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Abscisic acid content and the expression of genes related to its metabolism during maturation of triticale grains of cultivars differing in pre-harvest sprouting susceptibility

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ABSTRACT

Abscisic acid (ABA) is a plant hormone that plays a predominant role in the onset and maintenance of primary dormancy. Peak ABA accumulation in embryos of triticale grains was observed before any significant loss of water and was higher in Fredro, a cultivar less susceptible to pre-harvest sprouting (PHS), than in Leontino, a cultivar more sensitive to PHS. At full maturity, embryonic ABA content in Fredro was twice as high as in Leontino.

Two full-length cDNAs of 9-*cis*-epoxycarotenoid dioxygenase (*TsNCED1*, *TsNCED2*), an enzyme involved in ABA biosynthesis, and two full-length cDNAs of ABA 8′-hydroxylase (*TsABA8′OH1* and *TsABA8′OH2*), an enzyme involved in ABA catabolism, were identified in triticale grains and characterized. The maximum transcript level of both *TsNCED1* and *TsNCED2* preceded the peak of ABA accumulation, suggesting that both *TsNCEDs* contribute to reach this peak, although the expression of *TsNCED1* was significantly higher in Fredro than in Leontino. High expression of *TsABA8′OH2* and *TsABA8′OH1* was observed long before and at the end of the ABA accumulation peak, respectively, but no differences were observed between cultivars.

The obtained results suggest that mainly *TsNCED1* might be related to the higher ABA content and higher resistance of Fredro to PHS. However, Fredro embryos not only have higher ABA content, but also exhibit greater sensitivity to ABA, which may also have a significant effect on grain dormancy and lower susceptibility to PHS for grains of this cultivar.

1. Introduction

Seed development is a critical phase of the plant life cycle and allows the seed to enter the dormancy phase, which in turn enables seed propagation and restoration of growth under optimal conditions. One of the hormones that regulates key events during seed development as well seed germination is abscisic acid (ABA) (Finkelstein et al., 2002; Kermode, 2005; Nambara et al., 2010). ABA concentrations in seed tissues are maintained dynamically and are the cumulative result of ABA *de novo* synthesis in the seed itself, transport from the mother plant, and catabolism to inactive products (Nambara and Marion-Poll, 2005). Typically, ABA content in seeds is low during the early stages of seed development, increases to the maximum level around mid-development, when storage reserves are accumulated, and then declines as seeds undergo maturation drying (Quarrie et al., 1988; Romagosa et al., 2001; Walker-Simmons, 1987). Genetic studies using ABA-deficient mutants have shown that maternal ABA is crucial for promoting early embryonic growth, avoiding seed abortion, and proper seed pigmentation, and induces the synthesis of storage proteins, while ABA synthesized in the embryo just before any appreciable water loss has a predominant role in the induction and maintenance of primary dormancy (Cheng et al., 2002; Frey et al., 2004; Karssen et al., 1983).

Most of the genes engaged in ABA metabolism have been identified mainly through the characterization of the ABA-deficient mutants of *Arabidopsis* (Finkelstein, 2013; Nambara and Marion-Poll, 2005; Schwartz and Zeevaart, 2010), but in cereals, only some of the potential genes that participate in ABA metabolism have been described so far (Fidler et al., 2015). Additionally, knowledge of the temporal and spatial expression of these genes during cereal grain

Abbreviations: ABA, abscisic acid; ABA8′OH, ABA 8′-hydroxylase; CYP, cytochrome P450; DAP, days after pollination; UGT, uridine diphosphate glucosyltransferase; NCED, 9-*cis*-epoxycarotenoid dioxygenase; PHS, pre-harvest sprouting; ZEP, zeaxanthin epoxidase.

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development is very limited and incomplete. The first potential regulatory enzyme of the ABA biosynthetic pathway is zeaxanthin epoxidase (EC 1.14.13.90; ZEP), which converts zeaxanthin to *trans*-violaxanthin in a two-step reaction (Audran et al., 1998). Microarray analysis of the barley transcriptome indicated the presence of *HvZEP1,3,4* transcripts in the embryo and endosperm of maturing grains, although detailed temporal changes of transcript levels in relation to ABA content were not studied (Seiler et al., 2011). The expression of genes from the family encoding 9-*cis*-epoxycarotenoid dioxygenase (EC 1.13.11.51; NCED), which catalyzes oxidative cleavage of *cis*-violaxanthin and *cis*-neoxanthin to xanthoxin (Schwartz et al., 1997), and is considered rate-limiting in ABA synthesis in vegetative tissues (Iuchi et al., 2001), was analyzed only for barley grains. A high level of *HvNCED2* mRNA was observed in embryos during the early phases of grain development and preceded peak ABA accumulation, whereas the expression of *HvNCED1* increased at later phases of grain maturation and was relatively lower than that of *HvNCED2* (Chono et al., 2006). ABA catabolism occurs either by hydroxylation, mainly at C-8′ position, or conjugation with glucose. The 8′-hydroxylation of ABA is catalyzed by ABA 8′-hydroxylase (EC 1.14.13.93; ABA8′OH), a cytochrome P450 (CYP, P450) monooxygenase (Kushiro et al., 2004). Barley *HvCYP7074A1* and wheat *TaABA8′OH1* were mainly expressed in the middle to late stages of grain development, which correlates with the decrease in embryonic ABA content (Chono et al., 2006, 2013). The conjugation of ABA with glucose is catalyzed by ABA uridine diphosphate glucosyltransferase (EC 2.4.1.263, ABA-UGT) (Lim et al., 2005; Xu et al., 2002); however, no gene from this family has been identified in cereals so far.

