Reaction of garden pea (*Pisum sativum* L.) to inoculation and nitrogen fertilization in Eastern Croatia

Reakcija vrtnog graška (*Pisum sativum* L.) na bakterizaciju i gnojidbu dušikom u istočnoj Hrvatskoj

Irena RAPČAN, Zlata MILAKOVIĆ, Gabriella KANIŽAI ŠARIĆ*, Jurica ŠEPUT and Daria GALIĆ SUBAŠIĆ

Faculty of Agriculture, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, Osijek, Croatia, *correspondence: gkanizai@pfos.hr

Abstract

Biological nitrogen fixation represents the subject of numerous investigations of the scientific community. The advantages of this process, as already well known while scientific research trials attempts to clarify the interactions of symbionts in this type of nitrogen fixation as well as the influence of abiotic factors on its efficacy. A field experiment was conducted to investigate the influence of garden pea cultivars ('Alicia' and 'Miracle of America'), seed inoculation with nodule bacteria (*Rhizobium pisi* DSM 30132, and *Rhizobium leguminosarum* bv. *viciae* OS–103), and nitrogen fertilization (0, 30 and 60 kg N*ha^{-1}). The observed parameters are: stand density, number of pods, mass of 1,000 grains, mass of pods and grains and grain yield. It was established that all the investigated factors significantly influenced the traits. The seeds inoculated with the indigenous strain *R. leguminosarum* bv. *viciae* OS–103 had significantly increased numbers of pods per unit area, grain yield, and the weight of 1,000 grains, while a higher amount of applied nitrogen resulted only in an increase of grain yield. Cultivar 'Alicia' achieved a significantly higher grain yield compared to the 'Miracle of America' cultivars, while application of 60 kg N*ha^{-1} achieved statistically higher grain yield compared to control. Inoculation with *R. leguminosarum* bv. *viciae* OS-103 produced a significantly higher grain yield compared to inoculation with the reference strain *R. pisi* DSM 30132. The observed parameters were significantly influenced by the garden pea cultivar, seed inoculation with nodule bacteria, and nitrogen fertilization therefore further investigation are needed with new inoculant strains and new cultivars under different agroecological conditions.

Keywords: grain yield, nitrogen application, nodule bacteria, pea cultivars, yield components

Sažetak

Biološka fiksacija dušika predstavlja predmet mnogobrojnih istraživanja znanstvene zajednice. Prednosti ovog procesa, kao što je već dobro poznato, su mnogobrojne dok se znanstveno-istraživačkim pokusima nastoje razjasniti interakcije simbionata u ovoj vrsti fiksacije dušika kao i utjecaj abiotskih faktora na njenu djelotvornost. S ciljem ispitivanja utjecaja kultivara vrtnog graška ('Alicia' i 'Čudo Amerike'), inokulacije sjemena kvržičnim bakterijama (*Rhizobium pisi* DSM 30132 i *Rhizobium leguminosarum* bv. *viciae* OS–103) i gnojidbe dušikom (0, 30 i 60 kg N*ha^{-1}) proveden je poljski pokus. Promatrani parametri su uključili: sklop biljaka, broj mahuna, masu 1000 zrna, masu mahuna i zrna i prinos zrna. Rezultati istraživanja su potvrdili kako su svi ispitivani faktori značajno utjecali na ispitivana svojstva. Inokulacija sjemena sojem OS–103 značajno povećava broj mahuna po jedinici površine, prinos zrna i masu 1000 zrna, dok se povećanjem količine primijenjenog dušika dosljedno povećava samo prinos zrna. Kultivar Alicia je postigao značajno veći prinos od kultivara Čudo Amerike, dok je primjenom 60 kg N*ha^{-1} ostvaren statistički značajno veći prinos u usporedbi s kontrolom. Inokulacija s autohtonim sojem *R. leguminosarum* bv. *viciae* OS–103 ostvaren je značajno veći prinos zrna u usporedbi s inokulacijom s referentnim sojem *R. pisi* DSM 30132. Na promatrane parametre značajno su utjecali kultivar vrtnog graška, inokulacija sjemena kvržičnim bakterijama i gnojidba dušikom, stoga su poželjna daljnja istraživanja s novim sojevima kvržičnih bakterija, novim kultivarima i u drugačijim agroekološkim uvjetima.

