Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.)

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To understand the physiological and ecological aspects of plant growth, it is necessary to study the physiology of plant development, as well as the effects of certain environmental factors on plant growth depending upon the developmental stages when these factors act. The effect of a long-term (24 h) high-temperature (42 °C) shock on wheat seedlings (*Triticum aestivum* L.) was analyzed taking into consideration changes in the growth of wheat seedlings (maximum length of root and length of first leaf and coleoptile) and some changes on the molecular level, such as superoxide anion (O$_2^-$) production and malondialdehyde (MDA) content. The effect of high temperature was analyzed at the early (4-day-old) and late stages (7-day-old) of seedling development. The long-term high-temperature exposure strongly inhibited the development of roots and first leaves, but did not affect the growth of the coleoptile at the early stages of development. However, high-temperature exposure at the late stages of seedling development affected only the growth of the root system. This effect remained also two days after the high-temperature exposure. The increase of O$_2^-$ production, which was observed in the first leaf of wheat seedlings at all stages of development, led to an increase of MDA concentration. Material changes in the level of O$_2^-$ production were observed in the roots of wheat seedlings grown under high temperature exposure for 24 h at all stages of development, but MDA concentration in the roots of experimental and control seedlings almost did not differ at the early and late stages of development. The level of O$_2^-$ production in the coleoptile cells increased after a high-temperature exposure at the late stages of seedling development. A significant increase of MDA content in the coleoptile was observed immediately after the experiment at both early and late stages of seedling development and then decreased slightly within two days after treatment, though it was higher than MDA content in control seedlings.

Key words: heat shock, lipid peroxidation, malondialdehyde, oxidative stress, superoxide production, wheat seedlings

INTRODUCTION

The environment has a significant role in plant growth and development. Changes in atmospheric temperature are often very sudden, and plants cannot adjust to these changes and are damaged beyond recovery (Mavi, Tupper, 2004). Several plant species, including annual crop plants, are exposed to chill and heat stress during their lifetime. High temperature results in the desiccation of a plant and disturbs the balance between photosynthesis and respiration. At temperatures higher than the optimum cardinal, the physiological activity declines as a consequence of inactivation of enzymes and other proteins. The optimal intensity of temperature differs not only for individual organisms, but also for particular organs of the same organism; e.g., leaf functions are disturbed at about 42 °C, and lethal effects on active shoot tissues generally occur in the range of 50° to 60 °C (Mavi, Tupper, 2004).

The influence of high temperatures was investigated in different groups of plants. Doke (1997) showed that within 15 min after heat shock, potato leaf tissues produced an oxidative burst. More recently, exposure of whole tobacco seedlings to 40 °C for 1 h in the light induced a significant increase
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in H$_2$O$_2$ (Dat et al., 2000). A similar accumulation of H$_2$O$_2$ after a heat treatment was measured in mustard seedlings (Dat et al., 1998). In wheat crops, a major effect of high temperature appears to be accelerated senescence, including cessation of vegetative and reproductive growth, deterioration of photosynthetic activities, and degradation of proteinaceous constituents (Xu et al., 1995).

Exposure to low temperatures and high temperature induces reactive oxygen species (ROS) accumulation in plants, causing an oxidative stress (Larkindale, Huang, 2004; Worrall et al., 2007). The mechanisms by which ROSs can accumulate under such conditions are diverse. ROS generation is considered to be a primary event under a variety of stress conditions (Noctor, Foyer, 1998). ROSs are highly reactive and toxic, and can lead to the oxidative destruction of cells. The consequences of ROS formation depend on the intensity of stress and on the physicochemical conditions in the cell. It has been generally accepted that ROSs produced under stress are the decisive factor that causes lipid peroxidation (LP), enzyme inactivation, etc. (Blokhina, 2000).

