

## TOLERANCE OF EGGPLANT (*Solanum melongena* L.) SEEDLINGS TO STRESS FACTORS

<sup>1</sup>Agnieszka Sękara, <sup>2</sup>Renata Bączek-Kwinta, <sup>1</sup>Andrzej Kalisz, <sup>1</sup>Stanisław Cebula

<sup>1</sup>Department of Vegetable and Medicinal Plants, Faculty of Horticulture, University of Agriculture in Krakow  
29-Listopada 54, 31-425 Kraków, Poland

<sup>2</sup>Department of Plant Physiology, Faculty of Agriculture and Economics, University of Agriculture in Krakow  
Podłużna 3, 30-239 Kraków, Poland  
e-mail: a.sekara@ur.krakow.pl

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### Abstract

The aim of the present study was to describe eggplant (*Solanum melongena* L.) tolerance to stress factors in the seedling stage as a basis for future studies on cross-tolerance to other stressors in subsequent stages of growth. After germination (3 days / 26°C), 'Epic F<sub>1</sub>' seedlings were exposed to chilling stress (3, 6 and 9°C × 48 h<sup>-1</sup>), heat stress (35, 40 and 45°C × 2 h<sup>-1</sup>), osmotic stress (mannitol 0.2; 0.6 and 1.0 M × 2 h<sup>-1</sup>), and oxidative stress (H<sub>2</sub>O<sub>2</sub> 0.2; 0.4 and 0.6 M × 2 h<sup>-1</sup>). A linear measurement of seedling radicle growth, electrolyte leakage and external symptoms of radicle damage under the stress conditions, compared to the non-stressed control, were analyzed.

It was found that stressors in all experimental combinations caused a morphological and physiological response from eggplant seedlings. A significant reduction in linear growth of radicles, showed as an absolute length and as a percentage of the control, was found in the treatments exposed to chilling stress (3 and 6°C), heat stress (35, 40 and 45°C), osmotic stress (0.2, 0.6 and 1.0 M mannitol) as well as oxidative stress (0.2, 0.4 and 0.6 M H<sub>2</sub>O<sub>2</sub>). The changes in seedling length as a result of stress factors did not always correspond with the changes in seedling mass. Electrolyte leakage in the treatments exposed to the following stressors: 3 and 6°C as well as 0.6 M H<sub>2</sub>O<sub>2</sub>, was significantly greater than that observed in control plants. Based on the obtained results and microscopic observations of radicle damage, the following stressors can be identified as those which cause a physiological response without severe damage: 9°C × 48 h<sup>-1</sup> (chilling stress), 35°C × 2 h<sup>-1</sup> (heat stress), 0.2 M mannitol × 2 h<sup>-1</sup> (osmoticum), and H<sub>2</sub>O<sub>2</sub> 0.2 M × 2 h<sup>-1</sup> (oxidation factor). We propose these stressors as a basis for future studies on plant acclimation and hardening to other stresses.

**Key words:** eggplant, seedlings, heat stress, chilling stress, osmotic stress, H<sub>2</sub>O<sub>2</sub> stress

### INTRODUCTION

Warm climate plant species like eggplant, grown in the temperate climate zone, are subjected to environmental stress which limits crop productivity, its quality, and post-harvest life. Detrimental environmental factors induce biochemical, physiological and cytological alterations, which could be reversible or irreversible, depending on their duration and intensity. The primary sensor of physical stress is a cell membrane, because a direct reduction in its liquidity is observed (Chinnusamy et al. 2006). Biochemical and physiological acclimation leads to cell membrane stiffening and reorganization of microfilaments which may be followed by activation of Ca<sup>2+</sup> channels and an increased cytosolic Ca<sup>2+</sup> level (Örvär et al. 2000; Chinnusamy et al. 2006). The acclimation involves modification of plant calcium signalling to provide a "stress memory" (Knight et al. 1996). As a strategy for protection against chilling, plants change the composition of the cell membrane lipid fraction towards increased membrane liquidity. For many species, an increase in the concentration of osmoregulators, mainly sugars, potassium ions, betaine, and proline, was observed during acclimation, in order to prevent the loss of water and stabilize macromolecules and biological membranes (Chen and Murata, 2008). Low temperature also causes oxidative stress, and in this case the plant protection strategy is to increase the synthesis of antioxidants (i.e. glutathione, ascorbate, carotenoids, flavonoids, polyphenols, tocopherols) and of specialized enzymes decomposing reactive oxygen species, i.e. superoxide dismutase, catalase, peroxidase, and the enzymes involved in

glutathione metabolism (Bartosz, 1997; Bączek-Kwinta et al. 2005; Bączek-Kwinta and Kościelniak, 2009).

