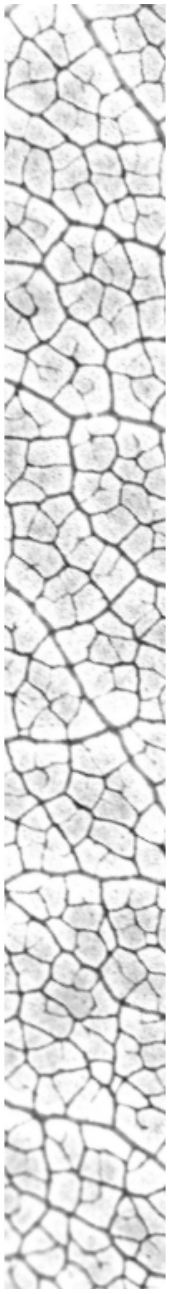

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Characterization of plant growth promoting rhizobacteria isolated from the rhizosphere of carob tree (*Ceratonia siliqua* L.) in Morocco

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Abstract
Plant growth promoting rhizobacteria (PGPR) are beneficial bacterial that colonize plant roots and enhance plant growth by a wide variety of mechanisms. Seven isolates of bacteria were successfully isolated and phenotypically, biochemically, physiological and molecular characterized. Subsequently to investigate the effect of PGPR isolates on the growth of carob tree, a pot culture experiement was conducted as Centre for Innovation and Technology Transfer (CITT), Meknes, Morocco. All the isolates were gram negative, rods, motile, able to grow on YDC medium and citrate simmons positive, degrade glucose, fructose and mannitol, capable of hydrolyzing esculin and starch. All strains are able to produce (74-150µgml^{-1}) of indole acetic acid, nitrogen fixatin and all isolate are unable to produce ammonia, chitinase and cellulase, the strains 2018-3, 2018-4 and 2018-5 showed protease activity. The pecentage of solibilization of phosphate varied between 15% to 45%, the maximum phosphate solubilization was identified by stain 2018-4. Sequencing of 16SrRNA genes led to the identification of two family of bacteria : *Bacillaceae and Pseudomonadaceae*. The species of bacteria are : *Bacillus thuringiensis*, two of *Bacillus flexus*, two of *Pseudomonas gessardii*, *Bacillus cereus* and *Bacillus thaonhiensis*. The analysis the result showed that the highest vegetative growth was obtained from stain 2018-4 such as plant height (13.6cm), root length (13.65cm), fresh weight of the aerial part (1.09g) and fresh weight of the root part (0.42g) in comparison to untreated plant.

Keywords: carob tree, PGPR, IAA, P-solubilization, N_2- fixation and plant inoculation

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INTRODUCTION

Carob tree (*Ceratonia siliqua* L.), belonging to the family of fabaceae, one of the most preciou socio-economic value trees, could be a choice plantation in regular refosestation programs to ensure the sustainable development of rural areas and contribute to the protection of soil and the fight against desertification (Konaté 2007). It is a typical species of mediterranean flora, well defined in the humid, sub-humid and semi-arid stage. In Morocco, it is found throughout the cuntru, mainly in the semi-aride stage and very excptionally in the arid stage in the south and in the upper part of Argania spinosa (Hmamouchi, 1999). Carob tress is an agro-forestry-pastoral species with significant socioeconomic and ecological intersts (Ait chitt et *al.* 2007).

At ground level microbial activity is intense, especially in the zone under the influence of roots, the rhizosphere, which contains more than one million microoorganisms per gram of soil. Microorganisms find in these medium energetic substrates released by the roots and necessary for their metabolism: sugars, amino acids, organic acids, hormones…. Some these microorganisms, mainly bacteria, are able to colonize effectively ; production of hormones (Idris et *al.* 2007), nitroge fixation (Sahin et *al.* 2004), solubilization of phosphorus (Wenxing et *al.* 2008) and potassium and /or protection against infections by phytopathogenic agents (production of antimicrobial enzymes) (Rahman et *al.* 2007, Reyes et *al.* 2004, Valdebenito et *al.* 2006). These bacteria of the rhizosphere are then referred to as plant growth promoting rhizobacteria (PGPR) (Somers et *al.* 2004).

