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Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (*Solanum Lycopersicum* L.) and characterization for direct PGP abilities in Morocco

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Abstract**—** *Plant Growth promoting rhizobacteria are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots. They benefit plants through Production of plant hormones, such as auxins, asymbiotic N_2 fixation, solubilization of mineral phosphates, antagonism against phytopathogenic microorganisms by production of antibiotics, siderophroes, Chitinase and other nutrients ability to effectively colonize roots are responsible for plant growth promotion. An experiment was conducted in the field of National Institute of Agronomic Research of Meknes. Morocco. The experiment was a completely randomized design with six replicates. There were four treatments viz. T1: (control; N0 -PGPR), T2: (N0 +2027-2), T3: (N0 +2066-7) and T_4: (N_0+2025-1). The results indicated that a remarkable increase in root growth, namely length, the diameter of the rod and the total chlorophyll. A total of three different bacteria colonies were isolated and proceed with in vitro screening for plant growth promoting activities; phosphate solubilization, nitrogen fixation, indole acetic acid (IAA), ammonia production and antimicrobial enzymes (cellulose, chitinase and protease) activity. Among the three bacterial strains, all bacterial strains are able to produce ammonia, IAA production and nitrogen fixation activity, one strain phosphate solubilizing activity, two strain are able to produce cellulase syntheses, Protease activity and Chitinase activity.*

Keywords**— Solubilization phosphate, nitrogen fixation, Production antimicrobial enzymes.**

I. INTRODUCTION

Plant nutrients are essential for the production of crops and healthy food for the world's expanding population. Plant nutrients are therefore a vital component of sustainable agriculture. Increased crop production largely relies on the type of fertilizers used to supplement essential nutrients for plants. The nature and the characteristics of nutrient release of chemical, organic and biofertilizers are different, and each type of fertilizer has its advantages and disadvantages with regard to crop growth and soil fertility [1].

Bacteria that are present in the rhizosphere and improve plant growth by some mechanism are called plants growth promoting rhizobacteria (PGPR) [2]. The association between organisms and roots can be beneficial (water uptake, soil stabilization, growth promotion, N_2 fixation, biocontrol, antibiosis, symbiosis), harmful (infection, phytoxicity) or neutral (nutrient flux, free enzyme release, attachment, alleopathy, competition) [6], PGPR most involved are: Pseudomonas, Bacillus, Rhizobium, Burkholderia, Micrococcus, Azotobacter and Erwinia. [7]. Or indirectly via their ability to remove a broad spectrum of bacterial, fungal and parasitic infections, also provide protection against viral disease. Many studies show the diversity of microbial agents involved in the biological control [8].

Some rhizobia strains have the ability to produce siderophores, biomolecules that act as specific iron chelating agents, often unavailable to living organisms and essential for achieving the vital functions such as DNA synthesis, respiration, photosynthesis and Biological nitrogen fixation [9 and 10].

This work was undertaken to evaluate and utilize the potential of rhizobacteria PGPR for plants tomato and characterize three PGPR, for their ability to produce metabolites IAA, solubilization of phosphorus, ammonia production, synthesis of enzymes (chitinase cellulase and protease) and nitrogen fixation.

II. MATERIALS AND METHODS

1. Plant Materials and Experimental Conditions

The experiment was conducted in the field of National Institute of Agronomic Research of Meknes. A field

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experiment was conducted to evaluate the effects of 3 treatments of bacteria (2027-2, 2025-1 and 2066-7) on tomato growth. The experiment was set up as a completely randomized design with six replications, one plantlet in each replicates. There were four treatments viz: T1: (control; N0 -PGPR), T2: (N0 +2027-2), T3: (N0 +2066-7) and T4: (N0+2025-1).

Leaf chlorophyll content of youngest fully expanded leaf (third leaf from the shoot) of each plant was indirectly measured by a chlorophyll meter at harvest, 45 DAI (days after inoculation). Measurements of morphological parameters, namely, the length of the aerial plant, the diameter of the stem, the length of the root system and Average yield of tomato in Kg/Plant were also taken.

2. Study on Plant Growth promoting activity of the Isolate

The isolated bacteria used were taken for studying its plant growth promoting activity. Plant growth promoting activity is studied for its determining it's: Biostimulant Activity, Biofertilization Activity and Biocontrol Activity.

Study on Biostimulant activity: In this the ability production of phytohormones indole acetic acid by the isolate was studied.

