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EFFECT OF PGPR INOCULATION ON DURUM WHEAT GROWTH VARIETIES

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Abstract – The purpose of this work is to isolate PGP-effect rhizobacteria and to evaluate their potential for durum wheat varieties. The isolation of the bacteria was done using the suspension-dilution process. Eleven bacterial isolates were isolated from the rhizosphere of hard wheat. The latter were described by molecular characterization, based on the sequencing of RNA 16s. Once established, these isolates were characterized by their traits of growth promotion, such as biological control through the testing of the anti-fungal activity which was performed to two species of *Fusarium*. The ability of stimulating growth of durum wheat varieties was assessed in vivo. The molecular identification of eleven isolates showed 98-99 % of our isolates to species of the following genera: *Enterobacter, Enterobacter asburiae, Paenibacillus glucanolyticus and Serratia sp.* Research on the plant growth promoting traits in our isolates, showed that 80% of isolates have shown positive results with qualitative and quantitative estimates of siderophores, as well as for solubilized phosphate. All of our isolates showed a similar result with production of ammonium, cellulase, pectinase and eight isolates released AIA at different concentrations; whereas only three isolates could release HCN, and four isolates did not produce the protease. Similarly, direct inhibition of the growth of Fusariumsp species was observed, with significant improvement in the different growth parameters of the durum wheat varieties being studied. This research concluded that the findings obtained so far demonstrated that our isolates have phytostimulative, and phytoprotective behaviors. The latter opens up the possibility of their use in future laboratory studies in order to generate biofertilizer inoculants.

INTRODUCTION

The soil is the habitat or cohabit the roots of plants, animals and microorganisms. As the rhizosphere is a part of the soil, it is known to be a very complex medium with intense microbial activity (Ameur, 2014).

Among microorganisms that lived in the soil, are the bacteria associated with herbal roots or rhizobactéries, which are usually very competitive strains capable of having beneficial or deleterious effects in plant growth and health (Kloepper, 1993).

The beneficial rhizobactories are a rather heterogeneous bacterial flora that stimulates plant development, known as PGPR (Plant Growth Stimulating Rhizobacteria). Indeed, several organisms are included in PGPR, affecting plant growth by many mechanisms, whether direct or indirect. They are capable of promoting plant growth by increasing the acquisition of soil elements, the production of phytohormones, and the development of inductive resistance to plants (Munees and Mulugeta, 2014), or by acting in the biocontrol of plants, by reducing the deleterious effects of plant pathogens, by synthesizing various antibiotics and plant defensive siderophors (Fischer *et al*., 2009; Kirdi, 2011; Glick, 2012).

In this context, this work was undertaken to

isolate PGP impact rhizobactéries from the rhizosphere of wheat and assess their ability on wheat varieties.

MATERIALS AND METHODS

Isolation of PGPR bacteria

In order to find stimulating bacteria for plant growth, a sampling was performed from rhizospheric soil of a hard wheat crop from Constantine region. Isolation was achieved by the suspension-dilution process. This test consists of 1 g suspended soil in a tube containing 9 ml of sterile physiological water. A series of decimal dilutions was produced, and from those suspensions, 0.1ml of each dilution was distributed over the crop medium Plat count agar (PCA) containing g / l: Glucose (1), Tryptone (5), yeast extract (2.5), Agar (12) and pH 7.0±0.2. The purified isolates were retained in the glycerol-added nutritional gelosis (50 %) to -20 °C.

Molecular characterization of isolates

Based on the sequencing of the 16S DNA, our isolates have been characterized by a molecular identification. For this the DNA extraction was carried out according to the instructions of the QIAGEN kit (DNeasy Blood and Tissue). The amplification was performed in a volume of 25 µL containing 5 µL DNA, using two universal initials 27f (5'-AGAGTTTGATCMTGGCTCAG- 3)' and 1492r (5'-GGT TAC CTT GTT ACG ACT T-3') according to Weisburg *et al.* 1991, the following programs were: 5 sec at 94 ºC initial disturbance followed by 35 cycles (1 esc at 94 ºC, 1 sec at 55 ºC, 1sec at 72 ºC), and a final extension to 72 °C for 7min.

