ACTA Acta Sci. Pol., Agricultura 10(2) 2011, 33-50

RESPONSE OF POLISH CULTIVARS OF SOYBEAN (*Glycine max* (L.) Merr.) TO BRASSINOSTEROID APPLICATION*

Anna Janeczko[1], Jolanta Biesaga-Kościelniak[1], Michał Dziurka[1], Jana Oklešťková[2], Maciej Kocurek[1], Grażyna Szarek-Łukaszewska[3], Zbigniew Janeczko[4]

[1] The *Franciszek Górski* Institute of Plant Physiology of Polish Academy of Sciences in Krakow
[2] Palacky University Olomouc & Institute of Experimental Botany Academy of Sciences of the Czech Republic in Olomouc
[3] The *Władysław Szafer* Institute of Botany of Polish Academy of Sciences in Krakow
[4] College of Medicine of Jagiellonian University in Krakow

Abstract. In the present study, we evaluated the effects of exogenously applied 24-epibrassinolide – one of the brassinosteroids – on the seed yield, seed chemical composition (content of soluble proteins, phytoestrogens, carbohydrates, minerals) and resistance to drought of soybean cultivated in Poland (cv. Aldana and Augusta). Quantitative and qualitative analysis of brassinosteroid content was made in seeds of plants treated with 24-epibrassinolide. Experiments were performed in the field and in greenhouse. Brassinosteroid was applied *via* presowing seed soaking (0,25 mg·dm^{-3}) and spraying of plants (1 mg·dm^{-3}). 24-epibrassinolide affected the increase of the weight of seeds per one plant (27-73%) depending on the cultivar as well as growth conditions (occurrence or lack of drought factor at the stage of seed setting). In the seeds, 24-epibrassinolide did not change content of proteins or soluble carbohydrates, but increased phytoestrogen genistein content in cultivar Aldana and decreased level of potassium in cultivar Augusta and calcium level in cultivar Aldana. In soybean seeds, the presence of brassinolide and castasterone was found. 24-epibrassinolide applied exogenously to plants was not accumulated in newly formed seeds. In drought-stressed soybean brassinosteroid showed protective effect on net photosynthesis and photosystem II efficiency.

Key words: 24-epibrassinolide, drought, genistein, presowing seed treatment, soybean cultivars, steroid hormones

---

Corresponding author – Adres do korespondencji: dr inż. Anna Janeczko, Department of Biology of Development of The *Franciszek Górski* Institute of Plant Physiology of Polish Academy of Sciences in Krakow, Niezapominajek 21, 30-239 Krakow, e-mail: ania@belanna.strefa.pl

* The research was conducted as part of the 2P06A01928 Project

INTRODUCTION

One of the significant causes of little interest in cultivation of plants from the legume family (*Fabaceae*) in Poland is their susceptibility to different types of environmental stresses e.g. droughts, water excess or the too high or too low temperatures in the growing period. Periodical droughts are especially dangerous at the stage of flowering and seed setting. At that time plants drop flowers and young pods, and their seeds are not well-filled. It opens the field for research on new exogenous growth regulators, which may be significant for agriculture. Especially interesting seem to be compounds of natural origin, which may be applied in preparations for plant protection against stress and improving the yield in cultivation exposed to unfavorable and not easily predictable environmental changes in the growing season. These types of regulators, studied in recent years also in Poland include steroid plant hormones, brassinosteroids [Janeczko et al. 2009, 2010]. Brassinosteroids (BR) were discovered 30 years ago, and today this group includes over 70 compounds commonly occurring in the plant kingdom [Grove et al. 1979, Bajguz and Tretyn 2003, Zullo and Kohout 2004]. BRs include among others: 24-epibrassinolide, brassinolide or castasterone. Brassinosteroids are present in all plant parts at a dose from <0.05-1600 $ng \cdot g^{-1}$ of fresh weight [Symons et al. 2008, Janeczko et al. 2010, Janeczko and Swaczynová 2010]. Brassinosteroid receptors are located in cell membranes [Wang et al. 2001], and the precursors in BR biosynthesis are sterols, mainly campesterole [Fujioka and Yokota 2003].

Brassinosteroids are characterized by multidirectional biological activity. The fundamental and at the same time first discovered effect of BR activity is stimulation of cell growth [Grove et al. 1979]. Brassinosteroids may also inhibit flowering induction in photoperiodical plants [Janeczko et al. 2003], stimulate photosynthesis and increase chlorophyll content [Yu et al. 2004, Holá 2011]. It was found that these compounds may accelerate metabolism of pesticides applied for example in cucumber cultivation [Xia et al. 2009b]. Favorable effect of BRs on the yield of plants of great economical significance (rice, potatoes, wheat, peanuts, beans and others) was proved [Ramraj et al. 1997, Fariduddin et al. 2008, Janeczko et al. 2010]. This effect in many cases was proved under conditions of drought and/or soil salinity, as well as under the influence of other stress factors [Upreti and Murti 2004, Ali et al. 2008, Hasan et al. 2008, Janeczko et al. 2010]. Many possibilities of practical application of BRs in agriculture and horticulture were patented.

The study included issues of testing the activity of one of the brassinosteroids (24--epibrassinolide) in soybean cultivation. Soybean (*Glycine max* (L.) Merr.; Augusta and Aldana cultivars) was chosen for research, as it is one of the most valuable plants in the world, among others with regard to nutritive and medicinal value of its seeds [Ziółek et al. 1996, Pisulewska et al. 1998, Birt et al. 2001, Krebs et al. 2004]. However, soybean cultivation in Poland is not widespread, as this plant coming from countries with warm climate, quite badly tolerates influence of stress factors occurring in the growing season in Poland. With regard to this, it was considered a convenient research object for testing the activity of a compound of potential anti-stress effect. In experiments 24--epibrassinolide was applied *via* presowing seed soaking as well as soybean plant spraying at the stage of seedling and flowering.

Research aims:

– determination of 24-epibrassinolide effect on soybean component yield in plot experiments carried out in two growing seasons differing significantly in weather conditions, that is in the year when drought occurred in the period of seed setting (2006) well as in the year which did not differ significantly in the amount and process rainfall in summer months from the mean value calculated for the long--term period 1961-1990 (2007),
– determination of 24-epibrassinolide effect on chosen chemical composition parameters of soybean seeds: content of soluble protein, minerals, soluble carbohydrates, phytoestrogens, as well as brassinosteroids; in the latter case careful attention was paid to quantitative and qualitative analysis of BR content in setting seeds in plants treated with 24-epibrassinolide,
– evaluation of physiological effect basis of 24-epibrassinolide in soybean through analysis of gas exchange intensity (photosynthesis and respiration) as well as efficiency of photosystem II in drought conditions and after plant rehydration.

MATERIAL AND METHODS

Plot experiment

Plant growth conditions, application of 24-epibrassinolide and evaluation of yield components

Experiments were carried out in the growing season 2006 and 2007 on experimental plots of the University of Agriculture in Krakow (Krakow-Mydlniki 50°03' N; 19°55' E) on brown soil of the good wheat complex class II. The site where the research was carried out had been uncultivated for five years.

Bioregulator 24-epibrassinolide (BR_{27} – according to Zullo and Kohout [2004]) at the amount of 2 mg was dissolved in 1 cm^3 of 50% ethanol in an ultrasound bath, obtaining a stock solution, and then through dilution, working water solutions were prepared at the required concentration, used for treating seeds and plants. In plot experiment two methods of treating plants with 24-epibrassinolide were used – pre-sowing seed soaking (24 hours at a temperature of 18-20°C; BR_{27} concentration of 0.25 $mg \cdot dm^{-3}$) or the twofold plant spraying (1 $mg \cdot dm^{-3}$) at the stage of seedling (2 developed threefold leaves and the setting of the third one) and at the stage of flowering (at the stage of flowers occurring on at least two nodes). Control objects were plants treated with water solutions containing trace amounts of ethanol, corresponding with the amount of ethanol in solutions with BR_{27}: in the case of seed soaking – 0.00625% ethanol (v/v), in the case of spraying – 0.025%.

