

Yield components of soybean cover crop regard to seed pre-treatment with bacteria and mycorrhiza

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Abstract: The aim of this study was to describe the importance of bacterization of soybean seeds and the use of preparations of mycorrhizal fungi in the sowing of soybeans on the family farm "Alduk" in 2020. Post-sowing of soybeans was done on 26th June 2020 as cover crop. Two very early varieties (00 maturity group): *Korana* (Agricultural Institute Osijek) and *Merkur* (NS seeds, Serbia) were used. Before sowing, the seeds were bacterized (Nitrobacterin – Faculty of Agrobiotechnical Sciences Osijek) or the addition of preparations of mycorrhizal fungi (VAM + *Azotobacter chroococcum* – Faculty of Agrobiotechnical Sciences Osijek). At harvest, yield components of soybeans in 2020 were determined. To determine the yield components from each treatment, 20 plants were selected and analysed separately. A total of 120 individual plants were analysed, and the following were determined: plant height (cm) and height to the first pod, number of fertile levels per plant from the central stem and per plant, number of pods per plant and seed mass of one plant (g), 1000 grain mass and at final, seed yield (t/ha). The height of the plants up to the first fertile pod was on average 7 cm, and varied from 5 cm (Mercury variety with VAM + AC treatment), to 9 cm (*Korana* on NB treatment). The number of fertile levels per plant averaged 11 on the main, central stem, while the total number of fertile levels per plant was 16. The number of pods per plant in this study averaged 42, with the seed weight of one plant being 10.48 g per plant. The highest mass of seeds per plant (g) had the *Korana* variety on the control treatment (14.95 g per plant). The *Korana* variety also had the lowest seed mass per plant (7.07 g per plant) with the application of Nitrobacterin. According to the results, *Korana* variety had the highest yield on the control treatment (1.19 t/ha), followed by the treatment with VAM + *Azotobacter chroococcum* (1.04 t/ha) and the lowest with the application of Nitrobacterin (0.84 t/ha). *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while with Nitrobacterin and VAM + *Azotobacter chroococcum* soybean yield increased by about 19% with Nitrobacterin and about 27% with VAM + *Azotobacter chroococcum*.

Keywords: Cover crop, soybean, bacteria, mycorrhizae, yield components

Received 27 July 2021, Revised 3 November 2021, Accepted 4 November 2021

Introduction

One of the biggest advantages of legumes is the symbiosis with bacteria that fix nitrogen from the atmosphere to the soil, which results in less nitrogen-fed mineral form, and over time reduces the cost of agricultural production. Legumes are a great pre-crop for small grains because they leave a lot of nitrogen in the soil that the next crop can use (Tucak et al., 2019). The most important representatives of legumes are soybeans, beans, peas and beans. Due to their nutri-

tional value, chemical composition and large amounts of protein grain and legume fruit are often used in human nutrition (Matoša Kočar et al., 2020).

Soybean, *Glycine max* (L.) Merrill, is a legume plant native to East Asia, where it has followed exceptional nutritional value for centuries as a food and medicine. It is grown from 20° to 60° north latitude. The agrotechnical significance of soybeans is in its symbiotic relationship with the nitrous nodule bacteria *Bradyrhizobium japonicum*, which through a natural process fixes inor-

ganic nitrogen (N_2) from the air and converts it into an ammoniacal form (NH_4^+) that approaches plants in exchange for carbohydrates (Lupwayi et al., 2000; Tokgöz et al., 2020). In this way, the need for mineral nutrition with nitrogen is reduced and enriched for the next crop in the crop rotation, which together significantly reduces production costs, and thus increases profits over time. Since biologically bound nitrogen is not leached from the soil, there is no leaching of nitrate into groundwater and eutrophication (Vratarić and Sudarić, 2000).

