Original article

Stimulatory effect of plants growth of wheat (Triticum aestivum) by PGPR (Plant Growth-Promoting Rhizobacteria) in Morocco

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ARTICLE INFO

Keywords:
PGPR, pathogens
shoot and root mass
plant hormones
bacterial strain
chlorophyll
fresh peas

ABSTRACT

Background: PGPR (Plant Growth Promoting rhizobacteria) stimulate plant growth in the absence of pathogens. These include the direct effects of air and increases root mass, strains root, and accelerated raising of seedlings. These increases are generally explained by better sampling and nutrient uptake by the plant, the production of plant hormones and the development of induced resistance in plants. Method: As part of this work, some PGPR bacterial strains were evaluated on the cultivation of wheat in the tunnel. Result: The isolates were closely related to Paenibacillus brasiliensis (2025-11), Pantoea agglomerans (2074-1), Bacillus cereus (2015-1), Bacillus cereus (2027-2), Serratia proteamculans (2025-1), Pantoea agglomerans (2066-7), Acinetobacter (2077-5) and Bacillus sp (2026-2). The results of this study demonstrated the ability of the Bacillus sp strain to increase the length of their system of the wheat plant at higher levels in comparison with the control plants. Conclusion: Also this stimulated the length of the root system of culture, while the bacterial strain Pantoea agglomerans showed satisfactory results in terms of plant growth parameter, namely chlorophyll and fresh pea’s plants of wheat.

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Introduction

Rhizosphere soil is there which is under the influence of root exudates. In this are there is a particular group of bacteria, that is name rhizobacteria. These are able to multiply and compete with other microorganisms to occupy this rich area with nutrients (Kloepper and al., 1992). Nearly 5% of rhizobacteria promote the growth of plant and protect against pathogens such as bacteria, fungi (Suslow1982; Weller1988) and nematodes (Kloepper and al., 1992). This rhizobacteria belong to different taxonomic groups of bacteria. They have been grouped under the name rhizobacteria that foster plant growth (PGPR). The groups of bacteria that are used belong including Bacillus (Holl and al., 1988), Pseudomonas (O’Neill and al., 1992), Enterobacter (Beall and al., 1989), but also rely Azospirillum, Azotobacter, Klebsiella, Rhizobium and Serratia spp. (Bakker and al., 1991; Kloepper1992).

The seed inoculation with these rhizobacteria usually results in yield increases about 10 to 30% (Suslow, 1982). Indeed they have been introduced into the ground to improve both growth by various mechanisms such as fixing of nitrogen (N2) and solubilization of trace elements such as phosphate (P) (Cakmakci and al., 2006; Orhan and al, 2006), the inhibition of the ethylene synthesis by the plant, the synthesis of plant hormones, and vitamins (auxins and gibberellins) (Dobbelare and al., 2003), and reducing the toxicity of heavy metals (Burd and al., 1998; Whipp, 2001). And also the health of plants and reduce the use of chemicals. This beneficial effect of bacteria on the growth of plant is often measured by the increase in weight of the roots and stems (Schipper and al., 1995; Gamalero and al., 2002).

In this work, we evaluated the influence of ten bacterial strains PGPR on wheat plants growth parameter sin tunnel in this case the collar diameter, shoot length, shoot fresh weight, root fresh weight and estimation of total chlorophyll content in leaf.

MATERIALS AND METHODS

Plant material

In this study the plant material use dare the seeds of Triticum aestivum of wheat were sown in pots tunnel less than 5 kg at the National Institute of Agronomic Research of Meknes (INRA).

Isolation and Storage Strains

Ten plant growth-promoting rhizobacteria, 2026-2, 2027-2, 2015-1, 2025-1, 2025-2, 2025-11, 2074-1, 2066-7, 2077-5, 2321-6 and 2328-B5, were used in the experiment. The strains were
Preparation of human erythrocytes:[14]

Fresh heparinized blood sample from both healthy controls and renal patients were centrifuged under cooled temperature for 5 min at 2 000 r/min. After removing plasma and buffy coat, the collected pellet was washed three times with phosphate buffered saline (PBS) at pH 7.4 and then re-suspended in PBS buffer to give 10% erythrocyte suspension.