Ključne riječi: gnojidba dušikom, komponente prinosa, kultivari graška, kvržične bakterije, prinos zrna

Introduction

Legumes have been used in agricultural production since the earliest civilizations. They are used as a primary source of nitrogen in many cultivation systems, as well as a source of food for humans and domestic animals (Power, 1987). Compared with the effect of other elements, nitrogen impacts both agricultural and biological yield growth almost in any agroecological condition. Additionally, it supports a higher production rate of organic matter of all kinds of plants. To achieve this, it is necessary to increase the amount of nutrients, especially nitrogen (Uher et al., 2007). Strunjak and Redžepović (1986) argue that a certain amount of energy must be spent to meet the needs of legumes for nutrients, which explains the efforts to provide legumes with a maximum nitrogen intake from the atmosphere, especially because solar energy as a renewable source is used for nitrogen reduction.

Marohnić (2006) claims that pea production is simple compared to soy production, which makes it one of the most important advantages of pea. Furthermore, pea grains can be stored more easily during the whole year because they do not contain oils that can get rancid. Finally, peas can be consumed directly without any prior treatment, and they can be easily harvested in the dry period at the grain moisture of 13%. Biological nitrogen fixation is an important aspect of the sustainable, environment-friendly food production and long-term crop productivity. Four elementary principles are used for control of the amount of fixed nitrogen: the efficiency of rhizobium-plant symbiosis, absorption capacity (i.e. the capacity of the host plant to store nitrogen), the quantity of the available nitrogen in the ground, and the limitations to nitrogen fixation present in the environment (Van Kessel and Hartley, 2000). Evers (2011) concludes that the rates of biological nitrogen fixation depend on the infection of root hairs of a certain legume by an efficient rhizobium strain. A primary advantage of biological nitrogen fixation by both grain and fodder legumes is the improvement of the yield potential without the need to apply any external nitrogen fertilizers. The ability to produce nitrogen fixation has resulted in the production of commercial inoculants in many countries and on every continent (Brockwell and Bottomley, 1995). The aim of this study is to determine the effect of inoculation and nitrogen fertilization on the stand density, yield components and grain yield of two different cultivars of peas.

Materials and methods

The research was conducted in eastern Croatia in Drenovci in the south of Vukovar-Srijem County in 2013 (latitude 44.92 ^{o}, longitude 18.85 ^{o}, located 90 m above the sea level). The soil is a pseudogley type on a plateau, a characteristic of which is the stagnation of surface water, a low amount of humus, low nitrification, shallow rhizospheric layer, and a pH value of 5–5.5. The climate is moderate continental, characterized by sunny and hot summers, and cold and snowy winters. The annual average for air temperature is 11.6 ^{o}C, and the average precipitation is 772.4 mm (according to Croatian Meteorological and Hydrological Service). This field experiment was set up within a random block design and it involved three repetitions. The following factors with regard to the two pea cultivars were researched: seed inoculation with bacteria, nitrogen fertilization in order to establish the stand density, the number of pods per unit area, the mass of pods and grains per unit area, grain yield per unit area, and the mass of 1,000 seeds. The cultivars used as first factor were 'Alicia' (GSN Semences, France) and 'Miracle of America' (Green Garden, Italy). Two inoculants were used for seed inoculations: *Rhizobium pisi* DSM 30132 (reference strain), and *Rhizobium leguminosarum* bv*. viciae* OS – 103 ('Nitrobakterin', The Faculty of Agriculture, Chair for Microbiology and Soil Resources) that contained $10x10^{9}$ CFU (colony forming unit) per gram of sterile peat, as the second factor alongside control (without inoculation). Nitrogen fertilization, as the third factor, included three variations: 0, 30 and 60 kg N*ha^{-1} of urea. Prior to sowing, 70 kg*ha^{-1} of phosphorus and 100 kg*ha^{-1} of potassium were applied to the whole experimental area. The size of the basic plot was 6 m^{2}. The peas were sown in rows of 20 cm, while the sowing distance and depth within a row were 5 cm, respectively. During the vegetation period the achieved stand density, the number of pods per unit area, and the weather indicators were established. After the harvest, the mass of pods and grains, grain yield, and the mass of 1,000 grains were determined. The data were statistically analysed and interpreted.

