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## THE NUTRIENT CONTENT IN SOYBEAN SEEDS (*GLYCINE MAX* L. MERR.) AFTER APPLICATION OF EXOGENOUS GROWTH REGULATORS

## ZAWARTOŚĆ SKŁADNIKÓW ODŻYWCZYCH W NASIONACH SOI ZWYCZAJNEJ (*GLYCINE MAX* L. MERR.) PO ZASTOSOWANIU EGZOGENNYCH REGULATORÓW WZROSTU

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**Streszczenie.** Dwuletnie wazonowe doświadczenie przeprowadzono w Hali Wegetacyjnej Zachodniopomorskiego Uniwersytetu Technologicznego w Szczecinie. W doświadczeniu badano wpływ egzogennych regulatorów wzrostu, tj. kwasu indolilo-3-masłowego (IBA), 6-benzyloaminopuryny (BAP) oraz ich mieszaniny na zawartość makroelementów (Na, K, Ca, P i Mg), azotanów (N-NO<sub>3</sub>) oraz aminokwasów siarkowych (metioniny i cysteiny) w nasionach trzech polskich odmian soi zwyczajnej, tj. Aldana, Progres, Jutro. Podczas wegetacji rośliny były opryskiwane dwukrotnie regulatorami wzrostu w stężeniu 30 mg · dm<sup>-3</sup>. Rośliny odmiany Jutro opryskiwane 6-benzyloaminopuryną (BAP) charakteryzowały się największą zawartością Na, K, Ca i Mg oraz aminokwasów siarkowych, ale jednocześnie najmniejszą zawartością azotanów. Regulatory wzrostu w innych wariantach miały również pozytywny wpływ na poprawę jakości plonu, jednak w mniejszym stopniu. Regulatory wzrostu miały istotny, ale zróżnicowany wpływ na zawartość makropierwiastków odżywczych w nasionach trzech odmian soi. Zawartość wapnia, potasu i magnezu w nasionach trzech odmian soi wzrosła niezależnie od rodzaju regulatora. Z kolei w przypadku azotu w formie azotanowej, stwierdzono jego spadek. BAP istotnie zwiększył zawartość cennych pod względem odżywczym pierwiastków takich jak Ca i Mg oraz egzogennych aminokwasów, szczególnie u odmian Jutro i Progres. Uzyskane wyniki badań świadczą o korzystnym wpływie zastosowanych regulatorów wzrostu na wartość biologiczną nasion badanych odmian soi.

**Key words:** growth regulators, macro-elements, nitrates, soybean, sulphur amino acids.

**Słowa kluczowe:** aminokwasy siarkowe, pierwiastki mineralne, regulatory wzrostu, soja.

## INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most valuable crop plants. Seeds of this plant contain about 40% protein of the best amino acid composition among plants, about 20% fat of the high content of essential polyunsaturated fatty acids, mineral elements, i.e. calcium, phosphorus and potassium, as well as valuable phytoestrogens, i.e. genistein and daidzein (Zeller 1999, Tapiero et al. 2002). Soybean seeds are used, for the production

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of diet supplements and feed components, as well as animal protein substitute (Lichtenstein 1998, Panthee et al. 2006). Among others, the high content of exogenous sulphur amino acids, including cysteine and methionine, as well as larger content of macro-elements than in other plants are evidence of high nutritive value of soybean seeds (Lampart-Szczapa 1997, Friedman and Brandon 2001). Deficit of these compounds reduces their nutritive value (McVey et al. 1995, Burton 1997). The largest consumption of soybean protein is found in some regions of India (from 30 to 50%) and South America (about 10%), where it constitutes a main source of local diet. It is estimated that demand for full complete protein will successively grow with increasing human population (Yazzie et al. 1994, Osman 2004).

Chemical composition of the seeds of leguminous plant is determined by many factors, such as – for example – genetic variation, environmental conditions or plant hormonal balance (Quiñtana et al. 1999, Moraghan et al. 2006).

In the opinion of many authors, phytohormones affect more intensive intake of nutrient elements and their distribution and accumulation in different plant parts (Nowak et al. 1997, Czapla et al. 2003). According to Svenson (1991), Meuwly and Pilet (1991) and Ali et al. (2008), plant hormones exert a significant effect on root elongation growth and root system development, which is connected with more intensive intake of nutrient elements from soil.