Soybeans first appeared in Croatia in 1876 in the area around Dubrovnik and in the north of the country, and were brought by the Austrian biochemist Friedrich Haberlandt (Vratarić and Sudarić, 2008). Until 1981, soybean production was at the level of the whole of Croatia only 3714 ha, which significantly depended on the year and the price on the world market. From 1987 can be considered the initial year of more stable soybean production in the country. In the period until 1997, there was a stabilization of soybean areas on about 20,000 ha, and the largest soybean areas in Croatia are in Slavonia and Baranja. Yields have been increasing from year to year, so since 2000 we have recorded an increase in yields up to 3.1 t/ha compared to the period until 2000, when yields ranged up to 1.4 t/ha. The largest sown area under soybean since 2000 was recorded in 2015, when 88,867 hectares of soybean area were sown. The highest yield was recorded in 2016, when it was 3.1 t/ha. The smallest area under soybeans was sown in 2008 just over 35,700 hectares, and the lowest yield in the period from 2000 to 2017 was recorded in 2003 when it amounted to a small 1.7 t/ha (Central Bureau of Statistics, 2020). The average soybean seed yield in the period from 2015 to 2019 was 3.2 t/ha and the harvested area was more than 83,000 ha (Figure 1).

Nitrogen is one of the most important elements in plant nutrition in agricultural pro-

duction (Basal and Szabó, 2019; Kristó et al., 2020). Nitrogen fixation is one of the five, and at the same time the last phase of nitrogen circulation in nature. It is preceded by nitrogen assimilation, ammonification, nitrification and denitrification. We know two types of nitrogen fixation, namely abiotic and biotic fixation. Biotic fixation is still divided into non-symbiotic and symbiotic. Plants can only absorb it in nitrate and ammonia form. Nitrogen fixation binds atmospheric nitrogen with the help of nitrogen fixing bacteria which are then used by leguminous plants. Nodule bacteria are not the only nitrogen fixatives. Mycorrhizae fungi can also fix nitrogen (Brundrett, 2009). Pre-sowing seed bacterization is a recommended measure in the cultivation of all legumes. It is especially important for soils where soybeans have never been grown or where soybeans have not been grown for a long time. The introduction of nitrogen fixing bacteria into the soil improves its structure, increases the protein content of soybeans, and saves nitrogen fertilizers for the next crop (Deaker et al., 2004). Pre-sowing bacterization is a standard practice in the production of legumes because it provides optimal conditions for creating a symbiotic relationship between nodule bacteria and legumes, which ultimately leads to the adoption of a significant amount of atmospheric nitrogen per hectare per year. Bacterization is considered successful if there are 15-30 well-developed nodules on each soybean plant (Milaković et al., 2012).

Milić et al. (2004) state that the annual share of fixed nitrogen in the yield is high, which justifies the use of highly effective strains in microbiological preparations for inoculation of legume seeds, allows the replacement of nitrogen from mineral fertilizers with biological nitrogen, and has economic and environmental justification. Microbiological preparations for inoculation do not pollute the soil, reduce the use of mineral nitrogen fertiliz-

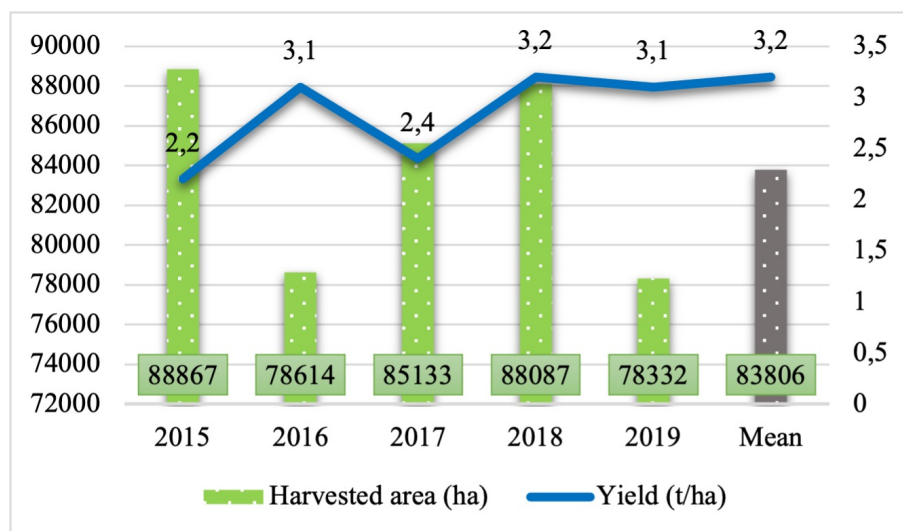


Figure 1: Soybean harvested area and seed yield in the Republic of Croatia (Central Bureau of Statistics, 2020).

