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Brassinosteroide analogue effect on lettuce grown at different moisture levels

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Abstract

Water deficit is one of the most adverse factors for plant growth and productivity. The experiments were conducted to investigate the influence of synthetic brassinolide analogue at concentrations 100 nmol L^{-1}, 1 nmol L^{-1}, 0.01 nmol L^{-1} and 0 nmol L^{-1} on lettuce seedling grown at two moisture levels (reduced, control). The plants were cultivated in growth chamber under day / night temperature of 20 °C/15 °C. The total quantities of irrigation water during the experiment: reduced – 28 mm; control – 39 mm. Brassinolide analogue were sprayed on the seedlings of two butter head lettuce cultivars (Mars and Maršálus) foliage at juvenile stage of growth. The plant parameters (length, fresh weight of shoots and roots) and dry matter content were measured on 21st and 28th day after sowing. Significantly higher values of the average fresh weight and length of above ground part and roots were recorded on 28th day for the cultivar Maršálus treated with brassinosteroide analogue at concentration 1 nmol L^{-1} in conditions with reduced irrigation. The results showed that treatment of plants at the initial stage of the development with 1 nmol L^{-1} solution can limit the consequences of reduced moisture conditions. Prior to use, the most effective concentration, plant parameter which is expected to be changed and sensitivity of the cultivars to the treatment, should be taken into account.

Key words: *Lactuca sativa* L. var. *capitata* L., seedling, growth, water deficit, brassinolide, vegetable, stimulant

Abbreviations: BA – synthetic analogue of the natural brassinosteroide; BRs – brassinosteroids; NS – not significant

Introduction

Water deficit is considered to be among the most severe environmental stresses and the major constraint on plant productivity; losses in crop yield due to water stress probably exceed the loss from all other causes combined (Kramer, 1980). The sensitivity of plants to drought differs among species, populations and varieties and also depends upon the physiological stage of the plant (Liu *et al*., 2011). Water shortage considerably reduces plant dry matter production and thus final yield (Wu *et al*., 2008). In this context, it is necessary to look for ways to reduce the negative impact of water deficit on production.

Brassinosteroids (BRs) were first discovered in oilseed rape pollen in 1979 (Grove *et al*., 1979). It has been well documented in the literature that these hormones act mainly in the meristem regions, causing cell lengthening and division (Mandava, 1988). BRs seem to be involved in the expression of critical development periods, from germination to the transition from plant vegetative to reproductive development (Suge, 1986). BRs stimulated callus proliferation in *Arabidopsis thaliana* and embryogenesis in conifers and rice (Hu *et al*., 2000; Pullman *et al*., 2003). Growth promotion due to BRs application was reported in increased growth of the geranium plant (Swamy and Rao, 2008), *Vicia faba* plants (Piñol and Simon, 2009) and maize (Holá *et al*., 2010). These phytohormones also reduce the effects of environmental stress on plant physiology, *e.g.* in relation to temperature (Ogweno *et al*., 2008) and water deficiency (Upreti and Murti, 2004; Jager *et al*., 2008; Behnamnia *et al*., 2009). Furthermore, BRs are type of environment-friendly (Kang and Guo, 2011) and non-toxic (Esposito *et al*., 2011) hormone.

The application of BRs could be a way to reduce the negative impact of water deficit in vegetable production. Therefore, the aim of this study was to examine how treatment with synthetic analogue of the natural brassinosteroide influences plant parameters of Butter head lettuce (*Lactuca sativa* L. var. *capitata* L.) seedlings grown at different moisture levels.