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PGPR are plant associated bacteria that aggressively colonize the rhizosphere/plant roots, imparting beneficial effects to plants (Panwar et *al.* 2014). PGPR enhance plant growth both directly and indirectly (Glick 2012). Generally, PGPR promote plant growth directly by either facilitatig nutient availability and acquisition via N2 fixation (Ahmed and Kibret 2014) and P-solubilization (Zaidi et *al.* 2009) or modulatinf plant growth by providing or regulating various plant hormones includinf indole acetic acid (IAA), abscisic acid, gibberellic acid and cytokinin (Panwar et *al.* 2014), siderophores (Glick 2012), and induced systemic resistance (Ramamoorthy et *al.* 2001).

Previous reviews have described the diversity of PGPR in multiple plant species, especaillt those with agronomical significance (Ahmed and Kibret 2014, Barriuso et *al.* 2008). *Azotobacter, Azospirillum, Bacillus, Enterobacter, Klebsiella, Paenibacillus, Pseudomonas* and *Serratia* (Ahmed and Kibret 2014) are genera that comprise common PGPR with known benefits on different crop plants. Research on PGPR has been increasing and many experimental studies have been carried out on different crops including maize (Zahid et *al.* 2015), wheat (Majeed et *al.* 2015) and rice (Sen and Chandrasekhar 2014). These studies have shown the potentiel of PGPR to increase the growth and yield of such crops with minor inputs of agrochemicals.

The objective of this study is the phenotypic, biochemical, physiological characterization and the identification by 16S ribosomal DNA of the PGPR strains isolated from the carob tree rhizosphere in a semi-arid area. On e the other hand, study the effects of these microoranisms on the growth and biomass of carob plants.

MATERIALS AND METHODS

Plant Materials and Experimental Conditions

A field experiment was conducted to evaluate the effects of seven treatments of bacteria (1, 2, 3, 4, 5, 6, 7) on carob growth. A pot experiment was conducted in the a fielde of Centre of Innovation and Transfer Technology, University Moulay Ismail Meknes Morocco, to evaluate the effects of rhizobacterial inoculation on different growth parameters of carob plants. Seven isolates were selected based on multiple plant grouwth promoting properties like nitrogen (N_2) fixation, indole acetic acid (IAA) production, and Phosphate solubilization. Seeds of carob were suface sterilized for 5 min in 90% (v/v) ethanol, then rinsed three times with sterile distilled water, followed by min in 10% (v/v) sodium hypochlorite, and finally were wached six times with sterile distilled water. Each pot (13 cmd diameter) was filled with 2 kg of autoclaved sandy soil. For inoculation treatments, pre-germinated seeds were soaked for 2h in the bacterial suspension of 10^8-10^9 cells ml^{-1} and placed at the same depth (approximately 2.5 cm below the sooil surface) in all inoculated pots. The uninoculated control treatment consisted of water treated seeds (without bacterial inoculation) was included. Experiment was set in a complete randomized design with four replicates. Meknes, situated at a latitude of 33°53'42''N, longitude of 3°33'17'' E and altitude of 560m. The climate of Meknes is semi-arid type with hot and dry summer and cold winter. The annual rainfall is about 600mm.

Determination of Plant Growth

Plant height: height of the plant from gound level of growing point of the stem was measured and the mean was calculated and expressed in centimeter.

Root length: plants were pullec carefully without damging ot the roots. The length of the roots from the point of attachment to the stem to tip of the roots were measured and expressed in centimeter.

Shoot dry weight: plants were pulled carefully without damaging to the shoots. The shoots cut into pieces and dried in a oven at 70°C till constant dry weight. The mean was worked out and expressed in g $plant^{-1}$.

Root dry weight: the root were cut into pieces of appromiately 5 centimeters length and dried in an oven at 70°C till constant dry weight. The mean was worked out and expressed in g $plant^{-1}$.

In-vitro Screening of Plant Growth Promoting (PGP) Activities

A screening of the PGP activities of all rhizobacteria for the purpose of select the most successful strains was carried out:

Analysis of phosphate solubilizing activity: bacterial isolates were screened for their tri-calcium phophate solubilizing activity on Pikovskayas agar (PVK). Isolates were spot inoculated on the centree of agar plate aseptically. All the plates were incubated at 28 °C for seven days. The zone diameter around the colony was measured and phosphate solubilization efficiency (PSE) was calculated by using folloing formula. (Gothwal et *al.* 2006, Nguyen et *al.* 1992, Pikovskaya 1948) **PSE= (Solubilization diameter/Colony diameter)*100**

Nitrogen fixation: the screening for nitrogen fixing rhizobacteria was evaluated by the ability to grow in semi-solide nitrogen-free NFb medium, Dobereiner, 1995. A halo of bacterial growth within the medium indicates nitrogen fixation.