The aim of carried out study was to evaluate the effect of exogenous growth regulators, i.e. indolilo-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) used separately and in mixture, on the content of selected macro-elements and sulphur amino acids in the seeds of three Polish soybean (*Glycine max* L. Merr.) cultivars.

## **MATERIAL AND METHODS**

### **Plant material and growth regulators**

The study object was three Polish cultivars of soybean (*Glycine max* L. Merr.), i.e. Aldana, Progres and Jutro, bred in the Institute of Plant Breeding and Acclimatisation in Radzików near Warsaw. In the experiment were used exogenous growth regulators: auxin, i.e. indolilo-3-butyric acid (IBA), and cytokinin, i.e. 6-benzylaminopurine (BAP), and their mixture (IBA + BAP). Growth regulators were purchased at Sigma-Aldrich Poland Co. Ltd.

### **Pot experiment**

In 2007–2008, a two-factor pot experiment was carried out in an experimental station of the West Pomeranian University of Technology, Szczecin. It was set up as randomised complete block design in six replications. In the aggregate, it consisted of 72 pots, with six pots per each variant and four plants per one pot. Soybean cultivars were the first factor, whereas growth regulators were the second one. Each pot was filled with 8 kg soil selected in accordance to soybean requirements. The soil was characterised by average abundance of essential elements (Table 1). The experiment was carried out under controlled humidity of the substrate, i.e. 60% of water field capacity.

Table 1. Soil properties and chemical composition  
Tabela 1. Właściwości i skład chemiczny gleby

Reaction – pH in KCl Odczyn – pH w KCl	Humus [%] Próchnica [%]	$C_{org}$ [g · kg <sup>-1</sup> ]	Dry matter [%] Sucha masa [%]	Moisture [%] Wilgotność [%]	Nitrogen content [mg · kg <sup>-1</sup> d.m.] Zawartość azotanów [mg · kg <sup>-1</sup> s.m.]		Content of macroelements (of available element) [g · kg <sup>-1</sup> d.m.] Zawartość makroelementów (składnika dostępnego) [g · kg <sup>-1</sup> s.m.]					Total content of microelements [mg · kg <sup>-1</sup> d.m.] Zawartość mikroelementów ogółem [mg · kg <sup>-1</sup> s.m.]				
					N-NO <sub>3</sub>	N-NH <sub>4</sub>	P	K	Mg	B	Mn	Cu	Zn	Fe		
7.0	3.0	17.2	88.9	11.8	1.4	63.6	0.49	0.15	0.10	2.3	195	8.9	22.9	1571		

Explanations: d.m. – dry mass.  
Objaśnienia: s.m. – sucha masa.

In each year, regular plant feeding with nitrogen (0.5 g N per pot in the form of NH<sub>4</sub>NO<sub>3</sub>), phosphorus (0.60 g P per pot in the form of KH<sub>2</sub>PO<sub>4</sub>), potassium (1.0 g K per pot in the form of K<sub>2</sub>SO<sub>4</sub>), and magnesium (0.5 g Mg per pot in the form of MgSO<sub>4</sub>) was applied both before sowing and during vegetation. The fertilisers were mixed with soil during pot filling. Plant feeding in the same quantity was repeated as topdressing at the end of June. Seeds of three soybean cultivars were sown each year in mid-May, when average air temperature exceeded 10°C, at a depth of 4–5 cm. After germination of seeds, 4 representative plants per each pot were left for further analyses. Each year during soybean vegetation, a double spraying with growth regulators was carried out, with the following concentrations: auxin (indolilo-3-butyric acid, IBA) – 30 mg · dm<sup>-3</sup>; cytokinin (6-benzylaminopurine, BAP) – 30 mg · dm<sup>-3</sup>; and auxin + cytokinin (IBA + BAP) – 30 + 30 mg · dm<sup>-3</sup>. The first spraying was made when plants reached a height of approximately 20 cm (tripartedcompound leaf stage). The second spraying was made at the beginning of the flowering stage. Control plants were sprayed with distilled water. Plants were sprayed until they were completely bedewed, using about 20 cm<sup>3</sup> of working fluid per pot.

