STUDY OF CHALCON ISOMERASE AND ANTHOCYANIDIN SYNTHASE DNA SEQUENCES IN WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

Anthocyanins, a group of flavonoid substances that is responsible for colored caryopses of common wheat (*Triticum aestivum* L.). Pigments formed in anthocyanin biosynthetic pathway are deposited in different parts of the caryopsis. Purple anthocyanins are accumulated in pericarp while blue anthocyanins are stored in aleurone layer of caryopsis. The spring wheat form of two genotypes with purple pericarp (Abyssinskaya arraseita and ANK-28B) and two genotypes with blue aleuron layer (Tschermaks Blaukörniger Sommerweizen and UC66049) were used in the experiments. Genotype with white caryopsis (Novosibirskaya 67) was used as a control. Total RNA was isolated from developing caryopses and transcribed into cDNA. Sequences for chalcon isomerase (98-100% similarity) and for anthocyanidin synthase genes were detected. The variability among genotypes in this study was due to insertion or deletion (indels). These candidate sequences were localized in the wheat genome and will be used for study of gene expression during maturation.

Key words: anthocyanins, chalcon isomerase, anthocyanidin synthase, wheat, *Triticum aestivum* L.

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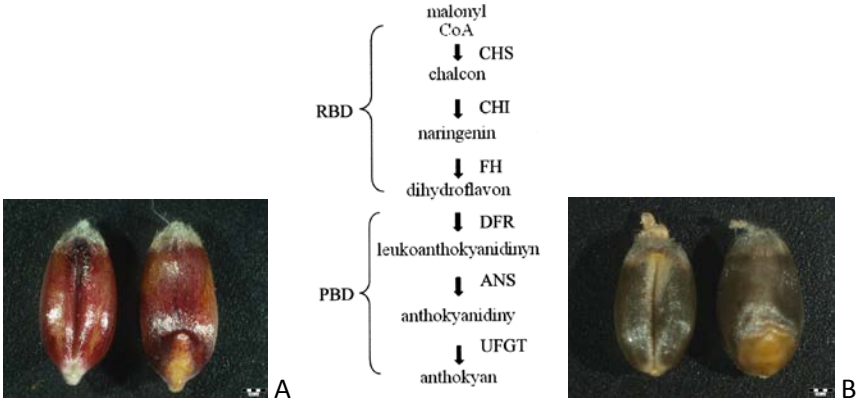
INTRODUCTION

Anthocyanins are natural pigments that discolor various parts of plants, e.g. flowers, leaves, fruits, seeds, and other tissues into blue, purple, or orange color. Anthocyanins are responsible among other things for different color grains in common wheat (*Triticum aestivum* L.). Blue anthocyanins are stored in aleurone layer of caryopsis, purple-colored anthocyanins are stored in pericarp. These flavonoid substances are highly prized for their sensory properties and also for their beneficial effect on health. The benefit is especially antioxidant activity and a positive effect on cardiovascular system (Wallace, 2011). Caryopsis with different color can be called as functional foods because its positive effects on human health have been scientifically proven (Mazza, 1998).

The genes for enzymes involved in anthocyanin biosynthetic pathway are often divided into two groups: early biosynthetic genes (CHS – chalcon synthase, CHI – chalcon isomerase / synonym chalcon-flavon isomerase / and FH – flavon hydroxylase) and late biosynthetic genes (DFR – dihydroflavonol reductase, ANS – anthocyanindin synthase and UFGT - UDP glucosoflavonoidglycosyl transferase) (Nesi et al., 2001) (Fig. 1).



Simplified diagram of anthocyanin biosynthetic pathway (modified from Ahmed et al., 2009).

RBD - early biosynthetic pathway, PBD - late biosynthetic pathway, CHS – chalcon synthase, CHI – chalcon isomerase, FH – flavon hydroxylase, DFR – dihydroflavonol reductase, ANS – anthocyanidyn synthase, UFGT - UDPG-flavonoidglucosyl transferase

Fig. 1 A- Abyssinskaya arraseita, purple pericarp, B- Tschermaks Blaukörniger Sommerweizen, blue aleurone

