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## Effects of plant growth promoting rhizobacteria (PGPRs) product IAA on the growth of two Moroccan wheat varieties (*Triticum durum Desf.*).

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### ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria that colonize the rhizosphere and, when applied to crops, enhance the growth of plants. These bacteria are known to produce Indole Acetic Acid (IAA), a hormone known to affect plant growth. In this study, we tested the effects of seven bacterial strains on two durum wheat varieties (*Triticum durum Desf.*) cultivated in Morocco: "Marzak" and "Karim" (concentration:  $10^5$  CFU. ml<sup>-1</sup>) in greenhouse conditions. Isolate S35 improved all the growth parameters of interest for the Karim wheat variety whereas isolate S50 was shown to promote the increase in stem length in the Marzak wheat variety. Isolate S48 was shown to favor root lengthening and the increase in the plants' wet and dry mass. In order to correlate the observed effects with IAA production, the different isolates were also tested for production of IAA and the results indicated that isolate S10 had the highest observed production (46.23 µg/ml). The results of this study constitute a starting point for a better control over the selection and usage of PGPRs in the agricultural sector in Morocco. Ultimately, we aim to improve the yield and quality of the crops without the use of chemical input and developing eco-friendly agricultural practices.

**Keywords:** PGPR, rhizosphere, IAA, *Triticum durum Desf.*, Morocco.

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## INTRODUCTION

Intensive agricultural practices aimed at a producing high yield and good quality harvests require the use of chemical inputs, which can be costly but also dangerous by accumulation for men and environmental ecosystems (Esitken et al., 2005). In order to achieve more sustainable agricultural practices, the use of biodegraded organic matter has become common practice due to its ability to provide a continuous supply in minerals that complements soil composition, but also for the large variety of beneficial microorganisms it provides to stimulate plant growth. Plant growth promoting rhizobacteria (PGPR) constitute a very important and diverse class of these microorganisms and contain numerous genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas* and *Serratia* adapted to different types of soils and climates. PGPRs were first described by Kloepper et al. (1989) as rhizospheric bacteria that contribute to the nutrition of the plants and their ability to take root. The mechanisms they employ to promote plant growth are very diverse. Some PGPRs are capable of producing plant endogenous phytohormones in the soil surrounding the roots, such as auxin (IAA) (Spaepen et al., 2007; Malhotra and Srivastava, 2008; Baniaghil et al., 2013), gibberillic acid (Mahmoud et al., 1984), cytokinin (Tien et al., 1979), ethylene (Steenhoudt and Vanderleyden, 2000; Galland; 2012) and abscisic acid (Forchetti et al., 2007). For all these hormones, the concentration of the signal molecule is crucial. For example, the dose response curve of the plant to exogenous auxin application is bell shaped (Taiz and Zeiger, 2010) and thus rhizobacteria can induce positive or negative effects depending on their level of auxin production (Barazani and Friedman, 1999). In addition to hormone production, studies have also shown that PGPRs are involved in Nitrogen fixation (Kennedy et al., 1997), ammonia (Samuel and Muthukkaruppan 2011), HCN (Ahmad et al., 2008) and siderophore production (Singh and Varma, 2015) as well as  $\beta$ -1,3-glucanase and chitinase activity (Renwick et al., 1991; Shahzad et al., 2013). They were also shown to produce antibiotics and improve the solubilization of mineral phosphates and other nutrients (De Freitas et al., 1997; Govindasamy et al., 2010). Wheat production is an essential part of the agricultural sector in Morocco and bread is the basis of most meals for the local population. The aim of this study is to isolate and characterize PGPRs found in the Haouz region and evaluate their effect, through inoculation, on the growth and development of two wheat varieties grown in the region with the ultimate goal of developing agricultural practices that are less reliant on chemical input.

## MATERIALS AND METHODS

### Bacterial isolation

PGPRs were isolated from the soil surrounding the roots of wheat grown in the Saada experimental domain of INRA, Marrakech (Institut National de Recherche Agronomique, Marrakech).

### IAA production assay

#### Qualitative assessment

The isolated strains were grown in 100ml flasks of LB medium containing L-tryptophan (1.02g / L). They were then incubated while being agitated at 28°C for 72 hours and each culture was then centrifuged at 7000rpm for 30min. 1mL of supernatant was added to 2ml of Salkowski's reactant ( 60% sulfuric acid and 3mL of 5 M ferric chloride) and 2 drops of orthophosphoric acid. The mixture was incubated at room temperature for 30 minutes; the appearance of a pink coloration is a positive test for IAA production by the bacteria (Loper and Scroth, 1986).