Climatic conditions

Compared to the multiannual average, the average air temperatures in April and July were lower by 1.7 ^{o}C and 0.8 ^{o}C, respectively (Table 1). On the other hand, the temperatures in May and June were aligned with the multiannual average. During the vegetation period the precipitation was 305.3 mm, which was consistent with the average of 308.7 mm for those months. However, the precipitation distribution was very different from the average. Compared with the average values, in April and June, the precipitation was lower by 24 mm and 48.1 mm, respectively. In May the precipitation reached 139.1 mm, which is 66.6 mm higher than the multiannual average. July was on the same level as the average value.

Table 1. The monthly average for air temperatures in 2013 and the multiannual average in the period of 1981–2010 for the vegetation months on Drenovci location

Tablica 1. Srednja mjesečna temperatura zraka u 2013. godini i višegodišnji prosjek (1981-2010) za vegetacijski period na lokaciji Drenovci

Month	Monthly average for air temperature (^{o}C)		Monthly precipitation (mm)	
	2013	1981-2010	2013	1981-2010
April	14	12.3	37.6	61.6
May	17.1	17.6	139.1	72.5
June	20.8	20.5	63.1	111.2
July	23.4	22.6	65.5	63.4

Results

Stand density

The peas were planted as a stand density 100 plants per m^2. A stand density of 43.56% to 54.22% was reached in dependence on the experimental design (Table 2). The lowest stand density was obtained with the fertilization of 30 kg N*ha^{-1}, whereas the highest stand density was reached in the case of seed inoculation with the reference bacteria strain. There was a significant difference between the cultivars (P=0.01%) in regard to achieved stand density. The inoculation with the reference bacteria strain produced better stand density results compared to the inoculation with OS–103 strain, while the experimental design without inoculation produced even worse stand density (P=0.01%). In regard to the nitrogen fertilization, there was a statistically significant difference of 1% in stand density with the best stand density achieved with 60 kg N*ha^{-1}, and the worst with 30 kg N*ha^{-1}. All the interactions of the investigated factors showed a statistically significant difference (P=0.01%).

Table 2. The justification of effects of the investigated factors

Tablica 2. Opravdanost djelovanja ispitivanih faktora

%		Cultivar K	Seed inoculation I	Nitrogen fertilization F
K_1/ I_0 / F_0		49.52	46.17	50.17
K_2/ I_1 /F_1		49.11	51.61	43.56
I_2 / F_2			50.17	54.22
LSD $_{0.05}$		0.23	0.28	0.28
LSD $_{0.01}$		0.3	0.37	0.37
Interactions	K x I	K x F	I x F	K x I x F
LSD $_{0.05}$	0.4	0.4	0.49	0.69
LSD $_{0.01}$	0.52	0.52	0.64	0.91

K = cultivar, K_1 = 'Alicia', K_2 = 'Miracle of America'; I = inoculation, I_0 = without seed inoculation, I_1 = inoculation with the reference strain, I_2 = inoculation with the strain OS – 103; F = nitrogen fertilization, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha^{-1}, F_2 = 60 kg N*ha^{-1};

K = kultivar, K_1 = 'Alicia', K_2 = 'Čudo Amerike'; I = bakterizacija, I_0 = bez bakterizacije, I_1 = bakterizacija s referentnim sojem, I_2 = bakterizacija sa sojem OS – 103; F = gnojidba dušikom, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha$^{-1,}$ F_2 = 60 kg N*ha^{-1}.

