Chilling stress in plants
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ABSTRACT: Chilling temperatures (1–10°C) lead to numerous physiological disturbances in the cells of chilling-sensitive plants and result in chilling injury and death of tropical and subtropical plants, e.g., many vegetable species. The literature review shows that the exposure of chilling-sensitive plants to low temperatures causes disturbances in all physiological processes – water regime, mineral nutrition, photosynthesis, respiration and metabolism. Inactivation of metabolism, observed at chilling of chilling-sensitive plants is a complex function of both temperature and duration of exposure. Response of plants to low temperature exposure is associated with a change in the rate of gene transcription of a number of low molecular weight proteins. The review analyzes historical aspects in the development of ideas about the nature of chilling damage of chilling sensitive plants and direction of modern research. Based on the authors’ own research and the literature data, the concept of cold damage was proposed, which highlighted the leading role of oxidative stress in the induction of stress response.

Key words: Chilling Stress, Low Temperatures, Physiological Processes.

INTRODUCTION

More than half of the 350 000 plant species on Earth are grown in the tropics and subtropics. In the course of evolution, they could not develop the ability to withstand low temperatures. Most of these species are damaged during storage at temperatures above the freezing point of tissues, but lower than 15°C (chilling temperatures). This damage is called chilling injury as opposed to damage during freezing (freezing injury) (Levitt, 1980; Raison and Lyons, 1986). Thus, chilling injury is damage to chilling-sensitive plant species during storage at temperatures above the freezing point of tissues, but lower than 15°C. Chilling-sensitive plants are the plants sensitive to chilling and damaged at chilling temperatures. The ability of plants in a vegetative state to survive the action of chilling temperatures without harm to the future growth and development is called cold resistance. In turn, chilling-sensitive plants are sensitive to chilling and after prolonged storage in these temperatures external symptoms of injury are developed and death of the organism occurs (Table). Plants, which have the visual injuries at temperatures above 15°C, are called “very sensitive to chilling” (Raison and Lyons, 1986). A number of tropical or subtropical plants, such as rice, maize, tomato, cucumber, cotton, soybeans, etc., introduced in the higher latitudes have not acquired substantial resistance to chilling, despite the long history of cultivation in temperate regions (Wilson, 1985).

Chilling temperatures effects on plants in temperate climates lead to a reduction or complete crop failure due to either direct damage or delayed maturation. Even a small drop in temperature, causing no visible damage to chilling-sensitive plants, caused to up to 50% reduction in their productivity. For example, chilling damage to young cotton plants in U.S. in 1980 resulted in the loss of 60 million dollars. In South and South-East Asia, high-yielding varieties of rice are not grown in areas of more than 7 million hectares, where they may be exposed to chilling temperatures (Wilson, 1985). Obviously, the problem of plant resistance to chilling temperatures, which often occur in spring and autumn in many countries, is important for practical plant breeding.
Table 1. The list of the vegetables, sensitive to chilling temperatures, the lowest safestorage/handling temperature andthe symptoms of chilling injury (DeEll, 2004)

<table>
<thead>
<tr>
<th>Crop</th>
<th>Lowest safe temperature °C</th>
<th>Chilling injury symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asparagus</td>
<td>0–2</td>
<td>dull, gray-green, limp tips</td>
</tr>
<tr>
<td>Bean (snap)</td>
<td>7</td>
<td>pitting and russetting</td>
</tr>
<tr>
<td>Cucumber</td>
<td>7</td>
<td>pitting, water-soaked lesions, decay</td>
</tr>
<tr>
<td>Eggplant</td>
<td>7</td>
<td>surface scald, Alternaria rot, seed blackening</td>
</tr>
<tr>
<td>Okra</td>
<td>7</td>
<td>discoloration, water-soaked areas, pitting, decay</td>
</tr>
<tr>
<td>Pepper</td>
<td>7</td>
<td>pitting, Alternaria rot, seed blackening</td>
</tr>
<tr>
<td>Potato</td>
<td>2</td>
<td>mahogany browning, sweetening</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>10</td>
<td>decay, especially Alternaria rot</td>
</tr>
<tr>
<td>Squash</td>
<td>10</td>
<td>decay, pitting, internal discoloration</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>10</td>
<td>water-soaking, softening, decay</td>
</tr>
<tr>
<td>Tomato (ripe)</td>
<td>7–10</td>
<td>poor colour when ripe, Alternaria rot</td>
</tr>
<tr>
<td>Tomato (mature-green)</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

