AGRONOMY SCIENCE
wcześniej – formerly
Annales UMCS sectio E Agricultura
VOL. LXXVII (2) 2022

http://doi.org/10.24326/as.2022.2.1

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The impact of foliar application of biostimulators on the morphological characteristics of the leaf rosette of winter rape plants

Wpływ dolistnego stosowania biostymulatorów na cechy morfologiczne rozety liściowej roślin rzepaku ozimego

Summary. The field experiment was caried out in three growing seasons (2013–2016) at the Agricultural Experimental Station – Zawady (52°03'N and 22°33'E) belonging to the University of Natural Sciences and Humanities in Siedlce, Poland. The experiment was set up in a split-plot configuration with three replications. The examined factors were: I – morphotypes of winter oilseed rape: population (Poznaniak), hybrid restored with a semi-dwarf type of growth (PX104), hybrid restored with a traditional type of growth (Konkret); II – four types of biostimulators: 1. control variant, 2. biostimulator Tytanit® (titan), 3. biostimulator Asahi®SL (sodium ortho-nitrophenol, sodium para-nitrophenol, sodium 5-nitroguaiacol), 4. biostimulator Silvit® (active silicon, potassium oxide, boron – form of pure element, zinc – form of pure element). The aim of the study was to evaluate the effect of biostimulators containing various active substances on the morphological characteristics of winter oilseed rape plants in the leaf rosette stage (number of rosette leaves, tap root length, height of the growth cone) and on the fresh and dry mass of the above-ground rosette and the root system of traditional and semi-dwarf winter oilseed rape morphotypes type of growth under changing climatic conditions. The genetic factor significantly influenced the morphological features of plants, marked in autumn by inhibition of vegetation. The population variety Poznaniak developed more rosette leaves, greater length of the tap root, and fresh and dry mass of the rosette and the root system as compared to the restored hybrids. The applied biostimulators significantly influenced plant parameters determined in the fall before the vegetation inhibition. After the use of a biostimulator containing the active substance in the form of sodium o-nitrophenol, sodium para-nitrophenol and sodium 5-nitroguajakol, a significantly higher value of the tested features was obtained compared to the control variant. Regardless of the type of biostimulator used, the height of the growth cone was the same as in the control object. The most favourable parameters of the leaf rosette were obtained in the first and second periods of summer-autumn vegetation and winter dormancy, while in extreme

drought conditions during sowing and a fairy dry period of emergence, its weakest features were obtained. Natural growth stimulants are becoming an increasingly common elements of agricultural plant agrotechnics. Their use has a positive effect on the growth and development of plants and increases the resistance of plants to the adverse effects of stress factors.

Key words: morphotypes, *Brassica napus* L., bioregulators, active substances, number of leaves in the rosette

INTRODUCTION

Biostimulators are products containing biologically active substances that influence the metabolism and contribute to increasing the quantity and improving the quality of the crop. According to Kocira et al. [2015], these preparations complement the application of plant protection products and mineral fertilizers. According to Babuška [2004], biostimulators are defined as preparations supporting the natural life processes of plants and increasing plant resistance to stress factors.

The development of plants through winter, taking into account the morphological features of the leaf rosette, determines the cold resistance of oilseed rape [Diepenbrock 2000, Velička et al. 2010]. In environmental conditions that are unfavourable for plant growth and development, the use of biostimulants results in faster and more even emergence of plants, stimulation of growth and development, and better overwintering. Applied at the beginning of vegetation, they allow plants to regenerate after winter and quickly grow in spring, while during the flowering period they help the plant to focus on flower development. Paradikovic et al. [2011] emphasize that the use of synthetic or natural biostimulators improves the biochemical, morphological and physiological processes taking place in the cultivated plant.

The aim of the study was to evaluate the effect of biostimulators containing various active substances on the morphological features of the leaf rosette (number of rosette leaves, tap root length, height of the growth cone) and on the fresh and dry mass of the above-ground rosette and the root system of traditional and semi-dwarf winter oilseed rape morphotypes type of growth under changing climatic conditions.

