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Fertilizer-dependent efficiency of *Mesorhizobium* strain for improving growth, nutrient uptake and grain yield of durum wheat (*Triticum turgidium*L.) variety

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Abstract – The search for microorganisms that improve soil fertility and enhance plant nutrition has continued to attract attention due to the increasing cost of fertilizers and some of their negative environmental impacts. Acquisition of nutrients by plants is primarily dependent on root growth and bioavailability of nutrients in the rooting medium. Most of the beneficial bacteria enhance root growth, but their effectiveness could be influenced by the nutrient status around the roots. In this study we aimed to investigate the effects of plant-growth-promoting rhizobacteria (PGPR) on durum wheat variety. To achieve this goal, the inoculation of durum wheat seeds by *Mesorhizobium* strains carried out in order to assess the positive impact of PGPR on growth, nutrientuptake and grain yield under different conditions of N fertilization (at 0%, 25%, 50%, 75%, and 100% of recommended doses). Results of field trials revealed that the efficacy of these strains for improving growth and yield of wheat reduced with the increasing rates of N added to the soil. In most of the cases, significant negative linear correlations were recorded between percentage increases in growth and yield parameters of wheat caused by inoculation and increasing levels of applied N fertilizers. It is highly likely that under low fertilizer application, the inoculation of wheat durum seeds by *Mesorhizobium* strain might have caused reduction in the synthesis of stress (nutrient)-induced inhibitory levels of ethylene in the roots through ACC hydrolysis into NH_3. The results showed also a synergetic effect of *Mesorhizobium* strain on nutrient uptake in wheat plant. The results of this study imply that these *Mesorhizobium* strains could be employed in combination with appropriate doses of fertilizers for better plant growth, better nutrient uptake and savings of fertilizers. The PGPR-based inoculants can be used as components of integrated nutrient management strategies.

Keywords: durum wheat, *Mesorhizobium*, PGPR, nutrient uptake, Nitrogen

1. Introduction

Durum wheat (*Triticum turgidum* L.var.durum, 2n=4x = 28; AABB), is the most extensively cultivated cereal in the Mediterranean area (Araus et al., 2003). It originated in the Fertile Crescent, and more particularly in the mountains regions that surround the fertile alluvial plains of the Tigris and Euphrates rivers (Braidwood, 1969). Durum wheat is the most produced crops, easily stored and transported, and an important nutritional source for humans (Arregui and Quemada, 2008). In Tunisia, wheat accounts for 60% of national cereal production and high yielding varieties need large and regulate supply of N to develop high photosynthetic capacity and maintain the proper nitrogen concentration in the leaves when N large rates are required for ear growth and grain filling period (Ayadi et al., 2012). In semi arid regions, fertilizer management is a key to successful durum wheat production. In Tunisia, nitrogen is the major limiting nutriment of durum wheat production because it affects plant growth, development and also grain yield. Several reports showed that N application increased significantly grain yield of wheat, number of fertile tiller per unit area, number of grain per spike and harvest index (Mandic et al., 2015). Dencic et al. (2011) and Flores et al. (2012) recorded increased grain yield and its components with increase in nitrogen level. The concern with having enough food with good quality has become widespread in the world. Therefore, healthy agriculture systems for food production without using agriculturalchemicals have become the main interest of the scientists to minimize the harmful effectsof intensive farming of fields (Turan, Ataoglu, and Sahin 2006; Savci 2012). It is admitted that over use of fertilizers can cause unanticipated environmental impacts (Kohler et al. 2009). Several studies reported that the biggest issue facing the use of chemical fertilizers is groundwater contamination (Yu et al. 2010;

