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RESEARCH ARTICLE

Effect of some *Pseudomonas* strains and *Agave americana* L. on wheat germination under salt stress

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Abstract

Currently, several efforts focus remedying the problem of agricultural soil salinity using eco-friendly strategies. This study aimed particularly the study of *Triticum durum* (durum wheat) seeds germination in the presence of Pseudomonas strains and hydro-alcoholic extract of *Agave americana* L. under saline stress conditions. The preliminary phytochemical screening of *A. americana*, phylogenetic identification and production of indole-3-acetic acid (IAA) by *Pseudomonas* strains, *in vitro* impact of hydro-alcoholic extract and *Pseudomonas* strains combination on salt stress resistance, preliminary effects of *A. americana* on *Triticum durum* germination and phytopathogenic fungi inhibition under salt stress were carried out using corresponding protocols. In *in vitro* trials, phytochemical screening revealed the richness of *A. americana* in polyphenols (1014.062±161.017 mM GA equivalent/g FW) and flavonoids (51.065±27.391 mg quercetin equivalent/g FW). The ability of *Pseudomonas* strains to produce the phytohormone indole-3-acetic acid (IAA) varied from 116.67±8.25 µg/ml to 857.14±80.50 µg/ml. The leaf extract of *A. americana* is an effective osmoprotectant that improves the resistance of the strain P1 *Pseudomonas plecoglissicida* to saline stress. In *in vivo* experiments, the extract of *A. americana* did not show any effect on the germination of wheat seeds. However, it effectively inhibited the contamination of seeds by phytopathogenic fungi during germination and saline conditions. Findings of the study revealed that *Pseudomonas plecoglissicida* and *A. americana* extract are very promising for the inhibition of phytopathogenic fungi and the alleviation of salt stress.

Keywords

Agave americana, germination, *Pseudomonas*, salt stress, *Tritium durum*

Introduction

One of the principal abiotic factors limiting plant productivity and hence agricultural production is salinity. High salt levels damage approximately 800 million hectares of land worldwide and have the potential to significantly reduce crop output (1). In particular, salinity threatens 3.2 million ha of agricultural land in Algeria (2). This salinization is typically seen in the arid and semi-arid regions. Numerous physiological mechanisms in plants are significantly affected by high levels of salt (primarily sodium chloride): germination, growth and availability of nutrients (3). Germination and other growth-related characteristics are greatly decreased when salinity reaches 100 mM (4). Since nutrients and saline ions are antagonistic to one another, growth under saline conditions enable plants to absorb Na^+ and Cl^-, which

can lead to a nutritional imbalance with potential impact on the foliage (5). The generation of reactive oxygen species, ionic imbalance and osmotic disruption are all strongly associated with salinity-induced cellular damage (6). Especially, salt stress caused oxidative stress in plants by producing reactive oxygen species (ROS), which degrade cellular membranes and cell organelle proteins, particularly those of mitochondria, chloroplasts and peroxisomes and alter the general integrity of the cell. Osmotic stress caused by salinity reduces the capacity of plant roots to absorb water and results in water loss from leaves which leads to an increase in the concentration of salts in salt-stressed plants (7). Other consequences of these impacts include the potential for membrane damage, changed level of growth regulators, enzyme inhibition and metabolic malfunction, the eventual impairment of photosynthesis and plant mortality (8, 9). As a result, it is also difficult for plants to absorb nutrients, produce phytohormones, control their roots and shoots and replicate DNA (10).

According to various studies, the resolution of this problem would lie in the improvement of management procedures and appropriate choice of plant cultivars, the selection and amelioration of osmotolerant PGPRs (Plant Growth Promoting Rhizobacteria), and the use of natural fertilizers that do not cause the accumulation of toxic ions (11, 12). Notably, the presence of PGPRs in the rhizosphere constitutes an undeniable interest in growth and development through various stimulating plant growth mechanisms (12). In this regard, it has been suggested that using plant growth-promoting bacteria (PGPB) may boost plants' resistance to salt stress while being eco-friendly and cost-effective (13). Especially, PGPR can improve plant salt tolerance through ion homeostasis, antioxidant generation, ACC deaminase, phytohormones, extracellular polymeric substance (EPS), volatile organic compounds, accumulation of osmolytes, activation of plant antioxidant enzymes and improved nutritional uptake (14). Some PGPR have the ability to generate cytokines, accumulate ABA and generate antioxidants that can detoxify ROS (15). Through supplying minerals like nitrate, phosphate and potassium, they can also colonize the surface of plant roots and reduce the effects of salt stress (16). The microbial mediated increase in soil enzyme activity, which was directly involved in the mineralization of key elements and hence controlled nutritional imbalance during salt stress, was highly linked with an improved nutrient profile under salt stress (17). Via lowering the maintenance of ionic homeostasis, boosting antioxidant machinery and controlling gene expression, salt-stressed plants inoculated with PGPR enhance crop growth and yield. Additionally, PGPR control plant tolerance to salinity as well as photosynthetic properties, notably, net photosynthetic rate, chlorophyll and carotenoid concentrations (18). The plant inoculation study revealed that bacterial inoculation significantly increases plant transpiration and stomatal conductance, which increases yield under saline environments (19).

