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RESPONSE TO CHILLING IN CUCUMBER (*Cucumis sativus* L.) PLANTS TREATED WITH TRIACONTANOL AND ASAHI SL

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Abstract

In pot experiments on cucumber cv. Śremski F_1, the effect of short-term chilling on plants earlier treated with triacontanol (TRIA) and Asahi SL was investigated. These plants were grown in a phytotron at an air temperature of 27/22°C (day/night), using fluorescent light with far flux density of 220 µmol × m^{-2} × s^{-1}, with a photoperiod 16/8. At the 4th true leaf stage, the respective experimental series were sprayed with: 1) H_2O – control, 2) TRIA 0.01, 3) TRIA 0.1, 4) TRIA 1.0 mg × dm^{-3}, 5) Asahi SL 0.2, 6) Asahi SL 0.3%. After 24 hours one half of the plants from each experimental series was treated for a period of 3 days at a temperature of 12/6°C, with all the other growth conditions unchanged. The obtained results have shown that short-term chilling stress caused a significant increase in electrolyte leakage, free proline content and in the activity of guaiacol peroxidase in leaves, but a decrease in chlorophyll a+b content, stomatal conductance, transpiration, photosynthesis, leaf area and in the activity of catalase in leaves. The application of TRIA or ASAHI SL on leaves in the pre-stress period reduced the values of the traits which had been increased as a result of chilling and increased those which had reduced. Generally, TRIA was most effective at a concentration of 0.1 mg × dm^{-3}, and Asahi SL at a concentration of 0.3%.

Key words: stress, biostimulators, electrolytes, proline, catalase, peroxidase, photosynthetic pigments, gas exchange

INTRODUCTION

Cucumber is a vegetable which is commonly grown in the conditions of Poland in field, in greenhouses and plastic tunnels. The optimal temperature for plant growth is 20-25°C. It is sensitive to ground frost and to persistent temperatures below 10°C, called chilling temperatures. The magnitude of chilling injury depends on air temperature, time of exposition, plant growth stage and it usually manifests itself after plants are transferred to a higher temperature. Chilling temperatures in leaves of thermophilic plants cause the degradation of membrane lipids and decreased integrity of cell membranes (De Kok and Kuiper, 1977; Chen and Lin, 1993), changes in the composition of photosynthetic pigments (Haldimann, 1998), decreased leaf stomatal conductance (Starck et al. 2000) and decreased photosynthesis (Foyer et al. 1994a; Haldimann, 1998; Starck et al. 2000; Jun – Sungsoo et al. 2001). The reduction in CO_2 fixation rate, induced by low temperature stress, leads to excessive accumulation of reactive oxygen forms (Robinson, 1988; Őquist and Huner, 1993) and increased activity of antioxidant enzymes (Graham and Patterson, 1982; El-Saht, 1998; Dong Hee Lee and Chin Bum Lee, 2000; Feng-Zhaozhong et al. 2003). Chilling stress also causes increased accumulation of free proline in plant leaves (Ait-Barka and Audran, 1997; Hare and Cress, 1997; Chen and Li, 2002).

The most frequent method used to increase plant tolerance to chilling stress is plant hardening which can be done before field planting, whereas during the growth of plants biostimulators can be used for this purpose.

Triacontanol (TRIA) is a primary alcohol [$CH_3(CH_2)_{28}CH_2OH$] naturally occurring in plant waxes. Existing research has shown that TRIA application stimulates dry matter accumulation in leaves (Borowski, 1992), increases the content of photosynthetic pigments (Kumaravelu et al. 2000) and the CO_2 assimilation rate (Mistra and Srivastava, 1991; Muthuchelian et al. 1995; Iwanow and Angelow, 1997; Blamowski et al. 1998).

