Effect of Indigenous *Pseudomonas chlororaphis* Strains on Morphological and Main Chemical Growth Parameters of Basil (*Ocimum basilicum* L.)

Aleksandra Stanojković-Sebić^{1*} · Zoran Dinić¹ · Renata Ilić² · Radmila Pivić¹ · Dragana Jošić¹

¹Institute of Soil Science, Teodora Drazera 7, 11000 Belgrade, Serbia

²Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

Summary: This study examined the effect of two indigenous plant growth promoting rhizobacterial strains of *P. chlororaphis* (Q16 and B25) on morphological (plant height; root length; number of leaves, buds and lateral branches) and main chemical (contents of N, P, K, Ca, Mg) growth parameters of two basil cultivars (Italiano classico and SRX 1920). The experiment was conducted in pots under glasshouse conditions from March to July 2014. Phosphorus was determined by spectrophotometer, potassium by flame emission photometry, nitrogen by using elemental CNS analyzer Vario EL III, while calcium and magnesium were determined by AAS. The obtained results showed that the treatment of basil cultivars with both *P. chlororaphis* strains had positive effect on all studied growth basil parameters in relation to the control, whereby the strain B25 was more effective than Q16. Concluding, tested *P. chlororaphis* strains have high potential in promoting the morphological and main chemical growth parameters of basil.

Keywords: basil, chemical growth parameters, growth, morphological characteristics, PGPR, plant growth stimulants, pot experiments, *Pseudomonas chlororaphis*, strains

Introduction

Basil (*Ocimum basilicum* L.) is the most common species in Lamiaceae family. This family includes 3500 species distributed between the genders as herbaceous plants, sometimes shrubs, but rarely as trees, with a significant content of essential oils. *Ocimum* genus includes about 150 species (Pushpangadan & Bradu, 1995) and within the species *Ocimum basilicum* L. there are a number of varieties that differ in the general morphological structure and texture, and in the content of chemical parameters (Grayer et al., 1996). The basil plant (*Basilici herba*) is used in traditional and homeopathic medicine to treat a number of diseases. The essential oil of basil (*Basilici aetheroleum*) is used in the food, perfumery and cosmetics industry and has expressed bactericidal, fungicidal, antiviral, repellent, antioxidant, antidiarrhoeal, chemopreventive and radioprotective activity (Opalchenova & Obreshkova, 2003; Pascual-Villalobos & Ballesta-Acosta, 2003; Chiang et al., 2005; Božin et al., 2006; Gutierrez et al.,

2008; Lukmanul Hakkim et al., 2008; Gajula et al., 2009; Dambolena et al., 2010; Runyoro et al., 2010).

The useful parts of the plants are leaves and seeds. Hot tea of basil plant leaves is good for treating nausea, dysentery, and flatulence. Externally, basil formulations can be used for different skin infections such as treatment of acne, snake bites and insect stings. In addition to these, basil has been used as a remedy for an enormous number of ailments, including cancer, convulsion, deafness, diarrhea, epilepsy, insanity, sore throat, toothaches, and whooping cough (Khatri et al., 1995).

Many medicinal and aromatic plants, including basil, are typically consumed without further processing after harvest and it is important that chemicals are not present in or on any part of the plant (Banchio et al., 2008). Therefore it is essential to identify the growth measurements of basil grown avoiding the chemical fertilizers and pesticides and achieving optimal growth and yields. Nowadays, the use of biofertilizers in production plays an important role as a supplement to improve the growth and yield of several agricultural, horticultural and medicinal plants (Murthy et al., 1998; Lugtenberg & Kamilova, 2009). This biological approach is nevertheless considered to be more environment friendly in the long term.

One of the emerging research areas for improving the plant growth and yield, as well as for the control of different phytopathogenic agents, is the use of plant growth promoting rhizobacteria (PGPR). They are able to improve plant growth by increasing the rate of seed

Corresponding author:
astanojkovic@yahoo.com

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germination and seedling emergence, minimizing the adverse effects of external stress factors, and protecting plants from soil-borne pests and diseases. In this respect, different isolates of fluorescent *Pseudomonas* species take prominent place. Consequently, these isolates have been intensively studied (Couillerot et al., 2009; Ivanović et al., 2009; Jošić et al., 2012; Jošić et al., 2015; Zdravković et al., 2015; Mrkovački et al., 2016).

Fluorescent *Pseudomonas chlororaphis* belongs to PGPR because of the ability to colonize the plants roots and stimulate growth by decreasing the frequency of diseases (Couillerot et al., 2009). It uses various mechanisms such as production of antibiotics, HCN, plant hormones, asymbiotic fixation of N₂, antagonism towards phytopathogenic microorganisms and the ability to solubilize mineral phosphates and other nutrients (Cattelan et al., 1999).