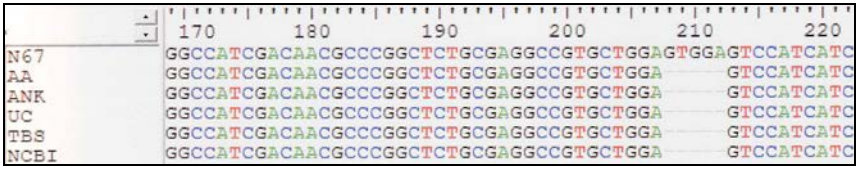
MATERIALS AND METHODS

Developing wheat caryopses (10-20 days post anthesis) of spring form with a different color were used in experiment. Genotypes UC66049 and Tschermaks Blaukörniger Sommerweizen have blue-aleurone layer. Genotypes Abyssinskaya arraseita and ANK - 28B are characterized by purple pericarp. Genotype Novosibirskaya 67 was used as the standard because it has white colored caryopsis. Seed samples were obtained from Agrotest fyto, Ltd,. Kroměříž, Czech Republic. Plants

were grown in a small-plot trial in the grounds of Botanical Gardens and Arboretum of Mendel University in Brno, Czech Republic, in the growing season 2011. Date of flowering corresponded to 65 BBCH (Biologische Bundesanstalt, Bundessortenant and Chemical Industry) and it has been different for studied genotypes: 13. 6. 2011 (UC66049), 15. 6. 2011 (ANK - 28B and Tschermaks Blaukörniger Sommerweizen) and 16. 6. 2011 (Abyssinskaya arraseita and Novosibirskaya 67). Total RNA was isolated from grains by phenol - chloroform extraction and transcribed into cDNA by kit Enhanced Avian Reverse Transcriptase (Sigma Aldrich, USA). Selected cDNA sequences from National Center for Biotechnology Information (NCBI) were used for primer design. For CHI cDNA sequence codenamed AB187026.1 in the database was selected. For ANS sequences codenamed AB247917.1, AB247918.1, AB247919.1 and AB247920.1 AB247921.1 were selected. Primers were designed by software Primer3. Gradient PCR was performed to determine optimal annealing temperature for the designed primers. Segments of sequences of CHI and ANS were amplified by PCR reactions. These sequences were after purification used for direct sequencing of PCR products. The sequencing was performed in specialized laboratory (Macrogen, Netherlands). The obtained candidate sequences were compared by software ClustalW2 to evaluate polymorphisms. Sequences were compared with each other and with the sequence from the NCBI database, according to which primers were designed. The obtained sequences were compared with sequences obtained from each chromosome arm of common wheat cultivar Chinese Spring.

RESULTS AND DISCUSSION

By sequencing of the PCR product for CHI sequences of DNA fragments of the size 335 bp (ANK-28B, Abyssinskaya arraseita, Tschermaks Blaköniger Sommerweizen and UC66049) were obtained, which is identical to the default sequence AB187026.1. For genotype Novosibirskaya 67 was the DNA sequence size 340 bp. Difference in number of nucleotide bases is given by a five nucleotide indel (insertion/deletions). This indel is located at a position between nucleotides 205 and 215 (Fig. 2). This indel interrupts the reading frame and may cause malfunction of the emerging protein. Ondroušková et al. (2012) detected the same insertion in winter variety Heroldo, which has white grain such as Novosibirskaya 67. Similarity between sequences was 98-100 %. Variability in sequences was not caused by single nucleotide polymorphisms, which have not been detected in CHI.



N67 - Novosibirskaya 67, AA - Abyssinskaya arraseita, ANK - ANK-28B, UC - UC66049, TBS - Tchermaks Blaukörniger Sommerweizen, NCBI - AB187026.1, sequence used to design primers

Fig. 2: Comparison of selected regions of CHI sequences using BioEdit

By sequencing of the PCR product for ANS sequence of the DNA fragment only in genotype UC66049 (20 days post anthesis) was obtained. Analysis of PCR products was repeated several times. Fragment size was 222 bp. The resulting sequence has 100% homology with a part of the sequence AB247920.1 in the NCBI database (Himi et al., 2006, unpublished).

Comparison of the sequences of CHI and ANS with sequences of chromosome arm of common wheat: In case of CHI identical sequences were found on the long arm of chromosome 5B. Similarity was 98-100 %. Data for chromosome 5DL are currently not available, and therefore it is

possible that a copy of the CHI gene is also in this region of the genome. For the ANS sequence 3 sequences with at least 90 % homology were found in the database. Two of them are located on 6AS chromosome arm and one on 6DS chromosome arm. One of the sequences from 6AS has 100 % homology with the sequence obtained from the UC66049 genotype. It is very interesting that no copy of the gene for ANS was found on the third homeologous arm 6BS.

CONCLUSIONS

We have obtained partial sequence of the genes for CHI and ANS by sequencing analysis. After comparison between genotypes mutual sequence homology of genes for CHI and ANS was found to be in the range of 98-100%. It was confirmed that the suggested primers amplified gene segments for CHI and ANS. Indels that disrupt reading frames and could have a great effect on the function of the emerging transcript were detect. The follow-up step in this work is to obtain complete sequences of transcribed genes by methods for rapid amplification of cDNA ends (5'RACE and 3'RACE). Furthermore, we want to compare the sequence of the complete cDNA with genomic DNA sequence to determine introns in genes. Finally, the data will be used for the design of qPCR primers to study gene expression during kernel maturation.

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