

Evaluation of the effect of synthetic brassinolide on the seedlings of lettuce in different moisture conditions

JITKA DOLEZALOVA, MARTIN KOUDELA, JOSEF SUS

Department of Horticulture
Czech University of Life Sciences in Prague
Kamycka 129, 165 21 Prague 6
CZECH REPUBLIC

dolezalovajitka@af.czu.cz

Abstract: The aim of this work was evaluation of synthetic brassinolide influence on growth of the lettuce seedlings (cultivar 'Maršálus') in optimal and reduced moisture conditions. The experiments were established in the growth chamber. Irrigation was based on EWC; 75 % for optimally irrigated trays (OPT), and 60 % for reduced irrigation (STR). The four variants of treatment were in both conditions: c0 – no application of synthetic brassinolide (SB), c11 - application of SB concentration 1.10^{-11} M, c9 - concentration 1.10^{-9} M, c7 - concentration 1.10^{-7} M. The plants were treated with SB 15th day after the sowing. The growth was assessed by harvesting 20 individuals per treatment (upper-part; root fresh weight; root length, root neck diameter; greater leaf length and dry matter content were measured. On 21st day the highest value of average leaf length (50.1 mm) was observed in STR, c9. The highest value of average length of roots (52.6 mm) was measured in OPT, c11. The parameters were not statistically significantly different from those measured in the control variants. On 28th day, the highest average value of length of upper-parts (65.5 mm) was found out for variant STR, c9. This value was statistically significantly different from the control value (c0), variants STR and OPT. The highest average value of length of roots (81.5 mm) was measured at OPT, c11 (same as 21st day). The maximum value of dry matter content (4.95 %) was found in variant STR, c9 treatment - statistically significantly different from other treatments in STR variant. The laboratory study indicates a tendency of treatment SB 1.10^{-9} M (c9) to support the aboveground plant size and the dry matter content in condition with reduced irrigation. But we cannot submit that the effect would be also valid for root growth of plants or root neck diameter.

Key-Words: lettuce, draught, brassinosteroids, vegetable, juvenile plant

Introduction

The insufficient water supply may cause wilting plants. The absence of accessible water in the soil profile may lead to irreversible wilting [1]. The most of vegetable species have a high transpiration rate (from 280 to 830 ml of water per 1 g dry matter). As limit value of efficient water capacity is reported at 50 %. If there is a drop below this level, vegetables suffer from lack of water [2]. Sensitivity of plants to drought differs among species, populations and varieties and depends upon physiological stage of the plant and duration of stress impact [3]. Water shortage considerably lowers plant dry matter production and thus final yield [4]. Drought stress as well as UV irradiation is the most adverse factors for plant growth and productivity [5]. In this context it is necessary to look for means to reduce the negative impact of water deficit for grower praxis.

The application of brassinosteroids could be one way to reduce the negative impact of water deficit in vegetable production. These hormones help by reducing of environmental stress impact of plant physiology, e.g. in connection to temperature [6] and to water deficiency [7, 8, 9]. This growth regulators on the basis of sterols not only promotes growth [10], but they also have the potential to increase yield and economic parameters in horticultural crops [11, 12, 13].

The hypothesis of this work was: application of synthetic brassinolide can minimize negative influence of water deficit in cultivation of head lettuce seedlings.

Material and Methods

The trials were carried out in the laboratory belonging to Department of Horticulture, CULS in Prague in 2012 and 2013. The experiments were established in the growth chamber BINDER KBW 400 with illumination, that allows to create constant lighting and temperature conditions. The synthetic analogue of the natural brassinosteroids - substance 2 α , 3 α , 17 β - trihydroxy - 5 α - androstan - 6 - one (SB) was used for testing (patent pending No. 252605 Industrial Property Office). The seeds of cultivar 'Maršálus' (seed category S; producer Semo a.s.) were sown (5 mm depth) in plastic seedling trays TEKU JP3050 160 (1 seed per cell; 20 cell per replication; four replications per treatment; 16 ml cell capacity) in ready-mixed seed-sowing compost based on peat (Agro CS a.s.).

