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GROWTH PROMOTION OF ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM* LAM.) BY APPLICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA

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SUMMARY

The object of this study was to evaluate the possible PGPR effects of ryegrass inoculation with *Pseudomonas* sp. strains as well as its co-inoculation with *Rhizobium trifolii* on the yield and quality of Italian ryegrassm, with the aim to select effective strains as biofertilizer. The inoculation effects of PGPR on the yield of Italian Ryegrass cultivar K-29t were examined in pot experiment under greenhouse conditions. The experiment was designed with 3 inoculated treatments with 3 replications in completely randomised system. Inoculation of ryegrass with two *Pseudomonas* sp. strains LG and L1K alone as well as strain co-inoculation with strains L1K and *Rhizobium trfolii* 459 represented treatments which were compared with control uninoculated treatment-Ø. The response of plant to the inoculation and co-inoculation was positive in comparison to uninoculated plants. Results showed a significant positive influence of co-inoculation with strains *R. trifilii* 459 and *Pseudomonas* sp. LG as well as *Pseudomonas* sp. L1K alone on shoot yield, N and P contents of Italian ryegrass. Results indicated that *Pseudomonas* sp. strains L1K and LG alone and in co-inoculation with rhizobial strains can be investigated in further researches as potential agent of biofertilizer for plant growth promotion of Italian ryerass.

Key words: PGPR, *Italian ryegrass*, *Pseudomonas*, *Rhizobium trifolii*

INTRODUCTION

Italian ryegrass, *Lolium multiflorum* Lam. is high yielding forage grass, one of the best in Serbia. It is grown all over the world as key forage grass. Its leaf-rich yields in spring and autumn are highly valued as they contain high levels of digestible energy and sugar. Owing to these traits Italian ryegrass is among the most palatable and highly digestible grasses for livestock. It establishes quickly and produces a lot of forage in a short period of time. Ryegrass plant was selected as a test plant due to its good growth in different soil types, diseases and insects resistance, rapid emergence and ability for cutting more than once and finally for its high depletion of the nutrients from soil (Sharma et al., 2004; Simic et al. 2009). The major essential macronutrients for plant growth and development are nitrogen (N) and phosphorus (P).

Plant growth-promoting rhizobacteria (PGPR) represent bacteria that colonize the rhizosphere and plant roots and have ability to enhance plant growth by different mechanisms; i.e. increasing N uptake (biological N fixation-BNF), phytohormone production (auxin, cytokinin), minerals solubilisation, nutrients mobility, production of siderophores that chelate iron and make it available to the plant root, controlling pathogens and stress alleviation can enhance plant growth (Zahir et al., 2004; Glick et al. 2007; Jalili et al. 2009; Abbas-Zadeh et al. 2010; Antoun and Prévost 2005; Mehboob et al. 2009; Egamberdiyeva et al. 2004). Due to ability of plant growth promotion, PGPR appeared as a promising alternative for mineral and organic fertilization (Halil er al., 2011). Therefore, in recent years there has been a growing interest in using bacterial inoculants as biofertilizers. The effect of PGPR belongs to lot of bacterial genera including *Pseudomonas* and rhizobial bacteria.

Pseudomonas is a genus of Gram-negative aerobic gammaproteobacteria, belonging to the family *Pseudomonadaceae* containing 191 validly described species including human and plant pathogen as well as the plant growth promoting strains. *Pseudomonas* is one of the most widely reported PGPR and it has been intensively studied with regard to their potential application in industrial biotechnology (Minorsky, 2008; Hayat et al., 2010).

Rhizobium leguminosarum bv. *trifolii* is soil Gram-negative aerobic bacteria (fam. *Rhizobiaceae)* well known to induces and colonizes nodules elicited on roots of clover (*Trifolium* spp.) in the process of symbiotic N fixation. Rhizobium previously well known as a symbiotic N fixer is reported as asymbiotic PGPR (associative and endophytic) microorganism in recent years and it can also solubilise organic and inorganic phosphate (Chabot et al., 1996; Biswas et al., 2000a, b; Hilali et al., 2001).