Products of this amplification were subjected to 1% agarose gel electrophoresis, followed by automated sequencing on DNA sequencer (Genetic Analyser 3500, Applied Device, HITACHI). The sequences obtained were then compared with those of the NCBI Blast-contig. (http://www.ncbi.nlm.nih.gov/Blast.cgi), using the blast software (Altschul *et al*., 1997).

Measurement of plant growth promotion activities

Production of siderophores: Based on the updated method described by Pérez-Miranda *et al*., (2007), the CAS medium (Chrome Azurol Sulfate) was used for siderophores synthesis detection.

The isolates inoculated in liquid King B being free from iron for 24 hours, followed by a spot deposit of 10 µL of each bacterial culture on the medium (CAS) suggested by Schwyn and Neilands, (1987). This blue colored medium allows the visualization of the development of siderophores, through the creation of an orange halo around the producing colony. The diameter of this halo is calculated.

In order to quantify the siderophores formed by these isolates, 100 µL of each bacterial suspension were inoculated in 10 mL of King B, then incubated 48 hours at 30 °C. After the incubation, these bacterial suspensions were centrifuged at 5000 g for 20 minutes. Only after 500 µL of supernatant had been combined with 500 µL of the CAS solution, the mixture was incubated to darkness for 30 minutes. The color changes from blue to orange according to the percentage of siderophores formed. The OD was measured by spectrophotometry (Heyios to thermo spectronic) at 630 nm. The percentage of siderophores was determined using the following formula (Gokan, 2010) :

$\% = (S_t - S_e) / S_t \times 100$,with:

S_t: DO of the CAS solution of intense blue (control)

S_e: DO of sample solution less blue to orange according to intensity of production.

Phosphate solubilization: This test helps us to determine the capacity of our isolates to solubilize phosphates in the medium of Pikovskaya (PVK) (Pikovskaya, 1948), this medium contains $Ca_3(PO_4)_2$ as the only source of phosphate.In this regard, the isolates were placed as spots on the solid PVK medium. After 10 days of incubation at 28 ° C, the total diameter (halo diameter + colony diameter) was measured. The solubilization halo diameter for each isolate was determined by subtracting the diameter of the colony from the total diameter.

For the quantitative estimate of phosphorus solubilized by these isolates, 100 µL of each bacterial culture has been inoculated into 5 mL of liquid PVK medium. At the end of the 11-day incubation at 30 °C, crops were centrifuged at 1000 g for 20 min. Based on the colorimetric method of John, (1970), 2 mL of supernagent have been blended to 8 mL in reactionary solution (mix 1.5 g of ascorbic acid with 100 mL in a stock solution prepared as follows: 450 mL of 10N H_2SO_4 and 100 mL of 5% Tartarate of antimony and potassium were added small by little in agitation to 300 mL of distilled water containing 20 g of $(NH_4)_6 Mo7O_{24}\ 4H_2O$. The DO was read at 880 nm after 10 minutes. The intensity of the blue

color is directly proportional to the concentration of phosphates in the sample. A typical calibration curve was developed with a KH_2PO_4 solution.

Ammonium production: Based on the Capuccino and Sherman process, (1992), NH3 production was tested on peptone water. This test involves inoculating 100 µL of bacterial culture in tubes containing peptone water, and incubating at 30 °C for 48 hours. The addition of 0.5 mL of the Nessler reagent gives a yellow to brown color that indicates the NH_3 production.

HCN Production: The production of hydrocyanic acid (HCN) was searched according to the method of Bakker and Schipperes, (1987). On a nutrient jelly added glycine (4.4g/L), the isolates were sown by streaks on the Petri-boxed jellyfish. A 9 cm diameter, Whatman N1 paper disk impregnated with a sodium picrat solution (5%picric acid and 2% anhydrous sodium carbonate) was placed at the bottom of the box's cover. It was covered with parafilm paper and incubated at 30 °C/96 h. The positive result is the transition from yellow to brown orange in Whatman's paper, showing the volatile production of HCN.

Production of Acetic Indole Acid: AIA production was conducted according to the method defined by Brik *et al*., (1991) and updated by Ahmad *et al.* (2008). It consisted of seeding 500 µL of a fresh bacterial culture in tubes containing 5 mL of Luria Bertani (LB) added 0.1% tryptophane. After incubation at 30 °C/ 96h under agitation, a colorimetric dosing was performed using Gorden and Weber, (1951) methods. Crops were centrifuged at 11000 g for 15 min to 4 °C. 2 mL of overlapping was added 4 mL of Salkowski reagent (1 mL of 0.5 M $FeCL_3$ in 50% of $HCLO_4$) and some drops of ortho-phosphoric acid. The OD was estimated at 530 nm after 30 minutes of dark incubation at 28 °C. AIA concentrations were calculated using a regular AIA calibration curve (Fluka).

