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# SENSITIVITY OF 'MONIKA' *CUCUMIS SATIVUS* SEEDLINGS TO LOW TEMPERATURE AND INDUCTION OF CHILLING TOLERANCE

## ABSTRACT

The aim of the research was to investigate the chilling sensitivity of cucumber seedlings and to alleviate its negative effects by short-term shock temperature applied during seeds imbibition, before radicle protrusion. The chilling sensitivity was investigated depending on initial root length (1, 3, 5 and 7 mm) and duration of chilling exposure (0, 1, 3, 7, 14, 21 days) at 0˚C and was evaluated by measurements of roots and hypocotyls growth, electrolyte leakage and dehydrogenases activity. To assess whether short-term shock temperature applied before radicle protrusion can increase chilling tolerance in cucumber seedlings the seeds were imbibed at 25°C for 16 h, exposed to temperatures of 0; 2,5; 5; 35; 40, 45°C for 0; 0,5; 1; 2; 4 h.

The obtained results indicated that cucumber seedlings sensitivity depended on duration of chilling exposure and initial root length during which the seedlings were subjected to these conditions. Chilling sensitivity were manifested by drop in the roots and hypocotyls elongation, reduction in dehydrogenases activity and membrane integrity expressed by electrolyte leakage. Seedlings with 1 mm roots was relatively tolerant to 3 days of chilling at 0°C. Their chilling sensitivity increased as roots elongated. Seedling with 3, 5, 7 mm of roots subjected to chilling suffered a subsequent 71, 75 and 81% of growth inhibition, respectively.

The presented results showed that a short-term of low (0; 2,5 or 5°C) or high (35; 40 or 45°C) temperature treatments applied before radicle protrusion i.e. after 16 h of seeds imbibition significantly counteracted the negative effects of chilling on seedlings. The best results expressed by increased length and the number of roots were obtained when imbibed seeds were exposed to 45°C for 2 and 4 h. The increased chilling tolerance of cucumber seedlings induced as early as at the stage of seed imbibition, before radicle protrusion indicates on the possibility of application of this method in practice. The positive response of such treatment on cucumber chilling tolerance are discussed.

Key words: chilling sensitivity, cucumber, thermal shock, temperature stress, chilling resistance

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## INTRODUCTION

Many plants in Poland are exposed to adverse environmental factors, such as spring frosts and prolonged cold weather. They cause inhibition of plant growth, yellowing of leaves, increasing prevalence of diseases, which in turn leads to death of plant, and in the best case, reduce very often inferior quality of the yield. It concerns particularly the cucumber (*Cucumis sativus* L.), which due to its origin belongs to the plants with high temperature requirements (Saltveit 1991).

Too low temperature during seed germination and seedling emergence interfere with the course of a number of physiological processes occurring at the tissue and cellular level. These include a reduction in seed germination and vigor, slow growth of seedlings and their dehydration, necrosis and discoloration, greater sensitivity of plants to disease. The reaction of sensitive embryonic roots to chilling is inhibition of their growth, damages the root tips and ultimately their decay. The end result of the harmful effects of the chilling are disturbances in the maturation of plants (Stark *et al.* 1993; Kopcewicz and Lewak 2002).

The adverse chilling effect observed at the cellular level revealed a loss of cytoplasm liquidity, a decrease in membrane integrity, changes in enzyme activity, accumulation of harmful compounds, increased release of carbon dioxide and ethylene and the reduction of photosynthesis. These symptoms increase with decreasing temperature and lengthening the time of its impact (Saltveit 1991; Saltveit 2001; Rab and Saltveit 1996; Mangrich and Saltveit 2000; Collins *at al.*1993; Jennings and Saltveit 1994; Kang and Saltveit 2001; Kang and Saltveit 2002). The extent of chilling injury is dependent on the tissue's susceptibility (which varies among tissues and during development), the duration of exposure, and the temperature. Prolonged exposure of meristematic tissue to lower temperatures usually produces the most severe injury.

Some approaches have been tried to reduce chilling injury of growing plants by exposure of seedlings to shock temperature (i.e. heat or cold shock) (Collins *et al.* 1993; Mangrich and Saltveit 2000; Kang and Saltveit 2001; Saltveit 2001). Currently, little attention is paid to the shock temperature applied during seeds imbibition and its subsequent effect on seedlings and plant resistance to chilling. It would be very useful if the positive effects of this method could be applied during seed imbibition, before the radicles protrude the seed coat.