The experiment was set up in the randomized block design. The seeds were sown on 25 April, in rows every 20 cm, the interrow width was 30 cm. Plants at the stage of seedling were treated with Nitragina preparation (Biofood, Polska). Soybean harvest was conducted at the end of August. Determined yield components included: the number of pods per plant, the number of seeds per plant, the weight of 1000 seeds, seed weight per plant. Yield structure analysis was conducted in total for 200 plants in 2006 and 80 plants in 2007.

Table 1 presents weather conditions in the growing season 2006 and 2007. In July 2006 and partly in May there was a drought (14.1 mm and 51.9 mm rainfall, respectively). 2007 did not differ with amount and process of rainfall in the summer months from the mean calculated for the long-term period 1961-1990.

Table 1. Rainfall and average monthly temperatures in the growing season 2006 and 2007 compared with data collected in the years 1961-1990 (Krakow-Mydlniki region)
Tabela 1. Warunki pogodowe w sezonie wegetacyjnym 2006 i 2007 na tle wielolecia 1961-1990 w rejonie Kraków-Mydlniki

Year Rok	Decade Dekada (1-3)	Month – Miesiąc					
		March marzec	April kwiecień	May maj	June czerwiec	July lipiec	August sierpień
Rainfall – Opady, mm							
2006	1	10.6	32.4	11.2	25.7	3.6	35.1
	2	29.8	11.6	18.1	35.1	0.4	10.3
	3	19.7	12.5	22.6	28.3	10.1	58.7
	Σ	60.1	56.5	51.9	89.1	14.1	104.1
2007	1	19.6	4.0	17.9	20.6	60.5	0.9
	2	29.3	6.5	18.0	15.4	1.4	65.7
	3	12.2	4.9	15.8	36.1	9.1	9.8
	Σ	61.1	15.4	51.7	72.1	71.0	76.4
1961-1990	$\bar{x}$	34.0	48.0	83.0	97.0	85.0	87.0
Temperature – Temperatura, °C							
2006	1	-0.8	3.8	9.4	9.8	17.1	16.5
	2	0.6	5.4	12.4	15.6	17.9	17.0
	3	0.8	7.8	10.9	19.7	20.7	13.5
	$\bar{x}$	0.2	5.6	10.9	15.0	18.6	15.6
2007	1	6.4	5.4	12.3	18.4	17.8	19.2
	2	5.9	9.7	15.8	18.7	21.5	20.0
	3	5.7	10.4	17.7	18.1	19.0	18.0
	$\bar{x}$	6.0	8.5	15.2	18.4	19.4	19.0
1961-1990	$\bar{x}$	2.4	7.9	13.1	16.2	17.5	16.9

Analysis of the chemical composition of seeds – chosen parameters

Analysis of the chemical composition of seeds was conducted in the material obtained in a plot experiment in 2006.

Determination of the content of soluble protein was carried out with spectrophotometric method according to Bradford [1976] in 0.02 g of samples of ground soybean seeds. The content of elements (potassium, calcium, magnesium, sodium, iron, copper) was determined in 0.5 g of seed samples with atomic absorption spectrometry AAS (Varian 220FS, USA). Composition of soluble sugars was analyzed with high-performance liquid chromatography in 2 g of samples of ground seeds (liquid chromatograph: Beckman System Gold 125NM Solvent Module (Beckman, USA) with ESA CoulochemII Analytical Cell 5040 detector with a gold electrode], Hamilton RCX-10 250 × 4.1 mm column (Hamilton, USA). Detailed description of the above mentioned analytical procedures is given in the paper of Janeczko et al. [2010].

Phytoestrogen content was determined with high-performance liquid chromatography. The sample (0.5 g) of ground seeds was extracted with methanol (50 cm^3) for 1 hour at a boiling point of the solvent in water bath under reflux condenser. The extract was filtered, and the material was extracted again under conditions described above. Extracts were combined (supplemented with methanol to 50 cm^3), and next 10 cm^3 was taken and methanol was distilled on a rotary evaporator under decreased pressure. Residue was hydrolyzed in 20 cm^3 5% water solution of hydrochloric acid at a boiling point of the water bath for 2 hours under reflux condenser. Hydrolyzate was cooled to room temperature, moved to separator of the capacity of 100 cm^3, and eluted twice with 25 cm^3 ethyl acetate (eluting the content every time for 5 minutes). Combined eluates were washed with water until neutral reaction, they were dried with anhydrous sodium sulfate and ethyl acetate was evaporated on the rotary evaporator. The residue in the flask was dissolved in methanol (5 cm^3) and analyzed with HPLC method. Analysis conditions: liquid chromatograph (Dionex, USA); PDA 100 UV-VIS detector (Photodiode Aray Detector), ASI 100 autosampler, p 680 HPLC pump, TCC 100 thermostat; operating system: Chromeleon® ver. 6.60; column: Hypersil BDS C-18 250 x 4.6 mm (THERMO EC); precolumn: Hypersil BDS C-18 5μm 10 x 4.6 mm (THERMO EC); solvents: - a: 0.1 % v/v H_3PO_4 in water: acetonitrile (20 : 80); column temperature 25°C; flow 1.0 $cm^3 \cdot min^{-1}$; injection: 0.02 cm^3; detection: 230 and 260 nm.

The content of brassinosteroids was determined with ultra-performance liquid chromatography – electrospray ionization-tandem mass spectrometry (UPLC(ESI)MS/MS – UPLC™ System (Waters, USA)) in seeds obtained from plants whose seed material was soaked before sowing in the solution of 24-epibrassinolide as well as in the control solution. Samples (1.5 g) of ground seeds were extracted and analyzed according to procedures described in the paper of Swaczynová et al. [2007] as well as Janeczko et al. [2010].

Analysis of phytoestrogen content (for both cultivars) and of brassinosteroids (for cv. Aldana) was conducted in 3 replications, one replication was the sample taken from the ground seeds collected from 5-7 soybean plants. Analysis of protein, carbohydrates and minerals was done for both cultivars in 5 replications, one replication was the sample taken from the ground seeds collected from 5-7 soybean plants. All analyses of the chemical composition of seeds were conducted on the air dry material.

Pot experiments

Conditions of plant growth and 24-epibrassinolide application

The experiment set up in 8 pots (40 × 20 cm; 15 plants per pot) tested:

- plants obtained from seeds which underwent soaking before sowing (12 hours at a temperature of 18-20°C) in a water solution of 24-epibrassinolide (0.25 $mg \cdot dm^{-3}$), which contained trace amounts of hormone solvent (ethanol 0.00625% v/v),
- plants obtained from seeds not soaked (dry) – control 1,
- plants obtained from seeds which underwent soaking before sowing (12 hours at a temperature of 18-20°C) in water – control 2,
- plants obtained from seeds which underwent soaking before sowing in a water solution of ethanol (0.00625% v/v) – control 3.

Seeds of Aldana and Augusta cultivars after sowing into the soil germinated in a growth chamber (darkness, 25°C) for 5 days, next the pots were moved to the greenhouse where the growth occurred under natural light conditions (May) at a day/night temperature, 24/20°C. On the 12th day of growing, the plants were watered for the last time, and next watering was ceased, which resulted in occurrence of drought symptoms in the following 10 days (in the last 5 days gradual plant wilting was observed). At that time only watering which compensated the possible irregular water loss between pots was used. On the 22nd day of growing, on the first leaf measurements of gaseous exchange were taken as well as of PS II efficiency, next the plants were watered and after 2 days measurements were taken again.