A number of studies have shown the existence of cross-tolerance in plants. The exposure of a plant to one type of moderated stress can enhance the resistance to other multiple stresses (Mei and Song, 2010). The seedling stage of development is characterized by the enhanced sensitivity to stress factors, concerned with the biochemical and physiological characteristic of meristematic tissues. The results of previous studies have revealed that treating seedlings with stress factors of sub-lethal intensity (hydrochloric acid, osmotic or heat stress) can increase tolerance to chilling (Jennings and Saltveit, 1994; Mangrich and Saltveit, 2000). Cucumber seedlings subjected to osmotic stress revealed increased tolerance to chilling (Mangrich et al. 2006). Kang et al. (2005) have shown that the exposure of cucumber seedlings to osmotic and heat stress caused their increased tolerance to chilling. The ability to use the controlled stress in eggplant seedlings has only been considered in a few papers. Gao et al. (2004; 2008) noticed a greater tolerance of grafted eggplant seedlings to low temperature (4 and 5°C), as compared to non-grafted ones, and revealed the key role of calcium ions in regulating the physiological mechanism of chilling tolerance. Qiong Qiu et al. (2005) explored the influence of cerium on seed germination and growth of eggplant seedlings under chilling stress (10 and 15°C). Ce decreased the symptoms of chilling, as a result of a change in cell membrane permeability, proline and sugar content and of increased hydrolase activity during germination. Kaizi and Chen (2005) compared the tolerance of seedlings of 14 eggplant cultivars to high temperature. Heat stress caused intensified electrolyte leakage and an increase in proline content. It seems that the leakage of electrolytes, reflecting the degree of damage to cell membranes, can be a good determinant of tolerance of eggplant seedlings. Although the temporary increase in permeability of the cell membranes under the stress conditions can be observed there, it may be one of the features acquired by the plants that helps to enhance their resistance to stress. The effect of this process is activation of the “stress memory” and cross resistance to another stressor.

The aim of the present investigations is to verify the tolerance of eggplant seedlings to selected stress factors on the basis of seedling radicle growth analysis and membrane status assayed as the electrolyte leakage. The intensity of stress factors, which are not lethal but cause a physiological response, is proposed. The results will provide the basis for future investigations on the enhancement of cross-tolerance of eggplant to stress factors during subsequent stages of growth.

## MATERIALS AND METHODS

### Plant material and experimental design

Eggplant (*Solanum melongena* L.) ‘Epic F<sub>1</sub>’ seeds, sterilized with Thiuram (Organica-Azot, Jaworzno, Poland), were placed on Petri dishes with a layer of paper moistened with distilled water. After germination in an incubator (3 days / 26°C), 30 uniform seedlings, with radicles initially 0.5-1.0 mm long, were selected as the material in the following experimental treatments:

1. Control – the exposure of seedlings to 26°C (optimal temperature) for 48 h.
2. Chilling stress – the exposure of seedlings to 3, 6 and 9°C × 48 h<sup>-1</sup>, and 26°C for the next 48 h.
3. Heat stress – the exposure of seedlings to 35, 40 and 45°C × 2 h<sup>-1</sup>, and 26°C for the next 48 h.
4. Osmotic stress – the exposure of seedlings to mannitol in concentrations of 0.2; 0.6 and 1.0 M × 2 h<sup>-1</sup>, and 26°C for the next 48 h.
5. Oxidative stress – the exposure of seedlings to H<sub>2</sub>O<sub>2</sub> in concentrations of 0.2; 0.4 and 0.6 M × 2 h<sup>-1</sup>, and 26°C for the next 48 h.

The experiment was conducted in darkness. All tests were conducted in three replications and repeated three times in 2010 (dates of beginning: April 05, May 10, June 16).

### Radicle length analysis

The method modified by Rab and Saltveit (1996) was used for this purpose. The linear length measurement of the seedling radicle exposed to stress conditions, compared to the control, was analyzed. The measurements of the radicle length of each seedling were carried out (i) before stress factor application, (ii) after stress factor application and 48 hours at a temperature of 26°C – to investigate the recovery capacity of seedlings during rewarming, based on the difference between parameters after and before stress application. The measurements were made using the Image Tool for Windows 3.0 software, after registration of the image using a SteREO LUMAR V12 microscope (Carl Zeiss AG, Germany). The seedling mass was evaluated by direct mass measurement using an analytical scale (Sartorius, Germany).