#### Quantitative assessment

Quantitative analysis of IAA production was conducted using the method described by Loper and Scroth (1986). Bacterial cultures in LB medium with or without the addition of 1% the L-tryptophan were incubated at 28°C for 72 hours, then centrifuged at 7000rpm for 3 minutes. 1 mL of the supernatant was added to 2 drops of orthophosphoric acid and 2ml of Salkowski's reactant, absorbance was then measured at 530nm using a spectrophotometer [UVmini-1240; SHIMADZU].

### Inoculation and sowing

The analysis was conducted on two wheat varieties (Marzak (V1) and Karim (V2)): the seeds were sterilized in ethanol for 30 seconds then rinsed with sterilized distilled water. The inoculation of the seeds was conducted by exposing them for 30 minutes to bacterial suspensions grown in LB medium at 28°C for 24hrs while being agitated. The seeds were then sown in trays containing sterile soil and 0.2ml ( $10^5$ CFU ml<sup>-1</sup>) of the medium containing the bacterial strain was added around the seed. Sterile LB medium served as a control and the germination was conducted in a greenhouse with daily irrigation.

### Wheat growth analysis

The wheat plants are collected after 30 days of growth and the length of the above-ground and root tissues was measured; the plants were also weighed in order to record the wet mass. The Dry mass was then measured after the plants are oven dried at 70°C for 72 hours.

### Statistical analysis

The data was analyzed using a two-factor analysis of Variance (ANOVA), the least significant difference test (LSD,  $p < 0.05$ ) was conducted in order to compare the different treatments and variations using SPSS for windows (Version 17, SPSS Inc., Chicago, IL, USA).

## RESULTS

### Bacterial isolation

Seven rhizobacterial strains were isolated from the soil collected in the Saada experimental domain affiliated with INRA (Institut National de la Recherche Agronomique), Marrakech. They were all identified as members of the *Bacillus* genus (Chrouqi et al., Submitted).

### IAA production assay

IAA production was detected using a colorimetric method using Salkowski's reagent, the color change from yellow to pink indicated the presence of IAA secreted by the isolated bacteria. The qualitative assay has shown that all the bacterial strains are capable of producing IAA although with varying intensities (Fig 1). Using a 1mg/ml IAA solution, a series of dilutions were made to prepare a standard curve which allowed the estimation of IAA production for each strain using a spectrophotometer. The S10 isolate has the highest observed production (46.23 µg/ml) while S35 has the lowest IAA production (1.98 µg/ml) (Fig.2).

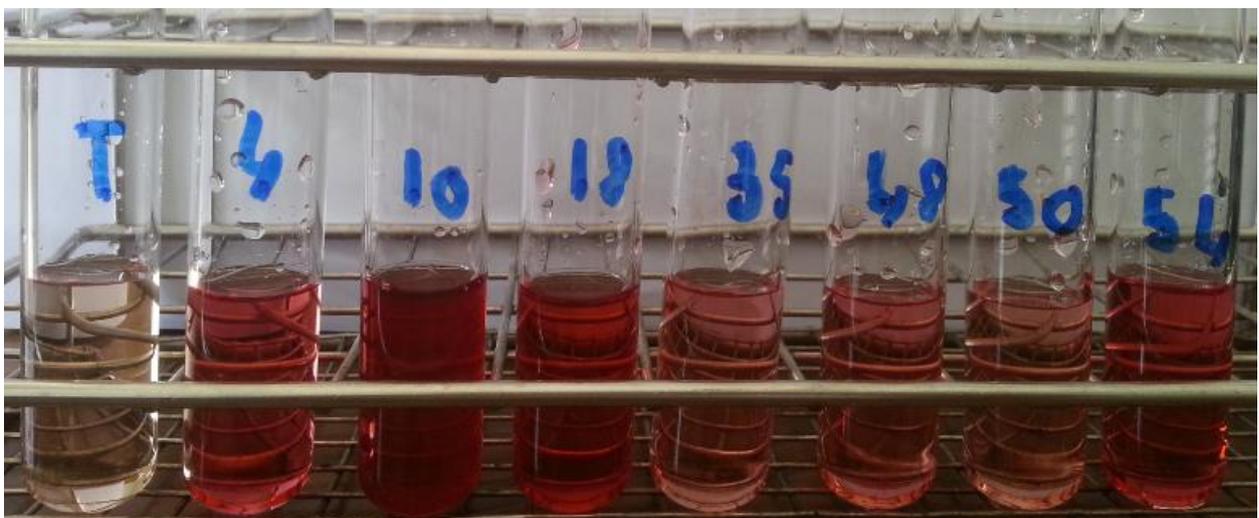


Figure 1: Qualitative assay results for IAA production for the different isolates

