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THE EFFECT OF THE METHOD OF APPLICATION AND CONCENTRATION OF ASAHI SL ON THE RESPONSE OF CUCUMBER PLANTS TO CHILLING STRESS

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Abstract

In pot experiments conducted on cucumber cv. Śremski F1, the effect was studied of short-term chilling stress on plants which had grown from seeds germinating in the solution of Asahi SL or treated with this biostimulator during the early growth period. The plants were grown in a phytotron at an air temperature of 27/22ºC (day/night), using fluorescent light with FAR flux density of 220 µmol × m^{-2} × s^{-1} and with a photoperiod of 16/8. The biostimulator was applied using the following methods: a) germination of seeds in 0.01% and 0.05% solution, b) watering of plants twice with 0.01% or 0.05% solution, c) spraying leaves with 0.3% or 0.5% solution. Plants sprayed with distilled water were the control. After 24 hours from foliar or root application of Asahi SL, one half of the plants from each experimental series was treated for a period of 3 days at a temperature of 12/6ºC, with all the other growth conditions unchanged. The obtained results show that short-term chilling stress caused a significant increase in electrolyte leakage, free proline content and in the activity of ascorbate peroxidase in leaves, but a decrease in the content of chlorophyll, its maximum fluorescence (Fm) and quantum yield (Fv/Fm), carotenoid content, stomatal conductance, transpiration, photosynthesis, leaf biomass and in the activity of catalase in leaves. Foliar or root application of Asahi SL in the pre-stress period decreased the values of the traits which increased as a result of chilling or increased those which decreased. Higher concentrations of the biostimulator solutions, applied using this method, were more effective. The application of the biostimulator during seed germination did not result in significant changes in the response of plants to chilling stress.

Key words: stress, Asahi SL, electrolytes, proline, catalase, peroxidase, Fm, Fv/Fm, photosynthetic pigments, gas exchange.

INTRODUCTION

Among vegetable plants commonly grown in Poland, cucumber is one of the most sensitive plants to chilling stress. Fruits for direct consumption come primarily from greenhouse production, in particular during the spring period. But processing cucumber is usually grown in field. Due to a rather unstable climate in our country, plants are exposed to the effect of chilling stress, especially in spring. This generally occurs at night and does not cause great damage if temperature drops are not large, but then a sunny morning is dangerous (Chaumont et al. 1995). The effects of stress, if it is short lived, are unnoticeable during its duration and they manifest themselves only after it has subsided. Cytoplasmatic membranes, in which there occurs the degradation of membrane lipids resulting in decreased integrity of cell membranes, are most exposed to chilling temperatures (De Kok and Kuiper, 1977; Chen and Lin, 1993). Stress also affects adversely photosynthetic pigment content (Haldimann, 1998; Borowski, 2009; Borowski and Blamowski, 2009), chlorophyll fluorescence (Huner et al. 1995; Misra et al. 2001; Borowski and Blamowski, 2009), and leaf gas exchange (Foyer et al. 1994a; Haldimann, 1998; Starck et al. 2000; Jun-Sungsoo et al. 2001; Borowski, 2009; Borowski and Blamowski, 2009). Such conditions usually lead to excessive accumulation in cells of reactive oxygen forms (Robinson, 1998; Öquist and Huner, 1993), proline (Ait-Barka and Audran, 1997; Hare and Cress, 1997; Borowski, 2009; Borowski and Blamowski, 2009), and increased activity of antioxidant enzymes (Graham and Petterson, 1982; El-Saht, 1998; Dong Hee Lee and Chin Bum Lee, 2000; Feng-Zhaozhong et al. 2003; Borowski, 2009).

In order to protect plants against the effects of environmental stresses, biostimulators, among others, are used in Poland, in particular Asahi SL which is also called Atonik. It contains natural substances

found in plants, such as 5-nitroguaiacolate as well as ortho- and para-nitrophenolate. The application of Asahi SL in plants increases photosynthetic pigment content (Mikos-Bielak and Michałek, 1999; Gawrońska et al. 2008; Borowski, 2009; Borowski and Blamowski, 2009) and maximum quantum yield of PS II (Fv/Fm) (Borowski and Blamowski, 2009), it stimulates leaf gas exchange (Gawrońska et al. 2008; Wróbel and Woźniak, 2008; Borowski, 2009; Borowski and Blamowski, 2009) and enhances the activity of antioxidant enzymes (Djanaguiraman et al. 2005; Gawrońska et al. 2008; Borowski, 2009).