Inoculation of economically important plants with plant growth-promoting rhizobacteria such as *Pseudomonas* or plant extract (20) is a biological method that is both efficient and cost-effective for recovering salt-affected soils and enhancing agricultural production (21). The principal aim of this work was to assess the effects of *Pseudomonas* strains and hydro-alcoholic extract of *A. americana* on wheat seeds germination under salt stress conditions. The process of seed germination, which is crucial to the development of total biomass and yield and involving complex phenomena of several physiological and biochemical changes that activate the embryo, is a criterion of the utmost importance (22). Salinity causes a variety of disorders and metabolic modifications during seeds germination, including solute leakage, K^+ efflux and α-amylase activity (23). For soil desalinization and seeds germination, the employment of biological strategies designed to integrate several treatments is particularly promising (24). In this context, the study was conducted in order to evaluate the effects of *Pseudomonas* spp. which are among the most abundant bacteria in the soil, probably because of their high growth rate compared to other bacteria. Moreover, they are known for their high metabolic diversity, biological control properties and resistance to salt stress. The combination of these bacteria with the extract of a thorny plant, *A. americana*, which is known for its tolerance to climatic conditions and not explored for this purpose yet, can present a promising alternative for alleviating salt stress during seed germination (a crucial step for the success of the crop culture) of wheat, representing, without doubt, a plant of worldwide economic interest. To solve the challenges affecting food security, it is essential thus to grow plants in high saline soils with the greatest amount of production (25).

Materials and Methods

Materials used in the study

Pseudomonas strains

In 2010, six (6) bacterial strains were isolated, purified and stored at -20°C in TSB+25% glycerol (Table 1). These strains were part of a collection aiming to screen and evaluate their PGP (Plant Growth Promoting) properties.

Table 1. Origins and identification of *Pseudomonas* strains

Code	Site	Department	GPS Localization	Plant
P1	Tizi	Mascara	35°18'N 0°04'E	*Allium cepa* L (B)
P2	Rocade Nord	Sidi Belabbes	35°13'N 0°37'W	(B)
P3	Rocade Nord	Sidi Belabbes	35°13'N 0°37'W	(B)
P4	Bendaoud	Relizane	35°42'N 0°31'E	*Pisum sativum* (R)
P5	Mohammadia	Mascara	35°35'N 0°03'E	*Hordeum vulgare* L (R)
P6	Mohammadia	Mascara	35°35'N 0°03'E	*Hordeum vulgare* L (R)

R: Rhizospheric soil, **B**: Balk soil

Agave americana L.

A. americana L. was collected from the department of Mascara. The aerial parts represented by the leaves of mature plant (herbarium specimen AA-2020-29) were recovered

after separation by cutting. The leaves were transferred directly to the laboratory for analysis (Fig. 1).

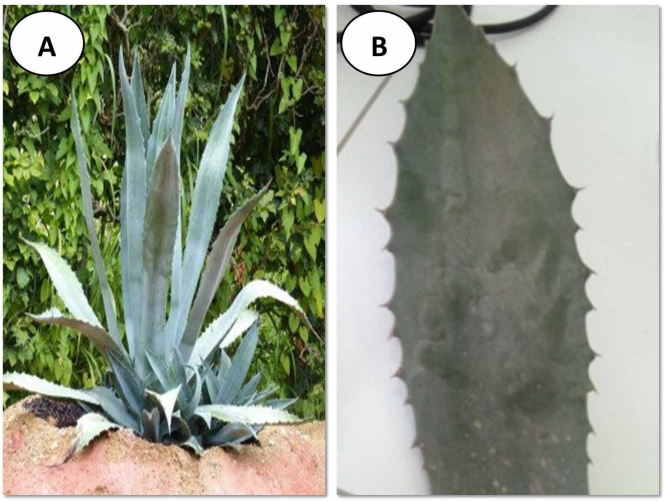

Fig. 1. Sample of *Agave americana* L. used in the study (Herbarium specimen AA-2020-29; **A**: Plant; **B**: Leaf.