ers, contribute to the production of ecologically healthy food, improve soil structure, increase the content of organic matter and positively affect the physical properties of the soil (Stevanović et al., 2017). Olsen (1982) recognizes the improvement of symbiotic nitrogen fixation in soybeans as part of an overall strategy to increase productivity and considers it necessary to pay attention to this in research work. Soybean and other legumes that have short root hairs, so association with mycorrhizal fungi is of paramount importance, resulting in better plant nutrition, such as, for example, the supply of phosphorus in the root (Fialho et al., 2016). Mycorrhizal symbiosis relationship implies that both participants benefit, in turn a particular plant supplies the fungi with photosynthesized food including sugar. In mycorrhiza, better nutrition (fungus promotes the absorption of water, carbon and nitrogen). Furthermore, fungi secrete enzymes that enable faster mineralization of soil organic matter and greater availability of nitrogen. In addition, fungi secrete acids that dissolve and absorb sparingly soluble minerals and transmit them from greater distances to the plant. The plant itself is more resistant and there is a greater

chance that the plant will survive in adverse climatic conditions because hyphae of the fungus act as a specific bioreservoir of water during drought.

The aim of the study was to describe the importance of bacterization of soybean seeds and the use of preparations of mycorrhizal fungi in the sowing of soybeans as a cover crop.

Materials and Methods

Field trail

An experiment of bacterization of soybean seeds and the use of mycorrhizal fungi in the sowing of soybeans was made at the family farm "Alduk" located in the Vukovar-Srijem County in Privlaka (Republic of Croatia). The family farm is managed by the owner of the same name, who took over the family farm founded in 2003 from his father Borislav. Currently, on about 210 ha, soybeans, wheat and corn are mostly sown. Soybeans have been sown for many years and have thus become an inevitable crop on this family farm. However, in 2020, for the first time, sown soybean as a cover crop.

Sowing of cover crop was done after the bar-

ley harvest. The soil was prepared for soybean sowing and sowing was done shortly after the barley harvest, on June 26, 2020. Two very early varieties (00 maturity groups) were used in sowing: *Korana* (Agricultural Institute Osijek, Republic of Croatia) and *Merkur* (NS seeds, Serbia). Before sowing, the seeds treatment were bacterized – NB (NitrobakterinS, Faculty of Agrobiotechnical Sciences Osijek) preparation of mycorrhizal fungi was added – VAM+AC (VAM, Faculty of Agrobiotechnical Sciences Osijek), in combination with *Azotobacter chroococcum* bacteria.

Varieties belonging to 00 maturity group, ie very early maturity group, were used in the experiment. *Korana* variety has a purple flower color, yellow hair color and a dark brown hilum color. According to the height of the stem belongs to the medium-high varieties. The sowing norm for the *Korana* variety is 135 - 145 kg/ha, and the recommended set is 700,000 - 750,000 plants/ha. Genetic yield potential is above 4 t/ha. The oil content in the seeds of the *Korana* variety is 22-23%, and the protein content up to 42% (Agricultural Institute Osijek, 2020). Variety *Merkur* is also a very early variety (00 group). The genetic potential for yield is 5 t/ha. In addition to a stable and high yield, *Merkur* is resistant to lodging, which is recommended so that it can even be irrigated if it is post-sowing. *Merkur* variety has a stem of medium height that is overgrown with brown hairs. The seeds are of medium size with a yellow seed and a hilum of brown color. It can be grown either as the first crop or at later sowing dates, and it is also good as a cover crop after peas and barley because sowing can be done by the end of June. It can also be suitable for late regular sowing, for growing in mountainous areas. The optimal set is 550,000 plants per hectare.

Weather conditions

According to the data of the Croatian Mete-

orological and Hydrological Service (2020), the long term mean temperature (°C) by decades for the Gradište station (1999 - 2018) (Table 1) differed from the average air temperatures (°C) in 2020 by decades for Gradište station. The highest average air temperature (°C) in 2020 per decade for the Gradište station was in August (23.8°C). The highest average rainfall (mm) in 2020 per decade for the Gradište station was in June and averaged 113.1 mm, and the lowest average precipitation in April was 16.7 mm, while in the long term mean of rainfall (mm) per decade for the Gradište station (1999-2018) was the highest in June at 70.93 mm as considers to soybean vegetation period.

