

Effect of Temperature Drop and Photoperiod on Cold Resistance in Young Cucumber Plants – Involvement of Phytochrome B

Marina I. Sysoeva^{1*} • Grete G. Patil² • Elena G. Sherudilo³ • Sissel Torre⁴ •
Eugenia F. Markovskaya⁵ • Roar Moe³

¹ Institute of Biology, Karelian Research Centre of Russian Academy of Sciences, Pushkinskaya st. 11, Petrozavodsk, 185910, Russia

² Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, PO Box 5003, N-1432 Ås, Norway

Corresponding author: * sysoeva@krc.karelia.ru

ABSTRACT

The aim of this work was to study the importance of day length and the involvement of phytochrome B in plant responses to temperature drop. Experiments were conducted with the cucumber (*Cucumis sativus* L.) phytochrome B deficient mutant (*lh*) and the wild type (WT) under two photoperiods: short day (SD, 10 h at a PPF of 180 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) and long day (LD, 16 h, 115 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$). The following temperature treatments were used under both photoperiods for 6 days at the stage of first true leaf: constant temperature of 20°C (control), 12°C (long-term treatment) and temperature drop to 12°C for 2, 4, 6 or 8 h at the end of night and for 2 h at the beginning of the day under LD (drop treatments). Temperature drop was more efficient than a constant low temperature in increasing cold resistance under both photoperiods. LD increased the potential of the plants to develop a high cold resistance in response to a temperature drop. Maximal effect of temperature drop on the petiole length decreasing in the night was accompanied by the maximal cold resistance increment. The *lh* mutant showed the same cold resistance as the WT when grown at 20°C, but developed systematically lower cold resistance in response to a drop treatment in the night period than the WT. There was no effect of temperature drop duration in the night on cold resistance in *lh* mutant under both photoperiods but in WT under SD the cold resistance decreased with increasing duration during the night, while under LD it increased. The results suggest that phytochrome B is required for a maximum increase in cold resistance in cucumber under drop treatment.

Keywords: *Cucumis sativus* L., *lh* mutant, petiole length, wild type

Abbreviations: LD, long-day; SD, short day; WT, wild type

INTRODUCTION

Day and night temperature alternations have a strong influence on stem elongation in many plant species (Went 1944), and such physiological responses have been used for introducing temperature strategies for elongation control in commercially important greenhouse plants. A positive difference between the day (DT) and night temperature (NT) (positive DIF) promote internode elongation, while a higher NT than DT (negative DIF) restrict elongation (Erwin *et al.* 1989; Myster and Moe 1995). Further, a temporary drop in temperature for two hours also restricts elongation in several plant species (Myster and Moe 1995). Cucumber plants respond in the described pattern to positive and negative DIF as long as the NT is kept above 15–18°C (Grimstad and Frimanslund 1993), and they also respond strongly to a temperature drop (Grimstad 1993; Sysoeva *et al.* 1997, 1999).

Temperature drop and cold resistance

In countries in the Northern hemisphere the risk of cold damage in crops transplanted in early spring is high. The plants are commonly hardened to tolerate hardening temperatures by exposing them to constant low positive temperatures prior to transplanting outside. In addition to reducing stem extension, a temperature drop may increase cold resistance since a series of experiments has shown that young cucumber plants gain higher cold resistance by a 2-h temperature drop from 20°C to 12°C than by a traditional hardening temperature of 12°C and the cold resistance last for a significant longer time during a de-acclimation period at 20°C (Sysoeva *et al.* 1999; Markovskaya *et al.* 2000, 2003;

Sysoeva *et al.* 2005). This hypothesis is supported by recent studies of *Populus*, which have shown substantially increased cold hardiness in response to fluctuating day and night temperatures (Mølmann *et al.* 2005).