Detection of IAA production: For production testing of the AIA, bacterial isolates were plated in Luria Bertani (tubes containing medium supplemented with tryptophan (T1: 0.5g/l and T2: 1g/l). After 72 hours of incubation at 28 ° C, 3 ml of the suspension was removed for the 4000tr to centrifugation at 4 °C for 10min. Then, in an Eppendorf tube 90 µl of supernatant was added to 60 µl of Salkowski reagent, the mixture was incubated in the dark for 30 min. using the spectrophotometer reading the OD at 530 nm were performed to estimate the quantity produced AIA. [11]

Study on Biofertilization activity: Ability of isolate to fix the atmospheric nitrogen and Phosphate solubilization was determined.

Phosphate solubilization: The bacterial strains were evaluated for their ability to solubilize inorganic phosphate. The PVK medium with or without BTB containing tribasic calcium phosphate was used in this trial. Each isolated culture was plated on Petri dishes and the dishes were incubated at 27 ° C for 7 days. The appearance of a clear halo around the colonies after four days has been marked as positive for the solubilization of phosphate [9]. The experiment was performed with three repetitions for each bacterial strain. [12]

PSE= (Solubilization diameter/Colony diameter)*100

Production of ammonia: Isolates were grown in peptone water at 28 ° C for 8 days. At the end of the incubation period, 1 ml of Nessler's reagent was added to each tube. The development of the pale yellow to dark brown said ammonia production. [9]

Nitrogen fixation: For the identification of fixing rhizobacteria nitrogen, nitrogen fixation activity was tested on medium Nfb. The binding activity was tested on both liquid and solid medium 0.5% bromothymol blue was used as a pH indicator. [13]

Study on Biocontrol activity: Ability of isolate to produce cellulase synthesis, protease synthesis and Chitinase synthesis was determined which can act against plant pathogens.

Cellulase synthesis: The ability to produce cellulase was measured on agar plates containing minimal medium with 2% (w/v) 1-carboxymethylcellulose as carbon source. Bacterial strains were grown on this medium and incubated at 30 °C for up to 8 days. The ability of bacterial isolates to hydrolyze cellulose was detected qualitatively by formation of a clear zone after the culturing period by adding 0.1% Congo Reed solution followed by de-staining with 1 M NaCl [14].

Protease synthesis: Protease activity was detected on 3% (w/v) powdered skim milk agar plates. A single bacterial colony of each strain was grown on this medium and incubated at 30 °C for up to 8 days. Protease activity was detected as a clear zone [15].

Chitinase synthesis: Activity of Chitinase was detected on 1% (w/v) colloidal chitin agar plates. A single colony of each bacterial strain was streaked on this medium and incubated at 30 °C for up to 8 days. Chitin hydrolysis was detected qualitatively after the culturing period by pouring 0.1% Congo Reed solution onto culture plates and checking for formation of a clear zone [16].

3. Statistical Analysis

Data from the infectivity and effectiveness tests were analyzed with the Excel2007 software.

III. RESULTS AND DISCUSSION

Effect of the bacterial strains on the parameters of the growth of tomato plants in a field

Agronomic measures namely the length of the overhead system, the diameter of the rod, the total chlorophyll and the length of the root system were used to develop the following figures (Figure 1a, b, c, and d). Indeed the length of the plants was very significantly stimulated by the three strains PGPR 2025-1, 2027-2 and 2066-7 in comparison with the inoculated plants (Figure 1a). For the length of the root system, the three strains were well stimulated length plants in comparison with other plants inoculated with the other strains and compared to the control (Figure 1b, 2).

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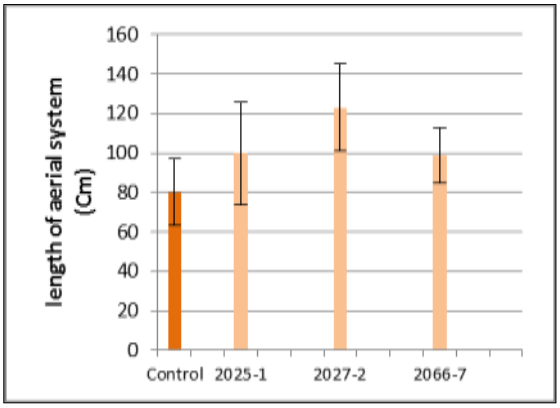
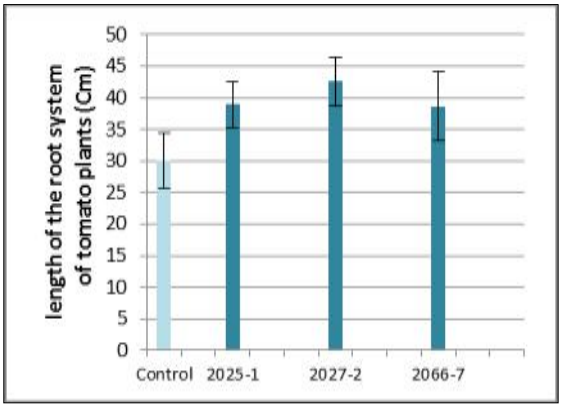
For the collar diameter of the plants, all the tested PGPR have yielded significant results in comparison to the control strain having 2025-1 including well stimulated plants compared to all treatments (Figure 3). While the total leaf chlorophyll was significantly stimulated by the three bacterial strains 2025-1, 2027-2 and 2066-7 strain (Figure 1d).