At the time of soybean germination in 2020, the highest precipitation fell in the 2nd decade of July 52.6 mm (Table 1). Later in vegetation, the amount of precipitation in 2020 was the highest in the 1st decade of August, 49.8 mm.

At the time of soybean germination in 2020, the average temperatures were 0.3 °C lower than the long term mean for Gradište station (1999-2018), and at the time of sowing in July 2020, the temperatures were for 1.3°C lower compared to the long term mean (Table 1).

Collecting of plant material

The harvest was done on 20th October, 2020. At the harvest, samples of plant material were taken to determine the components of soybean yield and to determine the yield per unit area.

Since the sowing of soybeans was performed at an inter-row spacing of 50 cm, samples were collected at 4 meters in length and from 3 rows (or repetitions) per treatment and separately for each variety. In this way, all plants were collected from one plot of 2 m² in 3 replications. All plants from one replicate that were collected from 4 m in length and from one replicate were placed in one bag next to the label.

Table 1: The mean air temperature (°C) and rainfall (mm) in soybean as cover crop vegetation period in 2020 by decades and the long term montly mean of Meteorological station Gradište (1999 – 2018) (Croatian Meteorological and Hydrological Service, 2020).

Decade	Air-temperature in 2020 (°C)				Rainfall in 2020 (mm)			
	June	July	August	September	June	July	August	September
I.	18.8	23.5	24.0	19.7	79.4	1.5	49.8	0
II.	20.3	19.8	23.9	20.9	8.1	52.6	1.1	0
III.	23.2	24.5	23.7	16.8	0	0	0	12.6
Average/Total	20.5	22.6	23.8	19.2	113.1	72.3	77.2	21.3
Long term mean of air-temperatures (°C)				Long term mean of rainfall (mm)				
Average/Total	21.1	22.9	22.5	17.3	84.28	64.5	50.1	66.4

Table 2: Analysis of Variance (ANOVA) of soybean yield components – model summary.

Source	DF	F-value	P-value	Significance
<i>Plant height</i>				
Genotype	1	10.06	0.002	$p < 0.01(**)$
Seed treatment	2	3.15	0.046	$p < 0.05(*)$
Genotype × Seed treatment	5	6.13	0.000	$p < 0.001(***)$
<i>Plant height to first fertile pod</i>				
Genotype	1	3.55	0.062	<i>ns</i>
Seed treatment	2	2.82	0.064	<i>ns</i>
Genotype × Seed treatment	5	3.82	0.003	$p < 0.01(**)$
<i>Number of pods per plant</i>				
Genotype	1	0.39	0.532	<i>ns</i>
Seed treatment	2	0.88	0.419	<i>ns</i>
Genotype × Seed treatment	5	2.31	0.048	$p < 0.05(*)$
<i>Seed mass per plant</i>				
Genotype	1	1.04	0.309	<i>ns</i>
Seed treatment	2	1.05	0.353	<i>ns</i>
Genotype × Seed treatment	5	3.71	0.004	$p < 0.01(**)$
<i>Thousand grain mass</i>				
Genotype	1	1.55	0.231	<i>ns</i>
Seed treatment	2	17.51	0.000	$p < 0.001(***)$
Genotype × Seed treatment	5	*	< 0.001	
<i>Seed yield</i>				
Genotype	1	9.99	0.006	$p < 0.01(**)$
Seed treatment	2	1.12	0.353	<i>ns</i>
Genotype × Seed treatment	5	*	< 0.001	

To determine the yield components from each treatment, 20 plants were selected and analyzed separately. A total of 120 individual plants were analyzed. The components of

