

ECOLOGICAL PERSPECTIVES IN PEA CULTIVATION

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Summary

The application of pre-sowing bacterization of leguminous plants is certainly one of the measures that can contribute to improving crop productivity and environmental sustainability by increasing soil fertility and reducing ecosystem damage. In this paper, we compare the efficiency of pre-sowing bacterization, biostimulators / soil conditioner application or their combination in relation to the control on garden peas of the variety Petit Provencal and Telephone. During this trial, influence on certain morphological parameters and yield of peas was recorded. The highest yield of 2.25 t ha⁻¹ has been found in the Telephone pea variety. Application of pre-sowing bacterization with *Rhizobium leguminosarum* 1001 or *Rhizobium pisum* strains as well and combination of *R. leguminosarum* 1001 with biostimulators / soil conditioner Humicraft showed the best results in peas yield. The applied inoculants showed perspective in pea cultivation for each measured trait as individual application or as the combination with biostimulator / soil conditioner showing a better result refer to non-inoculated variant, i.e. control. Inoculation with *Rhizobium* strains have positive effects on yield components of pea as an environmentally friendly way of growing in Herzegovina field's conditions.

Key words: *legumes, field trial, Rhizobium strains, ecology*

INTRODUCTION

Pre-sowing bacterization of pea seeds (*Pisum sativum* L.) with bacterial strains of *Rhizobium leguminosarum* can increase the yield using low fertilization rate and minimizing soil degradation (Ndakidemi *et al.*, 2006; Uher *et al.*, 2006; Rapčan *et al.*, 2017; Vasilj *et al.*, 2019). The symbiosis of *Rhizobium* spp. strains and legumes produces 50% of 175 million tons of N₂ per year in total biological fixation worldwide (Yadav & Verma, 2014). The nodules of the genus *Rhizobium* are present in the soil; however, their natural diversity and prevalence are dependable of various factors including crop types, crop rotation, soil properties, agricultural practice, extent and distribution of wild legumes (Sadowsky, 2005). Inoculation of legumes is recommended

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when the density of native population is very low or infectivity of strains decrease compared to alternative inoculants and / or is ineffective with some plant due to the nitrogen fixation (Hansen, 1994; Giller & Cadisch, 1995). Usually, populations of indigenous bacterial strains are very low if they are not compatible with a particular leguminous plant or in the stress environment due to soil pH reaction, osmotic stress, high temperatures, and presence of heavy metals harmful to nodule bacteria (Amarger, 1988; Catroux *et al.*, 2001). Ruisi *et al.* (2012) state that biological fixation can contribute to peas up to 50% higher yields and provide approximately 25 kg of N ha⁻¹ deposited in the soil for the next crop. According to Kellman *et al.* (2005) rhizobial strains including *Rhizobium leguminosarum* biovar *phaseoli* are widely used as inoculants to improve growth, symbiotic performance and grain yield of common bean under both glasshouse and field conditions. In addition to the use of inoculants in the production of leguminous plants, the use of biostimulators has been shown to be effective in increasing plant tolerance to various abiotic stresses, such as drought (Colla *et al.*, 2017), salinity (Matyjaszczyk, 2018) and thermal oscillations (Kocira *et al.*, 2018). Biostimulators applied to soil or plants contribute to plant health, quality and yield (Nardi *et al.*, 2018). Foliar application of amino acids has a positive effect on the yield of many plants, even their growth under stress conditions (Colla *et al.*, 2017). The area of Herzegovina has a long tradition in growing of all types of leguminous plants. However, the question arises how adaptable and responsive these plant species are in application of inoculants as ecologically acceptable alternatives during cultivation and how such measures could reflect on plant yield and other yield parameters. Long-term global climatic changes that we face from year to year (high temperatures, long drought periods, high input prices) require certain adaptations and innovations in agriculture production, which we will try to present through this research. In this paper, the aim is to determine the effectiveness of different inoculants and its adaptability in situ with two different pea cultivars. Effectiveness of the combination of inoculants with biostimulators / soil conditioners was also monitored regarding the yield and other morphometric performance of pea varieties Petit Provencal and Telephone in field conditions in Herzegovina (Bosnia and Herzegovina).