### Electrolyte leakage (EL)

The measurement of electrolyte leakage was based on the method of Markowski and Skrudlik (1995) and performed on seedlings taken after application of individual stress and an additional time period of 48 hours at a temperature of 26°C. Plant material was washed with deionized water and then shaken for 24 hours in tubes with 15 cm<sup>3</sup> of deionized water. The

measurements of conductivity of the deionized water ( $L_1$ ) and aquatic diffusate of the samples ( $L_t$ ) were made using a conductometer with automatic temperature compensation (Elmetron, Zabrze, Poland). Then, the samples were boiled at 100°C for 15 min, shaken for 24 h and the assay was repeated to obtain the total content of electrolytes ( $L_k$ ). The measurement of electrical conductivity of the deionized water was also repeated ( $L_2$ ). Electrolyte leakage was calculated as a percentage of total electrolyte content according to the equation:  $EL = [(L_t - L_1) / (L_k - L_2)] \times 100\%$ .

### External symptoms of stress damage on seedlings

The assessment of the external symptoms of damage after exposure to stress factors and additional time of 48 hours at a temperature of 26°C was made on the basis of observations of seedlings using a SteREO LUMAR V12 microscope (Carl Zeiss AG, Germany). The evaluation of damage caused by stress factors was assessed according to a grading system: 1 – severe injury, 2 more than 50% necrosis, 3 – partial injury with necrosis, 4 – partial injury, 5 – no injury.

### Statistical analysis

The presented results are the means of 30 seedling measurements, in three experimental replications. All data obtained were subjected to one-way ANOVA, and the differentiation of the means was compared by the Tukey test at  $P \leq 0.05$ . In all the figures, the data marked with the same letter do not differ significantly and the variability of data is shown as error bars representing standard errors of the means.

## RESULTS

The stressors in all experimental combinations caused a morphological and physiological response from eggplant seedlings. A significant reduction in linear growth of radicles, showed as an absolute length and as a percentage of the control, was found in the treatments exposed to chilling stress (3 and 6°C), heat stress (35, 40 and 45°C), and osmotic stress (0.2, 0.6 and 1.0 M mannitol) as well as oxidative stress (0.2, 0.4 and 0.6 M  $H_2O_2$ ) – Figs 1 and 2. Exposure of seedlings to 9°C caused a significant increase in radicle length by about 13% compared to the control (Fig. 1A). The action of 3°C caused the greatest reduction in radicle growth by about 50% compared to the control. Figure 1B shows that in the heat stress treatments a similar reduction of radicle growth was observed in seedlings exposed to 40 and 45°C (42–47% of the control). A temperature of 35°C caused a 36% reduction in radicle growth. Seedlings treated with increased solutions of mannitol and  $H_2O_2$  showed a com-

mensurate reduction in radicle growth (Fig. 2A, 2B). Exposure of seedlings to 0.2, 0.6 and 1.0 M mannitol solutions resulted in a decrease in radicle growth by 43, 51, and 79%, respectively. Seedlings treated with 0.2, 0.4, and 0.6 M  $H_2O_2$  exhibited a 36, 43, and 52% reduction in linear growth of the radicle in comparison to the control. It should be stated that the linear measurement of seedling radicle growth under stress conditions seemed to be a good parameter for the description of the response of eggplant seedlings to stress factors.

The changes in seedling length resulting from the stress response did not always correspond with changes in seedling mass. Exposure of seedlings to 3 and 6°C, and 1.0 M mannitol significantly reduced their mass by about 20, 40 and 20% respectively, as compared to the control (Figs 1C, 2C). A statistically significant increase in seedling mass was observed as a result of 0.2 M mannitol treatment (Fig. 2C). The mass of seedlings exposed to 9, 35, 40 and 45°C, 0.6 M mannitol, 0.2, 0.4 and 0.6 M  $H_2O_2$  was comparable to the control (Figs 1C, 1D, 2C, 2D).

The electrolyte leakage in the treatments with seedlings exposed to the following stressors: 3 and 6°C as well as 0.6 M  $H_2O_2$ , was significantly greater compared with the control (Figs 1E, 2F). There were no statistical differences in electrolyte leakage between the treatments exposed to different levels of heat stress (Fig. 1F). Seedlings treated with 0.2 and 1.0 M mannitol resulted in significantly lower electrolyte leakage from the tissues of stressed seedlings as compared to the control (Fig. 2E).

