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ORIGINAL RESEARCH PAPER

Application of biostimulants influences shoot and root characteristics of seedlings of winter pea (*Pisum sativum* L.)

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Abstract

In the cooler regions of Europe, the success of winter pea cultivation depends strongly on proper plant development before winter. Previous research has suggested that plants need to develop short internodes and at least their first two leaves before the arrival of frost. However, this stage of growth is sometimes not reached in the event of late sowing, due to factors such as unpredictable weather conditions or the delayed harvest of a previous crop. An effective solution may be the application of plant growth regulators before the sowing of seeds. The aim of this study was to assess the seedling developmental characteristics of winter pea dependent on biostimulator applications in low temperature conditions (4°C). Seven different winter pea cultivars were treated with three biostimulants: Asahi SL, Kelpak SL, and Primus B. After 21 days of seedling development, basic biometrical characteristics were measured (length and weight of shoots and roots). It was found that 'Enduro' and 'Aviron' showed greatest root development, regardless of the applied biostimulants. The highest germination was achieved by 'Aviron'. The efficiency of biostimulators on the cultivars tested was low, although slightly better results were found for Asahi SL in combination with 'Enduro' and 'Aviron'.

Keywords

shoot and root parameters; legume, Asahi SL; Kelpak SL

Introduction

The winter pea is becoming a more and more popular variety of pea in countries of Western and Southern Europe such as France, Germany, the Czech Republic, and Italy, where unpredictable weather conditions impose limitations on yields, especially in the spring cultivars of legumes. In the light of the newest results, winter form of legumes have greater yield potential when compared to spring form, owing to the use of available rainfall water in the fall and spring periods. Winter pea can be cultivated as an edible or fodder crop, but also as a catch crop to protect soil against erosion [1–3]. Annicchiarico and Iannucci [4] and Rapčan et al. [5] have indicated that winter pea is able to achieve its greatest seed yield (4–5 t ha^{-1}) and biomass yield (60 t ha^{-1}), as compared to the spring form. The winter form of the pea is characterized by its greater harvesting potential [reaching in postregistration variety testing (PDO) research about 4.15 t ha^{-1}], and stability of yields from the spring forms, due to the scale of the vernalization process [6]. The success of winter pea can be explained by higher productivity, however, the success of plants productivity itself depends on agronomic and morphological parameters such as time of sowing, fertilization, and the stage of plant development [7]. Andersen and Markarian [8] indicate that pea plants before the winter time have to develop compact and mostly short internodes of stems and splitting and sloping rosette. Lejeune-Henaut et al. [9] and Andrzejewska et al. [10] noted that a short photoperiod delays the initiation of flowering and allows plants to overwinter. Intense exposure to sunlight increases the concentration of sugars soluble in the plant and increases the tolerance to frost [11]. Prusiński [12] states that under the most suitable weather conditions in fall, plants are able to adapt to winter conditions by the reduction of evaporation and transpiration processes.

Despite the unsatisfactory breeding progress focused on winter pea frost resistance, farmers take a risk and sow the winter variety of pea suitable for fodder production without a reasonable chance of success. Another solution for the survival of the seedling in winter is the preparation of juvenile plants through the development of bigger biomass and stronger roots in a shorter period of time before winter, using biological techniques – biostimulations. The new approach to agriculture tends to use environmentally friendly and safe products such as biostimulators, which have a broad spectrum of activity [13]. The use of biostimulators is effective when plants are grown under unfavorable conditions [14,15]. The biostimulator used when the plants are still healthy should change the metabolism in such a way that they become stronger and more resistant to drought, drops in temperature, or the occurrence of a pathogen [16].

Harasimowicz-Herman and Borowska [14] and Budzyński et al. [15] suggest that Asahi SL improves the ability of winter oilseed rape to cope with stressors and so increase yield. Thus, we assume that biostimulation can also improve the characteristics of the winter varieties of pea seedlings before the onset of winter and so enhance their overwintering.

In Central Europe, the cultivation of the winter pea is limited, owing to insufficient seedling and juvenile plant development before winter. Plant adaptation may be adjusted on the basis of sowing time. Unpredictable weather conditions in fall can preclude winter pea sowing at the optimal time, in which case it is assumed that the biostimulators may be capable of preparing pea seedlings for winter survival.