Temperature drop and phytochromes

Although the process of cold acclimation under constant low temperature has been, and still is, subject to numerous studies, knowledge of the physiological and molecular mechanisms underlying the responses of plants to temperature fluctuations is limited. It has been shown that a change in the ratio between red and far-red light, sensed by the pigment phytochrome, interacts with the elongation response to DIF (difference between day and night temperatures) (Moe *et al.* 1991). It is suggested that phytochrome status are important for the DIF response in such a way that it is eliminated under a high amount of far-red relative to red light. In particular, the light stable phytochrome B is important for a thermomorphogenic response to DIF in cucumber (Xiong *et al.* 2002; Patil *et al.* 2003). Junttila *et al.* (1998) suggest that phytochrome may influence frost tolerance through several mechanisms. Smith (1995) shows that phytochrome A is involved in perception of day extensions in long-day plants and phytochrome B has a role in the perception of short days. Overexpression of phytochrome A in hybrid aspen prevented short day induced cold acclimatization (Olsen *et al.* 1997), while development of cold tolerance in response to low temperature was not disturbed under long day conditions (Welling *et al.* 2002). Crosatti *et al.* (1999) suggested that phytochrome might be involved in the control of *cor14b* gene expression in barley. Kim *et al.* (2002) demonstrated that phytochrome B is a primary pho-

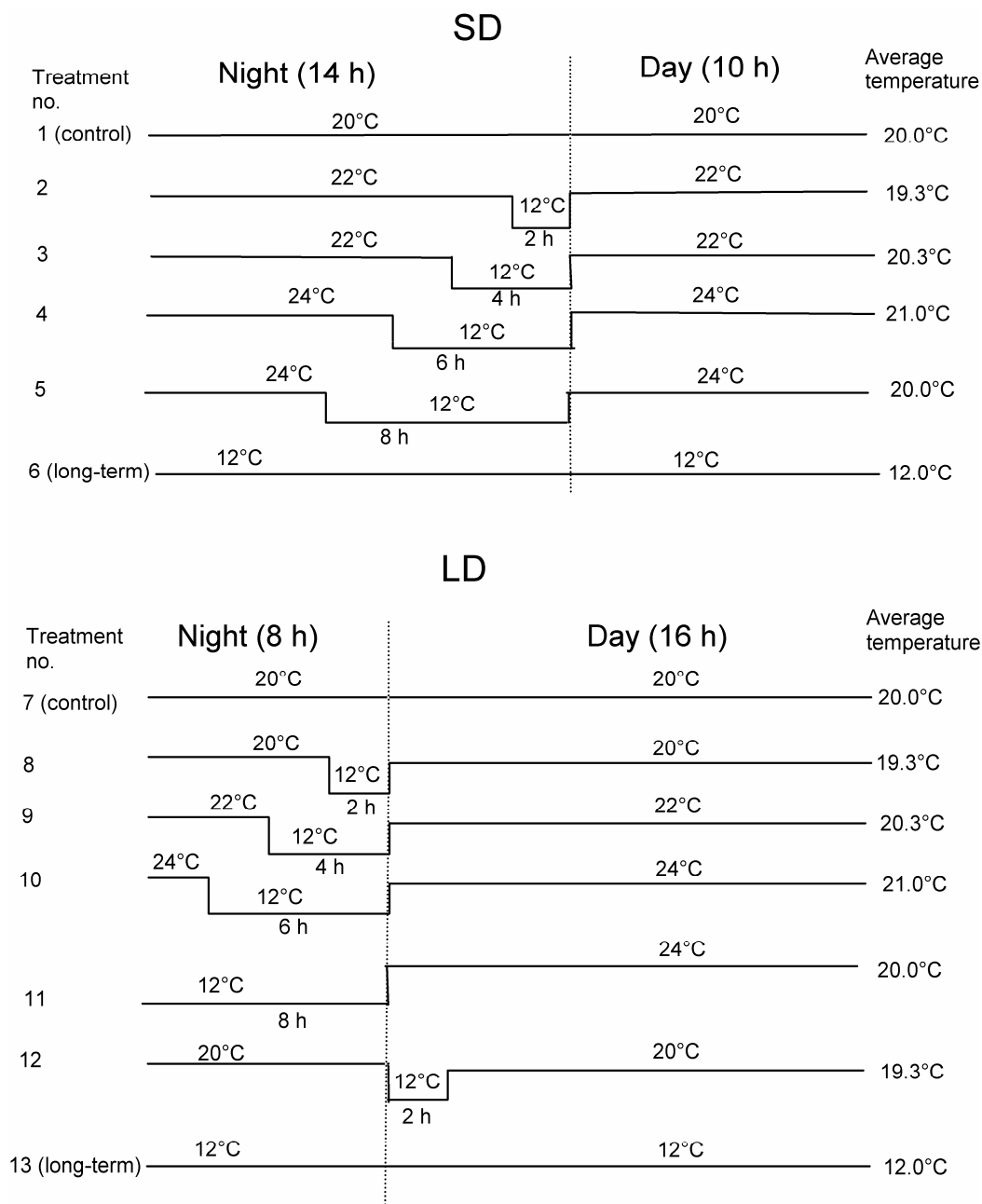


Fig. 1 Experimental set-up for the different treatments.

toreceptor responsible for activation of cold-stress signaling in response to light in *Arabidopsis*.