MATERIALS AND METHODS

The field experiment in Čapljina was set up in 2020 with two peas (*Pisum sativum* L.) varieties Petit Provencal and Telephone on which pre-sowing bacterization and biostimulator / soil conditioner were applied in five treatments. The treatments were as follows: inoculation by *Rhizobium leguminosarum* 1001 strain (I_{RL}), *Rhizobium pisi* strain (I_{RP}), biostimulator / conditioner Humicraft in combination with *Rhizobium leguminosarum* 1001 strain, biostimulator / soil conditioner Humicraft with the inoculum *Rhizobium pisi* strain and control (without inoculation). The application of 0.3% solution of the biostimulator / soil conditioner Humicraft Liquid has been carried out about 45 and 65 days after planting by foliar treatment. Humicraft Liquid is a suspension with 10% potassium humate (from Leonardite), 10% potassium alginate (from

Ascophyllum nodosum) and 10% amino acids, and has been used as a water-soluble high-quality plant growth stimulator and soil conditioner (made by Humitech, Germany). Before setting up the field experiment, soil samples have been applied for chemical analysis in order to determine the status of the basic parameters by standard methods. Standard soil analyses were performed before seed sowing: pH reaction by potentiometric method (BAS ISO 10390:2009) v/v 1:5; total nitrogen content in soil was detected by modified Kjeldahl method (BAS ISO 11261:2010); concentration of phosphorus (by spectrophotometer) and potassium (by flame photometer) in soil determined due to ammonium lactate method (Egner *et al.*, 1960); content of soil organic matter was analyzed by Kotzmann method and carbonates by volumetric method (JDPZ, 1966). For the chemical analysis of the soil, an average soil sample has been taken from the experimental field plot to a depth of 0-30 cm. The field experiment was set in a randomized complete block design (RCBD) in three replications. Sowing has been done manually in 20 cm wide rows with 5 cm distance between plants. The calculation plots was size of 2.0 m². The following parameters were monitored: number of nodules, number of pods, number of plants per basic plot, pod mass, plant mass, plant height and pea yield (t ha⁻¹) on the five random plants per plot. After harvest, protein contents in the grain dry matter and aboveground plant mass were determined by the Kjeldahl method (BAS ISO 11261: 2010). Meteorological data were used for interpretation of the results obtained from the experimental field. The data have been statistically processed by analysis of variance (ANOVA). The LSD tests have been performed for differences between average values according to inoculated bacterial strains; less than or equal to $P \leq 0.05$ being considered significant and their average values further tested by Student's t-test. The GENTSAT7 (GenStat for Windows 7th Ed) statistical program has been used for data analysis.

RESULTS AND DISCUSSION

Data from the Database and archives of the Federal Hydro meteorological Institute B&H, hydro meteorological station Mostar for the vegetation period in 2020 have been used for the elaboration of climatic conditions at the experimental location (Table 1). Mean annual air temperatures in 2020 were significantly above the standard normal value (1961-2010) in the entire observed area of Bosnia and Herzegovina (FHMZFBiH, 2020).

Table 1. Basic weather data for experimental site in 2020 for Mostar area

Monthly Average	January	February	March	April	May	June	Mean
Mean Air Temp. (°C) 2020 yr	6.8	9.7	11.3	15.6	18.9	21.9	14.03
Mean Precipitation (mm) 2020 yr	43.3	51.5	96.6	35.3	52.4	85.9	60
Mean temp for 1961-1990	4.8	6.6	9.6	13.3	17.9	21.5	12.28
Mean Perc. for 1961-1990	164.7	151.1	150.1	127.3	102.1	77.9	153

(Source: FHMZFBiH, 2020)

According to the data, the largest numbers of warm days were during the spring months. Monthly temperatures from the beginning to the end of the season varied and some maximum values were determined during the vegetation in March of 23.4°C