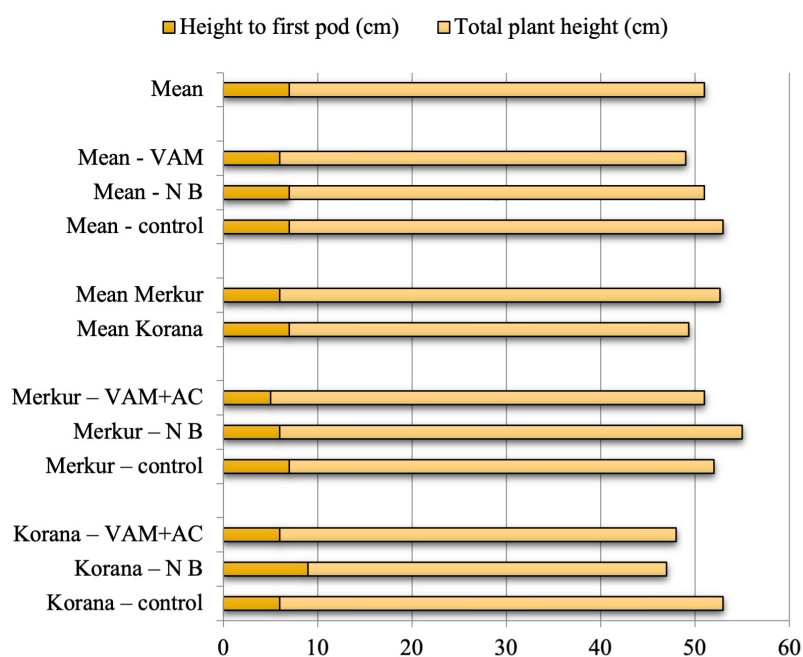


Figure 2: Plant height and height of first fertile pods of soybean cover crop in 2020 with bacteria and mycorrhiza seed pre-treatment.

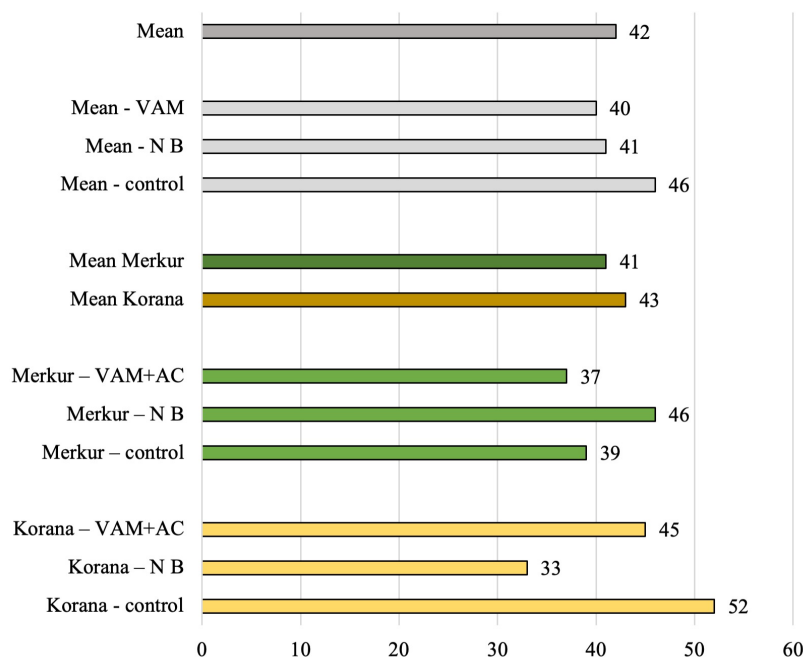


Figure 3: Number of pods per plant of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

soybean yield were determined in the Laboratory for Analysis of Field Crops of the Faculty of Agrobiotechnical Sciences Osijek,

the following components were determined separately for each variety and for each treatment: plant height (cm), plant height to the

