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Cold acclimation, de-acclimation and re-acclimation of spring canola, winter canola and winter wheat: The role of carbohydrates, cold-induced stress proteins and vernalization

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ABSTRACT

It is well established that a period of exposure to low temperature is required in order for temperate plants to achieve maximum freezing tolerance. During the cold acclimation process a large number of biophysical, biochemical and molecular changes occur that enable the plant to survive at below freezing temperatures. These include the alteration of carbohydrate and protein accumulation profiles, resulting large quantities of soluble sugars and cold induced stress proteins (dehydrins) thought to function in a cryoprotective role. When fully cold acclimated plants are exposed to warm temperatures (de-acclimation) a rapid turnover of these carbohydrates and proteins occurs, resulting in a plant that no longer possesses an elevated level of freezing tolerance. In certain species, re-exposure to cold acclimating temperatures (re-acclimation) results in re-accumulation of carbohydrates and proteins with a synergistic impact on freezing tolerance. In canola (*Brassica napus* L.), a full recovery of freezing tolerance is observed upon re-acclimation in both spring and winter cultivars. In contrast, the re-acclimation of winter wheat (*Triticum aestivum* L.) results in only a 39% recovery of freezing tolerance. Upon further analysis it was revealed that wheat does not accumulate carbohydrates during the re-acclimation period. While certain dehydrins also accumulate during re-acclimation, there is no clear relationship with freezing tolerance and we suggest it is the interaction of these proteins with soluble carbohydrates that is responsible for the development of freezing tolerance during re-acclimation. The role of vernalization in these processes is also discussed.

1. Introduction

Cold acclimation of winter annuals to survive prolonged periods of sub-zero temperatures is a complex multi-step process involving a series of concerted physiological and biochemical changes (Guy, 1990; Thomashow, 2001; Janská et al., 2010; Theocharis et al., 2012). Low temperatures (generally less than 8 °C) and short photoperiods are the general acclimation cues for temperate plants, but the conversion of these stimuli into a response is not fully understood. However, it is well established that exposure of temperate plants to low temperatures results in the development of freezing tolerance while a subsequent incubation at warm temperature promotes a loss of freezing tolerance (Levitt, 1980). It has been suggested that for cold acclimation to occur in winter cereals the plants must be in their vegetative state in order to respond to the environmental cues; however, this has not been proven unequivocally (Andrews et al., 1960; Mahfoozi et al., 2001b).

Vernalization is defined as the promotion of flowering by cold temperatures. This developmentally-controlled process is important for the acquisition of freezing tolerance in winter cereals (Mahfoozi et al., 2001a, 2001b). Winter annuals require four to ten weeks exposure to low non-freezing temperatures to signal the vegetative to reproductive transition (Mahfoozi et al., 2001a). In this manner, flowering is delayed during winter until favorable growth conditions in spring. Cold-acclimated plants of spring cereals can only tolerate −7 to −10 °C in contrast to −24 °C for the cold hardy winter wheat cultivar Norstar and −33 °C for the hardy winter rye cultivar Puma (Gusta and Fowler, 1976). As

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maximal cold hardiness is achieved during the vegetative phase, if vernalization saturation is either partially or fully fulfilled, winter cereals will respond similarly to spring cereals with respect to their ability to acquire freezing tolerance upon cold acclimation (Mahfoozi et al., 2001b).

Cold acclimation and the acquisition of freezing tolerance is associated with the accumulation of novel cold-induced stress proteins such as those encoded by the *Arabidopsis COR* and wheat *WCS/WCOR* genes (Guy, 1990; Sarhan et al., 1997; Thomashow, 1998, 1999). These proteins include the dehydrins, which belong to group 2 (D-11) of the late-embryogenesis abundant (LEA) proteins (Hundertmark and Hincha, 2008). Dehydrins are proteins that accumulate in plants in response to low temperature or other dehydrative cellular processes and have been found in a large variety of species (Close et al., 1993; Close, 1997; Kosová et al., 2007). These proteins are highly hydrophilic and are predicted to form amphipathic α-helicies that have been proposed to stabilize membranes under freeze-induced dehydration conditions and are thought to possess antifreeze activity (Thomashow 1999; Hundertmark and Hincha, 2008; Janská et al., 2010; Theocharis et al., 2012). In wheat, the WCS120 protein family consists of five members sharing homology with the D-11 dehydrin family (Houde et al., 1992a; Sarhan et al., 1997). In *Arabidopsis*, COR78 is a highly hydrophilic and boiling-stable protein expressed ubiquitously in all plant tissues in response to low temperatures, also thought to play a role in membrane stabilization (Horvath et al., 1993; Thomashow, 1998).

Previous studies have demonstrated that maximal expression of the genes encoding many cold-induced stress proteins only occurs in the vegetative phase during cold acclimation. The vegetative to reproductive transition, initiated by the fulfillment of the vernalization requirement, represents a critical developmental point which signals the down-regulation of these genes (Fowler et al., 1996a, 1996b, 2001; Mahfoozi et al., 2001a, 2001b). As levels of freezing tolerance are thought to be determined, in part, by the duration and degree of up-regulation of these genes, it is not surprising that plants in the reproductive phase have a limited ability to cold acclimate (Fowler et al., 2001; Danyluk et al., 2003). Thus, vernalization requirements allow for sustained gene expression at the temperatures required for cold acclimation (Fowler et al., 1996a, 1996b). Furthermore, plants that are still in the vegetative phase have the ability to re-acclimate following de-acclimation, whereas plants in the reproductive phase only have a limited ability to re-acclimate (Mahfoozi et al., 2001b). Gusta et al. (1983) reported fully cold acclimated winter cereals, prior to vernalization saturation could partially re-acclimate, following exposure to de-acclimating temperatures. They also documented that similarly treated plants collected in early spring when vernalization saturation was fulfilled, were unable to re-acclimate upon exposure to cold acclimating conditions (Gusta et al., 1983).