Measurements of gaseous exchange intensity

Measurement of gaseous exchange of a leaf was taken with the use of gas analyzer in infrared radiation (Li-6400, Portable Photosynthesis System, USA), at a concentration of CO_2 380-400 ppm. Respiration measurements were taken in darkness, whereas measurements of the net photosynthesis and transpiration were taken under conditions of photosynthetically active radiation (PAR) 600 μmol photons$\cdot m^{-2}\cdot s^{-1}$. The measurements were taken for cv. Augusta in five replications (1 replication = 1 leaf).

Measurements of chlorophyll *a* fluorescence (efficiency of energy flow in photosystem II)

Measurement of chlorophyll *a* fluorescence was taken according to methodology described in the paper of Janeczko et al. [2011] as well as Skoczowski et al. [2011] with Plant Efficiency Analyzer (Hansatech Ltd. King's Lynn, Angilia). After 30-minute adaptation of leaves to darkness, the following technical parameters of fluorescence were taken: F_o – fluorescence intensity at 50 μs point (it is assumed that all reaction centres of PS II are at that time open), Fm – maximum fluorescence (all reaction centres are closed), F_{300} – fluorescence intensity at 300 μs point (the so-called K point), F_{2ms} (F_J) – fluorescence intensity at 2 ms point (the so-called J point); $V_J = (F_{2ms} – Fo)/(Fm – Fo)$, V_J denotes a relative change of fluorescence at J point; $V_K = (F_{300} – Fo)/(Fm – Fo)$, V_K denotes a relative change of fluorescence at K point [Strasser et al. 2000, Kalaji and Łoboda 2009]. Obtained values were used to calculate the so-called phenomenological fluxes: energy absorption (ABS/CS = Fm); energy flux for trapping (TRo/CS = Fv/Fm · (ABS/CS)); energy flux for electron transport (ETo/CS = (Fv/Fm) · (1-V_J) · Fm); energy dissipation (DIo/CS = (ABS/CS)-(TRo/CS)), where CS is the cross section of the sample. Values of phenomenological fluxes inform about efficiency of energy flow in photosystem II (PSII) per cross section of the sample. Measurements of PSII efficiency were taken for both cultivars in 15 replications (1 replication = 1 leaf).

Significance of differences between particular mean values were estimated with Duncan test in Statistica 9.1 program (StatSoft, Polska). Detailed information concerning statistical analysis are given directly in captions under figures and tables.

RESULTS

Drought occurrence at the stage of seed setting in 2006 had an influence on decreasing the seed weight per one soybean plant compared to the harvest from 2007 (Tables 2 and 3). Observations conducted on experiment plots proved that cv. Augusta endured drought conditions worse than cv. Aldana. On plants of cv. Augusta premature leaf wilting was observed, and the plant growth was visibly inhibited. In this situation application of 24-epibrassinolide did not give positive effects any more (Table 3). Cv. Aldana, which survived the drought conditions better, was susceptible to hormone activity.

Table 2. The effect of 24-epibrassinolide (BR_{27} [$mg \cdot dm^{-3}$]) on the yield components of soybean in the growing season 2006

Tabela 2. Wpływ 24-epibrasinolidu (BR_{27} [$mg \cdot dm^{-3}$]) na komponenty plonu soi w sezonie wegetacyjnym 2006

Yield components Komponenty plonu	Cultivar Augusta – Odmiana Augusta				Cultivar Aldana – Odmiana Aldana			
	plant spraying oprysk roślin		seed soaking moczenie nasion		plant spraying oprysk roślin		seed soaking moczenie nasion	
	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25
Number of pods per plant Liczba strąków na roślinie	37 [a]	37 [a]	35 [ab]	31 [bc]	25 [c]	28 [c]	20 [d]	28 [c]
Number of seeds from plant Liczba nasion z rośliny	76 [a]	73 [a]	73 [a]	65 [ab]	53 [bc]	64 [ab]	43 [c]	63 [ab]
Weight of 1000 seeds, g MTN, g	86 [c]	85 [c]	85 [c]	87 [c]	123 [b]	131 [b]	151 [a]	147 [a]
Weight of seeds per plant, g Masa nasion z rośliny, g	6.6 [b]	6.3 [b] (-5%)	6.2 [b]	5.6 [b] (-10%)	6.6 [b]	8.4 [a] (+27%)	6.3 [b]	9.1 [a] (+44%)

mean values marked with the same letters (in rows, for each parameter separately) do not differ significantly according to Duncan's test; $P \leq 0.05$ – wartości oznaczone tymi samymi literami (oddzielnie dla poszczególnych parametrów) nie różnią się istotnie według testu Duncana, $P \leq 0,05$

in parentheses are given percentage values of changes of weight of seeds per plant in comparison to control – w nawiasach przedstawiono procentową zmianę masy nasion w stosunku do kontroli

Table 3. The effect of 24-epibrassinolide (BR_{27} [$mg \cdot dm^{-3}$]) on the yield components of soybean in the growing season 2007

Tabela 3. Wpływ 24-epibrasinolidu (BR_{27} [$mg \cdot dm^{-3}$]) na komponenty plonu soi w sezonie wegetacyjnym 2007

Yield components Komponenty plonu	Cultivar Augusta – Odmiana Augusta				Cultivar Aldana – Odmiana Aldana			
	plant spraying oprysk roślin		seed soaking moczenie nasion		plant spraying oprysk roślin		seed soaking moczenie nasion	
	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25
Number of pods per plant Liczba strąków na roślinie	53 [b]	74 [a]	51 [b]	76 [a]	36 [bc]	51 [b]	32 [c]	52 [b]
Number of seeds from plant Liczba nasion z rośliny	105 [b]	162 [a]	98 [b]	155 [a]	72 [c]	104 [b]	73 [c]	107 [b]
Weight of 1000 seeds, g MTN, g	110 [d]	124 [cd]	129 [c]	118 [cd]	160 [ab]	168 [a]	149 [b]	163 [ab]
Weight of seeds per plant, g Masa nasion z rośliny, g	11.6 [b]	20.1 [a] (+73%)	12.1 [b]	18.5 [a] (+53%)	11.9 [b]	17.3 [a] (+45%)	11.1 [b]	17.5 [a] (+58%)

mean values marked with the same letters (in rows, for each parameter separately) do not differ significantly according to Duncan's test; $P \leq 0.05$ – wartości oznaczone tymi samymi literami (oddzielnie dla poszczególnych parametrów) nie różnią się istotnie wg testu Duncana $P \leq 0,05$

in parentheses are given percentage values of changes of weight of seeds per plant in comparison to control – w nawiasach przedstawiono procentową zmianę wysokości masy nasion w stosunku do kontroli

The increase of seed weight per one soybean plant under the effect of BR27 was 27--44% (Table 2). In 2007 both cultivars yielded almost twice higher than the previous year (Tables 2 and 3). Under these conditions 24-epibrassinolide increased the seed weight per one soybean plant of cv. Augusta 53-73%, and cv. Aldana by 45-58% (Table 3). Increase of the weight of seeds collected from one plant resulted from the increase of the number of setting seeds.

Analyses of the chemical composition of seeds confirmed the intervarietal differences, however modifications resulting from the application of 24-epibrassinolide occurred only in some cases (Table 4). Seeds of cv. Augusta contained less soluble protein than seeds of cv. Aldana. Soluble protein content in seeds under the effect of BR_{27} did not change.

The level of phytoestrogen genistein in seeds of both cultivars was similar. Application of 24-epibrassinolide *via* presowing seed soaking resulted in the increase of genistein content in seeds of cv. Aldana by approximately 50%.

