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Effect of biostimulants on soybean seedlings

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A B S T R A C T

The aim of this study was to analyze the effect of biostimulants on the morphological characteristics of soybean seedlings. The testing was conducted in the laboratory of the Faculty of Biofarming in Bačka Topola. The experimental material included three soybean varieties ('Galina', 'Sava' and 'Rubin') selected at the Institute of Field and Vegetable Crops in Novi Sad. The study lasted for two years, 2015–2016, and identical biostimulant treatments were applied in both years. In order to determine the effect of biostimulants on soybean seedling root, hypocotyl and weight, the following commercial biostimulants were applied: EM Aktiv, Terra Green Hobby, Slavol and Bioplant Flora. In addition to the single application of biostimulants, two combinations of Slavol + Bioplant Flora and Slavol + Bioplant Flora + Epin Extra + Slavol S were used as treatments. EM Aktiv showed the greatest effect on root growth. The root was on average 12% longer than the control. Slavol S had the greatest influence on seedling hypocotyl and weight. The increase was 8.24% and 5.15%, respectively, compared with the control.

Keywords: biostimulants, seedlings, soybean, varieties.

И З В О Д

Циљ рада био је да се испита деловање биостимулатори на морфолошке особине клијанца соје. Тестирање је спроведено у лабораторији Факултета за биофарминг у Бачкој Тополи. Експериментални материјал су биле три сорте соје (Галина, Сава и Рубин) селекционисане на Институту за ратарство и повртарство у Новом Саду. Истраживање је трајало две године, 2015–2016, и у обе године примењени су идентични третмани са биостимулаторима. У сврху утврђивања утицаја биостимулатора на дужину корена, дужину надземног дела и масу клијанца соје примењени су различити комерцијални биостимулатори под називом: EM Aktiv, Terra Green Hobby, Slavol и Bioplant Flora. Поред појединачне примене биостимулатора, као третмани коришћене су и две комбинације Slavol + Bioplant Flora и Slavol + Bioplant Flora + Epin Extra + Slavol S. Резултати истраживања су показали да примена адекватног биостимулатора утиче позитивно на морфолошке особине клијанца соје, док неадекватна комбинација биостимулатора доводи до инхибиторног деловања. EM Aktiv је показао највећи ефекат на раст корена. Корен је у просеку био 12% дужи од контроле. Slavol S је показао највећи утицај на хипокотил и тежину садница. Повећање је било 8,24% и 5,15% у поређењу са контролом.

Кључне речи: биостимулатори, клијанци, соја, сорте.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is a major crop, not only in agricultural production but also in industrial processing. It ranks fourth in terms of production area in the world (Balesević and Miladinović, 2014). Worldwide, it is grown on an area of about 123.5 million hectares (http://faostat.fao.org, 2017). Using high quality seeds is a basic factor that determines high crop yields (Milošević et al., 1996). However, since soybeans are sown in different agro-ecological conditions, seed germination and vigor are influenced by various adverse environmental factors such as drought and extreme temperatures (Casenave and Toselli, 2007). Therefore, different methods are used to reduce the negative impact of environmental factors (Djukić et al., 2017; Miladinov et al., 2019). Biostimulants can be used to improve seed quality (Yildirim et al., 2007). Biostimulants are neither plant nutrients nor pesticides; they are organic materials that, when used in small amounts, improve plant growth and development, but to a degree different from the use of traditional plant nutrients (Yakhin et al., 2017).

Biostimulants can be divided into humic acid-containing biostimulants, hormone-containing biostimulants and amino acid-containing biostimulants, or biostimulants of plant growth and development depending on the type of biostimulant (Tkalec, 2010). Also, biostimulants must be able to penetrate the plant tissue. This is of great importance, especially in field conditions, where treated plants are exposed to different agroenvironmental conditions (Kolomazik et al., 2012). However, in addition to their positive effect on seed germination, biostimulants in some cases have the ability to reduce seed quality (Miladinov et al., 2014a) as well as to inhibit seedling growth (Miladinov et al., 2015).

There has not been much research on the effect of biostimulants on seedlings. Therefore, the aim of this paper was to investigate the influence of different biostimulants and their combinations on the morphological parameters of soybean seedlings.