The microscopic observations of seedling radicle damage were performed in order to describe the external symptoms of a plant's reaction to the stressors. The characteristic injury caused by the lowest of the applied temperatures (3 and 6°C) caused up to 50% necrosis (Figs 1G, 3A, 3B). Chilling with a temperature of 9°C resulted in a slight visual injury to the seedling radicles (Figs 1G, 3C). Heat stress (35 and 40°C) caused only a slight – but statistically significant when compared to the control – injury to the seedlings (Figs 1H, 3D, 3E). The result of 45°C treatment was partial damage to the radicles; namely up to 50% necrosis (Figs 1H, 3F). The treatment of seedlings with 1.0 M mannitol resulted in the most severe damage among all treatments investigated (Figs 2G, 3I). Mannitol, applied in concentrations of 0.2 and 0.6 M, caused a slight visual damage to the seedling radicles (Figs 2G, 3G, 3H). The hydrogen peroxide in concentrations of 0.4 and 0.6 M caused partial injury to the seedling radicles (Figs 2H, 3K, 3L). In a concentration of 0.2 M, the visual injury was significantly smaller (Figs 2H, 3J).

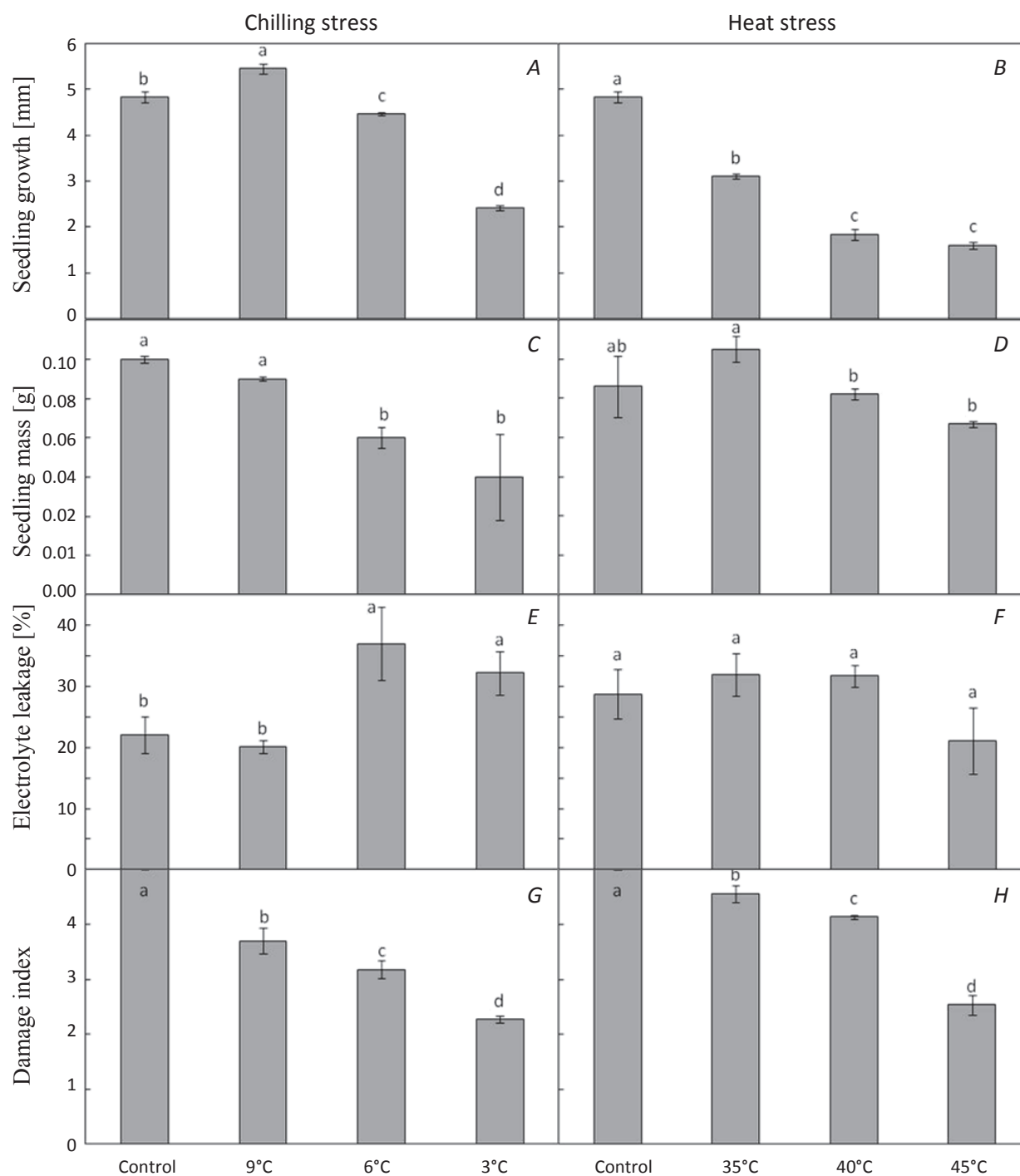


Fig. 1. The effect of chilling stress (9, 6, 3°C × 48 h<sup>-1</sup> and subsequent 26°C × 48 h<sup>-1</sup> treatment) and heat stress (35, 40, 45°C × 2 h<sup>-1</sup> and subsequent 26°C × 48 h<sup>-1</sup> treatment) on eggplant seedlings growth (A, B), mass (C, D), electrolyte leakage (E, F), damage index: 1 – severe injury, 2 – more than 50% necrosis, 3 – partial injury with necrosis, 4 – partial injury, 5 – no injury (G, H). Means marked with the same letter do not differ significantly (Tukey's test,  $P \leq 0.05$ ).