The most noticeable visual symptoms of chilling injury in herbaceous plants are leaf and hypocotyls wilting (Mitchell and Madore, 1992; Frenkel and Erez, 1996), which often precedes the appearance of infiltration (water saturated areas) (McMahon et al., 1994; Sharom et al., 1994), the appearance of surface pits and large cavities (Dodds and Ludford, 1990; Cabrera et al., 1992; Frenkel and Erez, 1996), discoloration of leaves and internal tissues (Sharom et al., 1994; Yoshida et al., 1996; Tsuda et al., 2003), accelerated aging and rupture of chilled tissues, slow, incomplete or uneven ripening (Dodds and Ludford, 1990), accompanied by a deterioration of the structure and flavor (Harker and Main donald, 1994; Ventura and Mendlinger, 1999); increased susceptibility to decay (Cabrera et al., 1992), drying of the edges or tips of leaf blades (Hahn and Walbot, 1989) and in the case of prolonged chilling – leaf necrosis and plant death (Mitchell and Madore, 1992; Frenkel and Erez, 1996). According to Skog (1998), potential symptoms of chilling injury are surface lesions, water soaking of tissues, water loss, desiccation or shrivelling, internal discoloration, tissue breakdown, failure of fruit to ripen, or uneven or slow ripening, accelerated senescence and ethylene production, shortened storage or shelf life, compositional changes, loss of growth or sprouting capability, wilting and increased decay due to leakage of plant metabolites, which encourage growth of microorganisms, especially fungi.

A characteristic effect of chilling temperature on chilling-sensitive plants is growth slowing, more pronounced in susceptible species and varieties in comparison with the tolerant species (Ting et al., 1991; Rab and Saltveit, 1996a; Venema et al., 1999). In addition, there is a delayed development and lengthening of the growing season (Skrudlik and Koscielniak, 1996). At the same time apical cone differentiation is delayed, reducing the number of newly formed plant organs and the rate of their occurrence, the structure of roots is changed, and flowering rate, fruit and seed filling are reduced (Buiset et al., 1988, Barlow and Adam, 1989; Rab and Saltveit, 1996b; Skrudlik and Koscielniak, 1996; Lejeune and Bernier, 1996).

**Effect of chilling on the physiological processes in chilling-sensitive plants**

Incubation of chilling-sensitive plants at low temperatures induces disturbances in physiological processes: water regime, mineral nutrition, photosynthesis, respiration and total metabolism (Levitt, 1980; Wang, 1982; Graham and Patterson, 1982).

Water regime. Chilling of sensitive plants affects all components of water regime and causes loss of water, resulting in strong wilting (Vernieri et al., 1991; Boese et al., 1997; Bloom et al., 2004). It is based on the two main factors: rapid decline in the ability of roots to absorb water and transport it to the shoots (Bolger et al., 1992) and reduced ability to close stomata in response to subsequent water deficit (Pardossi et al., 1992; Wilkinson et al., 2001; Bloom et al., 2004). Insufficient water supply provokes rapid drop in water potential of leaves during the first hours of cooling (Wolfe, 1991; Boese et al., 1997). The degree of chilling damage of plants can be reduced by means of preventing the disturbance of the water regime (Vernieri et al., 1991; Boese et al., 1997).

Mineral nutrition. Low temperatures have an effect on mineral nutrition of plants. Absorption of ions by roots is difficult, as well as their movement in the above ground parts of plants. The distribution of nutrients between the plant organs is disrupted, with general decrease in the nutrient content in the plant.