Materials and methods

Two investigations were conducted in 2013 (July-October) in the laboratory of Department of Horticulture, Czech University of Life Sciences Prague. Butter head lettuce cultivars Mars and Maršálus (produced by Semo Smržice, Czech Republic) were used for the investigation. A synthetic analogue of the natural brassinosteroide (BA) - substance 2α, 3α, 17β-trihydroxy-5α-androstan-6-one - was used for testing (patent pending No. 252605 Industrial Property Office, Czech Republic). The experimental factors were: different moisture levels (reduced irrigation and control), concentration of BA and the cultivar. The seeds were sown (5 mm depth) in plastic seedling trays TEKU JP3050 160 (1 seed per cell; 20 cells per replication; three replications per treatment; 16 mL of cell capacity) in ready-mixed seed-sowing compost based on peat (Profi Sowing Substrate; Agro CS a.s., Czech Republic). The plants were cultivated in growth chamber (KBW 400 Binder, Germany) under day/night temperature of 20 °C/15 °C (Petříková *et al*., 2012), day-illumination 13000 lx. The total quantities of irrigation water during the experiment: reduced variants – 28 mm; control variants – 39 mm. Irrigation was based on current values of available water content; the critical value was 75% for control, and 60% for treatments with reduced moisture conditions. The soil moisture was monitored using the ECH$_2$O sensor (Decagon, USA). The experiment was carried out in randomized design. Four variants were in both (reduced and control) conditions: 0 nmol L^{-1} – no application of BA, application of 0.01 nmol L^{-1}, 1 nmol L^{-1} and 100 nmol L^{-1} BA. The plants were treated with BA on 15th day after sowing. On 21st and 28th day – (BBCH 12 - 13, according to Meier, 2001) – the seedling growth was assessed by harvesting 30 individuals per treatment (ten plants per each replication). The juvenile plants were cleaned; lengths of above ground parts and roots (mm/plant), fresh weight (mg/plant) as well as the root neck diameter (mm/plant) were measured. Dry matter content was determined by drying at 105 °C in a Memmert UFP500 oven (Memmert, Schwabach, Germany) until constant weight was reached. Two months later, the entire experiment was repeated and results were evaluated together.

The data from two experiments were subjected to ANOVA. Means were compared by the Fisher´s LSD test with 0.05 level of probability to determine the statistical significance of the differences among treatments. All statistical evaluations were made with the STATISTICA CZ, version 12.0 software systems (Stat Soft CR S.R.O., Czech Republic).

Results and discussion

The results of measurements made on 21st day (Table 1, 2) shows significant effect of 1 nmol L^{-1} treatment on length and fresh weight of roots with cv. Mars in both moisture conditions. The length of above ground parts was positively influenced in conditions with reduced irrigation. Also the means of neck diameter, length of above ground parts and roots after treatment 0.01 nmol L^{-1} were significantly higher with cv. Mars in the same conditions.

The treatment 1 nmol L^{-1} significantly influenced length of roots and above ground parts with cv. Maršálus in reduced conditions and fresh weight of upper parts in both moisture conditions.

The measurement carried on 28th day (Table 3, 4) confirmed positive influence of 1 nmol L^{-1} in cv. Mars on length of above ground parts and roots in conditions with reduced irrigation and length of above ground parts in control irrigation. From the perspective of the grower, the most important category evaluated was fresh weight of plant. In the case of cv. Maršálus, treatment 1 nmol L^{-1} significantly increased fresh weight of above ground parts and length of roots in both moisture conditions.

Significantly higher values of the average fresh weight of

Table 1. Brassinosteroide analogue effect on the plant size, root length, neck diameter and dry matter content of lettuce seedlings grown at control level of irrigation, measured 21st day

Cultivar	Concentration (nmol L^{-1})	Above ground parts			Neck diameter (mm)	Roots		
		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})
Mars	0	50.4 ± 6.3^{abc}*	103.7 ± 17.6^{ac}	0.042 ± 0.006^{b}	0.579 ± 0.097^{abc}	32.7 ± 8.4^{b}	17.7 ± 4.4^{a}	0.053 ± 0.011^{a}
	0.01	50.0 ± 4.3^{abc}	88.3 ±16.6^{a}	0.045 ± 0.006^{ab}	0.557 ± 0.114^{a}	34.3 ± 7.6^{bc}	19.0 ± 4.2^{ab}	0.052 ± 0.011^{a}
	1	52.5 ± 6.1^{c}	106.0 ± 14.9^{abc}	0.046 ± 0.006^{ab}	0.573 ± 0.080^{ab}	40.3 ± 9.9^{de}	22.8 ± 4.3^{bc}	0.054 ± 0.013^{a}
	100	51.3 ± 6.0^{bc}	95.2 ±16.5^{a}	0.046 ± 0.008^{ab}	0.589 ± 0.102^{abc}	37.5 ± 8.0^{cd}	19.6 ± 3.9^{abc}	0.048 ± 0.010^{a}
Maršálus	0	49.1 ± 7.6^{ab}	117.9 ± 24.4^{bc}	0.050 ± 0.009^{a}	0.627 ± 0.143^{bcd}	45.7± 11.0^{af}	23.6 ± 4.5^{cd}	0.055 ± 0.013^{a}
	0.01	50.3 ± 6.1^{abc}	123.5 ± 26.7^{bd}	0.051 ± 0.008^{a}	0.670 ± 0.153^{d}	49.3 ± 10.6^{a}	27.7 ± 6.8 ^{de}	0.056 ± 0.014^{a}
	1	49.7 ± 4.0^{ab}	141.4 ± 22.2^{d}	0.045 ± 0.007^{ab}	0.632 ± 0.119^{cd}	42.8 ± 10.2^{ef}	21.5 ± 3.8^{abc}	0.052 ± 0.009^{a}
	100	48.1 ± 6.0^{a}	124.0 ± 22.4^{bd}	0.050 ± 0.007^{a}	0.660 ± 0.146^{d}	47.4 ± 11.0^{a}	29.9 ± 6.5^{e}	0.059 ± 0.012^{a}
A: Cultivar (*P*-value)		0.008	< 0.001	0.013	< 0.001	< 0.001	< 0.001	NS
B: Treatment (*P*-value)		NS	NS	NS	NS	NS	NS	NS
A × B (*P*-value)		NS	NS	NS	NS	< 0.001	0.002	NS

