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Importance of winter pea cv. Maksimirski rani in milk production on family farms

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Summary

Forage pea (*Pisum sativum* L.) is gaining importance as a forage legume in the Republic of Croatia. Pea seed contains 20-30 percent of protein, it is utilized without thermal treatment in feeding different types and categories of livestock, and with stable yield it provides an appreciable income per hectare. Two-year field trials (2005-2006) were carried out to determine the effect of winter pea seed inoculation and nitrogen top-dressing on the number and mass (g/plant^{-1}) of root nodules and also on the yield and quality of winter pea cv. Maksimirski rani in a mixture with wheat cv. Sana. Just before sowing, pea seeds were inoculated with the strain *Rhizobium leguminosarum bv. viciae* 1001 from the microbial collection of the Department of Microbiology, Faculty of Agriculture, University of Zagreb. The highest number of root nodules (43 nodules/plant), as well as the highest nodule mass (0.219 g/plant^{-1}) were determined in the inoculated variant. The highest number of pods (19.0) and seeds per plant (60) were determined in the inoculated variant as well. The highest 1000-seed mass (132 g) and seed mass per plant (7.93 g) were also determined in the inoculated variant. Average pea seed yield ranged from 2949 kg ha^{-1} (control) up to 3353 kg ha^{-1} (inoculation). The conclusion of this research is that the highest seed (3353 kg ha^{-1}) and crude protein yields (833 kg ha^{-1}) were obtained with inoculated forage winter pea cv. Maksimirski rani. Seed inoculation of the studied pea cultivar Maksimirski rani with the strain *Rhizobium leguminosarum bv. viciae* 1001 influenced also higher milk production per hectare compared to the control and the nitrogen top-dressed variant.

Key words: winter pea, inoculation, nitrogen top-dressing, seed yield, quality

Introduction

The former concept of agricultural production (high capital inputs and high yields) has caused a number of adverse effects in terms of soil degradation and contamination, increased ecological risks relating to groundwater contamination and eutrophication of open watercourses, thereby creating at first glance irreconcilable problems between economic and ecological efficacy of agriculture.

As the world population is increasing, it is estimated that the present generation is faced with the task to meet food requirements at the present supply level, which is known to be insufficient, and in the next 50 years produce food quantities equal to those produced in the past 8000 years of human history. There can be no illusion that this task can be fulfilled without a considerable increase in production per unit area, since the potentials of increasing production areas are very limited, on some continents practically non-existent, and on others possible changes in this direction (forest clearing) could cause serious changes in global ecological balance. For these reasons, the contemporary development concept is defined as sustainable agriculture, already called the fourth agricultural revolution, the basic tenets of which are some, still not standardized and not sufficiently exactly defined, plant production procedures, such as sustainable soil management, sustainable plant nutrition systems and integrated

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plant protection. At its historic crossroads, Croatia intends to base its economic recovery and development on a modern concept of sustainable development, based on agriculture and tourism as economic branches relying on renewable natural resources. This "pro-ecological" orientation requires certain changes and adjustments aimed at protection of natural resources of special importance to agriculture, primarily soil and water, a kind of "alliance" between the applied plant production practices and nature. In other words, increase of yield per unit area, that is, production per unit of capacity, demands application of modern, revised solutions in plant production.

Efficient inputs of nitrogen through biological fixation in agricultural practices lead to economically justified and ecologically acceptable agricultural production. Symbiotic fixation of molecular nitrogen fully satisfies the main requirements of soil management: productivity, safety, protection of natural resources, cost effectiveness and social acceptability of the system.

Biological nitrogen fixation at the global level provides $175x10^6$ tons of nitrogen, or more than 70% of total nitrogen fixed on the Earth each year. In this total quantity, symbiotic fixation with legumes accounts for 80×10^6 tons of nitrogen per year (Evans and Barber, 1977).

Of great importance for agricultural production is the symbiosis between nodule bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Synorhizobium* and *Azorhizobium* and legumes for biological binding of atmospheric nitrogen, which is promptly used for protein synthesis, thereby preventing the risk of groundwater contamination with nitrates, which otherwise appear in intensive application of mineral nitrogen fertilizers.

Due to the economic importance of biological nitrogen fixation and possible replacement of costly mineral nitrogen, as well as full omittance of nitrogen fertilizer application, in the last 20 years special attention has been paid on selection of efficient strains of bacteria from the genera *Rhizobium* and *Bradyrhizobium*, and finding the best symbiotic association of winter pea cultivars x *Rhizobium leguminosarum bv. viciae* strain in order to make the most of the natural process of symbiotic nitrogen fixation and increase the production of proteins in the aboveground mass and seed of winter pea.

In this way, soil supplies of biological nitrogen remaining after winter pea harvest could be increased, and subsequently used by the next crops in crop rotation (Bonnier and Brakel, 1969).

To bind nitrogen from the atmosphere, legumes use solar energy accumulated in host plant assimilates. Considering that, for instance, soybeans require four times as much nitrogen per unit yield as cereals (Hardy and Havelka, 1975) and that to bind this nitrogen industry has to consume a certain amount of costly fossil energy, which is limited, efforts to enable legumes to make maximum use of atmospheric nitrogen are understandable, all the more so as solar energy, a yearly renewable source, is used for its reduction (Strunjak and Redžepović, 1986).

