

Article

# Influence of Growth Regulators on Soybean Morphology Depending on Weather Conditions During the Vegetation Period

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**Abstract:** The aim of the research was to test the effect of application of plant growth regulators ascorbic acid (AsA), glycine betaine (GB) salicylic acid (SA), and water (H<sub>2</sub>O) on morphological characteristics of soybean plant (plant height, number of lateral branches, total number of nodules, number of fertile nodules, number of pods and weight of 1000 seeds). The field trial was conducted at an experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia in 2016–2017. The plant of the soybean cultivar ‘Sava’ was treated twice. The first treatment was done in the R1 reproductive stage and the second treatment was done in the R3 reproductive stage.

Research has shown that in a favorable year (2016), the application of GB had the best effect on the number of lateral branches and fertile nodules. The use of SA achieved a significantly higher 1000 seed weight in relation to the control, but also water, regardless of the weather conditions during the vegetation. In 2016, compared to the control, the 1000 seeds weight increased by 10.36%, while in 2017 the effect was slightly higher by 12.92%.

However, studies have shown that the use of growth regulators can also have a negative effect on the morphological characteristics of soybean plants, depending on the year and the weather conditions during the growing season.

**Keywords:** Growth regulators; soybean; plant height; 1000 seeds weight.

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## 1. Introduction

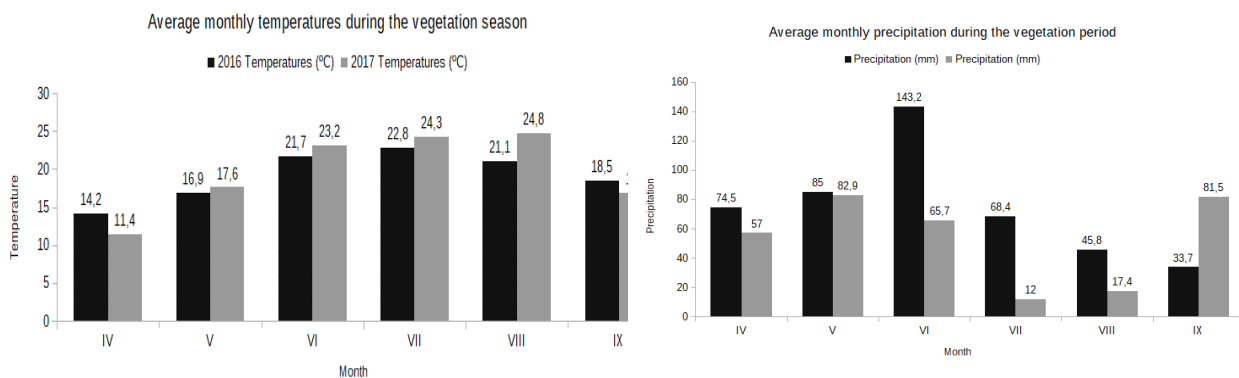
Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% [1]. Plants respond to environmental stress by induction of various morphological, biochemical and physiological responses [2]. Plant growth regulators can improve the physiological efficiency including photosynthetic ability and thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops [3]. Ascorbic acid is a major substance in a set of antioxidants that includes ascorbate, glutathione,  $\alpha$ -tocopherol and a group of antioxidant enzymes [4]. Ascorbic acid serves as an important co-factor in the biosynthesis of many plant hormones, including ethylene, gibberellic acid (GA), and abscisic acid (ABA) [5]. It is found in the cytoplasm, chloroplast, vacuoles and mitochondria of plant cells and plays a significant role in physiological processes such as: cell division, plant growth and cell

wall biosynthesis, metabolites and phytohormones. In addition, it plays an important role in chloroplast and mitochondrial membrane renewal [6]. Glycine betaine (GB) is an organic compound that occurs in plants, it is an amphoteric quaternary amine, plays an important role as a compatible solute in plants under various types of environmental stress, such as high levels of salts and high or low temperatures [7]. Glycine betaine protects plants from stress through different ways, including the contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins [8]. Salicylic acid is an endogenous growth regulator of phenolic nature and acts as a potential non-enzymatic antioxidant involved in the regulation of many physiological processes in plants, such as stoma closure, photosynthesis, ion consumption, ethylene biosynthesis inhibition, transpiration, and stress tolerance [9]. Salicylic acid is a natural plant hormone that acts as an important signaling molecule in tolerance against abiotic stress and plays a key role in plant growth, uptake, and ion transport [10].