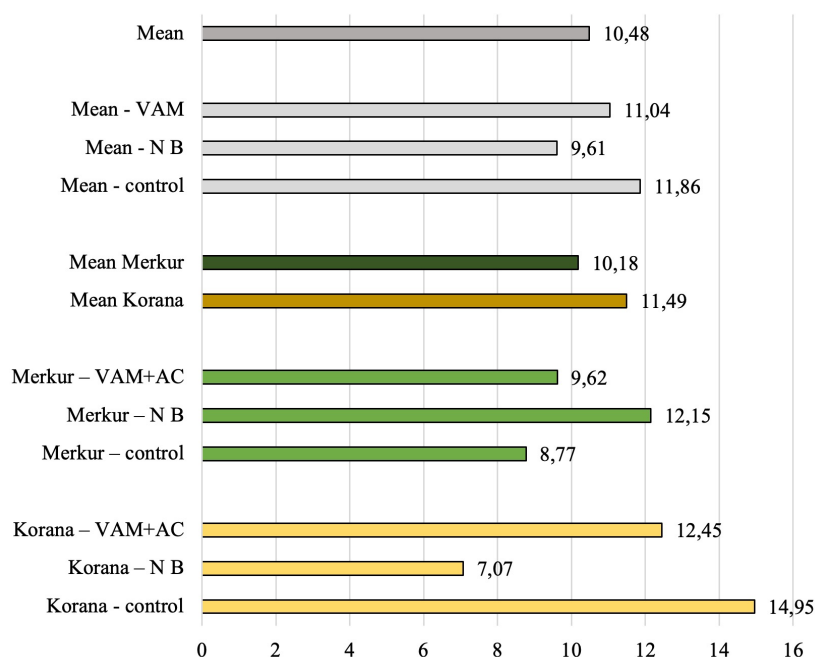


Figure 4: Seed mass per plant of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

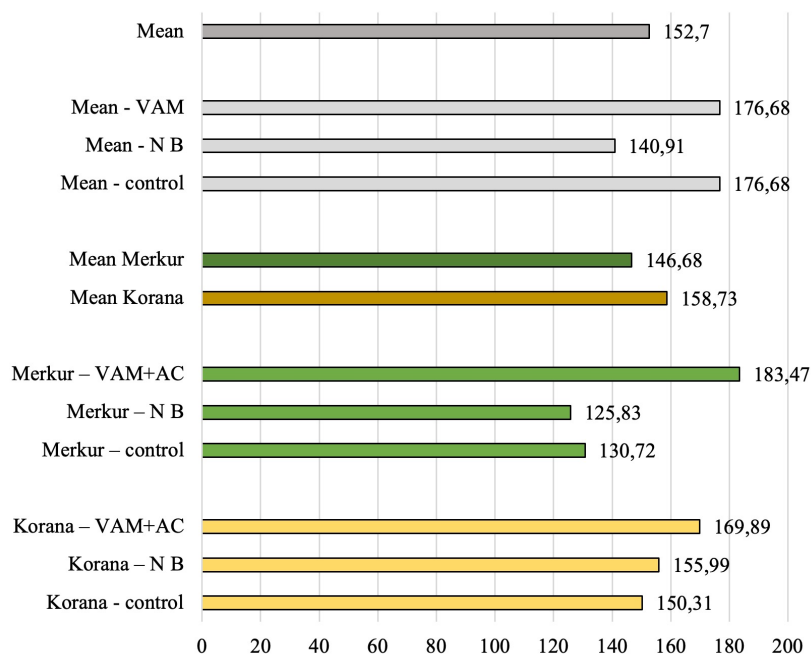


Figure 5: Thousand grain mass of soybean varieties depended of bacteria and mycorrhiza seed pre-treatment.

first pod (cm), number of pods per plant, determine soybean yield and calculated per mass of seeds of one plant and 1000 grains unit area. mass. The seeds of all plants were cleaned to

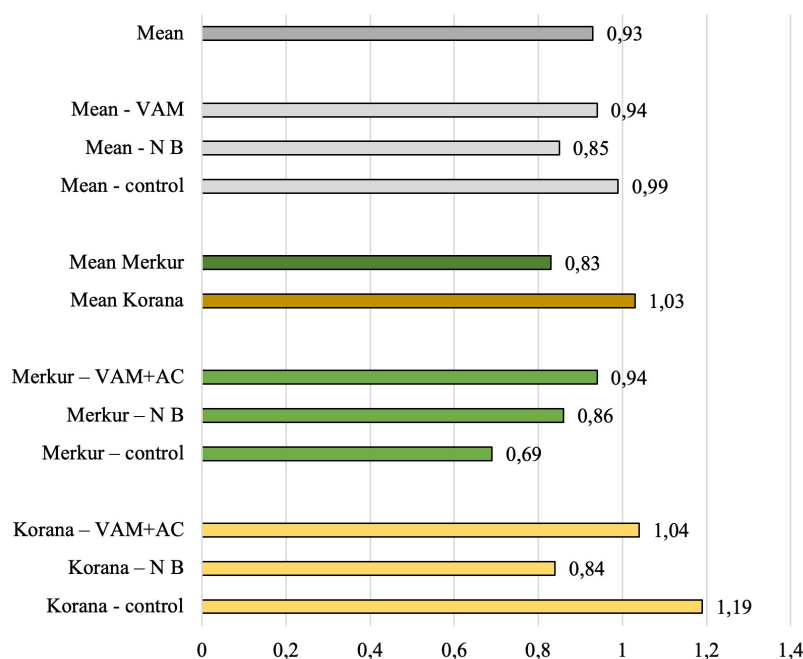


Figure 6: Soybean seed yield (t/ha) of two varieties depended of bacteria and mycorrhiza seed pre-treatment.