Irrigation was based on current values of efficient water capacity (EWC); the critical value of the EWC was 75 % for optimally irrigated trays (irrigation OPT), and 60 % for variants with reduced levels of irrigation (irrigation STR). The total quantities of irrigation water during test were as follows: the variant with optimal levels of irrigation (OPT) had 39 mm; the variant with reduced levels of irrigation (STR) had 28 mm. The irrigation was done by hand sprayer. Together with the first irrigation fungicide Previcur (concentration 0.25 %) was applied. The seedlings were cultivated in accordance with techniques recommended by Petříková et al. [14]: 20 °C /full illumination 13000 lx - 12 hours / day; 15 °C / 12 hours night.

The experiment was carried out in randomized design. Four variants were in both (OPT and STR) conditions: c0 - no application of SB, c11 - application of SB concentration 1.10^{-11} M, c9 - application of SB concentration 1.10^{-9} M, c7 - application SB concentration 1.10^{-7} M. The plants were treated with SB 15th day after sowing. Just before the using of SB, the controls out measurements of plants were carried. On 21st and 28th day (DC 19 - 29, according to Vogel et al. [15]) the seedling growth was assessed by harvesting 20 individuals per treatment (five plants per each replication). The juvenile plants were counted, cleaned, and upper-part and root fresh weight (g / plant) as well as the root length (mm / plant), root neck diameter (mm / plant) and the greater leaf length (mm / plant) were measured. For upper-part of plant dry matter content was determined [16].

The entire experiment was repeated again. The measured values were statistically analyzed using the STATISTICA CZ, version 12.0 software system for data analysis.

Results and Discussion

The first evaluation of plants was done 15th day. The differences between the lengths of upper-parts of plants (leaf length) were not significant. The average length of the plants in OPT was 33.1 mm and 31.4 mm in STR variant of irrigation.

The average values of the measured lettuce seedlings parameters are given in Table 1.

On 21st day (sixth day after the treatment) juvenile plants were in stage of two true leaves (DC 11 - 19). The highest value of average leaf length (50.1 mm) was observed in variant with reduced irrigation, treatment c9. The highest value of average length of roots (52.6 mm) was measured at optimally irrigated variant, treatment c11. The average root neck diameter (0.746 mm) has a maximum in the control (c0) variant STR. The maximum measured average parameters were not statistically significantly different from those measured in the control variants. The detailed statistical survey results are given in Table 1. The average values of the dry matter content of the upper parts of the plant are shown in Fig. 1. The highest average dry matter content (4.92 %) was found in variant STR, c9 treatment. Conversely, the lowest average dry matter contained samples of both non-treated (c0) variants (STR 3.78 %, OPT 3.85 %). There were not statistically significant differences between treatments. In case of the average fresh weight of upper-parts (Fig. 2), the highest average weight was observed at treatment c11 (0.145 g), irrigation OPT. The lowest average values were obtained for variant STR - treatment c7 (0.103 g).

On 28th day (13th day after the treatment) seedlings were in stage DC 21 - 29. When the measurement was carried out 28th day (Table 1), the highest average value of length of upper-parts (65.5 mm) was found out for variant STR, treatment c9. This value was statistically significantly different from the control value (c0), variants STR and OPT. The highest average value of length of roots (81.5 mm) was measured at optimally irrigated variant (OPT), treatment c11 - as in the case of measurements made 21st day. The average value of root neck diameter (0.970 mm) has a maximum in the variant OPT, treatment c9. The average values of the dry matter content in the upper-parts of the plant are shown in Fig.3. The highest average value of dry matter content (4.95 %) was found in variant STR, c9 treatment - as in the case of measurements made 21st day. This value is statistically significantly different from other treatments in condition with reduced irrigation. In case of average weight of fresh matter (Fig. 4) the highest

value (0.358 g) was found in variant OPT, c9 treatment. However, this value did not differ statistically significantly from the value for the treatment c9 (in STR) and all other treatments in conditions with optimum irrigation (OPT). In case of measurements carried out 21st day values of treated variants do not differ from the control (c0),

but during measurement carried out 28th day there are some differences already evident. It is mainly the average length of upper-parts (leaf length) with treatment c9, where the variants with reduced and optimum irrigation statistically significantly differed from the control variants.