In vitro RBC hemolysis assay:

The erythrocytes suspension was incubated with different concentrations of the hemolytic agents at 37 °C for 60 min. Aliquots of the hemolyzed mixture were taken at appropriate time intervals, diluted with 0.15 mol/L NaCl, and centrifuged at 2 000 r/min for 10 min. The supernatant was collected and its absorbance was measured spectrophotometrically at 415 nm. The extent of RBC hemolysis by the chemical agent was expressed as a percentage value relative to complete hemolysis of similar erythrocyte sample (blank) carried out in deionized water alone. Solutions of nitroso- amino acids were prepared freshly, as described previously.[14]

Antioxidants protection of RBC hemolysis:

For determining the protection effect of antioxidants, 10% of the RBC suspension was pre-incubated at 37 °C with 500μM antioxidant (uric acid or ascorbic acid or trolox) for 30 minutes. After centrifugation, the RBC pellet was re-suspended in 15mM cysteine and the suspension was incubated for one hour before assaying the hemolysis extent as described above. Similar hemolytic test with cysteine but lacking the anti-oxidant was used as a control.

Ferric Reducing/Antioxidant Power (FRAP) assay:

The FRAP assay [15], depends upon the reduction of ferric [Fe(III)-TPTZ] to [Fe(II)-TPTZ] complex that gives intense blue color absorbed at 593 nm. Due to possible interference of the endogenous antioxidants uric acid, it was necessary to consider the level of this interfering substance in plasma during the calculation of total reducing power by the FRAP method. In uremia patients, such discrepancy is particularly aggravated by the increase in tissue destruction which tends to elevate the concentration of uric acid in plasma (data not shown). Hence, we used the ratio Fe II/ uric acid concentration to express total antioxidant capacity by FRAP method instead of using the FeII concentration alone.

All experiments in this investigation were repeated in triplicate and values were expressed as the mean ± SD.

Statistical analysis

The values were expressed as means ± SD using SPSS software for data analysis. One way analysis of variance was used to assess group means and P <0.05 was considered statistically significant. Linear regression analysis was used to calculate the concentration of compound that gives 50% hemolysis of RBC (IC50).

Results:

Blood chemistry of uremia groups and control subjects

Blood obtained from uremia patients with CKD or under dialysis managements displayed high concentrations of urea; creatinine and uric acid, when compared with similar samples collected from healthy control (Table1). Also, these patients showed abnormal levels of electrolytes, in particular the CKD patients had significantly high concentration of plasma potassium (p < 0.05). The blood hemoglobin (Hb) scored another abnormal deviation, emphasizing the dominant association of anemia state with currently investigated uremia patients.

Susceptibility of uremia RBC for hemolysis:

Screening of hemolytic potency:

The hemolytic potential of various chemical compounds were screened in vitro against fresh preparations of erythrocytes from renal patients (Fig 1). During one hour exposure, the compounds SDS, nitrosocysteine, nitrosoarginine, cysteine, and salicylic acid produced strong hemolysis of more than 60% , but other compounds like spermidine, SNP, arginine, urea and methionine showed weak hemolytic power of less than 10% and their action is not pursued any further.

Comparison of strong hemolytic agents:

Despite their versatile chemical structure, all strong hemolytic agents exhibited distinctive action on uremia RBC when compared with the same hemolysis action on RBC of normal controls (Figs 2-6). This hemolysis was concentration dependent and its severity was more pronounced on RBC of CKD patients than on RBC of dialysis group. Further quantitative assessment of the hemolysis potency was susceptibility achieved by estimating the concentration of hemolytic agent that produces 50 % hemolysis of RBC (IC50 values). Data in table 2 show that IC50 values of all chemical agents determined from the hemolysis of uremia RBC is significantly different (p < 0.05) from similar values of RBC hemolysis in normal controls. This pattern of differential potency by the hemolytic agents was also observed between RBC of CKD patients and those under dialysis. In particular, the cytotoxic agent SDS scored the lowest IC50 values while salicylate expressed the highest IC50 on corresponding RBC of uremia patients.

Total antioxidant capacity (TAC):

Total antioxidant capacity (TAC) in plasma was determined by FRAP method, and expressed as FeII/ uric acid ratio. The highest value of this ratio was markedly detected in plasma of normal subjects followed by dialysis group and then CKD renal patients, respectively (Fig 7).

In vitro Protection of RBC hemolysis:

The in vitro protection of uremia RBC against cysteine hemolysis was attempted using the antioxidants ascorbic acid, uric acid and trolox chemical agents. Each antioxidant agent was pre-incubated with uremia RBC before the initiation of hemolysis by cysteine. A control of cysteine treated RBC sample from uremia patients but lacking the corresponding antioxidant was run in parallel. All tested antioxidants produced a decrease in cysteine hemolysis of renal RBC when compared with the control(Fig 8). The uric acid exhibited the highest potential of hemolysis protection (76 %), followed by ascorbic acid (68%) and then trolox (55%), respectively.

