THE EFFECT OF ENVIRONMENTAL FACTORS ON THE TRAITS OF SEEDS*

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The winter wheat cultivars (Estica, Samanta and Ebi), spring wheat cultivars (Mollera, AC Helena, Lucia), fodder mallow (*Malva verticillata* cv. Dolina) and *Fagopyrum* sp. were used in pot greenhouse experiments. Stress abiotic factors affect basic metabolic pathways (the rate of photosynthesis and respiration), chemical composition of seeds, yielding traits, seed traits and traits of sprouting plants. These changes are connected in the filial generation with tolerance to other abiotic stresses, root development and chemical composition of seeds (i.e result of change of root/shoot ratio).On the basis of so far obtained results it is possible to conclude that application of 24-epibrassinolide has substantial influence on the vigour of seeds, on the larger root system in filial generation and higher weight of the seeds. This phytohormon has a positive effect on the level of tolerance of plants against abiotic stresses and also in filial generation (influence across vigour of the seeds). The change of seed traits has statistically significant effect on change of the root system in the next generation. For each type of stress special type of change of every seed trait exists. The improved response of seed traits to stress conditions is accessible also via plant breeding.

24-epibrassinolide; seeds; quality of seeds; net energy; abiotic stresses

INTRODUCTION

Utilisation of seeds has large range. Seeds are used especially for protein production, for pharmacy, for production of carbohydrates, oil baked products, for livestock and for plant production.

The possibilities of understanding the relationship between seed development, environment and quality on the molecular, cellular, physiological and agronomical level are basic aim of seed science. The expected results of seed science methods are an increase in the efficiency and sustainability of crop and seed production, identification of factors determining the quality of the final product, especially stress factors and improvement of the economy of the production chain.

However, due to its complexity, research of seeds requires a multi-disciplinary approach ranging from breeding, seed physiology and molecular biology up to agronomy and processing technology. This can only be achieved by combining scientific expertise and resources from the different disciplines, i. e. development of sustainable production methods, biosynthesis on molecular, cellular, developmental and plant level, characterisation and modification of seed components.

Stress abiotic factors affect significantly growth and development of plants, root traits and seed traits. Through the seed traits and traits of sprouting plants stress abiotic factors affect in filial generation especially root morphology: length, surface, deep of penetration, weight, nutrient uptake, number of root tips, number of root hairs, number of lateral roots and density of roots. Which were genetic reasons for development of the seeds plants from scientific point of view? It seems that it is stability of population, possibility to survive in inconvenient climatic conditions and possibility to expand.

Brassinosteroids are one class of phytohormones controlling important developmental function, such a promotion of cell elongation and division, photomorphogenesis, fertility, seed germination, scenescence, retardation of abscission, promotion of ethylene biosynthesis… biological effects on plant growth and development.

In the present work the influence of abiotic stresses on the plant and seed traits and the influence of the application 24-epibrassinolide (Fig. 1) on the stress tolerance of the plants and seed traits are analysed.

MATERIAL AND METHODS

The winter wheat cultivars (Estica, Samanta and Ebi), spring wheat cultivars (Mollera, AC Helena, Lucia), fod-

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der mallow (*Malva verticillata* cv. Dolina) and *Fagopyrum* sp. were used in pot greenhouse experiments. The responses of wheat cultivars to different abiotic stresses and their combinations were analysed in pot experiments with mixture of soil (50% of soil, 50% of sand). Plant treatments during experiments were: standard – i.e. optimal control conditions for cultivation of analysed plants (C), and stress conditions i.e. drought + high temperature (S), that is to say 30% of water soil capacity and 30 °C at day and 20 °C at night. In all experiments the same seed stock was used and the chosen varieties had different pedigrees in order to avoid the influence of common parents. The basic experimental conditions, representing relatively strong influences of abiotic stresses are given in the following survey:

This method consists in detection of the change in concentration of carbon dioxide and water in the atmosphere surrounding assimilating object (Šesták, Čatský, 1966).

The influence of the spray application of the solution of 24-epibrassinolide (Fig. 1) at the beginning of plants flowering on the different traits of plants and especially seeds was also analysed. The 10^{-9} M concentration of the solution was used. In *Malva* sp. and *Fagopryum* sp. stepwise system of application was applied according to stepwise begin of flowering from low parts of plants to upper parts of plants.

The obtained results were statistically analysed by programme Statistica, version 6.1 Cz, statistical analysis ANOVA.