Enzyme production

Cellulase: Cellulase production was determined using the method defined by Cattelan *et al.* (1999). The isolates have been seeded on a nutrient gelosis applied with 1 % CMC (Craboxyl Methyl Cellulose). After incubation at 30 °C for 5 days, the boxes have been filled with a red Congo solution (1% w / v) for 20 min and then with a washing with a NaCl 1N solution. The creation of a light halo around the colonies indicates a positive response.

Protease : The protease production was determined by a clear halo developed in the round of the colonies, rising on a skim milk agar, obtained by mixing 1 g of Agar suspended in 50 mL of water distilled with 5 g of skim milk in powder diluted in 50 mL of distilled water (Chaiharn *et al.*, 2008)

Pectinase : Pectinase was found on nutritional agar with 0.5% pectin. The seeded boxes were incubated for 48 hours, then flooded with an iodine solution for 30 minutes. The appearance of a clear halo in the turn of the colonies indicates a positive reaction (Delarras, 2014).

In vitro antifungal activity: The isolate inhibitory action was assessed for two plant pathogens: Fus 1: *Fusarium culmurum* and Fus7: *Fusarium pseudograminiarum* (Sebihi *et al.*, 2016). According to the method described by Vincent *et al.*, (1991), the isolates were spread on half a box of PDA-containing petri, while a 4 mm fungal disk from a 5-day culture were placed, in the opposite direction, on the other half. Following a 7-day incubation at 28 °C, the results were reported with respect to mycelium growth in the control boxes (Hariprasad *et al.*, 2009).

The percentage of inhibition was calculated using the following formula: I= [(T-C)/T] X 100 and

I: percentage of fungal inhibition tested (%).

T: Average diameter of mycelium in the control box (mm).

C: Average diameter of mycelium in boxes inoculated by bacteria (mm)

Stimulation of wheatgrowth: Isolates have been tested to stimulate growth in the following wheat varieties: WAHA, SIMETO, GTE, HIDHAB and CIRTA.

In this regard, the following steps have been taken:

- Seeds of each variety have been sterilized on the surface, and then placed in petri dishes to germinate.
- The soil was autoclaved according to the Chao *et al.* protocol, 1986, and then divided into plastic pots previously disinfected by ethanol.
- Bacterial isolates were cultivated on nutritious broth for 24 hours.
- Three germ seeds were sown by pot, and then inoculated by 2 mL of the bacterial suspension for each seed.
- The witnesses were treated with sterile distilled water.

The test was conducted for 45 days in a growing chamber (phytotron) with average daily / nocturn

temperatures of 26 °C and 16 °C, respectively, and with a 16 hour lighting period. The height of the plant and the root length of each plant were retained.

Statistical analysis: The studied parameters were analyzed by the software Xlstat pro 2012, thus an analysis of the main components was performed to evaluate these parameters

RESULTS

Molecular Identification: Molecular analysis was performed by the partial sequence of RNA 16S gene, and BLAST analysis was used for the search of some similar homology with similar species existing in data banks (GenBank), the results were represented in Table 1.

Production of siderophores: The isolates showed an orange halo on CAS solid media indicating the production of steel fur with a maximum observed for insulates: E2, E6, E3, Pg1, E1, E5 and S1 as well as the production halo were between 19- 13 mm in diameter, followed by insulation E7 and E9, with a production halo between 11-10 mm. The E8 insulation showed a low 5 mm diameter production, while the E4 insulation revealed no colors on the CAS medium. As well the estimation of the concentration of siderophores produced in liquid medium, indicates that the first group of isolates E2, Pg1, E3, and E1 produce goodly at an interval of [94.05- 63.6], the second group includes isolates S1, E6, E9, E5 and E7 which have shown mean production at an interval of (40.6- 19.8), while the two isolates E8 and E4 did not mark any production (Table 2).