Number of pods per unit area

The number of pods per unit area in this experiment differed in dependence on the treatment and it ranged from 99.52 to 178.89 pods per m^2 (Table 3). The highest pod number (178.89) was achieved for the seed inoculation with the bacteria strain OS–103, whereas the lowest pod number of 99.52 pods was obtained for the 'Alicia' cultivar. All the treatments and their mutual interactions were on the significance level of 0.01%. An especially large difference in pod number (P=0.01%) per unit area was found for the cultivars, in which case 'Alicia' had 99.52 pods, while 'Miracle of America' had 160.63 pods per unit area. Compared to the seeds that were not inoculated, the seed inoculation with the reference bacteria strain increased the pod number per unit area by 2.33 pods, whereas the inoculation with the strain OS–103 increased the pod number per unit area by 74.39 pods (P=0.01%). Again, the fertilization with 30 kg N*ha^{-1} resulted in the lowest pod number per unit area, while the fertilization with 60 kg N*ha^{-1} produced the highest pod number, which accounted for a significant statistical difference of 0.01%.

Table 3. Justification of the effects of the investigated factors

Tablica 3. Opravdanost djelovanja ispitivanih faktora

pods*m^{-2}		Cultivar K	Seed inoculation I	Nitrogen fertilization F
K_1/ I_0 / F_0		99.52	104.5	129.56
K_2/ I_1 /F_1		160.63	106.83	125.78
I_2 / F_2			178.89	134.89
LSD $_{0.05}$		0.83	1.02	1.02
LSD $_{0.01}$		1.1	1.34	1.34
Interactions	K x I	K x F	I x F	K x I x F
LSD $_{0.05}$	1.44	1.44	1.77	2.5
LSD $_{0.01}$	1.9	1.9	2.33	3.29

K = cultivar, K_1 = 'Alicia', K_2 = 'Miracle of America'; I = inoculation, I_0 = without seed inoculation, I_1 = inoculation with the reference strain, I_2 = inoculation with the strain OS – 103; F = nitrogen fertilization, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha^{-1}, F_2 = 60 kg N*ha^{-1};

K = kultivar, K_1 = 'Alicia', K_2 = 'Čudo Amerike'; I = bakterizacija, I_0 = bez bakterizacije, I_1 = bakterizacija s referentnim sojem, I_2 = bakterizacija sa sojem OS – 103; F = gnojidba dušikom, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha$^{-1,}$ F_2 = 60 kg N*ha^{-1}.

The mass of pods and grains per unit area

Various experimental variations resulted in different mass amounts of pods and grains per unit area, as is shown in Table 4. The cultivar 'Miracle of America' reached a higher mass by 47.94 g*m^{-2} than the 'Alicia' cultivar. The strain OS–103 indicated a significantly higher pod and grain mass of 86.97 g*m^{-2} and 93.72 g*m^{-2} compared to the reference strain and the seeds that were not inoculated, respectively. The experimental variation without nitrogen fertilization resulted in a statistically higher pod and grain mass per unit area, which was higher by 56.43 g*m^{-2} and 33.84 g*m^{-2} than the fertilization with 30 kg N*ha^{-1} and 60 kg N*ha^{-1}, respectively. The highest pod and grain mass was achieved by the inoculation with the strain OS–103 (326.26 g*m^{-2}), and the lowest result was obtained by the experiment design which did not include seed inoculation (232.54 g*m^{-2}). All factors and their interactions reveal a statistically significant difference (P= 0.01%).

Table 4. Justification of the effects of the investigated factors

Tablica 4. Opravdanost djelovanja ispitivanih faktora

$g*m^{-2}$		Cultivar K	Seed inoculation I	Nitrogen fertilization F
K_1/ I_0 / F_0		242.06	232.54	296.12
K_2/ I_1 /F_1		290	239.29	239.69
I_2 / F_2			326.26	262.28
LSD $_{0.05}$		1.1	1.35	1.35
LSD $_{0.01}$		1.45	1.77	1.77
Interactions	K x I	K x F	I x F	K x I x F
LSD $_{0.05}$	1.9	1.9	2.33	3.3
LSD $_{0.01}$	2.51	2.51	3.07	4.34

K = cultivar, K_1 = 'Alicia', K_2 = 'Miracle of America'; I = inoculation, I_0 = without seed inoculation, I_1 = inoculation with the reference strain, I_2 = inoculation with the strain OS – 103; F = nitrogen fertilization, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha^{-1}, F_2 = 60 kg N*ha^{-1};

K = kultivar, K_1 = 'Alicia', K_2 = 'Čudo Amerike'; I = bakterizacija, I_0 = bez bakterizacije, I_1 = bakterizacija s referentnim sojem, I_2 = bakterizacija sa sojem OS – 103; F = gnojidba dušikom, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha$^{-1,}$ F_2 = 60 kg N*ha^{-1}.

