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The homoeologous genes encoding chalcone–flavanone isomerase in *Triticum aestivum* L.: Structural characterization and expression in different parts of wheat plant

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ABSTRACT

Chalcone–flavanone isomerase (CHI; EC 5.5.1.6.) participates in the early step of flavonoid biosynthesis, related to plant adaptive and protective responses to environmental stress. The bread wheat genomic sequences encoding CHI were isolated, sequenced and mapped to the terminal segment of the long arms of chromosomes 5A, 5B and 5D. The loss of the final *Chi* intron and junction of the two last exons was found in the wheat A, B and D genomes compared to the *Chi* sequences of most other plant species. Each of the three diploid genomes of hexaploid wheat encodes functional CHI; however, transcription of the three homoeologous genes is not always co-regulated. In particular, the three genes demonstrated different response to salinity in roots: *Chi-D1* was up-regulated, *Chi-A1* responds medially, whereas *Chi-B1* was not activated at all. The observed variation in transcriptional activity between the *Chi* homoeologs is in a good agreement with structural diversification of their promoter sequences. In addition, the correlation between *Chi* transcription and anthocyanin pigmentation in different parts of wheat plant has been studied. The regulatory genes controlling anthocyanin pigmentation of culm and pericarp modulated transcription of the *Chi* genes. However, in other organs, there was no strong relation between tissue pigmentation and the transcription of the *Chi* genes, suggesting complex regulation of the *Chi* expression in most parts of wheat plant.

1. Introduction

Chalcone–flavanone isomerase (CHI; EC 5.5.1.6.) catalyzing the conversion of chalcones to flavanones leads to the nine of twelve major subgroups of flavonoid compounds (Winkel-Shirley, 2001). CHI-like enzymes are known in certain bacteria and fungi (Gensheimer and Mushegian, 2004; Herles et al., 2004), but they are largely restricted to vascular plants (Ngaki et al., 2012). CHI activity is related to the plant's response to environmental stress. For example, the addition of fungal elicitors to bean cell cultures induced a rapid accumulation in *Chi* mRNA, just as occurs during both natural infection and wounding (Mehdy and Lamb, 1987); similarly, the transcription of a rice *Chi* gene was noticeably up-regulated in response to salinity stress (Walia et al., 2007). After its initial isolation from bean (Mehdy and Lamb, 1987), homologs have been identified in a number of other higher plant species (Druka et al., 2003; Grotewold and Peterson, 1994; Hong et al., 2012; Khlestkina et al., 2009b; Martin et al., 1991; Shirley et al., 1992). The *Chi* gene products fall into two classes depending on substrate specificity (Dixon et al., 1988): those found in non-legume species (type I enzymes) only isomerize 4,2′,4′,6′-tetrahydroxychalcone to 5,7,4′-trihydroxyflavanone, while type II enzymes, which are characteristically found in legume species, show activity with both 4,2′,4′-trihydroxychalcone and 4,2′,4′,6′-tetrahydroxychalcone (Shimada et al., 2003).

Bread wheat (*Triticum aestivum*, $2n = 6x = 42$, BBAADD) is an allopolyploid that was formed trough hybridization and chromosome doubling of three ancestral diploid species, related to nowadays existing *Triticum urartu* ($2n = 2x = 14$, AA), *Aegilops speltoides* ($2n = 2x = 14$, SS), and *Ae. tauschii* ($2n = 2x = 14$, DD) (Gill and Friebe, 2002). Thus,