Up until now, there have been few studies on the use of growth stimulators for the acceleration of seedling growth of winter legumes [17–19]. The aim of the study was to evaluate the seedling parameters of selected winter pea cultivars in low temperature conditions after the application of biostimulants.

Material and methods

Experimental design

The trial was carried out at the Laboratory of Plant Biology and Biotechnology of the University of Agriculture in Krakow, Poland. Two factors were considered. The first was biosimulator application (Asahi SL, Kelpak SL, or Primus B), and the second was winter pea cultivar. Asahi SL (Arysta LifeScience Polska Sp. z o.o.) is a composition of three basic phenolic compounds (sodium ortho- and para-nitrophenolate, sodium 5-nitro guaiacolate) which easily penetrate plant cells and are involved in many physiological processes. Kelpak SL stimulates the growth of pea seeds through a mechanism related to phytohormone activity (the ratio of auxins to cytokinins is 350:1 at a concentration of 11 and 0.031 mg L^{-1}, respectively) and Primus B enhances the growth of test plants by enriching them with macro- and micronutrients, required for the improvement of germination and effective and fast establishment, which consequently involves more of the above-ground parts of the plant.

Seven cultivars were obtained from different plant breeders and seed companies and were three Czech 'Arkta', 'Aviron', and 'Enduro', three German 'E.F.B. 33', 'Pandora', and 'James', and one French 'Isard' (according to the Common Catalogue of Varieties of Agricultural Plant Species, CCA: https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=OJ:C:2019:013:FULL&from=SV).

Seed preparation

The three biosimulators were applied at an equivalent dose rate of 2 L ha^{-1} under laboratory conditions. The seeds of each cultivar were soaked in biostimulator solution for 30 minutes. After this, the seeds were moved to germination boxes containing distilled water. This was subsequently used for the irrigation of seeds (3 min per day) during the whole period of germination and seedling growth (21 days).

Germination boxes with biostimulated seeds were maintained in a growth chamber (Simez Control s.r.o. Vsetin, the Czech Republic) for 21 days at 4°C, with a photoperiod 16/8 h day/night and a photon flux density of 250–280 μmol m^{-2} s^{-1} PAR. The humidity was not controlled.

Seedling measurement

Measurements were conducted 3 weeks after seed sowing in the growth chamber. They were selected morphometric traits including root and shoot lengths and biomass.

Statistical analysis

All data were analyzed in a two-factor analysis of variance (ANOVA) implemented by STATISTICA 13.1 software. The significance of differences between means ($n = 9$) were tested using Tukey's HSD test at the $p < 0.05$ probability level.

Results

No biostimulator had a significant impact on the stem/root lengths or biomass (Tab. 1) and cultivars differed significantly in stem and root lengths and biomass. Biostimulant source and cultivar also interacted significantly (Tab. 1). Asahi SL had a minor positive effect on the characteristics assessed when compared to Kelpak SL, which induced a reduction of stem and root weights. Primus B enhanced root weights of the pea seedlings (by about 20%) in comparison to the other biostimulators tested. Significantly longer

Tab. 1 Characteristics of seedlings dependent on biostimulation application and cultivar.

Traits	Root weight (g)	Root length (cm)	Stem weight (g)	Stem length (cm)	Germination (%)
Biostimulator					
Without (control)	0.0509	6.012	0.031	1.92	88.5
'Asahi SL'	0.051	6.502	0.042	2.00	86.5
'Kelpak SL'	0.051	5.740	0.035	1.69	76.9
'Primus B'	0.064	6.320	0.039	1.93	90.4
p value	n.s.	n.s.	n.s.	n.s.	n.s.
Cultivar					
'Enduro'	0.085 [b]	8.130 [b]	0.045 [ab]	1.85 [ab]	82.7
'E.F.B. 33'	0.030 [a]	4.970 [a]	0.029 [a]	2.13 [b]	90.4
'Arkta'	0.024 [a]	3.911 [a]	0.027 [ab]	1.71 [a]	73.1
'Aviron'	0.077 [b]	7.563 [b]	0.046 [ab]	1.86 [ab]	96.2
p value	**	**	**	*	n.s.
p value (Biostimulator × Cultivar)	**	*	**	**	n.s.