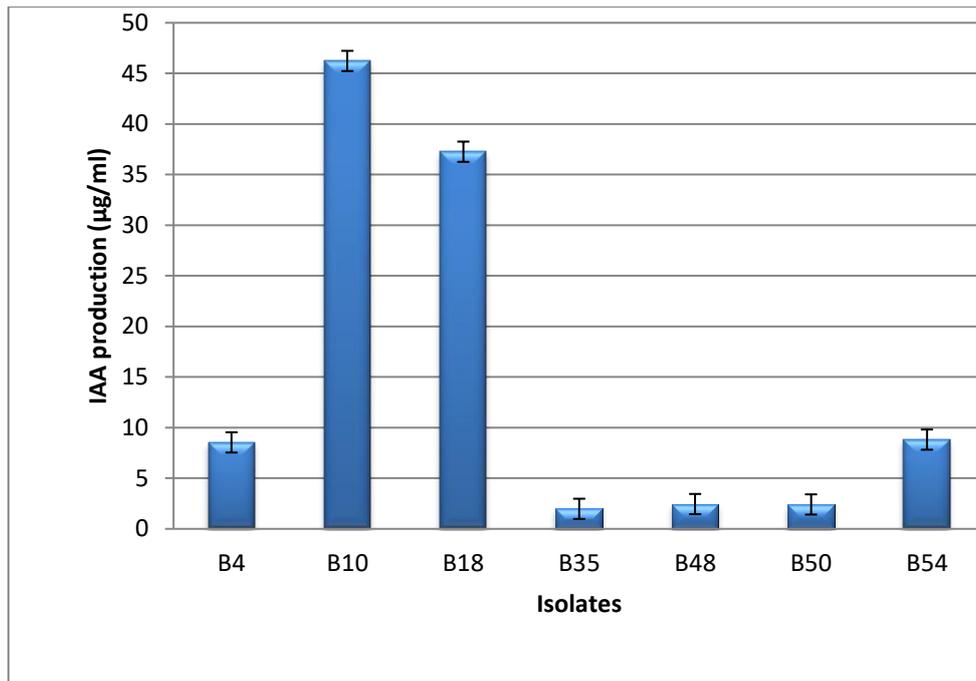


Figure 2: Quantitative assay for IAA production in the different isolates.

**Effects of PGPR inoculation on the growth of the different wheat varieties**

The effects of PGPR inoculation on the plant growth parameters, lengths of the stems and roots and their wet and dry mass are compiled in Table 1. The results indicate that isolate S50 was most efficient at promoting the increase in stem length in the V1 wheat variety, whereas S48 favors root lengthening and the increase in the plant’s wet and dry mass in the same. For the V2 wheat variety, isolate S35 had the biggest effect and improved all the growth parameters of interest.

**Table 1: Effects of the bacterial inoculation on the growth parameters for the Marzak (V1) and Karim (V2) wheat varieties.**

The presented values were averaged for three repetitions. Numbers followed by the same letter are not considered significantly different according to the Newman and Keuls method with a 0.05 threshold.

Wheat Variety	Bacterial isolate	Stem length (cm)	Root length (cm)	Above ground wet mass (g)	Above ground dry mass (g)	Root wet mass (g)	Root dry mass (g)
Marzak (V1)	Control	21,69±1,78a	25,04±1,53a	2,71±0,131a	0,39±0,04ab	3,30±0,18a	0,28±0,03a
	4	22,68±1,23cd	32,40±6,74a	2,32±0,136de	0,29±0,0173cd	3,95±0,08c	0,34±0,05b
	10	19,46±1,83b	23,76±5,11a	2,05±0,096c	0,27±0,02bc	3,48±0,36ab	0,31±0,01ab
	18	21,52±1,4bc	19,20±5,69a	2,29±0,361cd	0,32±0,04cd	3,00±0,29abc	0,28±0,064ab
	35	24,04±0,59d	24,50±3,65a	2,97±0,478 <sup>e</sup>	0,37±0,08cd	3,46±0,38bc	0,34±0,025b
	48	26,76±0,57d	<b>34,82±2,28a</b>	<b>3,30±0,064cd</b>	<b>0,43±0,02d</b>	<b>4,63±0,73bc</b>	<b>0,39±0,012ab</b>
	50	<b>27,53±0,37bc</b>	27,38±9,52a	3,24±0,295b	0,28±0,16a	3,28±0,53a	0,31±0,03a
	54	25,56±0,88b	24,70±1,74a	2,92±0,183ab	0,36±0,01a	2,99±0,20a	0,29±0,01a
Karim (V2)	Control	25,13±2,06a	28,03±7,43a	2,29±0,54a	0,36±0,09ab	3,57±0,42a	0,26±0,03a
	4	37,40±1,55cd	34,40±9,77a	6,28±0,47de	0,64±0,04cd	5,12±0,32c	0,36±0,03b
	10	34,93±2,57b	29,66±6,81a	5,29±0,69c	0,58±0,06bc	4,16±0,43a	0,28±0,006b
	18	35,43±3,23bc	35,66±6,81a	5,46±0,19cd	0,65±0,01cd	4,93±0,25abc	0,33±0,09ab