Tricicum durum

Three (3) varieties of *Triticum durum* (durum wheat) used in this work were recovered in 2019 from CCVD (Cooperative of Cereals and Vegetables Dry) of Mascara and Tiaret departments (Table 2).

Table 2. Origins of *Triticum durum* varieties

Code	Variety	Provenance	Origin
V1	Simeto B.D	CCVD of Mascara	Italy/Capeiti8/Valvona
V2	Vitron	CCVD of Mascara	Turkey/77/3/Jori/Anhinga/
V3	Chen's	CCVD of Tiaret	Bit 'S'CD 26406

V: varieties

Characterization of Pseudomonas strains

Phylogenetic identification

The method used for the extraction and purification of total DNA was based on the earlier report (26). DNA was extracted by precipitation with a phenol/chloroform/isoamyl alcohol mixture. Then, it was sequenced using the automatic sequencer ABI 377 (Applera, Foster City, USA). Sequence alignments were performed using Clustal W software (27). The homology of paired sequences was evaluated and the substitution rate per transformation was subjected to the two-parameter technique (28). The resulting substitution rate matrix was analyzed by the neighbour-joining method (29), utilizing Mega 7 (Molecular Evolutionary Genetics Analysis) software (30). The confidence levels of the phylogenetic tree topology obtained were estimated by the technique of data resampling analyses with 100 replications.

Production of indole-3-acetic acid (IAA)

IAA production was estimated using the standard method described (31). In 100 ml of TSB (Tryptic Soy Broth) supplemented with L-tryptophan, 10 % of exponential cultures of *Pseudomonas* bacterial strains were inoculated. After incubation at 30 °C/24 hr, the TSB was collected and centrifuged at 2700 g for 15 min. Then, quantitative IAA measurement using salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) was realized. Tubes containing IAA extract were kept at room temperature in the dark for 20 min. until the development of a pink color. Optical density was measured spectrophotometrically at 535 nm (32). A calibration curve (from 50 µg/ml to 800 µg/ml) was used to determine the concentration of IAA produced by each strain (33).

Agave americana L. *characterization*

Preparation of the hydro-alcoholic extract

200 g of *A. americana* leaves were washed and catted into small slices. Then, they were vortexed and homogenized in ethanol at 70 % (v/v). Agitation was performed at room temperature. The mixture obtained was filtered through filter paper in order to remove solid particles. The filtrate was evaporated to dryness under vacuum at 40 - 60 °C using a steam rotation. The dry extract obtained was weighed and recovered in 10 ml of distilled water (34).

Total polyphenol content

The Folin-Ciocalteu colorimetric method was used to establish the total phenolic content (35). Using a homogenizer, leaves (weighing 12 g) were mixed with 10 ml (80 %) of ethanol. After incubation at 4°C/2 hr in the dark, these samples were poured into tightly sealed 50ml plastic tubes and filtered. The pellets obtained were then soaked in ethanol (2.5 ml). Leaf extract (125 µl), Folin-Ciocalteu reagent (625 µl) and 250 µl (7.5%) Na_2CO_3 were used to make four repetitions. The contents were vortexed for a few seconds before being incubated for 15 min at 45 °C. The optical density of phenolic compounds was measured at 750 nm using a standard range of gallic acid (GA) as a standard. The results were given in mM GA equivalent/g FW (fresh weight) of plant material.

Total flavonoid content

Total flavonoid content was determined using the standard technique reported (36): 500 µl of plant extract were mixed with 1500 µl of methanol (95%), 100 µl of $AlCl_3$ (10%), 100 µl of sodium acetate (1M) and 2.8 ml of distilled water. The compounds were mixed and incubated at room temperature for 30 min in the dark. Through replacing the plant extract with methanol (95%), a blank was generated. A UV spectrophotometer was then used to measure absorbance at 415 nm. Using a quercetin calibration curve, the results were expressed as mg quercetin equivalent/g FW.