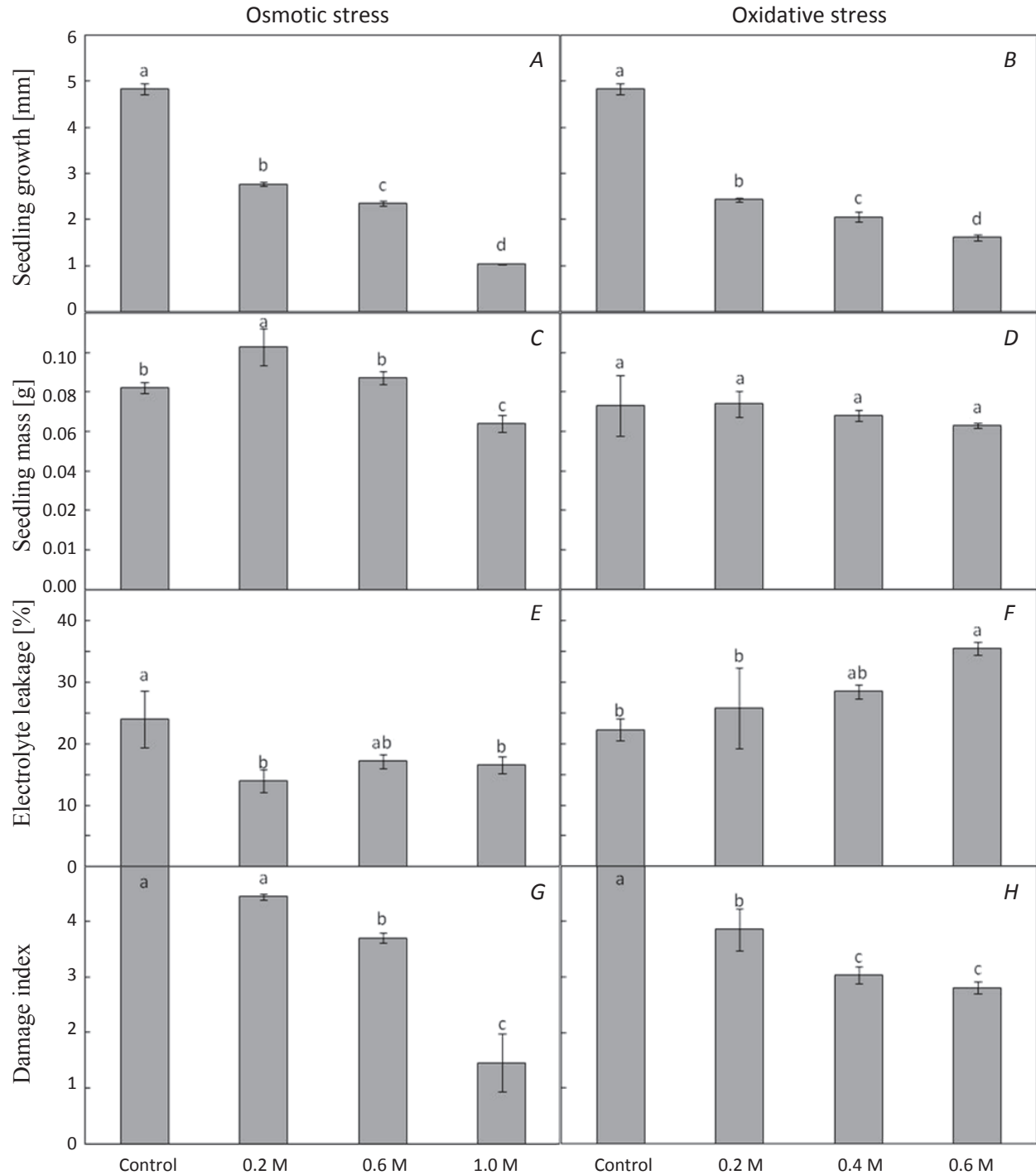


Fig. 2. The effect of osmotic stress (mannitol 0.2, 0.6, 1.0 M  $\times$  2 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment) and oxidative stress (H<sub>2</sub>O<sub>2</sub> 0.2, 0.4, 0.6 M  $\times$  2 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment) on eggplant seedling growth (A, B), mass (C, D), electrolyte leakage (E, F), damage index: 1 – severe injury, 2 – more than 50% necrosis, 3 – partial injury with necrosis, 4 – partial injury, 5 – no injury (G, H). Means marked with the same letter do not differ significantly (Tukey's test,  $P \leq 0.05$ ).