### Determination of mineral elements and sulphur amino acids in soybean seeds

After seed collection and their drying and grinding, the content of selected macro-elements, i.e. K, Ca, Mg, Na, P and N-NO<sub>3</sub>, as well as exogenous sulphur amino acids, i.e. methionine and cysteine, was determined. The measurements were made in four replications for each experimental variant.

In order to determine potassium, calcium, magnesium, sodium and phosphorus contents, the material prepared earlier was mineralised in a mixture of nitric (V) and chloric (VII) acids as 3 : 1 for 24 hours. The content of total phosphorus was determined by the vanadium-molybdenum method using a Specol spectrophotometer. Potassium, sodium, calcium and magnesium contents were determined by atomic absorption spectrophotometry (AAS), whereas nitrate content by the salicylic method using a DU 640 Beckman spectrophotometer, the USA production.

Sulphur amino acids, i.e. cysteine and methionine, were determined as their oxidised derivatives (cysteic acid and methionine sulphone) obtained after the performic acid oxidation of sample for 16 hours at 4°C. Proteins were liberated during acid hydrolysis for 24 hours at 110°C in 6 molar HCl. Hydrolysates were analysed by ion exchange chromatography using automatic amino acid analyser (AAA400) INGOS Company, Czech production.

### Methods for statistical evaluation of results

The findings of two-year experiment were statistically evaluated based on two-way analysis of variance. In order to determine the significance of differences between mean values, Tukey's confidence intervals were applied at a significance level  $\alpha = 0.05$  (LDS<sub>0.05</sub>). The findings were evaluated using Statistica 8.0 computer software package.

Results for the main factors, i.e. for cultivars and growth regulators were presented in tables. While results for the interaction between cultivars and growth regulators (C x R) were presented in figures.

## RESULTS AND DISCUSSION

Plant growth regulators activate the process of the intake of mineral elements intake and also participate in their distribution and accumulation (Svenson 1991, Meuwly and Pilet 1991, Czapla et al. 2003, Ali et al. 2008). However, the opinions on the role of exogenous growth regulators in the accumulation of chemical elements in plant seeds (Nowak et al. 1997).

In the authors' own experiment, K and Mg, were accumulated in significantly larger amounts after the application of growth regulators than under control conditions, in particular after BAP spraying. The same growth regulator had a significant impact on the lower content of N-NO<sub>3</sub> in soybean seeds.

The concentration of potassium was found to increase, on average by about 10%, as well as that of magnesium, by about 53%, and calcium, by about 20% (caused by the BAP and IBA + BAP). On the other hand, no significant changes occurred in Na and P levels under the influence of the growth regulators applied (Table 2).

Also, a significantly higher content of calcium was found in the seeds of the Jutro cultivar when compared to those of Progres and Aldana (Table 2). In the case of the content of other chemical elements, no differences were observed between soybean cultivars.

Insofar as the Na, K, Mg and Ca levels in seeds are concerned, the interactions between growth regulator types and soybean cultivars also proved to be significant (Fig. 1). The most sodium was accumulated in the seeds of the Aldana cultivar treated with IBA as well as in those of the Jutro cultivar sprayed with an IBA + BAP mixture, almost twice as much as in the control combination. The largest amount of calcium was accumulated in the seeds of the Jutro cultivar, irrespectively of the growth regulator type; however, there was significantly more calcium after BAP application than in the seeds of the Aldana cultivar. The same cultivar in the experimental combination with BAP was also characterised by the largest content of K and Mg. These results constitute evidence of a high ability of the Jutro cultivar to accumulate nutritionally valuable macronutrients under the conditions of 6-benzylaminopurine (BAP) application, which raises the biological value of the obtained seed yield.