Asahi SL (Atonik) contains natural substances found in plants, such as 5-nitroguaiacolate as well as ortho- and para-nitrophenolate. Its application in plants stimulates their growth (Djanaguiraman et al. 2005; Górnik and Grzesik, 2005;

Gawrońska et al. 2008), increases the content of photosynthetic pigments (Mikos-Bielak and Michałek, 1999; Gawrońska et al. 2008), facilitates transpiration and photosynthesis processes (Gawrońska et al. 2008; Wróbel and Woźniak 2008), as well as enhances the activity of antioxidant enzymes (Djanaguiraman et al. 2005; Gawrońska et al. 2008) and plant tolerance to chilling stress (Górnik et al. 2007).

Existing research on the application of TRIA and Asahi SL gives the basis for the supposition that both biostimulators may have a beneficial influence on plants subjected to chilling stress. Therefore, the aim of the present study was to determine the effect of chilling stress on cucumber (*Cucumis sativus* L.) plants earlier treated with different concentrations of TRIA and Asahi SL.

MATERIALS AND METHODS

The experiments were conducted in a phytotron of the University of Life Sciences in Lublin in the period 16 May – 9 June and 18 June – 16 July 2008. Seeds of cucumber cv. Śremski F_1 were sown into 84 pots with a diameter of 17 cm, filled with growing medium manufactured by the company Hollas from sieved and milled sphagnum peat with the addition of Hydro fertilizer, chalk and fine washed quartz sand. After emergence, unnecessary seedlings were removed, leaving 2 plants per pot. Until the 4th true leaf stage, the plants were grown in a room with an air temperature of 27/22°C (day/night), relative humidity of approx. 60%, using fluorescent light with far flux density of 220µmol × m^{-2} × s^{-1}, with a photoperiod 16/8 (day/night). The moisture content of the growing medium in the pots was maintained at a level of 70% (field water capacity – FWC), using "weight-based" watering. At the 4th true leaf stage, i.e., after 3 weeks of growth, the plants were divided into 6 experimental series (with 14 pots in each) differing in the type and concentration of the biostimulator applied: 1) control – H_2O; 2) triacontanol (TRIA) – 0.01; 3) TRIA – 0.1; 4) TRIA – 1.0 mg × dm^{-3}; 5) Asahi SL – 0.2; 6) Asahi SL – 0.3%. The biostimulators were administered by spraying the plants; the spraying was done in morning hours using a hand sprayer, applying ca. 5 cm^3 of the solution per pot. After 24 hours one half of the plants from each experimental series (7 pots) remained in the same conditions, whereas the other part (7 pots) was transferred to another phytotron and subjected to a temperature of 12/6°C (day/night) with relative air humidity of approx. 95%.

Immediately after the three-day period of the action of chilling stress, the following parameters were determined in leaves of the plants treated and not treated with chilling: electrolyte leakage (EL) in accordance with the methodology presented in the paper of Markowski and Skrudlik (1995), free proline content according to Bates et al. (1973), chlorophyll content according to Arnon (1949) and carotenoid content according to Britton (1985). Immediately after chilling stress, samples were also collected to determine the activity of catalase (CAT) and guaiacol peroxidase (POD). The plant material was homogenised in a homogeniser with the addition of 5 cm^3 of phosphate buffer, with a pH of 7.8 in the case of catalase and a pH of 7.0 in the case of guaiacol peroxidase, at a temperature of 4°C. The homogenate was poured into test tubes and centrifuged at 4°C at a speed of 10 000 g for 15 min. (catalase) or 10 min. (guaiacol peroxidase). In the solution obtained, extinction readings were made using the spectrophotometric method at a wavelength of 240 nm for catalase (Aebi, 1984) and at a wavelength of 480 nm for guaiacol peroxidase (Małolepsza et al. 1994). The activity of the enzymes was expressed in U × g^{-1} f.m., which means, in the case of catalase, the decomposition of 1 µmol H_2O_2 × min^{-1} × g^{-} f.m., and in the case of guaiacol peroxidase ΔE × min^{-1} × g^{-1} f.m. The abovementioned assays were performed in 4 replicates.