Regarding the preceding comments, the aim of this study was to examine the effect of two indigenous *P. chlororaphis* strains (Q16 and B25) on morphological (plant height, root length, number of leaves, number of the lateral branches, number of buds) and main chemical (content of N, P, K, Ca and Mg) growth parameters of two basil (*Ocimum basilicum* L.) cultivars.

Materials and Methods

The bacterial strains *Pseudomonas chlororaphis* Q16 and B25 were isolated from different host plants in Serbia. Taxonomic characterization of the isolates was based on tests for Gram staining, cytochrome oxidase, catalase, fermentation/utilization of glucose, lactose and sucrose, utilization of citrate, ability to degrade urea and 16S rDNA analyses (Ivanović et al., 2009; Jošić et al., 2012; Jošić et al., 2015). The bacteria were grown in liquid King's B medium (KB) for 24 h on orbital shaker at 120 rpm. Concentrations were determined spectrophotometrically (OD₆₀₀) and all experiments were conducted using 10⁶ CFUml⁻¹.

Plant material and pot experiments

The effect of PGP rhizobacteria was studied on two basil (*Ocimum basilicum* L.) cultivars: Italiano classico and SRX 1920, in pot experiments under semi-controlled conditions in the glasshouse of the Institute of Soil Science (Belgrade). The experiment was conducted during the first week of March until the first week of July in 2014, in plastic pots with 1.4 kg of homogenized soil - Fluvisol (WRB 2014). Three experimental variants for both cultivars were set up in five replications: 1. Control (V1); 2. *P. chlororaphis* - strain Q16 (V2); 3. *P. chlororaphis* - strain B25 (V3). In every plastic pot five basil seeds were sown and after emergence two plants per pot were left. The inoculation of the soil and basil plants in each pot with 10 ml of liquid inoculum of *P. chlororaphis* strains Q16 and B25 was performed during the last week of March in the phase of rooting, and another treatment

during the first week of May, in the phase of tree growth. Control was treated with the same volume of distilled water as V2 and V3 treatment.

Soil preparation and analysis

The soil samples were air-dried, crushed and passed through a sieve (≤ 2 mm). Before setting up the experiment soil physical and chemical properties were studied. Soil granulometric composition was analyzed by determination of particle size distribution in mineral soil material, using the standardized method by sieving and sedimentation (ISO 11277:2009(E) 2009). Chemical soil properties were determined using the following chemical analyses: soil acidity (pH in H₂O and 1M KCl, v/v - soil:H₂O=1:5, soil:1M KCl=1:5) was analyzed potentiometrically, using glass electrode (SRPS ISO 10390:2007 2007); total N and C content were analyzed on elemental CNS analyzer Vario EL III (Nelson & Sommers, 1996), whereby the content of humus was calculated on the basis of C content according to Džamić et al. (1996), using the following formula: humus (%) = C (%) $\times$ 1.724 (factor for conversion of organic C content to humus); available P₂O₅ (P) and K₂O (K) were analyzed by Al-method according to Egner-Riehm (Riehm 1958), where K was determined by flame emission photometry and P by spectrophotometer after color development with ammonium molybdate and stannous chloride. The total content of CaCO₃ was determined using the "rapid titration method" by Piper (van Reeuwijk, 2002).

Preparation and analysis of the plant material

Basil seedlings were grown according to the standard growing methods until 2 July, when all studied relevant parameters of the plant growth were measured, namely: plant height, root length, number of leaves, number of buds (if there were any) and number of the lateral branches. The basil plants were dried at 105°C for a period of 2 hours, using gravimetric method for determination of dry matter content of plant tissue (Miller, 1998), and weighed. For all plant samples of all treatments and replicates the following main chemical growth parameters of the aerial plant parts were analyzed: nitrogen (N) was analyzed on elemental CNS analyzer Vario EL III (Nelson & Sommers 1996); phosphorus (P) and potassium (K) contents were determined by method named as "wet" combustion, i.e. they were heated to boiling with the mixture of concentrated acids: H₂SO₄ and HClO₄ (in the obtained solution, P was determined by spectrophotometer with molybdate, and K - by flame emission photometry) (Jakovljević et al., 1985); calcium (Ca) and magnesium (Mg) were determined by Atomic Absorption Spectrometry (AAS) (Wright & Stuczynski, 1996), where the plant material was converted to a solution by method named as "dry" combustion, i.e., first by heating at 550°C (for several hours) and then by treating the obtained ash with hydrochloric acid (Miller, 1998a).