The aim of this work was to study the importance of day length and the involvement of phytochrome B in plant responses to temperature drop on the base of the comparison the effect of temperature drop on petiole length and cold resistance in young cucumber plants.

MATERIALS AND METHODS

Experiments were conducted in growth chambers with the cucumber (*Cucumis sativus* L.) phytochrome B deficient mutant (*lh*) (Lopez-Juez *et al.* 1992) and a near isogenic wild type line (WT) under two photoperiods with the same daily light integral ($6.5 \text{ mol} \cdot \text{day}^{-1} \cdot \text{m}^{-2}$): short day (SD, 10 h at a PPFD of $180 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$) and long day (LD, 16 h at a PPFD of $115 \mu\text{mol} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$). The *lh* mutant of cucumber is a photoreceptor mutant and contains <1% of the wild-type levels of at least one type 2 phytochrome, the *phyB*-like gene product (López-Juez *et al.* 1992).

Seeds were germinated on wet filter paper for 2 days, then planted in ceramic pots with damp sand and placed in a growth chamber. There was one plant per pot. The air temperature was maintained at $30 \pm 0.1^\circ\text{C}$ for germination and at $23 \pm 0.1^\circ\text{C}$ after emergence of seedlings until the end of cotyledon unfolding. At

the time of cotyledon expansion (4 days from sowing), the seedlings were selected for uniformity and the following temperature treatments were started under both photoperiods (Fig. 1): Constant temperature of 20°C (control), 12°C (long-term treatment) and temperature drop to 12°C for 2, 4, 6 or 8 h at the end of night period and for 2 h at the beginning of the light period under LD (drop treatments). The drop treatments were undertaken by using 4 chambers with 20, 22, 24 and 12°C at SD and LD photoperiods. At both photoperiods the temperature drop treatment was achieved by moving the plants from one growth chamber to another at a lower temperature. The same average daily temperature of $20 \pm 1^\circ\text{C}$ was maintained for all drop treatments by adjusting the temperature during the rest of the night and the photoperiod (Fig. 1). Plants were watered daily with a complete Knop nutrient solution (based on $1 \text{ g l}^{-1} \text{ Ca}(\text{NO}_3)_2$, $0.25 \text{ g l}^{-1} \text{ KH}_2\text{PO}_4$, $0.25 \text{ g l}^{-1} \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$, $0.25 \text{ g l}^{-1} \text{ KNO}_3$, trace quantity of FeSO_4 and pH 6.2-6.4).

All temperature treatments lasted for 6 days. At the end of the experiment plants had fully unfolded their first true leaf under the drop and control treatments and fully unfolded cotyledons under the long-term treatments. First true leaf petiole length and plant cold resistance were recorded at the end of the experiment.

Cold resistance of the plant was estimated by express freezing test (Drozdov *et al.* 1976). Leaf discs with ice crystals were placed into the micro refrigerator for 5 min at temperatures from -15 to $-$

10°C. The temperature that causes cytoplasm coagulation in 50% of the palisade parenchyma cells in leaf discs after short-term freezing was determined. The absolute value of the difference between temperatures that cause death in treated and control plant cells was accepted as an increment in plant cold resistance. Positive correlation (correlation coefficient 0.7-0.9) between the results obtained on the basis of estimation of cold resistance by freezing leaf discs and on the basis of the survival of seedlings after exposure of the whole plant at chilling injury temperatures has been suggested in previous study (Drozdov *et al.* 1980).

The experiment was run twice and means for the 10 plants with the same treatment within each run were subjected to analysis of variance (ANOVA) with the statistical package Statgraphics 7.0. T-test for least significant difference was used to determine whether differences between treatment means at the $P < 0.05$ level were significant.

