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Dynamics of cold acclimation and complex phytohormone responses in *Triticum monococcum* lines G3116 and DV92 differing in vernalization and frost tolerance level

Radomira Vanková[a], Klára Kosová[b], Petre Dobrev[a], Pavel Vítámvás[b], Alena Trávníčková[a], Milenka Cvikrová[a], Bedřich Pešek[a], Alena Gaudinová[a], Sylva Prerostová[a], Jana Musilová[b], Gabor Galiba[c], Ilja Tom Prášil[b,*]

[a] *Institute of Experimental Botany AS CR, Rozvojova 263, Prague 6 CZ-16502, Czech Republic*
[b] *Crop Research Institute, Drnovska 507, Prague 6 CZ-1606, Czech Republic*
[c] *Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunszvik 2, Martonvásár H-2462, Hungary*

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ABSTRACT

Cold stress response was compared in the crowns, leaves, and roots of *Triticum monococcum* DV92 spring line and G3116 winter line. The cold exposure was associated with a rapid increase of water saturation deficit, which resulted in a strong up-regulation of abscisic acid. Simultaneously, other stress hormones: salicylic acid, aminocyclopropane carboxylic acid (precursor of ethylene), and jasmonic acid decreased. The stress application resulted in a decrease of hormones associated with stimulation of cell growth and division (gibberellins, cytokinins, and auxin). During the acclimation phase of the stress response, the plants increased their frost tolerance and started the accumulation of dehydrins. Active gibberellin, cytokinins, and auxin were elevated; more rapidly in the spring line. Abscisic acid decrease was accompanied by a gradual increase of the other stress hormones. Simultaneously, the up-regulation of phenolic acids was observed, including ferulic and sinapic acids, which may be involved in the stabilization of auxin levels as well as antioxidative functions. After 21 days, the spring line DV92 exhibited its maximum of active cytokinins, which indicates the onset of the early stage of reproductive development. The winter line fulfilled its vernalization requirement after 42 days, as indicated by a decrease of frost tolerance and dehydrin levels, accompanied by similar growth hormone changes as in DV92. The similarities and differences between einkorn and common wheat in a long-term cold response are discussed.

1. Introduction

Cold is an important abiotic stress factor, affecting agricultural productivity in temperate climates (Sakai and Larcher, 1985). Cereals from the tribe *Triticeae* grown in temperate climates have evolved two major mechanisms of cold response – cold acclimation and vernalization. Cold acclimation represents a complex dynamic process directed at enhancement of the plant tolerance to low temperatures (frost tolerance = FrT), while vernalization represents an adaptation of plant development in order to prevent the plant premature transition from a cold-tolerant vegetative phase to a cold-sensitive reproductive phase (e.g., Kosová et al., 2008).

Einkorn wheat (*Triticum monococcum*) represents an ideal model for genetic studies of frost tolerance due to its diploid genomic makeup (A^mA^m). In the *T. monococcum* genome, both the major vernalization and frost tolerance associated loci (*Vrn1/Fr1* locus, *Fr2* locus, and *Vrn2* locus) all lie on the long arm of chromosome 5A (5AL; Dubcovsky et al., 1998). The *T. monococcum* lines G3116 and DV92 have been used for genetic mapping studies, leading to the identification of the *Fr2* locus (Vágújfalvi et al., 2003), as well as the deciphering the structure of the *CBF* cluster at this locus (Knox et al., 2008). They exhibit significant differences in acquired frost tolerance, but do not differ in the major *Vrn1/Fr1* frost-tolerance locus (Vágújfalvi et al., 2003). However, they do differ at the *Vrn2* locus, as line G3116 possesses a dominant *Vrn-A^m2* allele and has a vernalization requirement, while line DV92 possesses a recessive *vrn-A^m2* allele and lacks vernalization (Dubcovsky et al., 1998; Vágújfalvi et al., 2003). Genetically, the G3116 line corresponds to a winter growth habit due to a

Abbreviations: ABA, abscisic acid; ACC, aminocyclopropane carboxylic acid; CK, cytokinin; FA, ferulic acid; FrT, frost tolerance; GA, gibberellin; IAA, indole-3-acetic acid; JA, jasmonic acid; JA-Ile, jasmonate isoleucine; LT50, lethal temperature for 50% of the sample; OP, osmotic potential; PA, phaseic acid; SA, salicylic acid; SiA, sinapic acid; WSD, water saturation deficit; WCS, wheat cold-specific (protein).

* Corresponding author. Tel.: +420 702 087 686.
E-mail address: prasil@vurv.cz (I.T. Prášil).

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combination of a recessive *vrn1* allele and a dominant *Vrn2* allele, while the DV92 line can be considered a spring line due to the absence of a vernalization requirement (presence of a recessive *vrn2* allele). Due to the combination of recessive *vrn1* and *vrn2* alleles, DV92 can also be described as a facultative growth habit (Von Zitzewitz et al., 2005).

The detailed study of the genetic basis of frost tolerance of the G3116 and DV92 lines has led to the identification of 11 *TmCBF* genes, mapped to the *Fr2* locus (Knox et al., 2008). Quantitative differences were observed in *T. monococcum TmCBF* genes between the cold-treated lines. The G3116 line exhibited higher levels of *TmCBF12*, *TmCBF15*, and *TmCBF16* transcripts than the DV92 line did. Moreover, allelic differences between the two lines have been characterized in the *TmCBF12* gene, mapped to the central part of the *Fr2* locus. These differences indicate that the *TmCBF12* allele, which is present in the DV92 line, cannot bind to the CRT/DRE elements in the promoter of the *COR* genes, due to a mutation resulting in the deletion of the AP2 DNA binding domain. The inability of DV92 *TmCBF12* to bind to CRT/DRE binding elements in the promoter of the *COR* genes has been confirmed by electrophoretic mobility shift assays (EMSA; Knox et al., 2008). The authors hypothesize that the differences in the structure and ability of *TmCBF12* to bind to the CRT/DRE promoter elements may underlie the differences in acquired FrT between the two lines. Thus, *T. monococcum* lines G3116 and DV92 represent a very interesting model for the study of cold acclimation as well as vernalization response during a long-term cold treatment.

