FOLIA
HORTICULTURAE
Ann. 21/1, 2009, 39-48

The effects of triacontanol 'TRIA' and Asahi SL on the development and metabolic activity of sweet basil (*Ocimum basilicum* L.) plants treated with chilling

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Key words: chlorophyll, electrolyte leakage, fluorescence, growth, proline, transpiration, water deficit

ABSTRACT

In a potted experiment the influence of foliar application of triacontanol (TRIA) at the concentrations of 0.01; 0.10; 1.00 mg dm^{-3}, and Asahi SL at the concentrations of 0.1; 0.2; 0.3% on the growth and yielding of plants was studied. Electrolyte leakage, water saturation deficit, contents of proline and chlorophyll *a* + *b* in leaves, as well as the maximum quantum efficiency of chlorophyll (F_v/F_m) and gas exchange in plants which were treated for five days in temperatures of 15/7°C (day/night) were also examined.

The achieved results showed that periodic chilling decreased the value of all the analysed parameters of the plants to a significant degree, with the exception of electrolyte leakage, water saturation deficit and proline content, whose values under these conditions increased. Triacontanol and Asahi SL favourably influenced both the

plants treated and not treated with periodic chilling, but the effect of biostimulators on plants treated with chilling stress was clearly higher. The negative influence of chilling on the plants of *Ocimum basilicum* L. was decreased by TRIA in the concentration 0.10 mg dm^{-3}, and by Asahi SL in the concentration of 0.2 and 0.3%.

INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is one of the oldest medicinal and spice plants. The Latin name of this plant comes from the words *okimon* and *ocimum*, which mean an extraordinary taste and smell, while *basilicos* means royal (Senderski 2004). It is difficult to cultivate this valuable plant in Poland due to periodic chills occurring in the period of vegetation. One of the possible ways of appeasing the negative influence of the chilling stress on plants is the use of biostimulators. This group of compounds includes triacontanol and Asahi SL.

Triacontanol (TRIA) is a primary alcohol of a long 30-carbon chain [$CH_3(CH_2)_{28}$ CH_2OH], naturally occurring in plant wax. So far, studies have shown that TRIA application affects the increase of the dry weight of plants (Knowles and Ries 1981, Eriksen et al. 1982, Borowski 1992, Blamowski 1993), the content of reducing sugars (Sharma et al. 2002), aminoacids and proteins (Ries and Wert 1982, Kissimon et al. 1999), as well as the intensity of CO_2 binding (Mistra and Srivastava 1991, Ivanov and Angelov 1997, Blamowski et al. 1998). Triacontanol also had a positive effect on the content of photosynthetic pigments in the leaves (Kumaravelu et al. 2000) and on chlorophyll fluorescence (Chen et al. 2003).

Asahi SL (Atonik) is a biostimulator containing natural substances occurring in the plant world such as 5-nitroguaiacolan and ortho- and para-nitrophenolones. The studies showed that it stimulates the plants' growth (Vasudeva et al. 1981, Saniewska 1999, Kołodziej 2001, Górnik and Grzesik 2005), tolerance to temperature and drought stress (Górnik et al. 2007), and has a positive effect on the content of assimilation pigments and the photosynthetic activity of the leaves (Mikos-Bielak and Michałek 1999), as well as on the activity of some enzymes in the leaves (Sharma et al. 1984) and seeds (Górnik and Grzesik 2005).

The range of the effects of triacontanol and Asahi SL on plants allows one to predict that they can also have a positive influence on plants submitted to chilling stress. Hence, the purpose of the present study was to determine the effect of periodic chilling on *Ocimum basilicum* L. plants initially treated with different concentrations of triacontanol and Asahi SL.