Conditions	Type of soil	Temperature – night	Temperature – day	Water content	pH of soil	Environment
Control	clay loam	15 °C	20 °C	70%	6.5	greenhouse
Stress	clay loam	20 °C	30 °C	40%	6.5	greenhouse

The content of net energy was measured with automatic dry combustion calorimeter MS 10 A of Laget (Germany). The obtained values were recalculated to values per 1 g of dry matter ($kJ.g^{-1}$ of dry matter) according to ČSN ISO 1928.

Chemical composition-total starch and damaged starch granules were analysed by enzyme methods by Megazyme Kits, proteins content by Kjeldahl method and lipids by the Soxhlet method. Nutrient content was analysed with AAS-4 flow analyser.

After harvest the standard analysis of main yield traits was provided. The next step was analysis of development of the root system in the following generation after influence of abiotic stresses. The following stepwise analysis was applied for this purpose. Seeds surface was sterilised in 1% $Ca(ClO)_2$ for 5 minutes. Grains for experiments were rinsed with deionised water before use. Plants were cultivated under standard conditions in standard growth chamber. After 5 days of germination at low temperature (5 °C) in growth chamber with a day/night period 18/6 hrs an average standard sprouting plants were selected. Environmental conditions were maintained at 20 °C at day, 15 °C at night, 18 hrs light and 6 hrs darkness. The light source was sodium 400W lamp (light intensity was 490 $\mu mol.m^{-2}.s^{-1}$). Five replications of experiment were provided.

Thirty plants per replication were analysed. At harvest – two weeks after the beginning of growth in growth chamber – plant shoots were separated from the roots and various parameters of shoots and roots were measured by standard methods. The presented data are results of four independent experiments. The images of the root system were analysed by image analyser.

Immediate rate of photosynthesis and transpiration rate were measured gasometrically in open system by the apparatus LC Pro+ (Analytical Development Company Ltd., Caring for the Environment, Great Britain).

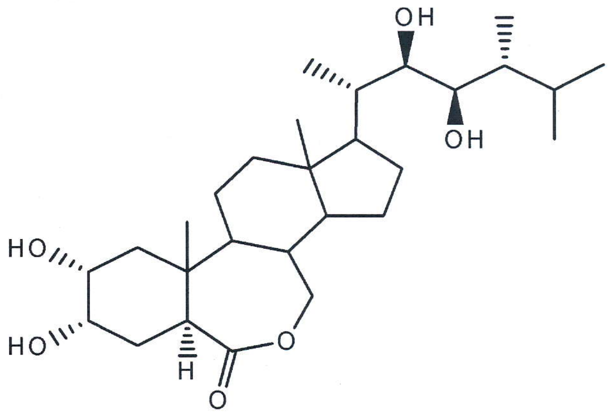

Fig. 1. Molecule of 24-epibrassinolide

RESULTS

The obtained results confirmed in all experiments statistically significant influence of abiotic stresses on the traits of seed ($P \leq 0.05$) and yield ($P \leq 0.01$) of plant. The traits of seeds have substantial effect on the tolerance to analysed abiotic stresses ($r = 0.86$**) in the filial generation. Very similar results between spring and winter wheat were obtained ($r = 0,78$*).

The change of seed traits has statistically significant ($P \leq 0.05$) effect on change of the root system in next generation. The combination of abiotic stresses (drought and high temperature) has a substantial influence on the analysed basic metabolic pathways as photosynthesis of winter and spring wheat. It is possible to conclude that the rate of photosynthesis was influenced by the experimental stress conditions. The stressed variant had the lowest rate of photosynthesis (7.11 $\mu mol\ CO_2.m^{-2}.s^{-1}$) and the control variant had the highest value (8.65 $\mu mol\ CO_2.m^{-2}.s^{-1}$). The variant of stresses reduced the rate of

