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Effect of Superior IAA Producing Rhizobia on N, P, K Uptake by Wheat Grown under Greenhouse Condition

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Abstract: A greenhouse study was conducted to determine the effects of the superior indole-3-acetic acid (IAA) producing Rhizobial and treatments of Ag (Ag^+ ion) and L-tryptophan (Trp) on uptake of N, P and K by wheat. Five strains with different ability of IAA production (belonging to genus *Rhizobium leguminosarum var. phaseoli* and *Rhizobium leguminosarum var. viciae*) and a non-inoculated control; two levels of L-tryptophan (Trp) (0 and 0.1 g/kg soil), three levels of Ag^+ ion (0, 10 and 100 µM) were used. Plants were grown for 90 days and plant root dry weight and the uptake of N, P and K were determined. Plants inoculated with the rhizobia together Ag^+ ion and L-tryptophan (Trp) gave the highest root dry weight and the uptake of N, P and K compared to non-inoculated control plants. The experiment indicated that rhizobia could used as bioenhancer and biofertilizer for wheat production and usage of both Ag and L-tryptophan (Trp) treatments together result in a significant increase on the uptake of N, P and K in comparison with using Ag^+ ion and Trp alone and also in comparison with the blank. Therefore, the rhizobia could uptake more nutrients (N, P and K) by producing IAA and subsequently the increase of plant root system.

Key words: Rhizobacteria · PGPR · Plant Growth Hormone (IAA) · wheat · NPK fertilizers

INTRODUCTION

In 1880 Charles Darwin proposed that some plant growth responses are regulated by 'a matter which transmits its effects from one part of the plant to another' [1]. Several decades later, this 'matter,' termed auxin (from the Greek 'auxein' which means 'to grow'), was identified as indole-3-acetic acid (IAA) [1,2]. IAA has since been implicated in virtually all aspects of plant growth and development [3-5]. It was later found that not only plants but also microorganisms including bacteria and fungi are able to synthesize IAA [1,6,7]. Now, it's completely proved that we can find strains among many more strains of each rhizobial group that can also do effective process in plant growth promoting as plant growth hormones production, in addition to their ability in N_2 fixation. Such strains can be useful not only for their special host, but also for other plants. Rhizobial bacteria are the best plant growth promotion among Rhizobactia [4-6,8,9]. The bacteria increase plant growth and yield by various methods [1]. Some PGPR strains, for example, able to enhance the growth and development of plants by interfering in the concentration of known phytohormones [9]. One of the most important ways that those bacteria affect growth and development is by producing Indole-3-acetic acid (IAA) that this hormone is led to plat root system development and subsequently uptake increase of nutrients by plant [1,10,11]. Many of rhizobial species enable to produce IAA [1]. In order to produce Indole-3-acetic acid (IAA), the bacteria use L-tryptophan (Trp) as precursor [12]. This substance can be converted to IAA by soil beneficial bacterial activities. An increased concentration of IAA can promote the production of 1-aminocyclopropane-1-carboxylate (ACC), precursor of ethylene production and result in inhibition of seed germination and root growth [13-15]. There is evidence that treatment with aminoethoxyvinyl-glycine (AVG) and silver (Ag^+ ions, inhibitors of ethylene biosynthesis, prevent ethylene inhibition of root elongation [14]. Application of ethylene inhibitors can also decrease the negative of stress symptoms in plants [13,15,16]. Based on the assumption that rhizobia containing IAA can play an important role in the processes of plant growth and an excess of IAA can also promote the production of hormone ethylene, the aim of present paper was to Assess the superior strains inoculums application effects on uptake of N, P and K by wheat that was completely essential to study.

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MATERIALS AND METHODS

Bacteria Inoculum: In this study the number of 5 rhizobial bacteria isolate (Table 1), belonging to genera *Rhizobium leguminosarum var. phaseoli* and *Rhizobium leguminosarum var. viciae* with different ability of IAA production, was selected and used as PGPR Rhizobial treatments in this study. To conduct each text, at first, fresh media inoculum for any bacterial isolate was provided as follows:

100-ml-Glassy Erlens were selected and prepared. The amount of 15 ml Yeast extract Mannitol Broth (YMB) media was added to any Erlen. Erlens were sterilized by autoclaving at 121 C º for 15 minutes. After cooling Erlens, broth media within each Erlen was inoculated by one loop of Rhizobial bacteria inoculum and medias were incubated and aerated for 72 hours (for bacteria with a fast growth) to 120 hours (for bacteria with a low growth) at 28°C on a shaker (~120 rev min $^{-1}$). After enough growth of bacteria within YMB media, at first, the optic density (OD) of Rhizobial suspensions was read using spectrophotometer (Model: Unico 1100, USA) at 570 nm. Then using growth curve (CFU – OD) and on the basis of Dilution Factor and by addition of the essential mount of sterilized distill water, the population of bacteria in all Rhizobial suspensions was regulated at about 2.4 * 10^9 cfu ml^{1-}.