(12.03.2020), April 27 °C (12.04.2020.), May 30.4 °C (16.05.2020.) and the second half of June 36.1 °C (29.06.2020.). In the pea germination period, the mean daily temperature was 11.3 °C, which is relatively lower than the optimal temperature for this phenological phase (18° to 25°C). The six-months mean temperatures for the measured period in 2020 showed oscillation in relation to thirty-year average (1961-1990) for 1.75°C. The six-month period in 2020 (from January to June) shows extremely low mean precipitation for 93 mm comparing to the thirty-year average. April was extremely dry, while in May and June approximately 10 precipitation days were recorded, so they are considered optimal, i.e., normal (FHMZFBIIH, 2020). According to Parađiković (2009), a required amount of precipitation for growing legumes varies in the range of 200 to 400 mm. Therefore, recorded data could be confirmed as sufficient amount of precipitation, but for Herzegovina, distribution is much more important than their monthly sum, so the months of March and April recorded 5-6 days with precipitation less than 1 mm that presents extremely dry period. The soil chemical analysis shown in table 2.

Table 2. Soil chemical analysis for location OPG „Pažin“

Soil depth	Soil pH reaction		CaCO ₃ (%)	Lime CaO (%)	Nitrogen N (%)	mg K ₂ O/ 100 g soil	mg P ₂ O ₅ / 100 g soil	Organic matter (%)
	H ₂ O	1M KCl						
0-30	7.9	7.2	31.13	4.48	0.24	31.21	99.8	5.91

Niste *et al.* (2013) found that the legumes require a neutral to acidic soil reaction for growth, especially when dependent on symbiotic nitrogen fixation. The bacteria of the genus *Rhizobium* according to Zhang *et al.* (2011) require a pH range of 6.5 to 7.5 that is the optimum for their development, which makes our experimental plot acceptable. Petit Provençal and Telephone pea varieties differed significantly in all measured traits as we expected without intention to compare varieties but to see their reaction on inoculation regarding the same growing condition. Protein content in the above ground part and number of nodules were similar for both varieties. This could lead us to conclusion that potential fixation and conversion of nitrogen to protein compounds and its distribution to vegetative plants parts was obtained in similar rate for both varieties. This was confirmed by similar number of formed nodules. Regarding the different proportion of protein content in pea pods could be due to the varietal characteristics as Telephone variety has longer canopy and higher pod and plant mass what could require longer period of redistribution of protein content from canopy to grain. Significant differences in yield were found between the Petit Provençal variety 1.36 t ha⁻¹ and the Telephone 2.25 t ha⁻¹ (Table 4). The treatments have significant impact on the yield (Table 3). Inoculation by *R. leguminosarum* 1001 strain in combination with Humicraft showed yield by 14.3%, *R. leguminosarum* 1001 by 29% and *R. pisi* by 32% higher compared to control. Application of both inoculants individually has obtained similar highest yield, without significant difference between, meaning a good compatibility with sown peas varieties and similar adaptability to soil conditions of Herzegovina. Although, lower yield in combination of Humicraft and *R. pisi* strain resulted similar as control variant. The obtained results of almost all measured traits showed that infectivity and efficiency of both strains with or without applied bio stimulator / soil conditioner

were similar, but improvements are evident compare to control. Influence of Humicraft on these measured traits is unclear, as it was not considered separately in this trial.

Table 3. Influence treatments on measured morphological parameters and pea yield

Measured parameters	Control	IRP	Humicraft + IRP	IRL	Humicraft +IRL	F test	LSD _s %	LSD _i %
Pea yield (t ha ⁻¹)	1.50 b	2.20 a	1.46 b	2.12 a	1.75 a	*	0.59	0.81
Pod protein content (%DW)	4.26 d	4.77 b	5.03 a	5.05 a	4.49 c	**	0.16	0.22
Protein content in aboveground plant part (% DW)	2.97 ab	2.78 b	2.78 b	2.97 ab	3.17 a	**	0.22	0.31
No. of plant per basic plot	63.3 ab	60.0 bc	52.3 c	68.3 ab	72.83 a	*	11.06	-
No of nodules	11.43 c	20.67 b	18.67 b	21.57 ab	27.20 a	**	6.20	8.50
Plant mass (g)	40.7	43.17	40.6	41.80	44.87	n.s.	-	-
Pod mass (g)	15.0	22.6	15.60	20.1	18.5	n.s.	-	-
Plant height (cm)	86.62	93.61	87.83	89.40	89.68	n.s.	-	-
No. of pods plant ⁻¹	2.5	3.03	2.3	3.0	2.97	n.s.	-	-

n.s.- non significant; * - significant; ** - very significant; values followed by the same letter aren't significant
 Different (p<0.05) according to Fisher LSD test.