The object of this study was to evaluate the possible effects of ryegrass inoculation with *Pseudomonas* strains as well as its co-inoculation with *Rhizobium trifolii* on the yield and quality of ryegrass with the aim to select effective strains as biofertilizer.

MATERIAL AND METHODS

Rhizobium trfolii strain 459 and two *Pseudomonas* sp. strains LG and L1K, from Collection of Institute of Soil Science were used for inoculation of Italian Ryegrass. The inoculation effects of PGPR on the yield of Italian Ryegrass cultivar K-29t were examined in pot experiment under greenhouse conditions. The pots were filled with 1.9 kg of non-sterile soil with following characteristics: pH (in H_2O) 6.9, NH_4-N 5.25 mg kg^{-1}, NO_3-N 5.5 mg kg^{-1}, P_2O_5 6.34 mg $100g^{-1}$, K_2O 8.25 mg $100g^{-1}$. The experiment was designed with 3 inoculated treatments with 3 replications in completely randomised system. Treatments were compared with one control treatment without inoculation-∅. *Rhizobium trfolii* strain in yeast manitol broth (YMB) *and Pseudomonas* sp. strains in King B medium were cultivated for 48 and 24h, respectively. Italian Ryegrass (*Lolium multiflorum* Lam.) seeds were surfaced-sterilized with 0.1% Hg Cl_2 solution (Vincent, 1970) and inoculated (2 ml $plant^{-1}$ of liquid culture) with *Pseudomonas* sp. single strains LG and L1K or with co-inoculated with LG and *Rhizobium trfolii* strain 459 in a ratio 1:1. Liquid culture of single strain contained $>10^9$ cells ml^{-1}. Ten seeds per plot were planted and after 2 weeks seedlings were thinned to 5 plants pot^{-1}. The pots were kept in greenhouse conditions for eight weeks in the first cut and for sixth weeks in the second cut.

Plant shoots were separated from roots and dried in an oven at 70 °C to constant weight and the average dry weight per plant was calculated. The percentage of shoot N was determined from dried and ground plant samples using the CNS analyser (Vario model EL III (ELEMENTAR Analysensysteme GmbH, Hanua, Germany) and it was used to calculate total N content in mg per pot. Plant P was determined by ammonium vandate-molybdate method after drying at 550EC and wet digestion. The data were statistically processed by the LSD and Duncan test using the statistical program SPSS 10.0. All references to significance in the text imply statistical significance at $P<0.05$, unless otherwise stated.

RESULTS

Influence of ryegrass inoculation with two *Pseudomonas* sp. strains (L1K and LG) and co-inoculation with strains LG and *R. trifolii* 459 was presented through plant performances (SDW, total N content and P content in plant shoot) in the first and second cuts of Italian ryegrass (Table 1). In the first cut ryegrass SDW was significantly higher in all inoculated treatments (3105-3554 mg pot^{-1}) as well as shoot N content (149-176 mg pot^{-1}) compared with the uninoculated control (2590 mg pot^{-1} and 127 mg pot^{-1}). The results indicated that co-inoculation and inoculation of ryegrass with applied PGPR strains positively influenced quantity of plant yield and also, shoot N content. Results showed that the greatest shoot P content was recorded in co-inoculation (11.63 mg pot^{-1}) and inoculation with strain L1K (11.46 mg pot^{-1}). The best shoot N and P content in co-inoculated plants highlighted significant PGPR potential of *R. trifilii* 459 applied in presented research. In addition, significantly greater shoot N content in plants inoculated with single strains in respect to control plants-∅ pointed at PGPR ability of strains L1K and LG.

In the second cut co-inoculation with LG and *R. trifoli* 459 and inoculation with L1K and LG alone showed their significant influence on SDW (290-331 mg pot^{-1}) and shoot P content (1.01-1.14 mg pot^{-1}) in comparison to control and there were no significant differences between inoculated treatments based on these parameters. However, based on shoot N content in this cut there were significant differences among treatments; significantly the highest total N content (about 16.4 mg pot^{-1}) had co-inoculated plants and plant inoculated with strain L1K alone.