Indole acetid acid (IAA) production: for detection and quantification of IAA production, bacterial colonies were inoculated into Luria-Bertani (LB) medium containing 0.5mg L-tryptophan/ml. the culture was incubated at 28°C with continous shaking at 125 revolution per minute (rpm) for 48h (Brick et *al.* 1991, Kumar et *al.* 2012, Rahman et *al.* 2010).

Ammonia production: bacterial isolates were tested for the production of ammonia (NH_3) in peptone water (Cappuccino and Sherman 1992).

Cellulase synthesis: the ability to produce cellulose was measured on agar plates containing minimal medium with 2% (w/v) 1-carboxymethylcellulose as carbon source (Zhang et al. 2009).

Protease synthesis: protease activity was detected on 3% (w/v) powdered skim milk agar plates (Chang et al. 2009).

Chitinase synthesis: activity of chitinase was detected on 1% (w/v) colloidal chitin agar plates (El haùshary et al. 2008).

Bacterial Characterization

Phenotypic characterization: Gram- staining and cell morphology was observed using an optical microscope.

Biochemical characterization: it is done for determining the biochemical characteristics of the isolate: the bacterial were identified by biochemical test including; levan test, KOH (3%), oxydase reaction, potato rot test, hydrolysis of tween 80, starch hydrolysis, gelatin hydrolysis hydrolysis lecithin, hydrolysis of esculin, motility test, indole test, uréase test, tryptophan desaminase test; simmons citrate test, gas production, H_2SO_4 production and growth in YDC medium.

Physiological characterization: different carbon source utilization is determined. The ability of the isolate to utilize different carbon sources was determined. Determination was done using the indicator bromothymol blue which shows acid or alkali production indicating carbon source utilization. Luria bertani liquid medium (LB) is used for determining carbon source utilization by the isolate. Different carbon sources used were mannitol, sucrose, glucose, fructose, lactose, sorbitol and cellubiose. It was the kept for incubation at 25°C for 48hours, after two days of incubation the flasks were observed for any color change.

Molecular Identification of Bacterial Isolates based upon 16S Ribosomal DNA (16SrDNA) Sequence

Isolation of strain bacteria genome was conducted with isolate ii genomic DNA kit. Quantification of DNA was performed using spectrophotometry. Primer Fd1 (CAGAGTTTGATCCTGGCTCAG) and RP2 (AGAGTTTGATCCTGGCTCAG) will amplify specific DNA fragment of 1500pb. The 25µl of PCR reaction mixture contained 5µl DNA, 2µl dNTPs mix (10mM), 0.125µl of each primers (100µM), 0.2µl Taq polymerase (5U/µl), 2µl buffer (10X) and 0.75µl $MgCl_2$ (50mM). The PCR amplification program was consisted of 95°C for 4min (Preheating), 95°C for 0.167 min, 53°C for 0.667min, 72°C for 2min (35cycles), 72°C for 10min (final extension) and stored at 4°C until used. PCR products were purified using EXOSAP-IT according to the following schedule: 37°C for 15min following bye 80°C for 15min prior to bi-directional sequencing using pA and pH primers. Determined sequences were compared with sequence availed in GenBank, EMBL, and DDBJ databases using the BLAST algorithm (McGinnis and Madden 2004). In order to assign isolate to a species, each derived sequence aligned by the BLAST algorithm, yielded at least 99% similarity score with identified species in the BLAST search and the high test S-ab value with identified species in the sequence match search.

Statistical Analysis

Data was analyzed by way analysis of variance (ANOVA) using SPSS and the mean difference was compared by the Duncan's test at 95 levels of probability. The graphs presented and the average groups of tables were constructed by the software excelstat 2008-2009.

RESULTS

Plant Growth Promotion in Carob

Plants treatment with bacterial strains plant growth promoting rhizobacteria (PGPR) applied significantly increased the size of the air and the root part, fresh weight of the aerial and root part, dry weight. Per cent increase of growth parameters over inoculated control is shown.