The CBFs directly affect metabolism of plant hormones gibberellins by stimulation of the expression of their main deactivating enzyme (GA 2-oxidases) (Achard et al., 2008). Down-regulation of the level of active gibberellins is associated with accumulation of their repressors – DELLA proteins, which results in the suppression of growth and increase in cold tolerance (Achard et al., 2008). Decrease in the growth rate at the beginning of the cold stress response was found to be associated with the down-regulation of also other hormones indispensable for cell division and growth – cytokinins and auxins (Kosová et al., 2012). Plant hormones generally are involved in the regulation of plant interactions with the environment. Abscisic acid (ABA) is the key plant hormone involved in the response to abiotic stresses associated with dehydration, which include not only drought, but also salinity and cold stress (Gusta et al., 2005). ABA mediates both fast responses (stomata closure), which are necessary for the control of water balance, as well as longer-term changes in the expression of many stress-associated genes. The function of ABA in the stabilization of water status in the early stage of the cold stress response has been repeatedly studied (Galiba et al., 1993; Janowiak et al., 2002). In addition, the participation of other "stress hormones" such as ethylene, jasmonic acid (JA), and salicylic acid (SA) in the cold response has been recognized (Majlath et al., 2012, Kosová et al., 2012).

Long-term cold treatment has been described in common wheat (*T. aestivum*) under both natural as well as controlled conditions (Gusta and Fowler, 1977; Kosová and Prášil, 2011; Kosová et al., 2011, 2012). These studies have shown differences in the time course of the FrT as well as of hormone levels, and enabled distinguishing of the different stages of the dynamics of the cold response: the alarm reaction, cold acclimation, phase of resistance, and the loss of high FrT. Tracking changes in a number of characteristics and properties then enabled the possibility to study the mechanisms influencing and controlling the dynamics of the FrT as well as of hormones in a greater detail (Vítámvás et al., 2010; Kosová et al., 2013).

The intent of our study lies in the study of the impact of a long-term (0–42 days) cold treatment on *T. monococcum* lines DV92 and G3116 at the level of plant development (days to heading), water relationships (water saturation deficit, osmotic potential), acquired frost tolerance (lethal temperature of 50% of the sample), dehydrin relative accumulation, phytohormone and phenolic acid levels. Comparisons of the data acquired on *T. monococcum* with our previous results on cold acclimation in the common wheat spring cultivar Sandra and the winter cultivar Samanta (Kosová et al., 2012) have been done in order to pinpoint both similarities and differences in a long-term cold response between the hexaploid and diploid wheats.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of einkorn wheat (*T. monococcum*), lines G3116 and DV92 were germinated on moist filter paper for 2 days at 22 °C in the dark. The germinated seeds were planted into pots filled with a mixture of field soil and sand (6:1), and grown under controlled conditions in a growth chamber (Tyler T-16/4, Budapest, Hungary) with a 12 h photoperiod, 350 μmol $m^{-2}s^{-1}$ irradiance, and regulated temperature. The temperature was set to 18–20 °C for the first three weeks. When the plants reached the three-leaf growth stage, the temperature was decreased to 4 °C. Leaf, crown, and root samples were taken in the middle of the light period at 0 (control, non-treated plants), 1, 3, 7, 21, 31, and 42 days of cold treatment for all analyses, except for the determination of days to heading. The leaf samples for hormone analyses consisted of a pool of middle parts of six youngest fully developed leaves. With the crowns, the non-green underground stem parts, containing shoot apical meristem, and the basal parts of the leaves from ca 10 to 15 plants were taken for the analyses; with the roots, the apical root parts (ca 10 cm) were taken from 6 to 10 plants.

2.2. Days to heading and shoot apex development

Days to heading, indicating the time of vernalization saturation, were determined for three plants of each line which were transferred from cold (4 °C) to control conditions (20 °C) at each subsequent week of the cold treatment (0, 7, 14, 21, 28, 35, and 42 days of cold) and let to grow until heading. The period of growth at 20 °C from the transfer from cold until heading was determined as the days to heading. The apical development was determined from the changes in the morphology of the shoot apex for three plants, sampled at the same time as the plants for heading day (for further details see Prášil et al., 2004).

2.3. Plant water relationships

Plant water status (tissue hydration) was determined as the water saturation deficit (WSD; %) and osmotic potential (OP) in the youngest fully developed leaf. WSD was determined on leaf segments 1 cm long, exposed to a water saturation treatment in a hydration chamber, according to Slavík (1963). Briefly, the WSD was calculated as the difference between the fresh weight of fully water-saturated leaf segments and the fresh weight of leaf segments sampled under the given experimental conditions, divided by the difference between the fresh weight of the fully water-saturated leaf segments and the dry weight of the same segments. Leaf OP was determined as the osmolality of thawed leaf segments, sampled in a syringe and stored at −25 °C using a VAPRO Dew Point Osmometer (WESCOR Inc., Logan, Utah, USA). OP values have been calculated from the osmolality values using the van't Hoff equation $\psi = -cRT$ (c is osmolality of the solution measured; R is an universal gas constant, 8.314 J mol^{-1} K^{-1}; T is a thermodynamic temperature in K).