In normal conditions, LP is a natural metabolic process. LP activation is one of the possible results of a rapid response to stress. One of LP products (MDA) was investigated in the present work. The content of MDA is often used as an indicator of lipid peroxidation resulting from oxidative stress (Malenčić et al., 2004).

In the present study, the effect of heat (42 °C) stress on the intensity of growth processes (length of the first leaf, coleoptile, and the maximum length of root) and on biochemical aspects (O$_2^-$ production and MDA concentration) of wheat seedlings was studied.

MATERIALS AND METHODS

Plant material and experimental conditions

The object of the study was etiolated seedlings of winter wheat (Triticum aestivum L., cv. Harmonia). Wheat is a convenient object for studying plant physiology and molecular biology, because the development of cereals is synchronous throughout ontogeny (Kirmos et al., 1997). Etiolated wheat seedlings grown at 26 °C were transferred to 42 °C for 24 h in the dark at the early (4 day-old) and late (7 day-old) stages of their development. Plant material (roots, coleoptiles and first leaves) was sampled for analysis immediately after heat shock and in two days after high-temperature exposure. Wheat roots were used because a high root temperature accelerates the senescence of the entire plant (Ferguson et al., 1990). The first leaf and the coleoptile were used because the first leaf is a developing organ of wheat, while the coleoptile is a senescent organ, and the processes that occur in these organs during high-temperature stress were expected to differ. High-temperature shock was analyzed taking into consideration changes in the growth of wheat seedlings (maximum length of root, first leaf and coleoptile length) (Shorning et al., 1999) and some changes on the molecular level, such as superoxide production and MDA content. Data shown in figures and tables are the mean of three independent experiments. To investigate morphological changes after high-temperature stress, the number of samples was 30 seedlings in each experiment.

Superoxide production

First leaves, coleoptiles and roots of four wheat seedlings were preincubated with or without 1 μl/ml superoxide dismutase (SOD) at 26 °C for 1 hour in an incubation buffer (with nitroblue tetrazolium (NBT)). Production of superoxide by first leaf and coleoptile cells was determined by the NBT reduction assay. NBT reduction was monitored spectrophotometrically at a 530 nm wavelength (Shorning et al., 2000).

Measurement of MDA content

MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Ali et al. (2005), with slight modifications. Approximately 0.2 g leaf, coleoptile and root segments were homogenized with 2 ml of 0.1% trichloroacetic acid (TCA) and centrifuged at 14,000 rpm for 15 min. After centrifugation, 1 ml of the supernatant was mixed with 2.5 ml 0.5% TBA in 20% TCA and incubated in hot water (95 °C) for 30 min. Thereafter, it was cooled immediately on ice to stop the reaction and centrifuged at 10,000 rpm for 30 min. Absorbance at 532 and 600 nm was determined, and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm, using an absorbance coefficient of extinction (155 mM$^{-1}$ cm$^{-1}$).

Statistical analysis

Data are presented taking into consideration the SE. Results were analyzed by one-way ANOVA to identify significant differences between the groups, and their significance levels (p < 0.05) were determined.

RESULTS AND DISCUSSION

Heat stress adversely affects the growth and development of wheat seedlings. Diverse environmental stresses differently affect the plant processes that lead to a loss of cellular homeostasis accompanied by the formation of ROS, which causes oxidative damage to membranes, lipids, proteins and nucleic acids (Srivalli et al., 2003).

In the present study, the effect of long-term (24 h) heat (42 °C) stress on the intensity of growth processes (length of the first leaf, coleoptile and maximum length of root) and on physicochemical aspects (O$_2^-$ production and MDA concentration) of wheat seedlings was studied. Recently, high-temperature effects (42 °C) on photosynthetic processes (Al-Khatib, Paulsen, 1999), programmed cell death (Fan, Xing, 2004), total protein degradation (Ferguson et al., 1990) etc. have been investigated in different organs of wheat seedlings. A longer exposure (48 h) to a high temperature (42 °C) almost killed the wheat tissue (Tewari, Tripathy, 1998).
Morphological changes