RESULTS

Cold resistance

There was no difference in cold resistance at 20°C under SD and LD (control treatments) for any of the genotypes or between the WT (-10.3°C) and the *lh* mutant (-10.2°C). All temperature drop treatments and constant low temperature caused an increase in plant cold resistance in both genotypes and under both photoperiods (Fig. 2). In general for all drop treatments, the increase in resistance was higher than under a constant low temperature, higher in the WT than the *lh* mutant (except for response to drop during the photoperiod), and higher in LD than SD conditions. Under SD, the highest increment in cold resistance was observed in WT exposed to a 2 h temperature drop at the end of night

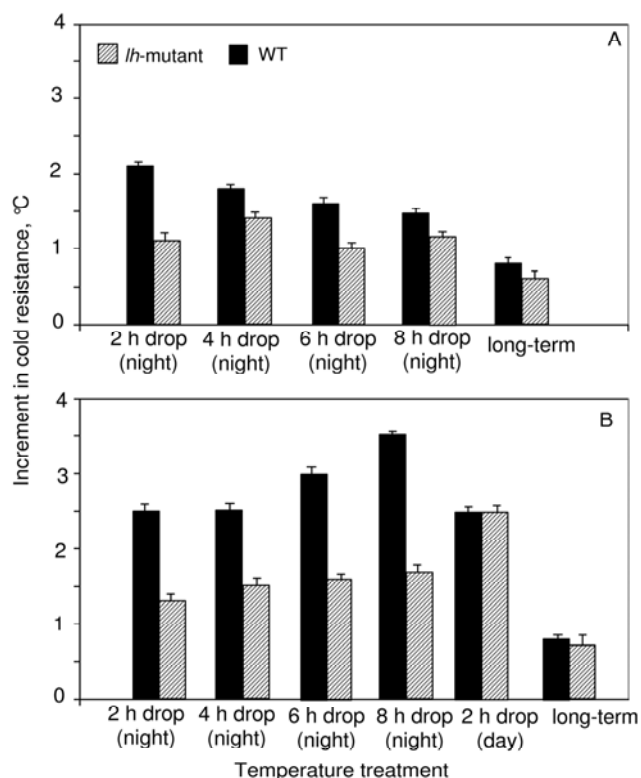


Fig. 2 Effect of constant low temperature of 12°C (long-term treatment) and temperature drop (to 12°C) of different durations at the end of night for a period of 6 days on cold resistance increment in young cucumber plants (WT and *lh* mutant) grown under short-day (A) and long-day photoperiod (B). Under long-day conditions a 2 h drop treatment at the beginning of the photoperiod was included. Increment in cold resistance is the absolute value of the difference between temperatures that cause death in treated and control plant cells. Control value of cold resistance was -10.2°C and -10.3°C for the *lh* mutant and the wild type, respectively under both SD and LD conditions. Error bars are standard deviation of the mean.

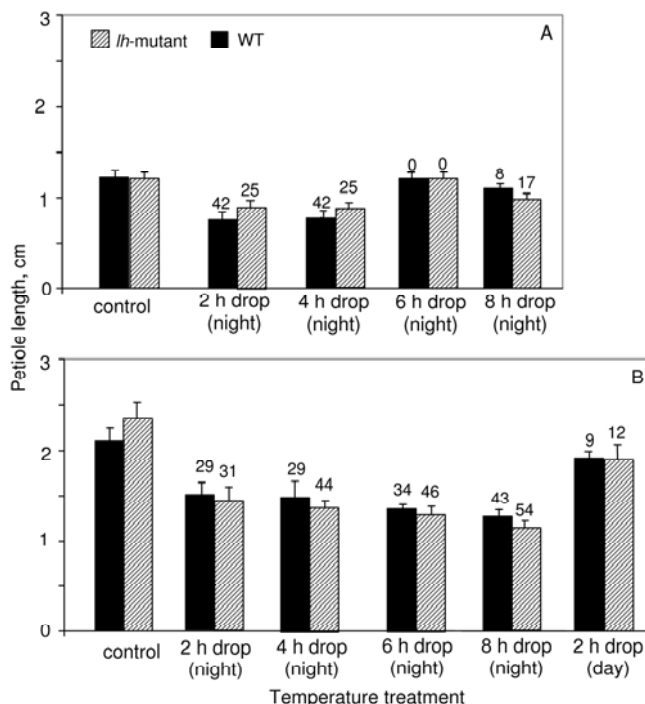


Fig. 3 Effect of temperature drop (to 12°C) of different durations at the end of night for a period of 6 days on first true leaf petiole length in young cucumber plants (WT and *lh* mutant) grown under short-day (A) and long-day photoperiod (B). Under long-day conditions a 2 h drop treatment at the beginning of the photoperiod was included. RI = response index: leaf petiole length under drop treatment / control $\times 100$ is given at the top of the bars. Error bars are standard deviation of the mean.