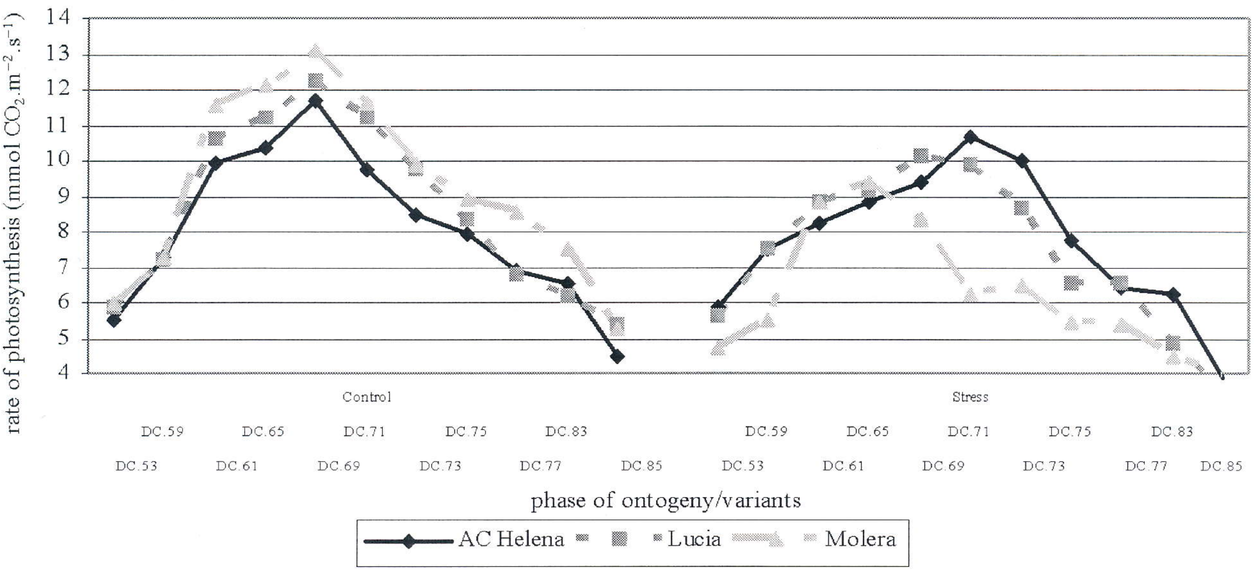

Fig. 2. The rate of photosynthesis (μmol $CO_2.m^{-2}.s^{-1}$)
DC – standard decadic code – phase of development (see Fig. 3)

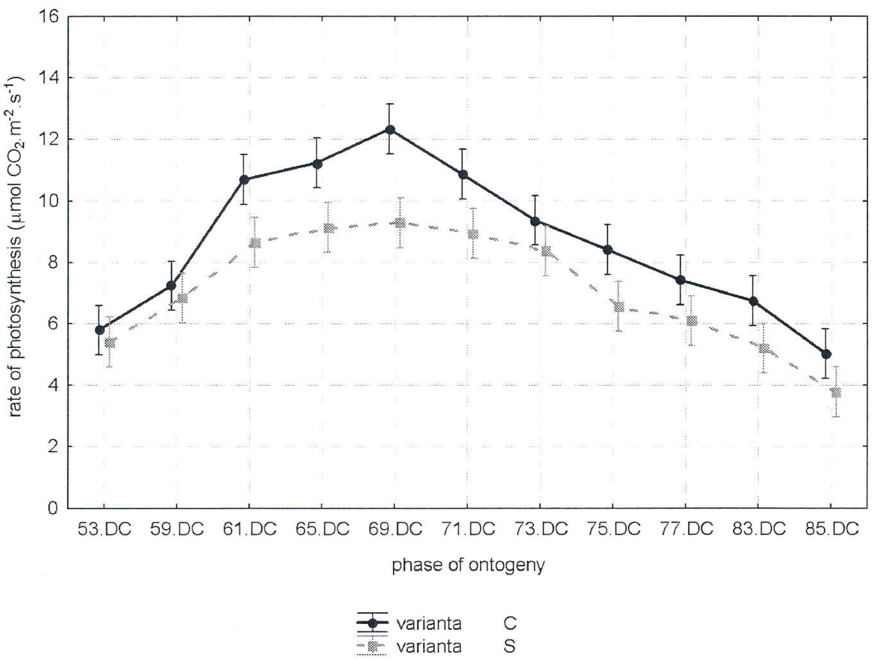

Fig. 3. The influence of the varieties, environment conditions and phases of ontogeny on content of net energy (0.95 intervals of reliability)

S – stress conditions, C – control – standard conditions
53.DC – heading, 59.DC – finish of heading, 61.DC – start of anthesis, 65.DC – anthesis, 69.DC – finish of anthesis, 71.DC – milk maturity, 73.DC – precocious of milk maturity, 75.DC – middle of milk maturity, 77.DC – late of milk maturity, 83.DC – precocious of wax maturity, 85.DC – wax maturity (growth stages according to Z a d o k s et al., 1974)

photosynthesis compared with the control by 17.74% (Fig. 2).