Grain yield per unit area

All the investigated factors achieved a statistically significant impact (P=0.01%) on grain yield per unit area (Table 5). The interactions of the investigated factors produced the same level of significance. The lowest grain yield of 170.23 g*m^{-2} was reached through the experimental design without nitrogen fertilization (0 kg N*ha^{-1}), whereas the highest grain yield of 209.78 g*m^{-2} was reached by seed inoculation with the strain OS–103. The cultivar 'Alicia' reached a significantly higher yield than 'Miracle of America', with the difference of 29 kg*ha^{-1} in favour of 'Alicia'. The inoculation with the reference bacteria strain produced a significantly lower grain yield, which was by 25.81 g*m^{-2} or 258.1 kg*ha^{-1} lower than the results of the inoculation with the strain OS–103.

The results of the experiment which excluded seed inoculation were lower by 32.23 g*m^{-2} compared to those achieved by the inoculation with the strain OS–103. The significance of nitrogen fertilization on grain yield reached the significance of 1%. The experimental variations without nitrogen fertilization resulted in 170.23 g*m^{-2}, while the fertilization with 30 kg N*ha^{-1} resulted in a grain yield higher by 26.05 g*m^{-2}.

However, the experimental variation with nitrogen fertilization with 60 kg N*ha^{-1} resulted in a yield higher by 34.56 g*m^{-2} (or 345.6 kg*ha^{-1}) compared to the variation without fertilization.

Table 5. Justification of the investigated factors

Tablica 5. Opravdanost djelovanja ispitivanih faktora

g*m^{-2}		Cultivar K	Seed inoculation I	Nitrogen fertilization F
K_1/ I_0 / F_0		191.88	177.55	170.23
K_2/ I_1 /F_1		188.98	183.97	196.28
I_2 / F_2			209.78	204.79
LSD $_{0.05}$		0.71	0.87	0.87
LSD $_{0.01}$		0.94	1.15	1.15
Interactions	K x I	K x F	I x F	K x I x F
LSD $_{0.05}$	1.23	1.23	1.51	2.14
LSD $_{0.01}$	1.62	1.62	1.99	2.81

K = cultivar, K_1 = 'Alicia', K_2 = 'Miracle of America'; I = inoculation, I_0 = without seed inoculation, I_1 = inoculation with the reference strain, I_2 = inoculation with the strain OS – 103; F = nitrogen fertilization, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha^{-1}, F_2 = 60 kg N*ha^{-1};

K = kultivar, K_1 = 'Alicia', K_2 = 'Čudo Amerike'; I = bakterizacija, I_0 = bez bakterizacije, I_1 = bakterizacija s referentnim sojem, I_2 = bakterizacija sa sojem OS – 103; F = gnojidba dušikom, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha$^{-1,}$ F_2 = 60 kg N*ha^{-1}.

The mass of 1,000 grains

The mass of 1,000 grains of the 'Alicia' cultivar was significantly higher by 64.85 g (P=0.01%) than the mass of 1,000 grains of the 'Miracle of America' cultivar (Table 6). The seed inoculation with the strain OS–103 resulted in the mass of 1,000 grains higher by 7.5% and 6.58% compared to the seeds that were not inoculated or those inoculated by the reference strand, respectively.

With regard to nitrogen fertilization, the mass of 1,000 grains for the experiment without fertilization was 363.44 g, which is significantly higher by 39.5 g and 27.11 g (P=0.01%) than the fertilization with 30 kg N*ha^{-1} and 60 kg N*ha^{-1}, respectively. A statistical analysis determined that all the interactions of the investigated factors were significant on the level of 0.01%.