2. Material and Methods

The research was carried out in the laboratory of the Faculty of Biofarming in Bačka Topola. Testing was performed on seeds of three soybean varieties: 'Galina', 'Sava' and 'Rubin' (Factor A). The varieties were selected at the Institute of Field and Vegetable Crops in Novi Sad, and research was conducted during 2015 and 2016 (Factor C). The seeds were treated with solutions of the following biostimulants (Factor B):

Treatment and concentration	Abbreviation	Active substance
EM Aktiv (50%)	EM	lactic acid bacteria, photosynthetic bacteria, ferment, cane molasses
Terra Green Hobby (100%)	FT	lactic acid bacteria, photosynthetic bacteria, ferment, cane molasses
Bioplant Flora (2%)	B	humic acids, fulvo acids, amino acids, phytohormones, macro and micro elements, N, K_2O
Slavol S (33%)	S	indole-3-acetic acid
Slavol S (20%) + Bioplant Flora (2%)	S+B	indole-3-acetic acid + humic acids, fulvo acids, amino acids, phytohormones, macro and micro elements, N, K_2O
Slavol S (20%) +Bioplant (2%) + Epin Ekstra (0.05%) +Slavol for soybean (10%)	S+B+E+SS	indole-3-acetic acid + humic acids, fulvo acids, amino acids, phytohormones, macro and micro elements, N, K_2O + auxins, cytokinins, gibberellins, ethylene and abscisic acid + symbiotic bacteria, associative and phosphorus mineralizing bacteria

The seeds treated with distilled water (C) were used as controls.

During the preparation of the solutions, given the negative effect of light on biostimulants containing active microorganisms, the whole process (from dosage to treatment of the seed and its comparison with the substrate) took place in a slightly darkened part (away from the light source) of the laboratory. The seeds were sprayed with biostimulant solutions. After 30 minutes, the seeds were spread on filter paper. They were evenly applied to filter paper measuring 580 x 580 mm (58 cm in length), and 580 x 290 mm (Factor D). Seed germination was performed under laboratory conditions using a standard laboratory test. Standard laboratory germination was tested for 4 × 100 seeds. The incubation period was eight days at 25 °C and 95% relative humidity (ISTA, 2008). After eight days of incubation, 4 × 10 average fresh soybean seedlings were taken from each treatment for the analysis of seedling root, hypocotyl, and weight.

The statistical significance of differences between treatment means was tested by four-way ANOVA with LSD test at two levels of significance (5% and 1%). Pearson's correlations between the tested properties were assessed for significance by the t-test.

3. Results and Discussion

The analysis of the effect of variety (A), treatment (B), year (C) and type of filter paper (D) on soybean seedlings showed that these factors had a very significant effect on root growth. All factors except year significantly influenced the hypocotyl growth and weight of seedlings (Table 1). Also, for all parameters, a very significant interaction was established between the factors, AxB, AxD, AxBxC and AxBxCxD.

Table1.
Effect of the factors under consideration on the parameters examined

Factors	Seedling root	Seedling hypocotyl	Seedling weight
Variety (A)	**	**	**
Biostimulants (B)	**	**	**
Year (C)	**	ns	ns
Length of filter paper (D)	**	**	*
AxB	**	**	**
AxC	*	*	ns
AxD	**	**	**
BxC	**	*	ns
BxD	ns	**	*
CxD	**	ns	*
AxBxC	**	**	**
AxBxD	**	**	**
AxCxD	**	**	ns
BxCxD	**	**	*
AxBxCxD	**	**	**

** – significant at 0.01; * – significant at 0.05; ns – not significant

The results of this study are in agreement with the research conducted by Vernieri et al*.* (2006) and Parađiković et al*.* (2008), who pointed out that the application of biostimulants in the germination stage can establish better conditions for the growth and development of seedlings, and especially the roots. Positive effects of biostimulants have been determined in the production of seedlings of vegetables, flowers, and medicinal, aromatic and spicy herbs (Jelačić et al., 2006; Gajc-Wolska et al., 2012; Zeljković et al., 2013; Palfi et al., 2017). However, in addition to having a positive effect, biostimulants can also have a negative effect on the seed if used at high concentrations (Türkmen et al*.,* 2004) or due to an improper combination of incompatible biostimulants (Miladinov et al., 2014b).