Solubilization of phosphates: After 10 days of incubation, the isolates chosen have produced a clear area around the colony, translating a solubilization of $Ca_3 (PO_4)_2$. Eight isolates deployed a solubilization index in an interval of [3.2 - 4.75], and two isolates solubilized phosphate with a solubilization index that ranges from 1.8 to 2.25.The

Table 1. Identification of Bacterialisolatesbased on 16s DNA sequencing

Isolates	Taxa	Similarity %	Species
E1	*Enterobacter sp*	99%	*Enterobacter sp.* PR5
E2	*Enterobacter sp*	99%	*Enterobacter sp.* PR5
E3	*Enterobacter sp*	99%	*Enterobacter sp.* B-8
E4	*Enterobacterasburiae*	98%	*E. asburiae* strain M-T-MRS_71
E5	*Enterobacter* sp	98%	*Enterobacter sp.* WXBRN3
E6	*Enterobactersp*	98%	*Enterobacter sp.* CTSP4
E7	*Enterobacterasburiae*	98%	*Enterobacter sp.* WXBRN3
E8	*Enterobactersp*	98%	*E. asburiae strain* R2-143
E9	*Enterobactersp*	99%	*Enterobacter sp.* WXBRN3
Pg1	*Paenibacillusglucanolyticus*	98%	*P. glucanolyticus* strain NBRC15330
S1	*Serratiasp*	99%	*Serratia* sp. OM17

Table 2. Phosphate, Siderophores, AIA, and NH3 production results.

TestsIsolates	siderophores Production		Phosphates Solubilization		AIA Production in µg/mL	NH_3 Production
	Ø Halo (mm)	%	Index	[P] µg/mL		
E1	14	63,57	0	152.34	30.13	+
E2	19	94,05	3	180.64	19.63	+
E3	15	74,95	4.75	316.18	17.88	+
E4	0	0,00	1.8	258.18	9.83	+
E5	13	48,17	3.17	109.82	14.75	+
E6	16	83,55	3.2	512.82	14.96	+
E7	11	19,76	3.5	43.00	11.04	+
E8	5	1,32	2.25	556.64	13.13	+
E9	10	26,81	3.2	180.27	13.88	+
Pg1	15	86,44	4	643.36	12.25	+
S1	13	40,59	4.25	575.00	10.83	+

(+) positive reaction, (-) negativereaction

halo was absent in E1. With respect to the quantitative estimate of liquid-solubilized phosphates, isolates showed concentrations ranging from 109.82 to 643.36 µg/ mL. Although there were no solubilization halos around the E1 insulation colony, on solid medium, the latter solubilized the phosphate at an amount of 152,34 µg/mL, and, conversely, the E7 insulation which revealed a solubilization index of 3.5 did not solubilize phosphate in the liquid environment with a limited amount of 43 µg/Ml (Table 2).

Ammonium Production: All our isolates presented a yellow-brown precipitate that was formed following the addition of the Nessler reagent on bacterial suspension, showing the production of ammonium (Table 2).

HCN Production: The change in the yellow color of the filter paper that has been permeated with picric acid in brown red indicates the HCN production. This result was observed in only three isolates: E4, E6, and S1 (Table 3).

Table 3. Results of the various enzymes production.

Isolates	Cellulase	Protease	Pectinase	HCN
E1	+	-	+	-
E2	+	+	+	-
E3	+	+	+	-
E4	+	+	+	+
E5	+	+	+	-
E6	+	-	+	+
E7	+	-	+	-
E8	+	-	+	-
E9	+	+	+	-
Pg1	+	+	+	-
S1	+	+	+	+

(+) positive reaction, (-) negativereaction

AIA Production: The development of a pink color following the addition of the Salkowski reagent on LB medium, with additional tryptophane (1g/L), revealed the ability of all of our isolates to produce AIA at concentrations that varied from 9.83 µg/mL to 30.13 µg/mL (Table 2).

Enzyme production: The occurrence of clear release zones around the colonies in the middle of the CMC and in the middle of the pectin indicated the production of cellulase, and pectinase by our isolates. Protease activity was observed in seven isolates on skim milk (Table 3).

Antifungal activity: The inhibitory activity of our isolates towards two pathogens in wheat, Fus1, *Fusarium culmorum* and Fus7, *Fusarium pseudograminearum*, was reported in the following results:

All of our isolates have had inhibitory power in Fus7, with an inhibition index reaching the range of [11.56-45.33], so the maximum Fus7 inhibition index has been observed in Isolate E5 (Figure 1).