Yan et al. 2000; Sun et al 2012). Nitrogen fertilizers break down into nitrates and travel easily through the soil. Because it is water-soluble and can remain in groundwater for decades, the addition of more nitrogen over the years has an accumulative effect. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth promoting rhizobacteria (PGPR). These rhizobacteria significantly affect plant growth not only by increasing nutrient cycling, also by suppressing pathogens by producing antibiotics and siderophores or by bacterial and fungal antagonistic substances and / or by other plant hormones. A divers array of bacteria including Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsilla, Entrobacter and Serratia have been shown to promote plant growth (Khalid et al., 2004). PGPR plays an important role in enhancing plant growth through a wide variety of mechanisms. The mode of action of PGPR that promotes plant growth includes (i) abiotic stress tolerance in plants;(ii) nutrient fixation for easy uptake by plant (iii) plant growth regulators; (iv) the production of siderophores; (v) the production of volatile organic compounds; and (vi) the production of protectio lytic-enzyme such as chitinase, glucanase and ACC-deaminase for the prevention of plant diseases (Choudhary et al. 2011; Garcia-Fraile et al. 2015). Chemical fertilizers often have low use efficiency, meaning that only a portion of the applied nutrients are taken up by plants. For example, P is precipitated after addition to soil, thus becoming less available to plants (Gyaneshwar et al. 2002). In addition, applied N can be lost through nitrate leaching, resulting in contamination of groundwater. Microbial inoculants have shown some promise in increasing nutrient availability. For example, previous reports have suggested positive impacts of microbes on N uptake involving non-legume biological fixation (Aseri et al. 2008). Also, inoculation with some microbes, including *Rhizobium,* resulted in P solubilization or enhanced plant uptake of fixed soil P and applied phosphate resulting in higher crop yield (Hemissi et al 2015). The main mechanism resulting in increased availability of inorganic P appears to be through the action of organic acids synthesized by inoculants (Hemissi et al 2015). The use of PGPR for improving plant nutrition and thus partially compensating the need of chemical fertilizers is becoming a popular strategy for sustainable agriculture (Karlidag et al. 2007). Nevertheless, there is a great deal of inconsistent information on the effectiveness of PGPR (such as *Azospirillum* species) on plant growth in soil amended with various levels of fertilizers (Lucy et al.2004).The objectives in this study were to determine (1) ifreduced rates of inorganic fertilizer (N) coupled with microbialinoculants (*Mesorhizobium*strain) will produce plantgrowth, yield, and nutrient uptake levels (N, P and k) equivalent to thosewith full rates of the fertilizer and (2) the minimum level towhich fertilizer could be reduced when inoculants wereused. To achieve these objectives, experiments weredesigned using *Mesorhizobium* strain coupled with differentfertilizer regimes. The*Mesorhizobium* strain studied was previously reported to elicit significant effects on root development, plant growth, biocontrol, and/or induced systemic resistance (Hemissi et al. 2011, 2015).

2. Materials and Methods

2.1. Bacterial strain and growth conditions

Mesorhizobium strain Pch Beja was obtained from INRAT (Hemissi et al., 2013, 2015). This PGPR possess ACCdeaminase activity, auxins production, phosphorus solubilization and antifungal activity. This strain was grown at 28°C on yeast extract mannitol (YEM) medium containing 0.08% of yeast extract (w/v) and 1% of mannitol (w/v). The bacterial isolate derived from single colonies.

2.2. Production of bacterial inoculum

Bacterial inoculum was prepared in 250 ml flasks containing YEM. After inoculation with bacteria, the flasks were incubated on a rotator shaker at 120 rpm at 28°C for 72 h. Before use, bacterial concentration was adjusted to 10^8cells ml^{-1} (OD $_{620}$ 0.8-0.9).100 ml of bacterial inoculum was added to each elementary plot.