After collecting leaf samples from the chilling-treated cucumber plants, the plants were returned to the previous conditions of 27/22°C in which, after 24-hour adaptation, measurements were made of leaf stomatal conductance, transpiration and photosynthesis rate. Determinations were made in 8 replicates using a LCA-4 leaf microclimate control system. During recording, the temperature in the measurement chamber was approx. 30°C, and far flux density 200 µmol × m^{-2} × s^{-1}. In the same leaves, measurements were also made of minimum fluorescence (F_o), maximum fluorescence (F_m) and maximum quantum yield of chlorophyll (F_v/F_m); however, due to the absence of significant differences these data are not presented in the present paper. After making the abovementioned measurements, total leaf area in the cucumber plants was also determined using a planimeter in 4 replicates. The data presented in this paper are the means from two experiments conducted; they were subjected to analysis of variance for double cross-classification. The significance of differences between the means was determined using Tukey's confidence half-interval, denoted in the present paper as LSD.

RESULTS AND DISCUSSION

The results presented in Tab. 1 show that the chilling-treated plants demonstrated a 68.2% higher electrolyte leakage from leaf cells than the plants not subjected to stress. Pre-stress spraying of the plants with

triacontanol evidently reduced the value of EL both in the untreated plants and plants treated with chilling. TRIA was most effective at a concentration of 0.1 mg × dm^{-3}, which reduced electrolyte leakage by 42.5% in the first case, and in the other case by 27.0% relative to the control treatment. Triacontanol at a concentration of 1.0 mg × dm^{-3} was least effective; in this case, analogous data for the untreated and chilling-treated plants were 13.9% and 5.6%, respectively. The value of the trait in question in the Asahi SL-treated plants was also at a similar level, since the application of the biostimulator in the plants not subjected to chilling stress reduced electrolyte leakage for both concentrations by 13.9%, on the average, and in the chilled plants by 21.4%. It seems that the partial dysfunction of cell membranes and their decreased integrity under the stress conditions are induced by the degradation of membrane lipids, which is indicated by the studies of De Kok and Kupier (1997) as well as of Chen and Lin (1993).

It was found that the beneficial influence, under the chilling stress conditions, of TRIA and Asahi SL on cells was multi-directional, being related, *inter alia*, to the effect of free proline on synthesis. The obtained results showed that the control plants subjected to chilling contained, compared to the plants not treated with chilling stress, over 5 times more free proline in their leaves, which is also confirmed by other authors (Ait-Barka and Audran, 1997; Hare and Cress, 1997; Chen and Li, 2002). The application of triacontanol increased the amino acid content in the cucumber leaves not treated with chilling by 6.3, on the average, relative to the control treatment, and the application of Asahi SL by 3.4 μg × g^{-1} f.m., on the average. The chilling-treated plants, and earlier sprayed with TRIA or Asahi SL before the application of stress, contained less free proline by 27.7 and 17.9 μg × g^{-1} f.m., respectively, relative to the control treatment. It indicates that both biostimulators used offer partial protection against the effects of stress, hence the decrease in free proline which performs the role of a protective substance also under stress conditions.

Antioxidant enzymes, such as e.g. catalase and guaiacol peroxidase, also perform a similar role as proline under stress conditions. The results obtained in the present experiments demonstrated that both in the control plants and biostimulator-treated plants chilling stress applied significantly reduced the activity of catalase by 17.0%, on the average, relative to the plants not subjected to chilling. But the application of triacontanol, both in the untreated and chilling-treated plants, significantly increased the activity of the enzyme by, respectively, 72.7% and 71.5%, on the average, compared to the control plants. In this respect, TRIA was most effective at a concentration of 0.1 mg × dm^{-3}, and least effective at a concentration of 1.0 mg × dm^{-3}. The influence of Asahi SL was analogous; in the plants not subjected to stress it increased the average activity of catalase by 57.5% and in the stress-treated plants by 64.5%, but the solution was more effective at a concentration of 0.3% (Tab. 2). However, the effect of chilling stress on the activity of guaiacol peroxidase in cucumber leaves was different, since in the control plants short-term chilling increased the activity of the enzyme more than twice, in the plants in which TRIA had been applied earlier more than 17 times, on the average, and in the plants with Asahi SL application – 8.5 times. But the earlier application of the biostimulators, in particular TRIA, in the plants not treated with chilling decreased the activity of peroxidase, whereas in the chilling-treated plants it clearly increased this activity, nearly three times, on the average, under the influence of triacontanol and four times under the influence of Asahi SL (Tab. 2). Dong Hee Lee and Chin Bum Lee (2000) as well as Graham and Patterson (1982) also note a significant increase in the activity of guaiacol peroxidase and a decrease in the activity of catalase under the influence of chilling stress, substantiating it with very low affinity of catalase for H_2O_2. But an increased activity of both enzymes under the influence of Asahi SL have been also observed by Djanaguiraman et al. (2005) and Gawrońska et al. (2008), whereas Górnik et al. (2007) have observed increased tolerance to chilling under these conditions.