Differences between means are significant at $p < 0.05$ (*) or at $p < 0.01$ (**).

and heavier roots were developed by 'Enduro' and 'Aviron'. Among the cultivars, the seedlings of 'E.F.B. 33' produced the longest stems (about 2.13 cm). However, 'Enduro' had the longest and heaviest roots (8.13 cm and 0.085 g, respectively).

Cultivar 'Aviron' had the greatest seed germination (96.2%), whereas only 73.1% of seeds germinated in 'Arkta'. The process of germination also varied between biostimulators used but the differences were not statistically significant. The application of Primus B resulted in the germination of 90.4% of seeds, whereas Asahi SL (86.5%) and Kelpak SL (76.9%) decreased the number of seeds germinated when compared to the control treatment (88.5%).

A more specific distinction between the cultivars in their reaction to the applied biostimulants is illustrated in Fig. 1A,B where both the lengths and the weights of roots were higher for 'Enduro' and 'Aviron'. The total weight of roots significantly varied between the cultivars and these was an interactive effect between cultivars and biosimulators. The heaviest roots were for 'Enduro' in combination with Primus B, which increased by 67% in relation to the mean value of the other cultivars treated with that biostimulator (Fig. 1A). The mean root weight for 'Enduro' and 'Aviron' tripled when compared to the average root weight of 'E.F.B. 33' and 'Arkta' regardless of the biostimulators applied. A similar result was observed for root lengths. The longest root length was attained by two cultivars 'Enduro' and 'Aviron', as both had 1.5 times longer roots on average, relative to 'E.F.B. 33' and 'Arkta' (Fig. 1B). However, we demonstrated that the combination of 'Enduro' with biostimulator Asahi SL was the most effective in improving root development of the winter pea cultivars tested in cool conditions (about 9.25 cm).