2.3. Field trials

Field trials were conducted at El Marja station which belongs to theNational Institute of the field Crop (INGC) in northern Tunisia at late October to early julyduring the cropping season 2015–2016., the annual mean rainfall is about 560 mm with amedian air temperature of 19°C; the soil is a vertisol with an average content of available. P and total N of 32 and 2.77mg Kg^{-1}. The durum wheat variety "Karim" and a *Mesorhizobium* strain previously tested in greenhouse were studied under field conditions. The field experiment consisted of 8 different treatments (Table1). The experiment was repeated 4 times in completely random block design. The surface of an experimental plot is 500 m^2 devised into 40 elementary plots of 8 m^2separated by 2 m. Seeds were hand sown on plots. Seed sowing density was

150 kg ha^{-1}. At emergence of seedlings stage, the number of seedlings emerging per m^2 was evaluated using a 1/2 m^2 iron ring. Plants in plots were harvested 220 days after sowing. Yield parameters evaluated were: grain yield (kg ha^{-1}), 1000-seed weight, number of spikes per m^2, and number of grains per spike. These parameters were determined after creating clearances of 1 m at the edges of each plot, and 2 sowing lines at each side. Nitrogen, Phosphorus, and Potassium contents in grain and straw samples were determined using the procedure described by Ryan et al. (2001).

Table 1. The ten treatments conducted under field conditions.

T1:	unfertized soil (control plants)
T2	N fertilizer was applied to soil at 25% of the recommended doses for durum wheat
T3	N fertilizer was applied to soil at 50% of the recommended doses for durum wheat
T4	N fertilizer was applied to soil at 75% of the recommended doses for durum wheat
T5	N fertilizer was applied to soil at 100% of the recommended dose for durum wheat
T6	unfertilized soil and plant inoculated with *Mesorhizobium* strain (100 ml of inoculants/ elementary plot)
T7	Plant inoculated with *Mesorhizobium* strain (100 ml of inoculants/ elementary plot) plus 25% dose fertilized
T8	Plant inoculated with *Mesorhizobium* strain (100 ml of inoculants/ elementary plot) plus 50% dose fertilized
T9	Plant inoculated with *Mesorhizobium* strain (100 ml of inoculants/ elementary plot) plus 75% dose fertilized.
T10	Plant inoculated with *Mesorhizobium* strain (100 ml of inoculants/ elementary plot) plus 100 % dose fertilized.

2.4. Statistical Analysis

The experimental design was a randomized complete block. Statistical analyses were performed by SPSS 20.0 software (SPSS, Chicago, IL, USA). The data were analysed using ANOVAs and subsequent comparison ofmeanswas performed using the Fisher's LSD test at 5% probability.

3. Results and Discussion

Developing crop cultivars with high grain yield has been the principal aim of durum wheat breeding programs worldwide. In this context, Garcia el Moral (2003) has mentioned that in the Mediterranean region, it is of special interest because of the low and erratic distribution of rainfall, which has explained as much as 75% of the variation in wheat yield (Blum and Pnuel, 1990). Under semi arid region, an optimal nutrient provision is an important factor to get high yield. Nitrogen is one of the most important elements of plant nutrition; It is also one of the most mobile plant nutrients in the soil. Today, Fertilizers are essential components of modern agriculture because they provide essential plant nutrients. However, overuse of fertilizers can cause unanticipated environmental impacts. In fact, applied N can be lost through nitrate leaching (Huang et al., 2018), resulting in contamination of groundwater. One potential way to decrease negative environmental impacts resulting from continued use of chemical fertilizers is inoculation with plant growth promoting rhizobacteria (PGPR). These bacteria exert beneficial effects on plant growth and development (Bakker et al. 2007). Recent studies have shown that some PGPR can be a great alternative to agrochemicals for increasing plant development (Sharma, 2013; Gupta, 2015) by increasing nutrient availability. The effectiveness of the bacterial inoculum is related to the characterization of multiple PGPR activities such as the production indole acetic acid (IAA), hydrogen cyanide (HCN), siderophores, of phosphate solubilization, as well as the antifungal activity. For example, previous reports have suggested positive impacts of microbes on N uptake involving non-legume biological fixation (Aseri et al. 2008). Therefore, this study was conducted to investigate the efficacy of *Mesorhizobium* strain Bj to promote root and shoot growth of wheat grown and to optimize nutrients uptake of wheat in soil amended with varied levels of N fertilizers. The results presented in this study support the hypothesis that PGPR can improve the nutrient use efficiency of fertilizers and the wheat growth. The performance of the plants was better in inoculated treatments in comparison to the control (Table 2).