An amount of accumulated energy in grains of wheat grains was significantly affected by cultivars and environment variants of trial. The control variant reached the highest value of net energy as compared with the stressed variant. From the combustion heat calorimetry follows, that seed stock produced from plants grown under abiotic stress conditions decreased very significantly amount of energy in 1 g of dry matter (decrease from 8.90% to 5.73%; $P \leq 0.05$), what means decrease of germinating power and changes in plants growth in the following generation (Fig. 3).

The basic changes were also confirmed by changes in anatomical construction of the caryopsis. We have found that the pericarp and seed coat layers of stressed grains are thicker and cuticle is more suberized. There are also palpable anatomical changes in the embryonal part of caryopsis. Cultivar differences in analysed traits were obtained.

There are essential differences at nutrient uptake between plants grown under standard and stress conditions. Under stress conditions the following decrease/increase of nutrient components were obtained: N = –1.79%, P = –0.223%, Ca = –42.7%, K = –32%, Mg = –29.1%, Na = –39.7%, B = –46.3%, Mn = +59,6.

Environmental conditions have substantial effect on the change of chemical composition of seed proteins, starch, starch damage and lipids. We have found that application of 24-epibrassinolide has a very positive influ-

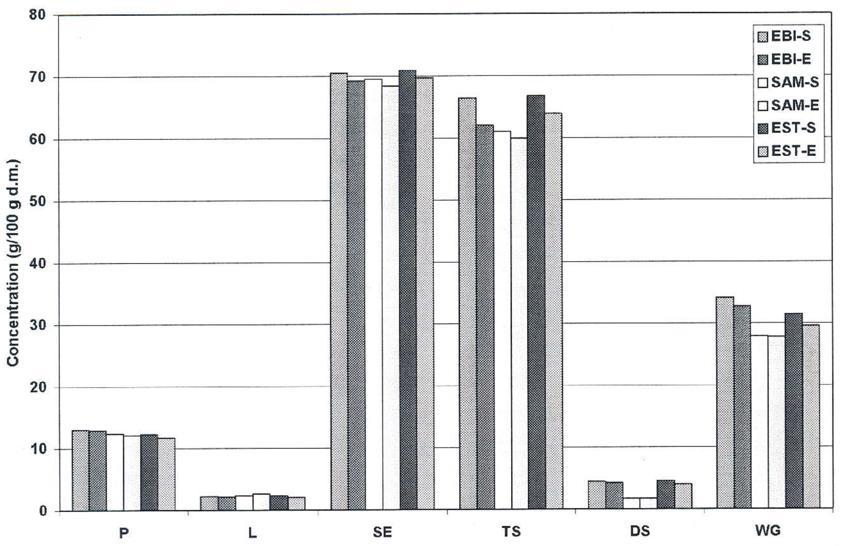

Fig. 4. Influence of 24-epibrassinolide on the chemical composition of the wheat seed; example of winter wheat

P – proteins, L – lipids, SE – starch according Ewers, TS – total starch, DS – damaged starch, WG – wet gluten
EBI-S cultivar Ebi, standard conditions EBI-E cultivar Ebi, stress condition SAM-S cultivar Samanta, standard conditions SAM-E cultivar Samanta, standard conditions EST-S cultivar Estica, standard conditions EST-E cultivar Ebi, standard conditions; d.m. – dry matter

ence on the seed vigour. This hormone has a positive effect on the tolerance of plants against abiotic stresses and also on the growth and development of the filial generation (influence across vigour of the seeds).

Chemical analysis of proteins, lipids, starch (total starch, damaged starch, Ewers), wet gluten and energy content confirmed that treatment with 24-epibrassinolide has no influence on the chemical composition of seeds. Decrease of all measured parameters under stress conditions was similar if this hormone was or was not applied: the content of proteins decreased to 96–99% of original values; lipids to 89–97%, total starch to 93–98%, damaged starch to 86–101%, starch according to Ewers to 98%, wet gluten to 94–99% and net energy to 84–95% of original values (Fig. 4).

The application of 24-epibrassinolide during flowering has a substantial effect on the traits of wheat sprouting plants (Table 1). It is very important for seed production because vigour of the seeds has a substantial effect on the growth of filial generation.

