

Molecular Responses of Soybean to Cold Acclimation

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Abstract: Low temperature damage is a common problem for early-planted soybean, because it is a tropical plant and is sensitive to low temperatures. Soybean (*Glycine max cv. Williams*) is a tropical crop, but is also grown in temperate regions in middle spring to late summer. This crop has an important role in human diet. Cold temperature damage is a common problem for this plant in temperate regions. Physiological responses to chilling, including, Malondialdehyde (MDA), total protein, Relative Water Content (RWC) and soluble sugar contents were investigated in soybean to identify mechanisms of chilling tolerance. Seedlings were exposed to 15°C (cold-acclimated) or 25°C (nonacclimated) for 24 h, under 250 $\mu\text{mol}/\text{m}^2/\text{sec}$ Photosynthetically Active Radiation (PAR). Then, all plants were exposed to chilling temperature at 4°C for 24 h and allowed to recover at 25°C for 24 h. Relative water content, MDA and total protein contents showed that cold-acclimated plants were less affected by chilling compared to nonacclimated plants. Cold-acclimated plants also recovered faster from chilling injury than nonacclimated plants.

Key words: Acclimation, chilling, MDA, RWC, protein, soluble sugar

INTRODUCTION

Low temperature is one of the abiotic stresses that are principal cause of crop failure world wide, dipping average yields for most major crops (Bray *et al.*, 2000). The plants which are native to warm habitat, such as maize (*Zea mays*), soybean (*Glycine max*) and cotton (*Gossypium hirsutum*), exhibit symptoms of injury when exposed to low non-freezing temperatures (Lynch, 1990). These plants are particularly sensitive to temperatures below 10-15°C and exhibit signs of injury (Lynch, 1990; Gilmour *et al.*, 1988; Hopkins, 1999). The symptoms of stress induced injury in these plants appear from 48-72 h, later, however, this duration varies from plant to plant and also depend upon the sensitivity of individual plant to cold stress (Shilpi and Narendra, 2005). Sugars appear essential in plant cold acclimation, as shown for example by the inability of an *Arabidopsis* sucrose synthase mutant to cold acclimation (Annikki and Palva, 2006) or the requirement for light in low-nonfreezing temperature-induced cold acclimation connected to sugar accumulation (Wanner and Junttila, 1999). Precise function of sugars is not known, but their high abundance in cold acclimated plants suggests a role in osmoregulation and less abundant sugars might also have a role in cryoprotection or as signaling molecules (Annikki and Palva, 2006). It is now known that exposure of chilling-sensitive plants, such as maize and tomato, to

temperatures slightly above chilling reduces chilling injury (Anderson *et al.*, 1995; Prasad, 1996; Scebbba *et al.*, 1999; Venema *et al.*, 2000). It has been reported that some chilling-sensitive plants acclimate if they are exposed to a low temperature slightly above the threshold chilling temperature, in a process analogous in some respect to the acclimation that occurs in perennial plants in the autumn (Daie and Campbell, 1981). Several factors involve in cold acclimation, such as plant hormones, especially Abscisic Acid (ABA), Ethylene (ET) and Gibberellic Acid (GA), proteins and carbohydrates (Annikki and Palva, 2006). Microtubules are key candidates for pronounced cold sensitivity of cell growth and depolymerize in response to low temperatures. During low temperature, ABA level raises. Elevated levels of ABA prevent microtubular destruction, which appears in response to chilling (Wang and Nick, 2001). Ethylene appears to be involved in cold acclimation in some plants, because of its increased levels during cold acclimation and ability of endogenous ethylene to induce number of antifreeze proteins (Yu *et al.*, 2001). Gibberellic acid has been suggested to function as an ABA antagonist during cold acclimation (Annikki and Palva, 2006).