Table 6. Justification of the investigated factors

Tablica 6. Opravdanost djelovanja ispitivanih faktora

g		Cultivar K	Seed inoculation I	Nitrogen fertilization F
K_1/ I_0 / F_0		373.67	325.94	363.44
K_2/ I_1/ F_1		308.82	347.39	323.94
I_2 / F_2			350.39	336.33
LSD $_{0.05}$		7.82	9.58	9.58
LSD $_{0.01}$		10.3	12.62	12.62
Interactions	K x I	K x F	I x F	K x I x F
LSD $_{0.05}$	13.55	13.55	16.6	23.48
LSD $_{0.01}$	17.84	17.84	21.85	30.9

K = cultivar, K_1 = 'Alicia', K_2 = 'Miracle of America'; I = inoculation, I_0 = without seed inoculation, I_1 = inoculation with the reference strain, I_2 = inoculation with the strain OS – 103; F = nitrogen fertilization, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha^{-1}, F_2 = 60 kg N*ha^{-1};

K = kultivar, K_1 = 'Alicia', K_2 = 'Čudo Amerike'; I = bakterizacija, I_0 = bez bakterizacije, I_1 = bakterizacija s referentnim sojem, I_2 = bakterizacija sa sojem OS – 103; F = gnojidba dušikom, F_0 = 0 kg N*ha^{-1}, F_1 = 30 kg N*ha$^{-1,}$ F_2 = 60 kg N*ha^{-1}

Discussion

Various authors have reached different conclusions with regard to different values necessary to attain stand density. Hampton and Scott (1982) report on a stand density attainment of 36%–89% in three experiments which included five lines of garden pea, while Castillo et al. (1993) report on an attainment of 39%–93% and 37– 98% in dependence on the sowing deadline. Rapčan et al. (2003) cite far higher values obtained for fodder pea during a two-year research (60–86% and 65–99%, for each year, respectively). Rapčan et al. (2006) report on a stand formation of 86%, as well.

The number of pods per unit area is a yield component that is affected by external factors, ranging from geographical position, agroecological factors, to agrotechnical factors. Rapčan et al. (2003) reached a pod quantity per unit area that is higher than the highest pod quantity in this experiment. It was 197.44–470.63 depending on the experiment. Ayaz et al. (2004) reached a three–time bigger pod quantity of 633 and 883 during a two–year time, respectively. Uher et al. (2006) made experiments with winter peas and achieved 10 pods per plant in controlled conditions, 11 pods per plant with nitrogen fertilization and 12 pods per plant with inoculation.

As is the case in this research, Gopinath et al. (2011) and JaiPaul et al. (2011) reached a higher pod yield when they applied inoculants. On the other hand, Mahanta et al. (2013) established a significantly higher pod yield of 3.42–9.12 $t*ha^{-1}$ compared to the findings of this experiment.

The grain yields in this experiment are somewhat lower than those cited by Thomson et al. (1997), which were established based on their research with and without irrigation (in a range from 104 to 279 $g*m^{-2}$). McPhee and Muehlbauer (1999) claim that during their three-year long experiments on three locations, various cultivars resulted in grain yields ranging from 121 to 346 $g*m^{-2}$. Significantly higher grain yields (ranging from 219 to 415 $g*m^{-2}$) than the ones established in this experiment were achieved by Popović et al. (2002) and Ayaz et al. (2004). Uher et al. (2006) conclude based on their experiments that inoculation increased grain yield by 21% compared to the control sample.

The results for the mass of 1,000 grains in this experiment are far above those obtained by Rapčan et al. (2004) during a two-year research on cultivars of dry peas performed on two locations, which ranged from 139.54 g to 209.37 g (an average of 126 g). The mass of 1,000 grains for inoculated seeds was higher by 9.65% and 2.1% compared to the control variable and the nitrogen fertilization, respectively (Uher et al., 2006).

