. 2022).

Furthermore, all the tested microbial species could produce IAA under all the tested salt concentrations. *B. subtilis* demonstrated the higher rate of IAA production under the higher concentration of NaCl (900 mM) followed by *B. paranthracis*, *B. invocatus* and *Z. alba*. The primary auxin in plants, IAA controls a variety of growth and developmental processes, including responses to light, gravity, and pathogens, apical dominance, tissue differentiation, and cell division and elongation (Feng et al. 2015). Our results, are in accordance with those reported by Sarwat et al. (2021).

In addition, Kondrasheva et al. (2022) showed that *Cladosporium* sp. has the capacity to produce IAA in extreme salinity conditions. reported that obtained data showed that the studied halotolerant

Furthermore, we noticed that all microbial strains can produce the siderophore up to the concentration 900 mM NaCl. However, *Zhihengliuella alba* and *Brevibacillus invocatus* demonstrated the higher percentage of production compared to the other strains at 600 and 900 mM NaCl. Furkan and Abdullah (2020) showed that *Zhihengliuella salsiginis* can produce 81.86 µL siderophore at an NaCl concentration of 200 mM.

Besides, maize (*Zea mays* L.) is highly impacted by salt stress, which has emerged as one of the main factors limiting maize output. Its productivity is particularly vulnerable to salinity stress at higher salinity levels (Muhammad et al. 2022).

In this study, when maize seed were subjected to concentrations of NaCl higher than 50 mM, no germination was observed, however at 50 mM different responses were reported depending on the microbial strain inoculated to the seeds.

We noticed that when maize seed are combined with all the tested microbial strains (separately) under normal conditions, except with *B. parenthracis*, the growth is increased which is evaluated through the measure of root and shoot lengths. Under salt stress we observed that maize seeds combined with all the tested microbial strains, except with *Z. alba*, demonstrated growth. *B. subtilis* inoculated seeds showed the best growth rate compared to the other strains. Similar results have been reported by Sarwat et al. (2021) that demonstrated under salt stress, both in lab and field conditions, plants inoculated by *Bacillus* sp. may retain their physio-morphological characteristics. However, strains of *Bacillus mojavensis* and *Bacillus subtilis* positively impacted the Arabidopsis growth parameter (Abdelkefi et al. 2024).

Bacillus strains that produce IAA and colonize roots are beneficial in enhancing seed germination and growth characteristics by enhancing several physiological activities, such as osmotic stress mitigation (Sarwat et al. 2021). Another research confirmed that in pot experiments, treatment with PHs from *Cladosporium* isolates have beneficial effects on growth and development of plants (Răut et al. 2021).

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Conclusion and perspectives

Conclusion and perspectives

Salt stress is a major environmental stress that affects plant growth and development. Several approaches are used to rehabilitate salt soils especially with the increasing demand of the growing population for food. Plant growth promoting microbes (PGPM) can enhance plant growth, speed up seed germination, improve seedlings emergence, and protect plant from various biotic and abiotic stress.

The present study aims to characterize the beneficial effects on plant growth of some endophytic and soil isolated microorganisms throughout in vitro and in vivo tests under normal and salt-stress conditions.

The in vitro studies have revealed that all microbial strains tested have ability to tolerate different concentrations of NaCl. *Bacillus subtilis*, *Brevibacillus invocatus*, *Cladosporium halotolrant* have shown the higher ability to growth in extreme salinity conditions with higher production of IAA and siderophores.

However, the in vivo tests have demonstrated that every microbial strain inoculated have positive impact on maize growth (root and shoot) both under normal and salt-stress conditions.

In general, our results showed that microbial inoculated plants are able to maintain their physio-morphological characters under salt stress.

It is very important to understand the dialog and sensing that occur between microorganisms and plants in order to manage very well the use of PGPM for salt soils rehabilitation.

Additional studies regarding the determination of the synthesis of other plant hormones and the ability to fixe and solubilize nutrients are also needed.

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