Earlier studies on the application of triacontanol and Asahi SL in cucumber plants treated with short-term chilling stress showed that foliar-applied Asahi SL was the most effective at the highest concentration of 0.3% (Borowski, 2009). The present study determined the effect of chilling stress on cucumber plants sprayed with this biostimulator at a concentration of 0.3% and 0.5% as well as on plants treated with root-applied Asahi SL and on those in which the biostimulator was applied during the seed swelling stage.

MATERIALS AND METHODS

The experiments were conducted in a phytotron of the University of Life Sciences in Lublin in the period 12 May – 3 June and 9 June – 2 July 2009. Seeds of cucumber cv. 'Śremski F1' germinated in dark on 2 layers of filter paper in Petri dishes, in distilled water or in 0.01% and 0.05% Asahi SL solution. After two days, when sprouts emerged, they 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 two plants per pot. Until the fourth true leaf stage, the plants grew in a room with an air temperature of 27/22ºC (day/night), relative humidity of approx. 60%, using fluorescent light with FAR flux density of 220 $\mu mol \times m^{-2} \times s^{-1}$ and with a photoperiod of 16/8 (day/night). The moisture content of the growing medium in the pots was maintained at a level of 70% of FWC (field water capacity), by using "weight-based" watering. At the initial phase of the fourth leaf stage, the plants were divided into 7 experimental series (12 pots in each) differing in the method of application of Asahi SL and its concentration: 1) control H_2O (watering and spraying); 2) germination – 0.01%; 3) germination – 0.05%; 4) watering – 0.01%; 5) watering – 0.05%; 6) spraying – 0.3%; 7) spraying – 0.5%. The biostimulator was administered twice to the growing medium, each time at an amount of 50 cm^3 of solution per pot, at an interval of 24 hours. Simultaneously with the second dose of root-applied Asahi SL, the plants of the respective series were sprayed with the biostimulator (one treatment), using approx. 5 cm^3 of solution per pot. After 24 hours, one half of the plants from each experimental series (6 pots) remained in the same conditions, whereas the other part was transferred to another phytotron and subjected to a temperature of 12/6ºC (day/night) with relative air humidity of approx. 95%.

Directly after the three-day period 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 by 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 ascorbate 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 sodium phosphate buffer (pH – 6.0 – 1% PVP-40 – 0.1M EDTA – 0.2 mM of sodium ascorbate) in the case of ascorbate peroxidase, at a temperature of 4ºC and centrifuged at 10 000 g for 15 min.

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 290 nm for ascorbate peroxidase (Nakano and Asana, 1981). The activity of the enzymes was expressed as $U \times g^{-1}$ FW, which means the decomposition of 1µmol of substrate $\times$ min^{-1} in optimal conditions. On the 3rd day of chilling stress duration, maximum fluorescence (Fm) and maximum quantum yield of chlorophyll (Fv/Fm) were also determined. The measurements were made using the Handy PEA fluorimeter (Hansatech) in accordance with the methodology presented in the paper by Schreiber et al. (1994). The abovementioned assays were performed in 4 replicates.

After collecting leaf samples and determining chlorophyll fluorescence, the chilling-treated plants were returned to the previous conditions of 27/22ºC in which, after 24-hour adaptation, measurements were made of leaf stomatal conductance as well as of transpiration and photosynthesis rates. Determinations were made in 10 replicates using a LCA-4 leaf microclimate control system. During recording, the temperature in the measurement chamber was approx. 30ºC, and FAR flux density 200 $\mu mol \times m^{-2} \times s^{-1}$. In the same leaves in which chlorophyll fluorescence (Fm, Fv/Fm) was determined earlier, measurements of gas

exchange were also made. 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.