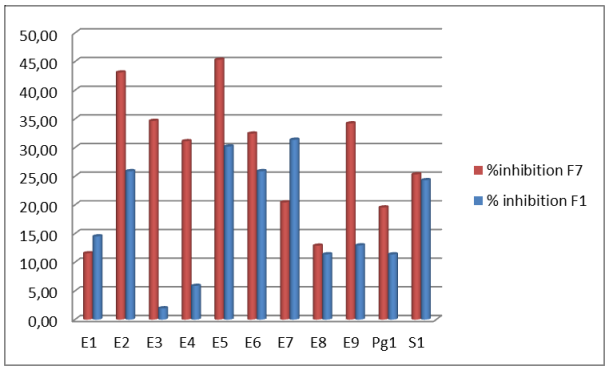

Fig. 1. Isolates inhibition index against two Fusariums.

However, the isolates did not have the same inhibiting power to Fus1. A maximum inhibition index of 31.37 % was observed for isolate E7, followed by isolate E5, E1 and E2 with an index varying in the range of [24.31- 30.20], so an inhibition mean with an index of [11.37- 14.51] was observed for isolates E1, E9, E8, and Pg1. Isolates E3 and E4 have no inhibitory power against Fus1.

Principal Component Analysis (PCA)

On the basis of the results obtained and with the objective of selecting the isolates performing, the PCA was used to compare the PGP activities expressed by the various isolates (Figure 2). PCA has revealed numerous observations between the activities (AIA, siderophores, phosphate solubilization and Fus1 and Fus7 inhibition) and the isolates:

- Based on the matrix obtained, there was avariability between the first and second components (CP1, CP2) of 71.17 % and 13.83 % respectively.
- Data analysis has revealed a negative correlation between the antifungal activity towards Fus1 and Fus7 and the production of AIA on one side, and a positive correlation of the phosphates solubilization on the other (axis 1). While the siderophores production formed a positive correlation with CAPaxis 2.

Regarding the behavior of the isolatesagainst the

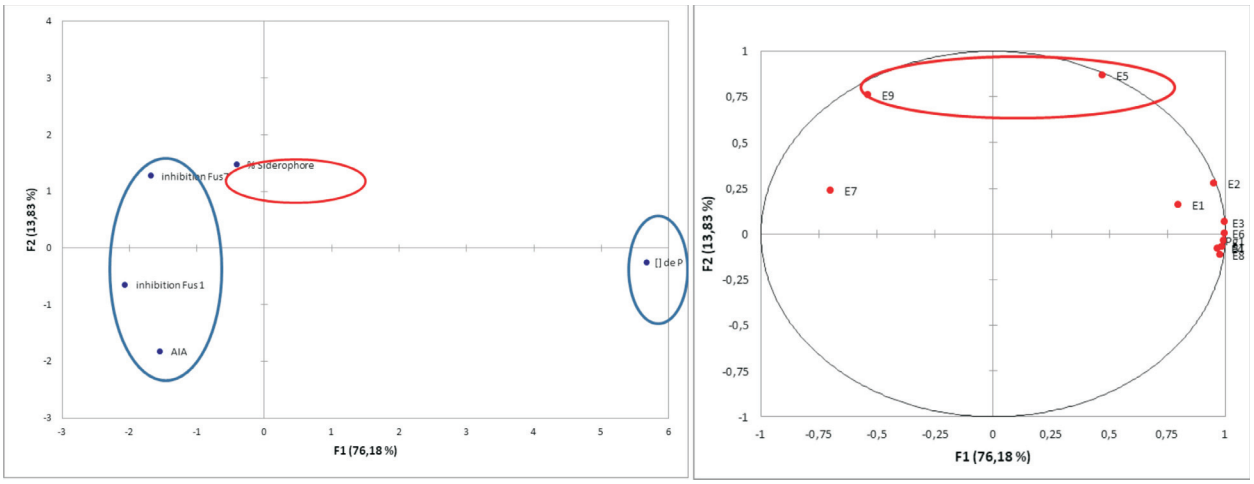

Fig. 2. CAP of activities generated by isolates

activities tested, three separate major groups were identified:

- The first was represented by isolates: E1, E2, E3, E4, E6, E8, Pg1, and S1 which solubilized the phosphates.
- The second group consisted of the E7 isolate, located opposite the first group, which showed a predominantly low activity pattern.
- Finally, the last group was composed by the two isolates E9 and E5 producing siderophores.