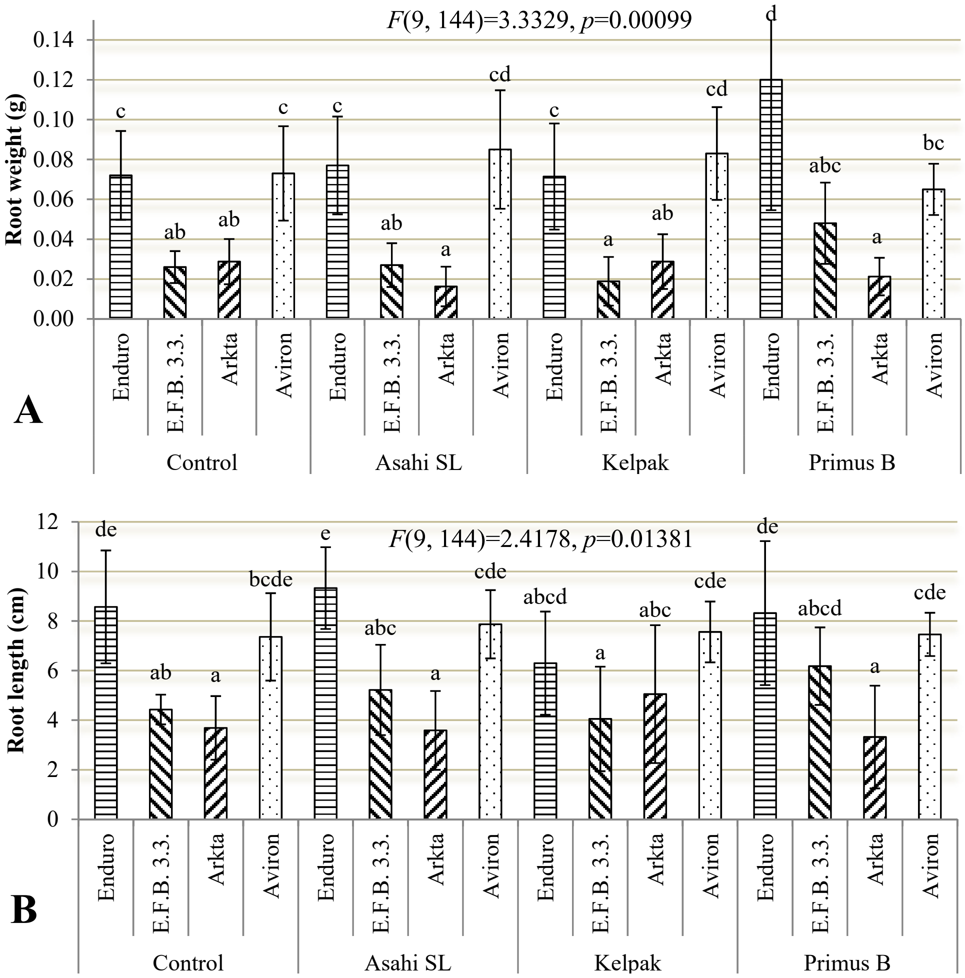

Fig. 1 The interaction of the cultivars and biostimulators in the assessment of the (**A**) weight (g) and (**B**) length (cm) of roots of winter pea seedlings. Different letters indicate differences between means significant at $p < 0.05$ according to Tukey's HSD test.

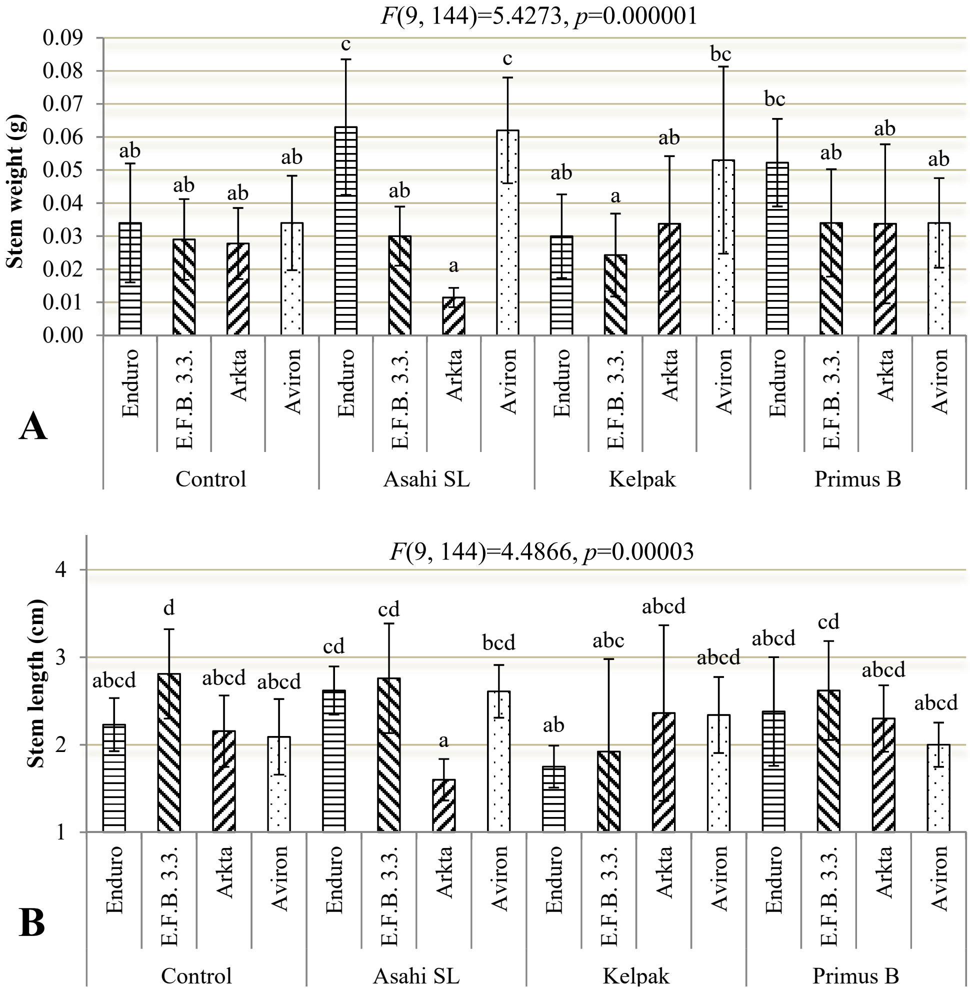

Fig. 2 The interaction of the cultivar and biostimulator in the assessment of the (**A**) weight (g) and (**B**) length (cm) of stems of winter pea seedlings. Different letters indicate differences between means significant at $p < 0.05$ according to Tukey's HSD test.