In vitro effect of hydro-alcoholic extract and Pseudomonas strains' combination on salt stress resistance

Screening of *Pseudomonas* strains for their tolerance to salt stress in the presence and the absence of *A. americana* hydro-alcoholic extract was performed by the standard method (37). Tubes containing 5ml of TSB supplemented with 200 mM of NaCl were prepared. The tubes were inoculated with standard inocula of each *Pseudomonas* strain, forming an equivalent concentration 2×10^5 CFU/ml. Four experiments were performed: tubes containing hydro-alcoholic extract of *A. americana*; tubes containing *Pseudomonas* strains and hydro-alcoholic extract of *A. ameri-*

cana; positive control tubes containing *Pseudomonas* strains and Glycine (5%) as an osmoprotectant; and negative control tubes containing *Pseudomonas* strains in TSB without NaCl. After incubatin at 30°C/24 hr, optical density was measured at a wavelength of 600 nm using an uninoculated medium representing the blank.

In vivo impact of Agave americana on Triticum durum germination

Preliminary effect of *Agave americana* on *Triticum durum* germination

A preliminary effect of the hydro-alcoholic extract *of A. americana* on wheat germination was tested using the method (38). Seeds were sterilized during three steps: they were first soaked in ethanol (70 %) for 1 min, then in sodium hypochlorite solution (12 %) for 15 min and finally, they were washed six times with sterile distilled water (39). The procedure involved Petri dishes previously containing water agar (8-9 %). Each wheat cultivar was implanted with 10 seeds per Petri plate. Then, 1 ml of hydro-alcoholic extract of *A. americana* was applied, generating the treated seeds and forming three repetitions. Untreated seeds were provided 1 ml of sterile distilled water as control. The Petri dishes were sealed with parafilm and germination was observed after incubation at 28 °C for 5 days in the dark. The findings were represented as the number of germinated seeds in each Petri dish at the end of the experiment and the germination percentage was calculated using the following formula:

$$PG = \frac{n}{N} \qquad \text{(Eqn 1.)}$$

PG: Percentage of germination; n: Number of germinated seeds; N: Total number of seeds.

Effect of A. americana on phytopathogenic fungi inhibition under salt stress

Petri dishes containing water agar (8-9 %) were inoculated with sterile wheat seeds. Each Petri dish contains 10 seeds of each variety forming 3 replicates. Then, 1 ml of *A. americana* extract was added to each Petri dish. The controls were inoculated with 1 ml of sterile distilled water. Incubation was performed at 28 °C for 5 days in the dark (40). At the end of the experiment, the percentage of infection by phytopathogenic fungi was determined as follows:

$$PI = \frac{I}{L} \qquad \text{(Eqn.2)}$$

PI: Percentage of infection; l: Number of infected seeds; L: Total number of seeds.

Results

Characterization of Pseudomonas strains

Phylogenetic identification

Sequencing of the 16S rRNA gene allowed for the phylogenetic identification of the 6 bacterial strains (Fig. 2). The construction of a phylogenetic tree by the Neighbour-Joining method had grouped the bacterial strains among species belonging to *Pseudomonas*.

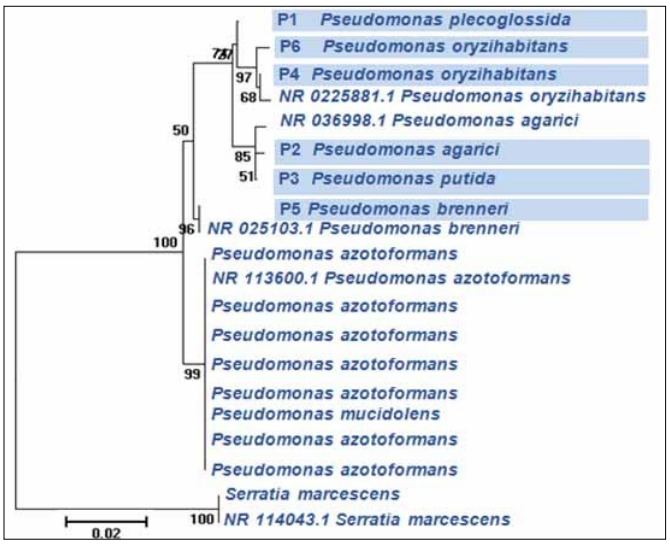

Fig. 2. Phylogenetic tree based on 16S rDNA identification of six bacterial strains.

Production of indole-3-acetic acid (IAA)

The phytohormone IAA was produced by all the bacterial strains on TSB supplemented with L-Tryptophan. The production of IAA varied from 607.14±31.13 µg/ml for strain P1 to 857.14±80.50 µg/ml for strain P3 (Table 3).

Table 3. IAA production by *Pseudomonas* bacterial strains.