MATERIAL AND METHODS

The experiment was conducted in a growth chamber at the Department of Plant Physiology of the University of Life Sciences in Lublin in 2007. Basil (*Ocimum basilicum* L.) seeds were sown in a window box filled with a mixture of universal soil and sand in the proportion of 1:1 (v/v). After 21 days, the seedlings with one pair of proper leaves were planted out one to each of 98 pots with a diameter of 14 cm, containing 1.5 kg of the mentioned medium. For the next 30 days the plants were kept in a room with an air temperature of 25/20ºC (day/night), relative air humidity of about 60%, fluorescent light with the stream density of PAR 220 µmol m^{-2} s^{-1} and a photoperiod of 16/8 hours (day/night). The medium humidity in the pots was controlled daily and was kept at the level of 70% (field water capacity). After this period the plants were divided into seven experimental series (14 pots each), differing with the kind and concentration of the biostimulator applied in the form of spraying: 1) control – H_2O distilled, 2) triacontanol (TRIA) – 0.01, 3) TRIA – 0.10, 4) TRIA – 1.00 mg dm^{-3}, 5) Asahi SL – 0.1, 6) Asahi SL – 0.2, 7) Asahi SL – 0.3%. The applied biostimulators were purchased from Sigma Chem. Co. USA (triacontanol) and Bio Gen Polska (Asahi SL). 24 hours after the spraying, one half of the plants from each experimental series (seven pots) remained in the same conditions, while the second half was placed in another growth chamber and exposed to the temperature of 15/7ºC (day/night). After a five-day period of chilling stress, the plants were returned back to the previous conditions, where – after an eight-hour adaptation – intensity of net photosynthesis (Pn) intensity of transpiration (E) and stomatal conductance for water vapour (gs) were measured. The measurements were made in seven repetitions on completely developed leaves of the third pair, using the LCA-4 apparatus for controlling the leaf microclimate. At the time when the temperature was registered, the temperature in the measurement chamber of the leaf was 25ºC, and the stream density of PAR was the same as during the plants' growth.

The content of chlorophyll $a + b$ in the leaves of the 3rd pair was determined according to the method by Arnon (1949). Chlorophyll fluorescence was determined after a 15-minute adaptation of the leaves in darkness using a Handy PEA fluorometer. The value of maximum quantum efficiency of chlorophyll (Fv/Fm) was analyzed after a second flash of light with the wavelength of 660 nm and the intensity of 2500 µmol m^{-2} s^{-1}. Parallel to these analyses, the leakage of electrolytes (E_L) from the leaf tissues was marked according to the method described by Kościelniak (1993), the content of free proline in the leaves according to Bates et al. (1973), and water saturation deficit according to the method described by Blamowski et al. (2001).

The final measurement of the plants' height and the herb harvest were carried out after a further 10 days of growth in the conditions accepted as standard (temperature 25/20ºC). The dry mass of the herb was determined after drying the aboveground parts of the plants at the temperature of 35ºC. Results concerning the plants' height, the yield of dry mass of the herb, the gas exchange of the leaves and fluorescence of chlorophyll were submitted to variance analysis for 3-factor experiments. The significance of differences between the compared mean values was evaluated using Duncan's confidence intervals at p = 0.05. Mean values that did not differ significantly were marked with the same letters.

RESULTS AND DISCUSSION

Indexes included in Table 1 point out that plants treated with periodic chilling were on average 22.5% shorter and gave 27.3% lower yields of herb dry mass as compared to the plants growing throughout the period at the temperature of 25/20ºC. The application of triacontanol and Asahi SL to the plants that were not submitted to the effect of stress did not have any influence on the plants' height or the dry mass yield of the aboveground parts. The exception was only after treatment with TRIA at the concentration of 1.0 mg dm^{-3}, which significantly lowered the values of both discussed plant parameters. In the case of plants treated with chilling, TRIA at the concentration of 0.10 mg dm^{-3} and Asahi SL at the concentration 0.2 and 0.3% significantly affected the height of the shoots and the yielding of plants (Table 1). This is in agreement with findings by other authors (Vasudeva et al. 1981, Saniewska 1999, Kołodziej 2001, Górnik and Grzesik 2005), who also found a positive effect of Asahi SL on the growth of the China aster, Californian grindelia, Antirrhinum plant and coffee as well as an analogous effect of triacontanol on the accumulation of dry mass in plants (Knowles and Ries 1981, Eriksen et al. 1982, Borowski 1992, Blamowski 1993). Górnik et al. (2007) also found that Asahi SL application in 0.2% increased grapevine tolerance to temperature and drought stress.

The obtained results point out that periodic chilling limits the growth and yielding of basil plants, mainly as a result of inhibiting the processes of gas exchange in the plants' leaves and changes in the photosynthetic activity of chlorophyll and its content in leaves. Injuries of the leaves, exhibited by electrolyte leakage and water deficit, were very small. On average, plants treated with chilling showed an increase in electrolyte leakage by 2.1% and water saturation deficit by 4.3% in comparison to the plants that were not submitted to the stress. At the same time, in these conditions, the content of free proline in the leaves increased about 3.5 times as compared to the control. The application of biostimulators only slightly decreased the negative effects of chilling (Table 2).