Cultivars 'Enduro' and 'Aviron' produced the greatest stem weights (Fig. 2A) at the cold temperature used (4°C). These two cultivars responded very positively to the use of all biostimulating compounds, especially to Asahi SL. This positive effect was also noted after Kelpak SL application to 'Aviron', and Primus B application to 'Enduro'. From Fig. 2B, it can be assumed that during the mild winter season (with approx. 5°C), cultivars such as 'E.F.B. 33' and 'Aviron' should develop the tallest above-ground parts after Asahi SL application, which may have adverse effects on their overwintering. There are only a few reports on this subject in the literature. According to Andrzejewska et al. [10], in terms of the requirements for overwintering well, pea seedlings should develop short stem internodes. The weights of the stems of the cultivars tested were similar to the control group. However, the most visible elongation was found in 'E.F.B. 33'.

The use of Kelpak SL resulted in shorter stem and root development, especially for 'E.F.B. 33' and 'Enduro', which would likely negatively impact on their winter survival, because the optimal height for 'Enduro' should be 5 cm, according to information provided by SELGEN a.s. The biostimulator Asahi SL had no influence on stem development of 'Arkta', as it prevented both the elongation and the increment grown. From these findings it can be generally concluded that the use of Asahi SL biostimulator is a beneficial option for both 'Enduro' and 'E.F.B. 33' in order to obtain a greater height and weight of the above-ground parts at a temperature of 4°C. Different results were observed for 'Arkta', for which an unfavorable impact of Asahi SL was observed, both in the height and weight of the stems of this winter pea cultivar. The use of Primus B biostimulator was beneficial for producing the longest stem (about 2.2 cm) for 'E.F.B. 33'.

Discussion

The research demonstrated that biostimulants affected pea seedling parameters by a variable magnitude (10–20%). Asahi SL biostimulator had a positive but relatively insignificant effect on the seedling parameters (root and stem length – 8% and 4%, respectively, and stem weight – 25%) compared to control. Kelpak SL application induced opposite reaction because it was observed inhibition of stems and roots traits (stem length – 15% and root length – 15%) and seed germination (about 15%) of winter pea. The third biostimulator, Primus B, stimulated only the root traits (length and weight) and the seed germination by about 20% compared to control and the other biostimulating compounds. Our findings only partly confirmed the previous published outcomes [20] related to biophysical techniques of biostimulations on plant morphological traits, because they were conducted on plants in advance morphological stages. The authors highlighted the positive effect of biophysical stimulation, which increases root mass by up to 24%, for leaf/stem mass between 10% and 45%, and increased yield in the range of 10–50%, compared to plants grown under normal conditions. Kozak et al. [21] proved that application of Asahi SL in soya increased the seed yield parameters. Similar outcomes were presented by Kocira et al. [22], who tested varied concentrations of Asahi SL on common bean yield.

Effectiveness of biostimulator is strongly dependent on the method of application, species, variety of crop, and concentration [23]. Our study proved a significant interaction between cultivars and biostimulator. 'Enduro' and 'Aviron' cultivars obtained significantly higher stem weight tested in low temperature (4°C), responding very positively to the use of biostimulation compounds, especially Asahi SL. This positive effect was observed after Kelpak SL was applied to *Peasum sativum* 'Aviron' and Primus B to 'Enduro'.

During the mild winter season (ca. 5°C), cultivars like 'E.F.B. 33' and 'Aviron' should develop the longest aboveground parts after Asahi SL application, which may have adverse effects in their overwintering. According to the available literature, there is visible limitation of papers regarding this issue. For the purpose of good wintering, winter pea seedlings should develop short stem internodes [10]. The weights of the stems among cultivars were aligned in the control group. However, the most visible decrease of stem weight was observed for 'E.F.B. 33'. Primus B as biostimulator had a strong but positive effect mainly on 'Enduro' seed germination and seedling development. Our findings are supported by previous studies of Jarecki et al. [24], who showed that Primus B significantly increased the seed germination. However, excessive stem elongation before winter time is not desirable. In the available literature, there are not many papers on the subject. According to Andrzejewska et al. [10], in terms of the requirements for wintering, winter pea seedlings should develop short stem internodes.