RESULTS AND DISCUSSION

The results presented in Table 1 show that chilling stress increased leaf electrolyte leakage by 66.9% in the control plants. Irrespective of the application method, Asahi SL distinctly decreased the value of the trait in question. Its effect on the plants not subjected to chilling stress was similar and did not depend on the application method and concentration used. But in the plants subjected to chilling stress, the lowest electrolyte leakage was shown by the plants sprayed with the biostimulator (on average 29.8%), the plants watered twice with Asahi SL solution demonstrated higher electrolyte leakage (33.3%), whereas EL was the highest in the plants which had germinated in the solution of this biostimulator (38.6%). The reduction in electrolyte leakage from leaves under the influence of Asahi SL in chilling conditions was observed in earlier studies on cucumber by Borowski (2009), while in the case of basil by Borowski and Blamowski (2009). Chilling stress, which increased the value of EL relative to the plants not subjected to its effect by 63% irrespective of the method of application and concentration of Asahi SL, causes the degradation of membrane lipids and thereby strongly disturbs membrane integrity (De Kok and Kupier, 1977; Chen and Lin, 1993).

The action of chilling in plants very quickly activates in cells resistance and protection mechanisms counteracting its effects. One of them is increased proline synthesis. The results presented in Tab. 1 show that, irrespective of the method of application and concentration of Asahi SL, the chilling-treated plants contained in their leaves about 4.6 times more proline than the unchilled plants, which also finds confirmation in the papers of other authors (Ait-Barka and Audran, 1997; Hare and Cress, 1997; Borowski, 2009; Borowski and Blamowski, 2009). Irrespective of the administration method, the application of the biostimulator did not exert an effect on amino acid content in the plants not subjected to chilling stress. But in the chilling-treated plants, there was a reduction in proline content by ca. 30%, relative to the control, in the plants watered and sprayed with Asahi SL solution. But we found no effect of the germination of seeds in the solution of this biostimulator on the value of the trait in question (Table 1). This indicates that Asahi SL, root- or foliar-applied in cucumber plants, performs the role of a protective substance, likewise endogenous proline synthesized under these conditions.

Treatment of cucumber plants with short-term chilling stress also changed significantly the activity of the antioxidant enzymes, since the activity of catalase clearly decreased relative to the plants not subjected to stress (a 34.2% decline), while the activity of ascorbate peroxidase increased nearly 3 times. Other researchers also observed decreased activity of catalase and increased activity of guaiacol peroxidase or ascorbate peroxidase under chilling conditions (Graham and Patterson, 1982; El-Saht, 1988; Dong Hee Lee and Chin Bum Lee, 2000; Feng-Zhaozhong et al. 2003; Borowski, 2009). Seed application of Asahi SL, but also root or foliar application at assumed concentrations, did not show an effect on the activity of catalase and a small, but at the same time ambiguous, effect on the activity of peroxidase in cucumber leaves not subjected to chilling stress. But in the chilling-treated plants, seed, root and foliar application of Asahi SL increased the activity of catalase by an average of 5.7%, 25.2% and 55.0%, respectively, compared to the control. A similar correlation was also found with respect to ascorbate peroxidase; in this case, the average increase in the activity of this enzyme was, respectively – 6.6%, 83.2% and 95.7%. However, both enzymes in question, but in particular ascorbate peroxidase, demonstrated higher activity in the conditions of application of a more concentrated solution of Asahi SL (Table 2).

As shown in the present study, chilling stress also affected negatively photosynthetic pigment content in cucumber leaves; chlorophyll (a+b) content decreased by 14.2% relative to the plants not treated with chilling, while carotenoid content by 6.3%. A similar correlation was also observed by Haldimann (1998), Borowski (2009), Borowski and Blamowski (2009). The application of Asahi SL, irrespective of its method and solution concentration, significantly increased the content of both photosynthetic pigments in leaves of the plants not treated and treated with chilling stress. Chlorophyll content in the plants not exposed to chilling, but treated with the biostimulator by using seed, root or foliar application, increased on average by 10.4%, 8.4% and 12.9%, respectively, relative to the control, whereas these figures for the chilling-treated plants stand at 4.0%, 9.7% and 12.6%, respectively. The method of Asahi SL application and solution concentration had a similar effect on carotenoid content in leaves. In this case, the values for unchilled plants were 3.3%, 6.7% and 16.7%, respectively, while for chilled plants 0.0%, 3.5% and 10.3%. Generally, higher concentrations of the solutions applied had a slightly more beneficial effect on the values of the traits concerned. The beneficial influ-