Mineral content in seeds of both cultivars was similar. Difference occurred only in the case of calcium content which was higher in seeds of cv. Augusta than in seeds of cv. Aldana. Application of 24-epibrassinolide decreased the calcium content by over 30% in seeds of cv. Aldana and by several percent the potassium content in seeds of cv. Augusta.

The effect of application of 24-epibrasinolide on the soluble carbohydrate content in seeds from both soybean cultivars was not confirmed (Table 5). However, intervarietal differences were found. The seeds of cultivar Augusta contained less sucrose and maltose than seeds of cv. Aldana. In seeds of cv. Augusta higher content of glucose and fructose was confirmed compared to seeds of cv. Aldana.

In soybean seeds of cv. Aldana two brassinosteroids were found, brassinolide and castasterone (Fig. 1), whereas occurrence of 24-epibrassinolide, 28-homobrassinolide, 24-epicastasterone and 28-homocastasterone was not confirmed. 24-epibrassinolide did not occur in seeds also in plants treated with this compound. However, exogenous application of BR_{27} caused changes in the proportion of brassinosteroids located in setting seeds, that is the decrease of castasterone content and increase of brassinolide content, the total brassinosteroid content remained on a similar level, approximately 800 $pg \cdot g^{-1}$ seeds.

In conducted pot experiment, the effect of 24-epibrassinolide was determined on changes of the intensity of photosynthesis and respiration as well as on the PS II efficiency at the time of drought and after watering soybean plants. In this experiment, apart from plants exposed to BR_{27} activity, three groups of control plants were used. The value of net photosynthesis for plants grown from seeds not soaked (control 1) was assumed to be 100% (Table 6). In this context, under drought conditions, almost twice as high values were obtained by plants whose seeds were soaked in water before sowing (control 2). In case of other treatments, net photosynthesis intensity did not differ significantly from the value obtained for control 1. Dark respiration intensity for all plants exposed to drought was on a similar level and did not undergo greater changes after watering. However, after rehydration, net photosynthesis value increased significantly. The highest photosynthesis intensity was found in plants grown from seeds soaked in the solution of BR_{27}. Transpiration intensity increased several times after watering the plants. Plants obtained from seeds treated with BR_{27} showed the highest transpiration.

Table 4. Chosen parameters of the chemical composition of soybean seeds [mg·g^{-1}] collected from plants treated with 24-epibrassinolide (BR_{27} [mg·dm^{-3}])
Tabela 4. Wybrane parametry składu chemicznego nasion [mg·g^{-1}] uzyskanych z roślin soi traktowanych 24-epibrasinolidem (BR_{27} [mg·dm^{-3}])

Seed chemical composition Skład chemiczny nasion	Cultivar Augusta – Odmiana Augusta				Cultivar Aldana – Odmiana Aldana			
	plant spraying oprysk roślin		seed soaking moczenie nasion		plant spraying oprysk roślin		seed soaking moczenie nasion	
	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0,25	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0,25
Soluble protein Białko rozpuszczalne	133 ^{d}	147 ^{d}	141^{d}	157 ^{cd}	183 ^{abc}	167 ^{bcd}	202 ^{a}	190 ^{ab}
Phytoestrogen (genistein) Fitoestrogen (genisteina)	0.495 ^{b}	0.553 ^{b}	0.510 ^{b}	0.597 ^{b}	0.675 ^{b}	0.712 ^{b}	0.653 ^{b}	0.962 ^{a}
Mineral components – Składniki mineralne								
Potassium – Potas	11.32 ^{a}	10.46 ^{c}	11.15 ^{ab}	10.55 ^{c}	10.82 ^{abc}	11.25 ^{a}	10.74 ^{abc}	10.45 ^{c}
Calcium – Wapń	5.65 ^{a}	5.18 ^{a}	5.20 ^{a}	5.39 ^{a}	3.84 ^{b}	2.44 ^{c}	3.42 ^{b}	2.15 ^{c}
Magnesium – Magnez	2.19 ^{a}	2.13 ^{a}	2.24 ^{a}	2.13 ^{a}	2.05 ^{ab}	2.04 ^{ab}	1.81 ^{b}	1.82 ^{b}
Sodium – Sód	0.123 ^{a}	0.120 ^{ab}	0.119 ^{ab}	0.113 ^{ab}	0.105 ^{b}	0.105 ^{b}	0.119 ^{ab}	0.110 ^{ab}
Iron – Żelazo	0.095 ^{ab}	0.101 ^{a}	0.096 ^{ab}	0.102 ^{a}	0.094 ^{ab}	0.091 ^{ab}	0.083 ^{b}	0.086 ^{b}
Copper – Miedź	0.021 ^{ab}	0.022 ^{a}	0.021 ^{ab}	0.020 ^{abc}	0.020 ^{abc}	0.020 ^{abc}	0.019 ^{bc}	0.018 ^{c}

mean values marked with the same letters (in rows, for each component separately) do not differ significantly according to Duncan's test; $P \leq 0.05$ – dane oznaczone tymi samymi literami (oddzielnie dla każdego składnika) nie różnią się istotnie wg testu Duncana; $P \leq 0,05$

Table 5. Comparison of the composition of soluble sugars [$\mu mol \cdot g^{-1}$] in seeds of two soybean cultivars – the effect of 24-epibrassinolide treatment on plants (BR_{27} [$mg \cdot dm^{-3}$])
Tabela 5. Porównanie składu cukrów rozpuszczalnych [$\mu mol \cdot g^{-1}$] w nasionach dwóch odmian soi – wpływ traktowania roślin 24-epibrasinolidem (BR_{27} [$mg \cdot dm^{-3}$])

Soluble sugars Cukry rozpuszczalne	Cultivar Augusta – Odmiana Augusta				Cultivar Aldana – Odmiana Aldana			
	plant spraying oprysk roślin		seed soaking moczenie nasion		plant spraying oprysk roślin		seed soaking moczenie nasion	
	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25	control kontrola	BR_{27} 1	control kontrola	BR_{27} 0.25
Sucrose – Sacharoza	110 ^{b}	113 ^{b}	104 ^{b}	108 ^{b}	141 ^{a}	136 ^{a}	143 ^{a}	144 ^{a}
Stachyose + rafinose Stachioza + rafinoza	82.77 ^{a}	87.41 ^{a}	80.43 ^{a}	84.10 ^{a}	83.59 ^{a}	79.71 ^{a}	83.71 ^{a}	87.47 ^{a}
Glucose – Glukoza	16.98 ^{a}	17.69 ^{a}	16.99 ^{a}	17.05 ^{a}	10.92 ^{b}	10.29 ^{b}	10.59 ^{b}	12.07 ^{b}
Fructose – Fruktoza	11.49 ^{a}	11.69 ^{a}	11.16 ^{a}	11.76 ^{a}	5.26 ^{b}	5.11 ^{b}	5.43 ^{b}	6.66 ^{b}
Maltose – Maltoza	0.643 ^{b}	0.829 ^{b}	0.770 ^{b}	0.698 ^{b}	1.62 ^{a}	1.53 ^{a}	1.32 ^{a}	1.44 ^{a}

mean values marked with the same letters (in rows, for each sugar separately) do not differ significantly according to Duncan's test; $P \leq 0.05$ – dane oznaczone tymi samymi literami (oddzielnie dla każdego cukru) nie różnią się istotnie wg testu Duncana; $P \leq 0.05$

Table 6. Characteristics of gas exchange of seedlings of soybean cultivar Augusta growing in drought conditions and two days after rehydration – effect of 24-epibrassinolide (BR_{27})
Tabela 6. Charakterystyka wymiany gazowej siewek soi odmiany Augusta w warunkach suszy oraz dwa dni po podlaniu – wpływ 24-epibrasinolidu (BR_{27})

