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Effect of short-term cold stress on oxidative damage and transcript accumulation of defense-related genes in chickpea seedlings

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ABSTRACT

Cold stress affects many plant physiological and biochemical components and induces cascades of alterations in metabolic pathways, amongst them the membrane fatty acid compositions, the activity of antioxidative enzymes and the regulation of gene expression. The present work aimed to characterize the changes of some of these factors in both cold acclimated (CA) and non-acclimated (NA) plants of chickpea (*Cicer arietinum* L.) to identify the role of the acclimation process in adjusting plant responses to severe cold stress. The results showed an increase in the unsaturated fatty acids (UFAs) ratio compared to saturated fatty acids, which was more obvious in CA plants. Defense enzymes had an important role in CA plants to create greater cold tolerance compared to NA ones in the cases of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) and lipoxygenase (LOX) activities. During cold stress, a high transcription level of *CaCAT* and *CaSOD* genes was detected in CA plants, but a low transcription of *CaLOX* gene was observed in CA plants compared to NA plants, which might have prevented the decline of UFAs (confirmed by double bond index (DBI) data). Moreover, the transcription level of the *Carubisco* gene, as an energy producing agent, was higher in CA plants than in NA plants and the transcription of the *Catubulin* gene, as a crucial substance of cell cytoskeleton, showed a decreasing trend in both CA and NA plants, but this decline was greater in NA plants. These responses showed the possible targets of cold stress as chloroplast and signal transduction to balance stress programs. The above results indicate the crucial role of FA compositions in creating cold tolerance in susceptible chickpea plants with possible responsive components and the possible interactions in protein and transcript levels even in facing extreme cold stress.

Introduction

Cold stress, which includes both chilling (less than 10 °C) and freezing injury (less than 0 °C), is one of the most significant abiotic stresses of agricultural plants, causing micro-organelle disruption, phase transition in cell membrane lipids, reactive oxygen species (ROS) production, as well as the inhibition of crop growth and development, which consequently reduces crop yield and production (Thakur et al., 2010; Kim et al., 2013). While freezing is lethal to most plants, winter annual and perennial plants in temperate climate zones mostly survive due to acclimation processes realized by exposure of plants to low but non-freezing temperatures (Bohn et al., 2007).

Numerous investigations of different aspects of cold acclimation and, in particular, the recently developed methods of metabolomic profiling, have detected changes in nearly all aspects of plant metabolism (Cook et al., 2004; Kaplan et al., 2004). Temperature reduction affects membrane-linked processes due to membrane fluidity and permeability (Los et al., 2013). To assess changes in membrane solute and electrolyte leakage, malondialdehyde (MDA) content as the final product of lipid peroxidation, lipoxygenases activity (LOX) as a responsible factor of membrane degradation and unsaturated fatty acid (UFAs) ratio compared to saturated (SFAs) ones as fluidity and stability preserving factor can demonstrate a reliable path to realize these processes.

Abbreviations: CA, cold acclimated; CAT, catalase; DBI, double bond index; ELI, electrolyte leakage index; FAs, fatty acids; FM, fresh mass; GC, gas chromatography; GPX, guaiacol peroxidase; JA, jasmonic acid; LOX, lipoxygenase; MDA, malondialdehyde; NA, non-acclimated; QPCR, quantitative reverse-transcriptase polymerase chain reaction; ROS, reactive oxygen species; SFAs, saturated fatty acids; SOD, superoxide dismutase; UFAs, unsaturated fatty acids; VLCFAs, very long chain fatty acids.

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Further, membrane damage leads to the production of reactive oxygen species (ROS), which cause oxidative stress (Senthil-Kumar et al., 2007). ROS affect many cellular functions by damaging carbohydrates, nucleic acids, oxidizing proteins, and causing lipid peroxidation (Foyer and Noctor, 2005). However, cells are equipped with excellent antioxidant defense mechanisms to detoxify the harmful effects of ROS (Gill and Tuteja, 2010). Therefore, studying the activity of these antioxidants, including superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (GPX), may help us to recognize this mechanism accurately. During stresses, proline acts as a cryoprotection, carbon and nitrogen storage, pH stabilizer, cell redox balancer, and stress-related growth-regulating signal so that plants with elevated proline levels have been reported to exhibit enhanced tolerance to abiotic stresses (Maggio et al., 2002; Rai and Penna, 2013). Changes in proline content can therefore be considered as an important factor in plant adaptation to abiotic stresses such as cold. The acclimation process also involves expression of diverse stress-responsive genes to maintain metabolic homeostasis during stress or to be able to re-establish subsequent to the stress period (Senthil-Kumar et al., 2007). It thus seems necessary to examine the program at the molecular, and particularly transcriptional level, surveying *SOD*, *CAT*, and *LOX* gene expressions. Furthermore, assessing *rubisco* and *tubulin* gene expression can be helpful in understanding the nature of the induced cold stress effect on plants. It is now well established that *in vivo* rubisco activity is rapidly regulated to control the flux through the photosynthetic carbon reduction cycle in response to fluctuations in the environment (Galmes et al., 2013). On the other hand, microtubules are key elements of the cytoskeleton and have recently come to be appreciated for their role in signaling and regulation. They are critical conduits for cellular trafficking and can serve as a template for the interaction of signaling proteins (Farajalla and Gulick, 2007).