Protein content in the pods and the aboveground parts were higher by 5 to 15% for applied inoculant treatments of both varieties compared to control. This implied-on effectiveness and competitiveness of inoculated *Rhizobium* strains regards to native ones. However, the protein content in the pods of the Petit Provencal variety was 38% higher than in the Telephone, while the protein content in the aboveground part of these two varieties did not show significant differences (Table 3). The Petit Provencal Variety has number of plants per plot higher by 35%. A variable number of plants on the basic plot may be the result of the germination of the seed itself or of birds, which often causes problems after sowing. A significantly higher number of nodules was found in the treatment with the applied combination of Humicraft and *R. leguminosarum* 1001 strain of 27.2, which showed deviations from the control by 57%, although other treatments also showed a significant deviation from control in the range of 39-47%. The formation of nodules on the roots of peas and other legumes were greatly influenced by environmental factors, but the relationship between the applied bacterial strains and the host plant is of great importance (Jarak *et al.*, 2006). Earlier, in the conditions of Herzegovina, Filipović *et al.* (2019) found the highest number of nodules per plant of pea in the treatment with combination of inoculants and bio stimulators ranging from 3.47 to 16.07 per plants that was lower than in this research. Research by JaiPaul *et al.* (2011) shows that different organic fertilizations and bio stimulators affected the number of pods per plant ranging from 12.5 to 26.8 meaning that except inoculation, the bio stimulants can affect the number of nodules per plants as in our case or it could be due to the soil or weather condition. A higher number of nodules per plant was found in studies (Rapčan *et al.*, 2017; Vasilj *et al.*, 2016; 2019) when applying pre-sowing bacterization of peas by indigenous strains compared to the control. In addition, Vasilj *et al.* (2019) reported that number of nodules varied from 18.83 to 27.83 for applied *R. leguminosarum* 1001 strain, at two different locations, for the same strain, which is very similar to our data. Our results were also similar to the findings of Ahmed *et al.* (2007)

in pea field experiment reported that *Rhizobium* inoculant significantly increased the number of pods by 14.45 pods per plant⁻¹ compare to 9.25 pods per plant⁻¹ in control. Influence depends on the microorganism used (Udovički & Jarak, 2005) as well as on its compatibility with the host plant. In terms of pod weight, it is evident that the Telephone variety (22.8 g) has a significantly higher mass of pods compared to the Petit Provençal variety (14 g) by 38.6% (Table 3).

Table 4. Influence of examined factors on measured morphological parameters and yield of pea Petit Provençal and Telephone varieties

Measured parameters	Petit Provençal	Telephone	F test	LSD _{5%}	LSD _{1%}
Pea yield (t ha ⁻¹)	1.36 b	2.25 a	**	0.12	0.51
Pod protein content (%DW)	4.90 a	4.54 b	**	0.10	0.14
Protein content in aboveground plant part (% DW)	2.88	2.95	n.s.	-	-
No. of plant per basic plot	77 a	50 b	**	7.0	9.59
No of nodules	19.6	20.21	n.s.	-	-
Plant mass (g)	19.67 b	64.79 a	**	4.02	5.51
Pod mass (g)	14.00 b	22.83 a	**	4.21	5.76
Plant height (cm)	45.82 b	133 a	**	3.57	4.89
No. of pods plant ⁻¹	3.53 a	1.97 b	**	0.44	0.61

n.s.- non significant; * - significant; ** - very significant; values followed by the same letter aren't significant Different (p<0.05) according to Fisher LSD test.