Table 1 Average values of measured parameters (21st and 28th day)

		21 st day			28 th day		
		Leaf length (mm)	Root length (mm)	Root neck diameter (mm)	Leaf length (mm)	Root length (mm)	Root neck diameter (mm)
c0	OPT	48.6 ^a	47.9 ^{ab}	0.644 ^{ab}	56.8 ^a	74.6 ^{ac}	0.875 ^{ab}
	STR	48.3 ^a	44.1 ^{abc}	0.746 ^b	51.6 ^b	68.8 ^a	0.854 ^{ab}
c11	OPT	49.4 ^a	52.6 ^b	0.705 ^{ab}	57.9 ^a	81.5 ^c	0.945 ^b
	STR	47.0 ^{ab}	41.3 ^{ac}	0.686 ^{ab}	51.6 ^b	67.0 ^{ab}	0.814 ^a
c9	OPT	49.4 ^a	42.7 ^{abc}	0.629 ^{ab}	58.8 ^{ac}	74.4 ^{ac}	0.970 ^b
	STR	50.1 ^a	45.6 ^{abc}	0.581 ^a	65.5 ^c	67.0 ^{ab}	0.848 ^{ab}
c7	OPT	47.4 ^{ab}	48.9 ^{ab}	0.690 ^{ab}	59.4 ^{ac}	67.2 ^{ab}	0.878 ^{ab}
	STR	45.5 ^b	37.6 ^c	0.662 ^{ab}	51.9 ^b	60.7 ^b	0.782 ^a

Legend: In each column; values followed by the same letter did not differ significantly ($P < 0.05$) according to Fisher's LSD test.

Fig. 1 Dry matter 21st day, upper-parts (in %)

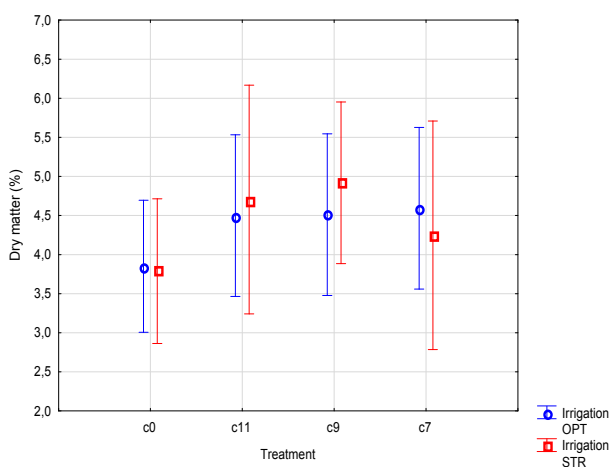


Fig. 2 Fresh weight 21st day, upper-parts (in g)

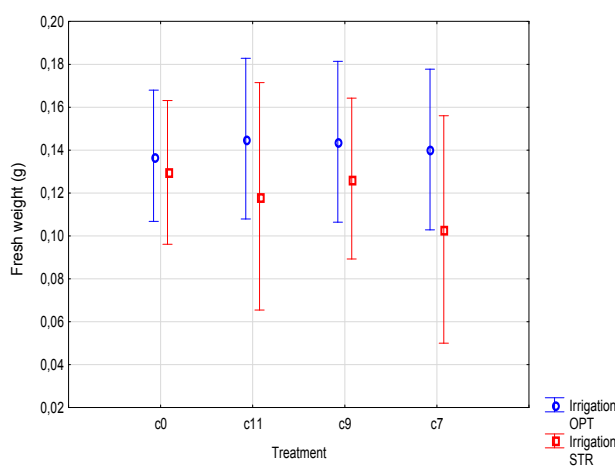
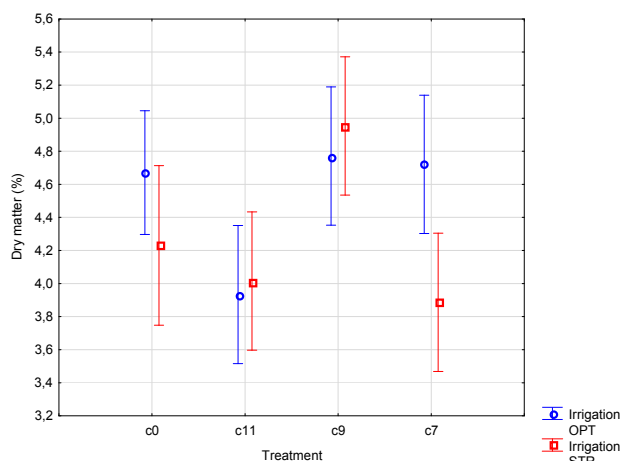