Table2. Effect of inoculation with *Mesorhizobium* strain on fresh biomass and 1000 grain of wheat at different level of N fertilizers in field trial (Average of four replicates)

Treatments	Fresh biomass (t ha^{-1})		1000 grain weight (g)	
N (kg ha^{-1})	Uninoculated	*Mesorhizobium* strain	Uninoculated	*Mesorhizobium* strain
T0 (control)	5.3 ± 0.58	6.9 ± 0.33	35.9 ± 1.41	40.9±0.70
25% of N fertilizer	6.4 ± 0.53*	8.3 ± 0.15*	46.3 ± 0.43*	49.4±2.30*
50% of N fertilizer	8.8 ± 0.23*	12.5 ±0.44*	48.7 ± 1.65*	52.9±0.92*
75% of N fertilizer	12.7± 0.92*	13.8 ±0.14*	50.7 ± 0.44*	54.8±0.71*
100% of N fertilizer	13.5± 0.28*	14.6 ±0.06*	55.1 ± 0.97*	55.0±0.42*

*Significantly different from the control at P < 0.05

However, the efficiency of the inoculation decreased with increasing fertilizers doses (Table2). Under unfertlized conditions, *Mesorhizobium* strain was relatively more efficient, as it increased biomass by 25% over respective uninoculated control. Under fertilized conditions, *Mesorhizobium* strain was relatively effective as it caused 23%, 30%, 8% and 8 % increase in biomass over respective uninoculated controls in the presence of 25%, 50%, 75% and 100% of fertilizer doses, respectively. When the percentage of recommended fertilizer was reduced and inoculants were used, plant height, shoot dry weight, root dry weight, yield, and nutrient uptake were comparable to those with the full rate of fertilizer without inoculants (Table 2, 3 and 4). Results also show that 100% fertilizer produced plant growth that was greater than all other lower rates if inoculants were not added (Table 2). This agrees with Biswas et al. (2000)] who suggested an interdependence of fertilizer N inputs and inoculants for optimal gain in rice productivity.Furthermore, the PGPR used in this study possess ACC deaminase activity, auxins production, phosphorus solubilization and antifungal activity. Importantly, auxin production and ACC deaminase activity of rhizobacteria are considered as one of the main plant growth-promoting attributes (Belimov et al. 2009; Pilet and Saugy 1987). Lowering of ethylene (Saleem et al. 2007) levels in plants through the synthesis of the enzyme 1-amino-cyclopropane1-carboxylate (ACC) deaminase that hydrolyzes the ethylene precursor ACC is another well-reported mechanism for growth promotion by PGPR (Glick et al. 2007; Shaharoona et al. 2007).On the other hand, after testing different reduced fertilizer rates, under these experimental conditions, 75% fertilizer was the stable minimum to which fertilizer could be reduced if supplemented with PGPR to achieve growth equivalent to 100% fertilizer without PGPR. Results also show that 100% fertilizer produced plant growth that was greater than all other lower rates if inoculants were not added (Table 2). Similarly, 1000 grain weight was significantly increased in response to inoculation with *Mesorhizobium* strain, but with decreased efficacy at increasing fertilizer doses (Table 2). Under unfertilized conditions, once again, *Mesorhizobium* strain performed better and caused 23% increase in one thousand kernels weight over respective uninoculated control.Grain yield was also significantly increased in response to inoculation with both the *Mesorhizobium* strain caused maximum increase in grain yield (25%) over respective uninoculated control (Table 3). Concerning, the concentration of macronutrients in wheat grain which presented in Table 4, data showed that inoculation with *Mesorhizobium* strain caused significant increase in the concentration of N, P and K elements as compared with the control plant. These findings are in agreement with those of Shaharoona et al (2008) who found that pre-inoculation with *Pseudomonas* led to significant improve in growth, yield and nutrient uptake of wheat, although the efficacy of these strains for improving growth and yield of wheat reduced with the increasing rates of NPK added to the soil. Under nutrient-deficient conditions, an efficient root system is particularly very critical for sustaining overall plant growth. Therefore, increased root growth in response to inoculation proved more beneficial for improving growth and yield of plants under low-nutrient conditions than the effectiveness of inoculation under nutrient rich matrices. Many researchers have reported that inoculation with rhizobacteria-containing ACC-deaminase decreased the negative impact of stresses-induced ethylene (Burd et al. 1998; Mayak et al. 2004a; Grichko and Glick 2001; Saleem et al. 2007). Previously, we have also reported that inoculation with *Mesorhizobium* containing ACC-deaminase promoted growth and yield of chickpea (Hemissi et al. 2013; 2015). Results of this study revealed that *Mesorhizobium* strain were effective in increasing biomass, one thousand grain weight and grain yield in field trial, but their efficacy decreased with increasing levels of N. Moreover, the results of this study imply that excessive use of expensive chemical fertilizer could be avoided to a significant extent without compromising the crop yields by employing these *Mesorhizobium* strain. Additionally, the use of PGPR with lower fertilizer application is also an environment-friendly strategy. Although inoculation was effective at all the N fertilizer levels, its positive impact decreased with increasing rates of fertilizer application. It could be concluded that PGPR technology should be employed with appropriate doses of fertilizers to get maximum benefit in terms of fertilizer savings and better growth.