Strain	Concentration of IAA (µg/ml) ± SD
P1	607.14±31.13
P2	397.62±04.12
P3	857.14±80.50
P4	438.10±04.12
P5	116.67±08.25
P6	242.86±0.00

P: *Pseudomonas*

Agave americana Characterization

Total polyphenol content

The results showed that *A. americana* contains a total phenolic content of 1014.062±161.017 mM GA equivalent/g FW (Table 4).

Table 4. Total polyphenol concentration of *Agave americana* L.

Concentration	Equivalent quantity (mM GA GA equivalent /g FW) ± SD
TPC	1014.063±161.017

TPC: Total Polyphenol Content

Total flavonoid content

The total flavonoid content of *A. americana* is 51.065±27.391 mg quercetin equivalent/g FW (Table 5).

Table 5. Total flavonoid concentration of *Agave americana* L.

Concentration	Equivalent quantity (mg quercetin equivalent/g FW) ± SD
TFC	51.065±27.391

TFC: Total Flavonoid Content

In vitro effect of hydro-alcoholic extract and Pseudomonas strains combination on salt stress resistance

Growth measurement of the six *Pseudomonas* strains under saline condition (200 mM of NaCl) revealed variability of each *Pseudomonas* strain alone, in the presence of Glycine, or in combination with hydro-alcoholic extract of *A. americana* compared to the control without NaCl (Fig. 3).

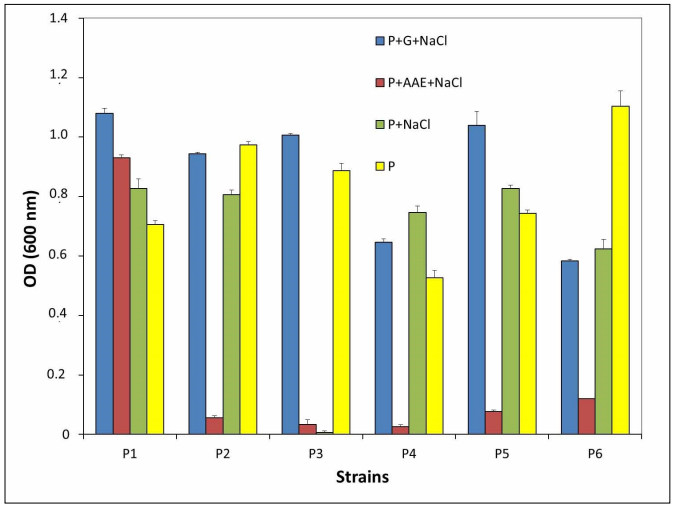

Fig. 3. Effect of hydro-alcoholic extract and *Pseudomonas* strains combination on salt stress resistance (P: *Pseudomonas*; G: Glycine; AAE: *Agave americana* L. extract).

In particular, the addition of the hydro-alcoholic extract of *A. americana* showed a stimulatory effect on growth of the strain P1 *P. plecoglicossicida* under salt stress condition. Growth was characterized by a high values in the presence of the strain P1 and hydro-alcoholic extract (OD=0.93±0.01), and in the presence of the strain P1 alone (OD=0.83±0.03). While, hydro-alcoholic extract of *A. americana* did not show any impact on other bacterial strains as growth was inhibited (OD<0.2) under salt stress condition. These findings stated that the hydro-alcoholic extract of *A. americana* improved the resistance of strain P1 *P. plecoglicossicida* to salt stress.

In vivo impact of Agave americana on Triticum durum germination

Preliminary effect of *A. americana* on *Triticum durum* germination

The hydro-alcoholic extract of *A. americana* has no positive effect on the % of germination of the variety V1 compared to the control (16.66±15.27), whereas, it reduced the percentage of germination of the control from 3.33±5.77 % to 0 % for the variety V2 and from 46.67±15.27 % to 6.67±5.77 % for the variety V3. Thus, the extract of *A. americana* seems to inhibit the germination of wheat seeds (Fig. 4).

Effect of Agave americana on phytopathogenic fungi inhibition under salt stress

During germination and salt stress condition (200 mM of NaCl), hydro-alcoholic extract of *A. americana* inhibited effectively the % of infection by phytopathogenic fungi compared to the control (Fig. 5). The highest reduction in the % of infection was observed for the variety V1 which was reduced from 40.00±20.00 % to 3.33±5.77 %. Similar reductions in the percentages of infection for the varieties