Since, soybean is sensitive to chilling temperatures, so, we investigated the effect of low temperature pretreatment on soybean's tolerance to chilling, which is one of the strategies to protect plants from chilling damage.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max cv. Williams*) were purchased from oilseeds center, Ardabil and were soaked in water for 6 h at 25°C and then were germinated in Petri dishes on 2 layers of filter paper for 48 h at 25°C in an incubator. Subsequently the seedlings were transferred to pots containing washed sand (4 seedlings per pot) and were watered with half-strength Hoagland nutrient solution. The plants were grown at 27/25°C (day/night) temperature, 70% relative humidity, with a 16/8 h day/night photoperiod under 250 $\mu\text{mol}/\text{m}^2/\text{sec}$ photosynthetic photon flux density. Seedlings at the 3-leaf stage were placed at 15°C (cold acclimated) or 25°C (nonacclimated) for 24 h. The acclimated and nonacclimated seedlings were then exposed to chilling at 4°C for 24 h and allowed to recover for 24 h at 25°C. Harvesting was done at the same time each day to avoid complications from diurnal fluctuations in biochemical processes. Experiments were conducted from May to July in 2007 at Biochemistry Lab, Department of biology, Faculty of Science, Urmia University, Iran. Means were separated by Tukey Multiple Range Test at $p = 0.05$. Values are the mean \pm SE of 3 replicates.

Total protein content: Total protein content was measured using Lowry method (Lowry *et al.*, 1951).

Relative water content: Chilling injury on leaves and roots was evaluated by changes in relative water content (RWC). Relative water content was calculated using the formula has become below (Yong *et al.*, 2003):

$$\text{RWC} = \frac{\text{One-dry weight of tissue}}{\text{Fresh weight of tissue}} \times 100$$

Soluble sugar content: Soluble sugars content was measured using phenol sulphoric method (Dubios *et al.*, 1956). The amount of 0.5 g of plant tissues (leaf or root) were sampled and homogenized with a mortar and pestle in 5 mL distilled water and then was filtered through Whatman No. 1 filter paper. Two milliliter of filtered extract transferred into tube and 1 mL of phenol 5% (w/v), 3 mL of sulphoric acid 98% were added. After 1 h the absorbance was recorded at 485 nm using UV-visible spectrophotometer (WPA model S2100).

Lipid peroxidation: Lipid peroxidation was estimated by the level of malondialdehyde (MDA) production by a slight modification of the thiobarbituric acid (TBA) method described by Buege and Aust (1978). Absorbance at 532 nm was recorded and corrected for nonspecific

absorbance at 600 nm. MDA concentrations were calculated by means of an extinction coefficient of 156/mM/cm and the following formula (Zhnyuan and Bramlage, 1992):

$$\text{MDA } (\mu\text{mol g}^{-1} \text{ fresh wt.}) = \frac{A_{532} - A_{600}}{156} \times 10^3 \times \text{dilution factor}$$

RESULTS AND DISCUSSION

The increase in chilling tolerance that occurs with cold acclimation is thought to involve the activation of multiple chilling tolerance mechanisms. Here, we showed that changes in multiple metabolites such as total protein, soluble sugars and MDA contents that are commonly observed to occur in plants during cold acclimation. There is evidence to indicate that each of these classes of biochemical alternations MDA (Kacperska, 1989; Williams *et al.*, 1988) and protein (Annikki *et al.*, 2002; Kee-Yong *et al.*, 2004) contribute to an enhancement of chilling tolerance. Cold acclimation proteins may play a physiological role similar to that of Heat Shock Proteins (HSPs) in protecting organisms from injury at low temperatures (Yeh *et al.*, 1997). Genes encoding Early Light-Induced Protein (ELIP) were found to be the most highly upregulated in cold acclimated plants (Wei *et al.*, 2005). HSPs in cells are vital for increasing thermotolerance (Yeh *et al.*, 1997; Kee-Yong *et al.*, 2004). Heat shock proteins are associated with plasma lemma and are thought to be physiologically important in reducing cellular leakage of solutes in soybean seedlings (Lin *et al.*, 1984). Like HSPs, cold acclimation proteins are associated with nuclei, mitochondria and ribosomes (Chen, unpublished data), which may explain why lower amount of amino acids and ions were found in the leakage of, chilled, cold acclimated seedlings than in that of chilled, nonacclimated seedlings. Chang *et al.* (2000) showed that proteins in the cell sap of cold acclimated mungbean seedlings were about 60% higher than the control seedlings. In this research, total protein content was increased in cold-acclimated and nonacclimated plants. Cold-acclimated plants recovered faster than nonacclimated plants in recovery phase (Fig. 1 and 2).