The aim of the study was to investigate the influence of growth regulators (AsA, GB, SA) on the morphological properties of the soybean plant. And to determine if the effect of the treatment is due to the effect of the active substance or water, since soy is a plant that responds well to air humidity, plain water was used as one of the treatments.

## 2. Materials and Methods

**Weather conditions.** During 2017, with a significant lack of rainfall, soybean was also exposed to high air temperatures during the June-August period. On average, temperatures were more than 1.5-3.7 °C (Table 1). Due to the very high temperatures from May to August and the lack of moisture in the period from June to the end of August, in 2017 there was a forced maturation of the plants and a shortening of the vegetation period by 10 days (Table 2), which is one of the factors that influenced the decrease in soybean yield as well as the decrease in seed germination.



**Table 1.** Main dates in soybean vegetation.

Year	Date				Length of growing season, (days)
	Sowing	Germination	Maturation	Harvest	
2016	10.04.	01.05.	06.09.	27.09.	128
2017	20.04.	05.05.	01.09.	13.09.	118

**Table 2.** Potential and actual evapotranspiration during soybean vegetation from 2016-2017. year

Year	SM	PV	PE	AE	PD	DS
2016	51.28	423.5	440	410	-30	18.08.
2017	29.63	191.8	475	200	-275	25.06.

SM- Soil moisture reserves during sowing time; PV- Precipitation during vegetation period; PE-Potential evapotranspiration; AE-Evapotranspiration; PD-Precipitation deficit; DS-Drought start

Soil winter moisture reserves were about 20 mm higher during 2016 (Table 3). During the vegetation period in 2016, there was 231.7 mm more rainfall, or 54.71% more than in 2017. Observed at the water needs of plants and the amount of rainfall by individual years, it is noticed that in 2016, the needs were 440 mm and 410 mm were available, while the water needs in 2017 were higher due to high temperatures and amounted to 475 mm, while only 200 mm of water was available to the plants. In 2016, soybeans lacked 30 mm of rainfall, while in 2017, soybeans lacked as much as 275 mm. The water deficit in 2016 occurred on 18.08. that is, 19 days before maturation, while in 2017, as many as 65 days of soybean were exposed to lack of rainfall.

**Plant material.** The plant material is a soybean cultivar ‘Sava’ developed at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The field trials were conducted during two vegetative seasons (2016 and 2017) at Rimski Sancevi experimental field, near Novi Sad, Serbia. The trial was set up on humus soil type as a randomized block design with four replications under the conditions of dry farming. Plot size was 10 m<sup>2</sup>. Inter-row spacing of 50 cm and intra-row spacing of 4.5 cm was applied. Plants were treated twice. The first treatment was done in the R1 reproductive stage and the second treatment was done in the R3 reproductive stage. The foliar application was used for treatment:

1. Control - without foliar spray
2. Water - H<sub>2</sub>O
3. Ascorbic acid AsA (400 mg/l)
4. Glycinbetanin GB (3kg/ha)
5. Salicylic acid SA (50 ppm)

In the phase of technological maturity, ten plants were taken from the central rows for morphological analyzes: plant height, number of nodules, number of fertile nodules, number of branches on a soybean tree, number of pods per plant and of 1000 seeds weight.

**Statistical analysis.** Data were analyzed using one-way analysis of variance (ANOVA). The mean values from treatments were compared using Tukey’s HSD (Honestly significant difference).

### 3. Results and Discussion

The application of growth regulators through foliar nutrition had a significant impact on plant height in both years of research. During the year with a higher amount of precipitation, only the plants treated with GB solution were higher than the control, while the use of AsA and SA resulted in lower plants. The average height of plants treated with GB was 120.2 cm, while the height of plants treated with AsA and SA solution was 117.2 cm and 116.3 cm. However, in years with insufficient rainfall, the use of growth regulators resulted in significantly lower plants compared to water control and use. This result indicates that the application of AsA, GB and SA had an inhibitory effect on plant height. In relation to the control, the plants were lower than 4.22-8.14%, depending on the regulator used. The lowest plants were achieved using AsA solution 86.54 cm, which is even 7.67 cm lower than the control. The best effect was achieved with the use of water, from 6.39-9.67%. The height of soybean plants is an important feature due to the possible lodging, which reduces the yield and increases the possibility of infection with pathogens. Higher plant height affects a larger number of fertile nodules, which affects the higher production of pods per plant. The height of the plant to the first fertile nodule is a very important feature on which the yield height directly depends [11]. If the first pods are too low, they will not be able to be harvested, which will increase the percentage of losses in the harvest.

