PREDICTION OF TECHNOLOGICAL QUALITY OF WHEAT BASED ON GENETIC MARKERS

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ABSTRACT

The importance of wheat with different grain colour in practice is increasing, mainly of those with purple pericarp and blue aleurone. Higher amount of phenolic compounds especially anthocyanins with demonstrable antioxidant effects make this wheat different from a standard wheat varieties. The research based on molecular methods with the use of PCR deals with detection of the allelic composition of *Glu-1* loci of high molecular weight glutenin subunits (HMW-GS) and presence or absence verification of secalin locus (*Sec-1*). For our research 8 wheat genotypes with blue aleurone and one genotype with white grain were used. At the respective loci these subunits and their combinations were identified: *Glu-A1* (Ax1, Ax2*, AxNull), *Glu-B1* (Bx7+By8*, Bx7*+By8, Bx7*+By20*, Bx7+By20*, Bx6+By20*), *Glu-D1* (Dx5+Dy10). Subunits Ax1, Ax2* and allelic combination Dx5+Dy10 are considered as markers of good bread-making quality. The presence of allele *Glu-D1d* which is a marker of good bread-making grain quality was proved in genetic resources with blue aleurone UC66049, Skorpion (RU 440-6) and RU 440-5 and they are therefore considered as perspective. The rye translocation 1BL/1RS was absent in the whole studied collection. Blue grain genotypes Barevná 9 and Barevná 25 showed higher resistance to fusarium head blight.

Key words: HMW-GS, DNA markers, AS-PCR, SPLAT**,** wheat, blue aleurone

INTRODUCTION

Nowadays traditional breeding methods are more and more complemented with genetic structure data of a studied object in early stage of ontogenesis. Point of interest are crops playing role in human diet where undoubtedly belongs wheat. Requirements for new registered cultivars have raising and demanding character and in agricultural companies crop characteristics have to meet profitability requirements. Eventual yield depression has to be compensated with better grain quality with appropriate technological characteristics depending on the purpose of use or with absolutely new characteristics which are attractive for producers and consumers as well. At the same time the new cultivars should resist to negative environmental effects like climate and weather changes, soil salinity, drought, insect and pathogen stress. The new problem of an overbreeded crop grown in monocultures and large areas is overcoming genes of resistance against new pathotypes causing fungal diseases.

Wheats with genetically different grain colour are potentially a source of natural colorants what makes them different from classical varieties. Some of the genetic resources (wild species, regional cultivars, genetic material in process, etc.) with different grain colour were preserved to present days and our aim is to study this material comprehensively and suggest possibility of their use in practical breeding. Especially interesting are the wheat genotypes with purple pericarp and blue aleurone which are characteristic for a high amount of fenolic compounds from a group of anthocyans (ZEVEN, 1991). These compounds are considered as antioxidants which have positive effects on health of consumers. Wild forms of these genetic resources and some of their derivatives could be important holders of genes of resistance (HANZALOVÁ et al., 2009).

Important information for breeders is gained from genetic analysis which can detect presence or absence of perspective genes and with their aid it is possible to select with high efficiency. Using DNA markers it is possible to apply selection in very early stages of breeding process with a minimum quantity of plant material.

The research is focused on molecular methods and DNA marker application to characterise alleles encoding high molecular weight glutenin subunits (HMW-GS) based on PCR with the aim to predict bread-making quality at wheats (LIU et al., 2008), mainly in genotypes characterised by blue aleurone in the grain.

MATERIAL AND METHODS

Nine genotypes of winter and spring wheat (*Triticum aestivum* L.) from the collection of Agricultural Research Institute Kromeriz, Ltd. harvested in 2009 were used. The collection contained 8 genotypes with blue aleurone and one genotype with white grain (tab. 1).

Allele specific DNA markers were used for the identification of HMW-GS alleles at *Glu-A1*, *Glu-B1*, *Glu-D1* loci and the presence of the secalin locus *Sec-1* in all 9 wheat genotypes based on SPLAT and AS-PCR method. Quality of isolated DNA was verified by gel electrophoresis.

For allele detection at *Glu-A1* locus primers PS1, PS2 (LAFIANDRA et al., 1997) and PS3 (DE BUSTOS et al., 2000) were used. Alleles of the *Glu-B1* locus were identified by PS4 (BUTOW et al., 2004), PS5, PS6 and PS7 (LEI et al., 2006) primers. Further primer cauBx642 for identification of HMW subunits Bx14 and Bx17 was used according to XU et al. (2008). For determination at *Glu-D1d* allele at *Glu-D1* locus primer combination („Primer D“) according to D'OVIDIO & ANDERSON (1994) was used. For identification of the secalin locus ω-sec primers according to CHAI et al. (2005) were used.

Total volume of PCR reaction mixture (Promega, USA) was 24 µl and contained: 1 µl of template DNA, 0,1 µl of *Taq* polymerase, 5 µl of buffer, 1 µl of each primer and 0,1 µl of dNTPs filled up with deionized water to the appropriate volume. PCR temperature and time profiles for PS primers followed the protocol of SALMANOWICZ & DYLEWICZ (2007). The rest of used primers (cauBx642, „Primer D" and ω-sec) followed temperature and time profiles according to appropriate protocols mentioned above. Electrophoretic separation was carried out under standard conditions on a 1.5% agarose gel stained by ethidium bromide. Final PCR products were compared with appropriate size standards: λDNA/Eco471/AvaII/ (MBI Fermentas), 100 bp DNA Ladder (Promega) and pBR322 DNA HaeIII (ABgene).