Chilling of plants leads to a decrease in the activity of nitrate reductase, reduction in the nitrogen incorporation into amino acids and proteins, and a drop in the proportion of organic phosphorus and an increase in inorganic P content (Holobrada et al., 1981; Zia et al., 1994), which is a consequence of a breach of phosphorylation and enhanced decomposition of organic compounds. Mechanisms to reduce the absorption of...
nutrients by chilling temperatures include depression of respiration and/or oxidative phosphorylation, impair enzymatic transport systems associated with conformational proteins changes in membranes, changes in membrane potential, reducing the supply of ATP to H⁺-transporting ATPase, as well as lowering the permeability coefficients for ions (Clarkson et al., 1988).

Respiratory rate. The consequence of keeping plants at chilling temperatures is a change in respiratory rate. There is evidence of its decline, occurring as a result of destruction of the mitochondria structure, the general lowering of kinetic energy, and the inhibition of some enzymes (Lyons et al., 1979; Yoshida et al., 1989; Prasad et al., 1994 a; Lawrence, Holaday, 2000; Munro et al., 2004). Other authors have observed that an increase in respiratory activity during chilling and prolonged elevation of the respiration rate after cold exposure may indicate irreversible metabolic dysfunction and accumulation of incompletely oxidized intermediates (Wilson, 1978; Steward et al., 1990; Yadegari et al., 2008). The mechanism of stimulation is unknown, but it is possible to assume that it was the result of uncoupling of oxidative phosphorylation (Wang, 1982). It is also possible that the increased respiration reflects a reaction to the transfer of plants from chilling temperatures to the higher temperatures (Zauralov, Lukatkin, 1997). As a result of decreased respiration and increased consumption of energy-rich phosphates at chilling temperatures is a reduction of ATP levels (Takeda et al., 1995; Lawrence, Holaday, 2000).

Cold-tolerant crop species have greater temperature homeostasis of leaf respiration than cold-sensitive species (Yamori et al., 2009). Chilling reduces the cytochrome path of the electron transport in seedlings (Prasad et al., 1994; Reyes and Jennings, 1997) and enhances alternative respiratory pathways (Or dentlich et al., 1991; Purvis and Shewfeld, 1993; Gonzalez-Meier et al., 1999; Ribascarbo et al., 2000). Perhaps these alternative pathways play an important role in plant adaptation to chilling (Steward et al., 1990). They are triggered at the chilling period and increase with decreasing temperature (Or dentlich et al., 1991). These alternative pathways induced by chilling caused a decrease in superoxide generated in mitochondria (Purvis and Shewfelt, 1993; Hu et al., 2008).

Rate of photosynthesis. During and after chilling, the rate of photosynthesis in the leaves of chillingsensitive plants decreased and this is more related to decreasing temperature and lengthening of chilling period (Smith et al., 1999; Venema et al., 1999; Van Heerden et al., 2003; Garstka et al., 2003). The physiological reasons for the suppression of photosynthesis are the inhibition of phloem transport of carbohydrates from the leaves, stomatal limitation, destruction of the photosynthetic apparatus, damage to water-splitting complex of photosystem I, inhibiting electron transport, and uncoupling of electron transfer and energy storage, changes in the activity and inhibition of synthesis of key enzymes of the Calvin cycle and C₄ way (Yordanov, 1992; Nie et al., 1992; McMahon et al., 1994; Gesch and Heilman, 1996; Yoshida et al., 1996; Terashima et al., 1998; Kingston-Smith et al., 1999; Venema et al., 1999; Van Heerden et al., 2003; Garstka et al., 2007). Cold-sensitive crop species have smaller temperature homeostasis of leaf photosynthesis than cold-tolerant species (Yamori et al., 2009).

The theory of chilling injury
In the initial period of studying, the influence of low temperatures on chilling-sensitive plants was widespread theory Sachs about the death of plants due to disorders of water regime. However, subsequent studies have shown one-sided interpretation of these data.

Changes in water regime were likely due to disturbances of other processes. In the middle of the 20th century it was found that the wilting of the aerial organs is not due to excessive transpiration over slow supply of water by roots, but is the result of lowering water-holding capacity due to disorganization of the cytoplasm structure and metabolic decompensation.