The number of branches and the shape of the branches is a varietal trait, but it varies depending on soil fertility, weather conditions, as well as the size of vegetation space and is a useful property in compensating for insufficient number of plants, which can occur due to a number of unfavorable factors [12]. Observing the influence of the growth regulator on the formation of lateral branches, it can be seen that the effect mostly depended on the weather conditions, i.e., on the year. In 2017, there

was no significant branching, regardless of the treatment. However, in 2016, significant branching was achieved, primarily by applying GB and AsA solutions, while the control variant had the weakest branching of plants. Yield oscillations and morphological characteristics in some years confirm that weather conditions during vegetation have a great influence on the yield and development of soybean plants [13, 14]. The greatest needs of soybeans for water are from the beginning of flowering to the end of pouring the grain, when the soybean crop consumes 60-90% of the total water needed. This, depending on the ripening group, refers to the time period from late June to early September. During flowering and grain pouring, drought and high temperatures lead to the decline of flowers and newly sprouted pods, with a decrease in the number of pods and the number of grains, and the grain remains small. The application of growth regulators has the ability to increase the yield depending on weather conditions [15]. In the year with a sufficient amount of precipitation using SA, the yield increased by 9.67%. However, in an unfavorable year, ie in a year with insufficient precipitation during the vegetation, the application of GB, although insignificant, reduced the yield.

**Table 3.** Effect of the growth regulators on plant height and number of lateral branches in soybeans.

Treatment	Plant height (cm)		Number of lateral branches	
	2016	2017	2016	2017
Control	118.63 <sup>a</sup>	94.21 <sup>a</sup>	0.33 <sup>c</sup>	0.17 <sup>a</sup>
H <sub>2</sub> O	113.43 <sup>c</sup>	95.8 <sup>a</sup>	0.57 <sup>b</sup>	0.21 <sup>a</sup>
Ascorbic acid	117.2 <sup>ab</sup>	89.68 <sup>b</sup>	0.83 <sup>ab</sup>	0.14 <sup>a</sup>
Glycin betanin	120.2 <sup>a</sup>	90.23 <sup>ab</sup>	1 <sup>a</sup>	0.12 <sup>a</sup>
Salicylic acid	116.3 <sup>b</sup>	86.54 <sup>b</sup>	0.67 <sup>b</sup>	0.22 <sup>a</sup>

Note. Different letters in each column represent significant difference at  $p \leq 0.05$  according to Tukey's HSD.

Pods and grains form on nodules in the leaf axils. The soybean forms flowers successively along the nodules starting from the first nodule above the soil surface towards the top. If the conditions are favorable at the time of flowering, a larger number of pods is formed on the nodule, and thus the total number of pods per plant will be higher. The results of the research showed that significantly more nodules were formed in a favorable year than in an unfavorable one, both in the total number of nodules and in the number of fertile nodules. The number of nodules with pods is a varietal trait, but this property certainly depends on the climatic conditions during the season [16]. In 2016, only the use of SA reduced the total number of nodules compared to the control. However, in the unfavorable 2017, the application of all regulators reduced the total number of nodules compared to the control, but also the application of water. The application of growth regulators can have a positive effect, but also a negative one, depending on the weather conditions during the year [17]. In the number of fertile nodules, the only improvement was achieved by applying the GB solution in a favorable year. However, in the unfavorable application of all growth regulators, it had a similar effect as water and control.