The effect of chilling on the processes of gas exchange in basil leaves was clearly greater – the decrease of photosynthetic intensity, transpiration and stomatal conductance of the leaves was 44.6%, 40.0% and 67.9%, respectively, as compared to the plants that were not exposed to temperatures of 15/7ºC (Table 3).

Table 1. Effect of chilling stress on growth and yielding of *Ocimum basilicum* L. plants treated with triacontanol and Asahi SL

Biostimulator	Concentration	Height of plants (cm)		Dry mass of herb (g plant^{-1})	
		without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling
Control (H_2O)	-	48.2 ghi	32.8 ab	2.1 cd	1.2 a
TRIA (mg dm^{-3})	0.01	51.2 i	32.8 b	2.5 d	1.5 ab
	0.10	48.2 ghi	42.5 defg	2.5 cd	2.1 cd
	1.00	37.1 bcd	26.7 a	1.6 ab	1.2 a
Asahi SL (%)	0.1	49.6 hi	36.3 bc	2.5 d	1.6 ab
	0.2	45.2 fgh	40.1 cde	2.3 cd	1.8 bc
	0.3	44.9 efg	40.4 cde	2.3 cd	2.2 cd
Mean	-	46.3	35.9	2.2	1.6

Table 2. Effect of chilling stress on electrolyte leakage (E_L), water saturation deficit (WSD) and proline content in *Ocimum basilicum* L. leaves treated with triacontanol and Asahi SL

Biostimulator	Concen-tration	E_L (%)		WSD (%)		Proline (µg g^{-1} f.m.)	
		without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling
Control (H_2O)	-	20.5	23.6	11.4	13.8	18.2	83.0
TRIA (mg dm^{-3})	0.01	19.2	21.4	11.6	11.7	18.2	62.9
	0.10	19.7	20.9	11.0	11.3	17.2	63.2
	1.00	19.4	20.3	11.0	11.4	17.7	72.6
Asahi SL (%)	0.1	18.2	17.2	11.7	11.7	20.3	59.0
	0.2	18.9	17.0	12.3	12.4	20.4	71.7
	0.3	19.6	17.4	12.2	12.5	20.1	72.3
Mean	-	19.3	19.7	11.6	12.1	18.9	69.2

Table 3. Effect of chilling stress on the intensity of photosynthesis (Pn), transpiration (E) and stomatal conductance (gs) in *Ocimum basilicum* L. leaves treated with triacontanol and Asahi SL

Biostimulator	Concen-tration	Pn (μmol CO_2 m^{-2} s^{-1})		E (mmol H_2O m^{-2} s^{-1})		gs (mol H_2O m^{-2} s^{-1})	
		without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling
Control (H_2O)	-	10.3 f	5.3 a	0.9 f	0.4 a	0.26 d	0.09 ab
TRIA (mg dm^{-3})	0.01	11.4 g	6.7 c	1.1 h	0.7 cd	0.24 cd	0.10 ab
	0.10	13.1 i	7.7 e	1.4 i	0.7 cde	0.29 e	0.10 ab
	1.00	10.3 f	6.1 b	0.8 f	0.5 b	0.22 c	0.08 a
Asahi SL (%)	0.1	12.4 h	6.7 c	1.0 g	0.6 c	0.30 e	0.11 b
	0.2	13.6 j	7.1 d	1.1 gh	0.8 e	0.34 f	0.10 ab
	0.3	14.0 k	7.3 d	1.1 h	0.7 de	0.31 e	0.09 ab
Mean	-	12.1	6.7	1.0	0.6	0.28	0.09

The application of biostimulators had a significant effect on the increase of photosynthesis intensity and transpiration in the leaves of basil plants, in both those treated and those not treated with periodic chilling. The only exceptions were plants which were not chilled and which were sprayed with TRIA at the concentration of 1.0 mg dm^{-3}. The application of triacontanol and Asahi SL, however, caused a mean increase of photosynthesis intensity by 12.6% and 29.1%, respectively, as compared to the control in plants that were not treated with chilling, whereas the increase was, respectively, 28.9% and 32.6% in plants submitted to chilling. The corresponding data concerning transpiration were 22.2% and 18.9% for the plants not treated with chilling, and 57.5% and 75.0% for those that were treated with it. The effect of the biostimulators used in the study on the stomatal conductance of the leaves was less distinct (Table 3). A positive effect of the application of triacontanol on the CO_2 binding in plants was also found by Mistra and Strivastava (1991), Ivanov and Angelov (1997), and Blamowski et al. (1998).