(2.1°C) but temperature drop duration did not significantly affect cold resistance increment in *lh* mutant. Cold resistance in WT grown under LD increased with increasing of temperature drop duration and maximum increment in cold resistance was observed under an 8 h temperature drop at the end of night (3.5°C). Duration of temperature drop in the night period under LD only slightly affects cold resistance increment in *lh* mutant and the maximum was observed under a 2 h temperature drop at the beginning of the light period (2.5°C). The long-term low temperature treatments caused similar cold resistance increment in the WT and *lh* mutant and under both photoperiods (0.8°C in WT and 0.6-0.7°C in *lh* mutant).

Leaf petiole length

Under SD conditions, a 2 or 4 h temperature drop treatment terminated at the end of the night period significantly reduced first true leaf petiole length in the WT cucumber plants and the *lh* mutant (by 42 and 25%, respectively) compared to constant 20°C temperature (control treatments) (Fig. 3A). There was no effect of a 6 h and 8 h temperature drop treatments (Fig. 3A) compared to control treatments.

Under LD all temperature drop treatments terminated at the end of the night reduced the petiole length in the WT and *lh* mutant (29-43 and 31-54% depending on the drop duration, respectively): the longer was the temperature drop duration, the more the reducing in petiole length (Fig. 3B). A 2 h temperature drop at the beginning of the light period had not significant effect on the leaf petiole length in the WT and *lh* mutant compared to the control (Fig. 3B). Under the LD there were no significant differences between WT and *lh* mutant in response to a temperature drop.

Plants exposed to the constant low temperature (12°C) were significantly delayed in development and the petiole length was not measured.

DISCUSSION

Our experiments indicate that 6 days with temperature drop increases cold resistance up to 3.5°C in young cucumber plants depending on the treatments (Fig. 2). A continuous 24-h low temperature treatment is significantly less efficient than any of the drop treatments applied and indicates that alternating temperature can be used to induce more high level of cold hardiness. This is in agreement with earlier reports in cucumber cv. 'Alma Atinsky 1' (Sysoeva *et al.* 1999; Markovskaya *et al.* 2003) and in wheat, cabbage and potato (Sysoeva *et al.* 2005). We found that photoperiod interacted with the efficiency of a drop treatment to induce cold resistance. Cold resistance increment in drop treatments was higher under LD than under SD, especially in WT, indicating that LD increases the potential of the plants to develop a high cold resistance in response to a temperature drop. Comparison of the effect of drop duration increasing under two different day lengths shows that there was no effect of temperature drop duration in night period on cold resistance increment in *lh* mutant under the both photoperiods but in WT under SD the cold resistance decreased with increasing duration during the night period, while under LD it increased. Opposite trend was found for the petiole length under LD. It was a typical quantitative response (Ihlebekk *et al.* 1995) to temperature drop duration: an increase in photoperiod requires an increase in temperature drop duration for an effect on petiole length. The results showed that under both photoperiods maximal thermomorphogenic effect in the night period was accompanied by the maximal cold resistance increment.

Temperature drop is often applied not only late in the night period but also in the morning to control stem elongation (Myster and Moe 1995), in particular in SD plants, since it is energy efficient when using the heat from the sun, and possibly from artificial lighting, for increasing the temperature after the drop treatment. Our results also indicate that a drop in the early morning is equally good in increasing cold resistance, but there is no effect on petiole length. This correspond with the results of Grimstad (1995) who found smaller effect of 2-hour temperature drop at the beginning of day on petiole length in comparison with a 2-hour drop application at time of change-over and at the end of night.