Biologically–fixed nitrogen by legumes is produced by solar energy, whereas nitrogen found in fertilizers is produced from non–renewable energy sources (Crews and Peoples, 2004). Agricultural production constantly aims at an environment-friendly production, as well as at decreasing the costs of energy production (Brkić et al., 2004). The main advantage of providing support for biological nitrogen fixation for grain and fodder legumes is the improvement of yield potential without the application of external nitrogen fertilizers. The ability to perform nitrogen fixation has resulted in the production of commercial inoculants in numerous countries and on every continent (Brockwell and Bottomley, 1995). Stephens and Rask (2000) claim that the *Rhizobium*/*Bradyrhizobium* inoculants have been in use for more than a century, and there has been a continuous effort to improve their performance. They also conclude that products with more strains should be investigated more deeply in order to be able to deal more effectively with the difficulties regarding the strain-soil-cultivar relationship.

Based on the field experiment conducted in eastern Croatia in 2013 on the impact of the cultivars, seed inoculation, and nitrogen fertilization the following conclusions can be made: the type of pea cultivar, seed inoculation with nodule bacteria, and nitrogen fertilization as well as their interactions had a significant impact on stand density, the amount of pods per unit area, pod and grain yield per unit area, grain yield and the mass of 1,000 seeds. The seed inoculation with the inoculant OS–103 significantly increased the number of pods per unit area, grain yield, and the mass of 1,000 grains compared to the control variable. An increased amount of the applied nitrogen resulted in only the grain yield increasing at a consistent rate.

Conclusions

Based on the field experiment conducted in Drenovci in 2013 on the impact of the cultivars, seed inoculation, and nitrogen fertilization the following conclusions can be made: the type of pea cultivar, seed inoculation with nodule bacteria, and nitrogen fertilization as well as their interactions had a significant impact on stand formation, the amount of pods per unit area, pod and grain yield per unit area, grain yield and the mass of 1,000 seeds. The seed inoculation with the inoculant OS–103 significantly increased the number of pods per unit area, grain yield, and the mass of 1,000 grains compared to the control variable. An increased amount of the applied nitrogen resulted in only the grain yield increasing at a consistent rate. Further investigation is required with the new inoculant strains and new cultivars under different agroecological conditions.

References

Ayaz, S., McKenzie, B.A., Hill, G.D., McNeil, D.L. (2004) Variability in yield of four grain legume species in a subhumid temperate environment. II. Yield components. Journal of Agricultural Science, 142 (1), 21–28. DOI: https://doi.org/10.1017/S0021859604004113

Brkić, S., Milaković, Z., Kristek A., Antunović, M. (2004) Pea yield and its quality depending on inoculation, nitrogen and molybdenum fertilization. Plant, Soil and Environment, 50, 39–45.

Brockwell, J., Bottomley, P.J. (1995) Recent advances in inoculant technology and prospects for the future. Soil Biology and Biochemistry, 27, 683–697.

Castillo, A.G., Hampton, J.G., Coolbear, P. (1993) Influence of seed quality characters on field emergence of garden peas (*Pisum sativum* L.) under various sowing conditions. New Zealand Journal of Crop and Horticultural Science, 21 (2), 197–205. DOI: http://dx.doi.org/10.1080/01140671.1993.9513768

Crews, T.E., Peoples, M.B. (2004) Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agriculture, Ecosystems and Environment, 102 (3), 279–297. DOI: http://dx.doi.org/10.1016/j.agee.2003.09.018

Evers, G.W. (2011) Forage legumes: forage quality, fixed nitrogen, or both. Crop Science, 51 (2), 403–409.

Gopinath, K.A., Mina, B. (2011) Effect of organic manures on agronomic and economic performance of garden pea (*Pisum sativum*) and on soil properties. Indian Journal of Agricultural Sciences, 81 (3), 236–239.

Hampton, J.G., Scott, D.J. (1982) Effect of seed vigour on garden pea production. New Zealand Journal of Agricultural Research, 25 (3), 289–294. Available at: http://www.tandfonline.com/doi/pdf/10.1080/00288233.1982.10417889 [Accessed 1 September 2015].

JaiPaul, Sharma S., Dixit A.K., Sharma, A.K. (2011) Growth and yield of capsicum (*Capsicum annum*) and garden pea (*Pisum sativum*) as influenced by organic manures and biofertilizers. Indian Journal of Agricultural Sciences, 81 (7), 637–642.

