Plant Physiology and Biochemistry 122 (2018) 31–39

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Effect of cold stress on oxidative damage and mitochondrial respiratory properties in chickpea

Samaneh Karami-Moalem, Reza Maali-Amiri*, Seyyedeh-Sanam Kazemi-Shahandashti

Department of Agronomy and Plant Breeding, University College of Agriculture and Natural Resources, University of Tehran, 31587-77871, Karaj, Iran

ARTICLE INFO

Keywords:
Alternative oxidase
Chickpea
Cold stress
Damage indices
Gene expression
Mitochondrial responses

ABSTRACT

The present work aimed to characterize some of the mitochondrial and defense responses involved in tolerance to cold stress (CS) in tolerant (Sel96Th11439) and sensitive (ILC533) chickpea (*Cicer arietinum* L.) genotypes. During CS, the tolerant genotype prevented the H_2O_2 accumulation significantly; led to a decrease in electrolyte leakage index (ELI), which was a sign of relative acclimation of this genotype compared to sensitive one. A significant positive correlation between ELI and H_2O_2 ($r_{0.01} = 0.86$) content confirmed these results. Under cold, a significant increase in the alternative oxidase (AOX) activity was observed in tolerant genotype compared to sensitive one. In parallel, the high activity of superoxide dismutase (SOD) accompanied with catalase (CAT) and ascorbate peroxidase (APX) activities and also the extreme amounts of ascorbate and proline certified the active reactive oxygen species (ROS)-scavenging systems. There was a significant negative correlation between damage indices like H_2O_2 content and the activity of AOX ($r_{0.01} = -0.79$) as well as significant positive correlation between AOX activity with CAT ($r_{0.05} = 0.61$), SOD ($r_{0.05} = 0.51$) and APX activity ($r_{0.05} = 0.52$). The increasing succinate dehydrogenase (*CaSDH*), *CaAOX* and cytochrome c oxidase (*CaCOX*) gene expression showed an enhancing response of respiration under CS in tolerant plants compared to sensitive ones. The increasing trend of phosphoenol pyruvate carboxylase (PEPC) activity in tolerant genotype particularly in the sixth day of CS indicated the recovered performance of metabolism pathways. Therefore, the increase of AOX activity along with other defensive mechanisms could be coordinately related to cold tolerance mechanisms in order to alleviate cold-induced oxidative stress in chickpea.

1. Introduction

The cold is one of the most common abiotic stresses which can be considered as a great threat for plants cultivation, productivity and even survive (Heidarvand and Maali-Amiri, 2010). Though, plants as the immobile creatures recognize the temperature seasonal changes, and then respond and conserve appropriate temperamental balance in their cells. This cellular equilibrium is created through morphological, physiological and biochemical adjustments which can result in cellular adaptation, derived of the alteration in their genes expression status.

Applying special strategies, tolerant crops can sustain their capacity for autumn sowing. Chickpea (*Cicer arietinum* L.) which is ranked as the third food legume is sown as an autumn or spring plant in Mediterranean climates (Heidarvand and Maali-Amiri, 2013). Earlier sowing of chickpea provides a more durable growth season, however a lack of cold tolerance (CT) mechanism causes reductions in growth, development and crop yield (Kazemi-Shahandashti et al., 2014). Therefore, recognizing the characterizations of plants cell responses during cold stress (CS) will help us improve CT development strategies.

As one of the major cell responses, mitochondria are briskly involved in the stress tolerance mechanism through expression, activity and interactions of many classical components, for instance the energy-dissipating elements like alternative oxidase (AOX) (Borecký and Vercesi, 2005; Rurek et al., 2015). In normal conditions, plant cell applies cytochrome pathway in its mitochondrial electron transfer chain (ETC) that leads to ATP production through proton motive force. Cytochrome c oxidase (COX) is the last respiratory complex of mitochondrial respiratory chain in this pathway (Dinakar et al., 2016). On the other hand, in stress conditions, AOX pathway protects the integration among respiration and other metabolic processes by forming the H_2O molecule from the intracellular oxygen-water concentration and prevents the blockade of electron flow. This fact has been proved by many studies in which flexibility of mitochondrial metabolism was enhanced sharply along with increase in AOX activity and its transcript