Results

In this research, an analysis of the components of soybean soybean yield on the family farm "Alduk" in 2020 was made. The statistical analysis show different significance to each parameter (Table 2). According to certain yield components, the average plant height was 51 cm (Figure 2). According to the average of the variety only, the *Korana* variety had a stem with an average height of 49 cm, and the height to the first pod was on average 7 cm. According to the average of all treatments, the variety *Merkur* had an average stem height of 53 cm. According to the treatments, the highest stem had *Merkur* variety with the use of Nitrobacterin S (average 55 cm), while the lowest stem was with the *Korana* variety with the treatment with mycorrhizal fungi and *Azotobacter chroococcum*. The height of the plants up to the first fertile floor was on average 7 cm (Figure 2), and varied from 5 cm (*Merkur* variety with VAM + AC treatment), to 9 cm (*Korana* on

NB treatment).

Interestingly, the number of fertile pods per plant averaged 11 fertile levels on the main, central stem, so this data was not shown in the figures. However, when we look at the total fertile floors on a plant, on average the plants formed 16 fertile levels. The *Korana* variety had the least fertile floors on the plant in the treatment with Nitrobacterin (14), and the *Korana* variety had the most fertile floors again, but in the VAM + AC treatment.

The number of pods per plant (Figure 3) in this study averaged 42, with the seed weight of one plant being 10.48 g per plant (Figure 4). According to the average, the *Korana* variety had a slightly higher seed weight per plant (11.49 g per plant) compared to the *Merkur* variety (10.18 g per plant). The highest mass of seeds per plant had the strain of the *Korana* variety on the control treatment (14.95 g per plant). The *Korana* variety also had the lowest seed weight per plant (7.07 g per plant) with the application of NitrobacterinS.

The average mass of 1000 grains in this study was 152.701 g (Figure 5). According to the average variety *Korana* had a slightly higher weight of 1000 grains (average 158.73 g), while the variety *Merkur* had an average weight of 1000 grains 146.68 g. If we look at individual treatments, the highest mass of 1000 grains had the variety *Merkur* with VAM and treatment with mycorrhizal fungi and *Azotobacter chroococcum* (183.47 g).

The average soybean seed yield in this study was 0.93 t/ha (Figure 6). According to the conducted research, the variety *Korana* had an average yield of soybean seeds in post-sowing on the family farm "Alduk" in 2020 of 1.03 t/ha, while the variety *Merkur* had an average yield of 0.83 t/ha. If we look at the individual impact of the treatment, the *Korana* variety had the best yield on the control treatment (1.19 t/ha), then on the treatment with mycorrhizal fungi and *Azotobacter chroococcum* (1.04 t/ha), and the lowest with the application of NitrobakterinaS (0.84 t/ha). The *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while the addition of NitrobacterinS and VAM preparation with *Azotobacter chroococcum* soybean yield increased by about 19% with NitrobacterinS, and by about 27% with VAM and *Azotobacter chroococcum* (Figure 6).

Discussion

Soybean as a cover crop can be produced for grain, silage, and in some conditions for green manure. For grain in lateral sowing, varieties of shorter vegetation 000, 00, and 0 maturity group are used at various inter-row sowing intervals depending on climatic conditions. Wang et al. (2011) state that soybean growth was significantly influenced by azotobacteria (*Glomus mosseae*) and rhizobium inoculation (*Bradyrhizobium* sp.), With higher dry matter mass and root-stem ratio being higher.

Siddiqui and Pichtel (2008) point out that mycorrhizae, which are indigenous organisms in the soil rhizosphere, have great potential in organic agricultural production. They have a positive effect on the growth of the root system, and can often control some plant pathogens. Fungal hyphae extend into the soil and secrete extracellular enzymes that efficiently absorb the maximum amount of nutrients available within root cells.