MATERIAL AND METHODS

The field experiment was carried out in three growing seasons (2013–2016) at the Agricultural Experimental Station – Zawady (52°03'N and 22°33'E) belonging to the University of Natural Sciences and Humanities in Siedlce, Poland. The experiment was set up in a split-plot configuration with three replications. The area of one plot for harvesting was 18 m^2. The examined factors were: I – morphotypes of winter oilseed rape: population (Poznaniak), hybrid restored with a semi-dwarf type of growth (PX104), hybrid restored with a traditional type of growth (Konkret); II – four types of biostimulators (used in accordance with the manufacturer's recommendations): 1. control variant – without the use of biostimulators, sprayed with distilled water, 2. biostimulator Tytanit® (titan), used in the fall in the 4–8 leaf stage (BBCH – BiologischeBundesanstalt, Bundessortenamt and Chemische Industrie 14–18) at a rate of 0.20 dm^3 ha^{-1}; 3. biostimulator Asahi®SL (sodium ortho-nitrophenol, sodium para-nitrophenol, sodium 5-nitroguaiacol), used in the fall in

the 3–5 leaf stage (BBCH 13–15) at a rate of 0.60 dm^3 ha^{-1}; 4. biostimulator Silvit® (active silicon, potassium oxide, boron – form of pure element, zinc – form of pure element), applied 3 weeks after emergence (BBCH 12–14) at a rate of 0.20 dm^3 ha^{-1}. In the 2013–2014 growing season, spring wheat was the forecrop for winter oilseed rape, and winter triticale for the remaining ones. The experiment was carried out on soil classified according to the IUSS Working Group WRB [FAO 2014], to the Haplic Luvisol group, sanded, belonging to the very good rye complex, valuation class IVb. In the years of the research, the reaction (pH in 1M KCl) of the soil was slightly acidic and ranged from 5.68 to 5.75. The soil was characterized by low abundance in assimilable forms of phosphorus, ranging on average from 75 to 81 mg P kg^{-1} and the average assimilability in potassium ranging on average from 200.0 to 205.0 mg K kg^{-1} and magnesium – on average from 59 to 61 mg kg^{-1}. Before sowing, phosphorus fertilization at the dose of 40 kg P ha^{-1} and potassium fertilization at the dose of 110 kg K ha^{-1} and the first dose of 40 kg N ha^{-1} were applied. Fertilization was applied in the form of Lubofos in a dose of 600 kg. The fertilization doses were supplemented with 55.9 kg ha^{-1} of ammonium nitrate, 29.6 kg ha^{-1} of triple superphosphate and 29 kg ha^{-1} of potassium salt.

The sowing of winter oilseed rape was performed with a row spacing of 22.5 cm, assuming a density of 60 pcs m^{-2}. Sowing was carried out at the optimal date recommended for this region (in 2013 on August 13th, in 2014 on August 11th, and in 2015 on August 14th).

In line with the recommendations of good agricultural practice, chemical protection was applied against weeds, diseases and pests. Immediately before the autumn vegetation was stopped, the following biometric features were determined on a randomly selected sample of 20 plants:

– number of rosette leaves before the inhibition of autumn vegetation (pcs.),
– height of the growth cone (cm),
– length of tap root (cm),
– fresh and dry weight of the above-ground part of the rosette (g),
– fresh and dry mass of the root system (g).

Research results were statistically analysed by ANOVA. The results of the study were statistically analysed using the analysis of variance. The significance of the sources of variation was tested by the Fischer-Snedecor "F" test, and the assessment of significance of differences at the significance level $p < 0.05$ between the compared averages used Tukey's multiple intervals. Statistical calculations were made based on our own algorithm written in Excel.