Inoculation of the young carob seddlings by PGPR bacteria increase the air and underground fresh weight for all bacterial strains except for two strains (2018-3 and 2018-5) giving a fresh weight of the lower aerial part compared to the control.

The dry weight of the air and the root parts give a better result after inoculation with the bacterial strains.

Measurement of fresh and dry root weight revealed a visible positive effect of inoculation by these strains PGPR.

The other morphological aspect of the effect of the inoculation is manifested by an increase in the size of the plant, root and foliar, for all bacteria when compared with the control. In this case, the strains (2018- 1, 2018-4 and 2018- 7) perform better than the other strains. Stem lengths show a decrease of 13.6 cm for strain 2018-4, 13.5cm for strain 2018-7 and only 12.75cm for strain 2018-1. However, root development is stimulated by bacterial inoculation in all strains, a length increase of 35.3 cm for strain 2018-7, 36.65 cm for strain 2018-4 and 39.5cm for strain 2018-1(**Fig. 1a-1f**).

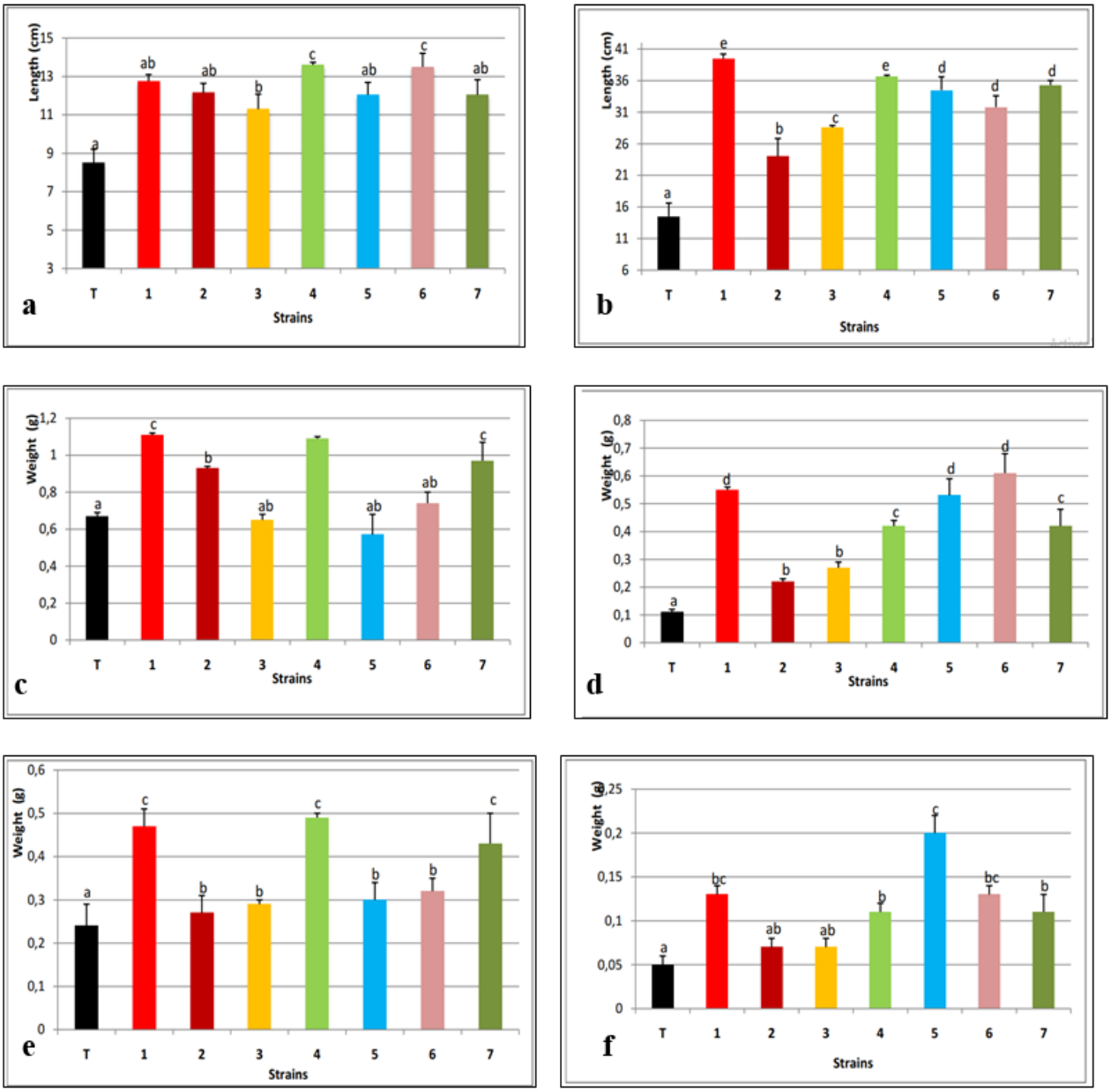

Fig. 1. Effect of Strains on the growth of Carob tree (*Ceratonia Siliqua* L.,): **a.** Size of the air part, **b.** Size of the root part, **c.** Fresh weight of the aerial part, **d.** Fresh weight of the root part, **e.** Shoot dry weight and **f.** Root dry weight

In-vitro Screening of Plant Growth Promoting (PGP) Activities

Out of seven strains screened for phosphate solubilization, only three strains had phosphate solubilizing ability, based on a clear zone on PVK medium. Figure shows the positive strains, showing a clear zone of phosphate solubilization around the bacterial colony along with the negative strains showing no zones. The percentage of solibulization of phosphate varied between 15% and 45%. The maximum phosphate solibulization was identified by strain 2018-4. All strains could produce indole acetic acide (IAA), and the quantifities of IAA produced ranged from 74 ± 2.10 to 150± 2.2 µg ml^{-1}. In this study all the bacterial stocks are able to produce of nitrogen, the ammonia is useful for plant as directly or