Table 1
Effect of chilling stress on electrolyte leakage (EL) and proline content in cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		EL (%)			proline (μg × g^{-1} FW)		
Control	H_2O	26.0	43.4	34.7	11.7	76.8	44.2
Seed germination	0.01	21.5	40.7	31.1	13.5	74.6	44.0
	0.05	21.6	36.5	28.7	11.4	79.7	45.5
Watering of medium	0.01	21.7	34.3	28.0	19.2	54.0	36.6
	0.05	21.2	32.3	26.7	18.3	55.2	36.7
Spraying of leaves	0.3	20.1	29.1	24.6	12.2	55.8	34.0
	0.5	19.5	30.5	25.0	11.9	51.8	31.8
Mean for B		21.6	35.2		14.0	64.0	
LSD for A				6.2			7.5
LSD for B				2.9			3.1
LSD for AxB				n.s.			12.3

Table 2
Effect of chilling stress on the activity of catalase and ascorbate peroxidase in cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		catalase (U × g^{-1}FW)			peroxidase (U × g^{-1}FW)		
Control	H_2O	198.1	106.8	152.4	2.22	4.22	3.22
Seed germination	0.01	196.5	112.2	154.3	2.05	2.52	2.28
	0.05	201.3	113.7	157.5	1.42	5.37	3.39
Watering of medium	0.01	201.2	131.0	166.1	2.43	5.22	3.82
	0.05	218.8	136.4	177.6	1.57	10.24	5.90
Spraying of leaves	0.3	198.0	139.3	168.6	2.88	4.68	3.78
	0.5	201.3	191.7	196.5	2.48	11.85	7.16
Mean for B		202.2	133.0		2.15	6.30	
LSD for A				15.3			1.14
LSD for B				5.3 24.9			0.40
LSD for AxB							1.87

Table 3
Effect of chilling stress on chlorophyll a+b and carotenoid content in cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	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} FW)			carotenoids (mg × g^{-1} FW)		
Control	H_2O	2.01	1.75	1.88	0.30	0.29	0.29
Seed germination	0.01	2.23	1.80	2.01	0.32	0.29	0.30
	0.05	2.22	1.84	2.03	0.31	0.30	0.30
Watering of medium	0.01	2.11	1.94	2.02	0.32	0.30	0.31
	0.05	2.25	1.91	2.08	0.33	0.30	0.31
Spraying of leaves	0.3	2.24	1.86	2.05	0.35	0.30	0.32
	0.5	2.30	2.08	2.19	0.35	0.35	0.35
Mean for B		2.19	1.88		0.32	0.30	
LSD for A				0.13			0.03
LSD for B				0.04 0.21			0.01
LSD for AxB							0.04

Table 4
Effect of chilling stress on maximum fluorescence (Fm) and maximum efficiency of PS II photochemistry (Fv/Fm) in cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	Growth conditions (B)		Mean for A	Growth conditions (B)		Mean for A
		without chilling	with chilling		without chilling	with chilling	
		Fm			Fv/Fm		
Control	H_2O	1585.3	1540.0	1562.6	0.811	0.767	0.789
Seed germination	0.01	1630.0	1561.7	1595.8	0.810	0.774	0.792
	0.05	1651.0	1628.3	1639.6	0.812	0.781	0.796
Watering of medium	0.01	1601.7	1558.7	1580.2	0.805	0.771	0.788
	0.05	1648.0	1610.3	1629.1	0.796	0.791	0.793
Spraying of leaves	0.3	1628.3	1630.3	1629.3	0.813	0.775	0.794
	0.5	1695.0	1634.0	1664.5	0.803	0.789	0.796
Mean for B		1634.2	1594.7		0.807	0.778	
LSD for A				n.s.			n.s.
LSD for B				35.5 n.s.			0.025 n.s.
LSD for AxB							