Another factor implicated in the cold acclimation process is the accumulation of soluble sugars, particularly sucrose (Steponkus and Lanphear, 1967; Levitt, 1980; Pollock, 1984; Olien and Clark, 1993; Bohnert and Sheveleva, 1998). Low temperatures induce the rapid accumulation of soluble carbohydrates and this may partly contribute to the acquisition of freezing tolerance during cold acclimation (Wanner and Junttila, 1999; Uemura and Steponkus, 2003; Rekarte-Cowie et al., 2008; Janská et al., 2010; Theocharis et al., 2012). While a role for these compounds in plants tolerance to exposure to subfreezing temperatures remains to a large extent unknown, they are thought to perform multiple functions as compatible osmolytes, cryoprotectants, scavengers of reactive oxygen species and signalling molecules (Janská et al., 2010; Theocharis et al., 2012). In many species, oligosaccharides of the raffinose and stachyose family accumulate during exposure to low temperature, supporting a role for these compounds in cold acclimation (Zuther et al., 2004; Janská et al., 2010; Theocharis et al., 2012). During cold acclimation, cereals and grasses accumulate large amounts of fructans, polymers of fructose derived from sucrose, which serve to stabilize cell membranes (Pollock, 1984; Pollock and Lloyd, 1987; Suzuki and Nass, 1988; Pollock and Cairns, 1991; Livingston, 1996; Livingston et al., 2006, 2009). Furthermore, trehalose, a non-reducing disaccharide of glucose, has been implicated in modulating levels of freezing tolerance and may also be involved in starch accumulation (Fernandez et al., 2010; Janská et al., 2010; Theocharis et al., 2012). In addition, a role for starch hydrolysis and concomitant increases in the concentrations of free saccharides in response to low temperature exposure has been proposed (Siminovitch et al., 1952; Pollock and Lloyd, 1987; Bohnert and Sheveleva, 1998; Kaplan et al., 2006).

In this study we compared the freezing tolerance, cold-induced protein accumulation and carbohydrate levels in a spring canola with no vernalization requirements to that of a winter canola and a winter wheat which have strong vernalization requirements. Plants were subjected to low temperature acclimation followed by de-acclimation at warm temperature and subsequent re-acclimation at low temperature. Both the spring and winter canola were able to cold acclimate to the same degree and re-acclimate to their initial levels of freezing tolerance following de-acclimation. The winter cereal was unable to re-acclimate to its initial levels of freezing tolerance. While a role for cold-induced proteins was equivocal, differential responses to re-acclimation may be in part reconciled by the accumulation of specific carbohydrates.

2. Materials and methods

2.1. Plant material and growth conditions

Spring (*Brassica napus* L. cv Quest) and winter (*Brassica napus* L. cv Express) canola, as well as winter wheat (*Triticum aestivum* L. cv Norstar), were seeded in Redi Earth (W.R. Grace and Co., Canada) at a rate of 15 seeds per pot. Pots were placed in controlled environment chambers (Conviron, Canada) maintained at 20/16 °C (light/dark) with a 16 h photoperiod under a 650 or 315 μmol m^{-2} s^{-1} photon flux density for canola and wheat, respectively. Canola plants at the two to three leaf stage (non-acclimated plants), were exposed to the following 23 d progressive cold acclimation protocol: 3 d at 10/7 °C with a 16 h photoperiod, 3 d at 7/5 °C with a 14 h photoperiod, 3 d at 5/2 °C with a 14 h photoperiod, 7 d at 2/0 °C with a 12 h photoperiod and 7 d at 0/−2 °C with a 12 h photoperiod. Irradiance values were decreased to 46% of their starting value by the end of the cold acclimation protocol. At this stage, the seedlings were considered as fully cold acclimated. Thereafter, the plants were de-acclimated at 20/16 °C under a 16 h photoperiod for 10 d, and subsequently exposed to the cold acclimation protocol as described above for re-acclimation.

Winter wheat was cold acclimated using the following 45 d progressive cold acclimation protocol: 5 d at 10/7 °C with a 16 h photoperiod, 5 d at 7/5 °C with a 14 h photoperiod, 5 d at 5/2 °C with a 14 h photoperiod, 15 d at 2/0 °C with a 12 h photoperiod and 15 d at 0/−2 °C with a 12 h photoperiod. Irradiance values were decreased to 41% of their starting value by the end of the cold acclimation protocol. Plants were de-acclimated at 23/23 °C under a 16 h photoperiod for 14 d, and subsequently exposed to the wheat cold acclimation protocol described above for re-acclimation. In our experiments, the de-acclimation/re-acclimation cycle for all cultivars was conducted when vernalization saturation was partially or fully fulfilled.

2.2. Analyses of freezing tolerance

Levels of freezing tolerance expressed as the lethal temperature for 50% of the plants (LT_{50}) were estimated for both cultivars of