The aim of the present study was to investigate the sensitivity of cucumber seedlings to chilling conditions by roots and cotyledons growth after chilling, dehydrogenases activity and electrolyte leakage and to evaluate the ability to improve plant tolerance to negative chilling effects by short-term shock temperature treatments applied during seed imbibition.

## MATERIAL AND METHODS

'Monika' commercial cucumber seeds (*Cucumis sativus* L.) were obtained from TORSEED S.A. – Garden Seed and Nursery Stock Company in Toruń. Cucumber seed lots were kept at 30% RH at 25°C until start of experiments and were considered as control seeds in the experiments.

The seeds were sown in 9,0 cm-diameter Petri dishes on cotton wool moistened with distilled water at 25°C at allowed to germinate and grow for various lengths of time to produce roots that were 1, 3, 5 and 7 mm long. The seedlings were chilled for 0, 1, 3, 7, 14 and 21 days at 0˘C and then allowed to grow for 3 days at 25°C in 16/8 photoperiod.

The extent of seedlings chilling sensitivity was investigated depending on initial roots length of seeds exposed to chilling (0°C) and duration of its negative impact. The chilling susceptibility was evaluated by measurements of roots and hypocotyls lengths, electrolyte leakage and dyhedrogenases activity.

To determine whether short-term shock temperature applied before radicle protrusion can induce chilling tolerance in cucumber seedlings, the seeds were sown in 9,0 cm-diameter Petri dishes on cotton wool moistened with distilled water at 25°C for 16 h and exposed to temperatures of 0; 2,5; 5; 35; 40 and 45°C for 0; 0,5; 1; 2 and 4 h (short-term shock temperatures treatments) and then returned to 25°C. When roots reached 3 mm in length the seedlings were transferred to Phytotoxkit Microbiotest and chilled at 0° C for 3 days (Persoone and Vangheluwe, 2000). After chilling the seedlings were subjected to growth of roots and hypocotyls, electrolyte leakage and dyhedrogenase activity evaluation.

Length of roots and hypocotyls was determined after seedlings were returned to 25°C for 72 h. The length was measured with a clear, transparent plastic ruler to the nearest mm.

Twenty five sunflower roots, in four replications were soaked in 3 ml of distilled water and incubated at 20°C for 4 h. The total conductivity of the solution was expressed as the percentages of the total leakage from seeds boiled for 10 min in water. Results correspond to the means of 4 measurements. A microcomputer conductivity meter CC-551 (ELMETRON, Poland) was used to measure the electrical conductivities of the leaches of roots.

For determination of dehydrogenase activity 0.2 g of root tips were placed in Eppendorf tubes, ground and incubated in 1 ml of 0.1 M sodium phosphate buffer, pH 7.2 containing 0.7% (w/v) of 2,3,5-triphenyl tetrazolium chloride at 25°C for 24 h. After that time samples were centrifuged (5 min; 5,000 x g) and the pellet was extracted six times with 1 ml of acetone. The solution absorbance was measured at 488 nm. A standard curve was prepared from known concentration of formazan. Each determination of root viability was made four times.

Each experiments was done twice. The experiment was designed in four replications, each comprising ten seedlings. The differences between the means were estimated by the Duncan multiple range test at a significance level of P = 0.05. Standard errors were calculated for figures presentation.

## RESULTS AND DISCUSSION

Table 1

**The effect of chilling on roots growth [mm]. Roots at different initial lengths were chilled at 0°C for 3 days and then incubated at 25°C for 72h**

| Chilling duration [days] | Initial length roots subjected to chilling [mm] | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| | Final roots length after 72 h at 25°C [mm] | | | |
| 0 | 37.9 d | 45.7 d | 47.7 d | 53 d |
| 1 | 36.7 d | 33.5 c | 32.1 c | 28.9 c |
| 3 | 25.6 c | 13.2 b | 11.7 b | 9.9 b |
| 7 | 9.6 b | 3.8 a | 6 a | 7 a |
| 14 | 2.6 a | 3.1 a | 5.4 a | 7 a |
| 21 | 1.3 a | 3 a | 5 a | 7 a |

Means within columns with the same letter are not significantly different at p = 0.05 according to the Duncan multiple range test