RESULTS

On the basis of own research, it was shown that the applied biostimulators increased the number of rosette leaves produced during the autumn vegetation (Tab. 1). The highest significant value of this characteristic, compared to the control object, was recorded after the use of a preparation containing sodium ortho-nitrophenol, sodium para-nitrophenol and sodium 5-nitroguajakol, while after the application of this preparation and a biostimulator containing titanium, the values of this characteristic were the same. The number of rosette leaves did not differ significantly in the control object and in object 4, where the active substance in the form of silicon was applied.

Among the examined cultivars, the highest number of rosette leaves – on average

10.2 pcs. was found in the Poznaniak cultivar, while the re-spotted hybrids (Konkret and PX104) were characterized by the same value of this feature.

The authors' own research showed no significant effect of the active substances used on the height of the growth cone (Tab. 1).

Table 1. Morphological features of plants after inhibition of autumn vegetation depending on the factors of the experiment

Factors of the experiment		Morphological features of plants after inhibition of autumn vegetation		
		number of leaves per rosette	height elevation of shoot apex (cm)	pile root length (cm)
Cultivars	Poznaniak	10.2 [a]	2.25 [a]	19.65 [a]
	Konkret	9.3 [b]	2.17 [b]	17.24 [b]
	PX104	9.0 [b]	2.21 [ab]	16.61 [c]
$LSD_{0.05}$		0.3	0.06	0.20
Types of biostimulators used	control variant	9.2 [a]	2.22 [a]	17.59 [a]
	biostimulant – active substance – titanium (Tytanit®)	9.7 [b]	2.20 [a]	17.76 [a]
	biostimulant – active substances: sodium orto nitrophenol, sodium para nitrophenol, sodium 5-nitroguaiacolate (Asahi®SL)	9.8 [b]	2.22 [a]	18.22 [b]
	biostimulant – active substances: active silicon, potassium oxide, boron – form of pure element, zinc – form of pure element (Silvit®)	9.3 [a]	2.19 [a]	17.75 [a]
$LSD_{0.05}$		0.3	n.s.	0.25
Years	2013–2014	10.1 [a]	2.27 [a]	19.00 [a]
	2014–2015	10.1 [a]	2.22 [a]	18.55 [b]
	2015–2016	8.3 [b]	2.13 [b]	15.94 [c]
$LSD_{0.05}$		0.3	0.06	0.20

n.s. – not significant; values marked with the same letter do not differ significantly at $p = 0.05$.

Our own research showed that the highest positioned growth cone was found in the population cultivar Poznaniak and the restored hybrid with a semi-dwarf growth type, while the differences in the value of this feature between the restored hybrids were statistically insignificant.

Based on the conducted research, it was shown that only after the use of a preparation containing sodium o-nitrophenol, sodium para-nitrophenol and sodium 5-nitroguajakol, a significant increase in the length of the tap root was found on average by 0.63 cm compared to the control variant (Tab. 1). As a result of the application of other biostimulators, the value of this feature was the same as on the object sprayed with water.

The population morphotype had the greatest length of the tap root, a significantly

lower value of this trait was demonstrated in the long-stemmed hybrid, and the lowest in the semi-dwarf form.

Own research showed that the highest fresh and dry mass of the aboveground part of the rosette and the root system was found after the application of a preparation containing sodium o-nitrophenol, sodium para-nitrophenol and sodium 5-nitroguajakol (Tab. 2). After the application of the silicon-containing biostimulator (object 4), the values of this characteristic were the same as on the control object.