Data analysis

The obtained data on soil properties represent the arithmetic means of three replicates and standard deviation values. The effects of V1, V2 and V3 treatments on the studied morphological and chemical parameters of the plants were evaluated using the analysis of variance (SPSS 20.0, Chicago, USA), followed by Duncan's Multiple Range Test (DMRT). Significant differences between means were tested by the LSD test at $P = 0.05$.

Results and Discussion*Soil properties*

Table 1 displays the results of soil physical and chemical properties. The studied Fluvisol is a sandy loam according to the soil textural class determined on the basis of particle size distribution, having alkaline reaction, low carbonates and medium humus content, with a high content of available phosphorus and medium provided with available potassium. It belongs to the third soil capability class, which includes the soils used for crop production, but with certain limitations. Improvement of the water, air and thermal regime, along with ensuring the available nutrients, high and stable yields of cultivated plants can be achieved on such soils (Škorić et al., 1985).

Morphological and chemical growth parameters of plants

Table 2 displays the data on morphological growth parameters of basil cultivars influenced by the treatment applied.

In general, certain effects of both strains on basil growth were noticed immediately after the second treatment while the plants were still in pots. The data on

studied morphological parameters at the end of experiment showed that the treatment of basil cultivars with both *P. chlororaphis* strains had positive effect on all parameters in relation to the control, whereby the B25 was more effective than Q16. The highest plants and number of leaves were obtained by applying B25 strain, but its influence was not statistically significant. Statistically significant positive effect of both strains was registered in root length, with better effect of B25 than Q16 (Table 2). The average values of V2 and V3 treatment were close to the control and did not influence the number of lateral branches. The buds were not observed. The promotion and increase of growth parameters of basil (plant growth and root length), when inoculated with PGPR strains, was also previously reported (Vinutha, 2005; Hemavathi et al., 2006).

Table 3 displays the data on chemical growth parameters (available macroelements) in basil cultivars influenced by the treatment applied.

The obtained results of N, P, K, Ca and Mg contents were in accordance with morphological parameters, meaning that the content of these elements was the highest in both basil cultivars treated with B25 strain. This influence was statistically significant regarding the content of N and P in both cultivars (Table 2). On the other hand, statistically significant positive effect of strain Q16 was registered only in N content in both basil cultivars (Table 2). This rationale is consistent with the observation that plants inoculated with PGPR take up N, P, K and microelements more efficiently from the soil (Cakmakci et al., 2005). Improved mineral nutrition would explain the promotion of root and plant growth. The increase in N, P and K content in *Ocimum basilicum* inoculated with

Table 1. Physical and chemical properties of Fluvisol

Granulometric composition	Value
Fraction (%)	
Bulky sand, 2-0.2 mm	0.30
Miniature sand, 0.2-0.02 mm	61.5
Dust, 0.02-0.002 mm	19.7
Clay, < 0.002 mm	18.5
Total sand, > 0.02 mm	61.8
Dust + clay, < 0.02 mm	38.2
Chemical properties	Value (means $\pm$ standard deviation)
pH in H ₂ O	7.89 $\pm$ 0.03
pH in 1M KCl	7.50 $\pm$ 0.03
Total content CaCO ₃ (%)	9.50 $\pm$ 0.05
Total N (%)	0.09 $\pm$ 0.01
Humus (%)	1.83 $\pm$ 0.02
Available P ₂ O ₅ (mg 100g ⁻¹)	22.06 $\pm$ 0.22
Available K ₂ O (mg 100g ⁻¹)	16.00 $\pm$ 0.83

Table 2. Morphological growth parameters of basil cultivars depending on the treatment

Variants	Plant height (cm)	Root length (cm)	Number of leaves	Number of lateral branches
Italiano classico				
V1 - control	11.52±3.48 ^a	6.41±0.63 ^a	11.00±2.12 ^a	4.80±0.76 ^a
V2 - strain Q16	13.69±1.58 ^a	7.51±0.39 ^b	11.60±0.55 ^a	4.90±0.22 ^a
V3 - strain B25	14.10±2.66 ^a	9.46±1.04 ^c	12.60±0.55 ^a	5.70±0.27 ^a
P value	NSD	***	NSD	NSD
LSD(0.05)	3.708	1.019	1.796	0.782
SRX 1920				
V1 - control	11.52±3.48 ^a	7.20±0.61 ^b	11.40±1.52 ^a	4.80±0.57 ^a
V2 - strain Q16	13.69±1.58 ^a	7.52±1.17 ^b	11.00±2.35 ^a	4.90±0.89 ^a
V3 - strain B25	14.10±2.66 ^a	9.45±1.45 ^a	12.00±0.71 ^a	5.20±0.57 ^a
P value	NSD	*	NSD	NSD
LSD(0.05)	3.708	1.559	2.292	0.763

LSD - least significant differences at P=0.05; *** - statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; NSD - statistically not significant; DMRT was used to compare different variants at P≤0.05, where values followed by the same letter in a column are not significantly different.