Chilling injury has been defined as damage sustained by plant tissue subjected to nonfreezing temperatures below 12°C (Lyons 1973). Symptoms of chilling injury include stunted growth, internal and external discoloration, development of necrotic areas and abnormal ripening of fruits (Khan *et al.* 1992). In the present study internal and external discoloration was the first signal of influence of unfavorable temperature for root tissue development but is not harmful for cucumber seedlings emergence and further growth of roots. Chilling sensitivity was evaluated by using four different methods: growth of roots and hypocotyls as well as electrolyte leakage and dehydrogenases activity. In performed experiment the chilling sensitivity of germinating seeds was minimal when the roots was 1 mm in length (Table 1). At this stage, 3 days of chilling at 0°C reduced growth of radicle by only 12,3 mm during subsequent incubation at 25°C for 3 days which is 6,1% inhibition of root growth in comparison to control (non-chilled seedlings). However, seedling with 3, 5, 7 mm roots in length subjected to the same period of chilling suffered a subsequent 71, 75 and 81% of inhibition of growth, respectively. Apparently, during growth of roots they changed from relatively tolerant to most sensitive and become more chilling sensitive as they elongated. Longer time of chilling that 3 days resulted in almost total inhibition of chilling. The obtained results indicated that cucumber root growth after chilling is a sensitive indicator of chilling stress. Similarly this parameter was frequently used for evaluation of chilling injuries severity in African violet (Saltveit and Hepler 2004), rice (Kato-Noguchi 2007; Saltveit 2002; Mangrich and Saltveit 2000) cotton, kenaf and okra (Mangrich and Saltveit 2000).

Table 2

**The effect of chilling on hypocotyls growth [mm]. Roots at different initial lengths were chilled at 0°C for 3 days and then incubated at 25°C for 72h**

| Chilling duration [days] | Initial length roots subjected to chilling [mm] | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| | Final hypocotyls length after 72 h at 25°C [mm] | | | |
| 0 | 5.8 b | 6.9 b | 7.6 b | 8.9 b |
| 1 | 5.3 b | 6.5 b | 7.2 b | 8.4 b |
| 3 | 2.4 a | 2.6 a | 3 a | 3.6 a |
| 7 | - | - | - | - |
| 14 | - | - | - | - |
| 21 | - | - | - | - |

Means within columns with the same letter are not significantly different at p = 0.05 according to the Duncan multiple range test

Similar response in relation to the roots elongation after chilling treatment was manifested in the case of hypocotyls growth (Table 2). Seedlings with 3, 5, 7 mm roots subjected to longer chilling conditions than 3 days prevented successive hypocotyls growth. Apparently, deleterious effects of chilling conditions was even more visible for hypocotyls than for roots.

Table 3

**The effect of chilling on electrolyte leakage of cucumber roots. Roots at different initial lengths were chilled at 0°C for 3 days and then incubated at 25°C for 72h**

| Chilling duration [days] | Initial length roots subjected to chilling [mm] | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| | Electrolyte leakage [% total electrolytes] | | | |
| 0 | 1.9 a | 2.0 a | 2.4 a | 2.4 a |
| 1 | 3.5 a | 4.3 a | 5.1 a | 5.0 a |
| 3 | 13.6 b | 35.6 b | 36.2 b | 37.9 b |
| 7 | 15.9 bc | 36.2 b | 37.2 bc | 38.2 b |
| 14 | 20 c | 40.3 c | 39.3 c | 42.3 c |
| 21 | 20.2 c | 40.5 c | 39.5 c | 42.5 c |

Means within columns with the same letter are not significantly different at p = 0.05 according to the Duncan multiple range test

Chilling negatively affected membranes integrity expressed by electrolyte leakage to the medium (Table 3). With longer period of chilling and higher initial length of roots subjected to these conditions increased electrolyte leakage to the medium. Similarly Saltveit (1989) reported that the rate of iron leakage from tomato pericarp discs was highly correlated with the degree of chilling injury. A significant increase of electrolyte leakage compared to control was visible after 3 days of chilling at 0°C (Table 3). The same pattern of chilling response was noted in the case of roots growth after chilling suggesting that electrolyte leakage to the medium are highly sensitive method of chilling injuries in cucumber seedlings. Posmyk *et al.* (2001) also demonstrated that this method can be used for evaluation of soybean chilling sensitivity. In the present study the most severe injures of membranes was caused after 14 and 21 days of chilling. Apparently, leakage of solutes from chilled germinating seeds suggests that membrane reorganization is impaired at low temperatures (Bramlage *et al.* 1978).