Table 1: Blood chemistry of uremia groups and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Blood test</th>
<th>Normal control</th>
<th>CKD</th>
<th>Dialysis</th>
</tr>
</thead>
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<tr>
<td>Urea</td>
<td>30± 5 mg/dl</td>
<td>193±25 *</td>
<td>144±31 *</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>1± 0.1 mg/dl</td>
<td>9.2±5.3 *</td>
<td>7.9±6.2 *</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>140± 2 mmol/L</td>
<td>136±2</td>
<td>132±2</td>
<td></td>
</tr>
<tr>
<td>K⁺</td>
<td>4.5± 0.5 mmol/L</td>
<td>6.5± 1 *</td>
<td>5.9±0.3</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13± 2 g/dl</td>
<td>11±3.5</td>
<td>9.2±2.5 *</td>
<td></td>
</tr>
</tbody>
</table>

(*) Indicates a significant difference between patient groups and normal controls (p < 0.05)
isolated from three types of composts (Composted manure, Forest soil and Earth worm compost). These bacteria were grown on LPGA (7g/l yeast extract, 7g/l peptone, 10g/l glucose and 18g/l Agar), and maintained in LP6 (7g/l yeast extract, 7g/l peptone), with 20% glycerol at ~80 °C for long-term storage. Preparation of pots and seed inoculation

The pots of (length: 35cm and diameter: 25cm) filled with 1/3 peat and 2/3 sand. The seeds of wheat (Triticum aestivum) were surface sterilized with 80 per cent ethanol and 0.1 per cent mercuric chloride and washed the seeds with sterile distilled water for 3 to 4 times. The seeds were mixed with carrier based plant growth promoting bacteria, either as individual organisms of organisms separately having a cell load of 108 cfu ml-1 and shade dried for 30 min. After shade drying, the seeds were sown at ten of seeds per pot and finally five seeds were maintained. A control pot without inoculation was also maintained. The experiment was conducted in completely randomized block design with three replications. The treatments are as follows:

T0: control
T1: 2025-11
T2: 2074-1
T3: 2015-1
T4: 2027-2
T5: 2025-1
T6: 2066-7
T7: 2328-B5
T8: 2321-6
T9: 2077-5
T10: 2026-2

The pots are placed under the tunnel after calculating field capacity (HCC) of the substrate. Irrigation is daily with a volume of 500 ml of water/pot. The second inoculation at the roots of the plants was made after 21 days of sowing.

Experiments were replicated (5 pots per treatment). Two month after planting, each plant was removed from its pot, roots were washed to remove vermiculite, and parameters reflecting growth-promoting effects of bacterial treatment were recorded: shoot length, shoot fresh weight, root fresh weight and estimation of total chlorophyll content in leaf.

Amplification and sequencing of 16S rDNA

This part of the work was performed in the Functional Genomics Platform, Technical Support Unit for Scientific Research, CNRST, Rabat, Morocco.

Total genomic DNA of each isolate was extracted using Gen Elute Mammalian Genomic Kit. The amount of DNA was quantified by recording the absorbance at 260 nm wavelength using UV/VIS spectrophotometer. DNA was stored at -20 °C for further use.

The PCR was done as follows: initial denaturation at 96°C for 4 min followed by 35 cycles consisting of a 30s denaturation at 95°C, 30s at annealing temperature of 52°C, followed by a Fd1 (C A G A G T T T G A T C C T G C T C A G ) a n d 2 P 2 (A G A T T G A C T G C T G C T C A G ) primer extension at 72°C for 2min, followed by an additional extension at 72°C for 4min. The PCR amplification was carried out in 0.2ml PCR tubes with 25 μl reaction volume consisting of following components: 2.5μl 10X Buffer, 2μl dNTP (10mm), 0.125μl of each primer (100 μM), 0.75μl MgCl2 (50 mM), 0.2μl Taq (5U/μl) and 5μl DNA. Then, the PCR products were run on a 1.5% agarose gel in TAE buffer. Sequencing was performed using a Big Dye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Foster City, USA) and reactions were analyzed on an automated DNA sequencer (Applied Biosystems model 310, Perkin Elmer Applied Biosystems).

The 16S rDNA sequences of different bacterial isolates were BLAST (Basic local alignment search tool) searched against the sequences of 16S rDNA of bacterial isolates available in the Gen bank Nucleotide Database (http://www.ncbi.nih.gov/BLAST).