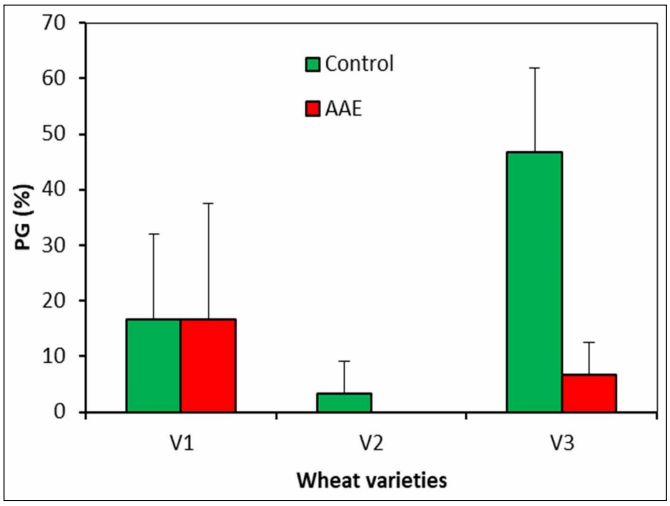

Fig. 4. Effect of hydro-alcoholic extract of *Agave americana* L on the percentage of germination (PG) of wheat seeds (AAE: *Agave americana* L. extract).

V2 and V3 were observed. The % of infection was reduced from 23.33±20.82 % to 3.33±5.77 % for the variety V2 and from 40.00±10.00 % to 20.00±34.64 % for the variety V3.

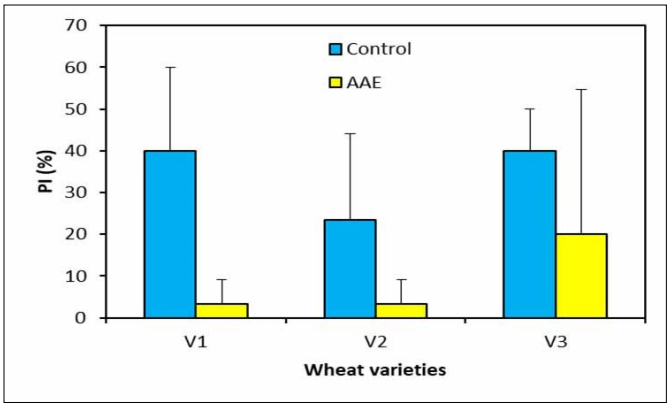

Fig. 5. Effect of hydro-alcoholic extract of *Agave americana* L. on the percentage of infection (PI) of the wheat seeds under salt stress conditions (AAE: *Agave americana* L. extract).

Discussion

Pseudomonas is one of the most diversified and successful bacterial groups yet described (41). The 16S rRNA gene amplicon sequencing has become the standard for culture -independent, taxonomic profiling of environmental microbial populations (42). Nevertheless, the 16S rRNA genes of closely related *Pseudomonas* species are remarkably similar, with less than 1 % nucleotide dissimilarity among several species (43). The genus *Pseudomonas* includes 144 species, making it the genus of Gram-negative bacteria with the most described species. Nowadays, the primary approach for determining phylogeny across species and genera is multilocus sequence analysis (MLSA). Partial gene sequences of housekeeping genes like 16S rRNA, *gyrB*, *rpoB* and *rpoD* can also be obtained from complete or draft genomes available in databases of strains related to the genus *Pseudomonas* (44).

Indole-3-acetic acid (IAA) synthesis has been widely reported in rhizobacteria (45, 46). Notably, *Pseudomonas* species are known to produce IAA (47). IAA is the most abundant auxin generated by plants and it is involved in a

variety of plant functions such as leaf growth, embryo development, root initiation and development, abscission (falling of leaves), phototropism, geotropism, fruit development etc (48). When compared to non-rhizospheric soil bacteria, these microorganisms produce and release more IAA as a result of the substrates secreted by the roots and directly boost plant development (49). Indole acetic acid is also known to play a significant role in plant tolerance to salt stress (50). Nevertheless, there appear to be little researches regarding the link between plant auxin amount and salt resistance. The variation of IAA content under stress conditions appears to be similar to that of abscisic acid (51) and also an increase of IAA level have been correlated with reduced growth (52). Recent research has shown that IAA-producing salt-tolerant bacterial strains can improve seeds germination and growth characteristics through enhancing several physiological processes, including reducing osmotic stress, boosting K^+ and lowering Na^+ absorptions and preserving proline content (53). IAA alone is not the only requirement for salt tolerance, since several *P. moraviensis* mutants improved resistance to applied NaCl compared to wild type and generated more protein (both extracellular protein and total cell protein) (54).