To conclude, it was found that biostimulation slightly improved the seedling parameters; however, in the interaction with winter pea cultivars, a significant effect on the selected parameters was indicated. The stem weight and length increased after the application of Asahi SL, mainly in 'Enduro' and 'Aviron', whereas the stem length of 'E.F.B. 33' decreased after the application of Kelpak SL. The cultivars 'Enduro' and 'Aviron' gained higher weight and length of roots in the cold thermal conditions (4°C), and we assume that these cultivars can be appropriate for use in Central Europe. The cultivar 'Arkta' exhibited the lowest seedling parameters of all the cultivars, which may be suitable in terms of optimal sowing conditions in warm winter conditions. The highest germination percentage (90.4%) was reported after the application of Primus B and the lowest value was noted after the application of Kelpak SL. Among the cultivars, 'Aviron' showed the highest seed germination percentage (96.2%).

References

1. Wilczewski E, Skinder Z, Lemanczyk G. Wartość wybranych roślin motylkowych uprawianych w międzyplonie ścierniskowym na glebie lekkiej. Część III. Wpływ następczy dla pszenicy jarej. Acta Scientarum Polonarum, Agricultura. 2007;6:45–56.

2. Księżak J, Staniak M. Ocena mieszanek strączkowo-zbożowych uprawianych ekologicznie jako surowca do produkcji kiszonek. Journal of Research and Applications in Agricultural Engineering. 2009;54:157–163.
3. Księżak J. Wybrane zagadnienia uprawy roślin strączkowych. Warszawa: Fundacja Programów Pomocy dla Rolnictwa FAPA; 2015.
4. Annicchiarico P, Iannucci A. Winter survival of pea, faba bean and white lupin cultivars in contrasting Italian locations and sowing times, and implications for selection. J Agric Sci. 2007;145:611–622. https://doi.org/10.1017/S0021859607007289
5. Rapčan I, Bukowić G, Grljušić S, Teklić T, Jurišić M. Yield of green mass, grain and other yield components of field pea (*Pisum sativum* L.) in dependence of agroecological conditions and seed maturity. Mljekarstwo. 2010;60:104–112.
6. Artyszak A. Pea yielding in PDO research in 2016 [Internet]. 2016 [cited 2017 Feb 25]. Available from: https://www.agropolska.pl/uprawa/straczkowe/plonowanie-grochu-w-badaniach-pdo-w-2016-r-,44.html
7. Strażyński P, Mrówczyński M. Metodyka integrowanej ochrony grochu dla doradców. Poznań: Instytut Ochrony Roślin – Państwowy Instytut Badawczy; 2016.
8. Andersen RL, Markarian D. The inheritance of winter hardiness in *Pisum*. III. Stem branching in autumn growth. Euphytica. 1968;17:473–478. https://doi.org/10.1007/BF00056250
9. Lejeune-Henaut I, Bourion V, Eteve G, Cunot E, Delhaye K, Desmyter C. Floral initiation in field-grown forage peas is delayed to a greater extent by short photoperiods, than in other types of European varieties. Euphytica. 1999;109:201–211. https://doi.org/10.1023/A:1003727324475
10. Andrzejewska J, Marciniak J, Skinder Z, Skotnicka E. Wartość gospodarcza ozimych rodów hodowlanych grochu siewnego (*Pisum sativum* L.) w warunkach regionu Kujawsko-Pomorskiego. Acta Scientarum Polonarum, Agricultura. 2005;4:5–15.
11. Bourion V, Lejeune-Henaut I, Munier-Jolain N, Salon C. Cold acclimation of winter and spring peas: carbon partitioning as affected by light intensity. Eur J Agron. 2003;19:535–548. https://doi.org/10.1016/S1161-0301(03)00003-0
12. Prusiński J. Proposals of new solutions in legumes production. Electronic Journal of Polish Agricultural Universities, Series Agronomy. 2011;14(2):14.
13. Glinicki R, Sas-Paszt L, Jadczuk-Tobjasz E. The effect of plant stimulant/fertilizer "resistim" on growth and development of strawberry plants. Journal of Fruit and Ornamental Plant Research. 2010;18:111–124.
14. Harasimowicz-Herman G, Borowska M. Effect of bio-stimulant Asahi SL in winter rapeseed depending on pluviothermic conditions. Rośliny Oleiste – Oilseed Crops. 2006;27(1):95–106.
15. Budzyński W, Dubis B, Jankowski K. Response of winter oilseed rape to the biostimulator Asahi SL applied in spring. In: Dąbrowski ZT, editor. Field crops. Warsaw: Editorial House Wieś Jutra; 2008. p. 18–24. (Biostimulators in Modern Agriculture).
16. Grzyś E, Demczuk A, Sacała E. Effect of Asahi SL on the activity of nitrate reductase, chlorophyll content in the leaves and yield of maize under nitrogen deficiency in the soil. In: Conference proceedings: "Biostimulators in modern agriculture"; 2008 Feb 7–8; Warsaw, Poland. Warszawa: Editorial House Wieś Jutra; 2008. p. 54.
17. Majchrzak B, Mikolajska J. Wpływ stresu temperaturowego i wilgotnościowego na kiełkowanie nasion i zdrowotność siewek grochu. Acta Academiae Agriculturae ac Technicae Olstenensis, Agricultura. 1992;184:93–102.
18. Prusiński J, Borowska M. Wpływ wybranych regulatorów wzrostu i ekolistu na skład chemiczny, żywotność i wigor nasion łubinu żółtego (*Lupinus luteus* L.). Acta Scientiarum Polonorum, Agricultura. 2002;1:81–97.
19. Zhelyazkova T. Influence of some plant growth regulators on the economic efficiency of winter pea variety Mir production. Agricultural Science and Technology. 2009;1(2):1–4.
20. Mosneaga A, Lozovanu P, Nedeff V. Investigation of biostimulation effects on germination and seedling growth of some crop plant species. Cellulose Chemistry and Technology. 2018;52:551–558.
21. Kozak M, Malarz W, Serafin-Andrzejewska M, Kotecki A. The effects of sowing rate and Asahi SL biostimulator on soybean growth and yield. In: Dąbrowski ZT, editor. Field crops. Warsaw: Editorial House Wieś Jutra; 2008. p. 77–84. (Biostimulators in Modern Agriculture).

