GENOMIC ANALYSES OF DIHYDROFLAVONOL REDUCTASE GENE IN GENOTYPES OF COMMON WHEAT (*TRITICUM AESTIVUM* L.) WITH NONSTANDARD COLOURED CARYOPSES

Štiasna K.[1], Sekanina P.[1], Vyhnánek T.[1], Trojan V.[1], Bartoš J.[2], Havel L.[1]

[1]Department of Plant Biology, Faculty of Agronomy, Mendel University in Brno, Zemedelska 1, 613 00 Brno, Czech Republic

[2]Centre of Plant Structural and Functional Genomics, Institute of Experimental Botany AS CR, Šlechtitelů 31, 783 71 Olomouc-Holice, Czech Republic

E-mail: xstiasna@node.mendelu.cz

ABSTRACT

Anthocyanins are responsible for the coloration in shades of blue, purple and red of various body parts of plants. Wheat caryopses with nonstandard coloration, specifically genotypes UC66049 and Tschermaks Blaukörniger Sommerweizen with blue aleurone, ANK28B and Abyssinskaya arraseita with purple pericarp were used in the experiment. Genotype Novosibirskaya 67 was used as a standard because it does not synthesize any pigments. Total RNA was isolated from caryopses by the phenol-chloroform method and transcribed into cDNA. In dihydroflavonol reductase (DFR) gene sequences obtained from direct sequencing of PCR product were detected several indels and single nucleotide polymorphisms. The similarity among all sequences of analyzed genotypes and sequence obtained from National Center for Biotechnology Information (NCBI) ranged between 94.59 to 100 %. The gene expression of DFR in samples varied during maturation. For the control genotype Novosibirskaya 67 there was highest DFR gene expression 25 days post anthesis (dpa) and the lowest 15 and 35 dpa. In Tschermaks Blaukörniger Sommerweizen was observed the lowest gene expression 40 dpa and the highest 10 dpa, while in genotype UC66049 was DFR gene expression almost the lowest 10 dpa and 40 dpa reached the lowest values. The highest DFR gene expression in Abyssinskaya arraseita was observed between 15 and 20 dpa and then rapidly decreases to its lowest level 25 dpa. Genotype ANK28B shows decreasing gene expression during maturation, the highest value was observed 10 dpa and the lowest 35 dpa, 40 dpa gene expression slightly increased again.

Key words: wheat, *Triticum aestivum* L., anthocyanins, dihydroflavonol reductase

Acknowledgments: This work was supported by IGA FA MENDELU IP No. 12/2013.

INTRODUCTION

Besides the commonly used varieties of common wheat (*Triticum aestivum* L.) with red coloured caryopses, which are considered the standard, there are many other genotypes with blue, purple, yellow or white caryopses. This is caused by anthocyanins, the natural pigments with flavonoid character, occurred in the aleurone layer of blue caryopses or in the pericarp in purple coloured caryopses. Generally, anthocyanins are responsible for the coloration in shades of blue, purple and red of various body parts of plants, for instance flowers and fruits or seeds, leaves, tubers and bulbs. Anthocyanins affect not only sensory properties of these plants, but they positively affect human health. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators and they can reduce the risk of cardiovascular diseases (Lin, Weng, 2006; Wallace, 2011). Overall, several of flavonoids appear to be effective anticancer promoters and cancer chemopreventive agents (Lin, Weng, 2006). Biosynthesis of the anthocyanins (Fig. 1) is often divided into early and late pathways and each is catalyzed by enzymes specific to a particular stage. The genes encoding the early biosynthetic enzymes are CHS (chalcone synthase), CHI (chalcone isomerase) and F3H (flavanone-3-hydroxylase), late biosynthesis genes are DFR (dihydroflavonol reductase), ANS (anthocyanidin synthase) and UFGT (UDP-glucose:flavonoid 3-o-glucosiltransferase) (Nesi et al., 2001).

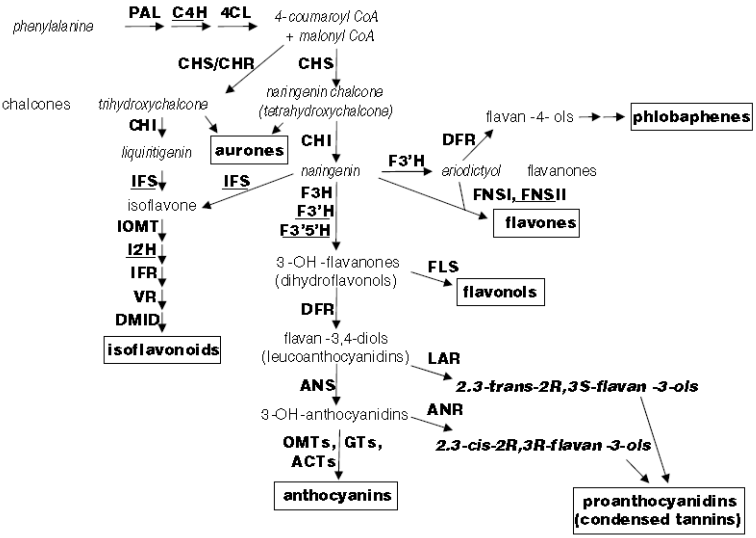

Fig. 1 Scheme of the flavonoid biosynthetic pathway (Winkel, 2006)