Materials and methods

Trial site

A field trial was set in Maksimir, at the experimental facility of the Department of Field Crop Production, Faculty of Agriculture in Zagreb, in crop years 2004/2005 and 2005/2006.

Soil of the trial area

The soil of the experimental facility of the Faculty of Agriculture in Maksimir is alluvial-colluvial cambisol, developed on alluvium. It is characterized by a noncalcareous surface horizon P of 0-20 cm depth and a 20-60 cm deep subsurface horizon (B). As regards its mechanical composition, the soil is of homogeneous stratigraphy, and texturally silty loam. High silt fraction content is characteristic of the surface horizon (68.2%), making the soil susceptible to crust formation. As per its physical properties, the soil is slightly porous in the surface P horizon (Vol. 41.4%) and of medium water holding capacity (Vol. 36.8%). Subsurface B horizon is slightly porous (Vol. 41.5%) and of low water holding capacity (Vol. 33.6%). Soil reaction is neutral, pH in nKCl is 7.0. The soil is poorly humous; it contains 1.8% of humus.

Based on the total nitrogen content, the soil is well supplied with nitrogen (0.14%). Soil contents of P_2O_5 and K_2O indicate that the Maksimir soil is moderately to well supplied with these nutrients; the plough-layer contains 35.7 mg P_2O_5 and 18.6 mg K_2O/100 g soil.

Table 1. Mean monthly air temperatures, precipitation and multi-year average (Meteorological Station Zagreb-Maksimir)

Tablica 1. Srednje mjesečne temperature zraka i količine oborina te višegodišnji prosjek (Meteorološka postaja Zagreb - Maksimir)

Month Mjesec	Mean monthly air temperature Srednje mjesečne temperature °C				Average Prosjek 1998-2007	Mean precipitation Proječne padaline mm				Average Prosjek 1998-2007
	2004	2005	2006	2007		2004	2005	2006	2007	
I	-0.1	0.1	-1.2	6.5	1.2	57.1	15.7	41.5	67.1	42.5
II	3.0	-1.7	1.5	6.9	3.1	42.0	61.8	42.2	44.9	37.5
III	5.6	5.0	5.6	8.8	7.3	60.1	29.7	53.0	71.7	49.3
IV	11.6	11.8	12.5	13.7	12.1	135.8	64.9	110.3	1.6	73.7
V	14.8	16.5	16.1	18.2	17.1	39.3	66.3	80.8	71.3	67.8
VI	19.1	19.9	20.5	22.2	20.7	102.2	68.6	40.3	96.6	83.2
VII	21.2	21.5	23.8	22.9	21.9	69.7	137.1	31.7	49.3	84.4
VIII	21.1	18.9	18.9	21.3	21.3	56.4	175.3	177.9	101.6	86.9
IX	16.2	16.9	17.7	14.5	16.2	80.6	67.8	67.6	136.1	107.7
X	13.1	12.3	13.2	10.4	12.0	186.2	26.7	16.9	104.4	86.8
XI	7.0	5.2	8.9	4.9	6.4	39.7	78.7	46.7	58.7	68.6
XII	2.1	1.5	4.0	0.5	1.4	49.3	113.4	37.3	54.1	62.9
Average Prosjek	11.2	10.7	11.8	12.6	11.7					
Total Ukupno						918.4	906.0	746.2	857.4	851.3

Source: Meteorological and Hydrological Service of Croatia
Izvor: Državni hidrometeorološki Zavod

Climate conditions

Data of the Meteorological Station Zagreb-Maksimir show that pursuant to Lang's rain factor (72.8) humid climate prevails in the Zagreb area. Mean ten-year air temperature is 11.7 °C, assigning this region to the moderately warm climate zone. Such climate is not an obstacle for growing the winter mixture of forage pea and wheat (Table 1). Average air temperature in 2005 was 10.7 °C, which is lower than the ten-year average (11.7 °C), while the average air temperature in 2006 was 11.8 °C, higher than the ten-year average (11.7 °C). Precipitation recorded in 2005 (906.0 mm) was higher than the ten-year average (851.3 mm), while the amount of precipitation in 2006 (746.2 mm) was lower than the ten-year average (851.3 mm).

Methods of work

The trial was laid up according to the randomized block scheme with four replications, fertilization being the main factor in the crop years 2004/2005 and 2005/2006:

Table 2. Number of root nodules per pea plant
Tablica 2. Broj kvržica na korijenu graška

Variant Varijanta	Number of root nodules per pea plant Broj kvržica na korijenu graška		
	Year/Godina		Variant average Prosjek varijanata
	2005	2006	
Control/Kontrola	40	33	36.5
Inoculation/Bakterizacija	44	42	43.0
Nitrogen Top-dressing Prihrana dušikom	36	31	33.5
Year Average Prosjek godina	40.0	35.3	
LSD 0.05			0.7 nodules/kvržice
LSD 0.05 †	1.1 nodules/kvržice		
LSD 0.05 ‡	1.9 nodules/kvržice		
			Significance Signifikantnost
Year/Godina			**
Variant/Varijanta			**
Year x variant/Godina x varijanta			**

†values for means within year comparison/za usporedbu srednjih vrijednosti unutar godine
‡values for means across years comparison/za usporedbu srednjih vrijednosti između godina

Factor I (fertilization) in 3 steps:

1. control (without topdressing and inoculation),
2. pea seed inoculation with the strain *Rhizobium leguminosarum bv. viciae* 1001,
3. nitrogen top-dressing (2 x 110 kg ha^{-1} calcium ammonium nitrate) at wheat tillering and forking.