Effect of biostimulants on soybean seedling root growth

The results showed that the application of a regulator can have a positive effect on soybean seedling root growth. The greatest effect is achieved by EM Activ treatment. Eight days after seed treatment, soybean seedlings had significantly longer roots relative to other treatments and controls. Root length averaged 15.5 cm, which is 12% longer than the control. Effective microorganisms found in EM Activ, in addition to nitrogen fixation and mineralization of organic forms of phosphorus in the soil, synthesize active substances such as enzymes, amino acids, vitamins, and fungicidal substances, thus directly or indirectly affecting the growth and development of plants (Cvijanović, 2017). Szymanski et al*.* (2003) reported that EM Activ led to an increase in seed germination and root development, enhanced flowering and fruit formation, and improved soil fertility. EM Activ was also found to have a positive effect on soybean yield. In two-year studies, the yield increased by an average of 10.84 % i.e. by 6.86 % and 14.81 % per year, respectively (Dozet et al., 2014). The effect of the other treatments on root growth was not significant, but they gave longer roots than the control. Compared with the control, Slavol S promoted root elongation by 7.27 %, Terra Green Hobby by 6.77%, S + B + E + SS by 5.61%, and Bioplant Flora by 5.28 %, whereas the combination of S + B increased root length by 4.82% (Figure 1).

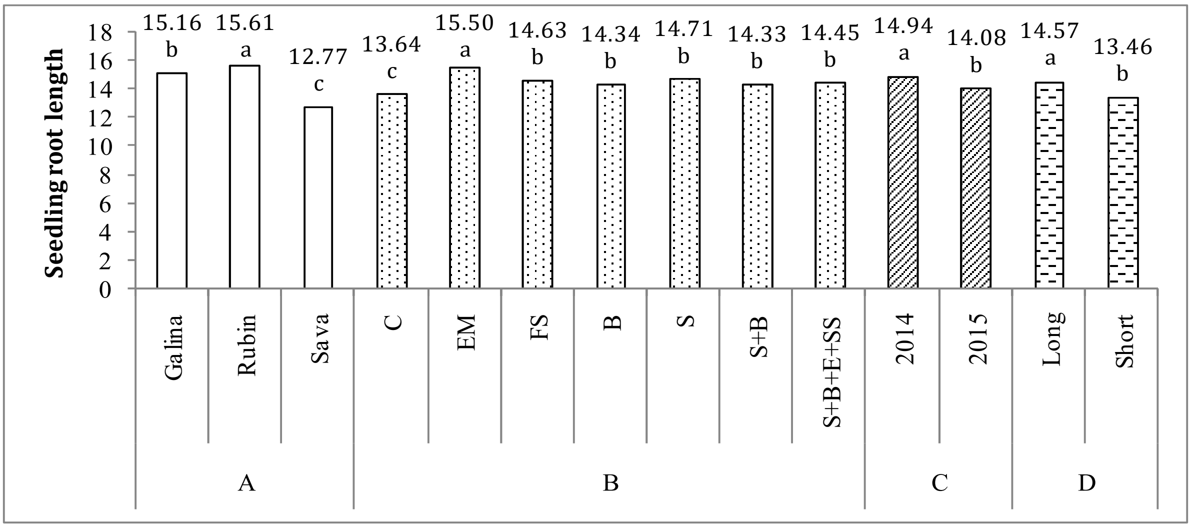

Figure 1. Influence of seed treatment on root length

The beneficial effect of Slavol S can be attributed to the active ingredient, which is indole-3-acetic acid. Indole-3-acetic acid is considered the most important natural auxin in higher plants. Auxins are phytohormones that affect plant growth by participating in stretching and cell division, inducing root growth (Normanly et al., 1995). Slavol S was also found to have a positive influence on sunflower seeds (Miladinov et al., 2014b; Miklić et al., 2016).