The measured parameters

Measures on the aerial and roots part
The parameters measured at the aerials part are plant height, collar diameter and total chlorophyll of the plant. While at the level of the root system the measured parameter is the length of the roots.

Destructive measurements
After harvest the whole plant, roots were separated from the aerial part of the measures to achieve the fresh plant biomass of wheat for the determination of their fresh weight.

Statistical Analyses
The data obtained were entered under the EXCEL software. The analysis of variance for a single (ANOVA one) was performed using SPSS version 20 of 5%. Hierarchical classification was under taken by the MVSP software (Multivariate Statistical Package) v

RESULTS AND DISCUSSION

Measures of the aerial part The height of the stem The average height of the stems of wheat plants treated with the strains 2026-2 and 2027-2 has recorded growth rates respectively about 35% and 27% compared to the untreated control (Fig.1).
Fig.2: Observed Length wheat plant stems inoculated with the strains 2026-2 and 2027-2 in comparison with the control.

Collar diameter: Measuring the diameter of the plants revealed the following figure (Fig.3). Bacterial strains 2026-2 and 2077-5 have shown an increase in diameter, in particular the strain 2026-2 having a diameter of 4.92 mm in comparison with the control which was 3.15 mm.

Chlorophyll

Measuring the total chlorophyll shows that plants treated with the bacterial strains 2066-7, 2027-2 and 2328 B-5 have a higher chlorophyll content relative to control plants, especially those treated with the Pantoea strain (2066-7) had a higher content compared with other treatments.

Custom root portion The length of the root system

The results obtained, in the heading stage, revealed that strains 2027-2, 2025-1 and 2066-7 strain showed an increase in root biomass of fresh wheat plants compared with the control.

Fig.3: Effect of ten bacterial strains tested on the collar diameter of the plant wheat, elongating.

Fig.4: Effect of ten bacterial strains tested in the plant chlorophyll wheat

 destruct measurements

The fresh weight of the plant

The plants treated with stem 2321-6, 2066-7, 2025-1 and 2027-2 showed the production of fresh biomass plants highly elevated compared to control plants.

Fig.5: The length of the root system of wheat plants inoculated with ten bacterial strains of PGPR.

Fig.6: The effect of the bacterial strains tested on fresh pea plant wheat the a d in tage

Indeed, the measurement of fresh pea plants treated with the four strains 2321-6, 2066-7, 2025-1 and 2027-2 showed a rate of increase respectively of the order of 99.8%, 76.9%, 55.64% and 38.78% compared with the control.

This study revealed the stimulatory effects of bacteria belonging to the genus Bacillus on the height of the stem and root collar diameter of plants growing wheat, this has been demonstrated by Iluseyin and al., 2007, who presented a significant increase on the growth of apple trees inoculated with the bacteria Bacillus genus. Inoculation basis of Bacillus bacterial strains has led to
more effective results in terms of growth and performance relative to other applications (Mycobacterium) and compared to the control (Huseyin et al., 2007) (Fig.7).

![Fig.7: Effect of bacterial applications (Bacillus and Mycobacterium) on yield and growth of apples (Huseyin et al., 2007).](image)

Furthermore, similar results were reported in previous studies, show that the application of the Bacillus can stimulate parameters yield and quality of sugar beet, barley (Caikmacei et al., 2001), apricot (Esitken et al., 2002, 2003), Franboise (Orhan et al., 2006) and apple (Aslanıaş et al., 2007).

The analysis of the hierarchical classification, showed the existence of two homo generous groups G1 (formed from plants treated with the genus Bacillus) and G2 (comprised of the treated plants with Pantoaea agglomerans strain and other strains T as tees with the control) (Fig.8).

![Fig.8: The hierarchical classification of ten bacterial strains tested on the cultivation of wheat in field.](image)

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 in 2011 on the cowpea, which showed that its bacteria has the ability to promote growth root by demonstrating a significant increase in the roots of cowpea plants after 21 days (Kerla, 2011).