Tab. 1 List of tested wheat genotypes

Genotype	**Form**	**Grain colour**
Novosibirskaya 67 (N 67)	spring	white grain
UC66049	spring	blue aleurone
Tschermaks Blaukörniger Sommerweizen (TBS)	spring	blue aleurone
Tschermaks Blaukörniger (TB)	spring	blue aleurone
48 M	winter	blue aleurone
RU 440-6 (Skorpion)	winter	blue aleurone
RU 440-5	winter	blue aleurone
Barevná 9	winter	blue aleurone
Barevná 25	winter	blue aleurone

RESULT AND DISCUSSION

Glu-A1b (Ax2*) allele was identified only in the genotype with white grain (Novosibirskaya 67) and *Glu-A1a* allele in five genotypes with blue aleurone (UC66049, Tschermaks Blaukörniger Sommerweizen, Tschermaks Blaukörniger, RU 440-6 and RU 440-5). At the *Glu-A1* locus three types of alleles were found. N 67 contained subunit Ax2* UC66049, TBS, TB, RU 440-6 and RU 440-5 subunit Ax1. In genotypes 48 M, Barevná 9 and Barevná 25 the subunit AxNull was identified. Obtained results for UC66049 and Barevná 25 genotypes correspond to results of the electrophoretic analysis of storage proteins published by CHŇAPEK et al. (2010) and the result of the genotype 48 M correspond to the study GREGOVÁ et al. (2011). According to LIU et al. (2008) both subunits Ax1 and Ax2* have positive influence on the bread-making quality and according to the qualitative evaluation based on *Glu-1* score (PAYNE et al., 1987) they obtained score value 3 which means the second highest evaluation in this scale. Vice versa in the case of subunit AxNull it is classified with the lowest value which is 1. Generally from 8 tested samples allele *Glu-A1a* was identified in 5 genotypes (tab. 2).

Within *Glu-B1* locus five allelic combinations was detected Barevná 9 was the only genotype having the subunit Bx6 which is a marker of worse bread-making quality (SCHWARZ et al., 2004). DONG et al. (1991) described negative effect of the allelic pair Bx6+By8 (*Glu-1* score = 1) on dough mixograph evaluation.

Glu-D1 locus positively and negatively influences bread-making quality as well. Subunit combination Dx5+Dy10 has positive effect on bread-making quality and has the highest *Glu-1* score 4 (from the *Glu-1* maximum of 10 points); and classify these wheat genotypes among the highest quality categories that means E and A (PAYNE et al., 1987). Contrarily Dx2+Dy12 subunit combination has negative effect on bread-making quality (D'OVIDIO & ANDERSON, 1994). Presence of *Glu-D1d* allele with Dx5+Dy10 subunits was identified in three genotypes: UC66049, RU 440-6 and RU 440-5 and they are considered as the most suitable for bread-making usage. RU

440-6 line was tested in official registration process in Austria and authorised as a new variety of winter wheat called „Skorpion“ (MARTINEK et al., 2012).

1BL/1RS translocation in wheat transferred from rye is possible to detect through presence of the secalin locus. Translocation carries genes of resistance to leave diseases mainly to rusts (HANZALOVÁ et al., 2009). Despite the absence of rye translocation in all tested genotypes high level of rust resistance during monitored vegetative seasons 2010 and 2011 was proved in most of genotypes except samples RU 440-5, Barevná 9 and Barevná 25 which were quite sensitive.

Materials Barevná 9 a Barevná 25 should be acceptable donors for fusarium head blight disease resistance in blue grained wheats.

Tab. 2 List of resulting alleles detection on Glu-1 and secaline loci

Genotype	**Alleles at *Glu-1* locus**			**Secaline locus**
	Glu-A1	***Glu-B1***	***Glu-D1***	***Sec-1***
White grain				
Novosibirskaya 67	*b*	*1a + 2o*	*	*
Blue aleurone				
UC66049	*a*	*1b + 2a*	*d*	*
Tschermaks Blaukörniger Sommerweizen	*a*	*1b + 2z*	*	*
Tschermaks Blaukörniger	*a*	*1b + 2z*	*	*
48 M	*c*	*1a + 2o*	*	*
RU 440-6	*a*	*1a + 2z*	*d*	*
RU 440-5	*a*	*1a + 2z*	*d*	*
Barevná 9	*c*	*1d + 2z*	*	*
Barevná 25	*c*	*1a + 2z*	*	*

Legend: *Glu-A1a* (encodes Ax1), *Glu-A1b* (Ax2*), *Glu-A1c* (AxNull), *Glu-B1-1a* (Bx7), *Glu-B1-1b* (Bx7*), *Glu-B1-1d* (Bx6), *Glu-B1-2a* (By8), *Glu-B1-2b* (By9), *Glu-B1-2o* (By8*), *Glu-B1-2z* (By20*), *Glu-D1d* (Dx5+Dy10), * – presence not proved

CONCLUSIONS

Analyses of HMW glutenin subunits for *Glu-A1*, *Glu-B1*, *Glu-D1* loci and secalin locus *Sec-1* (rye translocation marker) revealed that genotypes UC66049, RU 440-6 and RU 440-5 are the most acceptable for bread-making quality due to a presence of *Glu-D1d* allele. Because of missing rye translocation 1BL/1RS in genotypes Novosibirskaya 67, UC66049, TBS, TB, 48 M and RU 440-6 presence of other genes of resistance mainly to rusts can be supposed. With regard to the final quality of genotypes Barevná 9 and Barevná 25 lower occurrence of fusarium head blight was observed. These genotypes should be tested for resistance in provoking conditions to find out their potential to be possible donors of resistance against this pathogen in wheat.

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