indirectly. Ammonia production bye the PGPR helps influence plant growth indirectly. All strains are unable to produce ammonia, chitinase and cellulase. Protease activity was determined by clear zone on skim milk agar, the appearance of clear halo around colonies indicates protease production. The strains 2018-3, 2018-4 and 2018-5 showed protease activity (**Table 1**).

Bacterial characterization

From this seven strains of PGPR were isolated. The PGPR were confirmed on the basis of phenotypically, biochemically, physiological characters and molecular identification of bacterial strains: Gram cell morphology

Table 1. List of PGPR used in this study and their PGP characteristics **1 :** Solubilisation of phosphate efficiency (%) ; **2 :** Nitrogen fixation activity ; **3 :** AIA Production (µg/ml) 0,5g/l Tryptophane ; **4 :** Ammonia production ; **5 :** Protease activity ; **6 :** Cellulase activit ; **7 :** Chitinase activity **+ :** Positive reaction and **- :** Negative reaction

Strains Tests	2018- 1	2018- 2	2018- 3	2018- 4	2018- 5	2018- 6	2018- 7
1	-	-	42	45	15	-	-
2	+	+	+	+	+	+	+
3	74±2,1	138±3	150±2,2	100±3,1	120±1,9	102±0,9	120±2,6
4	-	-	-	-	-	-	-
5	-	-	+	+	+	-	-
6	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-

Table 2. Morphological, Biochemical and physiological characterization of carob-associated rhizobacteria

Strains Tests	2018- 1	2018- 2	2018- 3	2018- 4	2018- 5	2018- 6	2018- 7
KOH 3%	+	+	+	+	+	+	+
Gram	-	-	-	-	-	-	-
Form	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Oxydase	+	-	+	-	+	+	-
Levane	-	-	+	+	+	-	-
Urea	-	+	-	+	+	-	-
Indole	-	-	-	-	-	-	-
TDA	+	+	+	+	+	+	+
Motilité	+	+	+	+	+	+	+
Simmons citrate	+	+	+	+	+	+	+
Growth on YDC	+++	+++	+++	+++	+++	+++	+++
Gas production	-	-	-	-	-	-	-
H_2SO_4 production	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+
Gelatin hydrolysis	-	-	-	-	-	-	-
Lecithin hydrolysis	-	-	-	-	-	-	-
Pectine hydrolysis	-	-	-	-	-	+	-
Esculin hydrolysis	+	+	+	+	+	+	+
Tween80 hydrolysis	-	-	-	-	-	-	-
Use of sugars							
Glucose	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	-
Lactose	+	+	-	+	+	+	+
Sorbitol	-	+	+	-	-	-	-
Sucrose	+	+	+	+	+	-	+
Cellubiose	-	+	-	-	-	+	-