Table 2. Fresh and dry weight of the aboveground part of the rosette (g) and the root system of 1 rosette (g) depending on the factors of the experiment

Factors of the experiment		Morphological features of plants after inhibition of autumn vegetation			
		green matter of one plant (g)	dry matter of one plant (g)	green matter of the root system of one plant (g)	dry matter of the root system of one plant (g)
Cultivars	Poznaniak	42.13 [a]	9.21 [a]	9.49 [a]	2.34 [a]
	Konkret	33.61 [b]	7.39 [b]	8.09 [b]	1.95 [b]
	PX104	29.82 [c]	6.58 [c]	6.61 [c]	1.60 [c]
$LSD_{0.05}$		0.68	0.12	0.15	0.14
Types of biostimulators used	control variant	34.19 [a]	7.49 [a]	7.81 [a]	1.89 [a]
	biostimulant – active substance − titanium (Tytanit®)	35.47 [b]	7.77 [b]	8.08 [b]	1.96 [a]
	biostimulant – active substances: sodium orto nitrophenol, sodium para nitrophenol, sodium 5-nitroguaiacolate) (Asahi®SL)	36.40 [c]	8.00 [c]	8.36 [c]	2.09 [b]
	biostimulant – active substances: active silicon, potassium oxide, boron – form of pure element, zinc – form of pure element (Silvit®)	34.69 [a]	7.64 [a]	8.01 [b]	1.91 [a]
$LSD_{0.05}$		0.63	0.20	0.13	0.13
Years	2013–2014	38.45 [a]	8.30 [a]	9.22 [a]	2.18 [a]
	2014–2015	38.19 [a]	9.20 [a]	8.54 [b]	2.21 [a]
	2015–2016	28.92 [b]	5.68 [b]	6.43 [c]	1.50 [b]
$LSD_{0.05}$		0.68	0.12	0.15	0.14

Values marked with the same letter do not differ significantly at $p = 0.05$.

The largest fresh and dry weight of the above-ground part of the rosette and the root

system was characteristic for the population variety, significantly smaller for the restored hybrid with a traditional type of growth, and the smallest for the semi-dwarf hybrid PX104.

In the years of the research, in the periods of summer-autumn vegetation and winter rest, the climatic conditions were quite varied (Tab. 3–4). In the first year of research, during sowing, there was little rainfall (15 mm) compared to the multi-year average. The average daily temperature in this month averaged 18.8°C and was by 0.3°C higher than in many years. In September, rainfall was more than twice the long-term average, and the average air temperature was lower by 1.8°C on average. On the basis of the calculated Sielianinov coefficient (k = 2.63) it was shown that September was very humid. In October and November, precipitation totals were higher than for many years and warmed by 1.4°C and 1.1°C, respectively, while December was also warmer, but drier compared to the average for 1996–2010. Precipitation totals in the period from January to March had higher values compared to the long-term average, and in the case of average air temperatures, a similar trend was shown in February and March. In the 2014–2015 growing season, during sowing, significant amounts of precipitation occurred, exceeding the multi-year average by 45.8 mm, while the average daily air temperature was lower by 0.4°C. On the basis of the Sielianinov hydrothermal coefficient, September and October were very dry, while in November the total rainfall was higher by 12.3 mm, and the average temperature lower by 0.6°C compared to the multi-year average. In December, January and March, the amount of rainfall was recorded, significantly exceeding the amount of rainfall from the long-term period. In the last year of the research, August was exceptionally warm and quite dry (k = 1.2). During the sowing, the sum of rainfall was over five times lower, and the average air temperature was on average 2.5°C higher than for many years. In September, the average daily temperature was higher than the multi-year average, while October was humid and cooler by 1.4°C than in the multi-year period. In November, the total rainfall was twice as high as in many years, and the average air temperature was 0.7°C higher. December was much warmer compared to the multi-year period. In January, the sum of precipitation and the average daily air temperature were lower than the long-term average. February was exceptionally warm and humid. The average daily temperature in that month was higher than the long-term average by 4.8°C, respectively, and the sum of rainfall was by 13 mm. A similar tendency occurred in March, the total rainfall was greater by 15.2 mm, and the average daily temperature was 1.1°C higher than the average for the period 1996–2010.