**Table 4.** Effect of the growth regulators on the number of fertile nodules and the number of pods per plant.

Treatment	Total number of nodules		Number of fertile nodules	
	2016	2017	2016	2017
Control	19.30 <sup>a</sup>	15.3 <sup>a</sup>	14.23 <sup>b</sup>	11.7 <sup>a</sup>
H <sub>2</sub> O	18.73 <sup>b</sup>	15.9 <sup>a</sup>	14.33 <sup>b</sup>	11.9 <sup>a</sup>
Ascorbic acid	19.27 <sup>a</sup>	13.8 <sup>b</sup>	14.24 <sup>b</sup>	12.3 <sup>a</sup>
Glycine betanin	19.4 <sup>a</sup>	14.3 <sup>ab</sup>	14.83 <sup>a</sup>	12.2 <sup>a</sup>
Salicylic acid	18.63 <sup>b</sup>	13.6 <sup>b</sup>	14.20 <sup>b</sup>	11.8 <sup>a</sup>

Note. Different letters in each column represent significant difference at  $p \leq 0.05$  according to Tukey's HSD.

In the favorable 2016, significantly more pods were formed than in 2017. Regardless of the weather conditions, the plants from the control variant had the largest number of pods in both years.

In 2016, the plants from the control variant formed, on average, 50 pods per plant. A similar result was achieved using GB and AsA. However, with the application of SA, the number of pods was significantly reduced, on average by 6 per plant, i.e., by 12%. In the unfavorable 2017, the application of all regulators significantly reduced the number of pods per plant compared to the control. The largest decrease occurred with the use of GB, by as much as 15.38%.

The of 1000 seed weight represent a varietal characteristic. As a yield component, it is important in both mercantile and seed production. In seed production, seeds with a higher of 1000 seed weight have always been valued more [18]. It is considered that the seeds of varieties and hybrids with a higher of 1000 seeds weight are better for sowing because they contain more reserve substances and a higher germ. From such seeds, plants will develop faster, which is quite important in unfavorable climatic and edaphic conditions [19]. In both years of research, that is, regardless of the weather conditions, the use of SA had the best effect on the of 1000 seed weight. In 2016, compared to the control, the weight of 1000 seeds increased by 10.36%, while in 2017 the effect was slightly higher by 12.92%. Regarding foliar application of SA, the obtained results are similar to those describe sunflower [20]. The beneficial impact of SA on the plant can be attributed to its role in the process of nutrient uptake and transport [21] and flowering [22]. In 2016, a positive effect on 1000 seeds weight was achieved by applying all treatments in relation to the control. As with plant height and the number of pods per plant, these results also indicate that the increase in a given trait was not caused by the growth regulator but by water.

**Table 5.** Effect of the growth regulators on the number of seeds per pod and the weight of 1000 seeds.

Treatment	Number of pods per plant		1000 seeds weight	
	2016	2017	2016	2017
Control	50 <sup>a</sup>	39 <sup>a</sup>	154 <sup>c</sup>	128.1 <sup>c</sup>
H <sub>2</sub> O	46 <sup>ab</sup>	39 <sup>a</sup>	167.05 <sup>ab</sup>	141.4 <sup>b</sup>
Ascorbic acid	48 <sup>a</sup>	35 <sup>ab</sup>	163.9 <sup>b</sup>	143.9 <sup>ab</sup>
Glycine betanin	49 <sup>a</sup>	33 <sup>b</sup>	166.65 <sup>ab</sup>	130.1 <sup>c</sup>
Salicylic acid	44 <sup>b</sup>	35 <sup>ab</sup>	171.8 <sup>a</sup>	147.1 <sup>a</sup>

Note. Different letters in each column represent significant difference at  $p \leq 0.05$  according to Tukey's HSD.

#### 4. Conclusions

The application of growth regulators ascorbic acid, glycine betaine and salicylic acid through foliar spray had a significant impact on the morphological characteristics of soybean plants. Research has shown that the use of growth regulators can have both positive and negative depending on the year or weather conditions during the growing season. In a favorable year (2016), the application of GB had the best effect on the number of lateral branches and fertile nodules. On average, each GB-treated plant had one lateral branch as opposed to the control in which every third plant formed one lateral branch. The use of SA achieved a significantly higher of 1000 seeds weight in relation to the control, but also water, regardless of the weather conditions during the vegetation. In 2016, compared to the control, the weight of 1000 seeds increased by 10.36%, while in 2017 the effect was slightly higher by 12.92%.

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