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Complex phytohormone responses during the cold acclimation of two wheat cultivars differing in cold tolerance, winter Samanta and spring Sandra

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ABSTRACT

Hormonal changes accompanying the cold stress (4 °C) response that are related to the level of frost tolerance (FT; measured as LT50) and the content of the most abundant dehydrin, WCS120, were compared in the leaves and crowns of the winter wheat (*Triticum aestivum* L.) cv. Samanta and the spring wheat cv. Sandra. The characteristic feature of the alarm phase (1 day) response was a rapid elevation of abscisic acid (ABA) and an increase of protective proteins (dehydrin WCS120). This response was faster and stronger in winter wheat, where it coincided with the downregulation of bioactive cytokinins and auxin as well as enhanced deactivation of gibberellins, indicating rapid suppression of growth. Next, the ethylene precursor aminocyclopropane carboxylic acid was quickly upregulated. After 3–7 days of cold exposure, plant adaptation to the low temperature was correlated with a decrease in ABA and elevation of growth-promoting hormones (cytokinins, auxin and gibberellins). The content of other stress hormones, *i.e.*, salicylic acid and jasmonic acid, also began to increase. After prolonged cold exposure (21 days), a resistance phase occurred. The winter cultivar exhibited substantially enhanced FT, which was associated with a decline in bioactive cytokinins and auxin. The inability of the spring cultivar to further increase its FT was correlated with maintenance of a relatively higher cytokinin and auxin content, which was achieved during the acclimation period.

Introduction

Low temperatures (cold and frost) represent a severe threat to plant survival and economic crop productivity in many regions. The ability of plants to acclimate to low temperatures is crucial for their survival. Plant cold acclimation is a complex process accompanied by profound changes in the expression of multiple genes regulated by various phytohormones and resulting in the *de novo* biosynthesis of several stress-protective compounds and an enhancement of the acquired frost tolerance (FT) level. With regard to the general dynamics of the plant stress response, several phases can be distinguished: (a) a control, non-stressed phase; (b) an early alarm phase when the plant is not adapted and stress acts as a shock factor; (c) an acclimation phase, when the plant actively redirects its metabolism to deal with stress; (d) a resistance phase when the plant reaches its maximum stress tolerance level and a new, steady-state metabolic balance; and if the stress lasts too long (e) an exhaustion phase when the plant metabolism, adjusted to the stress conditions, collapses (Larcher, 2003). Each phase of the plant stress response is uniquely reflected at regulatory (phytohormone) as well as adaptive (water-relations, protein and metabolite) levels (Vankova, 2010; Kosova et al., 2011).

Changes in water relationships during plant cold stress response have been described in detail in Larcher (2003) and Gusta et al. (2005, 2009). Upon exposure to cold, hydraulic conductivity of the cereal roots decreases, which results in a decrease of water potential and induction of transient wilting of plant tissue, leading to

Abbreviations: ABA, abscisic acid; ACC, aminocyclopropane-1-carboxylic acid; CK, cytokinin; CKX, cytokinin oxidase/dehydrogenase; FT, frost tolerance; GA, gibberellin; IAA, indole-3-acetic acid; JA, jasmonic acid; SA, salicylic acid.

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stomata closure in the alarm phase. During the acclimation phase, the reduced water potential returns to levels similar to those before the cold exposure of plants due to the osmotic adjustment (accumulation of osmotically active compounds). As a consequence of osmotic adjustment, osmotic potential decreases in cereal organs during cold acclimation, allowing stabilization of turgor and opening of stomata for exchange of gases in leaves (Gusta et al., 2005; Prasil et al., 2007). However, the growth and development of plants are reduced (Larcher, 2003; Prasil et al., 2004).

Plant stress responses are regulated by a range of phytohormones, which may be divided into two groups: "positive growth regulators" [auxin, cytokinins (CKs), gibberellins (GA) and brassinosteroids] and "stress hormones" [abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA) and ethylene]. Due to intensive hormone cross-talk (positive or negative), individual processes are affected by multiple hormones. Nevertheless, different stress hormones play a key role in the response to a particular stress. Thus, ABA is a crucial hormone in the defense against abiotic stresses, SA plays a decisive role in the protection against biotrophs, while JA governs the response to wounding and necrotroph attack (Vankova, 2010).

One of the major groups of stress-protective proteins is COR/LEA proteins. This group includes the LEA-II family of proteins (dehydrins), which are elevated upon dehydration. In common wheat (*Triticum aestivum*), an important group of cold-inducible dehydrins is the WCS120 protein family (Sarhan et al., 1997), which is represented by at least five members (WCS200, WCS180, WCS120, WCS66, and WCS40) that differ in their relative molecular weights as well as their abundance. The most abundant member of this family in cold-treated wheat samples is the WCS120 protein. Previous studies (e.g., Vitamvas et al., 2007) have shown that differences in the relative abundance of WCS120 and its homologues in cold-acclimated *Gramineae* species are correlated with the level of acquired FT; *i.e.*, the WCS120 protein (and its homologues) can be regarded as markers of acquired FT in cold-acclimated cereals.

