Biostimulants and the antiradical activity of soybean seeds

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Abstract. In recent years, the interest in the application of biostimulants has increased, which positively affect the growth and development of plants and contribute to an increase in the yield and quality of crops. In Poland, the area of soybean cultivation is constantly increasing and thus the improvement of the quality of its seeds seems to be purposeful. In the available literature, there are few reports regarding the effect of biostimulants on the antiradical activity of plants. Therefore, studies on the influence of biostimulants on soybean seed antiradical activity seem to be justified. The study was carried out in 2014 - 2016 in Perespa, Poland. Annushka soybean seeds were sown in the third decade of April. During the growing season, four biostimulants: Kelpak SL, Terra Sorb Complex, Atonik, and Tytanit, were used in four combinations, using lower or higher concentrations and single or double spraying. After harvesting the plants, the antiradical activity of the seeds was evaluated by ABTS•+ assay. It has been found that the foliar application of biostimulants positively influenced the studied property. The highest antiradical activity of plants was found upon double spraying with lower concentrations of Atonik and Terra Sorb Complex.

1 Introduction

An increasing interest has recently been observed in the application of biostimulants, which positively affect the growth and development of plants and contribute to an increase in the yield and quality of crops. Their application is justified in the case of abiotic stress [1], especially in plants susceptible to adverse weather. Biostimulants used in crop cultivation are natural or synthetic origin and contain both organic and inorganic compounds. The natural biostimulants are based on free amino acids, humus compounds, extracts from fruit or marine algae, chitin and its derivative – chitosan, or effective microorganisms. In turn, the group of synthetic biostimulants includes preparations that contain growth regulators, phenolic compounds, inorganic salts, and nutrients (Al, Co, Na, Se, Ti, and Si) [2-6].

The use of biostimulants in the cultivation of crops with high thermal demands, like e.g. soybean, seems to be justified. In addition, the area of soybean cultivation in Poland is constantly increasing and therefore the possibility of improving the quality of its seeds is crucial. Literature data show that legumes are rich in antioxidants [7, 8], however only sparse reports are available on the effect of biostimulants on the antiradical activity of legumes [3, 4, 6]. Hence, research aimed at determining the effect of biostimulants on the antiradical activity of soybean seeds seems to be eligible.

2 Materials and Methods

2.1 Plant materials and growth conditions

The study was carried out in 2014-2016 in Perespa (50°66′N; 23°63′E), Poland. The soil type was characterized as Cambic Rendzic Phaeozems. It is alkaline (pH in 1M KCl 7.4–7.5) and rich in phosphorus, potassium, and magnesium. The experiment was established in a randomized block design in four replications with an elementary experimental plot area of 5 m^2. Seeds of soybean cultivar Annushka were sown in the third 10-day period of April with the spacing of 30 x 3.5 cm. The sowing rate was 80 plants·m^{-2} Over the growing season, four biostimulants: Kelpak SL (*Ecklonia maxima* extract, 11.0 mg/l auxin and 0.031 mg/l cytokinin), Terra Sorb Complex (20% free amino acids), Atonik (phenolic compounds, sodium para-nitrophenolate PNP (0.3%), sodium ortho-nitrophenolate ONP (0.2%) and sodium 5-nitroguaiacolate 5NG (0.1%)), and Tytanit (8.5 g Ti/l), were applied through leaf spraying according with the scheme presented in Table 1.

The biostimulants were applied with a GARLAND FUM 12B battery field sprayer (Lecher LU 120–03) at a pressure of 0.30 MPa, using 300 L liquid per hectare. All the results were compared to control, where plants were treated with the same volume of water (no biostimulant applied). Tillage of plants was done using good agricultural practices. No pesticides were used (pest

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number did not exceed the thresholds of harmfulness) and plants were hand weeding.

The average temperature and rainfall in the soybean growing season are shown in Table 2.

Table 1. Scheme of biostimulants application.

Biostimulants	Number of applications Plant's stage	Concentration
Atonik	single spraying BBCH 13-15	0.1%
		0.2%
	double spraying BBCH 13-15 BBCH 61	0.1%
		0.2%
Tytanit	single spraying BBCH 13-15	0.07%
		0.13%
	double spraying BBCH 13-15 BBCH 61	0.07%
		0.13%
Kelpak SL	single spraying BBCH 13-15	0.7%
		1%
	double spraying BBCH 13-15 BBCH 61	0.7%
		1%
Terra Sorb Complex	single spraying BBCH 13-15	0.3%
		0.5%
	double spraying BBCH 13-15 BBCH 61	0.3%
		0.5%

Table 2. Temperature (T) and precipitation during the soybean growing season 2014-2016.

