Journal of Plant Physiology 204 (2016) 44–53

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

Metabolic acclimation of tetraploid and hexaploid wheats by cold stress-induced carbohydrate accumulation

Negin Shahryar[a,b], Reza Maali-Amiri[a,*]

[a] Department of Agronomy and Plant Breeding, University College of Agriculture and Natural Resources, University of Tehran, 31587-77871, Karaj, Iran
[b] Department of Agronomy and Plant Breeding, Faculty of Agriculture, Science and Research Campus, Azad Islamic University, Karaj Branch, 31876-44511, Karaj, Iran

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form 13 June 2016
Accepted 13 June 2016
Available online 26 July 2016

Keywords:
Acclimation
Carbohydrate
Cold tolerance
Durum wheat
Gene expression
Protective metabolites

ABSTRACT

Metabolic acclimation of plants to cold stress may be of great importance for their growth, survival and crop productivity. The accumulation carbohydrates associated with cold tolerance (CT), transcript levels for genes encoding related enzymes along with damage indices were comparatively studied in three genotypes of bread and durum wheats differing in sensitivity. Two (Norstar, bread wheat and Gerdish, durum wheat) were tolerant and the other, SRN (durum wheat), was susceptible to cold stress. During cold stress (−5 °C for 24 h), the contents of electrolyte leakage index (ELI) in Norstar and then Gerdish plants were lower than that of SRN plants, particularly in cold acclimated (CA) plants (4 °C for 14 days), confirming lethal temperature 50 (LT_{50}) under field conditions. Increased carbohydrate abundances in the cases of sucrose, glucose, fructose, hexose phosphates, fructan, raffinose, arabinose resulted in different intensities of oxidative stress in bread (Norstar) plants compared to durum plants (SRN and Gerdish) plants as well as in CA plants compared to non-acclimated (NA) ones under cold, indicating metabolic/regulatory capacity along with a decrease in ELI content and enhanced defense activities. A significant decrease in these carbohydrates, particularly sucrose, under cold in NA plants showed an elevated level of cell damage (confirmed by ELI) compared to CA plants. On the other hand, an increase in hexose phosphates, particularly in NA plants, indicated sucrose degradation along with greater production of glucose and fructose compared to CA plants. Under such conditions, a significant increase in transcript levels of sucrose synthase and acidic invertase confirmed these results. Under cold, the high ABA-containing genotypes like Norstar and then Gerdish, which were obvious in CA plants, partly induced relative acclimation of cells for acquisition of CT compared to SRN. These results reveal an important role of carbohydrate metabolism in creating CT in durum wheats (particularly in Gerdish) as well as bread wheat with possible responsive components in metabolic and transcript levels.

1. Introduction

Many agronomic plants lose their production due to cold stress and day and night temperature fluctuations (Heidarvand and Maali-Amiri, 2010). Winter plants not only must survive in cold, but they also need to continue their growth in cold to be able to acquire cold tolerance (CT) and to maintain cell homeostasis (Trischuk et al., 2014). The most common cold stress effects include the production of reactive oxygen species (ROS). ROS are generally a regular basis of the cell in signal transduction. However, their excessive production or inefficient deactivation is translated into changes in membrane fluidity and stability, reduced photosynthesis, reduced carbon gain and primary and secondary compositions, which ultimately leads to low crop yield (Prasad et al., 1994; Kazemi Shahandashti et al., 2013; Nejadsadeghi et al., 2014; Rakei et al., 2016). The main site when plants get injured due to exposure to cold stress at the earliest moment is the plasma membrane (Heidarvand et al., 2011). Therefore, increased rates of solute and electrolyte leakage index (ELI) from membranes are the criteria used to evaluate damage due to cold stress in plants. In many cases there seems to exist a balance between ROS production and elimination with the degree of CT. In these situations, facultative adaptations, known as cold acclimation, that results in adjusted metabolic alterations. However, this capacity of plants to withstand cold stress, which usually is considered as the degree of tolerance, is not constant but increases

Abbreviations: ABA, abscisic acid; CA, cold acclimated; CT, cold tolerance; ELI, electrolyte leakage index; FM, fresh mass; F-6-P, fructose-6-phosphate; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; LT_{50}, lethal temperature 50; NA, non-acclimated; QPCR, quantitative reverse-transcriptase polymerase chain reaction; ROS, reactive oxygen species.

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

http://dx.doi.org/10.1016/j.jplph.2016.06.019
0176-1617/

noticeably upon exposure to progressive cold stress (Dominguez et al., 2010; Kazemi Shahandashti et al., 2013). On the other hand, after acclimation and at the end of vegetative growth, the ability of cold acclimation gradually decreases in a way that might make the plant vulnerable to damage by the early cold in spring, particularly in wheat (Trischuk et al., 2014). Therefore, the conversion of environmental stimuli like cold into a response in a temporary or fluctuating manner remains to a large extent unknown. However, cold acclimation as an effective process in production and yield depends mainly on survival or rapid recovery of plants that activate plant growth (Hurry et al., 1995b).