Promoter analysis of the wheat *Wcs120* gene, as well as its barley homologue *Dhn5*, revealed that their expression is regulated by both ABA-dependent and ABA-independent signalling pathways (Sarhan et al., 1997). The latter pathway involves C-repeat-binding transcription factors (*CBFs*).

In addition to the stimulation of the expression of stress-related genes, ABA exhibits other protective functions in cold stress defense, especially stabilization of membranes, protection against oxidative stress, improvement of water status by elevation of root hydraulic conductivity and stomata closure. The function of ABA in the early stage of the cold stress response coincides with a transient increase of ABA levels during chilling stress (Galiba et al., 1993; Veisz et al., 1996).

Recent reports suggest that, apart from the stimulation of defense mechanisms, an important component of the stress response is the modulation of plant growth and development. The cold-inducible CBF transcription factor was found to downregulate levels of bioactive GAs as well as to stabilize repressors of their signalling pathway, *i.e.*, DELLA proteins (Achard et al., 2008). Much less attention has been focused on CKs and auxins. Overexpression of the CK biosynthetic gene (*ipt*) was found to promote cold stress tolerance in *Arabidopsis* (Guo et al., 2010). However, Jeon et al. (2010) reported cold-stimulated expression of negative regulators of the CK signalling pathway (type-A response regulators), which indicated downregulation of the signal transduction of these hormones, at least during the initial phase of the cold stress response. A study by Shibasaki et al. (2009) on cold-induced changes in auxin transport suggested the involvement of auxin in cold stress responses.

The aim of the present study was to characterize individual hormone responses associated with the reaction to cold stress in a cold-tolerant winter wheat, cv. Samanta, and a cold-sensitive spring wheat, cv. Sandra, during 21 days of cold treatment (4 °C) and their phytohormone relationships to the FT level (measured as LT50) as well as changes in the content of the most abundant dehydrin, WCS120, in two different organs, leaves (green tissues) and crowns (non-green underground parts, not including roots). In addition to the main abiotic stress defense hormone ABA, the growth-promoting hormones CKs, auxin, GAs and their metabolites, as well as the stress hormones SA, JA and ethylene were evaluated.

Materials and methods

Plant material and growth conditions

Seeds of two wheat (*Triticum aestivum* L.) cultivars, winter cv. Samanta and spring cv. Sandra, were obtained from the breeding company Selgen a.s. (Prague, Czech Republic). The seeds germinated in the dark on moist filter paper at 20 °C for 2 days. The seedlings were planted in pots filled with soil (a mixture of field, soil and sand, 4:2:1) and grown at 18–20 °C; 12 h photoperiod and 350 $\mu mol\, m^{-2}\, s^{-1}$ in growth chambers (Tyler, type T – 16/4, Budapest, Hungary) until the three-leaf stage. Next, the growth temperature was decreased to 4 °C. During the entire experiment, the plants were watered regularly every 3rd day, with no fertilizer being supplied.

Leaf and crown tissues were sampled at 0 (control, non-treated plants), 1, 3, 7 and 21 days of cold treatment. The 3rd leaf (the youngest fully expanded leaf) was used for all analyses (frost tolerance test, determination of WCS120 content, determination of phytohormone levels). As crowns, non-green underground stem tissues not including roots were considered. During the entire experiment, neither of the cultivars entered the reproductive stage of development (a double-ridge stage of shoot apex) (Prasil et al., 2004).

Frost tolerance tests

Leaf and crown segments (1 cm) were exposed to a set of freezing temperatures in the range of −1.5 °C to −30 °C according to Prasil and Zamecnik (1998). The plant materials (two replicates per each frost temperature, consisting of 10 leaf segments or three crowns) were inoculated with ice crystals at −1.5 °C and held at 12 freezing temperatures for 20 min. The rate of cooling was 12 °C h^{-1}, and the frozen samples were thawed in a water bath at 1 °C for 12 h. The extent of freezing damage was determined conductometrically according to Prasil and Zamecnik (1998). Lethal temperatures (LT50), *i.e.*, the temperature causing 50% electrolyte leakage, were calculated according to Janacek and Prasil (1991).

Determination of WCS120 content

The third young, fully expanded leaves were frozen in liquid nitrogen and stored at −80 °C until protein extraction and boiling. The dehydrin extraction, 1D SDS-PAGE and immunoblot analyses were performed according to Vitamvas et al. (2007). The amount of WCS120 protein was analyzed densitometrically using Quantity One software (Bio-Rad, version 4.6.2, Munchen, Germany).

Quantification of phytohormones (CK, IAA, ABA, GA, SA, ethylene precursor ACC and JA)

The endogenous phytohormone concentrations were determined in the leaves and crowns of both wheat cultivars. All experiments were repeated three times. For analyses of endogenous CKs, extraction and purification were performed according to Novak et al. (2003), and CK levels were quantified by ultra