Based on observations of changes in protoplasmic viscosity at low temperatures, it has been suggested that this cell property plays a key role in the damage (Belehradek, 1935). The less tolerant plants to cold, the higher temperature at which cytoplasm gelling occurs and the faster increases the viscosity of the cytoplasm. At considerable increase in viscosity the rate of biochemical reactions in the cytoplasm is decreased, the metabolismis disturbed, which leads to dysfunction of physiological processes. However, it was shown that cucumber plants decreased viscosity with increasing chilling duration, and the worst after 2.5–4 days, and then increased gradually, reaching viscosity level of non-chilled plant, but shortly before the complete withering away could exceed this level. An increase in viscosity of highly damaged plants also continued after the transfer into heat. “Dying” increase protoplasmic viscosity and is the final stage of death, which has no relation to the first stage of damage, but only deepens it.
Among the hypotheses about the primary events that trigger the occurrence of reaction to lower temperatures, hypothesis of phase change prevailed in the 1970s, according to which the chilling-induced changes in the molecular ordering of membrane lipids may be the cause of chilling injury (Raison et al., 1971). These changes increase the damage by lowering the ATP levels, metabolic imbalances and increasing membrane permeability (Lyons, 1973). However, all these changes do not occur immediately after the start of chilling and are likely to be secondary disorders (Minorsky, 1985). The increase in membrane permeability due to the low-temperature exposure (“membranes leakage”) should be quick, registered in the first few minutes after placing the tissue at chilling temperatures, in accordance with the hypothesis of phase transitions. In reality, this does not happen, and often passive permeability is not increased (Patterson et al., 1979).

Moreover, the increase in electrolyte leakage is likely due to chilling-induced water stress, which has been revealed to be considerably weaker in an atmosphere saturated with water (Guinn, 1971). At the same time it is noteworthy that the low saturation of membranous phospholipides, which is generally determined, gives sensitivity to low temperatures to chilling-sensitive plants (Zhu et al., 2008). The data about the introduction of genes of fatty acid desaturases in a genome of chilling-sensitive plants confirm that this gives sensitive plants more pronounced chilling resistance (Kodama et al., 1994; Ishizaki-Nishizawa et al., 1996; Hamada et al., 1998; Murata and Tasaka, 1997).

In recent years, special attention of researchers has been drawn to two hypotheses to explain the induction of chilling damage to a rapid increase in the concentration of free cytosolic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{cyt}\)) (Minorsky, 1985) and the occurrence of oxidative stress upon chilling of chilling-sensitive plants (Hariyadi and Parkin, 1993). Minorsky (1985) proposed a hypothesis to explain most of the secondary effects of chilling shock, which suddenly increases (by 1–2 orders) in the concentration of [Ca\(^{2+}\)]\(_{cyt}\). It is assumed that the rapid increase in [Ca\(^{2+}\)]\(_{cyt}\) due to chilling, may serve as the primary physiological signal of cold exposure. It was shown that changes in intracellular calcium compartmentation in chilled plants, leading to an increase in [Ca\(^{2+}\)]\(_{cyt}\), stop cytoplasmic streaming and affect the subcellular structures (Woods et al., 1984). There is evidence that input of \(^{45}\)Ca\(^{2+}\) in maize root cells increased by 20–25% at a temperature of 2°C (Zocchi, Hanson, 1982). Changes in [Ca\(^{2+}\)]\(_{cyt}\) trigger cascade reactions in the cell, which leads to numerous disturbances at all levels of an organization. Our investigation shows that chilling induces abrupt reduction of Ca\(^{2+}\)-ATPase activity, which pumps out Ca\(^{2+}\) in apoplast and/or in intracellular deposits. So, this enhances the [Ca\(^{2+}\)]\(_{cyt}\) level in cytoplasm. During the growth of maize seedlings on nutrient media with different calcium status more intense chilling injury was observed at reduced or enhanced Ca\(^{2+}\) doses in comparison to optimal dose (Lukatkim and Isaikina, 1997). In recent years, the calcium hypothesis has been further developed in view of oxidative stress that occurs when cooling the chilling-sensitive plants. Oxidative stress occurs during cooling of chilling-sensitive plants plays a leading role in the transduction of chilling injury (Hu et al., 2008). The reason why production of free radicals and reactive oxygen species (ROS) increased is singlet oxygen, superoxide anion, hydroxyl radical, hydrogen peroxide (Suzuki and Mittler, 2006). These ROS cause considerable damage to membrane lipids and other cellular components (Lukatkim et al., 1995; Lukatkim, 2003). It was shown that [Ca\(^{2+}\)]\(_{cyt}\) changes are intimately connected to an oxidative stress. Oxidative stress causes an immediate increase in cytosolic calcium (Price et al., 1994), acting as the same as chilling shock (Knight et al., 1996).