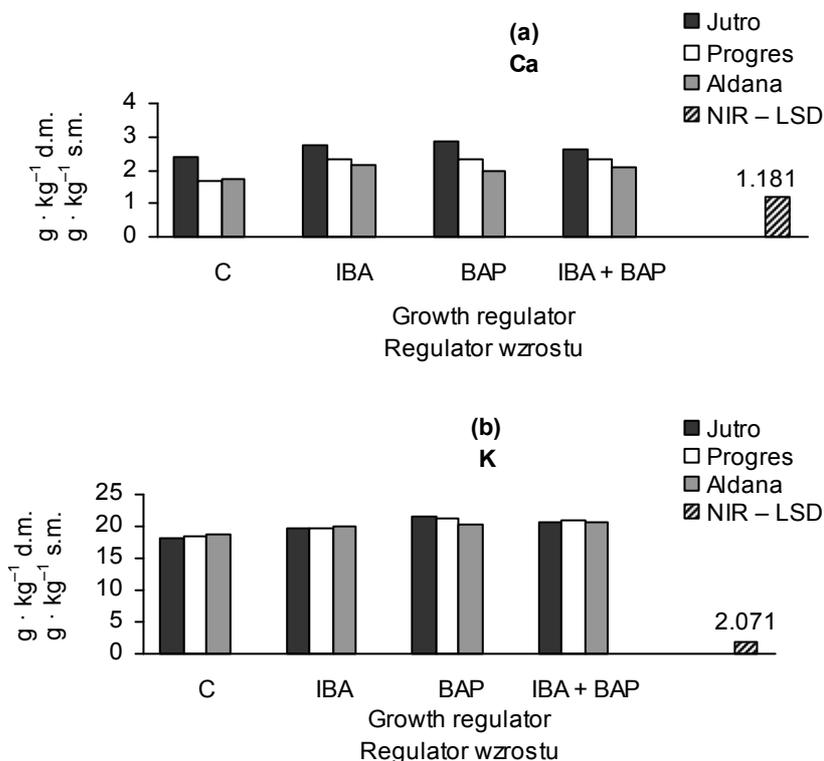
Table 2. The mean content of selected macroelements in seeds of tree cultivar soybean sprayed of growth regulator

Tabela 2. Średnia zawartość makroelementów w nasionach trzech odmian soi zwyczajnej po opryskiwaniu regulatorami wzrostu

Object Studies Objekt badań		Na	K	Mg	Ca	P	N-NO <sub>3</sub>
		[g · kg <sup>-1</sup> d.m.] [g · kg <sup>-1</sup> s.m.]				[mg · kg <sup>-1</sup> d.m.] [mg · kg <sup>-1</sup> s.m.]	
Cultivar	Jutro	0.33	20.14	2.71	2.66	3.40	18
Odmiana	Progres	0.23	20.17	2.76	2.16	3.51	20
	Aldana	0.30	19.91	2.60	1.98	3.57	17
Regulators Regulator	Control Kontrola	0.21	18.87	2.01	1.94	3.43	21.6
	IBA	0.32	19.77	2.71	2.40	3.54	20.1
	BAP	0.33	21.03	3.10	2.39	3.50	15.8
	IBA + BAP	0.30	20.63	2.93	2.34	3.49	18.2
LSD <sub>0.05</sub> for NIR <sub>0.05</sub> dla	Cultivar Odmiana	n.s. – r.n.	n.s. – r.n.	n.s. – r.n.	0.409	n.s. – r.n.	n.s. – r.n.
	Regulator Regulator	n.s. – r.n.	0.848	0.209	n.s. – r.n.	n.s. – r.n.	5.561

Explanation: IBA – indolilo-3-butyric acid (auxin), BAP – benzylaminopurine (cytokinin), IBA + BAP – mixture indolilo-3-butyric acid + benzylaminopurine (auxin + cytokinin); LSD<sub>0.05</sub> – least significant difference, n.s. – non-significant difference.

Objaśnienia: K – kontrola, IBA- kwas indolilo-3-masłowy (auksyna), BAP – benzyloaminopuryna (cytokinina), IBA + BAP – mieszanina kwasu indolilo-3-octowego + benzyloaminopuryny (auksyna + cytokinina); NIR<sub>0.05</sub> – najmniejsza istotna różnica, r.n. – różnica nieistotna.



