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Transcript and hormone analyses reveal the involvement of ABA-signalling, hormone crosstalk and genotype-specific biological processes in cold-shock response in wheat

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ABSTRACT

The effect of one-day cold-shock on the transcriptome and phytohormones (auxin, cytokinins, abscisic, jasmonic and salicylic acids) was characterised in freezing-sensitive (Chinese Spring), highly freezing-tolerant (Cheyenne) and moderately freezing-tolerant (Chinese Spring substituted with Cheyenne's 5A chromosome) wheat genotypes. Altogether, 636 differentially expressed genes responding to cold-shock were identified. Defence genes encoding LEA proteins, dehydrins, chaperons and other temperature-stress responsive proteins were up-regulated in a genotype-independent manner. Abscisic acid was up-regulated by cold accompanied by adherent expression of its metabolic genes. Data revealed the involvement of particular routes within ABA-dependent signalling in response to cold-shock in the examined genotypes. Cold-shock affected gene expression along carbohydrate metabolic pathways. In photosynthesis, cold-shock changed the expression of a number of genes in the same way as it was previously reported for ABA. Overrepresentation analysis of the differentially expressed genes supported the ABA-signalling and carbohydrate metabolism results, and revealed some pronounced biological process GO categories associated with the cold-shock response of the genotypes. Protein network analysis indicated differences between the genotypes in the information flow along their signal perception and transduction, suggesting different biochemical and cellular strategies in their reaction to cold-shock.

1. Introduction

Winter wheat varieties acquire their freezing-tolerance by exposure to decreasing temperature over a longer period [1]. The effects of this process, called cold-acclimation, on carbohydrates [2], amino acids and polyamines [3], the proteome [4,5], the phenolome [6], phytohormones [7,8] and gene expression [9–11] have been studied in wheat. These reports suggested that re-programming of metabolism occurs during cold-acclimation leading to reduced growth and development, and activation of defence processes.

In addition to cold-hardening, the exposure of winter wheat to a prolonged cold period is also required for transition from the vegetative to the generative developmental stage, while spring wheat does not need such vernalization. At the end of the vegetative/generative transition, flower primordia are in the irreversible double-ridge phase and ready to flower. Since the reproductive meristems in the developing flower primordia are more sensitive to cold than vegetative meristems and organs [10], the optimal timing of the reproductive development by vernalization is important and ensures that flowers and seeds develop in spring/summer. The key factors in vernalization are the *VRN1* genes with different alleles [12], which were mapped to the long arm of Chr 5 [13]. The Cheyenne (Ch) winter variety contains three homeologous recessive (*vrn1*) alleles, while the spring wheat Chinese Spring (CS)

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carries one recessive allele on each chromosomes 5A and 5B and possesses a single vernalization-insensitive dominant allele *(Vrn-D1)* on Chr 5D. We note here that the CS(Ch5A) substitution line was used to map the *Vrn-A1* allele and the freezing tolerance 2 locus (*Fr-A2*) on the long arm of Chr 5A [14].

In wheat, single chromosomes can be transferred from one variety to another using cytogenetic chromosome manipulation techniques, and hence their genetics can be examined in a different and homogeneous genetic background [15]. Previous studies revealed that a substitution line constructed by replacing the 5A chromosomes of the freezing-sensitive CS variety with the corresponding chromosome pair from the freezing-tolerant winter wheat Cheyenne, had a much higher freezing-tolerance than the recipient CS variety [16,17] but had no change in its spring habit. The enhanced freezing-tolerance of the substitution line CS(Ch5A) was associated with the increased transcription of the Cheyenne *CBF* (C-repeat binding factor) genes [18], which are clustered in the freezing tolerance locus (*FR2*) mapped to Chr 5A [19]. Of the *CBF* genes being partly responsible for the freezing-tolerance of cereals in the vegetative phase [20], *CBF14* was the most effective [21].

In nature, cold-acclimation and vernalization usually takes several weeks to months [22] and during this period plants can adapt to the gradually changing temperature. However, because of the recent climatic changes, plants can be exposed to very sudden weather changes. The average yearly number of extreme weather events, which have become more frequent and severe in the last few decades, has increased by 2.5-fold in the last 30 years [23]. Between 2000 and 2010, hydro-meteorological disasters caused 1070 billion USD losses worldwide, and in Europe alone the extreme climatic events (heat- and cold-waves, wildfire and drought) caused losses worth about seven billion USD [24]. Extreme weather has a large impact on economic systems including agriculture [25]. From a scientific point of view, extreme weather changes cause extreme stress to natural plant populations and crops. Amongst these extreme events, a number of cold waves and abnormally low temperature conditions have been reported [24].

A rapid drop in temperature is equivalent to a cold-shock for young wheat plantlets, which are in their early vegetative phase (both winter and spring varieties) or were not cold-acclimated yet (winter varieties). In cold-shocked wheat, a burst of gene activity was detected during the first day, and about 90% of all responsive genes were activated [9]. After 6 h, the induction response was very similar in spring and winter wheat cultivars, but after 24 h the transcript level of many transcription factors became significantly higher in winter than in a spring cultivars [9]. Similarly to this observation, our previous transcriptome analysis revealed the greatest changes in gene expression during the first day of a three weeks hardening period in wheat shoots [10]. In parallel to this phenomenon, the most rapid changes in freezing-tolerance occurred during the initial stages of acclimation in wheat [26,27].