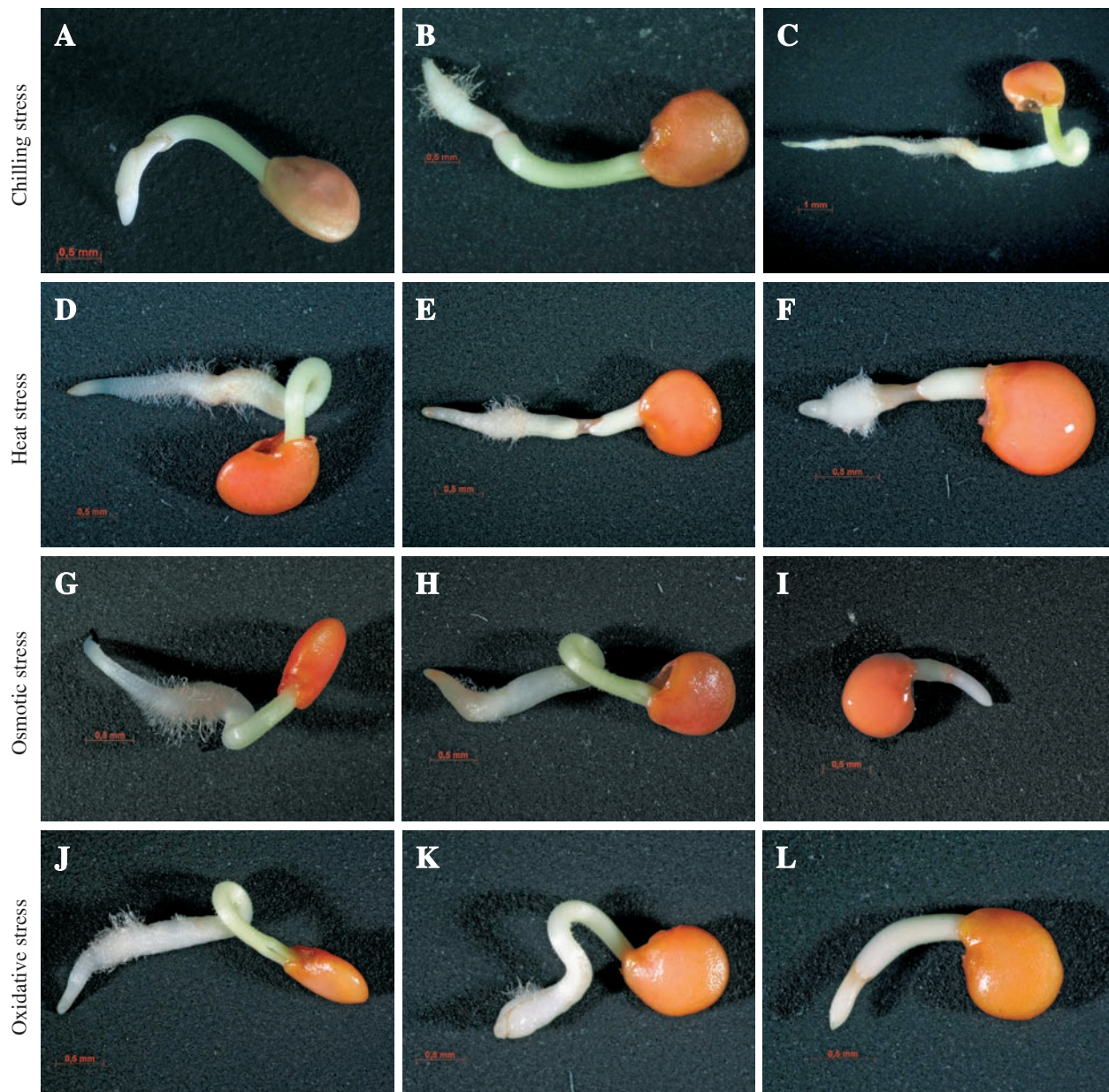


Fig. 3. Visual damage and growth disruption caused by chilling stress – temperature 3°C (A), 6°C (B), and 9°C (C)  $\times$  48 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment; heat stress – temperature 35°C (D), 40°C (E), and 45°C (F)  $\times$  2 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment; osmotic stress – mannitol 0.2 M (G), 0.6 M (H) and 1.0 M (I)  $\times$  2 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment; and oxidative stress – hydrogen peroxide 0.2 M (J), 0.4 M (K) and 0.6 M (L)  $\times$  2 h<sup>-1</sup> and subsequent 26°C  $\times$  48 h<sup>-1</sup> treatment. Photos representative to  $n = 25$ .

## DISCUSSION

The studies concerning the stress sensitivity of plants are often performed on seedling tissues or whole seedlings, because the response of young plants to stress is rapid and can be easily monitored. Kaizi and Chen (2005) screened the tolerance of seedlings of 14 eggplant varieties to heat stress. The authors proposed that, among others, electrolyte leakage might be suitable for selecting heat tolerant eggplant genotypes in breeding programmes.

The increase of electrolyte leakage in the case of seedlings exposed to 3 and 6°C, as well as 0.6 M H<sub>2</sub>O<sub>2</sub>, suggests considerable changes in cell membrane permeability of these plantlets. Saltveit (2002) proved the reduction in radicle growth of rice exposed to chilling (5°C). The presented results confirm a significant reduction in linear growth of eggplant radicles, shown as an absolute length and as a percentage of the control, in the seedlings subjected to chilling stress (3 and 6°C). It is interesting that treatment with 9°C

caused a significant increase in radicle length (about 13%), but its mass was comparable to the control. There was no impact of 9°C on electrolyte leakage. Thus, moderate chilling of eggplant seedlings with 9°C can be a possible way to enhance its tolerance to stress factors in subsequent growth stages.