Abbreviations: ABRE, abscisic acid response element; ACE, ACGT-containing element; *Ans*, Gene encoding anthocyanidin synthase; BAC, Bacterial artificial chromosome; bp, Base pair(s); cDNA, DNA complementary to RNA; CDS, Protein coding sequences; CHI, Chalcone–flavanone isomerase; *Chi*, Gene encoding CHI; *Chs*, Gene encoding chalcone synthase; cv, Cultivar; *Dfr*, Gene encoding dihydroflavonol 4-reductase; DNAse, Deoxyribonuclease; dNTP, Deoxyribonucleoside triphosphate; DRE, Dehydration responsive element; EST, Expressed sequence tag(s); F3h, Gene encoding flavanone 3-hydroxylase; in/del, Insertion or deletion of base(s); K_a, Number of non-synonymous substitutions; K_s, Number of synonymous substitutions; MRE, MYB-recognition element; mRNA, Messenger RNA; NCBI, National Center for Biotechnology Information; PDB, Protein data bank; RRE, R response element; *Rt*, Gene encoding rhamnosyltransferase; RT-PCR, Reverse transcription polymerase chain reaction; SE, Standard error; SNP, Single nucleotide polymorphisms; U, Unit(s); *Ubc*, Gene encoding ubiquitin; *Ufgt*, Gene encoding UDP-glucose:flavonoid 3-O-glucosyltransferase.

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there are commonly three representatives of each single copy gene present in bread wheat. They are referred as homoeologous genes. Although all three homoeologs of the majority of genes have been reportedly expressed in hexaploid wheat, transcriptional divergence up to epigenetic silencing of some of the homoeologous genes has been widely documented (Adams et al., 2003; Bottley et al., 2006; He et al., 2003; Himi and Noda, 2004; Hu et al., 2011, 2013; Kashkush et al., 2002; Shitsukawa et al., 2007; Wendel, 2000). The preferential expression/silencing of a specific homoeologue can be restricted to a particular tissue, organ or developmental stage (Adams et al., 2004; Bottley et al., 2006; Mochida et al., 2004).

Although flavonoids have a well-recognized role in the stress response of bread wheat (reviewed in Khlestkina, 2013; Liu et al., 2013), the sequence encoding CHI in wheat has not as yet been determined. DNA/DNA hybridization experiments have shown that the wheat genome harbors three copies of *Chi* (Li et al., 1999). The structural and functional divergence among them has yet to be characterized. Here, we describe the isolation and mapping of the genomic sequence of each of the three bread wheat *Chi* copies and provide a structural and functional comparison between them.

2. Materials and methods

2.1. Plant material and DNA/RNA extraction

The bread wheat *Chi* gene copies were extracted with the help of a BAC library made from the DNA of cv. 'Chinese Spring' (Allouis et al., 2003). The *T. urartu* and *Ae. tauschii Chi* genes sequences were determined using, respectively, accessions 'TRI 11499' and 'AE 145' from the collection of the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany). Chromosomal and intra-chromosomal location of the wheat *Chi* genes was assigned using initially the complete set of cv. 'Chinese Spring' nulli-tetrasomic lines (Sears, 1953) and subsequently a set of homoeologous group 5 chromosome deletion lines (Endo and Gill, 1996). The various other genetic stocks used to characterize *Chi* transcription are listed in Table 1. DNA was extracted from seven-day-old seedlings following Plaschke et al. (1995), and RNA was extracted using a QIAGEN Plant RNeasy kit (www1.qiagen.com), followed by DNAse treatment. RNA was extracted from the coleoptiles and roots of seedlings grown on filter paper in climatic chamber «Rubarth Apparate» (RUMED GmbH) at 20 °C under a 12 h photoperiod, as well as from culms, leaf sheaths, leaf blades and grain pericarp in greenhouse-grown plants.

2.2. Identification, cloning, and sequence analysis of wheat Chi copies

Based on the barley *Chi* cDNA sequence (AF474923), the BLAST algorithm (Altchul et al., 1990) was used to identify matching wheat ESTs deposited in the www.ncbi.nlm.nih.gov/Database/. Multalin version 5.4.1 (Corpet, 1988) was used to obtain multiple sequence alignments. Following the successful strategy described by Khlestkina et al. (2008), OLIGO software (Offerman and Rychlik, 2003) was then used to design a set of copy-specific primer pairs from the EST sequences (Supplementary Table S1, Fig. S1). One of the primer pairs and the amplicon it produced from a cv. 'Chinese Spring' template formed the basis of querying the BAC library, by respectively, PCR and membrane hybridization. The DNA of the selected BAC clone (2064 E19) was extracted using a Montage Plasmid Miniprep$_{HTS}$ kit (Millipore). The full length sequences of *T. urartu*, *Ae. tauschii*, and the various wheat *Chi* copies were reconstructed from a series of overlapping sequences produced by the amplification of genomic or BAC DNA, as well as with some BAC sequence itself. The sequences of primers (Supplementary Table S1, Fig. S1) used for sequencing the *Chi* copies were based on homologous sequences identified in CerealsDB (Wilkinson et al., 2012; www.cerealsdb.uk.net/CerealsDB/Documents/DOC_CerealsDB.php) (Table 2). Sequencing was conducted using resources of SB RAS Genomics Core Facilities (Novosibirsk, Russia, http://sequest.niboch.nsc.ru). Genomic nucleotide sequences of other plant species were found in databases