Basic tillage was carried out by ploughing to 30-cm depth. Presowing preparation was done using a tractor-mounted rototiller. Basic fertilization was done with 500 kg ha^{-1} NPK 8:26:26 or 40 kg ha^{-1} N, 130 kg ha^{-1} P_2O_5 and 130 kg ha^{-1} K_2O. Sowing was performed using a Wintersteiger drill with coulter set at inter-row spacing of 12 cm. Each plot was 10 m long and 1.20 m wide. Control variants were sown first, followed by nitrogen top-dressed variants (calcium ammonium nitrate), while the inoculated variants were sown last.

The sowing rate was 50 seeds/m^2 of winter pea cv. Maksimirski rani and 200 seeds/m^2 of wheat cv. Sana. The crop in variant 3 was topdressed two times with 110 kg calcium ammonium nitrate per ha, or a total of 60 kg ha^{-1} N at wheat tillering and forking. The number of nodules and dry matter mass of pea root nodules were determined on five plants at the initial stage of pod formation of winter pea cv. Maksimirski rani (on 30/05/2005 and 29/05/2006). After separating nodules from pea roots, their dry matter was determined by drying at 105 °C. Samples of pea plants were taken from the ground to 30 cm depth in each variant and replication.

Crops were harvested on 10/07/2005 and 12/07/2006. Upon harvest, the mixture was separated into pea and wheat, and then the yield was determined. Pea seed chemical composition (crude protein - Weende analysis) was determined in all variants on seed dry matter of cv. Maksimirski rani at the Department of Field Crop Production, Faculty of Agriculture.

Research results were processed using the statistical program SAS (SAS Institute, 1999).

Results

Number of root nodules per pea plant

The number of root nodules was determined on 5 pea plants and recalculated per pea plant (Table 2). In the first trial year, the highest number of root nodules was determined in the inoculated variant 2 (44), and the lowest number in the calcium ammonium nitrate (CAN) top-dressed variant 3 (36). In the second trial year, the highest number of root nodules was determined in the inoculated variant 2 (42), and the lowest number in the calcium ammonium nitrate top-dressed variant (31). In both trial years, the highest number of root nodules was determined in the inoculated variant 2, and the lowest number in the calcium ammonium nitrate top-dressed variant 3.

A significantly higher number of root nodules was on average recorded in the inoculated pea variant 2 (43) compared to other trial variants. Significant differences in the number of root nodules were also found between variant 3 (33.5) and variant 2 (43) (Table 2). Year x variant interaction in the number of root nodules was also significant. In the first trial year, all variants had a higher number of root nodules compared to the same variants in the second trial year.

Nodule dry matter mass (g/plant)

Dry matter mass of root nodules was measured on 5 pea plants by drying at a temperature of 105 °C to constant mass and expressed per pea plant (Table 3). The highest dry matter mass of root nodules in the first trial year was determined in the inoculated variant 2 (0.231 g), and the lowest mass in the calcium ammonium nitrate (CAN) top-dressed variant 3 (0.175 g). In the second year, the highest dry matter mass of root nodules was also determined in the inoculated variant 2 (0.208 g), and the lowest in the calcium ammonium nitrate top-dressed variant 3 (0.157 g).

Significantly higher dry matter mass of root nodules was on average recorded in the inoculated pea variant (0.219 g) compared to other variants. A significant difference in dry matter mass of root nodules was also recorded between variant 1 (0.182 g) and variant 3 (0.166 g) (Table 3).

Table 3. Nodule dry matter mass g/plant
Tablica 3. Masa suhe tvari kvržica (g/biljci)

Variant Varijanta	Nodule dry matter mass g/plant Masa suhe tvari kvržica g/biljka		
	Year/Godina		Variant average Prosjek varijanata
	2005	2006	
Control/Kontrola	0.198	0.167	0.182
Inoculation/Bakterizacija	0.231	0.208	0.219
Nitrogen Top-dressing Prihrana dušikom	0.175	0.157	0.166
Year average Prosjek godina	0.201	0.177	
LSD 0.05			0.006 g
LSD 0.05 †	0.009 g		
LSD 0.05 ‡	0.012 g		
			Significance Signifikantnost
Year/Godina			**
Variant/Varijanta			**
Year x variant/Godina x varijanta			**

†values for means within year comparison/za usporedbu srednjih vrijednosti unutar godine
‡values for means across years comparison/za usporedbu srednjih vrijednosti između godina