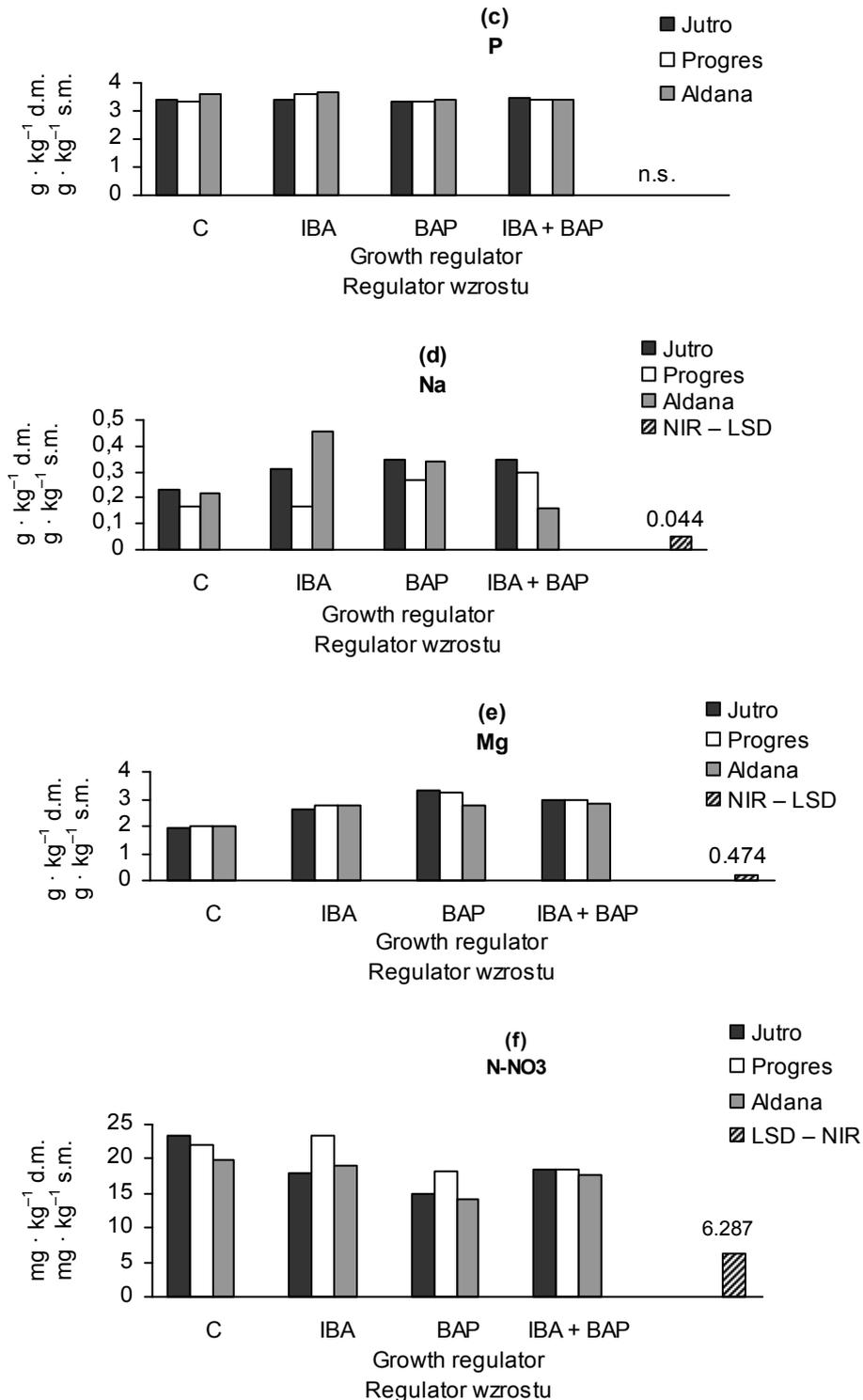


Fig. 1. The content of Ca (a), K (b), P (c), Na (d), Mg (e) and nitrates N-NO<sub>3</sub> (f) in the seeds of three soybean cultivars from plants sprayed with exogenous growth regulators

C – control (water treatment), IBA – indolilo-3-butyric acid (auxin), BAP – benzylaminopurine (cytokinin), IBA + BAP – mixture indolilo-3-butyric acid + benzylaminopurine (auxin + cytokinin); LSD<sub>0,05</sub> – least significant difference, d.m. – dry matter

Rys. 1. Zawartość Ca (a), K (b), P (c), Na (d), Mg (e) oraz azotanów N-NO<sub>3</sub> (f) w nasionach trzech odmian soi zwyczajnej opryskiwanej regulatorami wzrostu

C – kontrola (opryskiwane wodą), IBA – kwas indolilo-3-masłowy (auksyna), BAP – benzyloaminopuryna (cytokinina), IBA + BAP – mieszanina kwasu indolilo-3-octowego + benzyloaminopuryny (auksyna + cytokinina), NIR<sub>0,05</sub> – najmniejsza istotna różnica, s.m. – sucha masa.