22. Kocira A, Kocira S, Stryjecka M. Effect of Asahi SL application on common bean yield. Agriculture and Agricultural Science Procedia. 2015;7:103–107. https://doi.org/10.1016/j.aaspro.2015.12.045
23. Sultana V, Ehteshamul-Haque S, Ara J, Athar M. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. International Journal of Environmental Science and Technology. 2005;2(2):129–132. https://doi.org/10.1007/BF03325866
24. Jarecki W, Buczek J, Bobrecka-Jamro D. Response of spring wheat to diversified mineral fertilization. J Elem. 2017;22(3):881–891. https://doi.org/10.5601/jelem.2016.21.4.1328

Wpływ zastosowanych biostymulatorów na rozwój siewek grochu ozimego (*Pisum sativum* L.)

Streszczenie

W chłodnych regionach Europy czynnikiem warunkującym pomyślną uprawę grochu ozimego (*Pisum sativum* L.) jest odpowiedni przedzimowy rozwój roślin. Dotychczasowe wyniki badań wskazują, że do dobrego przezimowania, rośliny powinny wykształcić krótkie międzywęźla i przynajmniej dwa liście przed wystąpieniem pierwszych przymrozków, W przypadku opóźnionego terminu siewu, spowodowanego na przykład wahaniami pogodowymi lub opóźnionym zbiorem przedplonu, rośliny nie są w stanie rozwinąć się dostatecznie dobrze przed zimą. Rozwiązaniem tego problemu może być przedsiewne stosowanie regulatorów wzrostu. Celem przeprowadzonych badań było określenie wpływu biostymulatora na rozwój siewek grochu ozimego w warunkach niskiej temperatury (4°C). Siedem odmian grochu ozimego traktowano trzema biostymulatorami: Asahi SL, Kelpak SL i Primus B. Po 21 dniach rozwoju siewek grochu oceniono podstawowe cechy biometryczne (długość i masę części nadziemnych i korzeni). Najlepsze wartości parametrów korzeniowych odnotowano u odmian 'Enduro' oraz 'Aviron', niezależnie od zastosowanych biostymulatorów. Najlepiej kiełkowała odmiana 'Aviron'. Wpływ biostymulatorów na rozwój siewek był niewielki, jednakże stwierdzono zacznie lepsze efekty przy zastosowaniu preparatu Asahi SL u odmian 'Enduro' i 'Aviron'.