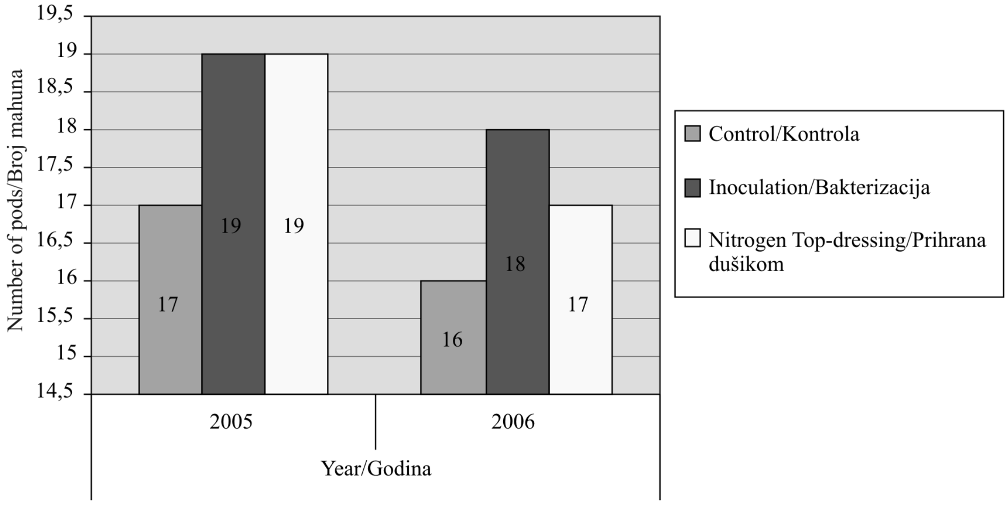

Fig. 1. Number of pods per pea plant
Grafikon 1. Broj mahuna po biljci graška

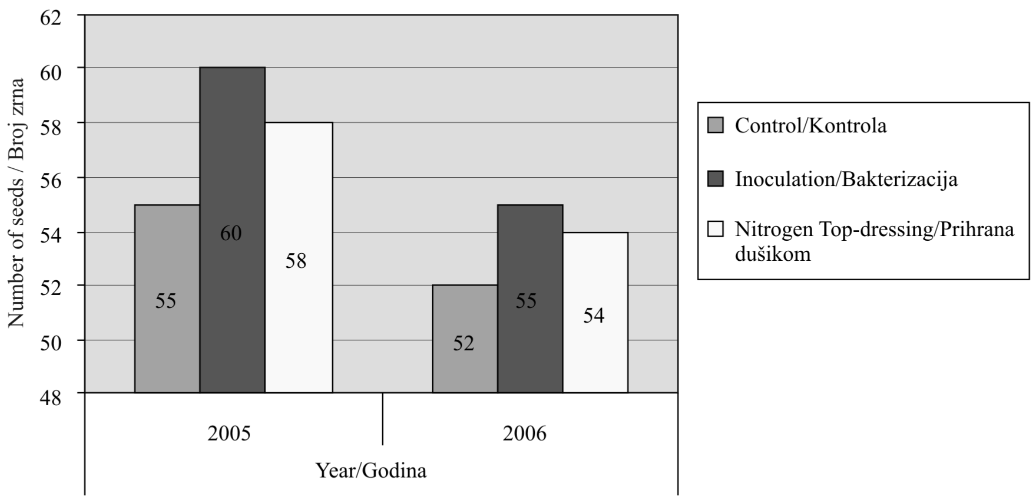

Fig. 2. Number of seeds per pea plant
Grafikon 2. Broj zrna po biljci graška

The year x variant interaction for dry matter mass of root nodules was significant. In the first year, all trial variants produced higher dry matter mass of root nodules compared to the same variants in the second trial year.

Number of pods per pea plant

In the first year, the inoculated variant 2 (19) had a higher number of pods per pea plant (Fig. 1) than control 1 (17) but not than the nitrogen top-dressed variant (19). In the second year, the inoculated variant 2 (18) had a higher number of pods per pea plant than control 1 (16) and the nitrogen top-dressed variant 3 (17).

Number of seeds per pea plant

In the first trial year, the inoculated variant 2 (60) produced a higher number of seeds per pea plant (Fig. 2) compared to control 1 (55) as well as compared to the nitrogen top-dressed variant (58).

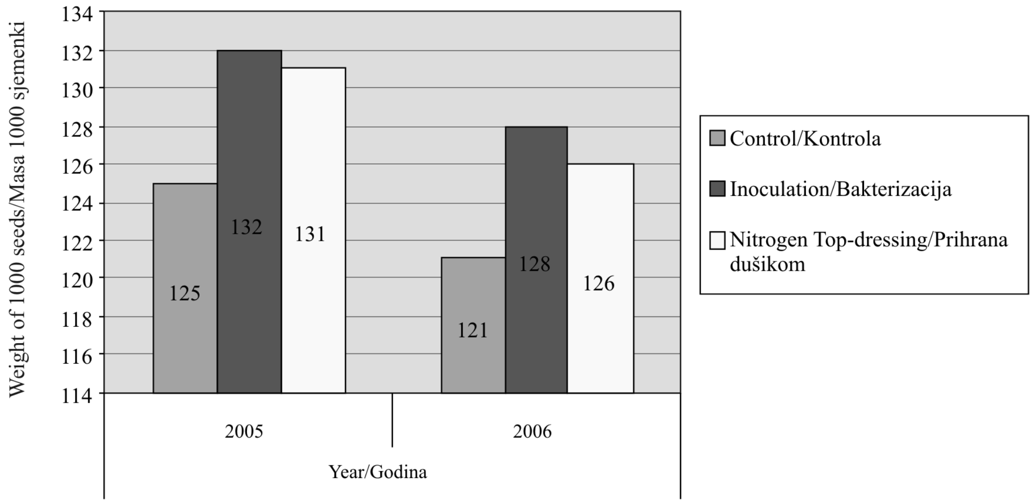

Fig. 3. Weight of 1000 seeds (g)
Grafikon 3. Masa 1000 sjemenki (g)