As indicated by the results contained in Tab. 3, chilling stress also affected significantly the content of chlorophyll a+b in cucumber leaves, whereas it had no effect on the carotenoid content. Irrespective of the type and concentration of the biostimulators used, the chilling-treated plants contained in their leaves, on the average, 15% less chlorophyll than the plants not subjected to chilling stress, which is also confirmed by Haldimann (1998). The application of the biostimulators in the pre-stress period had a beneficial effect on the content of both chlorophyll a+b and carotenoids in the cucumber leaves treated and not treated with chilling. The plants sprayed with TRIA contained, on the average, 11.9% more chlorophyll a+b relative to the control plants, and 7.9% in the case of those sprayed with Asahi SL, whereas the respective data for carotenoids were 19.0% and 26.2%. Triacontanol administered at a concentration of 0.1 mg × dm^{-3}, and Asahi SL at a concentration of 0.3%, had by far the most beneficial effect on the value of the trait in question. Other researchers also confirm the stimulating effect of TRIA (Kumaravelu et al. 2000) and Asahi SL (Mikos-Bielak and Michałek, 1999; Gawrońska et al. 2008) on the content of photosynthetic pigments in plants.

Table 1
Effect of chilling on electrolyte leakage (EL) and proline content in cucumber leaves treated with triacontanol (TRIA) and Asahi SL.

Biostimulator (A)	Concentration	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		EL (%)			proline (μ × g^{-1} f.m.)		
Control	H_2O	24.5	41.2	32.8	16.7	88.2	52.4
TRIA	0.01	15.1	36.5	25.8	16.6	63.4	40.0
(mg × dm^{-3})	0.1	14.1	30.1	22.5	23.4	64.2	43.8
	1.0	21.1	38.9	30.0	28.9	53.9	41.4
Asahi SL	0.2	19.5	34.2	26.8	19.7	71.6	45.6
(%)	0.3	22.7	30.7	26.7	20.6	69.0	44.8
Mean for B		19.5	35.3		21.0	68.4	
LSD for A			5.8			6.7	
LSD for B			2.6			2.6	
LSD for AxB			n.s.			11.1	

Table 2
Effect of chilling on catalase and guaiacol peroxidase activities in cucumber leaves treated with triacontanol (TRIA) and Asahi SL.

Biostimulator (A)	Concentration	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		catalase (U × g^{-1} f.m.)			peroxidase (U × g^{-1} f.m.)		
Control	H_2O	86.2	70.8	78.5	38.1	86.5	62.3
TRIA	0.01	149.5	105.4	127.4	13.0	216.6	114.8
(mg × dm^{-3})	0.1	175.9	159.7	167.8	10.8	215.8	113.3
	1.0	121.4	99.0	110.2	18.7	317.7	168.2
Asahi SL	0.2	129.0	105.4	117.2	50.7	361.1	205.9
(%)	0.3	142.7	127.7	135.2	32.8	347.3	190.0
Mean for B		134.1	111.3		27.3	257.5	
LSD for A			18.6			90.8	
LSD for B			7.2			45.5	
LSD for AxB			n.s.			194.7	

Table 3
Effect of chilling on chlorophyll a+b and carotenoid content in cucumber leaves treated with triacontanol (TRIA) and Asahi SL.