* The values followed by the same letter show no statistically significant differences (P<0.05). Mean values ± standard deviation. NS = not significant

Table 2. Brassinosteroide analogue effect on the plant size, root length, neck diameter and dry matter content of lettuce seedlings grown at reduced level of irrigation, measured 21st day

Cultivar	Concentration (nmol L^{-1})	Above ground parts			Neck diameter (mm)	Roots		
		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})
Mars	0	52.7 ± 6.5^{cd}*	99.7 ± 9.2^{a}	0.040 ± 0.003^{ab}	0.540 ± 0.085^{b}	34.6 ± 7.3^{b}	11.2 ± 2.8^{b}	0.070 ± 0.015^{ab}
	0.01	55.8 ± 4.8^{b}	108.9 ± 24.0^{ab}	0.037 ± 0.007^{a}	0.595 ± 0.115^{ac}	38.3 ± 7.1^{ac}	14.2 ± 3.4^{bcd}	0.069 ± 0.007^{a}
	1	57.2 ± 9.3^{b}	115.8 ± 21.2^{ab}	0.037 ± 0.008^{a}	0.548 ± 0.124^{bc}	44.2 ± 9.0^{d}	15.5 ± 3.6^{cde}	0.072 ± 0.009^{ab}
	100	55.4 ± 5.5^{bd}	106.2 ± 20.7^{a}	0.038 ± 0.005^{ab}	0.621 ± 0.103^{ad}	37.2 ± 7.6^{ab}	12.2 ± 3.1^{bc}	0.073 ± 0.010^{ab}
Maršálus	0	46.8 ± 4.9^{a}	113.5 ± 21.3^{ab}	0.041 ± 0.008^{ab}	0.664 ± 0.140^{de}	40.8 ± 10.1^{c}	17.6 ± 3.1^{ae}	0.080 ± 0.012^{ab}
	0.01	46.5 ± 5.5^{a}	126.4 ± 12.7^{bc}	0.038 ± 0.009^{a}	0.590± 0.148^{abc}	38.4 ± 7.6^{ac}	18.9 ± 4.6^{a}	0.081± 0.015^{b}
	1	52.2 ± 6.0^{c}	132.8 ± 27.7^{c}	0.045 ± 0.007^{b}	0.705 ± 0.166^{e}	45.4 ± 8.5^{d}	19.2 ± 4.9^{a}	0.073 ± 0.017^{ab}
	100	45.3 ± 5.7^{a}	117.5 ± 16.3^{abc}	0.036 ± 0.008^{a}	0.632 ± 0.153^{ad}	35.8 ± 6.1^{ab}	16.8 ± 2.7^{ade}	0.073 ± 0.010^{ab}
A: Cultivar (*P*-value)		< 0.001	0.001	NS	NS	< 0.001	NS	< 0.001
B: Treatment (*P*-value)		< 0.001	0.018	NS	NS	NS	< 0.001	0.042
A × B (*P*-value)		0.020	NS	NS	NS	< 0.001	0.012	NS

* The values followed by the same letter show no statistically significant differences (P<0.05). Each value is the mean ± SD. NS = not significant

Table 3. Brassinosteroide analogue effect on the plant size, root length, neck diameter and dry matter content of lettuce seedlings grown at control level of irrigation, measured 28[st] day