DISCUSSION

It is known that stress conditions affect the yield, seed and root traits (Bláha et al., 2003; Nilsen, Orcutt, 1996) and also vigour of sprouting plants by abscisic acid content. The change of the seed traits affects the rate of water uptake and water loss, i.e. in case of quick change of water soil content during seed sprouting in the field conditions. The vigour of seeds is not a result of only weight and chemical composition of endosperm but there is also a large influence of embryo vigour. Presented results confirmed that there are also great cultivar differences in the influence of drought and high temperature on the analysed traits and grain protein contents in the different cereal cultivars, i.e. testing seeds from different localities and cultivar differences are desirable (Torres et al., 1982; Welch, 1977).

We concluded that the grains from a stressed plant have significantly lower germination power activity and energy content compared to unstressed plants. Our results confirmed the theory of Paine (1971) that changes in chemical composition influenced also the content of energy, as the amount of energy is limited by portion and mutual combination of individual matters, which form individual plant organs.

We can conclude according to Golley (1961) and Hansen, Diepenbrock (1994) that energy value of plant material is the function of genotype and depends also upon external conditions. In the presented work we found that stress abiotic factors have substantial influence on the seed chemical composition. Cultivar differences in nutrient uptake and nutrient distribution for the N, P, K, Ca, Mg and Na to the roots and especially shoots exist. On the other hand, the cereals with higher nutrient uptake and with higher distribution of nutrients to the shoot are more tolerant to the different abiotic stress conditions (low change of root morphology).

The application of 24-epibrassinolide during vegetation period has a positive influence on the development

Table 1. Application of the 24-epibrassinolide at flowering. Influence on the sprouting plants – filial generation (average values across measured wheat cultivars)

Experimental conditions	Length of sprout (mm)	Length of root (mm)	Total length (mm)	Weight of sprout (g)	Weight of root (g)	Total weight (g)
Standard conditions	240.1	180.0	420.5	0.7	0.4	1.1
Stress conditions	210.1	175.2	385.3	0.6	0.3	0.9
Standard and 24-epibrassinolide	258.8	245.6	504.4	0.7	0.4	1.1
Stress and 24-epibrassinolide	263.1	267.3	530.3	0.7	0.4	1.1

Table 2. Example of Fodder mallow (*Malva verticillata*)

Experimental conditions	Dry weight of one plant matter (g)	Weight of 1000 grains (g)	Height of plants (cm)
Standard conditions	2.5	2.8	64.6
Stress conditions	2.1	2.6	60.5
Standard conditions + 24-epibrassinolide	1.9	3.3	58.7
Stress conditions + 24-epibrassinolide	1.9	3.2	59.9

Table 3. Example of Buckwheat (*Fagopyrum* sp.)

Fagopyrum tataricum – Czech provenancy			
Environment	Dry weight of one plant (g)	Weight of 1000 grains (g)	Height of plants (cm)
Standard conditions	2.1	13.9	103
Standard conditions and 24-epibrassinolide	4.7	15.9	120
Drought and high temperature	2.1	10.1	64
Drought and high temperature and 24-epibrassinolide	4.5	15.7	110
Fagopyrum tataricum – USA provenancy			
Environment	Weight of one plant (g)	Weight of 1000 grains (g)	Height of plants (cm)
Standard conditions	1.3	14.9	95
Standard conditions and 24-epibrassinolide	2.7	15.3	105
Drought and high temperature	1.4	12.2	50
Drought and high temperature and 24-epibrassinolide	2.5	14.7	95

of seeds from physiological point of view, i.e. 24-epibrassinolide has positive influence on the vigour of sprouting plants. Brassinosteroids (Zullo, 2003) are endogenous growth promoting hormones that have the structure similar to steroids-hydroxylated derivates of cholestane. It is known that brassinosteroids promotes germination of nondormant seeds (Metzger, 2003; Hayat, Ahmad, 2003).

To confirm our results in other plant species, the application of the solution of 24-epibrassinolide at the growth of *Malva verticillata* cv. Dolina was also studied. In both tested variants the lower content of proteins (93–95%) and lipids (88–95%) was detected by chemical analysis in comparison to the control, but a large increase of weight of seeds between stress conditions and stress conditions with application of 24-epibrassinolide (statistically significant at 5% level) was obtained (Table 2).