Basil plants treated with chilling – independently of the kind and concentration of the applied biostimulators – showed a lower content of chlorophyll *a* + *b* by 19.7%. Its maximum quantum efficiency (Fv/Fm) was 18.1% lower in comparison to the plants that were not chilled. The use of TRIA at the concentration of 0.01 and 0.10 mg dm^{-3}, and the use of Asahi SL at the concentrations of 0.2 and 0.3%, increased the mean content of chlorophyll in relation to the control by 17.2% and 4.8%, respectively. In the case of plants submitted to chilling, TRIA in the concentration of 0.10 mg dm^{-3} and Asahi SL at all applied concentrations had a positive effect on the content of chlorophyll. Triacontanol increased the content of chlorophyll by 31.6%, and Asahi by 18.7%, on average (Table 4). The biostimulators had also a significant positive influence on the maximum quantum efficiency of chlorophyll in plants submitted to chilling as well as those that were not treated with chilling; however, their effect in the case of plants treated with chilling was not significantly higher (Table 4). Similar data in relation to triacontanol were earlier documented by Kumaravelu et al. (2000) and Chen et al. (2003), whereas those related to Asahi SL, by Mikos-Bielak and Michałek (1999).

Table 4. Effect of chilling stress on content of chlorophyll *a* + *b* (Ch) and maximum quantum efficiency of chlorophyll (Fv/Fm) in *Ocimum basilicum* L. leaves treated with triacontanol and Asahi SL

Biostimulator	Concen-tration	Ch (mg g^{-1} f. m.)		Fv/Fm	
		without periodic chilling	with periodic chilling	without periodic chilling	with periodic chilling
Control (H_2O)	-	1.86	1.39	0.782 g	0.634 b
TRIA (mg dm^{-3})	0.01	2.26	1.39	0.788 h	0.642 c
	0.10	2.11	1.83	0.784 h	0.648 d
	1.00	1.66	1.26	0.780 g	0.630 a
Asahi SL (%)	0.1	1.72	1.60	0.796 ij	0.658 f
	0.2	1.96	1.64	0.798 j	0.657 f
	0.3	1.95	1.73	0.794 i	0.654 e
Mean	-	1.93	1.55	0.789	0.646

CONCLUSIONS

1. *Ocimum basilicum* L. plants showed a remarkable sensitivity to periodic chilling stress, which was reflected by a significant decrease of the plants' growth, gas exchange, content and photosynthetic activity of chlorophyll in the leaves and in a manifold increase of free proline in the leaves.
2. Foliar application of triacontanol and Asahi SL before chilling clearly appeased the negative consequences of chilling. TRIA was most effective at the concentration of 0.10 mg dm^{-3} and Asahi SL at the concentration of 0.2 and 0.3%.

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WPŁYW TRIAKONTANOLU (TRIA) I ASAHI SL NA ROZWÓJ I AKTYWNOŚĆ METABOLICZNĄ ROŚLIN BAZYLII POSPOLITEJ (*OCIMUM BASILICUM* L.) TRAKTOWANYCH CHŁODEM

Streszczenie: W doświadczeniach wazonowych badano wpływ dolistnej aplikacji triakontanolu (TRIA) w stężeniach 0,01; 0,10; 1,00 mg dm^{-3} i Asahi SL w stężeniach 0,1; 0,2; 0,3% na wzrost i plonowanie roślin, wypływ elektrolitów, deficyt wody, zawartość proliny i chlorofilu $a + b$ w liściach, a także maksymalną wydajność kwantową chlorofilu (Fv/Fm) i przebieg wymiany gazowej w roślinach poddanych 5-dniowemu działaniu temperatury 15/7°C (dzień/noc).

Uzyskane wyniki wykazały, że okresowy chłód w istotnym stopniu zmniejszył wartość wszystkich analizowanych cech z wyjątkiem wypływu elektrolitów, deficytu wody i zawartości proliny, których wartość w tych warunkach uległa zwiększeniu. Triakontanol i Asahi SL wpływały korzystnie na rośliny traktowane i nie traktowane okresowym chłodem, przy czym wpływ biostymulatorów na rośliny poddane działaniu stresu był wyraźnie większy. Najkorzystniej na ogół, negatywny wpływ chłodu na rośliny *Ocimum basilicum* L. łagodził TRIA w stężeniu 0,10 mg dm^{-3}, a Asahi SL w stężeniu 0,2 i 0,3%.

Received July 17, 2008; accepted April 02, 2009