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EFFECTS OF GENOTYPE AND *BRADYRHIZOBIUM* INOCULATION ON MORPHOLOGICAL TRAITS, GRAIN YIELD AND PROTEIN CONTENT OF SOYBEAN VARIETIES

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Soybean crop production in Serbia involves seed inoculation by N-fixing bacteria just before sowing time. The main objective of the current work was to assess the impact of the genotype and inoculation on range of morphological and yield traits of soybean (*Glycine max* L. Merrill), as well as the total protein content. The experiment was conducted on chernozem soil, where soybean was previously grown. The six local varieties were used, where each variety was sown, in three replicates for both inoculated and non-inoculated treatment. The following morphological traits were analysed: the plant height, number of lateral branches, distance to the first pod, number of pods per plant, pods (containing seeds) weight per plant, seed weight per plant, and the total grain yield. The total protein content in seeds was determined by standard analytical method, while subtle differences in qualitative protein composition were assessed using Raman spectroscopy. The total protein content varied from 39.6 to 42.15 %. Performance of inoculation resulted in an increase of the plant height and the distance to the first pod,

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although not in all tested varieties. The highest and the lowest plant height values were observed for non-inoculated variety Dana (59.23cm) and Sava (80.03cm), respectively. The effect of genotype was much more expressed causing differences in almost all tested characters, except for the total protein content. However, Raman spectroscopy analyses revealed distinct discrimination among surveyed varieties, and differences between inoculated and non-inoculated plants in qualitative composition of seed proteins.

Keyword: inoculation, *Glycine max*, varieties, yield, protein content, Raman spectroscopy

INTRODUTION

Soybean (*Glycine max* (L.) Merrill) is among the most grown legume crops, due to its significance in animal and human nutrition, as well as the use as a nutraceutical, i.e. as a source of the "phytoestrogens" and as an ornamental plant (PAGANO *et al.*, 2016). Soybean is an erect herbaceous annual short-day species, which can reach 40 to 100 cm of the total shoot height. The plant is much branched with well-developed taproots. The each plant produces a number of small pods containing 1-4 round-shaped, usually yellowish to black seeds. The three types of the growth habitus are found among soybean varieties: indeterminate, determinate and semi-determinate, which typically occur in both the early and the late maturity group (ALLIPRANDINI *et al.*, 2009; POPOVIĆ *et al.*, 2012; MANGENA, 2018).

Since the second half of the 20th century until now, there is a constant and increasing trend in cultivation of soybean worldwide. During the fifth decade of the 20th century, soybean was grown on 15 million hectares, while in 2019 the crop covered the 125 million hectares globally (https://usda.library.cornell.edu/). In the Republic of Serbia, the average area under the soybean crop amounted for 200.000 hectares in the period 2016-2019 (according to Statistical Bulletin of the R. of Serbia (https://www.stat.gov.rs/sr-cyrl/publikacije/?d=2&r).

Soybean is known as a leguminous oilseed, where the most prominent nutrition quality traits are the seed protein and oil content. Soybean grains contain between 45% and 53% of the high quality proteins rich in essential amino acids, especially the lysine and methionine, in addition to presence of up to 22% of fatty acids with high proportion of the linoleic and linolenic acid (WILSON, 2004; POPOVIĆ *et al.*, 2015; 2016; 2020; SOBKO *et al.*, 2020). The variations in seed chemical composition are inherent among soybean varieties as a consequence of the genotypic variability, but they are also affected by specific environmental conditions and performed management practices (BELLALOUI *et al.*, 2011; ASSEFA *et al.*, 2018).