However, this trait was attributed to varietal characteristics, more than to growing conditions. A similar happened with the height of the plant, considering that the Telephone (133 cm) is a later variety and forms a higher stem during vegetation. Stem of Telephone variety was by 65% higher. In all variants of the experiment, it is visible that a higher number of pods was achieved with the Petit Provençal variety, but the Telephone variety, as late variety, forms a longer pods and larger grain, which then results with higher pod mass and total yield. Number of pods per unit area is a component of yield affected by environmental factors, depending on geographical location, agro ecological factors and agro technical factors (Rapčan *et al.*, 2017). In these studies, the number of pods varied from 1.97 in the Telephone variety to 3.5 in the Petit Provençal variety. According to Borošić (1992) in addition to soil and agro-technical interventions, meteorological conditions, especially lack of precipitation and high temperatures, have a great influence on this property, before and during the flowering of legumes. Concerning the mass of pods per plant, number of pods and protein content in the grain, a significant interaction was found between the selected variety and the treatment, as shown in Table 4. Application of *R. pisi* strain showed a significantly larger difference in mass of pod in the Telephone variety compared to the Petit Provençal variety in the range of 70%. The highest number of pods of 4.33 was found in the Petit Provençal variety, 63% higher than in the Telephone variety. Interactions that occurred in terms of protein content in the grain were observed in the applied treatments, *R. leguminosarum 1001* strain, a combination of Humicraft and *R. leguminosarum 1001* strain, individual *R. pisi*, with significantly higher contents

observed in the Petit Provençal variety, of 11%, 8.7%, and 14%. Yield has been the highest in Telephone variety inoculated by strain *R. pisi*.

Table 5. Interaction of trial factors on some measured parameters of selected two pea varieties

Variety	Measured trait	Control	I _{RL}	Humicraft + I _{RL}	I _{RP}	Humicraft + I _{RP}	LSD _{5%}	LSD _{1%}
Petit Provençal	Mass of pod plant ⁻¹	13.53	15.8	18.33	10.44	11.73	8.9	12.2
Telephone		16.47	26.7*	18.73	34.8**	17.47		
Petit Provençal	Number of pods plant ⁻¹	3.67**	3.60*	4.33**	2.93	3.13**	0.99	1.4
Telephone		1.33	2.30	1.60	3.10	1.47		
Petit Provençal	Protein grain content %	4.28	5.34**	4.7**	5.14**	5.04	0.2	0.3
Telephone		4.25	4.74	4.29	4.4	5.03		
Petit Provençal	Yield t ha ⁻¹	1.35	1.58	1.63	1.07	1.17	0.84	1.14
Telephone		1.65	2.66	1.87	3.33**	1.75		

n.s.- non significant; * - significant; ** - very significant

The yield in the treatments with inoculation and used bio stimulators of Mahanta *et al.* (2013) show significantly higher pod yield of 3.42 - 9.12 t ha⁻¹ when seven different fertilization treatment applied compared to control. JaiPaul *et al.* (2011) achieved higher results with the pod yield of the Wonder of America pea variety ranging from 3.71 to 6.61 t ha⁻¹. Rapčan *et al.* (2010) obtained pea grain yields in the range from 0.93 to 3.42 t ha⁻¹, similar to our experiment, which was attributed to variety, weather conditions, location and seed age. McKenzie *et al.* (2001) observed increase of the yield up to 14% after inoculation of pea seeds with *Rhizobium* strains. Under favorable environmental conditions, the use of commercial inoculants with *Rhizobium* strains can be economically viable, especially on large planting areas, while poor conditions will affect the presence of *Rhizobium* population in the soil (Catroux *et al.*, 2001). The authors (Vasilj *et al.*, 2016, 2019) show that legumes usually respond to inoculation with *Rhizobium* strains on previously unused field areas where soils lack a sufficient population of compatible indigenous bacteria. On soils where legumes previously have been grown, a certain amount of effective *Rhizobium* population is usually present and that is sufficient to establish an effective symbiosis between the plant and bacterial strains (Huang *et al.*, 2017).