The lipid membrane is composed of a mixture of phospholipids and glycolipids that have fatty acid chains attached to carbon 1 and 2 of the glycerol backbone by an ester linkage. The peroxidation reactions differ among these fatty acids depending on the number and position of the double bounds on the acyl chain. Oxidation of unsaturated fatty acids by singlet oxygen produces distinctly different products (Kacperska, 1989), such as malondialdehyde (MDA). In this research, MDA content

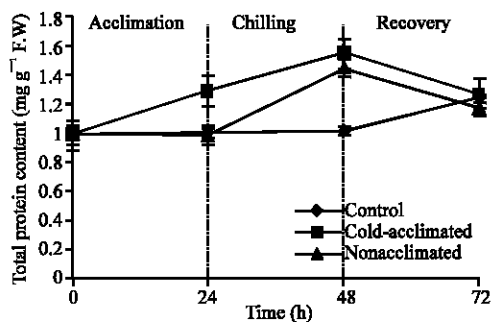


Fig. 1: Changes in root total protein content (mg g^{-1} F.W) in cold-acclimated and non-acclimated soybean roots during acclimation, chilling and recovery. Mean of 3 measurements \pm SE. $p = 0.05$

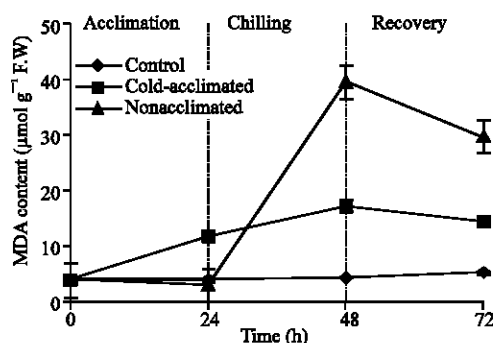


Fig. 4: Changes in root MDA content ($\mu\text{mol g}^{-1}$ F.W) in cold-acclimated and non-acclimated soybean leaves during acclimation, chilling and recovery. Mean of 3 measurements \pm SE. $p = 0.05$

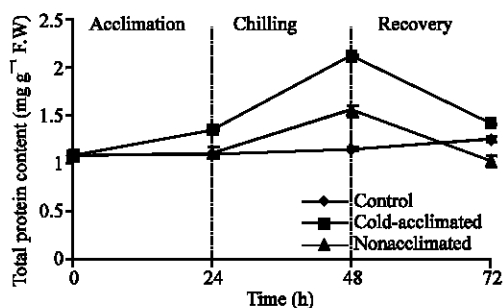


Fig. 2: Changes in leaf total protein content (mg g^{-1} F.W) in cold-acclimated and non-acclimated soybean leaves during acclimation, chilling and recovery. Mean of 3 measurements \pm SE. $p = 0.05$

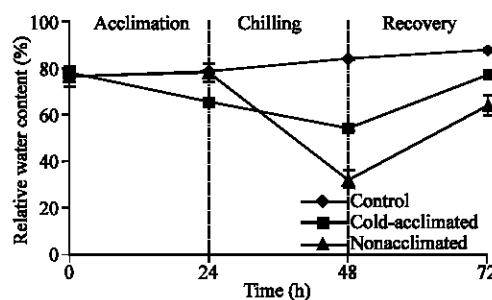


Fig. 5: Changes in root relative water content (%) in cold-acclimated and non-acclimated soybean roots during acclimation, chilling and recovery. Mean of 3 measurements \pm SE. $p = 0.05$

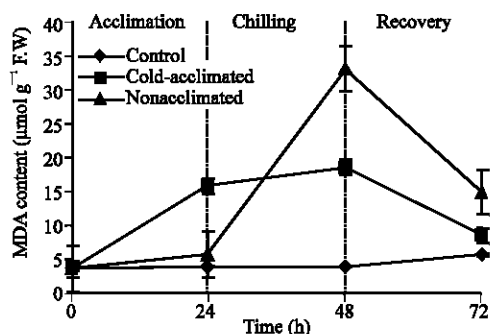


Fig. 3: Changes in leaf MDA content ($\mu\text{mol g}^{-1}$ F.W) in cold-acclimated and non-acclimated soybean leaves during acclimation, chilling and recovery. Mean of 3 measurements \pm SE. $p = 0.05$

of nonacclimated leaves was higher than cold acclimated ones in recovery phase. Cold acclimated plants recovered faster than nonacclimated leaves. It shows that pretreatment of cold temperature (15°C) can tolerate soybean plant against subsequent chilling temperature (4°C) (Fig. 3 and 4).