The high protein content and related nutrition value of the soybean is a typical feature of nitrogen (N)-fixing crops. The soybean roots attract the soil bacteria, primarily belonging to the genus *Bradyrhizobium* able to fix the atmospheric nitrogen (N_2), in the process of symbiotic N fixation supplying soybean plant with nitrogen compounds, while the plant supply *Bradyrhizobium* bacteria with carbohydrates, mainly in the malate form, in return (BISWAS and GRESSHOFF, 2014). During its life cycle, soybean varieties manage to fix on average 58-77% and up to 90% of the nitrogen provided through biological nitrogen fixation, placing soybean among the most efficient N-fixing legume crops (HERRIDAGE *et al.*, 2008). The most frequent, both natural (spontaneous) and intentional soybean inoculation is performed by specific symbiotic soil bacteria, *Bradyrhizobium japonicum* (CARCIOCHI *et al.*, 2019). The inoculation is an optional practice for achievement of the optimal grain yield of legumes on nitrogen deficient soils (e.g. BROCKWELL *et al*., 1989). Since the effects of inoculation with N-fixing bacteria on soybean yield and grain quality are still controversial (e.g. LEGGETT *et al.*, 2017) the main objectives of this study were to evaluate the effect of soybean inoculation strategy on the following traits: (1) morphology of the plant vegetative (total plant height, height up to first pod, and number of branches) and reproductive characters (number of pods, weight of pods and seeds), (2) the total grain yield, and (3) seed grain proteins. The hypothesis of this study is that possible differences in protein content and composition may occur as a consequence of the genotypic variability (i.e. soybean cultivars) and effect of *Bradyrhizobium* inoculation. To the best of our knowledge, the current study is the first report on use of the Raman spectroscopy in assessment of the soybean grain proteins. Raman spectroscopy is known as a non-destructive, rapid and prominent vibrational spectroscopy analytical tool (e.g. YANG and YING, 2011), enabling reliable and accurate information on physico-chemical and some other relevant properties of food and natural products (YANG and YING, 2011). Chemometrics-based Raman spectroscopy has been quite recently applied in food quality assessment in research studies and at industrial scale (QIN *et al*., 2010).

The main objective of the current study was to assess the impact of the genotype and inoculation on range of morphological and yield component traits, as well as the total protein content on chernozem soil type in Serbia.

MATERIALS AND METHODS

Plant material - soybean varieties

The six domestic soybean varieties created at the Institute of Field and Vegetable Crops in Novi Sad, and Delta Selsem Company were used in the experiment. The soybean varieties belong to two different maturity groups, i.e. 0 and I maturity group (MGs). Varieties of the Institute of Field and Vegetable Crops Novi Sad were: Galina, Princess (0 maturity group), and Sava (I maturity group). The varieties Dana, Dukat (0 maturity group), Galeb (I maturity group) were obtained from Delta Selsem company.

Site selection and experiment design

The experiment was set up in the area of south Banat district, at the experimental field of the Institute AIC "Tamiš" in April 2015. The experiment was performed on the chernozem soil type, which is characterized by high fertility and favourable physical and chemical properties. Sowing of 6 soybean genotypes was conducted in in experiment consisting of six inoculated treatmants and their control treatmats (without inoculation). Each genotype (variety) was inoculated with bacterial strain *Bradyrhizobium japonicum* (strain inoculant from Institute of Soil Science). Genotypes were sown in the three repetitions (experimental units) where each experimental. Experimental plot (plot size per genotype was 10 m^2) was structured in four rows: two central rows were used for experimental purposes, while the first and the fourth served for isolation. The distance between rows within each experimental plot and between the experimental units was 0.5 m. The distance between plants in the row was dependent on the soybean maturity group (MGs), where the distance between plants of MG 0 was 4 cm (125 plants per row), and of MG 1 was 4.5 cm (113 plants per row). The total plot area was 420 m^2.

Soil analyses

Chernozem belongs to automorphic soils of good water, air and temperature regime. Soil reaction (pH) was determined according to the potentiometric method; total nitrogen was determined by the Kjeldahl method, available nitrogen by the Bremner method and available P and K were determined by the Al-method of Egner-Riehm (EGNER & RIEHM, 1958). Humus content was determined as a modification of Turin Simakov (ISO 14235: 1998). Based on the analysis of the soil used for the experiment, the pH was 7.30. The soil contained significant humus content, high carbonate content, and it was well provided with nitrogen, phosphorus and potassium (Table 1). The analyses were performed in the Institute for Soil Science, Belgrade. The soil samples were randomly collected before soybean sowing and were treated as a bulk sample which was used for agrochemical and microbiological evaluation.