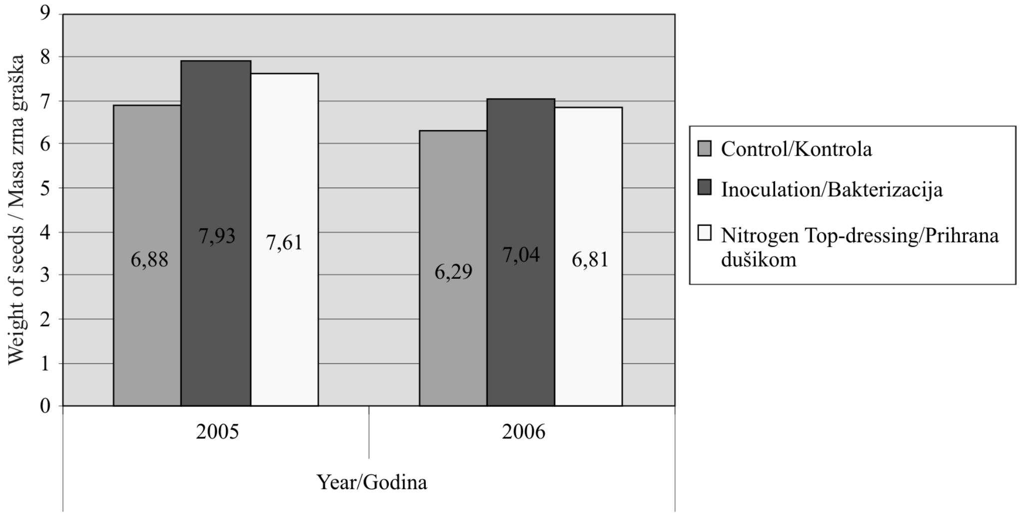

Fig. 4. Weight of seeds per pea plant (g)
Grafikon 4. Masa zrna graška po biljci (g)

In the second trial year, the inoculated variant 2 (55) produced a higher number of seeds per pea plant than control 1 (52) and the nitrogen top-dressed variant 3 (54).

Weight of 1000 seeds

In the first trial year, the inoculated variant 2 produced higher 1000-seed mass (132 g) compared to control 1 (125 g) as well as compared to the nitrogen top-dressed variant 3 (131 g) (Fig. 3). In the second trial year, the inoculated variant 2 produced again higher 1000-seed mass (128 g) than control 1 (121 g) and the nitrogen topdressed variant 3 (126 g).

Weight of seeds per pea plant (g)

In the first trial year, the inoculated variant 2 had higher seed mass per pea plant (7.93 g) compared to control 1 (6.88 g) as well as compared to the nitrogen top-dressed variant (7.61 g) (Fig. 4). In

Table 4. Winter pea seed yield, cv. Maksimirski rani, kg ha^{-1}
Tablica 4. Prinos zrna graška cv. Maksimirski rani u kg ha^{-1}

Variant Varijanta	Winter pea seed yield, cv. Maksimirski rani kg ha^{-1} Prinos zrna graška cv. Maksimirski rani u kg ha^{-1}		
	Year/Godina		Variant average Prosjek varijanta
	2005	2006	
Control/Kontrola	3129	2768	2949
Inoculation/Bakterizacija	3607	3098	3353
Nitrogen Top-dressing Prihrana dušikom	3514	2930	3222
Year average Prosjek godina	3417	2932	
LSD 0.05			72 kg ha^{-1}
LSD 0.05 †	121 kg ha^{-1}		
LSD 0.05 ‡	182 kg ha^{-1}		
			Significance Signifikantnost
Year/Godina			**
Variant/Varijanta			**
Year x variant/Godina x varijanta			**

†values for means within year comparison/za usporedbu srednjih vrijednosti unutar godine
‡values for means across years comparison/za usporedbu srednjih vrijednosti između godina

the second trial year, the inoculated variant 2 had again higher seed mass (7.04 g) than control 1 (6.29 g) and the nitrogen top-dressed variant 3 (6.81 g).

Seed yield of pea cv. Maksimirski rani

In the first trial year, the inoculated variant 2 gave a significantly higher seed yield per unit area (3607 kg ha^{-1}) compared to control 1 (3129 kg ha^{-1}) but not compared to the nitrogen top-dressed variant 3 (3514 kg ha^{-1}) (Table 4). Differences in seed yields between the nitrogen top-dressed variant 3 (3514 kg ha^{-1}) and control 1 (3129 kg ha^{-1}) were significant.

In the second trial year, the inoculated variant 2 gave again a significantly higher seed yield (3098 kg ha^{-1}) compared to control 1 (2768 kg ha^{-1}) as well as compared to the calcium ammonium nitrate (CAN) top-dressed variant 3 (2930 kg ha^{-1}). Significant differences in seed yields were also in that year determined between the nitrogen top-dressed variant 3 (2930 kg ha^{-1}) and the control (2768 kg ha^{-1}).

Inoculated variant 2 gave on average a significantly higher seed yield per unit area (3353 kg ha^{-1}) compared to the control variant (2949 kg ha^{-1}) as well as compared to the calcium ammonium nitrate top-dressed variant 3 (3222 kg ha^{-1}). Nitrogen top-dressed variant 3 gave a significantly higher seed yield (3222 kg ha^{-1}) than control 1 (2949 kg ha^{-1}). Year x fertilization interaction in pea seed yield was significant. Higher pea seed yield was obtained in 2005 compared to 2006.