MATERIAL AND METHODS

Wheat caryopses with nonstandard coloration, genotypes UC66049 (UC) and Tschermaks Blaukörniger Sommerweizen (TBS) with blue aleurone, ANK-28B (ANK) and Abyssinskaya arraseita (AA) with purple pericarp were used in the experiment. Genotype Novosibirskaya 67 (N67) was used as a standard because it does not synthesize any pigments, therefore, it has white caryopses. A seed samples obtained from the Agricultural Research Institute Kroměříž, Ltd., Czech

Republic, was sown in the Botanical Gardens and Arboretum Mendel University in Brno, Czech Republic, in the spring of 2011. The developing caryopses were sampled 10, 15, 20, 25, 30, 35 and 40 days post anthesis (dpa), thus after the appearance of anthers. Genotype UC66049 blossomed 13. 6. 2011, ANK-28B and Tschermaks Blaukörniger Sommerweizen bloomed 15. 6. 2011. The last bloomed genotypes Abyssinskaya arraseita and Novosibirskaya 67 started anthesis 16. 6. 2011. Isolation of total RNA from caryopses was performed by the phenol-chloroform method using RNA Blue (Top Bio, Czech Republic). Reverse transcription from RNA into cDNA was carried out using the Enhanced Avian HS RT PCR kit from Sigma Aldrich, USA. The success of transcription was checked by PCR with a housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers were designed by the Primer3 software according to sequence AB162138 obtained from NCBI database (National Center for Biotechnology Information). Gradient PCR was used to determine the optimal temperature of primers annealing and to optimalize the qPCR conditions. The protocol was optimized for the CFX96 Real Time Systems (Bio-Rad, USA) instrument. The gene expression was calculated using ΔCt, a difference between Ct value of house keeping gene and Ct of gene of interest, where Ct means threshold cycle, the number of cycles at which the fluorescence exceeds the threshold (Livak, Schmittgen, 2001). For sequence data, the DNA fragments of the candidate gene for the enzyme DFR were amplifed by PCR. After purification with Turbo DNA Free kit (Ambion, USA) were PCR products sent to a specialized laboratory company Macrogen (Netherlands) for sequence analysis. The obtained sequences were compared with sequences from each chromosome arm of wheat cultivar Chinese Spring.

RESULT AND DISCUSSION

DFR sequence data

DFR gene sequences obtained from the direct sequencing of PCR product were from 148 bp (Abyssinskaya arraseita, ANK-28B, UC66049, Tschermaks Blaukörniger Sommerweizen) to 153 bp (Novosibirskaya 67) long. The variation in the bases number is due to several single or double nucleotide indels (insertions/deletions) in Novosibirskaya 67 genotype in positions between 12th and 39th nucleotide (Fig. 2). Triple nucleotide indel at 13th to 15th position doesn't change the reading frame but the other two single nucleotide indels at position 27 and 42 change reading frame, what causes completely different protein translation. Several single nucleotide polymorphisms were observed, for example at positions 68, 75, 77, 101 and 119 (Fig. 2). The similarity existing among all sequences of analyzed genotypes and sequence obtained from NCBI ranged between 94.59 to 100 %. The sequence was localized in two copies on chromosome 3B and 3D. Our results correspond with the work Himi and Noda (2004).

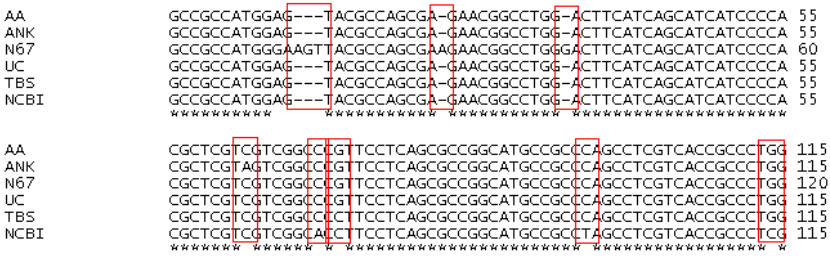

Fig. 2 Multiple alignment of selected section of DFR sequence using Clustal 2.1

AA – Abyssinskaya arraseita, *ANK – ANK-28B, N67 – Novosibirskaya 67, UC – UC66049, TBS – Tschermaks Blaukörniger Sommerweizen*