Month	Year 2014		Year 2015		Year 2016		Average from 2002 to 2013	
	T (°C) min max	Rainfall (mm)	T (°C) min max	Rainfall (mm)	T (°C) min max	Rainfall (mm)	T (°C)	Rainfall (mm)
April	9.4 -6.0 22.7	36.5	8.2 -1.7 24.3	30.1	9.2 -1.2 22.6	68.4	8.5	41.2
May	13.7 0.5 27.7	208.3	12.7 1.5 24.9	108.6	13.8 2.6 26.7	61.3	12.7	63.4
June	16.1 6.7 28.9	67.1	17.4 6.6 30.5	14.1	18.1 4.2 31.5	97.1	17.7	68.6
July	20.3 10.0 31.0	104.2	19.6 8.4 33.4	59.2	19.5 8.8 31.2	107.6	18.9	79.1
August	18.2 6.3 34.0	115.4	21.6 5.6 35.5	23.4	18.2 7.1 30.7	95.3	19.4	71.8
September	13.7 3.7 25.8	89.4	15.1 4.2 34.5	137.6	15.2 1.6 28.7	41.2	14.1	69.2
Average/ Total	15.1	620.9	15.8	373.0	17.1	470.9	15.2	393.3

2.2 Determination of the antiradical activity

After the harvest, seeds were dried, ground in a laboratory grinder, and sieved (mesh size 0.310 mm). Flour was stored at a temperature of -20°C until analyzed. The antiradical activity of soybean seeds against ABTS•+ was determined using an acetone extract prepared acc. to a modified method by Kumar et al. [9]. Ground soybean seeds (100 g) were weighed into a conical flask with a ground glass stopper, poured with 2 mL of 70% acetone, and shaken at a room temperature of 2 h. Next, the samples were centrifuged at the speed of 10,000 x g and temperature of 20°C for 15 min. The supernatant was collected and stored in the dark at a temperature of -50°C until analyzed.

2.2.1 Determination of the antiradical activity against ABTS •+cation radical

The antiradical activity was determined according to the method developed by Re et al. [10]. ABTS (diammonium 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) was dissolved in distilled water until it has reached the concentration of 7mM. The ABTS•+ cation radical was obtained as a result of ABTS reaction with 2.45 mM potassium persulfate (K2S2O8). The reaction mixture was incubated at a room temperature for 12 h. The ABTS•+ solution was diluted with distilled water until its absorbance of 0.700 (±0.02) has reached at the wavelength of λ = 734. Determination of the antiradical activity consisted in the measurement of absorbance decrease during ABTS•+ cation radical reduction under the influence of antioxidants contained in the analyzed extracts, resulting in the formation of a colorless ABTS. To this end, 250 µL of the ABTS•+ solution were added to 5 µL of the extract, and the mixture was thoroughly mixed. Absorbance of the resultant solution was measured after 2h at the wavelength of λ = 734, using an EPOCH 2 microplate reader (BioTek, USA). The antiradical activity was expressed as Trolox equivalent (TE) w mg/g d.m.

2.2 Statistical analysis

Data on the antiradical activity of soybean seeds from four replicates of each combination were subjected to the statistical analysis. The Shapiro-Wilk test was performed for the normal distribution of data. The results were analyzed using one-way analysis of variance, ANOVA. The significance of differences between evaluated mean values was estimated by means of Turkey's test intervals of confidence at a significance level of $p < 0.05$. The statistical analysis was performed with Statistica 12 (StatSoft, Inc.).

3 Results and Discussion

The study showed that foliar application of biostimulants were beneficially affected the antiradical activity of soybean seeds (Table 3). It was found that foliar application of Terra Sorb Complex in 2014, Atonik and Tytanit in 2015 or Kelpak SL in 2016 increased this property.

Table 3. Change in the antiradical activity of soybean cv. Annushka affected by the application of biostimulants (mg/g d.m.).

Year	Control	Application (B)	Biostimulant (A)				
			Atonik	Tytanit	Kelpak SL	Terra Sorb Complex	Mean
2014	5.65 bc	B1	5.60 ab	5.57a	5.54a	5.56a	5.57A
		B2	5.69c	5.68bc	5.72c	5.74c	5.71C
		B3	5.74c	5.75c	5.73c	5.68bc	5.72C
		B4	5.57a	5.69c	5.52a	5.72c	5.63B
		Mean	5.65AB	5.67AB	5.63A	5.68B	5.66
2015	5.18 de	B1	5.19de	5.30ef	5.25e	5.02abc	5.19C
		B2	5.41f	5.13cd	4.93a	5.10bcd	5.14AB
		B3	5.12cd	5.21de	4.96ab	5.07bc	5.09A
		B4	5.25e	5.27e	5.00ab	5.06bc	5.14AB
		Mean	5.24B	5.23B	5.04A	5.06A	5.14
2016	5.38 ab	B1	5.50cd	5.48bcd	5.48bcd	5.53de	5.50AB
		B2	5.41abc	5.44abc	5.66f	5.67f	5.54B
		B3	5.42abc	5.40abc	5.63ef	5.49cd	5.48A
		B4	5.40abc	5.35a	5.63ef	5.48bcd	5.46A
		Mean	5.43A	5.42A	5.60C	5.54B	5.50

Abbreviations: B1- single spraying with low concentration of biostimulant; B2- double spraying with low concentration of biostimulant; B3 - single spraying with high concentration of biostimulant; B2- double spraying with high concentration of biostimulant. Different letters within the same year denote significant differences between the treatments at $p<0.05$.