Presowing seed soaking Przedsiewne traktowanie nasion	Net photosynthesis Fotosynteza netto $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$		Transpiration Transpiracja $\mu mol \cdot m^{-2} \cdot s^{-1}$		Dark respiration Oddychanie ciemniowe $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$	
	measurement in drought conditions pomiar w warunkach suszy	measurement 2 days after rehydration pomiar 2 dni po podlaniu	measurement in drought conditions pomiar w warunkach suszy	measurement 2 days after rehydration pomiar 2 dni po podlaniu	measurement in drought conditions pomiar w warunkach suszy	measurement 2 days after rehydration pomiar 2 dni po podlaniu
Control 1 Kontrola 1	1.35 ^{b} (100%)	4.14 ^{c} (100%)	396 ^{ab} (100%)	739 ^{c} (100%)	-2.06 ^{a} (100%)	-1.60 ^{a} (100%)
Control 2 Kontrola 2	2.67 ^{a} (197%)	6.11 ^{b} (148%)	489 ^{a} (123%)	1371 ^{b} (185%)	-1.69 ^{a} (82%)	-1.74 ^{a} (108%)
Control 3 Kontrola 3	1.80 ^{ab} (133%)	4.43 ^{c} (107%)	283 ^{b} (72%)	930 ^{c} (126%)	-1.74 ^{a} (84%)	-1.32 ^{a} (83%)
BR_{27} 0.25 $mg \cdot dm^{-3}$	1.99 ^{ab} (147%)	7.49 ^{a} (181%)	508 ^{a} (128%)	1713 ^{a} (232%)	-1.75 ^{a} (85%)	-1.51 ^{a} (94%)

presowing seed soaking – przedsiewne traktowanie nasion: control 1 – kontrola 1 – seeds not soaked – nasiona nie moczone, control 2 – kontrola 2 – seeds soaked in water – nasiona moczone w wodzie, control 3 – kontrola 3 – seeds soaked in water with traces of ethanol – nasiona moczone w roztworze wodnym ze śladową ilością etanolu; BR_{27} – seeds soaked in water with 24-epibrassinolide and traces of ethanol – nasiona moczone w roztworze wodnym zawierającym 24-epibrasinolid i śladową ilość etanolu
mean values marked with the same letters (in columns) do not differ significantly according to Duncan's test; $P \leq 0.05$ – wartości oznaczone tymi samymi literami (w kolumnach) nie różnią się istotnie wg testu Duncana; $P \leq 0{,}05$
in parentheses are given percentage values in comparison to control 1 – w nawiasach przedstawiono wartości procentowe w stosunku do kontroli 1

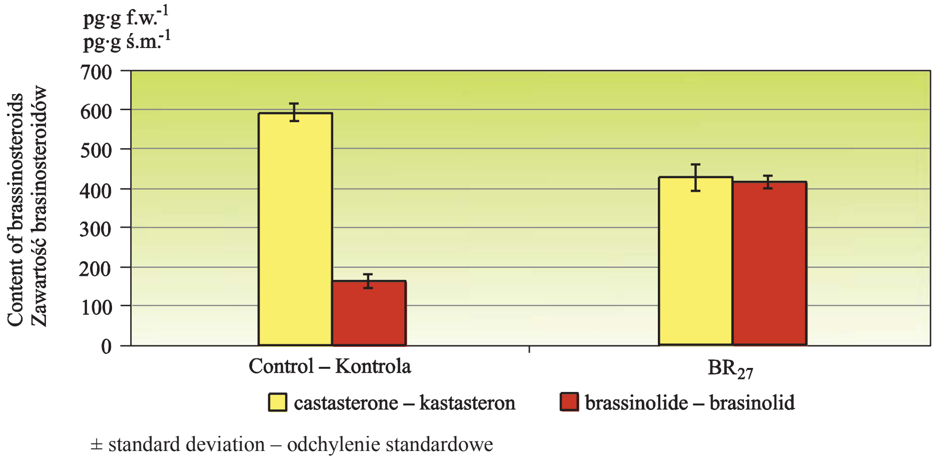

Fig. 1. Content of brassinosteroids in seeds of soybean cv. Aldana treated with 24--epibrassinolide (BR_{27})

Rys. 1. Zawartość brasinosteroidów w nasionach soi odmiany Aldana uzyskanych z roślin traktowanych 24-epibrasinolidem (BR_{27})

Under drought conditions, cv. Aldana showed greater efficiency of energy flow in photosystem II compared to cv. Augusta (Table 7). In soybean of cv. Aldana higher values of energy absorption (ABS), energy flux for trapping (TRo) as well as energy flux for electron transport were found. Additionally, energy dissipation (DIo) under drought conditions was also smaller in cv. Aldana than in cv. Augusta. Presowing seed treatment from control 2, 3 and BR_{27} did not influence values of mentioned parameters measured under drought conditions in cv. Aldana. However, after watering the plants, in control 1 of cv. Aldana decreased values of ETo were found, compared to other treatments. Relatively highest values of this parameter were characteristic of plants whose seeds were exposed to BR_{27} activity before sowing. In the case of cv. Augusta, presowing seed soaking in the solution of 24-epibrassinolide improved efficiency of energy flow under drought conditions, which resulted in the increase of the value of the most important factor, ETo. After watering the plants, this effect disappeared, and the values of phenomenological parameters of energy flow for all treatments were similar.

In the experiments the effect of trace amounts of ethanol (which was a steroid solvent in solutions used for plant treatment) was observed on some physiological processes, e.g. the decrease of net photosynthesis value and decrease of ABS and TRo parameters in the case of cv. Augusta exposed to drought (Tables 6 and 7). In these cases BR_{27} showed activity compensating this effect.

Table 7. Efficiency of photosystem II in drought-stressed seedlings of soybean cultivar Augusta – effect of 24-epibrassinolide (BR_{27})
Tabela 7. Sprawność przepływu energii w fotosystemie II siewek soi odmiany Augusta poddanych stresowi suszy – wpływ 24-epibrasinolidu (BR_{27})

Presowing seed soaking Przedsiewne traktowanie nasion	Measurement in drought conditions Pomiar w warunkach suszy				Measurement 2 days after rehydration Pomiar 2 dni po podlaniu			
	ABS	TRo	ETo	DIo	ABS	TRo	ETo	DIo
Cultivar Augusta – Odmiana Augusta								
Control 1 Kontrola 1	3091 ^{b} (100%)	2585 ^{b} (100%)	928 ^{b} (100%)	506 ^{a} (100%)	3122 ^{bc} (100%)	2636 ^{bc} (100%)	1152 ^{b} (100%)	485 ^{b} (100%)
Control 2 Kontrola 2	3051 ^{b} (99%)	2540 ^{b} (98%)	902 ^{b} (97%)	511 ^{a} (101%)	3157 ^{ab} (101%)	2670 ^{abc} (101%)	1234 ^{b} (107%)	487 ^{b} (100%)
Control 3 Kontrola 3	2932 ^{c} (95%)	2428 ^{c} (94%)	859 ^{b} (93%)	504 ^{a} (99%)	3047 ^{c} (98%)	2577 ^{c} (98%)	1203 ^{b} (104%)	470 ^{b} (97%)
BR_{27} 0.25 mg·dm^{-3}	3077 ^{b} (100%)	2588 ^{b} (100%)	1107 ^{a} (119%)	490 ^{ab} (97%)	3147 ^{abc} (101%)	2674 ^{abc} (101%)	1227 ^{b} (107%)	473 ^{b} (98%)
Cultivar Aldana – Odmiana Aldana								
Control 1 Kontrola 1	3340 ^{a} (100%)	2877 ^{a} (100%)	1273 ^{a} (100%)	463 ^{c} (100%)	3199 ^{ab} (100%)	2676 ^{abc} (100%)	965 ^{c} (100%)	524 ^{a} (100%)
Control 2 Kontrola 2	3283 ^{a} (98%)	2808 ^{a} (98%)	1256 ^{a} (99%)	475 ^{bc} (103%)	3197 ^{ab} (100%)	2726 ^{ab} (102%)	1241 ^{ab} (129%)	472 ^{b} (90%)
Control 3 Kontrola 3	3254 ^{a} (97%)	2794 ^{a} (97%)	1152 ^{a} (90%)	459 ^{c} (99%)	3244 ^{a} (101%)	2766 ^{a} (103%)	1252 ^{ab} (130%)	478 ^{b} (91%)
BR_{27} 0.25 mg·dm^{-3}	3328 ^{a} (100%)	2858 ^{a} (99%)	1183 ^{a} (93%)	470 ^{bc} (102%)	3243 ^{a} (101%)	2778 ^{a} (104%)	1390 ^{a} (144%)	466 ^{b} (89%)