Positive reaction (+); Negative reaction (–)

staining showed that all bacteria are Gram negative, All strains PGPR are rods, motile, able to grow on YDC medium and citrate$^+$, fructose$^+$, mannitol$^+$, TDA$^+$, starch hydrolysis$^+$, esculin hydrolysis$^+$, and degrade glucose oxidatively (glucose$^+$). In addition, they give a negative response to: production $H_2SO_4^-$, gelatin hydrolysis$^-$, lecithin hydrolysis$^-$, tween 80 hydrolysis$^-$, production of gas$^-$, indole$^-$, three from the mis (Urea$^+$ and levane$^+$) and four for them are oxidase$^+$. We found a single strain (2018-6) capable of hydrolyzing pectin$^+$. For the use of sugars as a source of carbon, six strains were found to be lactose$^+$, two strains were sorbitol$^+$, five strains were sucrose$^+$ and two strains were cellubiose$^+$ (**Table 2**).

Molecular Identification of Bacterial Isolates

Bacterial strains were examined for their ability to plant growth promoting rhizobacteria. In the present study: two strains of *Pseudomonas gessardii*, two strains of *Bacillus flexus,* one strain *Bacillus thuringiensis*, one strain of *Bacillus thaonhiensis* and one strain of *Bacillus cereus* were isolated from carob tree rhizosphere. Furthermore, the BLAST results of the 16SrRNA gene sequences allowed to classify the isolated strains from various soils into the family of Bacillaceae and Pseudomonadaceae. the four evaluated strains were aligned against sequences available from GenBank data; the seven strains: 1, 2, 3, 4, 5, 6 and 7 matched to *Bacillus thuringiensis, Bacillus flexus, Pseudomonas gessardii, Bacillus cereus, Pseudomonas gessardii, Bacillus flexus* and Bacillus thaonhiensis *respectively with 90%* of similarity percentage through GenBank data base as represented in **Table 3**.

DISCUSSION AND CONCLUSION

The use of plant growth promoting rhizobacteria (PGPR) promotes plant growth and development through a variety of mechanisms. The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are believed to be involved in plant growth promotion.

Rhizosphere soi lis a hot spot of microbial interaction and activity due to the presence of root exudates and rhizodeposits (Campant et *al.* 2010). In this complex ecosystem, now it is believed that, bacteria can positively improve plant growth while plants can « engineer » their microbiome in order to have beneficial

Table 3. Sequence analysis of 16S rRNA from PGPR representatives

Strains	Number of Base pairs	Closest relative species	Similarity (%)	Accession Number in GenBank
2018-1	500	*Bacillus thuringiensis*	99	NR112780
2018-2	500	*Bacillus flexus*	99	NR024691
2018-3	500	*Pseudomonas gessardii*	99	NR024928
2018-4	500	*Bacillus cereus*	97	NR074540
2018-5	500	*Pseudomonas gessardii*	99	NR024928
2018-6	500	*Bacillus flexus*	99	NR024691
2018-7	500	*Bacillus thaonhiensis*	90	NR125615

bacterial colonizers, including those living within the plant tissues « plant endophytes » (Marasco et *al.* 2012, Rachid et *al.* 2012).

Plants treatment with bacterial strains PGPR applied significantly increased the size of the air and the root part, fresh weight of aerial and the root part, dry weight. Per cent increase of growth parameters over inoculated control is shown (**Fig. 1a-1f**). These results were similar with the findings of Ibienne et *al.* (2012), who assessed the inoculation effect PGPR on growth of *Lycopersicum esculentus*. The results of the study by Sharifi et *al.* (2011) showed that seed priming with plant growth promoting rhizobacteria affected grain yield, plant height, number of kernel per ear, number of grains per ear row significantly. Maximum of these characteristics were obtained by the plots which seeds were inoculated with Azotobacter bacteria. Inoculation with bacterial strains possess multiple plant growth promoting traits, can ultimately benefit plant in terms of stimulating growth. In the plant inoculation assay, most of the tested isolates significantly improved plant growth compared to the uninoculated control. Innoculation with *Chryseobacterium* sp. NGB-29 and *Flavobacterium* sp. NGB-31 had the greatest potential to increase shoot and root fresh and dry weights. Plant growth promotion in response to PGPR applied alone or with N or P fertilizers has been frequently reported for different crops under different ecological and environmental conditions (Abdel Rahman et *al.* 2017, Naqqash et *al.* 2016). In consistent with our results (Zahid et *al.* 201), reported the positive growth promoting attributes of maize plants towards inoculation with Bacillus subtilus HJR3. Similarly Kuan et *al.* (2016) found that inoculation of maize with N2 fixing PGPR strains belonging to genera *Bacillus*, *Klebsiella* and *Acinetobacter* significantly increased to total N content and dry biomass of plants as well delayed plant senescence.