Rapid development in phytochrome research has opened new possibilities for studies on the physiological basis for photo- and thermoperiodic control of growth and cold acclimation. It has been found earlier that phytochrome B is needed for DIF to express itself in cucumbers indicating that phytochrome B is either directly involved in mediating the response or the extensive elongation hide any possibility to respond to this change in thermal environment (Xiong *et al.* 2002; Patil *et al.* 2003). We found no significant differences in phytochrome B deficient mutant and WT petiole length responses to temperature drop after 6 days exposure to drop treatment (Fig. 3). Further studies with a longer exposures period are needed to check the involvement of phytochrome B in the elongation response to a drop treatment in cucumber. But cold resistance increment was strongly higher in WT than in *lh* mutant and pilot experiments with cucumber plant have shown that with a prolonged drop treatment cold resistance increment was the same up to final unfolding of the first petiole (pers. comm. Markovskaya 2006) and was not depended on the time applied during the night period (Sysoeva *et al.* 1999). An interesting exception was no deviation in cold resistance increment in *lh* mutant from the WT when applying a drop in light under LD conditions. Light was shown to be required for enhanced cold tolerance induced by cold temperature in a variety of plants (Levitt 1980; Wanner and Junttila 1999). Elevated cold resistance of plants subjected to drop treatment was assumed to depend on photoassimilates (Markovskaya *et al.* 2007). Also carbohydrates alter different phytochrome-mediated responses that indicate a strong interaction between light- and sugar-signaling path-

ways (Short 1999). High level of cold resistance in *lh* mutant under light condition could be explained by involving of phy A-signaling mechanism modulated carbohydrate state of the cell.

Finally, a temperature drop induced higher cold resistance in WT than in *lh* mutant but under constant low temperature (long-term treatments) there was no differences in cold resistance increment between these genotypes (Fig. 2). This may indicate that different mechanisms are involved in the increment of plant resistance to long-term exposure to low temperature and temperature drop (Markovskaya *et al.* 2003). In particular, it seems that phytochrome B is involved in cold resistance formation under the drop but not under the constant low-temperature treatments.

CONCLUSION

In conclusion, a temperature drop in darkness or in light enhances cold resistance in both WT and *lh* mutant of young cucumber plants. The presence of functional phytochrome B in WT seems to be a prerequisite for complete development of cold hardiness in darkness, but other unknown growth factors are involved in the process.

ACKNOWLEDGEMENTS

The experimental work was financially supported by the Russian Foundation for Basic Research (project no 07-04-00063).

REFERENCES

* In Russian

- Crosatti C, Polverino de Laureto P, Bassi Rand Cattivelli L (1999) The interaction between cold and light controls the expression of the cold-regulated barley gene *cor14b* and the accumulation of the corresponding protein. *Plant Physiology* **119**, 671-680
- Drozdov SN, Kuretz VK, Budykina NP, Balagurova NI (1976) Determination of plant resistance to the frost. In: Udovenko GV (Ed) *Methods of Estimation of Resistance to Unfavorable Environmental Conditions*, Kolos, Leningrad, pp 222-229*
- Drozdov SN, Balagurova NI, Titov AF, Kritenko SP (1980) Methods for estimating of cold resistance in cucumber plant. *Russian Journal of Plant Physiology* **27**, 653-656
- Erwin JE, Heins RD, Karlsson MG (1989) Thermomorphogenesis in *Lilium longiflorum*. *American Journal of Botany* **76**, 47-52
- Grimstad SO (1993) The effect of a daily low temperature pulse on growth and development of greenhouse cucumber and tomato plants during propagation. *Scientia Horticulturae* **53**, 53-62
- Grimstad SO (1995) Low-temperature pulse affects growth and development of young cucumber and tomato plants. *Journal of Horticultural Science* **70**, 75-80
- Grimstad SO, Frimanslund E (1993) Effect of different day and night temperature regimes on greenhouse cucumber young plant production, flower bud formation and early yield. *Scientia Horticulturae* **53**, 191-204
- Ihlebekk H, Eilertsen S, Junttila O, Grindal G, Moe R (1995) Control of plant height in *Campanula isophylla* by temperature alternations; involvement of GAs. *Acta Horticulturae* **394**, 347-355
- Junttila O, Olsen JE, Nilsen J, Martinussen I, Moritz T, Eriksson M, Olsson O, Sandberg G (1998) Phytochrome overexpression and cold hardiness in transgenic *Populus*. In: Li PH, Chen THH (Eds) *Plant Cold Hardiness. Molecular Biology, Biochemistry and Physiology*, Plenum Press, New York, pp 245-255
- Kim H-J, Kim Y-K, Park J-Y, Kim J (2002) Light signaling mediated by phytochrome plays an important role in cold-induced gene expression through the C-repeat/dehydration responsive element (C/DRE) in *Arabidopsis thaliana*. *The Plant Journal* **29**, 693-704
- Levitt J (1980) *Responses of Plants to Environmental Stresses: Chilling, Freezing and High Temperature Stresses*, Academic Press, New York, 497 pp
- López-Juez E, Nagatani A, Tomizawa K-I, Deak M, Kern R, Kendrik RE, Furuya M (1992) The cucumber long hypocotyle mutant lack a light-stable PHYB-like phytochrome. *The Plant Cell* **4**, 241-251
- Markovskaya EF, Sysoeva MI, Kharkina TG, Sherudilo EG (2000) Influence of a night temperature drop on the growth and cold tolerance of cucumber plants. *Russian Journal of Plant Physiology* **47**, 445-448
- Markovskaya EF, Sherudilo EG, Sysoeva MI (2003) Influence of long-term and short-term temperature drops on acclimation and de-acclimation in cucumber cold resistance. *Acta Horticulturae* **618**, 233-236
- Markovskaya EF, Sysoeva MI, Sherudilo EG, Topchieva LV (2007) Differential gene expression in cucumber plants in response to brief daily cold