Table 1. Agrochemical properties of soil

Parameter	pH		$CaCO_3$	Humus	Total N	Available	
	nKCl	H_2O	%	%	%	P_2O_5 mg/100 g	K_2O mg/100 g
	7.4	8.1	12.1	3.43	0.23	19.7	16.4

Microbiological analysis of soil of experimental field confirmed the high number of free living of N-fixers (*Azotobacter* spp. and oligonitrophiles) within the entire microbial flora, which is a general feature of the chernozem soil, in addition to the fact that the parcel was continuously used for soybean production (Table 2).

Table 2. Microbiological properties of chernozem soil (values expressed per gram of absolutely dry soil)

Parameter	Total number of microorganisms ($x10^6$ CFU g^{-1})	*Azotobacter* (x MPN g^{-1})	Free N-fixing microorganisms ($x10^5$ CFU g^{-1})
	10.33	173.75	49.00

Meteorological data

Meteorological data for the year when experiment was conducted (2015), including the rainfall pattern were downloaded from www.hidmet.sr.gov.rs (Table 3).

Table 3. Basic meteorological data for soybean vegetation period in year 2015

Month	April	May	Jun	July	August	September	Average values
Precipitation (mm)	25	88.2	20.1	4.8	69.1	86.4	48.93
Temperature (°C)	11.9	18.5	23.3	27.5	25.5	20.9	21.27

The sum of precipitation during the vegetation period from April to September was 293.6mm. Such amount of precipitation was significantly lower from required amounts, since was stated that 430-450 mm are optimal for soybean cultivation (SREBRIĆ AND PERIĆ *et al*., 2014). Average temperatures during the vegetation period April-September were above the multi-year average value. Based on meteorological conditions, it is clear that the plants were exposed to drought stress during the June and July.

Plant morphological traits

To examine the morphological parameters, 30 plants per genotype (10 plants, 3 replications) were harvested at the end of the vegetation period, in the phase of the full grain maturity. The following traits were evaluated: the plant height (cm), plant height to the first pod (cm), number of branches per plant, number of internodes, number of pods per plant, weight of pods per plant (g plant^{-1}), weight of seeds per plant and total grain yield (kg ha^{-1}).

Total protein content in soybean seeds

Determination of protein content in soybean seeds was performed by the method of Kjeldahl (AOAC 976.05).

Raman spectroscopy analysis

Raman microspectroscopy was performed using XploRA Raman spectrometer (Horiba Jobin Yvon), with laser at a wavelength of 532 nm equipped with a 600 lines/mm grating. Spectra were accumulated from 5 scans, during 5s. In order to consider possible sample inhomogeneity, at least twenty Raman spectra were recorded using single point Raman measurements at 50x objective lens for each sample. The spectra were recorded in the range between 1200 and 1700 cm^{-1}. All recorded measurements were with 6 cm^{-1} spectral resolution. The Raman spectra acquisitions were managed by the LabSpec 6.0 software (Horiba Jobin Yvon). All spectra are smoothed (13 smoothing points), linear baseline corrected and unit vector normalized. Pre-treatment analysis of the spectra was performed using the software The Unscrambler X version 10.4 (Camo Software, Oslo, Norway).

Statistical analysis

The obtained results were expressed as mean values ± standard deviation (SD). In order to understand if there is an effect of factors (genotype and treatment) and their interactions (genotype x treatment) on dependent variables, the two-way Analysis of variance (ANOVA) was performed. ANOVA was followed by Duncan post-hoc test for multiple comparisons ($p<0.05$). Data were analysed with SPSS 26.0 (SPSS, Inc., Chicago, IL) software. Intensity of Raman bands are compared by t-test for independent samples.