Crude protein content of cv. Maksimirski rani seed in g kg^{-1}

In the first trial year, the inoculated variant 2 had a significantly higher seed crude protein content (246.6 g) compared to control 1 (235.7 g) as well as compared to the nitrogen top-dressed variant (246.4 g) (Fig. 5). In the second trial year, the inoculated variant 2 (250.4 g) had again a higher seed protein content than control 1 (239.5 g) and the nitrogen top-dressed variant 3 (249.5 g).

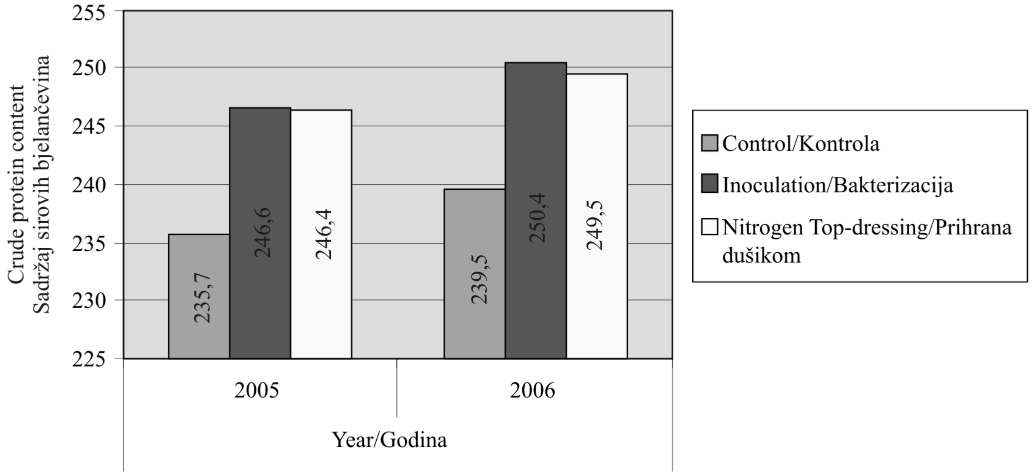

Fig. 5. Seed crude protein content of cv. Maksimirski rani in g kg^{-1}
Grafikon 5. Sadržaj sirovih bjelančevina u zrnu cv. Maksimirski rani u g/kg

Table 5. Crude protein yield of cv. Maksimirski rani (kg ha^{-1})
Tablica 5. Prinos sirovih bjelančevina cv. Maksimirski rani u kg ha^{-1}

Variant Varijanta	Crude protein yield of cv. Maksimirski rani (kg ha^{-1}) Prinos sirovih bjelančevina cv. Maksimirski rani (kg ha^{-1})		
	Year/Godina		Variant average Prosjek varijanata
	2005	2006	
Control/Kontrola	738	663	701
Inoculation/Bakterizacija	889	776	833
Nitrogen Top-dressing Prihrana dušikom	866	731	799
Year average Prosjek godina	831	723	
LSD 0.05			90 kg ha^{-1}
LSD 0.05 †	73 kg ha^{-1}		
LSD 0.05 ‡	127 kg ha^{-1}		
			Significance Signifikantnost
Year/Godina			**
Variant/Varijanta			**
Year x variant/Godina x varijanta			**

†values for means within year comparison/za usporedbu srednjih vrijednosti unutar godine
‡values for means across years comparison/za usporedbu srednjih vrijednosti između godina

Crude protein yield of cv. Maksimirski rani (kg ha^{-1})

In the first trial year, the inoculated pea variant gave a significantly higher yield of crude proteins (889 kg ha^{-1}) compared to control 1 (738 kg ha^{-1}) but not compared to the nitrogen top-dressed variant 3 (866 kg ha^{-1}) (Table 5). Differences in crude protein yields between the nitrogen top-dressed variant 3 (866 kg ha^{-1}) and control 1 (738 kg ha^{-1}) were significant. In the second trial year, the inoculated variant 2 gave a significantly higher yield of crude

Table 6. Seed yields of cv. Maksimirski rani potentially cover the needs of production of milk with 4% fat and 3.4% proteins in kg ha^{-1}

Tablica 6. Utvrđeni prinosi zrna graška cv. Maksimirski rani potencijalno namiruju potrebe za proizvodnju mlijeka s 4% mm i 3,4% bjelančevina u kg ha^{-1}

Variant Varijanta	Crude proteins Sirove bjelančevine			Energy, MJ NEL Energija, MJ NEL		
	Year/Godina		Variant Average	Year/Godina		Variant Average
	2005	2006		2005	2006	
Control/Kontrola	10086	9061	9580	7173	6345	6759
Inoculation/Bakterizacija	12150	10606	11378	8269	7102	7685
Nitrogen Top-dressing Prihrana dušikom	11836	9991	10913	8056	6717	7386
Year average Prosjek godina	11357	9886	10621	7833	6721	7277

proteins (776 kg ha^{-1}) compared to control 1 (663 kg ha^{-1}), but not compared to the calcium ammonium nitrate (CAN) top-dressed variant 3 (731 kg ha^{-1}). No significant differences in crude protein yields of cv. Maksimirski rani were determined in that year between variant 3 and the control. Inoculated variant 2 had on average a significantly higher yield of crude proteins (833 kg ha^{-1}) compared to the control variant (701 kg ha^{-1}) but not compared to the calcium ammonium nitrate (CAN) top-dressed variant 3 (799 kg ha^{-1}).