Pre-harvest sprouting (PHS) is characterized as germination of grains while still in the ear of the mother plant (Fang and Chu, 2008). This problem occurs mostly in years with prolonged rainfall preceding the harvest period and causes significant economic losses. Particularly susceptible to this adverse phenomenon are wheat, rye and triticale, which are characterized in general by short grain dormancy (Jiang and Xiao, 2005; Masojć et al., 2007; Wu and Carver, 1999; Trethowan et al., 1993). Seed dormancy and PHS are very complex and closely interrelated features, controlled jointly by a number of genes and environmental factors (Finkelstein et al., 2008; Gao et al., 2013; Gubler et al., 2005; Graeber et al., 2012; Rodríguez et al., 2015). It is assumed that an increase in ABA content precedes the induction of seed dormancy, while breaking seed dormancy is accompanied by a decrease in ABA content and/or sensitivity. Because of the enormous economic importance of cereals and the steadily increasing percentage of triticale in crop structures, an investigation of mechanisms responsible for maintaining adequate levels of ABA during maturation of seeds of species with short dormancy may contribute to our understanding of the mechanism behind pre-harvest sprouting.

In the present study, the nucleotide sequences of potential genes engaged in ABA synthesis and catabolism during maturation of triticale grains were identified and characterized. The expression profiles of the cloned genes in relation to changes in embryonic ABA content and the germinability of grains of two triticale cultivars that are extreme in terms of their resistance to pre-harvest sprouting during grain maturation were analyzed. The impact of ABA levels and sensitivity of embryos to ABA on the susceptibility of triticale grains to pre-harvest sprouting is discussed.

2. Material and methods

2.1. Plant material and experimental conditions

Grains of two hexaplioid winter triticale (x *Triticosecale* Wittm.) cultivars (Fredro and Leontino) that differ in their susceptibility to pre-harvest sprouting were provided by the Plant Breeding Station in Laski (Danko Plant Breeders Ltd., Poland). Fredro is considered one of the most resistant cultivars to PHS, while Leontino is one of the most susceptible. Field experiment was conducted at the Plant Breeding Station in Laski under standard fertilization and crop protection. Monthly rainfall and average temperature after pollination (June 3rd 2013) were 100 mm/18 °C in June and 20 mm/19 °C in July. Grains were harvested 25, 30, 35, 40, 45, and 50 days after pollination (DAP).

2.2. Germination tests

Freshly harvested grains were sterilized in 70% Et-OH for 1 min and in 5% NaClO for 5 min, then rinsed three times with distilled water after each step. Grains were placed on 9 cm diameter Petri dishes (thirty per dish in triplicate) containing two Whatman No. 2 filter papers wetted with 5 mL of sterile water and incubated at 23 °C in darkness. Grains were considered germinated when the coleorhiza was visible beyond the seed coats.

2.3. ABA sensitivity tests

Sensitivity of the embryo to ABA was measured as the capacity of exogenous ABA to block its germination. 50 DAP half grains containing embryo (embryo-half grains) were imbibed in water (control) or ABA solution for 30 min, sterilized as above and placed on Petri dishes (thirty per dish in triplicate) containing two filter papers wetted with 5 mL of sterile water (control) or ABA solution. Grains were then incubated at 23 °C in darkness and the germination percentage was calculated.

2.4. Determination of ABA content

ABA was extracted from the embryos of maturing triticale grains as described by Zdunek and Lips (2001) and was analyzed using the Phytodetek ABA enzyme immunoassay test kit (Agida).

2.5. Total RNA extraction and cDNA synthesis

Total RNA was isolated from the embryos of maturing triticale grains using the PureLink RNA Mini Kit (Life Technologies) according to manufacturer protocols. Based on the total RNA, cDNA templates were obtained using the Reverse Transcription System (Promega).

2.6. Amplification of the internal regions of genes

Internal fragments of the analyzed triticale genes (*TsZEP1*, *TsNCEDs*, *TsABA8′OHs*, *TsUGT1*) were amplified using Q5 High-Fidelity DNA Polymerase (New England BioLabs) and primers designed from known sequences of rice *ZEP* (AB050884.1), barley *NCEDs* (AB239297.1, AB239298.1), barley *ABA8′OHs* (AB239299.1, DQ145933.1) and wheat *GT* (EU568801.1). Primers for the 1st PCR reactions are listed in Table 1. PCR conditions were as follows: 30 s at 98 °C; 40 cycles of 10 s at 98 °C, 20 s at 62 °C (*TsZEP1*), 60 °C (*TsNCED1*, *TsUGT1*), 58 °C (*TsNCED2*), or 57 °C (*TsABA8′OH1*, *TsABA8′OH2*), 30 s at 72 °C, with a final 10 min extension at 72 °C. PCR products were cloned into the pJET1.2/blunt cloning vector (Thermo Scientific), amplified in E. coli JM109 cells and then sequenced using BigDye Terminator v3.1 chemistry kit and ABI3730XL genetic analyzer at DNA Sequencing and Oligonucleotide Synthesis Laboratory at The Institute of Biochemistry and Biophysics, Polish Academy of Sciences. At least nine clones of each PCR product were sequenced and analyzed.