Cultivar	Concentration (nmol L^{-1})	Above ground parts			Neck diameter (mm)	Roots		
		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})
Mars	0	58.5 ± 7.4^{abc}*	293.6 ± 53.3^{a}	0.044 ± 0.001^{abc}	0.782 ± 0.134^{abc}	59.0 ± 12.6^{a}	33.9 ± 4.8^{a}	0.053 ± 0.004^{ab}
	0.01	61.4 ± 6.0^{cd}	267.9 ± 49.5^{a}	0.045 ± 0.003^{bc}	0.774 ± 0.136^{ab}	64.6 ± 13.3^{abc}	35.8 ± 6.4^{a}	0.043 ± 0.004^{a}
	1	62.8 ± 8.2^{d}	281.5 ± 54.9^{a}	0.043 ± 0.001^{abc}	0.751 ± 0.123^{a}	64.6 ± 14.2^{abc}	30.2 ± 5.1^{a}	0.054 ± 0.008^{ab}
	100	60.4 ± 5.4^{bcd}	268.1 ± 38.8^{a}	0.043 ± 0.001^{abc}	0.813 ± 0.133^{abc}	61.3 ± 12.2^{ab}	29.8 ± 5.0^{a}	0.053 ± 0.009^{ab}
Maršálus	0	56.8 ± 8.6^{a}	279.1 ± 58.0^{a}	0.047 ± 0.007^{c}	0.848 ± 0.167^{cd}	59.6 ± 11.5^{a}	55.8 ± 12.6^{b}	0.069 ± 0.013^{c}
	0.01	58.0 ± 7.9^{ab}	270.4 ± 51.4^{a}	0.039 ± 0.007^{a}	0.957 ± 0.200^{e}	70.6 ± 16.2^{d}	66.2 ± 13.4^{c}	0.071 ± 0.014^{c}
	1	59.9 ± 7.2^{abcd}	350.6 ± 72.3^{b}	0.044 ± 0.009^{abc}	0.913 ± 0.178^{de}	69.0 ± 13.5^{cd}	56.9 ± 9.0^{bc}	0.062 ± 0.007^{bc}
	100	59.3 ± 8.5^{abc}	261.2 ± 55.7^{a}	0.042 ± 0.009^{ab}	0.827 ± 0.159^{bc}	66.8 ± 10.8^{bcd}	64.9 ± 14.1^{bc}	0.065 ± 0.014^{c}
A: Cultivar (*P*-value)		0.006	NS	NS	NS	< 0.001	0.005	< 0.001
B: Treatment (*P*-value)		0.019	0.029	NS	NS	NS	< 0.001	NS
A × B (*P*-value)		NS	NS	NS	NS	0.001	NS	NS

* The values followed by the same letter show no statistically significant differences ($P<0.05$). Each value is the mean ± SD. NS = not significant

Table 4. Brassinosteroide analogue effect on the plant size, root length, neck diameter and dry matter content of lettuce seedlings grown at reduced level of irrigation, measured 28[st] day

Cultivar	Concentration (nmol L^{-1})	Above ground parts			Neck diameter (mm)	Roots		
		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})		Length (mm)	Fresh weight (mg)	Dry matter content (g g^{-1})
Mars	0	57.8 ± 9.1^{ab}*	254.0 ± 45.6^{a}	0.044 ± 0.001^{ac}	0.698 ± 0.138^{a}	52.8 ± 10.7^{a}	18.7 ± 4.5^{a}	0.088 ± 0.016^{a}
	0.01	58.6 ± 6.9^{abc}	230.9 ± 49.4^{ab}	0.048 ± 0.005^{ab}	0.736 ± 0.110^{a}	53.9 ± 10.5^{a}	19.6 ± 4.8^{a}	0.089 ± 0.019^{a}
	1	61.4 ± 4.9^{c}	251.7 ± 22.8^{a}	0.046 ± 0.003^{abc}	0.743 ± 0.134^{a}	63.3 ± 12.0^{c}	24.6 ± 5.0^{a}	0.076 ± 0.013^{ab}
	100	58.8 ± 5.5^{abc}	234.7 ± 58.3^{a}	0.049 ± 0.002^{ab}	0.749 ± 0.111^{a}	57.7 ± 12.3^{ab}	22.4 ± 5.0^{a}	0.079 ± 0.013^{ab}
Maršálus	0	51.6 ± 6.6^{d}	191.1 ± 17.5^{c}	0.051 ± 0.010^{b}	0.817 ± 0.132^{b}	57.7 ± 9.0^{ab}	23.6 ± 5.9^{a}	0.088 ± 0.016^{a}
	0.01	56.3 ± 11.4^{ae}	231.8 ± 38.8^{ab}	0.040 ± 0.004^{c}	0.822 ± 0.176^{b}	62.8 ± 13.0^{bc}	32.0 ± 8.6^{b}	0.087 ± 0.014^{a}
	1	60.6 ± 8.3^{bc}	301.6 ± 65.5^{d}	0.049 ± 0.008^{ab}	0.853 ± 0.177^{b}	63.0 ± 13.4^{c}	38.5 ± 9.6^{c}	0.078 ± 0.012^{ab}
	100	54.3 ± 7.5^{de}	189.5 ± 36.3^{c}	0.041 ± 0.004^{c}	0.818 ± 0.175^{b}	61.8 ± 12.0^{bc}	32.6 ± 4.7^{bc}	0.070 ± 0.015^{b}
A: Cultivar (*P*-value)		< 0.001	NS	NS	NS	< 0.001	< 0.001	0.015
B: Treatment (*P*-value)		< 0.001	< 0.001	NS	NS	NS	< 0.001	NS
A × B (*P*-value)		NS	< 0.001	< 0.001	< 0.001	NS	NS	NS