It is a well-known fact that growth inhibition is natural in plants and is related to physiological and metabolic changes, which in turn could increase the ROS generation and induce an oxidative stress (Asada, Takahashi, 1987). The morphological features of seedlings revealed that a long-term high-temperature stress in wheat seedlings caused an inhibition ($p < 0.001$) of the first leaf and root growth (Fig. 1). The length of the first leaf of experimental seedlings at the early stages of development immediately after exposure was inhibited by 43% and the maximum length of roots by 46% compared to control seedlings (Fig. 1a). As is shown in Fig. 1b, this effect was present also two days after a 24-h exposure (55% and 38%, respectively).

Although a long-term high-temperature exposure inhibited the development of roots and first leaves at the early stages of seedling development, the coleoptile length was the same ($p < 0.05$) compared to control seedlings (Fig. 2a). This trend was present also two days after a long-term exposure ($p > 0.05$). However, in spite of a relative stability of the mor-

![Fig. 1. Length of the first leaf and root at the early stages of wheat seedling development ($p < 0.001$) in response to the 24-h high-temperature immediately after stress (a) and in two days after stress (b). Each value is mean ± SE of three replicates.](image1)

![Fig. 2. Length of the coleoptile at the early (a) and at the late (b) stages of wheat seedling development in response to the 24-h high-temperature stress (ns – not significant differences ($P > 0.05$), * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). Each value is mean ± SE of three replicates.](image2)
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Phenological features of the coleoptile, an increase of oxidative activity in the coleoptile, i.e. high MDA content (Table), was observed. The possible reason is the function of the coleoptile which protects the first leaf against injury during the process of seed germination. High-temperature exposure at the late stages of seedling development did not affect (p > 0.05) the first leaf and coleoptile length (Fig. 3, b), but it decreased (p < 0.001) the growth of the root system of experimental seedlings by 36% immediately after a high-temperature exposure (Fig. 3). This effect was present (p < 0.01) also two days after a long-term exposure (30%).

Superoxide production

The development of etiolated wheat seedlings is accompanied by a cyclic formation of superoxide anions which are essential for plant growth and development (Shorning et al., 2000). ROS are always produced in the leaf tissue, but under non-stress conditions ample detoxication mechanisms are available. Under stress conditions, the highly toxic ROS production increases dramatically and overwhelms the detoxication system. The negative effect of the various environmental stresses is, at least partially, due to ROS generation (Shalata, Tal, 1998).

Changes of O$_2^-$ production after a high-temperature exposure in the root, coleoptile and first leaf were investigated. No increase in O$_2^-$ production after a long-term high-temperature stress at the early stages of development was observed in the coleoptile (Fig. 4a). Moreover, O$_2^-$ production in the experimental coleoptile was lower than in the control coleoptile. It is possible that the antioxidant system of the coleoptile functions more effectively under stress conditions to protect the first leaf from damaging. However, the level of O$_2^-$ production in the coleoptile cells increased (p < 0.001) by 71% (Fig. 4b) compared to control after a high-temperature exposure at the late stages of seedling development. This effect was still present (p < 0.01) two days after a high-temperature exposure (33%). According to our data, growth inhibition of the root system could be connected with a powerful oxidative stress, evidenced by a significant increase (68%) of O$_2^-$ production (p < 0.001) in root cells at the early stages of seedling development.