Literature reports on very different results as regards the content of macro-elements under discussion in the seeds of leguminous plants or in cereal grains. It is suspected that the reason for this is diverse techniques and conditions of growing plants. Czaplą et al. (2003) report on a decrease in the potassium content, on average by 9%, as well as on a slight decrease in calcium and magnesium contents in soybean seeds after spraying with exogenous auxins, i.e. IBA, NAA and IBA + NAA, at a concentration of  $20 \text{ mg} \cdot \text{dm}^{-3}$ . Prusiński and Borowska (2002), when applying IBA and BAP separately as well as their mixture at different concentrations (20, 40, 50  $\text{mg} \cdot \text{dm}^{-3}$ ) on lupin plants, demonstrated a downward trend in the K content in seeds as affected by an increasing in growth regulators doses. On the other hand, IBA and BAP applied separately significantly influenced the increase in the calcium content in lupin seeds, whereas increasing doses of the mixture of these compounds had a significant influence on greater accumulation of magnesium. Wierzbowska (2006), when applying kinetin and auxin on wheat plants, observed a clear increase in potassium content in grains, doses by 16.5% and 10.5% respectively, as well as in the calcium content after applying kinetin, on average by 14%.

In the authors' own experiment, the growth regulators applied did not influence the phosphorus content in the seeds of three soybean cultivars, which confirms the findings of Klasa et al. (1996), Nowak et al. (1997), Prusiński and Borowska (2002) and Czaplą et al. (2003). These authors demonstrated a lack of significant dependence in accumulation of that chemical element on exogenous auxins and cytokinins in field bean, soybean and lupin seeds.

Both the authors' own study and those of the authors mentioned above show a significant, favourable role of exogenous cytokinins and auxins in the accumulation of nutritionally valuable macro-elements in the seeds of leguminous plants. This is explained, amongst other things, by the effect of growth regulators on root system development, in particular by elongation of the root-hair zone (Meuwly and Pilet 1991, Svenson 1991, Ali et al. 2008), which leads in consequence to a more intensive nutrient intake from soil. In particular, the role of auxins is emphasised here as they are signals that inform about the course of physiological processes in acceptors and the increasing demand of plants for food materials. Moreover, cytokinins together with auxins stimulate the activity of cambium and the formation of vascular tissues which facilitate the penetration of different food material types.

Another important parameter that determines the biological value of the obtained plant crop is the amount of accumulated nitrates in plant edible parts. Excessive accumulation of the nitrate form of nitrogen ( $\text{N-NO}_3$ ) significantly decreases the nutritional value of the crop (Van der Bonn et al. 1990).

In the authors' own experiment, a generally favourable effect of exogenous growth regulators on the biological value of seeds of the three soybean cultivars examined, decreasing the accumulation of  $\text{N-NO}_3$  in seeds, was demonstrated (Table 2). However, 6-benzylaminopurine (BAP) significantly decreased their content in the Jutro cultivar seeds, almost by 30%, when compared to control plants (Fig. 1). These results confirm the usefulness of further studies referring to proper selection of exogenous growth regulators decreasing the accumulation of nitrates in the seeds of leguminous plants with simultaneous high intake and accumulation of nutritionally valuable mineral elements. This is because

growth stimulants, apart from activation of physiological processes and increasing plant productivity, should also influence improvement of the biological value of the obtained crop.

Next, an unusually important element in the evaluation of crop biological value is plant ability to increase the synthesis of sulphur amino acids and accumulate them in plant edible parts. Li et al. 2005 and Sexton and Shibles (1999) clearly indicate that a low content of sulphur amino acids, i.e. cysteine and methionine, reduces the nutritive value of protein in soybean seeds.

In the own study, soybean seeds contained definitely more cysteine than methionine, regardless of the soybean cultivar and the growth regulator type (Table 3).