On the basis of own research, a significant influence of the climatic conditions in the years of the experiment was found: morphological features of the leaf rosette, such as: number of rosette leaves, tap root length, height of the growth cone and fresh and dry mass of the above-ground part of the rosette and the root system (Tabs 1 and 2).

In the 2013–2014 and 2014–2015 growing season, the number of leaves produced in the rosette was the same, while in the last period of the summer-autumn vegetation, which was quite dry during sowing and emergence, the value of this feature was much lower. A similar tendency was recorded with regard to the height of the growth cone.

The greatest length of the tap root was found in the first test period, which was extremely dry during sowing and very humid in emergence. In the second year of the research, it was quite wet during sowing and very dry during emergence, the value of this feature was lower on average by 0.45 cm, while in the last growing season the length of the tap root was the smallest, on average 15.94 cm.

Table 3. Characteristics of weather conditions in the years 2013–2016 (Zawady Meteorological

Station, Poland)

Months	Rainfall (mm)				Air temperature (°C)			
	multiyear sum	monthly sum			multiyear mean	monthly mean		
	1996–2010	2013–2014	2014–2015	2015–2016	1996–2010	2013–2014	2014–2015	2015–2016
August	59.9	15.0	105.7	11.9	18.5	18.8	18.1	21.0
September	42.3	94.3	26.3	47.1	13.5	11.7	14.1	14.5
October	24.2	32.8	3.0	37.0	7.9	9.3	8.5	6.5
November	20.2	34.7	32.5	42.2	4.0	5.1	3.4	4.7
December	18.6	15.4	90.4	16.5	-0.1	1.2	0.1	3.7
January	19.0	28.6	51.4	10.9	-3.2	-4.5	0.6	-4.5
February	16.0	34.0	0.7	29.0	-2.3	0.7	0.7	2.5
March	18.3	29.6	53.1	33.5	2.4	5.8	4.6	3.5
Sum/Mean	218.5	284.4	363.1	228.1	5.1	6.0	6.3	6.5

Table 4. Sielianinov hydrothermic coefficients in the years 2013–2016

Sielianinov hydrothermic coefficients*			
Months	2013–2014	2014–2015	2015–2016
August	0.31	1.87	0.20
September	2.63	0.66	1.20
October	1.01	0.22	2.15
March	1.48	4.63	3.49
Mean	1.35	1.84	1.76

* Index value [Skowera 2014]: extremely dry $k \leq 0.4$; very dry $0.4 < k \leq 0.7$; dry $0.7 < k \leq 1.0$; rather dry $1.0 < k \leq 1.3$; optimal $1.3 < k \leq 1.6$; rather humid $1.6 < k \leq 2.0$; humid $2.0 < k \leq 2.5$; very humid $2.5 < k \leq 3.0$; extremely humid $k > 3.0$.

No statistically significant differences were found in the growing seasons 2013–2014 and 2014–2015 in the fresh and dry matter of the above-ground part of the rosette and the dry weight of the root system. The lowest values of the examined traits were found in the last summer and autumn growing season.

DISCUSSION

The results of own research are consistent with the results of Gugała et al. [2017]. After using the Asahi SL biopreparation, the authors also noted its highest value, while Sikorska et al. [2019a], after the application of the biostimulant containing amino acids, noted the same number of leaves in the rosette and in the control object.

Own research and the research of many other authors showed a greater number of rosette leaves in population cultivars. Gugała et al. [2017] obtained higher values of the discussed traits in the population variety Monolit compared to the heterotic ones: PR44D06

and PT205, Kotecki et al. [2007] in the population variety Lisek compared to the heterotic hybrids Baldur and Titan, Wielebski and Wójtowicz [2018] found the highest value of this trait in the population from Starter, significantly lower in restored hybrids Poznaniak and PR45D03. Velička et al. [2010] who recorded an average of 15.3% more rosette leaves of the Kronos restored hybrid compared to the population variety. Whereas Sikorska et al. [2019a] showed that the genetic factor did not significantly affect the number of leaves in the rosette.