Dehydrogenases activity of roots declined with duration of chilling period (Table 4). A significant drop in dehydrogenases activity was observed after 3 days at 0°C suggesting that low temperature inhibited activity of respiratory enzymes in chilled roots. Presumably, a decrease of the respiration rate due to exposure of root tips to 0°C impaired cucumber chilling tolerance. In Kasai *et al.* (1998) study, an increase of the respiration rate was suggested as an adaptive strategy for stress in *Triticum aestivum* seeds germinating under salinity. Dehydrogenase activity is caused by a wide group of endocellular enzymes, which are present in all living cells and are essential in catalyzing the biological oxidation of organic compounds. Activity of dehydrogenases (respiratory enzymes) is reported as an index of tissue respiration and metabolism by Kasai *et al.* 1998; Białecka and Kępczyński 2010; Farooq *et al.* 2010.

Table 4

**The effect of chilling on dehydrogenases activity of cucumber roots. Roots at different initial lengths were chilled at 0°C for 3 days and then incubated at 25°C for 72h**

| Chilling duration [days] | Initial length roots subjected to chilling [mm] | | | |
|---|---|---|---|---|
| | 1 | 3 | 5 | 7 |
| | Dehydrogenases activity [mg formazan × g roots$^{-1}$] | | | |
| 0 | 0.85 d | 0.93 d | 0.97 d | 1.08 d |
| 1 | 0.83 d | 0.89 d | 0.89 c | 0.81 c |
| 3 | 0.70 c | 0.68 c | 0.70 b | 0.65 b |
| 7 | 0.59 b | 0.62 b | 0.61 a | 0.60 a |
| 14 | 0.48 a | 0.53 a | 0.59 a | 0.58 a |
| 21 | 0.46 a | 0.53 a | 0.60 a | 0.61 a |

Means within columns with the same letter are not significantly different at p = 0.05 according to the Duncan multiple range test

For the next experiment seedlings with 3 mm roots were selected since at that length they exhibited a significant increase in chilling sensitivity expressed by roots and hypocotyls growth, electrolyte leakage and dehydrogenases activity.

A short-term (0; 0,5; 1; 2 or 4 h) of low (0; 2,5 or 5°C) or high (35; 40 or 45°C) temperature treatments applied after 16 h of seeds imbibition in distilled water, before radicle protrusion significantly counteracted the negative effects of chilling on seedlings (Fig. 1). The best results was observed when imbibed seeds were exposed to 45°C for 2 and 4 h. Such treatments stimulated growth of roots by approximately 250% compared with control plants. Apparently, plants develop the ability to withstand lethal temperatures upon exposure to sublethal temperatures (referred as induction stress) (Senthil-Kumar *et al.* 2008).