Furthermore, the BLAST results of the 16S rRNA gene sequences allowed to classify the isolated strains from various compost into the family of Enterobacteriaceae, Pseudomonaceae, Bacillaceae and Moraxellaceae. The four evaluated strains were aligned against sequences available from GenBank data; the eight PGPR 2025-11, 2074-1, 2015-1, 2027-2, 2025-1, 2066-7, 2077-5 use as biological fungicides or as stimulators of plant growth.

and 2026-2 matched to Paenibacillus brasiliensis, Pantoaea agglomerans, Bacillus cereus, Bacillus cereus, Serratia proteamucalis, Pantoaea agglomerans, Acinetobacter and Bacillus sp respectively with 99% of similarity percentage through GenBank database as represented in Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage of Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paenibacillus brasiliensis</td>
<td>99%</td>
</tr>
<tr>
<td>Pantoaea agglomerans</td>
<td>99%</td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td>99%</td>
</tr>
<tr>
<td>Serratia proteamucalis</td>
<td>99%</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>99%</td>
</tr>
<tr>
<td>Bacillus sp</td>
<td>99%</td>
</tr>
</tbody>
</table>

These rhizobia producing indole-3-acetic acid (IAA) which are known by their ability to increase growth and root length, AIA is involved in the initiation of cell division at the root level, and their enlargements (Salisbury, 1994). This effect results in a greater root surface and accessibility for more nutrients to the plant (Kerla, 2011).

Moreover, much research has focused on these strains of bacteria because they are common inhabitants of the rhizosphere and have agreed at activity in the biological control of soil-related illnesses. They have the ability to produce many antibiotics and they are easy to grow in vitro or manipulate in the laboratory (Cavaglileri et al., 2005).

In addition, gender bacillus have an advantage over other bacteria due to their ability to form endospores which are resistant to changing conditions also benefit medium for product formulation (Raaijmakers and al., 2002).

The plant chlorophyll content was positively affected by the bacterial strains of Bacillus genus and species Pantoaea agglomerans, stimulating the photosynthetic activity of plants. In addition, these two types of bacteria show higher fresh weight of seedlings which is due to the production of Avery large amount of dry matter. These results corroborate the results of the study Huseyin et al., 2007 have shown that treatment P. agglomerans has significantly increased plant growth and yield of rice. All parameters (such as fresh and dry weight of shoot fresh weight and dry root, stem length, stem diameter, leaf area, chlorophyll content, the content of macronutrients in the sheet, the number tillers, number of panicles per hill, the percentage of grains per panicle terse, dry weight of 1000 grains and grain weight per hole) of treated plants plant growth and the yield was significantly higher than those of the untreated control plants (P<0.05).

The total chlorophyll content was significantly stimulated by P. agglomerans strain compared with other treatments, these results are similar to those demonstrated by Khalimi and al., 2012 on the increase of the absorption of nutrients by plants of the rice treated with the formulation of compost Pseudomonas which resulted in an increase in the growth of leaves, stems, roots and increase the chlorophyll content.

The increase in chlorophyll content in the leaves of plants treated with P. agglomerans can be caused by ACC desaminase activity (Teng and al., 2010) that slow down the degradation of chlorophyll (Silva and al., 2004). The total chlorophyll content in rice treated with strains belonging to the genus Pantoaea, P and PB were 38.25, 39.12 and 39.32 SPAD units, which increased by 23.30%, 26.11% and 26.76%, respectively compared to control (Khalimi and al., 2012). Indeed, similar results were obtained by Hanand Lee, 2005, in which the lettuce inoculated with the strain P. agglomerans contained 13.91% more chlorophyll than the untreated control. Nadeem et al., 2006 have shown that treatment of corn seed through dipping the suspension based on the same strain at a concentration of 108 UFC/ml could increase chlorophyll content of 102.22% compared to the untreated control.
Prospect

In order to enhance these results, it will be necessary to use these strains in vitro against some plant pathogens (bacteria and fungi). Should be optimized cropping parameters to improve the production of antifungal and identify the active ingredient of these strains and to assess their effects in the field or in storage for

<table>
<thead>
<tr>
<th>Strains</th>
<th>Number of base pairs</th>
<th>Closest relative species</th>
<th>Similarity (%)</th>
<th>Accession number in Gen Bank (NCBI) of the strain</th>
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<tbody>
<tr>
<td>2025-11</td>
<td>1373</td>
<td><em>Paenibacillus brasilensis</em></td>
<td>99%</td>
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<td>2074-1</td>
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<td><em>Pantoaea agglomerans</em></td>
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<td>WAB1925</td>
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<tr>
<td>2015-1</td>
<td>1461</td>
<td><em>Bacillus cereus</em></td>
<td>99%</td>
<td>JMG-01</td>
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<tr>
<td>2027-2</td>
<td>1461</td>
<td><em>Bacillus cereus</em></td>
<td>99%</td>
<td>JMG-01</td>
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<tr>
<td>2025-1</td>
<td>872</td>
<td><em>Serratia proteamaculans</em></td>
<td>100%</td>
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<tr>
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<tr>
<td>2321-6</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>2328 BS</td>
<td>-</td>
<td>Not yet identified</td>
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REFERENCES