### Table. MDA content (μmol*g$^{-1}$FW) in organs of wheat seedlings at the early (I) and at the late (II) stages of development in response to the 24-h high-temperature stress. Each value is mean ± SE of three replicates

<table>
<thead>
<tr>
<th></th>
<th>First leaf</th>
<th>Coleoptile</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>2.509 ± 0.032</td>
<td>1.834 ± 0.146</td>
<td>0.549 ± 0.022</td>
</tr>
<tr>
<td>24 h</td>
<td>1.545 ± 0.013</td>
<td>2.505 ± 0.001</td>
<td>2.478 ± 0.159</td>
</tr>
<tr>
<td>Differences</td>
<td>***</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>Control</td>
<td>1.567 ± 0.035</td>
<td>0.438 ± 0.02</td>
<td>0.297 ± 0.003</td>
</tr>
<tr>
<td>24 h</td>
<td>1.015 ± 0.044</td>
<td>1.052 ± 0.018</td>
<td>1.292 ± 0.116</td>
</tr>
<tr>
<td>Differences</td>
<td>***</td>
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<td>***</td>
</tr>
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</table>

Note. 1 – immediately after stress; 2 – in two days after stress; ns – not significant differences (P > 0.05), * P < 0.05; ** P < 0.01; *** P < 0.001

Fig. 3. Length of the first leaf (p > 0.05) and root (p < 0.001 for A; p < 0.01 for B) at the late stages of wheat seedling development in response to the 24-h high-temperature immediately after stress (a) and in two days after stress (b). Each value is mean ± SE of three replicates.
development and a insignificant increase (6%) of $O_2^-$ production ($p > 0.05$) two days after a high-temperature exposure, as compared to control seedlings (Fig. 6a). The increase of $O_2^-$ production was observed also in roots after a high-temperature stress at the late stages of development ($p < 0.05$), and this effect ($p < 0.001$) was present also two days after a high-temperature exposure (6% and 42%, respectively) (Fig. 6b). Moreover, $O_2^-$ production in the roots two days after the experiment at the late stages was more intensive than at the early stages of development (79% and 22%, respectively). It is possible that activity of the antioxidant system decreased in the senescent coleoptiles and roots, and they could not combat the negative environmental factors which led to an oxidative stress. This oxidative stress is likely to be due to the increased generation of ROS at a high temperature. The production of ROS, mediated by alternate $O_2$ reduction and subsequent oxidative damage in heat-stressed plants, has been reported by several researchers (Larkindale, Huang, 2004; Sairam et al., 2000; Almeselmani et al., 2006; Wahid et al., 2007).

A significant increase (75%, $p < 0.01$) of $O_2^-$ production was observed in the first leaf of wheat seedlings at the early stages of development compared to control seedlings (Fig. 5a). In two days, the $O_2^-$ production in the experimental first leaf started decreasing ($p > 0.05$) and was similar to control (Fig. 5a), possibly because of the increased antioxidant activity in the first leaf cells. However, an increased $O_2^-$ production ($p < 0.001$) was observed in the first leaf cells after high-temperature exposure at the late stages of seedling development (Fig. 5b) immediately after exposure (65%) and two days after exposure (34%). Possibly it is connected with senescence of the coleoptile. The senescent coleoptile loses its protective properties because it is subjected to a strong oxidative stress caused by the high $O_2^-$ production (Fig. 4b).
and high MDA content (Table). Therefore, the first leaf can rely only on its own antioxidant defense system. Besides, the increased \( \text{O}_2^- \) production can possibly be connected with the increased level of MDA, which was observed in the first leaf at all stages of development two days after a heat shock (Table). As is known, LP activation is a response to ROS formation. In the present work, only one of the reactive oxygen species – superoxide anion (\( \text{O}_2^- \)) – was investigated. Perhaps other ROSs are accumulated in the first leaf cells, leading to LP activation, i.e. \( \text{O}_2^- \) is relatively unstable, being either converted back to \( \text{O}_2 \) or, in reaction with a proton, to \( \text{H}_2\text{O}_2 \), either spontaneously or in a reaction catalysed by the enzyme superoxide dismutase. \( \text{H}_2\text{O}_2 \) is one of the major and the most stable ROSs, which regulates the basic acclimatory, defensive and developmental processes in plants (Slesak et al., 2007). Hydrogen peroxide is more likely to be a long-distance signalling molecule than \( \text{O}_2^- \) (Vranová et al., 2002). Membrane water channels, known as aquaporins, may facilitate \( \text{H}_2\text{O}_2 \) transmembrane movement together with water (Henzler, Steudel, 2000), and it easily reacts with other molecules, such as lipids, nucleic acids and proteins, which can be damaging or even fatal for the cell (Rao et al., 1997; Matés, 2000; Mittler, 2002).