- treatments. *Russian Journal of Plant Physiology* **54**, 686-691
- Moe R, Heins RD, Erwin J** (1991) Stem elongation and flowering of the long-day plant *Campanula isophylla* Moretti in response to day and night temperature alternations and light quality. *Scientia Horticulturae* **48**, 141-151
- Myster J, Moe R** (1995) Effect of diurnal temperature alternations on plant morphology of some greenhouse plants. A mini review. *Scientia Horticulturae* **62**, 205-215
- Mölmann JA, Asante DK, Beck Jensen J, Krane MN, Ernstsen A, Junttila O, Olsen JE** (2005) Low night temperature and inhibition of gibberellin biosynthesis overrides phytochrome action and induce budset and cold acclimation, but not dormancy in PHYA overexpressors and wild/type of hybrid aspen. *Plant, Cell and Environment* **28**, 1579-1588
- Olsen JE, Junttila O, Nilsen J, Eriksson M, Martinussen I, Olsson O, Sandberg G, Moritz T** (1997) Ectopic expression of phytochrome A in hybrid aspen changes critical day length for growth and prevents cold acclimation. *The Plant Journal* **12**, 1339-1350
- Patil G, Alm V, Junttila O, Moe R** (2003) Interaction between phytochrome B and gibberellins in thermoperiodic responses of cucumber. *Journal of the American Society for Horticultural Science* **128**, 642-647
- Short TM** (1999) Overexpression of Arabidopsis phytochrome B inhibits phytochrome A function in the presence of sucrose. *Plant Physiology* **119**, 1497-1505
- Smith H** (1995) Physiological and ecological function of the phytochrome family. *Annual Review of Plant Physiology and Plant Molecular Biology* **46**, 289-316
- Sysoeva MI, Markovskaya EF, Kharkina TG** (1997) Optimal temperature drop for the growth and development of young cucumber plants. *Plant Growth Regulation* **6**, 1-5
- Sysoeva MI, Markovskaya EF, Kharkina TG, Sherudilo EG** (1999) Temperature drop, dry matter accumulation and cold resistance of young cucumber plants. *Plant Growth Regulation* **28**, 89-94
- Sysoeva MI, Sherudilo EG, Markovskaya EF, Obshatko LA, Matveyeva EM** (2005) Temperature drop as a tool for cold tolerance increment in plants. *Plant Growth Regulation* **46**, 189-191
- Welling A, Moritz T, Palva TE, Junttila O** (2002) Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. *Plant Physiology* **129**, 1633-1641
- Wanner LA, Junttila O** (1999) Cold-induced freezing tolerance in Arabidopsis. *Plant Physiology* **120**, 391-399
- Went FW** (1944) Plant growth under controlled conditions. II. Thermoperiodicity in growth and fruiting of the tomato. *American Journal of Botany* **31**, 135-150
- Xiong J, Patil G, Moe R** (2002) Effect of DIF and end-of-day light quality on stem elongation in *Cucumis sativus*. *Scientia Horticulturae* **94**, 219-229