RESULTS AND DISCUSSION

Impact of the genotype and Bradyrhizobium inoculation on soybean morphological traits and total grain yield

Many studies indicated that continuous several-years cultivation of inoculated soybean usually did not result in any significant differences in morphological traits and yield between inoculated and non-inoculated soybean plants, due to the already established structure of the symbiotic bacteria populations in the soil (ALBAREDA *et al*., 2009; DE BRUIN *et al*., 2010; DE LUCA *et al.*, 2014; CARCIOCHI *et al.*, 2019). However, the effects of inoculation are still debatable, implying the possible mutual relations among specific rhizobium strains and certain host genotype (e.g. ALTHABEGOIT *et al*., 2008), in addition to the sensitivity of symbiosis to different environmental conditions, soil characteristics and management practices (e.g. FEREIRA *et al*., 2000). For example, in tropic regions with already established *Bradyrhizobium* populations, soybean cultivars didn't exhibit considerable yield responses, while in the experiments where soybean was sown for the first time, the yield was significantly higher due to the effect of inoculation (RUIZ DIAZ *et al*., 2009; DE LUCA *et al*., 2014).

The highest average number of pods per plant was observed in the non-inoculated variety Galeb (63.10). Inoculation did not significantly affect the number of pods per plant in none of the studied varieties (Table 4). According to the study of MORETTI *et al*. (2018) there were no differences in the number of pods per plant compared between inoculated and non-inoculated soy plants, similarly to our results. Nevertheless, LAMPTEY *et al.* (2014) showed significantly higher values in number of pods in inculcated soybean variety, similarly to results of NTAMBO *et al*. (2017) and EZEKIEL (2017). It was shown that soybean inoculation may improve some yield components if crop was not previously cultivated on the experimental plot (SOLOMON *et al*., 2012).

The highest value of the pods weight (containing the seeds) per plant was noticed in the non-inoculated variety Galeb (the value of 27.88) (Table 4). Similarly to our results, it was shown that inoculated plants haven't exhibited higher values of pod weight compared with the non-inoculated plants in experiment conducted on previously grown soybean (ADEYEYE *et al*., 2017). However, some studies revealed the positive effects of inoculation on the seed pod weight (EZEKIEL 2017; LAMPTEY *et al*., 2014; ALAM *et al*., 2015; NTAMBO *et al*., 2016).

The inoculated variety Galeb had the highest average value of the seed weight per plant (19.30) (Table 4). Significant differences among the tested varieties were not determined for this trait, as well as for the inoculation effect. Contrary to our results, the experiment of ADEYEYE *et al*. (2017), showed that seed weight per plant in inoculated plants has a significantly higher value compared to non-inoculated plants. STEVANOVIĆ *et al.* (2016) showed that with seed inoculation with NS Nitragin achieved the highest average weight seed per plant (7.59 g) for growing localities, in variants with the use of nitrogen fertilizers from 100 kg ha^{-1}. Without seed inoculation achieved the highest average weight seed per plant (7.28 g), for tested localities growing, in variants with the highest quantities of nitrogen, 150 kg ha^{-1}.

The highest average value of the soybean height was obtained in the non-inoculated variety Sava (value of 80.03 cm) (Table 4). Significant differences in plant height were found among varieties Galina, Dana and Princess, where inoculation had statistically significant effect on plant height. However, for varieties Dukat, Sava and Galeb no differences in plant height were observed, independently on inoculation. Some recent investigations indicated that differences in plant height were predominately related to genotype rather to inoculation (ADEYEYE *et al.*, 2017; SAMUDIN *et al.*, 2018). It was shown that in case of former continuous soybean production there are not remarkable effects of inoculation on the plant height (MORETTI *et al.*, 2018; CARCIOCHI *et al.*, 2019). The controversial effects of inoculation could be confirmed by report of ALAM *et al.* (2015) showing that inoculation significantly influenced the plant height of all inoculated soybean varieties.