At the time of harvesting, it was found that the average total yield of tomatoes treated with the bacterial strains was significantly higher than that of the control. In fact, the plants inoculated with the bacterium 2027-2 recorded a yield Doubled compared to the control plants (Figure 1e).

The results of this study, based on inoculation 3 PGPR bacterial strains revealed the stimulatory effect of bacteria belonging to the genus *Bacillus* on the stem height and collar diameter of plants of tomato cultivation this was demonstrated by Huseyin et al., 2007, who experienced significant increase on the growth of apples inoculated with bacteria of the genus *Bacillus*.



(a)



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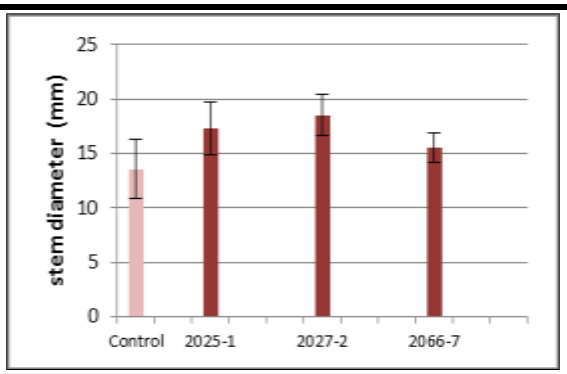

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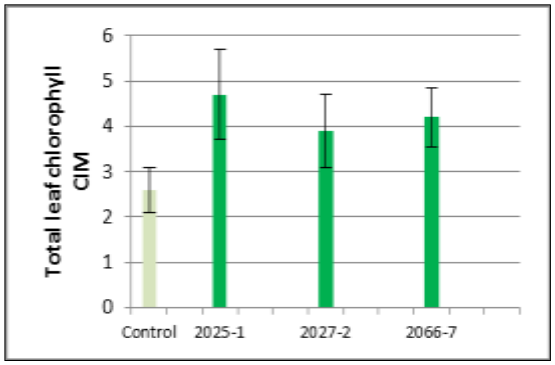
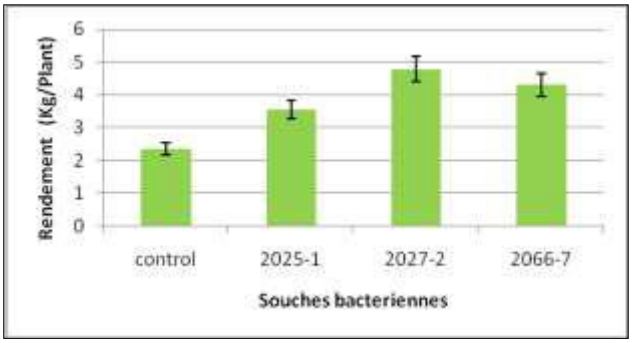


(d)



(e)

Fig.1: Effect of three isolates on the growth of tomato: a. Size of the air part, b. Size root part, c. stem diameter and d. Total leaf chlorophyll and e. Yield of tomato in Kg/Plant

(a) *(b)*

Fig.2: comparison between plant roots inoculated with strain 2027-2 (a) and those control (b)

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In addition, inoculation based on Bacillus bacterial strains resulted in more efficient results in terms of growth and yield compared to other applications (Micobacterium) and compared to control [7]. Similar results were reported in Previous studies show that application of Bacillus can stimulate yield and quality parameters in sugar beet, barley [17], apricot [18], franboise [19] and apple [20].

Inoculation based *Bacillus* bacterial strains has resulted in more effective results in terms of growth and yield compared to other applications (Mycobacterium) and compared to the control [21] .From addition, *Pseudomonas* species, *Bacillus* [22], and other endophytic bacteria such as *Enterobacter*, *Klebsiella*, *Burkholderiaet Stenotrophomonas*, attracted the attention of many researchers in recent years because of their association with important crops and their potential to improve plant growth [23] .