A major contributor to cold acclimation is the metabolic regulation of carbohydrate compounds like their precursors, intermediates and different products that act as osmoregulators, cryoprotectants, and ROS scavengers along with their direct relationships with photosynthesis, translocation and respiration (Nishizawa et al., 2008; Wang et al., 2013). Numerous reports have shown a positive correlation between sugar contents and the degree of CT in cereals so that the altering pattern of the studied carbohydrates illustrates a possible complementary function mechanism and shows the importance of cooperative activity at the transcriptional and translational levels in response to cold stress (Streb et al., 2003). However, there are different types of carbohydrates that may accumulate during cold in plant species. Sucrose, as the primary translocatable carbohydrate in plants, is vital to the initiation of increased CT (Chen et al., 2012). In many plants, the genes for sucrose synthase and invertase are the best-understood genetic system with a role in sucrose regulation and other sugars under cold (Zhang et al., 2011). The change in these genes and metabolic profiling of carbohydrates has been studied in plants under cold stress. However, such processes could be different in tetraploid (durum) wheat with A and B genomes and in hexaploid (bread) wheat with A, B and D genomes. Since durum wheats genetically are sensitive to cold stress, how durum genotypes modulate carbohydrate metabolites and use them in downstream reactions could be of great interest in new varieties tolerant to cold (Majláth et al., 2016).

Many studies have shown the involvement of plant hormones, particularly abscisic acid (ABA), in maintaining plant growth and development and in response to environmental stresses like cold (Danquah et al., 2014). It was suggested that ABA as a signal not only plays physiological roles such as stomatal closure, water transpiration and accumulation of osmoprotectants, but is also involved in many CT controlling genes in transcription, transcript processing, and stability levels triggers partly acclimation against cold-induced oxidative stress (Cutler et al., 2010; Maruyama et al., 2014). Therefore, targeted analyses of this hormone can be considered one of the criteria to characterize signaling and metabolic networks and genetically screen genotypes under cold stress.

Studies have shown extensive genetic variability for CT in durum genotypes in Iran that is considered to be a major wheat genetic pool. However, these data are generally based on field and morphological studies and not many physio-biochemical studies associated with development mechanisms of CT in durum have been conducted. Only moderate improvement has eben realized using conventional plant breeding. In our previous studies, we assessed physio-biochemical characteristics of cold-tolerant wheat genotypes, Norstar (bread wheat) and Gerdish (durum wheat), and a susceptible genotype to cold stress, SRN (durum wheat) (Nejadsadeghi et al., 2014, 2015). Unique carbohydrate networks provided by metabolic enzyme genes with a central role in acclimation to cold stress, along with the previous systems biology approaches in relation to defense and damage indices, metabolism regulation of fatty acid and hormones could be targets for future CT breeding programs, which may enhance crop production, extend agricultural production areas and ultimately increase economic efficiency.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of cold-susceptible (SRN) and tolerant (Gerdish) durum wheats as well as tolerant bread wheat (Norstar) provided by Dryland Agriculture Research Institute (DARI) of Iran in Maragheh city of Azerbaijan province, were sterilized with 10% sodium hypochlorite for 5 min, soaked in distilled water, and then germinated in Petri dishes on filter paper for 72 h at 25 °C in a thermostat. After germination, uniform seedlings were transferred to pots in a growth chamber (Arvin Tajhiz Espadana, Iran) at 25 °C, an irradiance of 200 $\mu mol\ m^{-2}\ s^{-1}$ from white light luminescent lamps, a 16/8 h light/dark photoperiod, and 75% relative humidity for 14 days. Plants were irrigated with distilled water as necessary. Fourteen-day-old plants were divided into two groups. One group was maintained under control conditions (25 °C) and the other group was moved from control conditions immediately into an acclimation period 4–5 °C for 14 days with the same photoperiod and irradiance (Nejadsadeghi et al., 2014, 2015). Irradiance values were decreased to 45% of their starting value by the end of the cold acclimation period. By the end of the twenty-eight days, both groups of plants were placed into a climatic chamber chilled initially to 0 °C and the temperature was lowered gradually to −5 °C (at a rate of 0.5 °C min^{-1}) and both groups of plants, cold acclimated (CA) and non-acclimated (NA) plants faced cold stress for one day. Thus, in this study, our experiments were focused on four groups of wheat plants: samples from unstressed plants as control plants (25 °C), plants exposed to the acclimation period (in the end of acclimation period), CA plants that faced cold stress and NA plants that faced cold stress. Samplings occurred at the end of each period. In thermal treatments, leaves were harvested immediately after removing the plants from the cold exposure room for physiological analysis. All measurements were made on 2 cm in the middle parts of the first leaves of wheat seedlings.

2.2. Electrolyte leakage index

Electrolyte leakage index (ELI) as a damage index of leaf tissues harvested in thermal treatments was assessed according to our previous study (Nazari et al., 2012).

2.3. Lethal temperature 50 (LT_{50})

The temperature at which 50% of the plants are killed by freezing stress (LT_{50}) was determined for each genotypes according to the procedure outlined by Mahfoozi et al. (2001). The seeds of genotypes were sown in early September under field conditions. The seedlings growth continued until early-November when the temperature decline stunted their growth. At late tillering stage (mid-November), the growth of seedlings was dramatically suppressed because of declining minimum temperature to −5 °C. After two months of acclimation under field conditions (mid-December), the crowns were detached from the plants and covered in moist sand in aluminum weighing cans and placed in a programmable freezer that was held at −3 °C for 18 h. After this period, they were cooled at a rate of 2 °C h^{-1} to −17 °C and then cooled at a rate of 8 °C h^{-1} to a minimum of −22 °C. Five crowns were removed for each of the tested temperatures in each genotype. Samples were thawed overnight at 4 °C. Thawed crowns were planted into flats containing soil, sand, and soft mold leaves (2:1:1) that were kept moist. The flats were placed in a growth room maintained at 20 °C with a 16/8 h light/dark photoperiod. Plant recovery was rated