For the morphological parameter number of lateral branches, the highest value was observed for the inoculated variety Dukat (average value is 3.63) (Table 4). However, significant differences for that parameter were not determined among varieties and inoculation had no an impact as well. Similar results were obtained by BEKERE *et al.* (2013) and ADEYEYE *et al.* (2017), while some other studies indicated an increase of lateral branches by inoculation (SHAHID *et al.*, 2009).

Table 4. Factorial analysis of variance of tested parameters. Data are the mean ± standard deviation of the intensity of selected, smoothed, baseline corrected and normalized bands during maturation.

Tested parameters (mean ± standard deviation)

Treatment	Non-inoculated					
Variety	Galina	Dana	Princeza	Dukat	Sava	Galeb
Height (cm)	71.97±7.89^{aC}	59.23±6.58^{aA}	65.97±6.68^{aB}	77.53±4.06^{aD}	80.03±5.43^{aD}	76.70±5.99^{aD}
Distance to the first pod (cm)	8.45±1.38^{aBC}	7.70±1.21^{aA}	7.93±0.91^{aAB}	8.13±1.22^{aABC}	9.13±0.94^{aDE}	9.45±0.89^{aEF}
Number of branches per plant	2.45±0.74^{aA}	2.23±0.90^{aA}	3.23±0.86^{aBC}	3.30±0.92^{aBC}	2.50±0.51^{aA}	3.40±0.75^{aBC}
Number of internodes	13.00±1.20^{aB}	13.73±1.46^{aBC}	13.37±1.19^{aBC}	14.20±1.61^{aC}	13.97±1.32^{aC}	13.75±1.65^{aBC}
Number of pods per plant	49.03±13.37^{aA}	52.43±17.19^{aAB}	51.87±13.01^{aAB}	51.60±19.85^{aAB}	48.97±7.25^{aA}	63.10±23.94^{aC}
Weight of pods per plant (g)	21.92±6.91^{aA}	23.73±8.73^{aAB}	22.96±7.18^{aAB}	21.34±9.58^{aA}	22.58±5.47^{aAB}	27.88±12.02^{aB}
Weight of seed per plant (g)	14.20±4.45^{aAB}	15.42±5.84^{aABC}	15.35±4.70^{aABC}	13.62±6.11^{aA}	15.29±3.58^{aABC}	19.30±8.42^{aD}
Total grain yield (kg ha^{-1})	1250.0±180.3^{aAB}	1116.7±175.6^{aA}	1438.3±108.0^{aB}	1129.0±131.4^{aA}	1452.0±59.7^{aB}	1186.7±21.5^{aAB}
Treatment	Inoculated					
Height (cm)	77.20±5.74^{bD}	63.67±4.27^{bB}	69.70±4.71^{bC}	77.63±5.30^{aD}	78.80±4.33^{aD}	78.41±3.45^{aD}
Distance to the first pod (cm)	9.27±1.60^{bDE}	7.97±0.89^{aAB}	7.77±0.77^{aA}	8.40±1.10^{aBC}	9.47±0.90^{aE}	9.20±0.85^{aDE}
Number of branches per plant	2.4±0.621^{aA}	2.43±0.858^{aAB}	2.8±0.925^{aBC}	3.63±0.669^{aDE}	2.63±0.556^{aABC}	3.3±0.702^{aD}
Number of internodes	13.33±0.96^{aC}	13.93±0.87^{aCD}	13.77±0.94^{aCD}	14.30±1.53^{aDE}	13.77±0.86^{aCD}	14.72±1.19^{bE}
Number of pods per plant	51.47±13.04^{aAB}	49.60±10.77^{aA}	51.13±10.06^{aAB}	49.97±13.52^{aA}	46.93±8.22^{aA}	60.55±18.87^{aBC}
Weight of pods per plant (g)	22.35±6.90^{aAB}	21.51±6.76^{aAB}	23.22±5.73^{aABC}	19.87±5.34^{aA}	19.96±5.06^{aA}	25.00±10.59^{aBC}
Weight of seed per plant (g)	14.50±4.52^{aABC}	13.91±4.32^{aAB}	14.66±3.75^{aABC}	13.04±3.16^{aA}	14.37±3.86^{aAB}	16.12±7.08^{aABC}
Total grain yield (kg ha^{-1})	1196.7±225.0^{aA}	1166.7±208.2^{aA}	1351.0±122.9^{aAB}	1126.0±186.0^{aA}	1334.0±175.2^{aAB}	1133.3±90.7^{aA}

The highest average value of grain yield was obtained for the non-inoculated variety Sava (1452 kg ha^{-1}), Table 4. There were no significant differences in grain yield by inoculation, but the effect of the genotype was expressed.