The root growth was stimulated very significantly by the genus *Bacillus* in comparison with other strains and compared to the control. These results corroborate the biological tests performed in 2011 on the cowpea, which showed that its bacteria have the ability to promote root growth by demonstrating a significant increase in the roots of cowpea plants after 21 days [24].

The total chlorophyll content was significantly stimulated by souches *P. agglomeranset Proteamaculansen* comparison with other treatments; these results are similar to those demonstrated by [25]. on the increase in the absorption of nutrients by plants of the rice treated with the compost formulation *Pseudomeunas* resulting in increasing the growth of leaves, stems, roots and the increase in the total chlorophyll content. Also the treatment of rice plants based on the increased strain *pantoeaa* macro-nutrient such as nitrogen, phosphorus and potassium, and increased chlorophyll content [26]. Furthermore, similar results have shown the ability of the strain *Serratia* increased total chlorophyll content relative to other therapies [27].

Since the bacterial strains tested *bacillus cereus* (2027-2), *pantoea agglomerans* (2066-7) and *serratia proteamaculans* (2025-1) gave satisfactory results compared to the control plants in terms of aerial system length, diameter of Stem, root length, chlorophyll content and mean yield. These three bacteria were chosen to study their mode of action on the basis of their characteristics of improvement and promotion of the growth of tomato plants in the field.

Since bacterial strains tested *B.cereus* (2027-2), *P. agglomerans* (2066-7) and *S.proteamaculans* (2025-1) gave satisfactory results compared to control plants in terms of air system length, diameter stem, root length and chlorophyll content these three bacteria were selected to study their mode of action on the basis of their characteristics to improve and promote the growth of tomato plants in a field.

Solubilization of phosphate production of indole acetic acid (IAA), the production of ammonia (NH_3), the biological nitrogen fixation and production of cellulase, chitinase and protease. In our study, among the three strains of *P. agglomerans* a single strain could solubilize phosphate in vitro in Petri dishes as positive the formation of a clear halo around the colony (fig. 6).

Table.1: Characterization of selected bacteria PGPR in different in vitro tests

Tests strains	***solubilization phosphorus***	***production of ammonia (NH_3)***	***Nitrogen fixation***	***Production of cellulase***	***Production of chitinase***	***Production of the protease***
2025-1	-	+	+	-	-	+
2066-7	+	+	+	+	+	+
2027-2	-	+	+	+	+	-

Regarding production of the IAA, all strains are producing indole acetic acid with different ranges for each strain to be the first treatment (0.5 g/l of tryptophan) or to the second treatment (1g/l tryptophan). Products isolates significant amounts of IAA from 4.32 g/l to 6.60 g/l for Serratia, to 4,27g/l to 6,58g/l for Pantoea and 6.37 g/l to 7,84g / l bacillus having shown the highest range of the production of IAA. Similar observations for IAA production have been reported by others [28] (Fig. 3).

Another important feature of the PGPR is the production of ammonia which indirectly affects the growth of plants. All selected isolates were positive for the production of ammonia (Fig. 3).

Nitrogen is an essential nutrient known for the growth and development of plants and its fixation by soil bacteria is considered one of the main mechanisms by which plants benefit from the microbial association. In our study all selected bacteria gave positive results for the nitrogen fixing activity by changing the green color of the medium based on malic acid in a blue environment (Fig. 3). Studies have shown that among the non-fixing bacteria symbiotic nitrogen the most important property of many species: *Azoarcus*sp.,*Gluconacetobacterdiazotrophicus*, *Herbaspirillium*sp., *Azotobacter* sp., *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*,

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Corynebacterium, *Derxia*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas*et *Xanthobacter*. *Azospirillum* is the representative of PGPR, capacities were evaluated in experiments around the world [29 and 30].

Table.2: phosphorus solubilization efficiency

Strains	***phosphorus solubilization efficiency %***
2025-1	*0*
2066-7	*64,28*
2027-2	*0*

Bacterial strains having protease activity must be highly resistant to environmental stress, mechanical and chemical. All selected strains were positive for the production of the protease enzyme except for *Bacillus cereus* strain that showed negative results.

Furthermore, cellulase activity and chitinase was well observed in both strains and *Pantoea bacillusen* showing a clear zone around the colony on agar colloidal medium, with the exception of *Serratia* strain that showed negative results in production of enzymes chitinase and cellulase.