	<b>35</b>	<b>40,76±0,67d</b>	<b>37±5,41a</b>	<b>6,42±0,46<sup>e</sup></b>	<b>0,66±0,03cd</b>	<b>5,44±0,33bc</b>	<b>0,37±0,015b</b>
	<b>48</b>	35,93±2,39d	34,80±7,08a	5,00±0,62cd	0,64±0,04d	4,09±0,49bc	0,22±0,06ab
	<b>50</b>	30,10±0,9bc	31,83±2,41a	2,98±0,03b	0,33±0,01a	3,49±0,35a	0,18±0,02a
	<b>54</b>	27,70±1,82b	37,83±10,70a	2,42±0,29ab	0,27±0,04a	4,42±0,70a	0,27±0,01a

### DISCUSSION

Plant growth promoting rhizobacteria can promote plant growth by associating with the roots of the plant host (Suarez et al., 2014). Some of the mechanisms involved in this plant- bacteria association involve the production or degradation of phytohormones that regulate plant growth and development (Hayat et al., 2010). Previous studies have shown that IAA concentration in rhizospheric *Bacillus* species can reach a value of 20,8µg/ml (Akhtar, 2013) whereas other species such as *S. marcescens* can reach a value of 19.68µg/ml (Shahzad et al., 2013). IAA concentrations in our isolates varied between 1.98 µg/ml and 46.23µg/ml. Previous studies have also clearly demonstrated a positive effect of PGPRs on plant growth in a wide variety of plants. Specifically, PGPRs were shown to promote the development and improve the quality and texture of tomato plants (Hortencia et al., 2007) and have also been shown to improve the yield and quality of sugar beets (Cakmakci et al., 2001), apricots (Esitken et al., 2002; Esitken et al. 2003 ) and cherries (Esitkena et al., 2006). The vast majority of plant growth promoting rhizobacteria isolated in this study had a positive effect on all the examined growth parameters, notably on stem and root length and the wet and dry mass of the stems and root. These beneficial effects on the growth, yield and quality of the plants can be explained by the ability of PGPRs to solubilize phosphate and produce more IAA and ammonia (Cakmakci et al., 2001; Esitken et al., 2002; Esitken et al., 2003; Tsavkelova et al., 2007; Goswami et al., 2014). They are, however, highly dependent on the level of IAA production (Barzani et Friedman, 1999; Nehl et al., 1996; Vacheron et al., 2013). Although over 80% of rhizobacteria can produce IAA (Loper and Schroth, 1986), the dose response of the plants to IAA, in the form of a bell curve, shows that as the concentration of IAA exceeds a certain level, its effects decrease (Taiz and Zeiger, 2010). At lower concentrations, it can enhance plant growth (Patten and Glick, 2002) while the accumulation of IAA past its concentration range of action can inhibit root growth (Xie et al., 1996). In our study, we have shown that the bacterial isolates that produce low concentrations of IAA are the ones with the most beneficial effects on plant growth whereas a negative effect was noted at the highest IAA concentration (46.23µg/ml). The concentration of bacteria in the inoculate also seem to have an effect on the growth parameters. In order to optimize the positive effects of the inoculate, we used a concentration of 10<sup>5</sup>CFU/ ml. In a 2001 study on the effect of IAA-producing *pseudomonas* on *Arabidopsis thaliana*, favorable effects were observed at a lower concentration (10<sup>5</sup>CFU/ ml) while at higher concentration (10<sup>6</sup>CFU/ ml), the inoculation had undesirable effects (Persello-Cartieaux et al., 2001). A 2014 study also reported similar effects in the same plant (Suarez et al., 2014). Although the properties and characteristics of PGPRs are a determining factor in the improvement of the plant host's growth, health and yield, the genotypic and physiological properties of the host plant itself can determine the span and intensity of the effects of these PGPRs and the hormones they produce on the plant (Nehl et al., 1996, Persello-Cartieaux et al., 2003). In our study, we have noticed a stronger response to the IAA treatment in the V2 (Karim) variety compared to the V1 variety. The results of this study constitute a first step towards a better understanding and control over the use of PGPRs in the agricultural sector in Morocco. The proper selection of PGPRs and most adequate plant hosts can improve the quality and yield of different crops without the use of chemical input.

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