Analysis of variance (ANOVA-factor analysis) showed that the factor "Genotype" affects all parameters except the total grain yield, while the factor "Inoculation" had not the impact on morphological traits, except for the plant height. Furthermore, the interaction between two tested factors (genotype and inoculation) did not have statistically significant effect (Table

5). Since the factor "Genotype", i.e. soybean variety, had a significant impact on most of the studied morphological traits, the differences among soybean varieties analyzed by post-hoc tests have been already presented separately (Table 4).

Table 5. Factor analysis for morphological parameters and the total grain yield (p<0.05)

Source of variability	Dependent variable							
	Height (cm)	Distance to the first pod (cm)	Number of branches per plant	Number of internodes	Number of pods per plant	Weight of pods per plant (g)	Weight of seed per plant (g)	Total grain yield (kg ha^{-1})
Genotype	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.09
Inoculation	0.020	0.020	0.630	0.085	0.578	0.904	0.525	0.393
Genotype x Inoculation	0.051	0.066	0.071	0.312	0.352	0.285	0.163	0.944

Impact of the genotype and Bradyrhizobium inoculation on the soybean grain proteins content
Total protein

The results of total protein content using a standard analytical method indicated that there were no significant differences between the examined soybean varieties. The highest value of the total protein content was obtained for variety Galeb, while the lowest value was observed for varieties Sava and Dana (Table 6). Obtained results are in accordance by KOLARIĆ *et al*. (2009) and POPOVIĆ *et al*. (2015). YOSEPH *et al*. (2014) and FUSKAH *et al*. (2019) examined the influence of inoculation and application of nitrogen and phosphorus fertilizers on the content of the total proteins in soybean seeds. According to these reports, the inoculation had no effect on the total proteins, similarly to results of our study.

On the contrary, some other reports showed that inoculation with several different strains of nitrogen-fixing bacteria and sulphur, nitrogen and organic fertilizers strongly increased the content of the total seed proteins in all tested varieties (PANTORA *et al*., 2018; ABERA *et al*. 2019).

Table 6. Total protein content of seeds of soybean variety (%)

Varietes	Treatment	
	Inoculated	Non-inoculated
Galina	40.21±1.20	40.75±1.94
Dana	39.60±2.00	39.26±1.35
Princeza	40.40±1.34	41.13±1.12
Dukat	40.40±1.56	40.57±1.44
Sava	39.73±1.88	40.11±1.21
Galeb	42.15±1.26	42.07±1.29

Data are the mean ± standard deviation.

Characterisation of soybean seed proteins using Raman spectroscopy

The obtained spectra in the spectral region of 1230-1680 cm^{-1} are divided in the following regions: amide I region between 1680 and 1600 cm^{-1} (stretching vibration of C=O), amide II region observed in the range between 1580 and 1480 cm^{-1} and amide III region observed in the range between 1300-1230 cm^{-1} (both associated with coupled C-N stretching and N-H bending vibrations of the peptide group) (SCHULZ and BARANSKA, 2007) (Figure 1). These bands intensities can be used to evaluate contribution of each band of the protein spectrum. Since intensities of amide III and amide II regions were significantly higher in non-inoculated Galina and Sava varieties, it is possible that these secondary protein structures are more prevalent in the total protein spectrum (Table 7).