Considering the modes of biostimulants application, the best effects were achieved after double plant spraying with the lower concentration of the preparations in 2014 and 2016, when the antiradical activity of the seeds increased by 1.1 - 3.0% compared to the control seeds (Table 3). However, single spraying with the lower concentration of biostimulants in 2015 increased this property and gave a similar results as control. Both the double spraying with Atonik, Kelpak SL and Terra Sorb Complex in the lower concentration and Tytanit, Terra Sorb Complex in the higher concentration as well as single spraying with Atonik, Tytanit and Kelpak SL in the lower concentration increased the antiradical activity of soybean seeds in 2014. Also the best effect on this property had the double spraying with Atonik in the lower concentration in 2015 and the same mode of Kelpak SL and Terra Sorb Complex application in 2016.

It was no significant effect of the foliar application of the biostimulants on the antiradical activity of soybean seeds (means for 2014-2016) (Figure 1). However the plants double sprayed with Atonik and Terra Sorb Complex in the lower concentrations achieved the same and, simultaneously, the highest antiradical activity of the seeds. Single spraying with Atonik, regardless of its concentration, caused a similar antiradical activity of the seeds, but double spraying with this biostimulant in the higher concentration reduced it. Of all tested combinations, the lowest antiradical activity was noted upon single spraying with Terra Sorb Complex in the lower concentration. In turn, the higher concentration of this biostimulant increased the activity, however differences in the antiradical activity between the single and double sprayed plants were negligible. Plants single sprayed with Tytanit responded with an enhanced antiradical activity compared to the double sprayed plants, but still the highest value of the activity was obtained upon double application of the biostimulant in the lower concentration. Both the single spraying with Kelpak SL in both concentrations as well as double spraying with this biostimulant in the lower concentration had a positive effect on the antiradical activity of soybean seeds compared to the double spraying with Kelpak SL in the higher concentration.

The control plants were characterized by a lower antiradical activity which was, however, higher than that achieved upon single spraying with Terra Sorb Complex in the lower concentration and upon double spraying with Kelpak SL in the higher concentration. In addition, the single spraying of the plants with biostimulants in the higher concentrations resulted in a smaller scatter of results.

The positive effect of biostimulants on the quality of legume seeds was confirmed in earlier studies. Kocira et al. [3] demonstrated double application of Kelpak SL, in both lower and higher concentration, to significantly increase the antioxidative activity of seeds of *Phaseolus vulgaris* Toska cv. (with red seed coat) against ABTS•+. In the case of bean of Aura cv. (white seed coat), this activity was more beneficially affected by double spraying with Kelpak SL in the higher concentration. Single application of a biostimulant based on nitrophenolic compounds (Atonik) in the lower concentration had a positive effect upon antiradical activity (ABTS) of Aura cv. common bean seeds, causing a significant increase in the activity (by 64%). In turn, common bean of Toska cv. was more positively responding to the single spraying with Atonik in the higher concentration; the antiradical activity of its seeds increased by 9%, though differences were statistically insignificant [4]. A 5-6% increase in the antioxidative activity of seeds of common bean of Toska cv. was also obtained upon the application of an organic growth stimulant Nano-Gro, regardless of the form of its application [6].

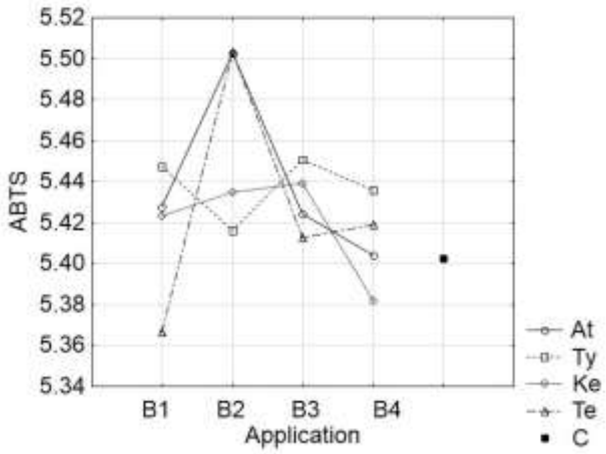

Fig. 1. Antiradical activity of soybean cv. Annushka affected by the application of biostimulants (means for 2014-2016) (mg/g d.m.). Abbreviations: see table 2; At-Atonik; Ty-Tytanit; Ke-Kelpak SL; Te-Terra Sorb Complex; C-Control.

4 Conclusions

The foliar application of both natural and synthetic biostimulants improved the quality of soybean seeds. In addition, it positively influenced the antiradical activity of soybean seeds, especially upon the double spraying with lower concentrations of the preparations.

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