Abbreviations: AOX, alternative oxidase; APX, ascorbate peroxidase; CAT, catalase; CS, cold stress; CT, cold tolerance; COX, cytochrome c oxidase; ELI, electrolyte leakage index; ETC, electron transfer chain; PEPC, phosphoenol pyruvate carboxylase; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SOD, superoxide dismutase; TCA, tricarboxylic acid cycle

* Corresponding author.
E-mail address: rmamiri@ut.ac.ir (R. Maali-Amiri).

https://doi.org/10.1016/j.plaphy.2017.11.011
Received 5 September 2017; Received in revised form 17 November 2017; Accepted 17 November 2017
Available online 21 November 2017
0981-9428/ © 2017 Elsevier Masson SAS. All rights reserved.

under environmental stresses (Wang et al., 2011; Vanlerberghe, 2013). Plant AOXs are encoded by a multigene family and are different depending on plant species. In chickpea, *AOX3* gene encodes mitochondrial AOX enzyme.

As an important biochemical change under CS, reactive oxygen species (ROS) production is observed in vital processes like respiration and photosynthesis. Plant cells cope with this oxidative damage by applying ROS-scavenging systems, including both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and non-enzymatic antioxidants like ascorbate and proline (Erdal et al., 2015; Zouari et al., 2016). The activity of ROS-scavenging systems have been correlated with CT in plants. SOD detoxifies $O_2^{-\bullet}$ into H_2O_2, a double role molecule that acts in both signaling process of cold perception and in toxifying cell activities that is finally scavenged by CAT and APX activities in cells (Das and Roychoudhury, 2014). During stresses, ascorbate plays important roles in plant biochemical processes such as photosynthesis, cell cycle, cell expansion, mitochondrial ETC and ROS scavenging (Szarka et al., 2013). Therefore, such functional relationships may specify the alteration of its metabolic networks, as an important component of CT. Proline accumulation increases cell osmolarity, membrane and subcellular structures stability, and as a cell redox balancer, it can protect cells against oxidative stress under environmental stresses (Liu et al., 2016). Besides the crucial role of antioxidant system in reducing ROS damages, AOX pathway can also dampen ROS generation in tolerant plants to environmental stresses. This process leads effectively to minor cell damages, verified by lower electrolyte leakage index (ELI), as an important measure of cell membrane permeability (Panda et al., 2013). This issue may demonstrate a kind of defensive cooperation between antioxidant system and AOX pathway against ROS production.

On the other hand, when we consider mitochondrial responses against thermal changes, succinate dehydrogenase (SDH) as the second enzymatic complex in ETC and also as an involved enzyme in tricarboxylic acid cycle (TCA) plays an effective role. New investigations showed that SDH is a direct source of ROS production in plants followed by the induction of ROS production that is accompanied by the up-regulation of stress-related genes (Jardim-Messeder et al., 2015). However, the increase of SDH activity can result in more active AOX pathway, revealing its double role.

Besides the important role of enzymes and their cellular pathways in creating CT, plants need other metabolic pathways not only to survive cold conditions but to promote their recovery after CS, and this may lead to stable yields. Preserving cellular balance through the acceptable performance of photosynthesis provides plant stability. The phosphoenol pyruvate carboxylase (PEPC) activity, as one of the carboxylase family enzymes possesses important roles in carbon fixation and also in biosynthesis flux of TCA with the special act in plant adaption to environmental stresses (Gonzalez et al., 2007). Although PEPC does not affect cellular tolerance mechanisms directly, it can protect energy efficiency under stress conditions by playing essential roles in metabolism pathways.

In our previous research, genotypic screening of cold tolerant genotypes with high yield led to identification of a high-performance accession with better performance (Sel96Th11439) under field conditions (Heidarvand et al., 2011; Heidarvand and Maali-Amiri, 2013). However, CT mechanisms in chickpea still remains unknown so that different inducible pathways in response to CS should be clarified. There are few studies with regard to the importance of AOX pathway in CT mechanisms of chickpea. In this study, we investigated a set of biochemical and molecular responses in tolerant genotype (Sel96Th11439) compared to sensitive one (ILC533) to elucidate the role of AOX pathway and its coordination with antioxidant defense system in CT. Complex networks of integrating metabolic pathways that are involved in energy and defense metabolism with a central role in acclimation to CS could be targeted for future CT breeding programs in chickpea production areas.