Table 7. Factorial analysis of variance of characteristic Raman band intensities. Data are the mean ± standard deviation of the intensity of selected, smoothed, baseline corrected and normalized bands during maturation

		Observed Raman region		
Treatment	Variety	1230-1300 cm^{-1}	1480-1580 cm^{-1}	1600-1680 cm^{-1}
Non inoculated	Galina	0.1700±0.0036^{aE}	0.04965±0.0135^{aC}	0.1628±0.0033^{aA}
	Dana	0.1646±0.0011^{aCDE}	0.03093±0.0175^{aA}	0.1821±0.0028^{aD}
	Princeza	0.1581±0.0018^{aC}	0.01001±0.0936^{aB}	0.1708±0.0036^{aC}
	Dukat	0.1656±0.0042^{aCDE}	0.0325±0.0052^{aA}	0.1614±0.0027^{aA}
	Sava	0.1668±0.0005^{aDE}	0.0308±0.0063^{aA}	0.1766±0.0080^{aC}
	Galeb	0.1508±0.0083^{aB}	0.0312±0.0043^{aA}	0.1871±0.0008^{aD}
Inoculated	Galina	0.1614±0.0040^{bCD}	0.0445±0.0010^{a}	0.1678±0.0016^{bC}
	Dana	0.1498±0.0017^{bB}	0.0472±0.0180^{bC}	0.1895±0.0749^{aB}
	Princeza	0.1610±0.0024^{aCD}	0.0326±0.0120^{bA}	0.1860±0.0028^{bD}
	Dukat	0.1621±0.0063^{aCDE}	0.0493±0.0305^{bC}	0.1671±0.0085^{bC}
	Sava	0.1586±0.0046^{bC}	0.0218±0.0031^{bD}	0.1705±0.0022^{aC}
	Galeb	0.1411±0.0086^{aA}	0.03108±0.0161^{bA}	0.1890±0.0078^{bB}

Means with the same small superscript letters within the same column between the same varieties are not significantly different ($p < 0.05$).
Means with the same capital superscript letters within the same column between all varieties are not significantly different ($p < 0.05$).

Analysis of variance showed that individual factors (“Genotype” and “Inoculation”), as well as their interaction did not have statistically significant effect on the total seed proteins, in difference to their effects and interaction on Raman bands intensity ($p<0.05$), where statistically significant differences were determined (Table 8).

Such results may indicate the possible effect of genotypic variability, i.e. the effect of a soybean variety, on qualitative protein characteristics – the protein chemical composition and therefore their further nutritional relevance, as already shown (ZAWORSKA-ZAKRZEWSKA *et al.*, 2020). Results of our study confirmed the controversial effects of inoculation revealing that even such practice is performed on soils of previously grown legumes and in conditions of water deficit, certain impact of inoculation on some morphological traits and qualitative protein features might occur. Furthermore, the relevance of non-destructive, novel and rapid analytical tool, such as Raman spectroscopy in assessment of differences in chemical composition among soybean genotypes was pointed out.

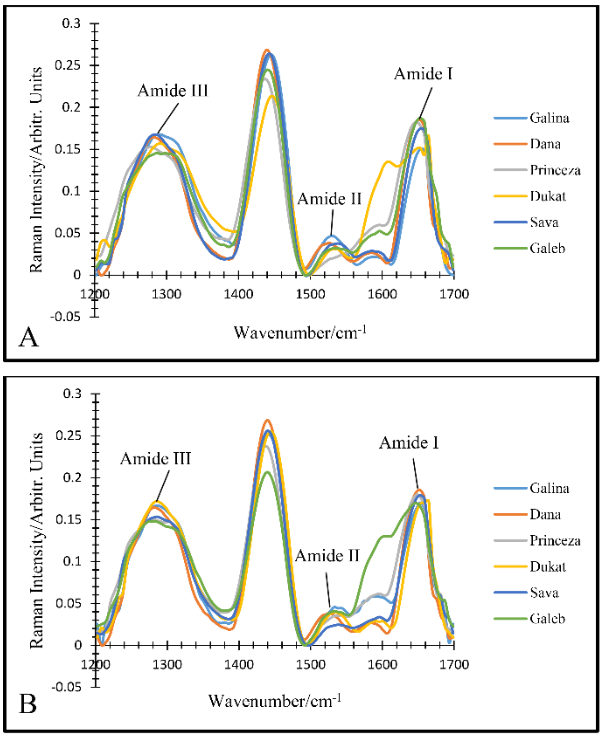

Figure 1. Smoothed, baseline corrected and normalized Raman spectra of non-inoculated (A) and inoculated (B) soybean varieties