Topdressed variant 3 gave a significantly higher yield of crude proteins (799 kg ha^{-1}) than control 1 (701 kg ha^{-1}). Year x fertilization interaction in pea crude protein yield was significant. Higher crude protein yield of cv. Maksimirski rani was recorded in 2005 compared to its yield in 2006.

Milk quantity produced from pea seed, cv. Maksimirski rani, in kg ha^{-1}

Pea seed digestibility is high (82 % DLG 1997) and its energy value is 8.53 MJ NEL. In the two-year average, pea seed yields satisfy the digestible protein requirements of 10621 kg ha^{-1} milk, and energy requirements of 7277 kg ha^{-1}. As per its proteins and energy, the seed yield of the inoculated variant can potentially produce more milk with 4% fat and 3.4% proteins compared to the nitrogen top-dressed variant and control.

Discussion

The constant growth of world population increases the demand for food. To satisfy higher food demand, better quality and higher-yielding cultivars are created, and more rational, fossil energy saving solutions are sought. To achieve high and good quality yields, large amounts of oxygen should be provided for forage plants. As plants of the legume family live in symbiosis with bacteria of the family *Rhizobium*, which bind atmospheric nitrogen, of which there is about 6400 kg per each hectare (FAO 1984), they satisfy their nitrogen requirements by this fixation, using solar energy. This symbiotic nitrogen fixation is attracting increasing interest and numerous investigations are conducted worldwide in order to select the most efficacious symbiotic association of legume cultivars and bacterial strains. For this purpose, research was carried out at the Faculty of Agriculture with the strain *Rhizobium leguminosarum bv. viciae* 1001 from the collection of the Department of Microbiology, which was used to inoculate winter pea seed aiming to determine the efficacy of the symbiotic relationship of that strain and the new winter pea cultivar Maksimirski rani.

The highest number of root nodules per plant (43) of the new winter pea cultivar was on average determined in the inoculated variant, which is in accord with the results of Uher et al. (2006abcd), who also recorded the highest number of root nodules of cv. Maksimirski ozimi inoculated with the strain *Rhizobium leguminosarum bv. viciae* 1001. On two studied soil types (humogley, semigley-chernozem luvic) Brkić et al. (2004) determined an increase in the number of root nodules of spring pea cv. Sobel in the control variant (variant without seed inoculation with *Rhizobium leguminosarum bv. viciae*) up to increasing the nitrogen rate of 80 kg ha^{-1}. At the nitrogen rate of 120 kg ha^{-1}, the same authors recorded a decrease in the number of pea root nodules. They determined the highest number of pea root nodules in the variant inoculated with *Rhizobium leguminosarum bv. viciae* on humogley fertilized with 40 kg ha^{-1} nitrogen, and up to increasing the nitrogen rate of 80 kg ha^{-1} on semigley-chernozem luvic.

Uher et al. (2006e) determined the highest number of nodules of G_4 pea genotype in the inoculated variant (40) in the first trial year, 37 in the second and 40 in the third trial year. Jarak (1989) determined from 16 to 44 nodules on the roots of 1 pea plant. Peenstra (1980) determined 16 do 59 nodules per plant of the same pea cultivar inoculated with different strains of *R. leguminosarum*. Nutman (1976) counted 13 to 85 nodules, and Lie (1981) 16 do 68 nodules per plant also in dependence on the *R. leguminosarum* strain.

Using *R. leguminosarum* strains, Lie (1969) determined from 90 to 170 nodules per plant, while Sprent (1976) found from 78 to 111 nodules, and Winarno (1979) from 14 to 100 nodules.

Inoculated variant had on average the highest dry matter mass of root nodules per plant (0.219 g) compared to other variants, which is in agreement with two-year research done by Uher et al. (2006abcd), who determined on average the highest dry matter mass of nodules in the inoculated variant compared to other trial variants. Santalla et al. (2001) found that the dry matter mass of nodules was lower (0.16 g) when pea was fertilized with 150 kg ha^{-1} of nitrogen compared to the nitrogen rate of 30 kg ha^{-1} (0.18 g). Brkić et al. (2004) determined the highest dry matter mass of root nodules of spring pea cv. Sobel in the control variant on two soil types (humogley, semigley-chernozem luvic) up to increasing the nitrogen rate of 80 kg ha^{-1}. At the nitrogen rate of 120 kg ha^{-1}, these authors recorded a decrease in dry matter mass of root nodules. In the variant inoculated with *Rhizobium leguminosarum bv. viciae*, the highest dry matter mass of root nodules was determined on humogley up to increasing the nitrogen rate of 40 kg ha^{-1}, and up to increasing the nitrogen rate of 80 kg ha^{-1} on semigley-chernozem luvic.

Inoculated variant 2 (18.5) had on average a higher number of pods per pea plant compared to the control variant 1 (16.5), but the same number of pods (18) as the nitrogen topdressed variant 3, which is also in agreement with the results of Brkić et al. (2004), Uher et al. (2006abcd).

Inoculated variant 2 had on average the highest seed number (57.5) and the highest 1000-seed mass (130 g) as well as seed mass per pea plant (7.49 g) compared to other trial variants, which is also in agreement with the results of Brkić et al. (2004), Uher et al. (2006abcd).