Biostimulator (A)	Concentration	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		chlorophyll a+b (mg × g^{-1} f.m.)			carotenoids (mg × g^{-1} f.m.)		
Control	H_2O	1.87	1.65	1.76	0.19	0.24	0.21
TRIA	0.01	1.91	1.81	1.86	0.20	0.25	0.22
(mg × dm^{-3})	0.1	2.37	1.84	2.10	0.27	0.26	0.26
	1.0	2.20	1.74	1.97	0.28	0.24	0.26
Asahi SL	0.2	1.98	1.72	1.85	0.26	0.26	0.26
(%)	0.3	2.11	1.79	1.95	0.25	0.29	0.27
Mean for B		2.07	1.76		0.24	0.26	
LSD for A			0.11			0.03	
LSD for B			0.04			0.01	
LSD for AxB			0.19			0.04	

Table 4
Effect of chilling on stomatal conductance and transpiration rate of cucumber leaves treated with triacontanol (TRIA) and Asahi SL.

Biostimulator (A)	Concentration	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		conductance (mol × m^{-2} × s^{-1})			transpiration (mmol × m^{-2} × s^{-1})		
Control	H_2O	0.15	0.07	0.11	2.15	1.10	1.62
TRIA	0.01	0.18	0.09	0.13	2.32	1.24	1.78
(mg × dm^{-3})	0.1	0.20	0.12	0.16	2.47	1.35	1.91
	1.0	0.18	0.08	0.13	2.24	1.15	1.69
Asahi SL	0.2	0.21	0.12	0.16	2.45	1.40	1.92
(%)	0.3	0.22	0.12	0.17	2.52	1.47	1.99
Mean for B		0.19	0.10		2.36	1.28	
LSD for A			0.02			0.21	
LSD for B			0.01			0.08	
LSD for AxB			n.s.			n.s.	

Table 5
Effect of chilling on photosynthetic rate and total leaf area in cucumber plants treated with triacontanol (TRIA) and Asahi SL.

Biostimulator (A)	Concentration	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		photosynthesis (μmol × m^{-2} × s^{-1})			leaf area (dm^2 × $plant^{-1}$)		
Control	H_2O	5.22	3.00	4.11	7.34	5.28	6.31
TRIA	0.01	6.36	4.14	5.25	7.43	5.85	6.64
(mg × dm^{-3})	0.1	6.85	4.85	5.85	7.65	5.94	6.79
	1.0	6.08	3.84	4.96	8.12	6.02	7.07
Asahi SL	0.2	6.74	4.78	5.76	8.27	6.14	7.20
(%)	0.3	7.05	4.82	5.93	9.53	6.61	8.07
Mean for B		6.71	4.24		8.06	5.97	
LSD for A			0.53			n.s.	
LSD for B			0.21			0.87	
LSD for AxB			n.s.			n.s.	

Chilling stress inhibited particularly strongly leaf gas exchange processes in cucumber, as the plants treated with a temperature of 12/6°C, compared to those growing at 27/22°C (day/night), showed on the average nearly twice lower leaf stomatal conductance, which has been also observed by Starck et al. (2000) in tomato under the influence of chilling stress. Such behaviour of stomata in leaves, for understandable reasons, resulted in a nearly 46% decline in transpiration and a 37% decline in photosynthesis (Tab. 4 and 5). The rapid decrease in CO_2 assimilation in plant leaves under chilling conditions has also been found by Foyer et al. (1994a), Haldimann (1998), Starck et al. (2000) and Juan-Sungsoo et al. (2001). The reduction in the CO_2 assimilation rate induced by low temperature stress leads to insufficient supply of natural electron acceptors, in particular NADP; in this situation, O_2 becomes such acceptor, which leads to the generation of RFT in cells (Robinson, 1988; Öquist and Huner, 1993). The application of the biostimulators in the pre-stress period clearly mitigated the negative effect of chilling on the gas exchange processes in question. The use of TRIA in the untreated and chilling-treated plants increased the average leaf stomatal conductance by 27.3% relative to the control treatment, and the application of Asahi SL increased this parameter by as much as 50%. The respective data for transpiration are 10.5% and 20.4%, whereas for photosynthesis 30.2% and 42.1%. The beneficial effect of triacontanol on CO_2 assimilation in plants has also been found by Mistra and Srivastava (1991), Muthuchelian et al. (1995), Iwanow and Angelow (1997) as well as Blamowski et al. (1998), whereas the same has been found by Gawrońska et al. (2008) as well as Wróbel and Woźniak (2008) with respect to the biostimulator Asahi SL. TRIA mitigated, to the greatest extent, the negative effects of chilling on the gas exchange processes in cucumber leaves at a concentration of 0.1 mg × dm^{-3}, whereas Asahi SL at a concentration of 0.3% (Tab. 4 and 5).