Table 8. Factorial analysis of variance for total proteins and Raman spectroscopy amide bands (p<0,05)

Source of variability	Dependent variable			
	Total proteins	1230-1300 cm^{-1}	1480-1580 cm^{-1}	1600-1680 cm^{-1}
Genotype	0.437	0.000	0.000	0.002
Inoculation	0.325	0.000	0.000	0.000
Genotype x Inoculation	0.436	0.01	0.000	0.000

CONCLUSION

Results presented in the current study showed that *Bradyrhizobium* inoculation of soybean seeds did not have statistically significant effects on number of morphological traits, as well as on the yield components and the total seed protein content. The exception was the plant height and the distance to the first pod, but such effects were not observed for all tested varieties. The experiment was performed on the soil where soy was previously grown, confirming slight effects of inoculation under such conditions. It was assumed that the soil was "saturated" in N-fixing microorganisms, enabling an efficient nodulation process also for plants which weren't inoculated before sowing. Such findings are in accordance with similar recent studies. However, inoculation itself and the consequent impact on nodulation efficiency and related agronomic and yield component traits are very complex and still contentious. As expected, the effect of the genotype (the variety) was considerable, resulting in differences in most of the studied traits, except for the total seed proteins. Performance of chemometrics-based Raman spectroscopy allowed the qualitative protein discrimination among the tested soybean varieties, both inoculated and non-inoculated. The first report on use of the Raman spectroscopy in assessment of soybean variability highlights the need of further research on N-fixation symbiosis and nodulation impact on qualitative traits and chemical composition of legume varieties.

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EFEKAT GENOTIPA I INOKULACIJE BAKTERIJAMA IZ RODA *BRADYRHIZOBIUM* NA MORFOLOŠKE OSOBINE, PRINOS I SADRŽAJ PROTEINA IZABRANIH SORTI SOJE

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Izvod

Proizvodnja soje u Srbiji uključuje inokulaciju setvenog materijala azotofiksirajućim bakterijama pre setve. Glavni cilj ovog rada je procena uticaja genotipa i inolulacije na morofoške parametre i prinos soje (*Glycine max* L. Merrill) kao i na sadržaj ukupnih proteina. Eksperiment je sproveden na zemljištu tipa černozem na kojem je prethodno uzgajana soja. Za setvu je korišćeno šest domaćih sorti soje, gde je svaka sorta sejana u tri ponavljanja sa dva tretmana (inokulisano i neinokulisano seme) i praćeni su sledeći morfološki parametri: visina biljke, broj bočnih grana, broj spratova, broj mahuna po biljci, masa mahuna sa semenom po biljci, masa semena po biljci bez mahune i ukupni prinos. Sadržaj ukunih proteina u semenu određen je standardnom analitičkom metodom dok su fine razlike u kvalitativnim osobinama proteina procenjene Ramanovom spektroskopijom. Sadržaj ukunih proteina bio je uniforman i kretao se do 39.6-42.15 %. Inokulacija je dovela do povećanja visine biljke i visina biljke do prve mahune. Najveća i najmanja prosečna visina biljke je uočena kod neinokulisanih sorti Dana (59,23 cm) i Sava (80,03 cm), respektivno. Efekat sorte je bio izraženiji i izazvao je efekat kod svih parametara osim sadržaja ukupnih proteina. Međutim, primena Ramanove spektroskopije je pokazala jasne razlike između ispitivanih sorti u kao i razlike između inokulisanih i neinokulisanih sorti u pogledu kvalitativnog sastava proteina semena.

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