Mahanta, D., Bhattacharyya, R., Gopinath, K.A., Tuti, M.D., Jeevanandan, K., Chandrashekara, C., Arunkumar, R., Mina, B.L., Pandey, B.M., Mishra, P.K., Bisht, J.K. (2013) Influence of farmyard manure application and mineral fertilization on yield sustainability, carbon sequestration potential and soil property of garden pea-french bean cropping system in the Indian Himalayas. Scientia Horticulturae, 164, 414–427.

Marohnić, I. (2006) Grašak – buduće glavno bjelančevinasto krmivo Europe. Krmiva 48 (6), 363–368. Available at: http://hrcak.srce.hr/26500 [Accessed 17 September 2015].

McPhee, K.E., Muehlbauer, F.J. (1999) Variation for biomass and residue production by dry pea. Field Crops Research, 62, 203–212. Available at: https://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=10761&content=PDF [Accessed 15 September 2015].

Popović, S., Stjepanović, M., Grljušić, S., Čupić, T., Tucak, M., Buković, G. (2002) Prinos i kakvoća zrna jarog stočnog graška. Krmiva, 44 (4), 191–197. Available at: http://hrcak.srce.hr/139892 [Accessed 15 September 2015].

Power, J.F. (1987) Legumes: Their potential role in agricultural production. American Journal of Alternative Agriculture, 2 (2), 69–73. DOI: https://doi.org/10.1017/S0889189300001600

Rapčan, I., Jurišić, M., Rozman, V. (2003) Pea (*Pisum sativum* spp. *arvense* L.) yield and some fenotyp characteristics in depending on the sowing term, stand and nitrogen fertilization at Osijek region. Sjemenarstvo, 20 (3–4), 131–145. Available at: http://hrcak.srce.hr/169803 [Accessed 19 September 2015].

Rapčan, I., Jurišić, M., Grljušić, S., (2004) Reakcija graška (*Pisum sativum* spp. *Arvense* L.) na rok sjetve, sklop i gnojidbu dušikom na području Osijeka. Agronomski glasnik, 1–2, 33–45. Available at: http://hrcak.srce.hr/103509 [Accessed 19 September 2015].

Rapčan, I., Bukvić, G., Grljušić, S., Teklić, T., Jurišić, M. (2006) Produkcija biomase krmnog graška (*Pisum sativum* L.) u zavisnosti od starosti sjemena i agroekoloških uvjeta uzgoja. Poljoprivreda, 12 (2), 29–35. Available at: http://hrcak.srce.hr/7687 [Accessed 23 September 2015].

Stephens, J.H.G., Rask, H.M. (2000) Inoculant production and formulation. Field Crops Research, 65 (2–3), 249–258.

Strunjak, R., Redžepović, S. (1986) Bakterizacija leguminoza – agrotehnička mjera u službi štednje energije. Agriculturae Conspectus Scientificus, 72, 109–115.

Thomson, B.D., Siddique, K.H.M., Barr, M.D., Wilson, J.M. (1997) Grain legume species in low rainfall Mediterranean-type environments I. Phenology and seed yield. Field Crops Research, 54, 173–187.

Uher, D., Štafa, Z., Blažinkov, M., Kaučić, D. (2006) Utjecaj bakterizacije i prihrane dušikom na prinose zrna ozimog graška u smjesi s pšenicom. Sjemenarstvo, 23 (2), 115–130. Available at: http://hrcak.srce.hr/1920 [Accessed 25 September 2015].

Uher, D., Štafa, Z., Redžepović, S., Blažinkov, M,. Sikora, S., Kaučić, D. (2007) Utjecaj bakterizacije i prihrane dušikom na prinos i krmnu vrijednost ozimog graška cv. Maksimirski ozimi u smjesi sa pšenicom cv. Sana. Mljekarstvo, 57 (2), 101–117. Available at: http://hrcak.srce.hr/13480 [Accessed 25 September 2015].

Van Kessel, C., Hartley, C. (2000) Agricultural management of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Research, 65 (2–3), 165–181.