Results expressing the total polyphenol content of *A. americana* are non-similar to the earlier work reported (55), who characterized the ethanolic extract of *A. americana* leaves in West Algeria. TPC (Total Phenolic Content) values ranged from 37.42 mg GAE/g DW to 62.44 mg GAE/g DW. These results varied also from another study (56) who reported a value of 7.7 g total phenols/kg DM for *A. americana* leaves from the same region. In addition, total polyphenols was reported to be 659.53± 4.90 mg Gallic Acid Equivalent (GAE)/100 g DW in *A. americana* leaves from Tunisia (57). Likewise, studies conducted in different regions of the world on *A. americana* indicated that its total phenol content differs from that of *A. americana* from Algeria. This involves the influence of environmental conditions and regions on the composition of those compounds.

The recovery of polyphenols from plant material is influenced by their solubility in the extraction solvent, the type of solvent, the degree of polymerization of phenolic compounds, and the interaction of phenols with other plant constituents as well as the formation of insoluble complexes (58). They can also be affected by many other factors. According to one report (59), the progression of extraction time can decrease the yield of plant extract and cause the degradation of some natural substances such as polyphenols. Ethanol is generally known to be a good solvent for polyphenols extraction and it is safe for humans (60). Polyphenols play an important role in the interaction of the plant with its physicochemical and biotic environment, especially in the relationship with symbiotic or pathogenic microorganisms (61). In particular, polyphenols (anthocyanins) have an important function in pollination and seed dispersal, plant development and the adaptation of plants to biotic (pathogen attack) and abiotic (salt, drought, UV, blue light, high-intensity light and sugar and nutrient deficiency) stress conditions (62). In a first investigation, the varied responses of genotypes, the antioxidant and protective effects of polyphenols against salt stress-induced oxidative damage were demonstrated (63). In a similar study, reactive oxygen species (ROS) accumulation caused by tea polyphenols (TP) in wheat seedlings was shown to be accompanied by a decrease in REC (relative electrical conductivity), no change in MDA (molondialdehyde) content and an increase in Ca^{2+} level. This suggests that TP treatment alone might provide a protective mechanism different from salt stress against ROS accumulation (64).

Compared with results (65), who studied different fractions of *A. attenuata*, the values of total flavonoid content were very high, ranging from 43.35±2.99 (CE mg/100 g) for the hexane fraction to 304.8±5.02 (CE mg/100 g) for the methanolic fraction. These variations may be due to harvesting time and season, climatic conditions, extraction and measurement methods. The total flavonoid content of *A. americana* leaf extract from Tunisia was estimated to be 5.15 ± 0.18 mg RE/g FW (66). While, the total flavonoid content of *A. Americana* leaf extract from Algeria varied from 1.24 ± 1.31 to 34.32±3.31 mg/100g catechin equivalent (67). Thus, significant differences in flavonoid content of *A. americana* were observed between different locations. These are probably due to various factors such as soil type, microclimatic conditions, geographical position, site, age and vegetative stage of the plant.

Flavonoids play many roles in vital plant processes: defense against predators, attraction of pollinators, pigmentation of organs, growth and protection against ultraviolet light (68). Furthermore, one of the most highlighted functions of flavonoids is plant protection against abiotic stresses like drought, salinity, UV radiation and heat and biotic stresses including insects and pathogen attacks (69-71). For instance, the importance of flavonoids and proline during salt stress for the prevention of photosynthetic apparatus activities and plant adaptability to various high salt levels is recognized (72). Through control of transcriptional and hormonal pathways, increased flavonoid accumulation in the nagdong rice cultivar can decreases combined salt and heat stress (73). Following treatment with 150 mM and 200 mM NaCl, a dose-dependent rise in flavonol concentration was seen for wheat genotypes with more significant purple-blue pigmentation (74).

Bacteria resistant to salt concentrations ranging from 200 mM to 1200 mM belong to slightly halophilic microorganisms (75, 76). Halotolerant rhizobacteria can develop intrinsic molecular mechanisms to survive and grow under increasing salinity (77). In particular, bacteria affiliated with the genera *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Azotobacter*, *Azospirillum* and *Achromobacter* are among the most dominant PGPRs in saline soils (78, 79). In addition, the composition of an extract in bioactive molecules is directly or indirectly involved in osmoprotection like the supply of amino nitrogen and proteins (34). The increase in intracellular amino acid content as a function of osmotic stress resistance has been observed in several bacterial species (80). Among these, most studied amino acids, proline, trehalose and betaines are dominant in a

wide variety of plants and halophilic bacteria (81, 82). For example, a number of studies reported the successful restoration of wheat growth under saline conditions after inoculation with *Azospirillum brasilense* NH and concomitant application of aqueous extracts of *Ulva lactuca* that provide a new approach to the formulation of seed inocula and improvement of wheat growth under salt stress (83).