Inoculation with *Rhizobium* strains significantly increased pod yield and plant growth performance of the Petit Provençal and Telephone pea varieties indicating a poor symbiotic competitiveness and effectiveness of native rhizobia on Herzegovinian soils and seasonally weather condition, while affirming the symbiotic superiority of the introduced strain (Table 2). According to Martin *et al.* (1994) in moderately dry areas, water stress is a major factor in yield reduction. The amount of precipitation in this trial during the vegetation period of peas is not satisfactory. The initial period of germination and growth was extremely dry especially during April. Amount of precipitation in the range of 175–316 mm was sufficient for stable and good pea yield (Uzun *et al.*, 2005). However, their interaction with extreme daily temperatures during vegetation may be

crucial to achieve optimal yields. Dornbos & Mullen (1991) also point out the negative impact of drought accompanied with high air temperatures on inoculation. Regarding the metrological data, we can say that these inoculated strains had good respond in water stress condition that happened in vegetation season 2020 for Herzegovina area. In terms of agrochemical properties of the soil on our experimental plot, all soil parameters were satisfactory, while the content of phosphorus and carbonate has too pronounced.

CONCLUSIONS

The use of strains *R. pisi*, *R. leguminosarum* or *R. leguminosarum* strains 1001 in combination with the biostimulator / soil conditioner Humicraft contributed to a highest yield. Larger number of plants, protein content in the aboveground plants part, pod mass, as well as a larger number of nodules per plant, with pronounced varietal variability were established in application of individual *Rhizobium* strains and *R. leguminosarum* strains 1001 in combination with the biostimulator / soil conditioner Humicraft. Protein content in pea pods were lager in *R. pisi* and Humicraft combined treatment, while lager pod, mass and number of pods per plant were highest by *R. pisi* inoculation. The individual inoculants used or their combination with Humicraft differ in their effect on measured traits. It is evident that each trait either in the individual application of the inoculant or its combination with the biostimulator / soil conditioner shows a better result compared to the non-inoculated variant, i.e., the control. The Telephone variety achieves a higher yield (2.25 t ha⁻¹) in relation to the Petit Provencal variety; however, when choosing the variety, it should be considered that they are of different morphological characteristics. Inoculation by *Rhizobium* strains, with or without combination by bio stimulator/soil conditioner have positive effects on yield components of pea especially showing a good respond dry and hot weather season.

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EKOLOŠKE PERSPEKTIVE UZGOJA GRAŠKA

Rezime

Primjena predsjetvene bakterizacije leguminoza zasigurno je jedna od mjera koja može doprinijeti poboljšanju produktivnosti biljke i održivosti okoliša povećanjem plodnosti tla i smanjenjem štetnog utjecaja na ekosustav. U ovom radu je provjeravana učinkovitost predsjetvene bakterizacije i kombinacija bakterizacije i biostimulatora/poboljšivača u odnosu na kontrolnu na vrtnom grašku sorte Mali provansalac i Telefon. Tijekom ovog ispitivanja zabilježen je utjecaj na određene morfološke parametre i prinos graška. Najveći prinos od 2,20 t ha⁻¹ zabilježen je kod sorte graška Telefon. Primjena predsjetvene bakterizacije sojevima *Rhizobium leguminosarum* 1001, *Rhizobium pisum*, te kombinacija *R. leguminosarum* 1001 s biostimulatorima/kondicionerom tla Humicraft pokazala je najbolje rezultate u prinosu graška. Primijenjeni inokulanti razlikuju se u svom učinku na pojedinačna izmjerena morfološka svojstva, ali je evidentno da svako svojstvo bilo u pojedinačnoj primjeni inokulanta ili njegovoj kombinaciji s biostimulatorom/kondicionerom tla pokazuje bolji rezultat u odnosu na necijepljenu varijantu, tj. kontrolu. Cijepljenje s *Rhizobium* bakterijama ima pozitivan učinak na komponente prinosa graška kao jedan ekološki prihvatljiv način uzgoja ove kulture na području Hercegovine.

Ključne riječi: *leguminoze, poljski pokus, Rhizobium bakterijski sojevi, ekologija*