2. Materials and methods

2.1. Plant materials and cold treatments

Two genotypes of chickpea, sensitive (ILC533) and tolerant (Sel96TH11439) to CS were prepared by Dryland Agriculture Research Institute (Maragheh, Iran). At first, chickpea seeds were sterilized by sodium hypochlorite 10% for 5 min and then washed by sterile water. Then, the seeds were planted in Petri dishes with suitable moisture. Petri dishes were placed in dark conditions with 25 °C temperature and after 3 days of germination, seedlings were transferred to pots contained potted soil (soil, sand and farm yard manure). Indirect transform of seedlings to pots was due to germination uniformity of pot seedlings so that seedlings with similar physiological age were considered in cold stress experiment. They were kept in growth chamber with 25 °C temperature, 75% relative humidity and 200 μmol m^{-2} s^{-1} irradiance and a 16/8 h light/dark photoperiod for 3 weeks. Three-week-old seedlings were approximately 20 cm height with at least five branches of 5–8 cm. These seedlings were transferred to chilling chamber (Arvin Tajhiz Espadana, Iran) with 4 °C for 6 days. Our experiments were focused on four groups of chickpea plants: control condition samples (25 °C), samples collected in the first, third, and sixth days after exposing the seedlings to CS. For each treatment, three pots with five plants were prepared and each pot was considered as a single replicate. After sampling, plants from each pot were collected and mixed to create a sample. All measurements were made on the middle leaves from the apex of all plants in each treatment. Collected samples were immediately flash frozen in liquid nitrogen and stored at −80 °C for further studies.

2.2. Damage indices (electrolyte leakage index and hydrogen peroxide)

Electrolyte leakage index (ELI) was assessed according to Popov et al. (2005) using fresh leaves. In addition, hydrogen peroxide content was measured according to Loreto and Velikova (2001). The content of H_2O_2 was expressed in μmol g^{-1} fresh mass (FM).

2.3. Enzymatic antioxidants

SOD was assessed according to Beyer and Fridovich (1987). The activity of this enzyme assayed based on the photochemical reduction of nitroblue tetrazolium and the ability of SOD enzyme in the inhibition of this reaction. The enzyme activity was reported in enzyme unit min^{-1} mg^{-1} protein. CAT activity was measured with spectrophotometer (Shimadzu UV-160, Kyoto, Japan) according to Scebba et al. (1998). The activity of enzyme was expressed in nmol of H_2O_2 decomposed min^{-1} mg^{-1} protein. APX activity was determined according to Ranieri et al. (2003) and expressed in nmol oxidized ascorbate min^{-1} mg^{-1} protein.

2.4. Non-enzymatic antioxidants (proline and ascorbate)

The analysis of proline content was determined according to Bates et al. (1973). Leaf samples were homogenized in 3% (w/v) sulfosalicylic acid and centrifuged at $4000 \times g$ for 20 min. 2 mL glacial acetic acid, 2 mL ninhydrin reagent and 2 mL of supernatant were the components of the reaction mixture. After applying reaction mixture, samples were incubated for 1 h at 90 °C, then after stopping the reaction with ice, 4 mL of toluene was added and mixed by vortex. The absorbance of upper toluene phase was measured in glass cuvette at 520 nm. Proline content was expressed in μmol g^{-1} FM for each sample. The ascorbate content was assessed as described by Wang et al. (2017). At first, 0.5 g of leaf samples were ground in 2 mL 1 M $HClO_4$, and then centrifuged at $12{,}000 \times g$ at 4 °C for 15 min. Then saturated K_2CO_3 neutralized the supernatant to pH 5.6. The assay mixture included 100 mM sodium-phosphate buffer, pH 5.6, 1 unit of ascorbate oxidase

Download English Version:

https://daneshyari.com/en/article/8353591

Download Persian Version:

https://daneshyari.com/article/8353591

Daneshyari.com