Table 1
Genetic stocks of wheat used to characterize *Chi* transcription. NIL: near-isogenic line, SCSL: single chromosome substitution line, IL: introgression line. * RNA was extracted upon appearance of anthocyanin pigment on the organ, ** RNA was extracted simultaneously with the sister lines 'i:S29Pp1Pp2' and 'i:S29Pp1Pp3'.

Name	Description	Organ/stage for *Chi* expression examination—non coloured/coloured (gene determining coloration)	Reference to genes determining coloration
'Saratovskaya 29' ('S29')	Russian spring wheat	coleoptile/4-day-old—light-red color (*Rc-A1*) culm/**—light-purple color (*Pc-A1*) leaf blade/**—light-purple color (*Plb-A1*) leaf sheath/**—light-purple color (*Pls-A1*) pericarp/**—non-coloured roots/4-day-old—non-coloured	Khlestkina et al., 2010
'i:S29Pp1Pp2'	wheat NIL developed on 'S29', donor— 'Purple Feed' (Arbuzova et al., 1998)	coleoptile/4-day-old—dark-red color (*Rc-A1* + *Rc-D1*) culm/*—dark-purple color (*Pc-A1* + *Pc-D1*) leaf blade/*—dark-purple color (*Plb-A1* + *Plb-D1*) leaf sheath/*—dark-purple color (*Pls-A1* + *Pls-D1*) pericarp/*—dark-purple color (*Pp3* + *Pp-D1*) roots/4-day-old—non-coloured	Tereshchenko et al., 2012a
'i:S29Pp1Pp3'	wheat NIL developed on 'S29', donor— 'Purple' (Arbuzova et al., 1998)	coleoptile/4-day-old—dark-red color (*Rc-A1* + *Rc-D1*) culm/*—dark-purple color (*Pc-A1* + *Pc-D1*) leaf blade/*—dark-purple color (*Plb-A1* + *Plb-D1*) leaf sheath/*—dark-purple color (*Pls-A1* + *Pls-D1*) pericarp/*—dark-purple color (*Pp3* + *Pp-D1*) roots/4-day-old—non-coloured	Tereshchenko et al., 2012a
'Chinese Spring' ('CS')	Chinese spring wheat	coleoptile/4-day-old—non-coloured	–
'CS(Hope 7A)'	wheat SCSL developed on 'CS', donor— 'Hope' (Gale and Flavell, 1971)	coleoptile/4-day-old—dark-red color (*Rc-A1*)	Gale and Flavell, 1971
'CS(Hope 7B)'	wheat SCSL developed on 'CS', donor—'Hope' (Gale and Flavell, 1971)	coleoptile/4-day-old—light-red color (*Rc-B1*)	Gale and Flavell, 1971
'Golubka'	Russian spring wheat	coleoptile/4-day-old—non-coloured	Khlestkina et al., 2009a
'Mironovskaya 808'	Ukranian winter wheat	coleoptile/4-day-old—dark-red color (*Rc-D1*)	Khlestkina et al., 2002
'Novosibirskaya 67'	Russian spring wheat	coleoptile/4-day-old—dark-red color (*Rc-D1*)	Khlestkina et al., 2009a
'Purple'	Australian spring wheat 'k-46990'	coleoptile/4-day-old—dark-red color (*Rc-D1*)	Tereshchenko et al., 2012a
'Purple Feed'	Canadian spring wheat 'k-49426'	coleoptile/4-day-old—dark-red color (*Rc-D1*)	Tereshchenko et al., 2012a