Kumar et al. (2010) suggested that under heat stress the growth inhibition depended on the genotype and temperature of treatment. Sato et al. (2001) pointed out that heat shock can protect rice seedlings against chilling injury. Esemine et al. (2010) observed that very high temperatures may provoke cellular injury and subsequent cell death, but moderately high temperatures can provoke considerable damage, after a long-term exposure. According to the cited authors, direct injuries include protein denaturation and increased fluidity of membrane lipids. Indirect injuries, due to a lower heat level, include inactivation of enzymes, inhibition of protein synthesis, protein degradation and loss of membrane integrity. In the present study, no significant differences were found in electrolyte leakage between the seedlings exposed to heat stress. The temperatures of 40 and 45°C dramatically reduced seedling growth and caused visual damage. Interestingly, as a result of a temperature of 35°C, we obtained a significant increase in seedling mass (although radicle growth was not altered); therefore, it could be possible to study eggplant cross-tolerance to stress after exposure of seedlings to this temperature.

Many studies have used mannitol as an osmotic component generating osmotic stress (Sadeghian and Yavari, 2004). Sadeghian and Yavari (2004) applied 0.0, 0.2 and 0.3 M mannitol to assess the rate of seed germination and early seedling growth in sugar beet under water deficit stress conditions. Seedling growth and germination rates severely declined at the highest concentration of mannitol. In the present study, it was interesting that the exposure to 0.2 and 1.0 M mannitol resulted in significantly lower electrolyte leakage from the stressed seedlings, compared to the control. When analyzed, the external symptoms of damage displayed no differences between control and mannitol-treated seedlings, therefore a low level of tissue damage was confirmed in the seedlings in this part of the experiment. Seedlings exposed to 0.2 M mannitol were characterized by a higher mass compared to the control, but the radicle elongation was reduced. Therefore, when applied in a concentration of 0.2 M, mannitol can be an effective factor enhancing the tolerance of eggplant to stress factors in subsequent growth stages. For example, Kang et al. (2005) showed that exposure to osmotic (0.6 M mannitol) or heat (2 min at 45°C) stress enhanced chilling tolerance of cucumber seedlings.