The minimal impact of chilling on relative water content (RWC), high photosynthetic efficiency of cold-acclimated plants and the full recovery of carbon fixation capability of cold-acclimated plants after chilling. Typical symptoms of chilling injury are wilting, yellowing of leaves and inhibition of growth. Leaf fresh weights of cold-acclimated and nonacclimated soybean seedlings were reduced in chilling period (Fig. 5 and 6). Leaf and root fresh weight of nonacclimated plants rapidly declined when exposed to chilling temperature and did not recover from the chilling treatment properly. Leaf and root fresh weight of cold-acclimated plants also declined significantly 24 h after exposure to chilling temperature. Although, the cold-acclimated plants did not completely recover from chilling injury compared with untreated control plants, the cold-acclimated plants showed a general increase in leaf and root weight compared with nonacclimated plants during the recovery period.

Soluble sugars function as cryoprotectants and osmolites that protect cells from freezing damage (Xin and Browse, 2000). Huixia Shou *et al.* (2004) showed that 24 and 48 h cold acclimations significantly increased the

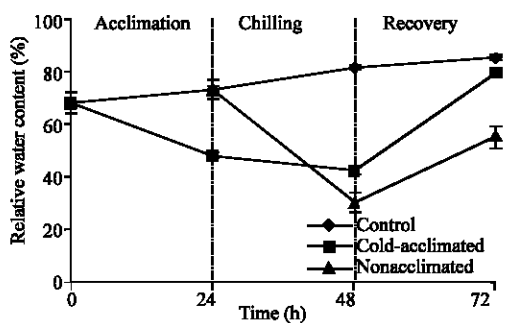


Fig. 6: Changes in leaf relative water content (%) in cold-acclimated and non-acclimated soybean leaves during acclimation, chilling and recovery. Mean of 3 measurements±SE. p = 0.05

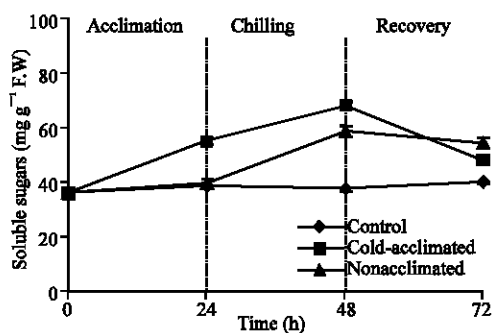


Fig. 7: Changes in root soluble sugars (mg g⁻¹ F.W) in cold-acclimated and non-acclimated soybean roots during acclimation, chilling and recovery. Mean of 3 measurements±SE. p = 0.05

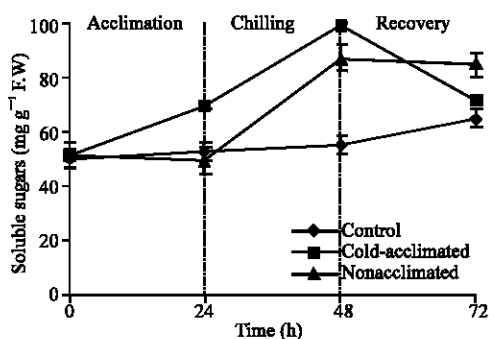


Fig. 8: Changes in leaf soluble sugars (mg g⁻¹ F.W) in cold-acclimated and non-acclimated soybean leaves during acclimation, chilling and recovery. Mean of 3 measurements±SE. p = 0.05

soluble sugar levels in plants. Accumulation of sucrose and other simple sugars that occurs with cold acclimation also contributes to the stabilization of membrane as these molecules can protect membranes against freeze-damage

because increase in sugar content lowers the freezing point of cell solution (Shilpi and Narendra, 2005). Especially sucrose can increase the effectiveness of the heat stable proteins in preventing protein denaturation (Robertson *et al.*, 1994). Soluble sugar levels were increased in roots. In cold-acclimated roots, soluble sugars content were started to increase in acclimation phase and downed in recovery phase. But in nonacclimated roots they increased in chilling phase suddenly (Fig. 7). In leaves changes were significant (Fig. 8). Soluble sugars content in nonacclimated leaves, in acclimation phase, decreased, then increased suddenly in chilling phase and subsequently decreased in recovery phase.

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