Stimulation of wheat growth: The test of inoculated growth stimulation of sprouts of wheat of different varieties (compared with controls) showed the following results on plant height and length of root.

- For the SIMETO: the 11 isolates tested have shown growth stimulus, and the isolates S1, E9, E8, E1, E4, E6 and E7 have promoted a better increase in plant height as well as the length of their roots.
- For the CIRTA variety: S1, Pg1, E1, E3, and E4 isolates have improved the development of the height of plants and their root.
- For the HIDHAB variety: E1, E2, E6, E5, and E4 showed excellent growth in seedlings.
- For the GTE variety: the E1, E4, S1 and E7 isolates also have the proper plant development.
- Finally for the variety WAHA: E2, E5, E9, E4, E7 and E8 also proved the obvious evolution of the wheat plants.

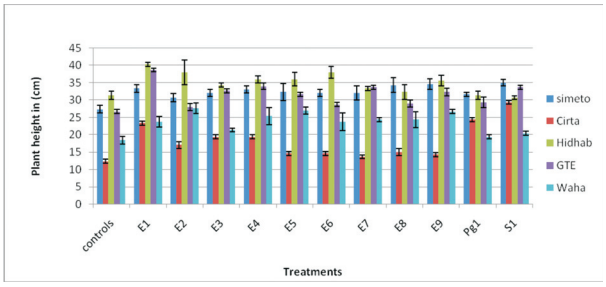

Fig. 3. Inoculation effect on stem height of varieties.

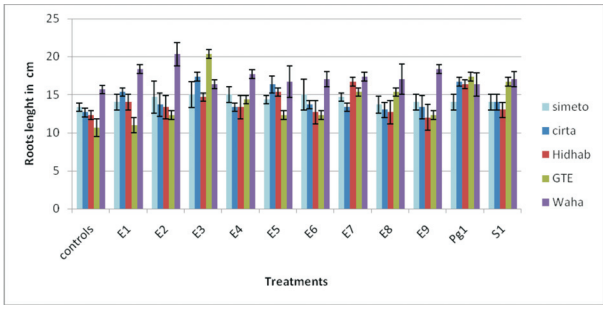

Fig. 4. Inoculation effect on roots length of the varieties.

DISCUSSION

Our work has been done to isolate PGPR-bacterial strains from the rhizosphere of wheat, and then to explore their stimulating growth activities on five hard wheat varieties. 11 bacterial isolates were isolated and part of: seven isolates an *Enterobacter* sp, two isolates an *Enterobacter asburiae Sp*, one isolate of *Paenibacillus* sp, and one isolate from *Serratia sp*.

In the rhizosphere PGPR were found at the surface of the roots or in combination with the roots due to the richness of available nutrients (Ahmad *et al.*, 2008). Several bacterial species have been identified as PGPR because of numerous studies of several plant species. Currently, PGPR contain a large range of taxa, including *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Arthobacter, Burkholderia, Serratia, Bacillus* and

Paenibacillus (Lucy *et al.,* 2004; Kloepper *et al.,* 2004; Hayat *et al .,* 2010).

The production of siderophores represents an important biocontrol mechanism of the PGPR-group; in fact siderophores have become molecules excreted by microorganisms, and have the capacity to trap the ion of the iron. All of our isolates produced siderophors at varying rates and the most efficient isolate was E2. Several subsequent research work has shown that PGPR may produce and secrete these iron fixing molecules (Meyer *et al.,* 2002), and *Pseudomonas, Azotobacter, Bacillus*, *Enterobacter*, *Serratia, Azospirillum* and *Rhizobium* secrete various siderophorous types at different levels (Glick *et al.,* 1999; Loper andHenkels, 1999).