Plant Growth Condition: The enough number of seeds of wheat (variety pishtaz) were separated and disinfected with 95% ethanol within 30 seconds and then washed by sterilized distill water at least 8 times and germinated within Petri dishes, containing distill water and the solution of 10 and 100 µl silver sulfate, at 28°C. After reaching the length of all roots to 0.5 cm, they were transferred into pots.

Owing to the need to soil with very low bacterial population and with low NPK percent in this stage, of a poor soil, located in around Karaj, also remained as fallow land in several years, was sampled. And samples were poured into nylon bags and transferred to laboratory. After soil samples were air-dried and sieved (4-mm), they were uniformly mixed together and then selected a soil sample and were tested of some soil chemical and physical properties. Soil chemical and physical properties were measured by common methods in soil laboratory. Plastic pots with 30 cm height and 25 cm spout diameter were used in this test. After washing pots with 2% sodium chlorite hypos for 15 min and disinfecting, they well scrubbed. Each empty pot weighs 50 g. After putting drainage in bottom of pots and pouring the amount of 200 g of mixture of sand (2mm) and Ao perlit to each pot, weight of any pot exactly reached 300 g. Inoculum was provided from the superior strains selected in Y.M.B media. In each pot the number of 5 seedlings (in the depth of 2-cm) was sowed in an equal distance and any seedlings was inoculated with 1 ml of inoculum. After appearing seedlings of soil, the number of wheat seedling has been reduced to 3 seedlings. Then 0.1 g of L-tryptophan (Trp) per kg soil after being solving in water was added to any pot. Pots were maintained in growth chamber (with light intensity of 1000 lux and maximal temperate of 27-28°C and minimal temperature of 18-19°C for 3 months. All pots by weight were irrigated with distill water every day. The content of nitrogen applied in all pots (including positive control pots) was 200 kg/ha urea that was solubly added to soil of pots along with irrigation water during 3 periods (time of sowing, the end of one and two months). For supplying the need of potassium and phosphor of plants, 150 kg/ha k as K_2SO_4 and the same content of P as super phosphate were applied. This content of K and P fertilizers was added to pots during two periods along with irrigation water (time of sowing and the end of one month). Also in a period 200 ml of Hoagland solutions was added to soil of pots to obviate the plant need to micronutrients. At 100 days of growth plants was harvested.

Table 1: The characteristics of the used bacteria in the greenhouse study

Class I		Class II		Class III	
(very high production)		(high production)		(moderate production)	
HD/CD >3		HD/CD =2.5-3		HD/CD= 2-2.5	
R1	297Rlp	R2	284Rlp	R4	336Rlv
		R3	254Rlp	R5	494Rlv

Rlp: *Rhizobium leguminosarum var. phaseoli*

Rlv: *Rhizobium leguminosarum var. viciae*

Experimental Design: This test was performed by factorial tests in a randomized complete block design with four replications on a non-sterilized soil with sandy texture, neutral $_P$H and low available content of NPK. Test treatments were including: (i) 5 levels of Rhizobial strains (R_1, R_2, R_3, R_4 and R_5) + a non bacterium control, (ii) 3 levels of Ag^+ ion (Ag10 µM, Ag100 µM and Ag_0), (iii) 2 levels of L-tryptophan (L-Trp) (L $_{1}$= 0.1 g/kg and $L_{0=0.0}$ g/kg).

Measurements: The effects of different treatments on root dry weight were measured and registered. In addition, the above ground organs of plant were analyzed and the concentration of N, P and K in them was separately measured. The plant parts were dried in the oven at 70°C till a constant weight reached and the dry weights measured. The dried samples were ground using mechanical grinder with 0.5 mm sieve and digested by concentrated sulphuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) using Block Digestion following the Kjeldhal method. N, P and K were determined using an Auto analyzer (AA). Data were analyzed by analysis of variation using of the statistical analysis system (SAS, version 6.12, 1989). Mean separation showed by Duncan range test (P=0.05).