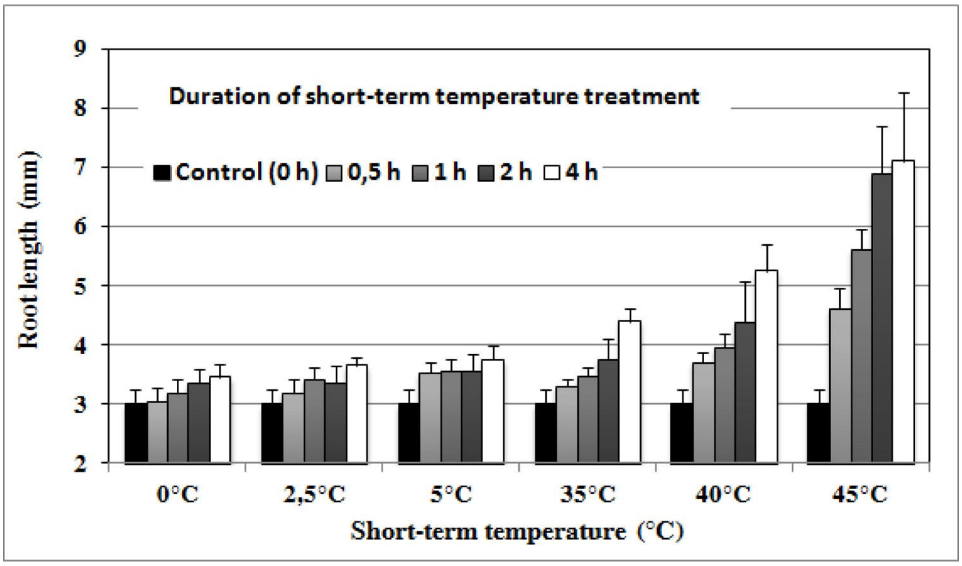


Fig. 1. Root growth of cucumber after seed were moistened in water for 16 h at 25°C, subjected to temperature of 0; 2,5; 5; 35; 40, 45°C for 0 - control; 0,5; 1; 2; 4 h at and returned to 25°C. Seedlings with 3 mm roots were chilled at 0°C for 3 days. Root length was determined after seedlings were returned from chilling condition to 25°C for 72 h. The vertical bar atop each bar represents the standard error

Similarly, short-term temperature treatments increased the number of roots (Fig. 2). Rab and Saltveit (1996) reported that the development of lateral roots decreased with prolonged chilling of corn, mung bean, tomato and okra. Presumably, in the present experiment short-term heat shock treatment reversed the negative effects of chilling. The obtained results showed that roots and cotyledons growth after chilling, electrolyte leakage as well as dehydrogenases activity are methods which can be widely used for evaluation of chilling sensitivity of cucumber seedlings. From presented data is also clearly visible that chilling tolerance of cucumber seedlings can be increased by short-term heat shock applied as early as at the stage of seed imbibition, before radicle protrusion. The positive response of such treatment indicates on the possibility of application of this method in practice.

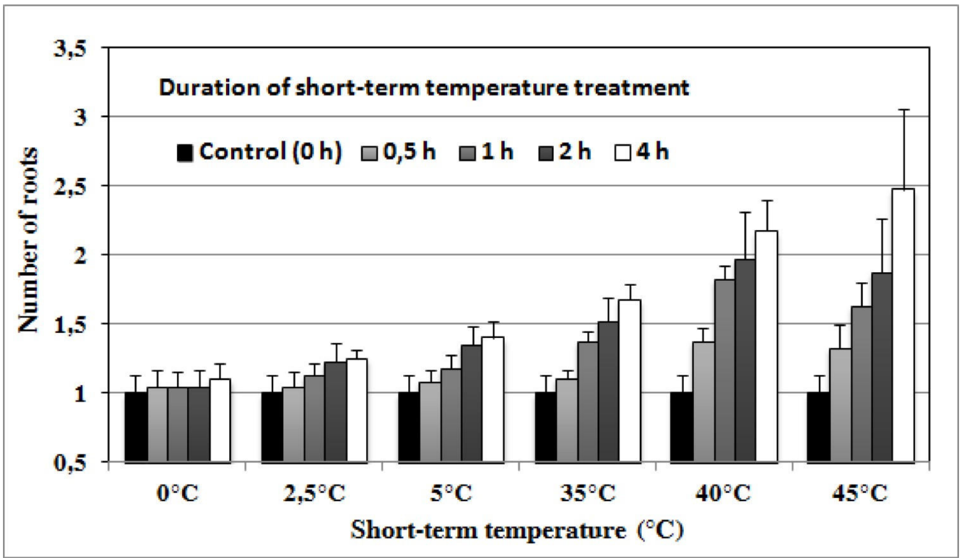


Fig. 2. Number of roots of cucumber after seed were moistened in water for 16 h at 25°C, subjected to temperature of 0; 2,5; 5; 35; 40, 45°C for 0 - control; 0,5; 1; 2; 4 h at and returned to 25°C. Seedlings with 3 mm roots were chilled at 0°C for 3 days. Root length was determined after seedlings were returned from chilling condition to 25°C for 72 h. The vertical bar atop each bar represents the standard error

CONCLUSIONS

- Cucumber chilling sensitivity are manifested by drop in the root and hypocotyls elongation, reduction in dehydrogenases activity and membrane integrity expressed by electrolyte leakage.
- Cucumber chilling sensitivity increases with roots elongation. A significant increase in chilling sensitivity reveals when seedlings roots reach 3 mm in length.
- Induction of cucumber chilling tolerance occurs after 16 h of seeds imbibition. The best effects is visible when imbibed seeds is exposed to 45°C for 2 and 4 h.
- A short-term of low (0; 2,5 or 5°C) or high (35; 40 or 45°C) temperature treatments applied before radicle protrusion i.e. after 16 h of seeds imbibition counteracted the negative effects of seedlings chilling.

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