This reaction is transient, and finishes within 1–2 minutes. Inturn, [Ca\(^{2+}\)]\(_{cyt}\) influences a level of free radicals, inhibiting activity of SOD (Price et al., 1994). So, increasing the concentration of ionized calcium causes increased oxidative stress (Price et al., 1994; Lock and Price, 1994), i.e., is the signal amplification cascade that causes chilling damage.

**Ways to improve chilling tolerance of chilling-sensitive plants**

At the present time, to improve the chilling tolerance of sensitive plants, various techniques are used, which can be divided into several groups: the thermal effect, chemical treatment, the use of cellular and genetic engineering.

Thermal effects includes low-temperature hardening, thermal conditioning, intermediate arming, and the effect of heat stress. The basis of seed and seedling hardening of chilling-sensitive plants to cold, which has long been used in practical plant breeding, is the adaptation of the organism in the early stages of development, accompanied by the emergence of specific structural and functional re arrangements. Low-temperature hardening process is associated with the protein-synthesizing system and is accompanied by a rearrangement of the hormonal system of plants.

Intermediate warming is another way of thermal regulation of chilling injury. Transfer of the chilled plants in the warm afternoon prevented the appearance of visible symptoms of damage, impaired inhibition of photosynthesis and transpiration, reduced leaf osmotic potential (Koscielniak et al., 1996b; Koscielniak and Biesaga-
Koscielniak, 2000; Skrudulik et al., 2000). Intermediate warming is often used for storage of chilling-sensitive plants’ fruits (Wang, 1993). It is assumed that the temporary placement in heat allows the chilled tissues to metabolize toxic substances that accumulate during the chilling process, or helps to restore the compounds in tissues that are depleted during chilling (Lyons, 1973).

Cytokinins and ABA were most effective of all plant growth regulators (Duncan and Widholm, 1991; Mitchell and Madore, 1992). Non-hormonal growth regulators are used also in order to improve the chilling tolerance of cultivated plants. These include pkl obtutrazol, chlocholin chloride, me fluidid „unikonazol and other triazoles (Lurie et al., 1994; Feng et al., 2003). The treatment by antioxidants and freeradicals quenching (ethoxyquin, sodium benzoate, glutathione, tyrene, formate, ascorbate, diphenylamine, α-tocopherol, propyl gallate) can slow down the degradation of unsaturated fatty acids and reduce chilling damage in chilling-sensitive plants, leaves and fruits (Lukatkin and Levina, 1997; Michaeli et al., 1999; Xu et al., 2000; Kocsy et al., 2001).

Cellular and genetic engineering is a new trend, which allows fundamental changes in the chilling resistance of chilling-sensitive plants. They are based on a large genetic variability in components, controlling sensitivity, on the one hand, and on the development of gene transfer technology, transformation and selection markers, on the other hand (Greaves, 1996). So, screening the surviving cells during chilling of callus and suspension cultures and subsequent plant regeneration yielded plants with increased epigenetic resistance to chilling temperatures (Dix, 1979; Lukatkin, 2010).

**CONCLUSION**

The literature review shows that the exposure of chilling-sensitive plants to low temperatures leads to disturbances in all physiological processes – water regime, mineral nutrition, photosynthesis, respiration and metabolism. Inactivation of metabolism, observed at chilling of chilling-sensitive plants is a complex function of both temperature and duration of exposure. Response of plants to low temperature exposure is associated with a change in the rate of gene transcription of a number of low molecular weight proteins. Based on the authors’ own research and the literature data, the concept of cold damage was proposed, which highlighted the leading role of oxidative stress in the induction of stress response. According to this concept, there were distinguished possible ways to improve cold tolerance, which were combined into several groups: the thermal effect, chemical treatment and the use of gene and cell engineering.

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