presowing seed soaking – przedsiewne traktowanie nasion: control 1 – kontrola 1 – seeds not soaked – nasiona nie moczone, control 2 – kontrola 2 – seeds soaked in water – nasiona moczone w wodzie, control 3 – kontrola 3 – seeds soaked in water with traces of ethanol – nasiona moczone w roztworze wodnym ze śladową ilością etanolu, BR_{27} – seeds soaked in water with 24-epibrassinolide and traces of ethanol – nasiona moczone w roztworze wodnym zawierającym 24-epibrasinolid i śladową ilość etanolu
energy flow in PS II – przepływy energii w PS II: ABS – energy absorption by antennas – absorpcja energii przez anteny, TRo – energy flux for trapping (energy transferred to reaction center) – energia przekazana na centrum reakcji, ETo – energy flux for electron transport – energia przekazana na łańcuch przenośników elektronów, DIo – energy dissipation (energy lost as heat) – strata energii w postaci ciepła
mean values marked with the same letters (in columns, for each parameter separately) do not differ significantly according to Duncan's test; $P \leq 0.05$ – wartości oznaczone tymi samymi literami (w kolumnach, oddzielnie dla każdego parametru) nie różnią się istotnie wg testu Duncana; $P \leq 0,05$
in parentheses are given percentage values in comparison to control 1 – w nawiasach przedstawiono wartości procentowe w stosunku do kontroli 1

DISCUSSION

In agricultural experiments carried out among others in India and China, effect of BR on the yield of plants from legume family was studied. Using 28-homobrassinolide, less often 24-epibrassinolide and brassinolide, in cultivation of groundnut, bean, lentil and chick-pea, yield increase was obtained [Ramraj et al. 1997, Vardhini and Rao 1998, Fariduddin et al. 2003, Hayat and Ahmad 2003, Hasan et al. 2008]. Yield increase resulted in the increase of the pod number, increased number of produced seeds, while much less frequently the increase of the weight of 1000 seeds was observed. As a result of BR activity, also the increase of the number of root nodules as well as of the length or weight of roots was observed [Vardhini and Rao 1999, Upreti and Murti 2004]. Also in own research, the results were obtained which confirmed the increase of the weight of seeds collected from soybean plants after application of 24-epibrassinolide, however it was dependent on the cultivar and environmental conditions occurring in the growing season. The newest research also points to the positive effect of brassinosteroid on the yield of lupine and pea [Biesaga-Kościelniak et al. 2011].

From the physiological-biochemical point of view in BR effect four main directions may be listed: effect on the efficiency of photosynthesis, especially fixation and conversion of CO_2 [Fariduddin et al. 2003, Yu et al. 2004, Janeczko et al. 2005], stimulation of the activity of antioxidation system [Hasan et al. 2008, Li et al. 2008, Xia et al. 2009a], effect on nitrogen management [Vardhini and Rao 1999, Hayat and Ahmad 2003, Upreti and Murti 2004, Hasan et al. 2008], as well as effect on synthesis of other hormones connected, among others, with stimulation of flower production and regulation of bud abortion [Carlson et al. 1987, Nagel et al. 2001, Upreti and Murti 2004].

In the present paper higher net photosynthesis intensity was observed in soybean plants of cv. Augusta treated with BR_{27}, exposed to drought, compared to control plants (pot experiment). Increase of CO_2 fixation is probably caused by the increase of the activity of carboxylase/oxygenase ribulose-1.5-bisphosphate enzyme [Yu et al. 2004]. Additionally after withdrawal of the drought factor in plants treated with BR_{27}, significantly higher transpiration level was observed than in controls. It may present the effect of BR_{27} on water management of the plant, which is also related to photosynthesis efficiency.

Generally higher values of phenomenological parameters of energy flow in cv. Aldana, measured in the pot experiment may be considered as an indicator of its greater resistance to drought than in cv. Augusta [Kościelniak et al. 2005]. As a result of BR_{27} effect, efficiency of energy flow of PSII under drought conditions was improved (cv. Augusta, pot experiment). The phenomenon of improvement of functioning of PSII *via* BR_{27} application was also observed earlier in spring rape growing under stress conditions caused by medium contamination with cadmium as well as by pathogen infection [Janeczko et al. 2005, Skoczowski et al. 2011]. Improvement of efficiency of PSII *via* BR_{27} application in a pot experiment was not observed in a more drought-resistant cultivar Aldana. However, it is possible that effects of 24-epibrassinolide would occur at a higher intensity of the studied stress factor. Attention should be paid to the fact that under field conditions, where at the stage of seed setting for about 30 days there occurred water deficiency, this cultivar responded to the applied BR_{27} with an increase of the weight of seeds collected from the plant. For soybean of cv. Augusta, drought intensity under field conditions (unlike the drought, which greenhouse plants were exposed to) may have been too high and then BR_{27} activity proved insufficient.

In conducted experiment also changes in chemical composition of seeds caused by 24-epibrassinolide were confirmed, however these changes were minor and also dependent on the cultivar. In soybean seeds collected in the plot experiment, effect of BR_{27} on the content of protein and soluble sugars was not confirmed. The effect of BR_{27} (although dependent on the cultivar) was visible in the case of analysis of minerals and genistein. In earlier scientific papers [Janeczko et al. 2009], it was confirmed that under the effect of BR, β-carotene content increases and the composition of phytosterols (campesterol, stigmasterol and β-sitosterol) in soybean changes. Vardhini and Rao [1998] proved that brassinolide and 24-epibrassinolide affect the increase of fat and carbohydrate content in legume plant seeds.

Analyses conducted in the paper of the brassinosteroid content in soybean seeds are, according to the available literature, the first ones for this species. Castasterone and brassinolide (beside other BRs) had been determined so far in pea, common bean and broadbean as well as in several other more exotic legume plants (mainly qualitative analyses) [Bajguz and Tretyn 2003]. Quantitative analysis of BR occurrence in immature seeds of *Cassia tora* L. proved that both these compounds are present in a smaller amount than in mature seeds of the studied soybean; it concerns especially brassinolide [Park et al. 1994]. In soybean, presence of BRs belonging to one group, C_{28} (i.e. BRs, which in a molecule have 28 carbon atoms), proves that in seeds of this species occurs one – the first discovered and at the same time best known – type of biosynthesis of BR leading from castasterone to brassinolide [Choe et al. 2002]. However, the seeds lack brassinosteroids of C_{29} type (e.g. 28-homobrassinolide) as well as brassinosteroids of 24-epi type (e.g. 24-epicastasterone). Exogenous 24-epibrassinolide applied *via* presowing soaking of the seed material did not accumulate in setting soybean seeds. It corresponds with previous research on wheat which proved that BR_{27} applied *via* presowing seed soaking appears in leaves only after application of higher concentrations, and in the seeds does not accumulate at all [Janeczko et al. 2010, Janeczko and Swaczynová 2010]. Although in the present paper the BR content was not studied in seeds of plants sprayed with BR_{27}, according to Symons and Reid [2004], BRs applied onto leaves of another legume plant, bean, remain immobile though probably they get inside tissues, where they cause physiological effects.