In our study, the Nitrobacterin treatment have positive for *Korana* variety, but it was not the same case for *Korana* variety. Milić et al. (2002) examined the potential for biological nitrofixation in eight different soybean genotypes with the aim of establishing a correlation between symbiotic community efficiency indicators and soybean grain yield. The results showed a positive correlation between grain yield, plant dry matter mass, nitrogen content in the aboveground part of the plant and in nodules, and nodule dry matter mass, from which they concluded that soybean grain yield depends not only on the efficiency of the micro symbiont but also on the genetic potential of the host plant. Differences between soybean genotypes in nitrofixation potential were also confirmed by Sudarić et al. (2008) who in their research also indicate a highly significant positive effect of bacterization on grain yield and soybean grain quality. Brevedan et al. (1978) found that in soybeans, with the increase in the amount of nitrogen available to the plant in the period from the beginning to the end of flowering, the grain yields also increased (in experiments in the greenhouse by 33% and in field experiments by 28-32%).

Keyser and Li (1992) write in their paper that a fully compatible symbiosis of legumes and nodule bacteria arises from the recognition, penetration, stimulation of host plant cells, differentiation of nodule bacteria into bacteroids, synthesis of leghemoglobin and nitrogenase, and nitrogenase activity. The amount of nitrogen bound by symbiotic ni-

trofixation varies considerably and can range from 0% to as much as 97% of the total nitrogen in the plant, but most estimates range from 25 to 75%. Biological nitrogen fixation is improved by selection to improve symbiotic nitrofixation, selection for the ability to nodulate and fix nitrogen in soils with high nitrogen content, development of soybean genotypes that have the ability to limit nodulation by indigenous strains of *B. japonicum*, but which allow nodulation by introduced by inoculation, and the development of soybean genotypes that form a symbiotic community with indigenous strains of *B. japonicum*.

Mycorrhizal fungi play a very important role in terrestrial ecosystems. They preserve the favorable structure of the soil, the circulation of matter in nature, regulate the cycles of carbon and other elements. In natural communities, mycorrhizae are thought to provide up to 80% of nitrogen and phosphorus to plants, and many plant species depend on this association.

According to Wani et al. (2013) bacteria of the genus *Azotobacter* synthesize auxins, cytokinins, and substances similar to gibberellic acid, which are the primary substances that control plant growth. These hormonal substances originating from the rhizosphere or root surface affect the growth of closely related higher plants. In order to guarantee the high efficiency of inoculants and microbiological fertilizers, it is necessary to find compatible partners, i.e. a certain genotype of the plant and a certain strain of *Azotobacter* that will make a good association.

Conclusions

The aim of the study was to describe the importance of soybean seed bacterization and the use of mycorrhizal fungus preparations in post-sowing soybean as a cover crop in 2020

and to determine the impact of seed pre-treatment on yield and yield components. Two varieties *Korana* and *Merkur*, 00 maturity groups were used in the study.

The weather conditions in 2020 did not differ significantly from the multi-year average. The average height of the plant was 51 cm. According to the treatments, the highest stem had the strain of the *Merkur* variety with the use of Nitrobacterin S (average 55 cm), while the lowest stem was with the *Korana* variety with the treatment with mycorrhizal fungi and *Azotobacter chroococcum*. The height of the plants up to the first fertile pod was on average 7 cm, and varied from 5 cm (*Merkur* variety with VAM + AC treatment), to 9 cm (*Korana* on NB treatment). The number of pods per plant averaged 42, with the seed weight of one plant being 10.48 g per plant. In the case of the *Korana* variety, the average number of pods per plant increased by 3.20 for all treatments by increasing one fertile floor per plant, while in the *Merkur* variety the increase was smaller, and with the increase in the number of fertile floors per plant increased by 2.05.

The average soybean seed yield in this study was 0.93 t/ha. According to the conducted research, on average for all treatments, the variety *Korana* had an average yield of soybean seeds as cover crop in 2020 of 1.03 t/ha, while the variety *Merkur* had an average yield of 0.83 t/ha. *Merkur* variety had the lowest soybean yield on the control treatment (0.69 t/ha), while the addition of NitrobacterinS and VAM preparation with *Azotobacter chroococcum* soybean yield increased by about 19% with NitrobacterinS, and by about 27% with VAM and *Azotobacter chroococcum*.

Generally, probably due to low rainfall after sowing and in the summer period, the real effect of mycorrhizae and bacteria missed out due to lack of water.

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