Table 3. The mean content of sulphur amino acid in seeds of tree cultivar soybean sprayed of regulator growth

Tabela 3. Średnia zawartość aminokwasów siarkowych w nasionach trzech odmian soi potraktowanych regulatorami wzrostu

Object Studies Objekt badań		Cysteine Cysteina	Methionine Metionina
		[g · (100 g) <sup>-1</sup> d.m.] [g · (100 g) <sup>-1</sup> s.m.]	
Cultivar Odmiana	Jutro	0.77	0.55
	Progres	0.76	0.54
	Aldana	0.73	0.56
Regulators Regulator	Control Kontrola	0.69	0.50
	IBA	0.76	0.55
	BAP	0.81	0.60
	IBA + BAP	0.76	0.56
LSD <sub>0.05</sub> for NIR <sub>0.05</sub> dla	Cultivar Odmiana	0.029	0.028
	Regulator Regulator	0.042	0.036

Explanation: IBA – indolilo-3-butyric acid (auxin), BAP – benzylaminopurine (cytokinin), IBA + BAP – mixture indolilo-3-butyric acid + benzylaminopurine (auxin + cytokinin); LSD<sub>0.05</sub> – least significant difference, n.s. – non-significant difference.

Objaśnienia: K – kontrola, IBA- kwas indolilo-3-masłowy (auksyna), BAP – benzyloaminopuryna (cytokinina), IBA + BAP – mieszanina kwasu indolilo-3-octowego + benzyloaminopuryny (auksyna + cytokinina); NIR<sub>0.05</sub> – najmniejsza istotna różnica, r.n. – różnica nieistotna.

Significant differences were found in the content of both amino acids in seeds, both between soybean cultivars and growth regulators types. The application of growth regulators, in particular BAP, significantly influenced the increase in cysteine and methionine levels in soybean seeds when compared to control plants, respectively by 17% and 20%. The Jutro and Progres cultivars contained significantly more cysteine than the Aldana one. On the other hand, the latter was characterised by the highest content of methionine. BAP showed the highest effectiveness in increasing the amount of methionine and cysteine in the seeds of soybean cultivars under examination (Fig. 2).

There are not many reports in specialist literature on the synthesis of sulphur amino acids in the seeds of leguminous plants as affected by exogenous growth regulators. They usually refer to changes in the total protein content in the seeds of various leguminous plants after the application of cysteine. Such studies were carried out, among others, by Prusiński and Borowska (2002) who demonstrated an increase in the protein content as influenced by exogenous cytokinins. Chwil (2001) stated that a good magnesium supply to plant organs

favours an increase in the protein content. In the present study, a considerably higher content of this chemical element was found in soybean seeds treated with cytokinin (BA) and the auxin + cytokinin mixture (IBA + BAP), which could also contribute to an increase in the amount of sulphur amino acids that build high-value proteins. Based on the findings presented, it can be concluded, in particular, about a favourable effect of 6-benzylaminopurine (BAP) on the nutritive value of soybean seeds of the Jutro cultivar. Other growth regulator combinations also had a favourable effect on qualitative traits of the soybean cultivars examined but not such a significant one like BAP.