Mittler et al. (2004) underlined the dual role for reactive oxygen species (i.e. H<sub>2</sub>O<sub>2</sub>) in plant biology,

as toxic byproducts of aerobic metabolism and key regulators of growth, development and defence pathways. Also Wan and Liu (2008) emphasized that H<sub>2</sub>O<sub>2</sub> plays a dual role in plants as the toxic by-product of normal cell metabolism and as a regulatory molecule in stress perception and signal transduction. Excessive H<sub>2</sub>O<sub>2</sub> generation resulting in an oxidative stress in plants was observed as a result of chilling, salinity, heavy metals, drought and other biotic and abiotic stress factors (Baczek-Kwinta, 2005; Šlesak et al. 2007). On the contrary, exogenous H<sub>2</sub>O<sub>2</sub> can enhance the tolerance of plants to salt stress (Li et al. 2010), chilling (Lin and Saltveit, 2005; Feng et al. 2008), drought (Qiu et al. 2010), heat (Wahid et al. 2008). In this study, exposure of eggplant seedlings to 0.2 M H<sub>2</sub>O<sub>2</sub> resulted in a significant decrease in radicle linear growth, but it had no significant influence on its mass and cell membrane status. Visual damage was also slight in this case. A strong correlation between the effect of H<sub>2</sub>O<sub>2</sub> on plant growth and the decrease in ABA was observed by Barba-Espin et al. (2010). Lin and Saltveit (2005) tested the hypothesis that moderate oxidative stress offers protection against chilling injury of mungbean seedlings. They also showed that chilling inhibits the subsequent radicle growth and indicated a possible role of moderate stress in inducing partial tolerance to the chilling of that species.

## CONCLUSIONS

The presented results provide a new concept for eggplant seedling development under stress environments. Exposure of seedlings to stress factors in the manner proposed can be a way to increase eggplant tolerance to subsequent environmental stressors during ontogenesis. The proposed stressors triggered the physiological response of eggplant seedlings without significant injury, so we can postulate that the results provide a foundation for future investigations on cross-tolerance or subsequent tolerance of eggplant to stress factors and possibilities to enhance its tolerance to environmental stresses.

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### **Tolerancja siewek oberżyny (*Solanum melongena* L.) na czynniki stresowe**

#### **Streszczenie**

Celem przeprowadzonych badań była analiza tolerancji oberżyny (*Solanum melongena* L.) w stadium siewek na czynniki stresowe. Po skiełkowaniu (3 dni / 26°C), siewki oberżyny 'Epic F<sub>1</sub>' poddano działaniu stresu chłodowego (3, 6 i 9°C × 48 h<sup>-1</sup>), cieplnego (35, 40 i 45°C × 2 h<sup>-1</sup>), osmotycznego (mannitol 0,2; 0,6 i 1,0 M × 2 h<sup>-1</sup>), i oksydacyjnego (H<sub>2</sub>O<sub>2</sub> 0,2; 0,4 i 0,6 M × 2 h<sup>-1</sup>). Analizowano wzrost elongacyjny korzonka zarodkowego, wyciek elektrolitów oraz zewnętrzne objawy uszkodzeń korzonka zarodkowego w warunkach stresu, w porównaniu do nie stresowanej kontroli. Stwierdzono, że stresory we wszystkich

eksperymentalnych kombinacjach wywołały morfologiczną i fizjologiczną reakcję siewek oberżyny. Istotny spadek przyrostu liniowego korzonków zarodkowych, wykazany w postaci ich bezwzględnej długości, jak i procentowo w stosunku do kontroli, stwierdzono w obiekcie eksponowanym na stres chłodowy (3 i 6°C), cieplny (35, 40 i 45°C), osmotyczny (0,2; 0,6 i 1,0 M mannitol) oraz oksydacyjny (0,2; 0,4 i 0,6 M H<sub>2</sub>O<sub>2</sub>). Zmiany długości korzonka zarodkowego siewek pod wpływem czynników stresowych nie zawsze odpowiadały zmianom ich masy. Wyciek elektrolitów w obiektach eksponowanych na następujące czynniki stresowe: 3 i 6°C oraz 0,6 M H<sub>2</sub>O<sub>2</sub> był istotnie większy, niż obserwowany w obiekcie kontrolnym. Na podstawie otrzymanych wyników oraz mikroskopowych obserwacji zewnętrznych uszkodzeń powierzchni korzonka zarodkowego, wytypowano następujące stresory, które wywołują odpowiedź fizjologiczną ze strony siewek oberżyny bez poważnych uszkodzeń tkanek: 9°C × 48 h<sup>-1</sup> (stres chłodowy), 35°C × 2 h<sup>-1</sup> (stres cieplny), 0,2 M mannitol × 2 h<sup>-1</sup> (stres osmotyczny), i H<sub>2</sub>O<sub>2</sub> 0,2 M × 2 h<sup>-1</sup> (stres oksydacyjny). Zaproponowano te czynniki stresowe jako bazowe do dalszych badań nad zwiększeniem tolerancji oberżyny na inne stresory działające w kolejnych fazach wzrostu i rozwoju ontogenetycznego.