Similar results were obtained at buckwheat where especially substantial (statistically significant on 5% level) increase of weight of grains was obtained (Table 3).

The change of root morphology influenced by seed provenance has in the filial generation effect on physiological properties of plants, i.e. different level of tolerance to the abiotic stresses during the vegetation period and to the biotic stresses. Importance of the root system is still neglected .

Starting with epoque of Charles Darwin it is known a special idea – Darwin view on the root system – this part of plant is "the brain" of plants. On the basis of contemporary knowledge for signals transfer of from the roots protoplasmatic connections and molecules of auxins exist. Activity of root system is very similar to "brain" with a quick transfer of information to the shoot root system. Every new information from the environment a new synapse construct – which are stable – "memory of plants". According to this "memory" future reaction to this similar stress of environment is defined. At present large group of synapses is known. There is possibility to conclude with a caution that the root system is information centre of plants (Baluška et al., 2003).

CONCLUSION

Stress abiotic factors affect the basic metabolic pathways (photosynthesis, respiration), chemical composition of seeds, yielding traits, seed traits and traits of sprouting plants. These changes are connected in the filial generation with tolerance to other abiotic stresses, root development and chemical composition of seeds (= result of the change of root/shoot ratio).

On the basis of so far obtained results it is possible to conclude that application of 24-epibrassinolide has a substantial influence on the vigour of seeds, on the larger root system in filial generation and higher weight of the seeds. This phytohormone has a positive effect on the tolerance level of plants against abiotic stresses and also in filial generation (influence across vigour of the seeds).

The change of seed traits has a statistically significant influence on change of the root system in the next generation. For each type of stress a special type of change of every seed trait exists. The improved response of seed traits to stress conditions is accessible also via plant breeding.

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Vliv vnějšího prostředí na vlastnosti semen.

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U pšenice jarní (odrůd Molera, Lucia a AC Helena) a pšenice ozimé (Samanta, Estica, Ebi), slézu krmného odrůdy Dolina a pohanky (populace českého původu) byly sledovány vybrané kvalitativní ukazatele rostlin a jejich semen a obsah energie v semenech rostlin ve standardních a ve stresových (sucho a vysoká teplota) kultivačních podmínkách. Rostliny byly pěstovány v řízených skleníkových podmínkách. Jednalo se o plnou zálivku se 70 % polní vodní kapacity. Ve stresovém případě se jednalo o omezenou zálivku (stres sucha) během celé vegetace, kdy rostliny dostávaly pouze 35–40 % vody z celkové polní vodní kapacity, navíc v kombinaci se stresem vysoké teploty, kdy v průběhu dne byla teplota v průměru 30 °C a v noci 20 °C. U standardního pokusu se teplota pohybovala od 20 do 25 °C přes den a 15 °C přes noc. Délka dne činila 18 hodin, přisvětlování bylo prováděno sodíkovými 400W výbojkami, a délka noci byla 6 hodin. Rostliny byly kultivovány v kultivačních nádobách s homogenizovanou půdou a o stejném objemu půdy. Do nádob bylo vždy vyseto 20 semen hodnocených plodin. Pokusy byly rozděleny na čtyři varianty: kombinace stresu sucha a vysoké teploty, standardní podmínky a kromě toho obě tyto varianty spojené s postřikem 24-epibrassinolidu (10^{-9} M) formou spreje v době kvetení za účelem snížení vlivu stresu (sucha a vysoké teploty) na vlastnosti rostlin, zejména semen. Jako doplňkové pokusy byly testovány i výše uvedené pokusné varianty u dalších plodin (proso, fazole, hrách). Z výsledků vyplývá, že stres suchem a vysokou teplotou má negativní vliv na fotosyntézu, hodnoty spalného tepla generativních orgánů, chemické složení semen a růstové vlastnosti klíčních rostlin.

Aplikace 24-epibrassinolidu během vegetace v době kvetení u většiny plodin a odrůd významně ovlivňuje vlastnosti rostlin – zejména jejich odolnost vůči negativním podmínkám prostředí, a současně také vlastnosti semen sklizených z takto ošetřených rostlin, resp. klíčních rostlin. Vzhledem k nízkým koncentracím postřikové látky je možné předpokládat i praktické a finančně nenáročné využití uvedené látky v produkci semen.

24-epibrassinolid; semena; kvalita semen; netto energie; fotosyntéza; biotické stresory

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