RESULTS AND DISCUSSION

Plant Growth: Results showed that there was a significant response of rhiziobia incubation, Ag^+ ion, Trp and interaction of three factors on root dry weigh. The treatments improved the growth parameters compared to the non-inoculated control plants (Table 2). inoculated plants with Ag (100µM) and Trp (0.1g/kg) produced significantly (p<0.05) higher growth parameters when compared with inoculated plants with Ag (10 µM) and Trp (0.0g/kg). *Rhizobium leguminosarum var. phaseoli* (R_1) inoculated with Ag (100 µM) and Trp (0.1g) produced the highest plant root weight, which show that Ag has increased the production of stress ethylene [6,11,13,14]. The inoculation process also enhanced plant growth of the wheat which could be related to enhancement of root growth and higher nutrient uptake. Previous studies showed that the proper use of Ag and L-tryptophan (Trp) simultaneously in combination with PGPR produced significantly higher plant growth than those from L-tryptophan (Trp) alone under field condition [5,1,10,17]. Rhizobial inoculation and TRP without Ag significantly (p<0.05) increased the root wight compared to the control (Table 2), which show that in some rhizobia the production ability of IAA was low and could also be the cause of production of the enzyme ACC deaminase that in the last few years, it has been found in a number of plant growth-promoting bacteria [13,18-20]. In general, rhiobial inoculation improved the root weight compared to non-inoculated control, with R1 inoculation producing the highest root yield. Application 100 µM of Ag generally increased yield compared to 10 µM of Ag and non-inoculated treatments. Control plant without Ag produced the lowest root weight. Highest yield was observed in plants inoculated with R1 and R2 together with Ag and L-tryptophan (Trp). Khalid *et al*. [10] found that, inoculated wheat with PGPR and L-tryptophan (Trp), produced higher root yield and plant growth of wheat

Table 2: The effect of rhizobial inoculation, Ag and Trp on root weight and wheat shoot nutrient concentration

Treatment				Nutrient concentration (%)		
Rhizobial isolates	Trp (g/kg)	Ag (µM)	root dry weight (g)	N	P	K
R0(control)	0	0	0.56 d	1.6 d	0.119 l	2.5 f
	0.1	10	0.66 c	2 g	0.147 hi	3.07 e
		100	0.68 c	2 fg	.015 h	3.07 e
R1	0	0	0.87 b	2.5 cd	0.185 fg	3.58 cd
	0.1	10	0.91 b	3.1 b	0.27b bc	4.72 b
		100	1.08 a	4.02 a	0.325 a	5.54 a
R2	0	0	0.74 c	2.8 cd	0.182 fg	3.56 cd
	0.1	10	0.9 b	3.1 b	0.244 cd	4.45 b
		100	1.07 a	4.02 a	0.325 a	5.54 a
R3	0	0	0.73 c	2.4 cde	0.181 fg	3.51 cd
	0.1	10	0.9 b	3.1 b	0.237 de	4.39 b
		100	1.07 a	4.0 a	0.323 a	5.52 a
R4	0	0	0.73 c	2.3 def	0.173 gh	3.16 de
	0.1	10	0.88 b	2.7 c	0.212 ef	3.86 c
		100	0.94 b	3.4 b	0.275 b	4.77 b
R5	0	0	0.67 c	2.1 efg	0.16 gh	3.07 e
	0.1	10	0.87 b	2.7 c	0.211 ef	3.86 c
		100	0.94 b	3.3 b	0.272 bc	4.72 b
Significant due			*	*	*	*
to rhizobia Ag			*	*	*	*
Rhizobia * Ag			*	*	*	*
Rhizobia * Trp			*	*	*	*
Rhizobia*Ag*Trp			*	*	*	NS

*: significant difference at (p<0.05) and NS: Non significant. Means in columns followed with same letter(s) are not significantly different (p>.05)

plants than non-inoculated without of Trp. The benefit effects could probably be due to production of plant growth promoting substance such as IAA and other metabolic activates by bacteria. Plant growth promoting substance (IAA) play an important role in root elongation and shoot growth. This suggests that IAA may be partly responsible for increase in yield [1,5,12,20,21].

Nutrient Concentration in Plant Biomass: There was a significant effect of rhiziobia, Ag and Trp on N, P and K concentrations in plant biomass. Wheat shoot nutrient concentration increased at Ag (100, 10 µM) and Trp (0.1g) with all rhizobia compared to the control. Plant inoculated with R1 and Trp (0.1 g) and Ag (100) showed higher N, P and k concentrations compared to the control plants without Ag and Trp (Table 2). N, P and k concentrations showed higher with R1 and Ag application of 100 µM compared to 10 µM of Ag. The result show that shoots N, P and K concentration increased with application of Ag and Trp compared to the control. However, there is no interaction effect of R inoculation, Trp and Ag on K concentration of shoots (Table 2). Yasmin *et al*. [22] found that growth promoting effects of PGPR inoculation are mainly derived from morphological and physiological change of enhancement of nutrient concentration in plant inoculated plant [5,21,22]. Application of rhizobial together with the proper rate of Ag and Trp improved the root system [13,14,23-25]. The increase in yield could be due to the beneficial effects of the applied PGPR which stimulated root growth and enhanced nutrient uptake. There were different in the performance of the bacterial species on growth and yield under the conditions. In general, inoculation of wheat with beneficial bacteria has the potential to increase the yield of wheat and improve the higher plant growth.

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