However, when combined with other biostimulants, its effect is reduced, and therefore the root is shorter than when treated with Slavol S alone, which is in agreement with the results conducted by Miladinov et al*.* (2015). Miladinov et al*.* (2014b) have also indicated that the effect of biostimulants is also significantly dependent on genotype, which is consistent with the research on soybeans. The results showed that the use of biostimulants had the greatest effect on root growth in 'Rubin'. 'Rubin' had a significantly longer root system than the other varieties. The root length of 'Rubin' was 15.61 cm, which is 2.97% and 18.19% longer than that of 'Galina' and 'Sava', respectively. The rapid development of the root system is of great importance to the plant, especially under water deficit conditions during germination, because it allows plants to quickly reach soil moisture (Gupta et al*.,* 2008). Using alonger strip of filter paper, a significantly higher root length (14.57 cm) was observed, which is a 7.62 % increase. This is understandable, because the root had no physical barriers, and could therefore unfold vertically downward, which is of great importance for the initial growth of the seedling.

Effect of biostimulants on soybean seedling hypocotyl growth

The biostimulants Slavol S, Terra Green Hobby and EM Aktiv significantly influenced hypocotyl growth. Compared with the control, Slavol S increased hypocotyl length by 8.24 %, Terra Green Hobby by 6.52 %, and EM Aktiv by 4.97 %. By contrast, the combination of S + B + E + SS reduced the length of the hypocotyl by 4.67% (Figure 2).

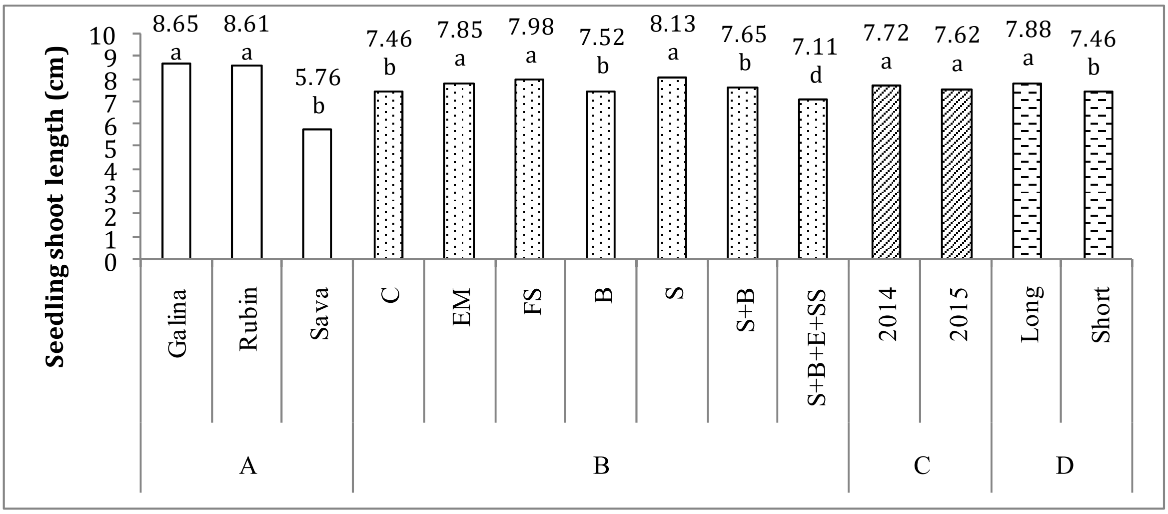

Figure 2. Influence of seed treatment on hypocotyl length

Similar results were reported on sunflower by Miladinov et al. (2014b). They found that the combined effect of Slavol S and Bioplant Flora resulted in an inhibitory effect on sunflower seedling hypocotyl, which was reduced by as much as 9.09% over control. Although many studies have indicated the positive effect of humic acids – the active substance of Bioplant Flora – on germination, plant growth and development (Russo and Berlyn, 1990; Parađiković, 2008; Zeljković, 2013), their effect on hypocotyl length has not been established. The results of the present study showed that the effect of biostimulants is significantly dependent on genotype, which is in line with the results on soybean (Cindrić, 2019) and sunflower (Miladinov et al., 2014b). The results showed that the use of biostimulants had a significantly better effect in 'Galina' and 'Rubin'. The hypocotyls of 'Galina' and 'Rubin' were, respectively, 33.41% and 33.10% longer than those of 'Sava'.