As a result of its direct effect on growth processes as well as through the inhibition of CO_2 assimilation, chilling stress significantly restricted plant growth. The results contained in Tab. 5 indicate that, irrespective of the earlier application of the biostimulators, the chilling-treated plants developed a 25.9% smaller total leaf area compared to the plants grown at a temperature of 27/22°C. The pre-chilling application of the biostimulators, in particular Asahi SL, increased the leaf area growth rate; however, it was not confirmed statistically. The beneficial effect of triacontanol on plant growth has also been confirmed by Borowski (1992), whereas in the case of Asahi SL by Djanaguiraman et al. (2005), Górnik and Grzesik (2005) as well as Gawrońska et al. (2008).

CONCLUSIONS

1. The three-day period of treatment of young cucumber plants with a temperature of 12/6°C (day/night) caused a significant increase in electrolyte leakage, free proline content and in the activity of guaiacol peroxidase in leaves, but a decrease in chlorophyll a+b content, stomatal conductance, transpiration, photosynthesis, leaf area and in the activity of catalase in leaves.
2. The application of triacontanol or ASAHI SL in leaves in the pre-stress period reduced the value of the parameters which had been increased as a result of chilling and increased those which had decreased. Generally, TRIA was most effective at a concentration of 0.1mg × dm^{-3}, and Asahi SL at a concentration of 0.3%.
3. In the light of the results obtained, foliar application of biostimulators (TRIA, Asahi SL) may be an efficient method of mitigating the negative effects of chilling stress in thermophilic plants.

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Reakcja na chłód roślin ogórka traktowanych triakontanolem i Asahi SL

Streszczenie

W doświadczeniach wazonowych prowadzonych na ogórku odm. Śremski F_1 badano wpływ okresowego chłodu na rośliny wcześniej traktowane triakontanolem (TRIA) i Asahi SL. Rośliny rosły w fitotronie w temp. powietrza 27/22°C (dzień/noc), korzystając ze światła fluorescencyjnego o gęstości strumienia FAR 220 µmol × m^{-2} × s^{-1}, przy fotoperiodzie 16/8. W fazie 4-tego liścia właściwego odpowiednie serie doświadczalne opryskano: 1) H_2O – kontrola, 2) TRIA 0.01, 3) TRIA 0.1, 4) TRIA 1.0 mg × dm^{-3}, 5) Asahi SL 0.2, 7) Asahi SL 0.3%. Po 24 godzinach połowę roślin z każdej serii doświadczalnej traktowano przez okres 3 dni temp. 12/6°C przy niezmienionych pozostałych warunkach wzrostu. Uzyskane wyniki wykazały, że okresowy chłód wywołał istotny wzrost stopnia wypływu elektrolitów, zawartości wolnej proliny i aktywności peroksydazy gwajakolowej w liściach, spadek zaś zawartości chlorofilu „a+b", przewodności szparkowej, transpiracji, fotosyntezy, powierzchni liści i aktywności w nich katalazy. Aplikacja na liście w okresie przedstresowym TRIA lub ASAHI SL obniżyła wartość tych cech, które w wyniku chłodu uległy podwyższeniu, a podwyższyła te które uległy obniżeniu. Na ogół najbardziej skuteczny był TRIA w stężeniu 0.1 mg × dm^{-3}, a Asahi SL w stężeniu 0.3%.