### Malondialdehyde concentration

The ROS-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Jain et al., 2001). An enhanced level of lipid peroxidation, as indicated by MDA content, was observed in wheat organs in response to a high temperature in the present study, clearly indicating an oxidative stress under the effect of a high temperature. Heat stress increased MDA content in all organs of wheat seedlings. MDA levels were detected and quantified in plant tissues, including wheat (Király, Czövek, 2002; Sofó et al., 2004; Yin et al., 2008). One can see that the influence of high temperature on MDA content differs not only depending on the stages of development, but also for particular organs of wheat (Table). MDA content in the first leaf of wheat seedlings decreased immediately after treatment and increased two days after a high-temperature exposure. MDA content decreased (\( p < 0.001 \)) by about 38% immediately after a high-temperature exposure in the first leaf at the early stages of seedling development (Table). This effect was still present (\( p < 0.001 \)) at the late stages of development (35%). However, MDA content increased (\( p < 0.01 \)) by 27% in the first leaf in two days after exposure at the early stages of seedling development, and this trend remained (\( p < 0.001 \)) also at the late stages of development (58%) as compared to control seedlings, in spite of a high \( \text{O}_2^- \) production in the first leaf, which was recorded immediately after a high-temperature stress. Our data show that high-temperature exposure immediately after stress did not affect LP in the first leaf. The damaging influence of ROS in the first leaf is possibly of an accumulating character which causes LP in the membrane only two days after a high-temperature stress.

By contrast, the MDA content in the coleoptile of wheat seedlings significantly increased (\( p < 0.001 \)) immediately after treatment and then decreased slightly within two days after treatment (Table). A significant increase of MDA content was observed (\( p < 0.001, p < 0.01 \)) in the coleoptile immediately after the experiment at the early (78%) and late stages (77%) of seedling development, but MDA concentration in the coleoptile decreased in two days after exposure, compared to MDA content in the coleoptile immediately after exposure, although it was higher than in control seedlings. As mentioned above, LP activation is a response to ROS formation; besides, LP is an inherent feature of a senescent cell (Bhattacharjee, 2005). Probably it is connected with more intensive oxidative processes in the coleoptile in that period (Fig. 4), because the coleoptile is a senescent organ and is unable to protect itself against the oxidative stress. Senescence is a developmental process in plants (Nooden, 1988), and high-temperature stress may accelerate some aspects of senescence and inhibit others.
Our data show that a long-term high-temperature exposure has the most powerful influence on the root system, but, despite an intensive O$_2$ production and a significant growth inhibition after a high-temperature exposure in the root at all stages of seedling development, MDA concentration in the roots of experimental and control seedlings almost did not differ at the early and late stages of development (Table). The possible reason may be activity of antioxidant enzymes in the root. Ali et al. (2005) showed that the influence of catalase and peroxidase activities in the root was most substantial, implying that they play an important role against the ROS caused by stress.

The results of this study provide an evidence that a long-term high-temperature stress interferes with the premature degradation of tissues, which is expressed in the inhibition of growth processes and in the changed intensity of oxidative processes (increased ROS level and accumulation of LP products). Besides, we found the strongest effect of long-term high-temperature exposure on the intensification of oxidative processes in the senescent organ – coleoptile – at all stages of development, whereas the intensive O$_2$ production in the developing organ – the first leaf – had an accumulating character, and LP was more intensive two days after a high-temperature treatment. The influence of high temperature on the inhibition of growth processes was strongest in the root, whereas, despite a relatively high O$_2$ production, these was no increase in MDA content.

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