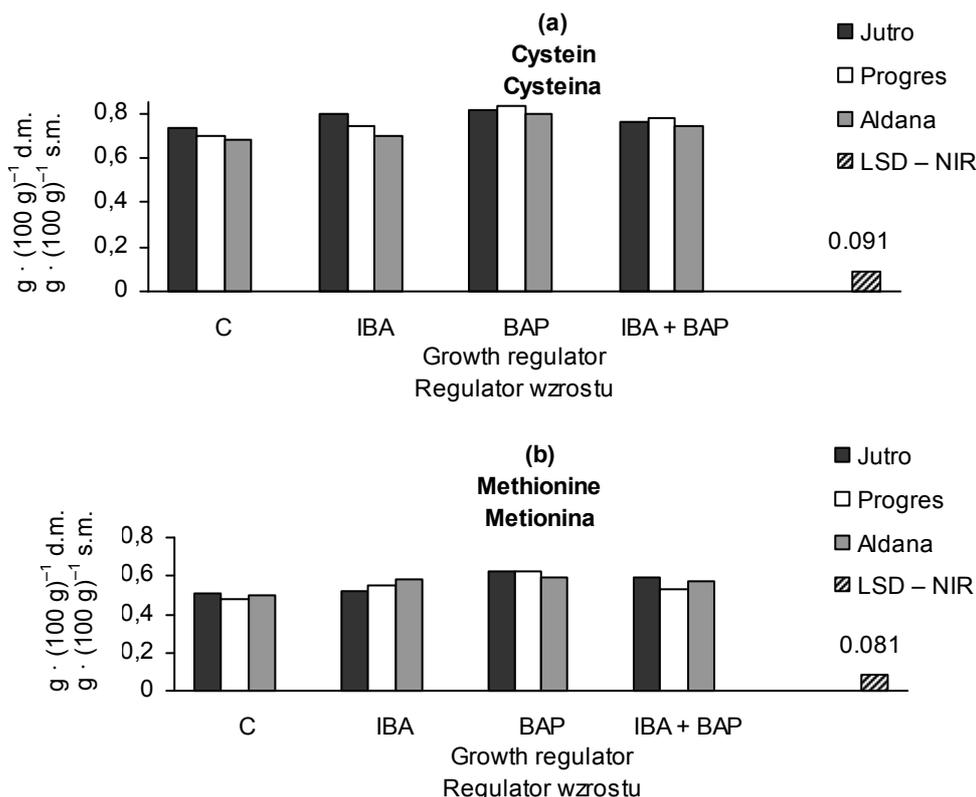


Fig. 2. The content of cystein (a) and metionin (b) in the seeds of three soybean cultivars from plants sprayed with exogenous growth regulators

C – control (water treatment), IBA – indolilo-3-butyric acid (auxin), BAP – benzylaminopurine (cytokinin), IBA+BAP – mixture indolilo-3-butyric acid + benzylaminopurine (auxin + cytokinin);  $LSD_{0.05}$  – least significant difference, d.m. – dry matter.

Rys. 2. Zawartość cysteiny (a) i metioniny (b) w nasionach trzech odmian soi zwyczajnej po zastosowaniu egzogennych regulatorów wzrostu

C – kontrola (opryskiwane wodą), IBA – kwas indolilo-3-masłowy (auksyna), BAP – benzyloaminopuryna (cytokinina), IBA + BAP – mieszanina kwasu indolilo-3-octowego+benzyloaminopuryny (auksyna + cytokinina),  $NIR_{0.05}$  – najmniejsza istotna różnica, s.m. – sucha masa.

## CONCLUSIONS

1. Growth regulators significantly but variably influenced on the nutritional content of macronutrients in seeds of three soybean cultivars. The content of calcium, potassium and magnesium have increased regardless of the type of growth regulators. On the other hand, the decrease in the nitrate form of the nitrogen content was observed.

2. BAP significantly increased the content of magnesium, and essential amino acids, especially in the Jutro and Progres cultivars.
3. The results indicate a positive effect of growth regulators on the biological value of the tested cultivars of soybean seeds.

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**Abstract.** A two-year pot experiment was carried out at the Experimental Hall, West Pomeranian University of Technology in Szczecin. The experiment examined the effect of exogenous growth regulators, i.e. indolilo-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) used separately and in a mixture, on the content of nutritionally valuable macronutrients (Na, K, Ca, P and Mg) and sulphur amino acids (methionine and cysteine) in the seeds of three Polish soybean (*Glycine max* L. Merr.) cultivars, i.e. Aldana, Progres and Jutro. During plant vegetation, double spraying with growth regulators at a concentration of  $30 \text{ mg} \cdot \text{dm}^{-3}$  was applied. Plants from the Jutro cultivar sprayed with 6-benzylaminopurine (BAP) were characterized by the highest content of Na, K, Ca and Mg and sulphur amino acids but also the lowest content of nitrates. Other variants of growth regulators also had a positive effect on the improvement of qualitative traits in the soybean cultivars examined but to a lesser extent. Growth regulators significantly but variably influenced the nutritional content of macronutrients in the seeds of three soybean cultivars. The content of calcium, potassium and magnesium increased regardless of the type of growth regulators. On the other hand, a decrease in the nitrate form of nitrogen content was observed. BAP significantly increased the content of nutritionally valuable elements such as calcium and magnesium, and essential amino acids, especially in the Jutro and Progres cultivars. The results indicate a positive effect of growth regulators on the biological value of the tested cultivars of soybean seeds.