Production of phytohormones is one of the main mechanisms responsible for the direct PGP effect of rhizobacteria on plants. Indole acetic acid (IAA) is generally considered the most important phytohormone that coordinates different developmental processes in plants; a low concentration of IAA promotes primary roots elongation, where as a high IAA concentration stimulates lateral and adventitious roots formation (Duca et *al.* 2014). In nature, IAA is commonly synthesized by plants and PGPR from amino acid tryptophan, a common precursor in root exudates, through transamination and decarboxylation biochemical reaction (Apine and Jadhay 2012). In thi study, *Pseudomonas gessardii* and *bacillus flexus* were identified as the high efficient IAA producers, in both L-tryptophan supplemented (150 and 138 $\mu g ml^{-1}$) (**Table 1**). The variation of IAA productio by rhizobacteria species and strains belonging to the same genera has been previously reported (Zahid et *al.* 2015). This variation is attributed to various biosynthetic pathways, genetic regulation and environmental factors involved in IAA biosynthesis (Duca et *al.* 2014).

Phosphorus is a primary essential nutrient element for carob tree production. To enhance phosphorus uptake efficiency, PGPR play an important role in supplying phosphate to plants, which is environment friendly and sustainable approach (Amanullah and Khan 2015). In present study, two *Pseudomonas gessardii* and *Bacillus cereus* were able to solulibize phosphate (**Table 1**). Majeed et *al.* (2015) reported a range of P-solubilization varied from 2 to 19 $\mu g\ ml^{-1}$ among nine bacterial strains isolated from wheat rhizosphere and root-endoshpere that belonging to genera *Acetobactor*, *Bacillus* and *Stenostrophomonas*. The group of bacteria belonging to *bacillus sp*p., *Pseudomonas sp*p., and *Acinetobacter sp*p., already reported as effective phosphate, as well as IAA producer (Moreno-Ramirez et *al.* 2014, Ndeddy aka and Babalola 2016).

The presence of fixing nitrogen bacteria in the ground and their insulation as well as the possibility of their conversion into biofertilisants is an important strategy and an alternative solution with the use of the expensive and polluting artificial fertilisers in particular in the grounds low in nutrients. In this study all the bacterial stocks are able to produce of nitrogen (**Table 1**). Lwin et *al.* (2012) showed that isolates rhizospheric identified like *Serratia sp*p. and *Bacillus sp*p. had a capacity to fix nitrogen on solid medium G-NFM with the BBT like indicator. *Bacillus sp*p. and *Burkholderia cepacia* were also brought back to fix atmospheric nitrogen (Habib et *al.* 2016, Hongritipun et *al.* 2014).

The production of ammonia is an important characteristic of the PGPR. This property influences indirectly the growth of plant (Yadav et *al.* 2011). In the study showed that all the bacterial stocks are unable to produce ammonia (**Table 1**). Singh et Jha, 2016, reported that an identified PGPR as *Serratia marcescencs* had the capacity to produce ammonia.

Furthermore, the Blast results of the 16S rRNA gene sequences allowed to classify the isolated strains from various soils into the family of bacillaceae and

pseudomonadaceae. The seven evaluated strains were aligned against sequences available from GenBank dat: *Bacillus thuringiensis* (2018-1), *Bacillus flexus* (2018-2), *Pseudomonas gessardii* (2018-3), *Bacillus* cereus (2018-4), *Pseudomonas gessardii* (2018-5), *Bacillus flexus* (2018-6) and *Bacillus thaonhiensis* (2018-7) (**Table 3**). All of the espece identified in this study have been associated with plant rhizosphere and their plant growth promoting activities has also been reported earlier (Eleonora et *al.* 2016, Holajjer et *al.* 2018, Jiaheling et *al.* 2016, Mia et *al.* 2009, Van Pham and Kim 2014).

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