Effect of biostimulant on soybean seedling weight

As regards the influence of biostimulants on soybean seedling weight, Slavol S gave the greatest increase. In comparison with the control, seedling weight was increased by 5.15 %. The results are consistent with the research conducted by Jelacic et al. (2006). Kaludjerovic and Mirecki (2013) determined that Slavol S increased the weight of lettuce seedlings by an average of 35.74 %. The weight was increased regardless of the substrate used for seedling growth Bioplant Flora reduced seedling weight by 2.17 % over control, which is statistically insignificant. However, the results contradict the research conducted by Türkmen et al. (2004) on tomatoes. They found that humic acid based products, such as Bioplant Flora, increased the content of macro and microelements, resulting in improved growth. However, a positive effect was only present when the product was used at certain concentrations. At high concentrations, its effect was negative. The combination of two biostimulants, S + B, did not have a significant effect on seedling weight relative to the control. However, the combination of three biostimulants, S + B + E + SS, gave a significantly lower seedling weight, compared with the control of 8.70% (Figure 3).

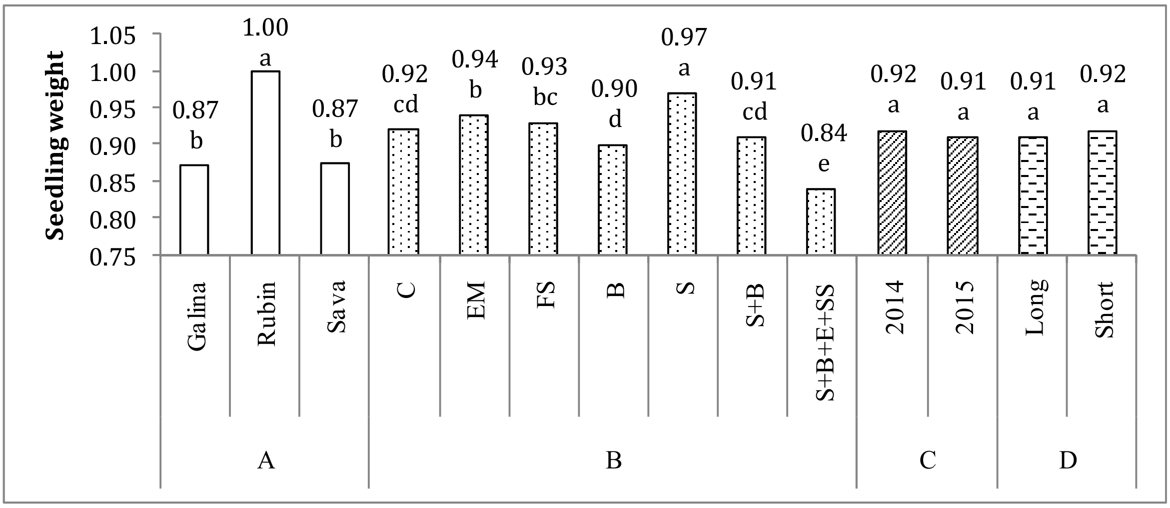

Figure 3. Influence of seed treatment on seedling weight

Correlation between tested parameters

The application of Pearson's correlation coefficient revealed the highest positive correlation between hypocotyl length and seedling weight (r = 0.703**). The smallest positive correlation was found between root length and seedling weight (r = 0.314 **), which is understandable, since most nutrients and water are taken up for building aboveground vegetative biomass and creating favorable conditions for the start of autotrophic feeding of a new young plant (Table 2).

Table 2.
Correlation between tested parameters

Correlation	Seedling roots	Seedling hypocotyl	Seedling weight
Seedling roots	1	0.529**	0.314**
Seedling hypocotyl	0.529**	1	0.703**
Seedling weight	0.314**	0.703**	1

4. Conclusion

Soybean varieties responded differently to biostimulant treatments. The most significant difference was found in the influence of biostimulants on root growth. The greatest effect was found in 'Rubin'.

Pre-sowing seed treatment with EM Aktiv showed the greatest effect on root growth. The root was on average 12% longer than the control.

Slavol S had the greatest influence on seedling hypocotyl and weight. The increase was 8.24% and 5.15%, respectively, compared with the control.

The biostimulants were more effective on large-sized filter paper, which was an expected finding because the seedling had no physical barriers and could develop smoothly.

The year of production had an effect only on root growth, whereas it did not have a significant effect on the other morphological parameters.

However, unlike the positive effects of certain biostimulants when used alone, their improper combination can inhibit the morphological properties of soybean.

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