Table3. Effect of inoculation with *Mesorhizobium* strain on grain yield of wheat at different level of N fertilizers in field trial (Average of four replicates)

Treatments	Grain yield (t ha^{-1})	
N (kg ha^{-1})	Uninoculated	*Mesorhizobium* strain
T0 (control)	2.3 ± 0.58	3.1 ± 0.33
25% N fertilizer	2.8 ± 0.53	3.8 ± 0.15
50% N fertilizer	3.5 ± 0.23*	5.5 ±0.44*
75% N fertilizer	4.7± 0.92*	5.7±0.14*
100% N fertilizer	5.5± 0.28*	5.8±0.06*

*Significantly different from the control at P <0.05

Table 4. Effect of inoculation with *Mesorhizobium* strain on macronutrient concentration (%) ingrain of wheat plant at different level of N fertilizers in field trial (Average of four replicates)

9	N (%)		P (%)		K (%)	
N (kg ha^{-1})	Uninoculated	*Mesorhizobium* strain	Uninoculated	*Mesorhizobium* strain	Uninoculated	*Mesorhizobium* strain
T0 (control)	1.32	1.52	0.032	0.041	0.055	0.068
25% N	1.62	1.67	0.049	0.059*	0.063	0.071
50% N	1.71	1.82*	0.041	0.064*	0.067*	0.078*
75% N	2.01	2.30*	0.051*	0.065*	0.074*	0.089*
100% N	2.41	2.88*	0.055*	0.069*	0.073*	0.092*

*Significantly different from the control at P <0.05

4. Conclusion

Our results showed that pre-inoculation with *Mesorhizobiu m* strain Bj led to significant improve in growth, yield and nutrient uptake of wheat. The productive efficiency of a specific PGPR may be further enhanced with the optimization and acclimatization according to the prevailing soil conditions. In future, they are expected to replace the chemical fertilizers and artificial growth regulators which have numerous side-effects to sustainable agriculture. Further research and understanding of mechanisms of PGPR mediated-phyto-stimulation would pave the way to find out more competent rhizobacterial strains which may work under diverse agro-ecological conditions

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