BRs may be used in agricultural, horticultural and pomicultural practice as bioregulators in order to improve yield, stress resistance, plant growth regulation etc. In plants these compounds, because of their natural origin, undergo metabolic conversions and utilization as part of the existing homeostasis. Results of the research carried out on rats indicate that even a dose of 5 g of brassinosteroid per kilogram of body weight is not harmful [Ramraj et al. 1997]. BR amounts used in plant treatment are several times smaller, and the research carried out in Poland on wheat [Janeczko et al. 2010] and soybean additionally proved that exogenously applied brassinosteroid is not accumulated in seeds. Compared to auxins or gibberellins, which also show a positive effect on the legume plant yield [Prusiński and Borowska 2002], brassinosteroids have additional properties – they protect plants much better against unfavorable effect of environmental factors. Thus, functionally they are closer to the well-known anti-stress hormone, ABA. Considering the possibility of a practical use of BRs in Poland, attention should be paid to the economic issue, which is related to two directions of activity: maximum decrease of the applied concentration (including selection of the application method – presowing seed soaking uses less of this compound than e.g. plant spraying) or elaboration of cheaper than available now methods of chemical synthesis of BRs. The presented paper depicts issues connected with BR use in agricultural practice in Poland, though it does not exhaust the subject matter and opens new fields for research.

CONCLUSIONS

1. 24-epibrassinolide positively affected components of soybean yield (especially the seed weight per one plant), though dependence was found of this steroid's activity on weather conditions and on the cultivar.

2. BR_{27} effect on the content of protein and soluble carbohydrates in soybean seeds was not proved. Effect of BR_{27} (though dependent on the cultivar) was visible in the case of analysis of minerals and genistein. In soybean seeds two brassinosteroids were found: brassinolide and castasterone. Exogenously applied 24-epibrassinolide did not accumulate in seeds.

3. Application of 24-epibrassinolide increased net photosynthesis intensity in plants exposed to drought. After withdrawal of the drought factor, plants treated with BR_{27} were characterized by increased transpiration. The effect of the studied steroid on the photosystem II efficiency was less visible. Effect of BR_{27} on the dark respiration of plants was not confirmed.

Acknowledgements

Authors express their thanks to prof. dr hab. Kazimierz Klima (University of Agriculture in Krakow) for making accessible weather data from Krakow-Mydlniki Meteorological Observatory as well as to dr. Ondřej Novák for help with UPLC/MS measurements.

REFERENCES

Ali Q., Athar H., Ashraf M., 2008. Modulation of growth, photosynthetic capacity and water relations in salt stressed wheat plants by exogenously applied 24-epibrassinolide. Plant Growth Regul. 56, 107-116.

Bajguz A., Tretyn A., 2003. The chemical characteristic and distribution of brassinosteroids in plants. Phytochemistry 62, 1027-1046.

Biesaga-Kościelniak J., Mirek M., Janeczko A., Dziurka M., 2011. Poprawa wybranych cech ilościowych i jakościowych plonu roślin strączkowych poprzez zastosowanie nowych bioregulatorów [Improvement of chosen quantitative and qualitative features of the legume plant yield *via* application of new bioregulators]. Mat. konf. Nauka dla hodowli i nasiennictwa roślin uprawnych, Zakopane, 107 [in Polish].

Birt D.F., Hendrich S., Wang W., 2001. Dietary agents in cancer prevention: flavonoids and isoflavonoids. Pharmacol. Ther. 90, 157-177.

Bradford M.M., 1976. A rapid and sensitive for the quantitation of microgram quantitites of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72, 248-254.

Carlson D.R., Dyer D.J., Cotterman C.D., Durley R.C., 1987. The physiological basis for cytokinin induced increases in pod set in IX93-100 soybeans. Plant Physiol. 84, 233-239.

Choe S., Schmitz R.J., Fujioka S., Takatsuto S., Lee M.O., Yoshida S., Kenneth A., Feldmann K.A., Tax F.E., 2002. *Arabidopsis* brassinosteroid-insensitive *dwarf12* mutants are semidominant and defective in a glycogen synthase kinase 3β-like kinase. Plant Physiol. 130, 1506-1515.

Fariduddin Q., Ahmad A., Hayat S., 2003. Photosynthetic response of *Vigna radiata* to pre-sowing seed treatment with 28-homobrassinolide. Photosynthetica 41, 307-310.

Fariduddin Q., Hasan S.A., Ali B., Hayat S., Ahmad A., 2008. Effect of modes of application of 28-homobrassinolide on mung bean. Turk. J. Biol. 32, 17-21.

Fujioka S., Yokota T., 2003. Biosynthesis and metabolism of brassinosteroids. Annu. Rev. Plant Biol. 54, 137-164.

Grove M.D., Spencer G.F., Rohwedder W.K., Mandawa N., Worley J.F., Warthen J.D., Steffens G.L., Flippen-Anderson J.L., Cook J.C., 1979. Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus* pollen. Nature 281, 216-217.

Hasan S.A., Hayat S., Ali B., Ahmad A., 2008. 28-Homobrassinolide protects chickpea (*Cicer arietinum*) from cadmium toxicity by stimulating antioxidants. Environ. Pollut. 151, 60-66.

Hayat S., Ahmad A., 2003. Soaking seeds of *Lens culinaris* with 28-homobrassinolide increased nitrate reductase activity and grain yield in the field in India. Ann. Appl. Biol. 143, 121-124.

Holá D., 2011. Brassinosteroids and photosynthesis. [In:] Brassinosteroids: a class of plant hormone, S. Hayat, A. Ahmad (eds.), Springer Dordrecht, 143-192.

Janeczko A., Biesaga-Kościelniak J., Dziurka M., 2009. 24-Epibrassinolide modifies seed composition in soybean, oilseed rape and wheat. Seed Sci. Technol. 37, 625-637.

Janeczko A., Biesaga-Kościelniak J., Oklešťková J., Filek M., Dziurka M., Szarek-Łukaszewska G., Kościelniak J., 2010. Role of 24-Epibrassinolide in wheat production: physiological effects and uptake. J. Agron. Crop Sci. 196, 311-321.

Janeczko A., Filek W., Biesaga-Kościelniak J., Marcińska I., Janeczko Z., 2003. The influence of animal sex hormones on the induction of flowering in *Arabidopsis thaliana*: comparison with the effect of 24-epibrassinolide. Plant Cell Tiss. Org. Cult. 72, 147-151.

Janeczko A., Kościelniak J., Pilipowicz M., Szarek-Łukaszewska G., Skoczowski A., 2005. Protection of winter rape photosystem II by 24-epibrassinolide under cadmium stress. Photosynthetica 43, 293-298.

Janeczko A., Oklešťková J., Pociecha E., Kościelniak J., Mirek M., 2011. Physiological effects and transport of 24-epibrassinolide in heat-stressed barley. Acta Physiol. Plant. (in print), DOI: 10.1007/s11738-010-0655-y.

Janeczko A., Swaczynová J., 2010. Endogenous brassinosteroids in wheat treated with 24--epibrassinolide. Biol. Plant. 54, 477-482.