The aim of this study was to compare the effect of one-day cold-shock on gene expression and the levels of different hormones in a model system of freezing-tolerant and freezing-sensitive genotypes in their early vegetative growth phase, and to integrate the obtained data. We were also interested in identifying possible key factors, system differences between tolerant and sensitive wheat genotypes, and the effect of Chr 5A in response to cold shock.

2. Materials and methods

2.1. Plant material

Seeds of the CS and Ch varieties, and the CS(Ch5A) substitution line were germinated on wet filter paper in Petri dishes (1 day at 25 °C, 3 days at 4 °C and 2 days at 25 °C). Seedlings were then grown in half-strength modified Hoagland solution under 16 h illumination at 270 μmol m^{-2} s^{-1}, 20/15 °C day/night temperature and 70–75% relative humidity in a growth chamber (Conviron PGV-15; Controlled Environments Ltd., Winnipeg, Canada) for ten days. Half of the plants were then kept for one day at 4 °C for cold-shock, while the other half of the plants were kept for a further day at the control temperature. Illumination and humidity conditions for both groups during the additional day were the same that were used in the ten days growing period. No vernalization treatment was carried out for the plantlets. The ten days old plantlets subjected to cold-shock had two fully developed leaves and the presence of the single ridge shoot apex and the absence of the tillers indicated that the plants were in their vegetative phase. The shocked plants did not show any cold-related symptoms, such as senescence or retarded growth, after one day chilling. The shoots of the plantlets were collected for the microarray and hormone studies.

2.2. Microarray experiments

RNA was isolated in two biological replicates using a standard TRI reagent (Sigma-Aldrich, Budapest, Hungary) method according to the instruction of the manufacturer and applying DNase I (Promega, Madison, WI, USA) treatment. Preparation of Cy5- and Cy3-labelled cDNA using RNA isolated from the control and cold-shocked samples, respectively, and microarray hybridisation to a stress-specific 15k wheat oligonucleotide microarray [28] were performed as described [29]. An Agilent scanner (Agilent, Santa Clara, CA, USA) was employed for microarray scanning and data collection.

To validate microarray data, the expression of seven genes (about 1% of all differentially regulated genes) was investigated by qRT-PCR in three biological replicates. RNA was transcribed into cDNA using M-MLV Reverse Transcriptase and oligo$(dT)_{18}$ primer (Thermo Scientific/Life Technologies, Budapest, Hungary) and amplification was performed using the KAPA SYBR® FAST qPCR kit (Kapa Biosystems, London, UK) and a CFX96 thermo-cycler (Bio-Rad, Budapest, Hungary) with the following cycle: denaturation at 95 °C for 3 min followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C. The primer sequences are shown in Supplementary Table S1. The melting curve was recorded between 60 and 95 °C in 0.5 °C increments. The relative quantities of the individual transcripts were calculated by the ΔΔCt method using a phosphogluconate-dehydrogenase (UniGene ID: Ta30797) housekeeping gene for normalisation [30]. The ratio between the treatment and control data and their Student's *t*-test were calculated in Excel.

2.3. Hormone analysis

Extraction and analysis of plant hormones were performed according to established methods [31,32] in three biological replicates. Briefly, samples were homogenized and extracted with methanol/water/formic acid (15/4/1, v/v/v). The following labelled internal standards (10 pmol per sample) were added: $^{2}H_6$-ABA, $^{2}H_3$-PA, $^{13}C_6$-IAA, $^{2}H_2$-OxIAA, $^{2}H_5$-$^{15}N_1$-IAA-Asp, $^{2}H_4$-SA, $^{2}H_5$-JA, $^{2}H_5$-*trans*Z, $^{2}H_5$-*trans*ZR, $^{2}H_5$-*trans*Z7G, $^{2}H_5$-*trans*Z9G, $^{2}H_5$-*trans*ZOG, $^{2}H_5$-*trans*ZROG, $^{2}H_5$-*trans*ZRMP, $^{2}H_3$-DHZ, $^{2}H_3$-DHZR, $^{2}H_3$-DHZ9G, $^{2}H_6$-iP, $^{2}H_6$-iPR, $^{2}H_6$-iP7G, $^{2}H_6$-iP9G, $^{2}H_6$-iPRMP (Olchemim, Olomouc, Czech Republic). Extracts were purified using an SPE-C18 column (SepPak-C18, Waters, Milford, MA, USA) and separated on a reverse phase-cation exchange SPE column (Oasis-MCX, Waters, Milford, MA, USA). The hormone fractions eluted with methanol or with 0.35 M NH_4OH in 70% methanol were separated by HPLC (Ultimate 3000, Dionex/Thermo Fisher Scientific, Vienna, Austria) and hormones were quantified using a hybrid