DFR expression

The expression of DFR in individual samples varied during maturation. For the control genotype Novosibirskaya 67, which does not synthesize any anthocyanins, there was highest DFR gene expression 25 dpa and the lowest 15 and 35 dpa. Expression of the CHS gene in the same genotype showed rather decreasing trend during maturation (Trojan et al., 2014). Blue coloured Tschermaks Blaukörniger Sommerweizen was observed the lowest expression 40 dpa and the highest 10 dpa, while in genotype UC66049 was DFR gene expression almost the lowest 10 dpa and 40 dpa reached the lowest values. Expression trend for the genotype Tschermaks Blaukörniger Sommerweizen during maturation is deceasing. Genotype UC66049 shows an increasing trend at first, but then decreasing again. Yang and collective (2003) mentions that the genotypes with blue aleurone had the highest mRNA level 18 dpa, then decreases rapidly and completely disappears 33 dpa. The highest DFR expression in Abyssinskaya arraseita, the genotype with purple pericarp, was observed between 15 and 20 dpa and then rapidly decreases to its lowest level 25 dpa. Genotype ANK28B shows decreasing expression during maturation, the highest value was observed 10 dpa and the lowest 35 dpa, 40 dpa expression slightly increased again. Quantitative and qualitative analysis of anthocyanins performed by the high-performance liquid chromatography method (Chabinová et al. 2011) is linked with expression data and will be carried by Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Czech Republic.

CONCLUSIONS

Comparing the DFR gene sequences obtained from NCBI and from various wheat genotypes with nonstandard coloured caryopses gives high degree of similarity (94.59 – 100 %). Both indels, which do not affect the reading frame and those that change reading frame were observed. Moreover several single nucleotide polymorphisms were detected. qPCR analyses of candidate DFR sequence provided primal results of gene expression during development of various wheat genotypes. It should be noted that the whole experiment will be repeated and optimized for accurate results. Last but not least it will be supplemented by the results of quantitative and qualitative analyses of anthocyanin.

AKNOWLEDGEMENTS

The authors are grateful for the support of Ing. Petr Martínek, CSc. from Agricultural Research Institute Kroměříž, Ltd., Czech Republic, for the seed samples and Ing. Tomáš Koloušek, a head of Botanical Gardens and Arboretum Mendel University in Brno, Czech Republic, for the cultivation of experimental plants

REFERENCES

CHABINOVÁ, J., ZÍTKA, O., HÚSKA, D., KLEJDUS, B., KIZEK, R., 2011: Stanovení antokyanů ve vzorcích barevné pšenice v průběhu zrání zrna. *Nové poznatky z genetiky a šľachtenia poľnohospodárskych rastlín*. 1. vyd. Piešťany, Slovensko: Centrum výskumu rastlinnej výroby Piešťany, 1003-1010.

HIMI, E., NODA, K., 2004: Isolation and location of three homoeologous dihydroflavonol-4-reductase (DFR) genes of wheat and their tissue-dependent expression. *Journal of Experimental Botany*, 55, 365-375.

LIN, J.-K., WENG, M.-S., 2006: *Flavonoids as nutraceuticals* in GROTEWOLD, E. *The Science of Flavonoids*. Springer, 213-238.

LIVAK, K. J., SCHMITTGEN, T. D., 2001: *Analysis of Relative Expression Using Real-Time Quantitative PCR and the 2-$^{\Delta\Delta C}{}_{T}$ Method*. Methods 25, 402-408.

NESI, N., JOND, C., DEBEAUJON, I., CABOCHE, M., LEPINIEC, L., 2001: The Arabidopsis TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed.*The Plant Cell*, 13, 2099-2114.

TROJAN, V., MUSILOVÁ, M., VYHNÁNEK, T., KLEJDUS, B., HANÁČEK, P., HAVEL, L., 2014: Chalcon synthase expression and pigments deposition in wheat with colored caryopsis. *Journal of Cereal Science*.

WALLACE, T.C. Anthocyanins in Cardiovascular Disease. *Advances in Nutrition*. 2011, 2, 1-7.

WINKEL, B. S. J., 2006: *The Biosynthesis of flavonoids* in GROTEWOLD, E. *The Science of Flavonoids*. Springer, 71-95.

YANG, G. H., ZHAO, X. Q., LI, B., LIU, J. Z., ZHENG, Q., TONG, Y. P., LI, Z. S., 2003: Molecular cloning and characterization of a DFR from developing seeds of blue-grained wheat in anthocyanin biosynthetic pathway. *Acta Botanica Sinica*, 45, 1329-1338.