Isolates producing PGPR plant hormones (indole acetic acid), solubilizing the phosphate and ammonia significantly improve plant growth. The maximum improvement was observed in treatments with the *Bacillus cereus* strain involving the average root length recorded with 42,6 cm. The average length of the plants was recorded with 123 cm compared to control plants.

Table.3: Production of indole acetic acid in g/l by bacterial isolates

Test Strains	***Production AIA in g/L***	
	0,5 g of Tryptophane	*1g of Tryptophane*
2025-1	*4,32*	*6,60*
2066-7	*4,27*	*6,58*
2027-2	*6,37*	*7,84*

IV. CONCLUSION

This study illustrates the importance of rhizobacteria in vitro conditions for several PGPR traits and their evaluation in controlled conditions in a tomato field trial. This led to the selection of effective PGPR namely *Serratia proteamaculans* (2025-1), *P. agglomerans* (2066-7) and *Bacillus cereus* (2027-2) which, because of its multiple Traits PGPR, could prove effective in enhancing the growth and vigor of plants and in the stimulation of plant root system.

This type of study is necessary because it advocates the use of PGPR biofertilizer or as an inoculant is an effective approach to replace chemical fertilizers and isolates of these PGPR can be used as organic fertilizers to improve growth and productivity trade to grow the plants in the local agro-climatic conditions.

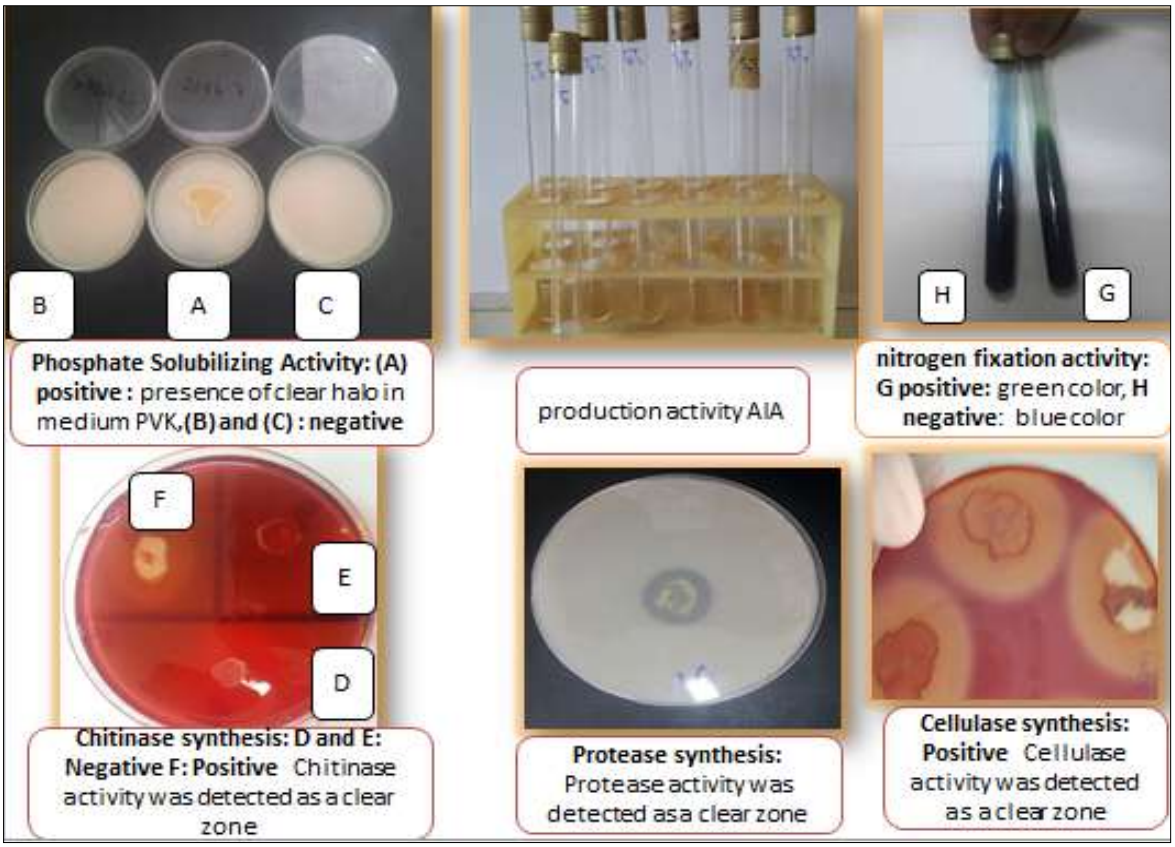

Fig.3: Strains and their PGP traits and production of antimicrobial substances

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