Table 5
Effect of chilling stress on stomatal conductance and transpiration rate in cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	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	0.17	0.08	0.12	2.27	1.15	1.71
Seed germination	0.01	0.17	0.09	0.13	2.30	1.20	1.75
	0.05	0.18	0.09	0.13	2.40	1.24	1.82
Watering of medium	0.01	0.18	0.12	0.15	2.44	1.32	1.88
	0.05	0.20	0.14	0.17	2.52	1.44	1.98
Spraying of leaves	0.3	0.22	0.14	0.18	2.68	1.46	2.07
	0.5	0.22	0.15	0.18	2.63	1.58	2.10
Mean for B		0.19	0.11		2.46	1.34	
LSD for A				0.03			0.23
LSD for B				0.02			0.09
LSD for AxB				n.s.			n.s.

Table 6
Effect of chilling stress on photosynthetic rate and fresh weight of above-ground parts of cucumber plants treated with Asahi-SL by using different application methods

Asahi-SL (A) application method	Concentration of solution in %	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})			fresh weight (g/pot)		
Control	H_2O	5.35	3.16	4.25	9.26	5.61	7.43
Seed germination	0.01	5.43	3.27	4.35	9.20	6.10	7.65
	0.05	5.70	3.35	4.52	9.13	5.96	7.54
Watering of medium	0.01	5.87	3.98	4.92	10.03	6.96	8.49
	0.05	6.42	4.23	5.32	10.58	6.80	8.69
Spraying of leaves	0.3	7.15	4.35	5.75	11.41	7.43	9.42
	0.5	7.24	4.28	5.76	11.93	9.16	10.54
Mean for B		6.16	3.80		10.22	6.86	
LSD for A				0.57			1.05
LSD for B				0.23			0.37
LSD for AxB				n.s.			n.s.

ence of Asahi SL on photosynthetic pigment content in leaves is also confirmed by Mikos-Bielak and Michałek (1999) as well as Gawrońska et al. (2008), while under chilling stress conditions by Borowski (2009) as well as Borowski and Blamowski (2009).

The results presented in Tab. 4 indicate that chilling temperatures resulted not only in a decrease in the content of photosynthetic pigments, but they also significantly decreased their photosynthetic activity as determined by the measurement of maximum fluorescence (Fm) and maximum quantum yield of chlorophyll (Fv/Fm). The obtained data are confirmed in the papers by Huner et al. (1995), Misra et al. (2001), Borowski and Blamowski (2009). However, we found no significant effect of Asahi SL on the values of chlorophyll fluorescence, in spite of the fact that such effect had occurred in earlier studies conducted on basil plants (Borowski and Blamowski, 2009).

In the present experiments, chilling stress inhibited particularly strongly the process of gas exchange in cucumber leaves. This applied both to the process of transpiration and photosynthesis at a similar degree, and was undoubtedly related to the effect of this factor on leaf stomatal conductance. In the pre-stress period, chilling reduced stomatal conductance by 42.1%, transpiration by 45.5% and photosynthesis by 38.3% relative to the plants which had not been earlier subjected to stress. A similar response of plants to chilling conditions was also observed by many authors (Foyer et al. 1994a; Haldimann, 1998; Starck et al. 2000; Jun-Sungsoo et al. 2001; Borowski, 2009; Borowski and Blamowski, 2009). Irrespective of the application method and concentration, Asahi SL increased stomatal conductance in leaves and, undoubtedly through this, it also increased their transpiration and photosynthesis in the plants both subjected and not subjected to chilling stress. However, the influence of this biostimulator on each of the traits in the plants which were not under the conditions of chilling stress was distinctly smaller than in the plants exposed to its effect. Asahi SL applied in the form of foliar spraying increased gas exchange most effectively; in the second place, when applied to the growing medium, in particular at a concentration of 0.05%. But no significant effect of Asahi SL was found on gas exchange in cucumber plants when it was applied during seed germination (Tables 5 and 6). The stimulating effect of this biostimulator on plant gas exchange, which may result, as suggested by Gawrońska et al. (2008), from better water uptake in such conditions, is also confirmed by Wróbel and Woźniak (2008), Borowski (2009), Borowski and Blamowski (2009).