The percentage of N was very high in the both cuts (4-6.5%) because shoot yield of ryegrass growing in the pots was consisted mainly of leaves.

Table 1. Average shoot dry weight, shoot N and P content from inoculated Italian ryegrass, Lolium multiflorum Lam. indicating PGPR of trains.
Tabela 1. Prosečne vrednosti težine suve nadzemne mase, sadržaja N i P u nadzemnoj masi italijanskog ljulja, Lolium multiflorum Lam. koje ukazuju na PGPR sojeva.

Treatments of PGPR	The first cut of Italian raygrass					The second cut of Italian raygrass				
	Shoot dry weight (mg)	Shoot N (%)	Shoot total N content (mg pot^{-1})	Shoot P (%)	Shoot total P content (mg pot^{-1})	Shoot dry weight (mg)	Shoot N (%)	Shoot total N content (mg pot^{-1})	Shoot P (%)	Shoot total P content (mg pot^{-1})
L1K	3183 a	4,669	149 b	0.36	11.46 a	331 a	4.96	16..44 a	0.34	1.13 a
LG	3105 a	4.836	151 b	0.27	8.39 b	290 a	5.02	14.61 ab	0.35	1.01 a
LG+Rhizobium	3554 a	4.980	176 a	0.33	11.63 a	300 a	5.49	16.47 a	0.38	1.14 a
Ø	2590 b	4.606	127 c	0.34	9.38 b	162 b	6.48	10.47 b	0.37	0.6 b
LSD 0.05	423.52		16.91		1.15	99.02		5.41		0.35

DISCUSSION

Our results indicated that effect of co-inoculation with strains LG and *R.* trifolii 459 was dominant in the first while in the subsequent cut effect of this co-inoculation was equalized with effect of *Pseudomonas* strains alone. It can be assumed that *R. trifolii* 459 reduced his influence on plant performances in second cut due to some unexpected abiotic factors. It is known that *R. trifolii* strains behave like PGPR since significant increase was observed in wheat SDW and grain yield (Hilali et al., 2001; Yanni et al., 1995). In presented pot experiment, performed in semi-controlled conditions, plant co-inoculation with LG and *R.* trifolii 459 increased SDW by 37% in the first cuts which is in agreement with results by Zahir et al. (2004) in more controlled conditions. According to some authors in field conditions it has been obtained significant SDW increase (7-8%) by inoculating maize, wheat and barley with *R. trifolii* (Höflich et al., 1994). In addition, co-inoculation in presented research increased shoot N and P content highly; by 39% and 24% in the first cut, respectively. The ability of rhizobia to solubilise both inorganic and organic phosphate has been the subject of many investigations. These bacteria may also have one or several of the characters attributed to PGPR, such as secondary metabolite production, siderophore, cyanide and antibiotic production. The rhizobia influence crop growth and development by changing the physiological status of inoculated roots which favours improved nutrient uptake (Afzal and Bano, 2008).

Pseudomonas spp. showed many plant growth promotion activities, among them the most important are: production of ACC deaminase, IAA like products as well as P solubilization. PGPR *Pseudomonas* spp. strains have been shown to colonize the roots of various plants and increase the height and plant yield (Minorsky, 2008; Zabihi et al., 2010). Presented results also confirmed that two *Pseudomonas* strains L1K and LG increased SDW in the first cut by 37 and 23 %, respectively while shoot N content by about 18%. These strains applied did not significantly differ in their ability to enhance plant yield. However, strain LG behaved differently in the cuts. In the first cut strain LG did not influenced P elevation in plant shoot but did elevation of shoot N content, while in the second cut there was inversed situation with these parameters suggesting further investigation to LG as PGPR of ryegrass.

The presented study showed a significant positive influence of co-inoculation with strains *R. trifilii* 459 and *Pseudomonas* sp. LG as well as *Pseudomonas* sp. L1K alone on shoot yield, N and P contents of Italian ryegrass. Results indicated that *Pseudomonas* sp. strains L1K and LG alone and in co-inoculation with rhizobial strains can be investigated in further researches as potential agent of biofertilizer for plant growth promotion of Italian ryerass.

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