* The values followed by the same letter show no statistically significant differences ($P<0.05$). Each value is the mean ± SD. NS = not significant.

roots were recorded on 28[th] day for the cultivar Maršálus in all of the a brassinosteroide analogue treatments in reduced irrigation conditions. On the other hand, cv. Mars did not react significantly for treatment for this parameter. The influence of a brassinosteroide analogue treatment 1 nmol L^{-1} with the cultivar Maršálus was significant on length of above ground parts in same conditions. Moreover, positive influence of treatment 0.01 nmol L^{-1} on length and fresh weight of above ground parts was also noticed with cv. Maršálus in reduced irrigation conditions.

As regards the influence of BRs on plant height or leaf number, previous studies with various plant species usually reported positive results. The increase in plant size could be a direct effect of BRs, since Nakaya *et al*. (2002) found that mutants of *Arabidopsis thaliana* with known defects in the perception of BRs develop small leaves. Treatment of the mutants with BRs reversed the mutation and restored the potential for growth to that of the wild type. BRs play a role in regulating cell expansion and cell proliferation in the leaf (Kim *et al*., 2008; Oh *et al*., 2011). Arora *et al*. (2008) found the positive effect of the addition of 1 nmol L^{-1} (but not 100 nmol L^{-1} or 0.01 nmol L^{-1}) solution of 28-homobrassinolide to the growth medium on the shoot length of maize. Similarly, repeated foliar spray on *Arachis hypogaea* plants with brassinolide or 24-epibrassinolide solutions of 1 µmol L^{-1} concentration had positive effect on the shoot length (Vardhini and Rao, 1998). These results are also confirmed by the data we found about the positive influence of 1 nmol L^{-1} on the size of the plants of lettuce in the reduced irrigation conditions. The values we measured are also supported by the results of Serna *et al*. (2012), who reported a significant positive change in the size of the head of the lettuces (diameter and length) after the application of an analogue of a brassinosteroide along with the agent Tomex Amin. The findings of Holá *et al*. (2010) show that the effective concentration apparently depends also on plant genotype.

Other authors who simultaneously examined the effect of exogenous application of BRs on plants of more than one genotype of the same species have often found that the response of individual genotypes can be markedly different (Pipattanawong *et al*., 1996; Janeczko and Swaczynová, 2010; Doležalová *et al*., 2016 In Press.).

It can be said generally that the application of BRs helps plants, which are exposed to unfavorable environmental conditions (Kang *et al*., 2007; Shahbaz *et al*., 2008). This is also confirmed by our results with cv. Maršálus in reduced irrigation conditions. Nevertheless, the different tolerance of the cultivar to water deficit must be taken into consideration, as presented by Ghane *et al*. (2012) or Pazderů and Koudela (2013).

The assumption that it is possible to reduce partially the negative impact of a water deficit in growing of lettuce seedlings with a brassinosteroide analogue was confirmed. The results show that the spray treatment of the lettuce plants in the initial stage of the development with 1 nmol L^{-1} solution of an analogue of synthetic brassinolide can be used to limit the consequences of reduced moisture conditions. Although the exogenous application of BRs to plants can certainly change lettuce growth and consequently, influence plant growth parameters. The most effective concentration, the plant parameter which is expected to be changed and sensitivity of the cultivars to the treatment, should be taken into account.

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