Chickpea (*Cicer arietinum* L.), the world's second most important food legume, is globally cultivated on an area of 13.20 million hectare with an annual production of 11.62 million tons. The global demand for chickpea in 2020 is projected to be 17.0 million tons (Varshney et al., 2014). In the Mediterranean area, sowing earlier or as autumn or spring cropping provides chickpea with a more durable growth season, efficient use of soil moisture and higher yield (Clarke and Siddique, 2004). However, a lack of cold tolerance causes yield losses in different chickpea growth stages. Many plants increase cold tolerance due to the cold acclimation process that allows plants to develop essential tolerance for cold stress survival through multiple levels of biochemical and cell biological changes. These responses are due to reprogramming of gene expression that results in the adjusted metabolic alterations (Heidarvand and Maali Amiri, 2010). Our aim was to evaluate the cold acclimation effect on severe cold shocks so that winter sowing of this plant in warm climates can be possible. According to the results of our last study, cold acclimation has a positive effect on chickpea plants during long-term cold stress (2 days in 4 °C) (Kazemi Shahandashti et al., 2013). However, information about plant responses involved in defense during short-term cold stress is not available. Generally, the responses of genes to cold stress are grouped into two categories, the "early" and "late" (Schade et al., 2004; Yun et al., 2010). The early response, which is a rapid-transient response in the short-term, is the determinative factor in the late response, which sustains the plant during long-term cold stress and helps it to survive (Hannah et al., 2005). Although responses under short-term cold stress can be different in the medium- or long-term, significant changes in gene expression occur in the short-term (Hannah et al., 2005). Also, it was assumed that in addition to long-term stress, short-term stress and physio-biochemical assays can be used to evaluate cold stress tolerance of chickpea profitably in a short time and at a low cost. Thus, monitoring the dynamic of plant responses throughout the short-term cold stress provides an opportunity to discover and study targets in response to the stress (Heidarvand and Maali-Amiri, 2013). In this study, we evaluate a set of responses (physiological, biochemical and molecular) in acclimated and non-acclimated chickpea Jam plants (local cultivar) during short-term cold stress (15 min in −10 °C), which is called cold shock, on plants. Such an experiment can likely illustrate the genetic tolerance of chickpea in response to cold environmental stress.

Materials and methods

Plant material and growth conditions

Sterilized (by 10% (v/v) sodium hypochlorite for 10 min) seeds of chickpea Jam (local cultivar) from the Maraghe institute were germinated for 3 days in the dark at 23 °C on filter paper soaked with distilled water. After germination, seedlings were transferred to pots in a growth chamber (day/night regime: 16/8 h, temperature: 23 °C, light intensity: 200 $\mu mol\ m^{-2}\ s^{-1}$ irradiance and relative humidity: 75%. Chickpea seedlings used in the experiment were 20 days including the time for germination. These seedlings were approximately 20 cm height with at least five branches of 5–8 cm. All measurements were made on the middle leaves from the apex of all plants in each treatment. The seedlings were considered to be of similar physiological age (Hurry and Huner, 1991). Twenty-day-old plants were divided into two groups. Inducing thermal treatment, one group was maintained under control conditions (23 °C) and the other group was moved to another growth chamber with 10 °C as acclimation phase for five days. By the end of the fifth day, the temperature of growth chamber was shifted to −10 °C and both groups of plants (acclimated and non-acclimated) faced cold stress for 15 min. Thus, in this study, our experiments were focused on four groups of chickpea plants: Samples from unstressed plants were collected as control plants (23 °C), acclimated plants (in the end of acclimation phase), the acclimated plants faced cold stress (CA plants) and non-acclimated plants faced cold stress (NA plants). Samplings occurred at the end of each phase. Physiological experiments consisting of electrolyte leakage index (ELI) and MDA were conducted using fresh leaves, while other experiments were performed using samples flash frozen in liquid nitrogen and stored at −80 °C.

Electrolyte leakage index (ELI)

Cell membrane permeability was assessed by ELI in damaged tissues harvested in thermal treatments according to our previous study (Nazari et al., 2012).

Lipid peroxidation analysis

The measurement of lipid peroxidation in leaves, the thiobarbituric acid test, which determines MDA as an end product of lipid peroxidation, was conducted according to our previous study (Heidarvand and Maali-Amiri, 2013) and expressed in $\mu mol\ g^{-1}$ fresh mass (FM).

Soluble protein content and antioxidant activities

Total protein content was determined according to Bradford (1976) and expressed in $mg\ mL^{-1}$ protein. SOD activity (EC-number: 1.15.1.1) was assayed according to our previous study (Kazemi Shahandashti et al., 2013) and expressed in $U\ min^{-1}\ mg^{-1}$ protein for each sample. CAT activity (EC-number: 1.11.1.6) was determined by monitoring the initial rate of disappearance of H_2O_2 according to our previous study (Kazemi Shahandashti et al., 2013) and expressed in nmol of H_2O_2 decomposed $min^{-1}\ mg^{-1}$ protein assuming extinction coefficient of $39.4\ cm^{-1}\ mM^{-1}$. GPX