Fig. 3 Dry matter 28th day, upper-parts (in %)

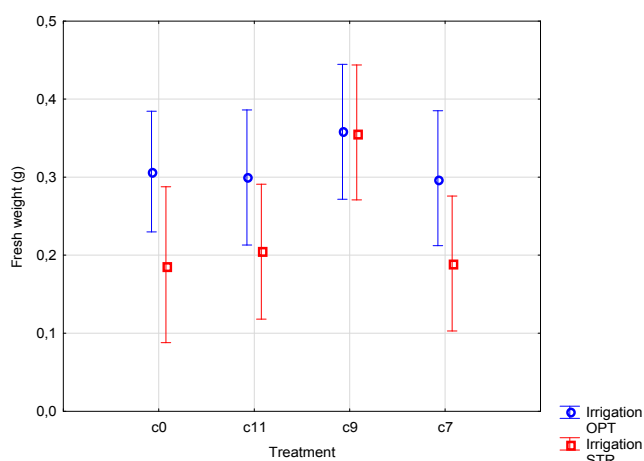
Also in this case, the treatment c9 variant STR is showed promising. However, the value of dry matter - treatment c9 in variant STR is not statistically significantly different from the values of c0, c7 and c9 treatments in optimum irrigation conditions (OPT).

As indicated by numerous studies, application of brassinosteroids can reduce negative environmental impacts, e.g. negative influence of pesticides [17] or biotic stressors [18]. The positive effect of synthetic brassinolide is reflected by increase of value of leaf length and together with increase of dry matter content. The obtained data show a positive effect of treatment c9 in both - reduced and optimal irrigation conditions.

Although there are not always statistically significant differences, the tendency is evident. This finding is consistent with data of Procházka et al. [19], who reported effective concentration from 10^{-8} to 10^{-11} M. We can also agree with the conclusions of Mussig [10] and Pavlová et Fischer [20], who talks about supporting effect of brassinosteroids on growth and the size of plant, respectively.

Conclusion

The study indicates a tendency of treatment with synthetic brassinolide concentration 1.10^{-9} M (c9) to support the aboveground plant size and thus the growth of the plant. We cannot, however, clearly state that the effect would be valid completely for root growth of plants or root neck diameter. The positive effect of treatment c9 was observed in both - reduced and optimal irrigation conditions. The effect of application of synthetic brassinolide was reflected in measuring carried out 28th day (i.e. 13th day after the treatment). The highest average value of length of upper-parts (65.5 mm) was find out for variant with reduced irrigation, treatment c9. This

Fig. 4 Fresh weight 28th day, upper-parts (in g)

value was statistically significantly different from the control value (c0), both variant - with optimal and reduced irrigation. It is known that lack of water in the cultivation leads to a decrease of dry matter content [4]. The results (treatment c9, variant STR), however, show that this effect can be limited by application of synthetic brassinolide.

It would be appropriate to verify results, obtained in the laboratory, in cultivation of seedlings in the greenhouse.

Acknowledgement

The research was financially supported by the project No. NAZV QJ 1210165.