Table 3. Available macroelements in basil cultivars depending on the treatment

Variants	N (%)	P ₂ O ₅ (%)	K ₂ O (%)	Ca (%)	Mg (%)
Italiano classico					
V1-control	3.31±0.08 ^a	0.61±0.02 ^b	3.64±0.17 ^a	3.08±0.04 ^a	0.50±0.04 ^a
V2-strain Q16	3.54±0.11 ^b	0.61±0.04 ^b	3.72±0.07 ^a	3.18±0.19 ^a	0.55±0.06 ^a
V3-strain B25	4.22±0.12 ^c	0.70±0.04 ^a	3.82±0.09 ^a	3.24±0.10 ^a	0.57±0.06 ^a
P value	***	**	NSD	*	NSD
LSD(0.05)	0.15	0.05	0.18	0.15	0.07
SRX 1920					
V1-control	3.14±0.12 ^a	0.62±0.02 ^b	3.48±0.11 ^a	3.09±0.07 ^a	0.52±0.03 ^a
V2-strain Q16	3.42±0.06 ^b	0.65±0.03 ^b	3.59±0.09 ^a	3.18±0.14 ^a	0.54±0.04 ^a
V3-strain B25	4.52±0.09 ^c	0.88±0.09 ^a	3.60±0.02 ^a	3.27±0.15 ^a	0.55±0.05 ^a
P value	***	***	**	NSD	NSD
LSD(0.05)	0.09	0.08	0.84	0.17	0.05

LSD - least significant differences at P=0.05; *** - statistical significant differences at the P<0.05, P<0.01 and P<0.001 levels, respectively; NSD - statistically not significant; DMRT was used to compare different variants at P≤0.05, where values followed by the same letter in a column are not significantly different.

PGPR *Pseudomonas putida* and *Azotobacter chroococcum* was previously reported (Ordookhani et al., 2011).

In addition, as stated by the other authors (Zdravković et al., 2015), and as it was done in this study, it is suggested that bacterial suspension should be applied during the growth phase of plants when the treatment has the greatest impact, i.e. when enzyme activity is the strongest.

Conclusions

The data on the studied morphological and chemical growth parameters of basil cultivars showed that their treatment with both *P. chlororaphis* strains had positive effect on all parameters in relation to the control, although this influence was statistically significant only regarding the root length and the content of N and P in both cultivars, with better effect

of B25 strain than Q16 strain. Also, statistically significant positive effect of strain Q16 was registered in N content in both basil cultivars. In addition, studied *P. chlororaphis* strains have high potential in promoting the morphological and main chemical growth parameters of basil. Also, bacterial suspension should be applied during the plants growth phase when an enzyme activity is the strongest.

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Uticaj autohtonih sojeva *Pseudomonas chlororaphis* na morfološke i osnovne hemijske parametre rasta bosiljka (*Ocimum basilicum* L.)

Aleksandra Stanojković-Sebić · Zoran Dinić · Renata Ilić · Radmila Pivić · Dragana Jošić

Sažetak: Ispitivan je uticaj dva autohtona soja *Pseudomonas chlororaphis* (Q16 and B25) na morfološke (visina biljke, dužina korena, broj listova, broj pupoljaka i broj bočnih grana) i osnovne hemijske (sadržaj azota, fosfora, kalijuma, kalcijuma i magnezijuma) parametre rasta dve sorte bosiljka (*Ocimum basilicum* L.): Italiano classico i SRX 1920. Ogled je izveden u vegetacionim sudovima u periodu od marta do jula 2014. godine. Fosfor je određen spektrofotometrijski, kalijum plamenom emisionom fotometrijom, azot na elementarnom CNS analajzeru Vario EL III, dok su kalcijum i magnezijum određeni atomskom apsorpcionom spektrometrijom. Dobijeni rezultati su pokazali da je tretman testiranih sorti bosiljka sa oba soja *P. chlororaphis* imao pozitivan uticaj na sve ispitivane parametre rasta ove biljne vrste u odnosu na kontrolu, pri čemu je soj B25 bio efikasniji od Q16 soja. Može se zaključiti da testirani sojevi *P. chlororaphis* imaju veliki potencijal u promovisanju morfoloških i osnovnih hemijskih parametara rasta bosiljka.

Ključne reči: bosiljak, hemijski parametri rasta, morfološke karakteristike, PGPR, *Pseudomonas chlororaphis*, rast, stimulant rasta biljaka, sojevi, vegetacioni ogled

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