Different investigations have shown that plant extracts can have either stimulation or inhibition effects on the germination of plants. For instance, the aqueous extract of *Ruta montana* inhibits (100 %) the germination of canary seeds, while, it only inhibits (4 %) the germination of lettuce seeds (84). The inhibitory effect of *Ruta graveolens* was also highlighted (85), who showed that the aqueous extract of this plant inhibits (80 %) the germination of foxglove seeds (*Digitalis purpurea* L.). The content of those extracts in active molecules capable to preventing seed germination is most likely responsible for this activity (86). Plant toxins include alkaloids, terpenes and phenolics, which are considered secondary compounds found in various plants (87). These molecules can limit plant germination and seedling development (88). Possibly, owing to interference with indole acetic acid metabolism, protein synthesis and ion absorption (89). Recently, the occurrence of several bioactive components was qualitatively determined using phytochemical screening in the aqueous extract of *Calotropis procera* (alkaloids, phenolics, flavonoids, tannins, saponins, sterols and terpenoids). Wheat germination was reduced dramatically by higher doses of *C. procera* extract (7 % and 10 %) as compared to control (90).

Some researchers looked at the antifungal properties of the genus *Agave*. For example, using the media poisoning method, crude extracts of 5 different *Agave* species (*A. americana*, *A. ferox*, *A. montana*, *A. scabra* and *A. marginata*) were found to effectively inhibit 6 plant pathogenic fungi, namely *Alternaria porii*, *Macrophomina phaseolina*, *Aspergillus awamorii*, *A. niger*, *Fusarium solani* and *F. udum* (91). Different bioactive chemicals from *Agave* plants are linked to various biological functions (92). The ethanolic plant extracts from *A. lechuguilla* demonstrated 100 % inhibition of *F. oxysporum* proliferation, providing an approach for controlling *F. oxysporum* (93). *Agave lechuguilla* exhibits mycelia growth suppression against a variety of phytopathogenic fungi including *Colletotrichum gloeosporioides, Rhizopus stolonifer* and *Penicillium digitatum* (94). Leaf extracts from *A. americana* supposedly have antifungal properties. Depending on the characteristics of the isolated molecule, the bioactive potential is selective. *A. oryzae* α-amylase was more inhibited by the isolated compounds puerarin and apigenin from this plant species (95).

Inhibition of phytopathogenic fungi by hydro-alcoholic extract of *A. americana* under salt stress can be attributed to the bioactive substances (polyphenols and flavonoids). In plants, the synthesis and accumulation of polyphenols are generally stimulated as a response to stresses such as salinity (96, 97). Polyphenols are also constituents of the natural defenses of plants against pathogen attack. They are involved in different lines of defense. They are involved in phytoanticipins following physical barriers and their presence in the plant tissues before infection. Phytoanticipins can include phenolic acids, flavonols and isoflavones (98). Phenolic molecules seem to prevent the spread of plant diseases by a variety of strategies, including inhibition of extracellular fungal enzymes (cellulases, pectinases, laccase, xylanase etc.) and suppression oxidative phosphorylation of fungi. Also, the limitation of substrates used by pathogens and antioxidant activity in plant tissues (99). Some phenolic acids (chlorogenic acid, quinic acid, ferulic acid etc.) are reported to be efficient inhibitors of various fungal crop pathogens (98).

Conclusion

Based on the findings of the present study, it is possible to conclude that selected *Agave americana* L. plant has potential total phenolic and flavonoid contents; as the chosen *Pseudomonas* spp. bacteria show high level of IAA production. *In vitro*, these results suggest also that the combination of hydro-alcoholic extract of *A. americana* and the strain P1 *Pseudomonas plecoglicossicida* act synergically to alleviate salt stress. *In vivo*, hydro-alcoholic extract of *A. americana* had an adverse influence on seeds germination of wheat, whereas, it inhibit effectively the development of phytopathogenic fungi during germination and salt stress condition. Nonetheless, further researches are needed to optimize the amount of *A. americana*, identify the active compounds and investigate the implicated mechanisms in the plant bacteria interaction.

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Authors contributions

In the current study, SM initiated, planned, carried out experiments, analyzed data and wrote the manuscript. NH guided and edited the manuscript, followed by resolving the reviewers' edits.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interests to declare.

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