Kalaji M.H., Łoboda T., 2009. Fluorescencja chlorofilu w badaniach stanu fizjologicznego roślin. Wyd. SGGW Warszawa [in Polish]. Chlorophyll fluorescence in studies of the physiological condition of plants.

Kościelniak J., Janowiak F., Kurczych Z., 2005. Increase in photosynthesis of maize hybrids (*Zea mays* L.) at suboptimal temperature by selection of parental lines on the basis of chlorophyll *a* fluorescence measurements. Photosynthetica 43, 125-134.

Krebs E.E., Ensrud K.E., MacDonald R., Wilt T.J., 2004. Phytoestrogens for treatment of menopausal symptoms: a systematic review. Obstet. Gynecol. 104, 824-36.

Li K.R., Wang H.H., Han Q.J., Fan J., 2008. Effects of brassinolide on survival, growth and drought resistance of *Robinia pseudoacacia* seedlings under water-stress. New Forests 35, 255-266.

Nagel L., Brewster R., Riedell W.E., Reese R.N., 2001. Cytokinin regulation of flower and pod set in soybeans (*Glycine max* (L.) Merr.) Ann. Bot. 88, 27-31.

Park K.H., Park J.D., Hyun K.H., Nakayama M., Yokota T., 1994. Brassinosteroids and monoglycerides in immature seeds of *Cassia tora* as the active principles in the rice lamina inclination bioassay. Biosci. Biotech. Biochem. 58, 1343-1344.

Pisulewska E., Pisulewski P., Hanczakowski P., Szymczyk B., 1998. Wykorzystanie współczesnych metod oceny białek roślinnych do określenia jakości białka nasion jadalnych roślin strączkowych [Use of contemporary methods of evaluation of legume plant proteins for determination of the quality of protein of edible legume plant seeds]. Mat. konf. Strączkowe rośliny białkowe, Lublin, 71-75 [in Polish].

Prusiński J., Borowska M., 2002. Potencjał biologiczny roślin strączkowych i jego wykorzystanie. Cz. I. Zastosowanie regulatorów wzrostu w uprawie roślin strączkowych [Biological potential of legume plants and its utilization. Part I. Application of growth regulators in legume plant cultivation. Seed plant cultivation]. Hod. Rośl. Nasienn. 2, 2-7 [in Polish].

Ramraj V.M., Vyas B.N., Godrej N.B., Mistry K.B., Swami B.N., Singh N., 1997. Effects of 28--homobrassinolide on yields of wheat, rice, groundnut, mustard, potato and cotton. J. Agric. Sci. 128, 405-413.

Skoczowski A., Janeczko A., Gullner G., Tóbias I., Kornaś A., Barna B., 2011. Response of brassinosteroid-treated oilseed rape cotyledons to infection with the wild type and HR-mutant of *Pseudomonas syringe* or with *P. florescence*. J. Therm. Anal. Cal. (in print), DOI: 10.1007/s10973-010-1204-z.

Strasser R.J., Srivastava A., Tsimilli-Michael M., 2000. The fluorescence transient as a tool to characterise and screen photosynthetic samples. [In:] Probing photosynthesis: mechanisms, regulation & adaptation, M. Yunus, U. Pathre, P. Mohanty (eds.), Taylor & Francis London, UK, 445-483.

Swaczynová J., Novák O., Hauserová E., Funksová K., Šíša M., Strnad M., 2007. New techniques for estimation of naturally occurring brassinosteroids. J. Plant Growth Regul. 26, 1-14.

Symons G.M., Reid J.B., 2004. Brassinosteroids do not undergo long-distance transport in pea. Implications for the regulation of endogenous brassinosteroid levels. Plant Physiol. 135, 2196-2206.

Symons G.M., Ross J.J., Jager C.E., Reid J.B., 2008. Brassinosteroid transport. J. Exp. Bot. 59, 17-24.

Upreti K.K., Murti G.S.R., 2004. Effects of brassinosteroids on growth, nodulation, phytohormone content and nitrogenase activity in French bean under water stress. Biol. Plant. 48, 407-411.

Vardhini B.V., Rao S.S.R., 1998. Effect of brassinosteroids on growth, metabolite content and yield of *Arachis hypogaea*. Phytochemistry 48, 927-930.

Vardhini B.V., Rao S.S.R., 1999. Effect of brassinosteroids on nodulation and nitrogenase activity in groundnut (*Arachis hypogea* L.). Plant Growth Regul. 28, 165-167.

Wang Z.Y., Seto H., Fujioka S., Yoshida S., Chory J., 2001. BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature 410, 380-383.

Xia X.J., Wang Y.J., Zhou Y.H., Tao Y., Mao W.H., Shi K., Asami T., Chen Z., Yu J.Q., 2009a: Reactive oxygen species are involved in brassinosteroid-induced stress tolerance in cucumber. Plant Physiol. 150, 801-814.

Xia X.J., Zhang Y., Wu J.X., Wang J.T., Zhou Y.H., Shi K., Yu Y.L., Yu J.Q., 2009b: Brassinosteroids promote metabolism of pesticides in cucumber. J. Agric. Food Chem. 57, 8406-8413.

Yu J.O., Huang L.F., Hu W.H., Zhou Y.H., Mao W.H., Ye S.F., Nogués S., 2004. A role for brassinosteroids in the regulation of photosynthesis in *Cucumis sativus*. J. Exp. Bot. 55, 1135--1143.

Ziółek W., Kulig B., Pisulewska E., Wir-Konas E., 1996. Wpływ terminu i sposobu zbioru na zawartość oraz plon białka i tłuszczu w nasionach dwóch odmian soi [Effect of the date and method of harvest on the content and yield of proteins and fat in seeds of both soybean cultivars]. Rośl. Oleiste XVII(2), 415-420 [in Polish].

Zullo M., Kohout L., 2004. Semisystematic nomenclature of brassinosteroids. Plant Growth Reg. 42, 15-28.

REAKCJA POLSKICH ODMIAN UPRAWYCH SOI (*Glycine max* (L.) MERR.) NA STOSOWANIE BRASINOSTEROIDU

Streszczenie. Badano oddziaływanie brasinosteroidu – 24-epibrasinolidu – na plonowanie, skład chemiczny nasion (zawartość białek, cukrów, fitoestrogenów i składników mineralnych) oraz odporność na suszę uprawianej w Polsce soi odmian Aldana i Augusta. Przeprowadzono ilościową i jakościową analizę zawartości brasinosteroidów w nasionach roślin traktowanych 24-epibrasinolidem. Eksperymenty wykonano w warunkach poletkowych oraz w szklarni. Brasinosteroid aplikowano poprzez przedsiewne moczenie nasion (0,25 mg·dm^{-3}) i oprysk roślin (1 mg·dm^{-3}). 24-Epibrasinolid wpływał na wzrost masy nasion w przeliczeniu na jedną roślinę (od 27-73%) w zależności od odmiany oraz warunków wegetacji (wystąpienie lub brak czynnika suszy w okresie zawiązywania nasion). Nie oddziaływał na poziom białek i cukrów rozpuszczalnych w nasionach, ale zwiększał zawartość fitoestrogenu genisteiny (odmiany Aldana) oraz zmniejszał poziom potasu (odmiany Augusta) i wapnia (odmiany Aldana). W nasionach soi stwierdzono obecność dwóch ważnych brasinosteroidów: brasinolidu i kastasteronu. 24-Epibrasinolid, którym traktowano rośliny, nie był akumulowany w nasionach. Wykazywał natomiast działanie ochronne na proces fotosyntezy u soi rosnącej w warunkach suszy.

Słowa kluczowe: 24-epibrasinolid, genisteina, hormony steroidowe, odmiany soi, przedsiewne traktowanie nasion, susza

Accepted for print – Zaakceptowano do druku: 20.05.2011