Different bacteria of which the most powerful are the *Bacillus, Enterobacter, Erwinia* and *Pseudomonas* genera have the potential to solubilize phosphorus by the action of phosphatase for its organic form, or by releasing organic acids for its inorganic shape (Lugtenberg *et al .,* 2013). The ability of these micro-organisms to convert insoluble phosphorus into accessible form is an important feature of the PGPR, thus rhizospheric bacteria solubilizing phosphate may be a promising source of biofertilizer in agriculture (Sharma *et al.,* 2007). No correlations were observed during the liquid and solid phosphate solubilization test of our isolates. Indeed, some isolates showed high concentrations of solubilization of phosphate, whereas no clear halo was formed in the colonies. The same result was observed in the genus Azotobacter by Silini, (2012).

Another common property of PGPR is the NH_3 production, which were common in our tested isolates (100%) and was comparable to the reported one by numerous authors (Joseph *et al.,* 2007; Ahmed *et al.,* 2008). PGPR produce different biological molecules in very low concentrations, such as auxins, which directly affect plant growth (Srivastava *et al.,* 2002). The AIA is the most important hormone for the plant and its production was found in over 80 % of the rhizobacteria (Klopper *et al.,* 2007). According to Barazani and Friedman (1999), bacteria capable of secreting more than 13.5 ìg/mL of indolic compounds are considered to be PGPR. In this study, a remarkable production of this hormone was revealed in all isolates. Auxine synthesezation can be used for the testing of effective PGPR strains, and in particular, AIA output is a trait of promoting the growth of the most widespread plant in the RMPs (Khalid *et al .,* 2004).

Another parameter required for the detection of PGPR is the production of enzymes, which give the plants resistance against phytopathogens. Hydrogen cyanide (HCN) is a secondary metabolite in the cyanide family, involved in the elimination of various pathogens. HCN produced through PGPR and its antagonist effect on pathogens plays an important role for the plant (Defago and Haas, 1990). As regards our work, only three isolates were able to generate this metabolite, although they showed strong hydrolytic enzymes such as cellulase, pectinase, and protease. The capacity of PGPR to dissolve fungal cell walls through the development of hydrolytic enzymes is another benefit in their use as an efficient biofertilizer (Glick, 2012). Further, direct inhibition of pathogen growth through the development of antifungal and/or antibiotic products is another method used by PGPR to restrict the penetration of pathogens into plant's tissues. This has been well illustrated by the antifungal behavior of our isolates against *Fusarium culmorum* and *Fusarium pseudograminiarum*.

The biocontrol of pathogens by rhizobacteria could reduce the use of chemical pesticides to control plant pathogens (Giroux, 2015). Thus biocontrol agents can use several mechanisms to promote growth of plants such as production of antifungal metabolites, antibiotic, cell wall enzyme, HCN production, and siderophores production (Martinez-Viveros *et al.,* 2010 ;, Singh and Singh, 2013 ; Parvatha Reddy, 2013)) .

The beneficial effects of bacterial inoculation only occur if certain conditions are met. In fact, our results were consistent with PGPR data. Bacteria associated with roots (PGPR) can act strongly on plants nutrition through numerous mechanisms involved in mutualistic relationships.

Inoculation of cultivated plantswith certain strains of PGPR, at an early stage in their development, improves the production of biomass by direct effects on growth of roots and on the air (Saharan and Nehra, 2011). The effect of PGPR will be linked to the development of Phytohormones that are important for plant growth. (Benmati, 2014).

The bacterial inoculation stimulation test has been performed with our 11 isolates on 5 different varieties of durum wheat. The results of daily monitoring of plants development showed considerable improvement in the various parameters of growth such as stem length and root length (as compared with the neutral control), and a

substantial difference in growth among the varieties used..

Previous studies of PGPR-inoculation have shown that the tested strains significantly increase growth parameters such as plant height, lengths of fresh roots and weight, and root and leaf dry sprouts (Kloepper *et al.* 1978; Kirdi, 2011; Silini, 2012; Cherif, 2014; Benmati, 2014)).

The heterogeneous growth promotion activities of our isolates such as: development of AIA phytohormone, biofertilization through phosphate solubilization, and bio-control through the processing of steel fur and antifungal molecules have helped to boost growth in wheat varieties.

CONCLUSION

Tentative observations made so far indicate that our isolates exhibit the possess phytostimulation and phytoprotective activities, so the inoculation of hard wheat seeds substantially improves the morphological growth parameters for the five wheat varieties. This indicates the possibility to use them in future laboratory studies with the goal of developing bio-fertilizing inoculants.

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