In own and other authors' research, no significant effect of the active substances used on the height of the growth cone. Sikorska et al. [2019a], after using the Aminoplant biostimulator, showed the same value of this feature as in the control study. Gugała et al. [2017] showing the greatest increase in the height of the growth cone elevation under the influence of the Asahi SL biostimulator.

The results of own research concerning the height of the growth cone are consistent with the results by Kotecki et al. [2007]. Whereas Malarz et al. [2006], Gugała et al. [2017] and Sikorska et al. [2019a] showed no significant differences between the studied morphotypes in the height of the growth cone. Wielebski and Wójtowicz [2018] obtained a higher growth cone by an average of 1.3 cm compared to a restored hybrid with a traditional type of growth.

Research results concerning the influence of biostimulants on the tap root length is consistent with the results of the research by Gugała et al. [2017]. The authors obtained the highest value of the discussed feature – on average 17.2 cm after spraying Asahi SL, and the lowest on the plots where Tytanit and Silvit were applied (on average 17.0 cm). Similar research results were obtained by Aisha et al. [2014] and Albayrak and Camas [2005] after the application of humic acids. Whereas Sikorska et al. [2019a], as a result of using the Aminoplant biostimulator, obtained an increase in the length of the tap root by only 0.19 mm.

The results of own research are consistent with the results of the research by Gugała et al. [2017], who in all the years of the study received the highest value of this trait in the population cultivar Monolit. Different research results were obtained by Sikorska et al. [2019a], who, among the compared cultivars, noted the highest tap root length in a restored hybrid with a traditional type of growth, significantly lower in a semi-dwarf hybrid, and the lowest in the population cultivar Monolit.

Research results concerning the influence of biostimulants on fresh and dry mass of the aboveground part of the rosette and the root system are consistent of the research of Sikorska at el. [2017] and Gawrońska at al. [2008]. The authors also obtained the highest value of the discussed feature on objects where the Asahi SL biostimulator was used. Albayrak and Camas [2005], Soheir et al. [2012] and Aisha et al. [2014], under the influence of stimulation with humic acids, obtained higher fresh and dry mass of the above-ground part of the rosette compared to the object on which no biostimulators were used. Sikorska et al. [2019b] received a slight increase in the value of this feature under the influence of a biostimulator with amino acids.

The results of own research are consistent with the results of the research by Sikorska at al. [2017, 2019b], who showed that among cultivated varieties, the highest fresh and dry weight of the rosette was recorded in the population form in a restored hybrid with a traditional type of growth, and the lowest in a restored hybrid with a semi-dwarf type of height. On the other hand, according to Jankowski and Budzyński [2007], it was heterotic forms that produced a rosette with a larger fresh and dry mass of 1 plant.

CONCLUSIONS

1. The genetic factor significantly influenced the morphological features on plants, marked in autumn by inhibition of vegetation. The population variety Poznaniak developed more rosette leaves, greater length of the tap root, and fresh and dry mass of the rosette and the root system as compared to the restored hybrids.

2. The applied biostimulators significantly influenced plant parameters determined in autumn before the vegetation inhibition. After the use of a biostimulator containing the active substance in the form of sodium o-nitrophenol, sodium para-nitrophenol and sodium 5-nitroguajakol, a significantly higher value of the tested features (number of leaves per rosette, pile root length, green and dry matter of one plant, green and dry matter of the root system of one plant) was obtained compared to the control variant. Regardless of the type of biostimulator used, the height of the growth cone was the same as in the control object.

3. The most favourable parameters of the leaf rosette were obtained in the first and second periods of summer-autumn vegetation and winter dormancy, while in extreme drought conditions during sowing and a fairly dry period of emergence, its weakest features were obtained.

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The source of funding. The results of the research carried out under the research theme No. 32/20/B were financed from the science grant granted by the Ministry of Science and Higher Education.

Received: 12.02.2022
Accepted: 05.05.2022