Fresh weight yields of the above-ground organs of plants are a kind of summary of the effect of short-term chilling as well as different application methods and concentrations of Asahi SL on plant growth. They indicate that chilling stress strongly reduces the accumulation of biomass in the above-ground parts of plants, which is also confirmed by the earlier studies of the present author (Borowski, 2009; Borowski and Blamowski, 2009). Asahi SL, applied in the pre-stress period by spraying or watering twice, significantly decreased the effects of chilling. It is difficult to assess the results relating to the effectiveness of different methods of application of this biostimulator due to the absence of appropriate comparative studies in this area; however, the paper by Przybysz et al. (2008) shows that the effects of foliar and root application of Asahi SL on *Arabidopsis thaliana* plants were similar.

CONCLUSIONS

1. The three-day period of treatment of young cucumber plants with a temperature of 12/6ºC (day/night) caused a significant increase in electrolyte leakage, free proline content and in the activity of ascorbate peroxidase in leaves, but a decrease in the content of chlorophyll (a+b), its maximum fluorescence (Fm) and quantum yield (Fv/Fm), carotenoid content, stomatal conductance, transpiration, photosynthesis, leaf biomass and in the activity of catalase in leaves.
2. The application of Asahi SL in the pre-stress period decreased the values of the parameters which increased as a result of chilling and increased those which decreased.
3. Foliar application of Asahi SL in the form of spraying and root application in the form of plant watering proved to be an effective method of application of this biostimulator. The best effect was obtained when the solutions of 0.5% and 0.05%, respectively, were used. The germination of seeds in Asahi SL solutions proved to be ineffective compared to foliar and root application of this biostimulator.
4. In the light of the present study results, foliar and root application of Asahi SL may be an efficient method of mitigating the negative effects of chilling stress in cucumber plants.

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Wpływ sposobu aplikacji i stężenia Asahi SL na reakcję roślin ogórka na chłód

Streszczenie

W doświadczeniach wazonowych prowadzonych na ogórku odm. Śremski F_1 badano wpływ okresowego chłodu na rośliny wyrosłe z nasion kiełkujących w roztworze Asahi SL lub traktowane tym biostymulatorem w okresie młodocianym. Rośliny rosły w fitotronie w temp. powietrza 27/22ºC (dzień/noc), korzystając ze światła fluorescencyjnego o gęstości strumienia FAR 220 µmol × m^{-2} × s^{-1}, przy fotoperiodzie 16/8. Biostymulator podano poprzez a) kiełkowanie nasion w roztworze 0.01% i 0.05%, b) dwukrotne podlanie roślin roztworem 0.01% lub 0.05%, c) oprysk liści roztworem 0.3% lub 0.5%. Kontrolę stanowiły rośliny opryskane wodą destylowaną. Po 24 godzinach od dolistnej lub dokorzeniowej aplikacji Asahi SL połowę roślin z każdej serii doświadczalnej traktowano przez okres 3 dni temp. 12/6ºC przy niezmienionych pozostałych warunkach wzrostu. Uzyskane wyniki wykazały, że okresowy chłód wywołał istotny wzrost stopnia wypływu elektrolitów, zawartości proliny i aktywności peroksydazy askorbinianowej w liściach, spadek zaś zawartości chlorofilu, jego fluorescencji maksymalnej (Fm) i wydajności kwantowej (Fv/Fm), zawartości karotenoidów, przewodności szparkowej, transpiracji, fotosyntezy, biomasy liści i aktywności w nich katalazy. Aplikacja roślinom w okresie przedstresowym Asahi SL w sposób dolistny lub dokorzeniowy obniżyła wartość tych cech, które w wyniku chłodu uległy podwyższeniu, a podwyższyła te które uległy obniżeniu. Bardziej skuteczne były wyższe stężenia zastosowanych tą drogą roztworów biostymulatora. Podanie biostymulatora w okresie kiełkowania nasion nie wywołało istotnych zmian w reakcji roślin na chłód.