Inoculated variant 2 (3353 kg ha^{-1}) gave on average the highest pea seed yield compared to other trial variants, which is in agreement with the results of Brkić et al. (2004), who recorded higher pea yields on inoculated variants compared to variants inoculated and top-dressed with nitrogen.

Inoculated variant 2 (833 kg ha^{-1}) had on average the highest yield of crude proteins compared to other trial variants, which is in agreement with the results of Brkić et al. (2004), who obtained higher crude protein yields in inoculated variants compared to variants inoculated and top-dressed with nitrogen. Inoculated variant 2 (11378 kg ha^{-1}) rendered on average higher milk production than the control and nitrogen top-dressed variants.

In dependence on the cultivar and growth conditions, pea seed contains 10.9% water, 20-30% proteins, 60.7% carbohydrates, 1.4% fat, 1.4 fibers, 2.7% ash (Duke, 1981). Like other legumes, pea contains essential amino acids: lisine, methionine, tryptophane, threonine, valine, phenylalanine, leucine and isoleucine (Gatel et al., 1990). These amino acids are indispensable in human and animal diets. Due to its high protein content and balanced amino acid composition, pea makes valuable forage, the use of which has greatly increased over the last forty years (Zhang, 2004). The value of pea in the current economic ambience of the Republic of Croatia depends mainly on yields, environmental conditions and its acceptance by local farmers as feedstuff for the needs of dairy farms.

Conclusions

Two-year investigations of the efficacy of inoculating winter pea seed, cv. Maksimirski rani, with *Rhizobium leguminosarum bv. viciae* 1001 in a mixture with wheat as carrier, conducted at the Faculty of Agriculture in Zagreb, point to the following conclusions:

Seed inoculation of the pea cultivar cv. Maksimirski rani with the strain *Rhizobium leguminosarum bv. viciae* 1001 had a significant effect on the number of nodules (43), root nodule dry matter mass per plant (0.219 g/plant), seed yield (3353 kg ha^{-1}), pea crude protein content (250.4 g/kg) and crude protein yield of cv. Maksimirski rani (833 kg ha^{-1}) compared to the control variant.

Nitrogen top-dressing had a significant effect on the reduction of the number of root nodules (33.5) and nodule dry matter per plant (0.166 g) compared to the inoculated and control variants.

Seed inoculation of the studied pea cultivar with the strain *Rhizobium leguminosarum bv. viciae* 1001 influenced also a higher number of pods (18.5), seeds (57.5), 1000-seed mass (130 g) and seed mass per pea plant (7.49 g) compared to the control and the nitrogen top-dressed variant.

Seed inoculation of the studied pea cultivar with the strain *Rhizobium leguminosarum bv. viciae* 1001 influenced also higher milk production per hectare compared to the control and the nitrogen top-dressed variant.

Značenje zrna ozimog graška cv. Maksimirski rani u proizvodnji mlijeka na obiteljskim gospodarstvima

Sažetak

Krmni grašak (*Pisum sativum* L.) postaje sve zastupljenija krmna mahunarka na oranicama Republike Hrvatske. Zrno graška sadrži 20-30% bjelančevina, koristi se bez termičke obrade u ishrani različitih vrsta i kategorija stoke, a uz stabilan prinos osigurava značajan dohodak po hektaru. Dvogodišnjim istraživanjima (od 2005. do 2006.) utvrđivan je utjecaj učinkovitosti bakterizacije sjemena ozimog graška cv. Maksimirski rani i prihrane dušikom na broj i masu suhe tvari kvržica na korijenu graška (g/biljka^{-1}), prinose i kvalitetu zrna graška cv. Maksimirski rani u smjesi s pšenicom cv. Sana. Prije sjetve izvršena je predsjetvena bakterizacija sjemena graška sojem *Rhizobium leguminosarum bv. viciae* 1001 iz zbirke Zavoda za mikrobiologiju Agronomskog fakulteta Sveučilišta u Zagrebu. Ukupno najveći broj kvržica na korijenu graška utvrđen je na bakteriziranoj varijanti (43 kvržica/biljka), kao i masa suhe tvari kvržica (0,219 g/biljka^{-1}). Najveći broj mahuna (19,0) i zrna po biljci graška (60) utvrđen je na bakteriziranoj varijanti. Najveća masa 1000 zrna (132 g) i masa zrna po biljci graška (7,93 g) također je utvrđena na bakteriziranoj varijanti. Prosječni prinosi zrna graška cv. Maksimirski rani iznosili su od 2949 kg ha^{-1} (kontrola) do 3353 kg ha^{-1} (bakterizacija). Na temelju ovih istraživanja možemo zaključiti da je najveći prinos zrna (3353 kg ha^{-1}) i sirovih bjelančevina (833 kg ha^{-1}) ostvaren bakterizacijom zrna ozimog graška cv. Maksimirski rani. Bakterizacija sjemena istraživanog kultivara graška Maksimirski rani sojem *Rhizobium leguminosarum bv. viciae* 1001 utjecala je i na veću proizvodnju mlijeka po hektaru u odnosu na kontrolu i varijantu prihranjivanu dušikom.

Ključne riječi: ozimi grašak, bakterizacija, prihrana dušikom, prinos zrna, kakvoća

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