References:

- [1] Šebánek J, Gréc L, Javor A, Švihra J, Kupka J, Procházka S, *Fyziologie rostlin*, SZN Praha, 1983.
- [2] Malý I, Bartoš J, Hlušek J, Kopec K, Petříková K, Rod J, Spitz P, *Polní zelinářství*, Agrospoj, 1998.
- [3] Liu C, Liu Y, Guo K, Fan D, Li ., Zheng Y, Yu L, Yang R, Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China, *Environmental and Experimental Botany*, Vol.71, No.2, 2011,pp. 174 – 183.
- [4] Wu F, Bao W, Li F, Wu N, Effects of drought stress and N supply on the growth, biomass partitioning and water-use efficiency of *Sophora davidii* seedlings. *Environmental and Experimental Botany*. Vol.63, No.1 – 3, 2008, pp. 248 – 255.
- [5] Rajabbeigi E, Eichholz I, Beesk N, Ulrichs, C, Kroh LW, Rohn S, Huyskens-Keil S Interaction of drought stress and UV-B

- radiation - impact on biomass production and flavonoid metabolism in lettuce (*Lactuca sativa* L.), *Journal of Applied Botany and Food Quality*. Vol.86, 2013, pp. 190- 197.
- [6] Ogweno J O , Song XS , Shi K, Hu WH, Mao WH, Zhou YH, Yu JQ, Nogues S, Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*, *Journal of Plant Growth Regulation*, Vol.27, No.1, 2008, pp. 49 – 57.
- [7] Jager CE, Symons GM, Ross JJ, Reid JB, Do brassinosteroids mediate the water stress response? *Physiologia Plantarum*, Vol.113, No.2, 2008, pp. 417 – 425.
- [8] Behnamnia M, Kalantari K M, Ziaie J, The effects of brassinosteroid on the induction of biochemical changes in *Lycopersicon esculentum* under drought stress, *Turkish Journal of Botany*, Vol.33, No.6, 2009, pp.417 – 428.
- [9] Mousavi EA, Kalantari KM, Jafari SR, Change of Some Osmolytes Accumulation in Water-stresses Colza (*Brassica napus* L.) as Affected by 24 – Epibrassinolid, *Iranian Journal of Science and Technology Transaction A – Science*. Vol.33, No. A1, 2009, pp. 1 – 11.
- [10] Müssig C, Brassinosteroid - promoted growth. *Plant Biology*. Vol.7, No.2, 2005, pp. 110 – 117.
- [11] Cutler HG, Advances in the use of brassinosteroids, *Acs Symposium Series*, Vol. 551, 1994, pp. 85-102 Leslie JF, Summerell BA, Bullock S, *The Fusarium Laboratory Manual*, Blackwell Publishing, 2006.
- [12] Khripach V, Zhabinskii V, De Groot A, Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century, *Annals of Botany*, Vol.86, No. 3, 2000, pp. 441 – 447.
- [13] Koudela M, Hnilička F, Martinková J, Svozilová L, Doležalová J, Yield and Quality of Head Lettuce after 24-epibrassinolide Application under Optimal and Reduced Irrigation, *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, Vol. 60, No.11, 2012, pp. 93 – 99.
- [14] Petříková K, Hlušek J, Jánský J, Koudela M, Lošák T, Malý I, Pokluda R, Poláčková, J, Rod J, Ryant P, Škarpa P, *Zelenina pěstování, výživa, ochrana a ekonomika*. Profi Press, 2012.
- [15] Vogel G, Hartmann HD, Krahnstöver K, *Handbuch des speziellen Gemüsebaues*. Eugen Ulmer GmbH & Co, 1996.
- [16] Javorský P, *Chemické rozborý v zemědělských laboratořích*, Díl 1. Výstavnictví zemědělství a výživy, 1987.
- [17] Xia, XH, Huang Y Y, Wang L, et al., Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L, *Pesticide Biochemistry and Physiology*, Vol. 86, No. 1, 2006, pp. 42 – 48.
- [18] Masuda D, Ishida M, Yamaguchi K, et al. Phytotoxic effects of trichothecenes on the growth and morphology of *Arabidopsis thaliana*, *Journal of Experimental Botany*, Vol.58, No.7, 2007, pp. 1617 – 1626.
- [19] Procházka S, Macháčková I, Krekule J, Šebánek J, *Fyziologie rostlin*, Academia, 1998.
- [20] Pavlová L, Fischer L, *Růst a vývoj rostlin*. Karolinum. 2011.