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IDENTIFICATION OF *Glu-B1* ALLELES IN BREAD WHEAT CULTIVARS USING PCR

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High dough strength is used as a predictor of good quality bread wheat and it has been attributed largely to the type of allele present at the *Glu-D1* locus, where the *Glu-D1*d allele is most favorable. Recently, it has been observed that cultivars with over expressed subunit Bx7^{OE} at the *Glu-B1* have enhanced dough strength. In order to implement recently developed PCR assay for *Glu-B1* x-type allele discrimination in marker-assisted selection, a set of wheat cultivars from 11 countries was analyzed. The PCR results matched the known *Glu-B1* HMW GS genotypes of the cultivars possessing subunits Bx7 or Bx17. It was shown that this molecular marker also differentiated some other *Glu-B1* alleles: Bx6, Bx13, Bx20 and Bx22. These results create the opportunity for an improved method of polymorphism scoring at the *Glu-B1* in bread wheat germplasm.

Key words: wheat, HMW GS, *Glu-B1*, marker-assisted selection

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INTRODUCTION

The bread-making quality of wheat (*Triticum aestivum* L.) is primarily influenced by its protein content and gluten quality. The major gluten fractions are glutenin proteins composed of high molecular weight glutenin subunits (HMW GS) and low molecular weight glutenin subunits (LMW GS).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is an efficient method for profiling HMW GS in wheat. In some cases, however, difficulties arise when different subunits of the same mobility cannot be discriminated on the gel. Besides SDS-PAGE, RFLP based markers were used in our wheat breeding programs (VAPA *et al*., 1995). Wide range of PCR markers for detection of *Glu-1* alleles were developed (AHMAD, 2000, De BUSTOS *et al.*, 2000, SCHWARZ *et al.*, 2004). In the case of alleles of the *Glu-B1*-encoded subunit Bx7, only small and unclear differences in electrophoretic mobility between subunit Bx7 and Bx7* were found (MARCHYLO *et al.*, 1992). To overcome this problem a PCR marker, originally designed to discriminate *Glu-1* Bx7 and *Glu-1* Bx17 (MA *et al.*, 2003) was used to differentiate two allelic variants of Bx7 HMW GS Bx7* and $Bx7^{oe}$ (BUTOW *et al.*, 2003). The importance of these findings was confirmed by strong association between lines with an over expression of $Bx7^{oe}$ and high dough strength.

The aim of the present paper was to evaluate PCR assay for *Glu-B1* alleles discrimination in order to implement it into bread making quality oriented wheat breeding programs.

MATERIALS AND METHODS

Twenty-eight wheat cultivars (Tab. 1) from 11 countries represent a part of the Core Collection of Novi Sad (Serbia) wheat breeding centre. Cultivars 'Cheyenne', 'Red River' and 'NS 100' (1B/1R substitution) were used as references in the PCR analysis.

PCR analysis: Genomic DNA was isolated from seeds (PLASCHKE *et al.*, 1995). DNA fragments encoding the part of the *Glu-B1-1* were amplified by PCR using a set of locus-specific primers (MA *et al.*, 2003). PCR products were separated on 6% non-denaturing polyacrylamide gels in 1X TBE buffer and visualized by silver staining (SANGUINETTI *et al.*, 1994).

RESULTS AND DISCUSSION

DNA fragments encoding the part of central repetitive domain and a part of carboxyl termini of *Glu-B1-1* were amplified by PCR using a set of locus-specific primers. The reference cultivar 'Cheyenne' (Bx7*, normal expression level) yielded two bands of approximately 650bp and 750 bp, whereas the second reference 'Red River' gave two bands of 670 bp and 770 bp in length. A single amplification product for cultivars containing the Bx17 gene and two bands for Bx7 cultivars were generated (Fig. 1). The PCR results matched the known *Glu-B1* HMW GS genotypes of the cultivars with subunits Bx7 or Bx17 in complete

agreement with the report by BUTOW *et al.* (2003). Comparison of known cultivar HMW glutenin genotypes (Tab. 1) and the results obtained by PCR analysis of *Glu-B1* showed that 4 cultivars possessed 7+8 glutenin subunits, while a cultivar 'Centurk' had the over-expressed Bx7^{oe} allele.

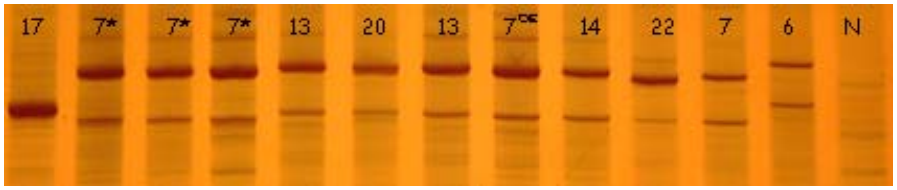

Figure 1. PCR assay for the Bx HMW-GS
(from the left to the right: S. Cerros, Bezostaya 1, Pobeda, Cheyenne, Lerma Rojo, Kraljevica, Timson, Red River, KG 56, Klein Toledo, C. Desprez , Avalon, NS 100)

Table 1. The Glu-B1 HMW GS and the PCR results for selected cultivars.

Cultivar	Origin	*Glu-B1*/ PCR*		Cultivar	Origin	*Glu-B* / PCR*	
Agent	USA	7+9	**4**	Lerma Rojo	MEX	13+16	**2**
Auburn	USA	7+9	**4**	Ljiljana	SCG	7	**4**
Avalon	UK	6+8	**1**	Mina	SCG	6+8	**1**
Bankut 1205	HUN	7+9	**4**	Odeska 51	UKR	7+8	**4**
Bezostaya 1	RUS	7+9	**4**	Pesma	SCG	7+9	**4**
Brigant	UK	6+8	**1**	Pobeda	SCG	7+9	**4**
Cajeme 71	MEX	7+9	**4**	Rana niska	SCG	7	**4**
C. Desprez	FRA	7	**4**	Sava	SCG	7+8	**4**
Centurk	USA	7+8	**3**	S. Cerros	MEX	17+18	**6**
Cook	AUS	17+18	**6**	Sremica	SCG	7+9	**4**
Highbury	UK	17+18	**6**	Timson	AUS	13+16	**2**
Ivanka	SCG	7+8	**4**	ZG 1011	CRO	22	**5**
Jugoslavija	SCG	7+9	**4**	Reference cultivars			
KG 56	SCG	14+15	**3**	Red River	USA	7+8	**3**
Klein Toledo	ARG	22	**5**	Cheyenne	USA	7+9	**4**
Kraljevica	SCG	20	**2**	NS 100	SCG	21	**N**

The Bx PCR products are marked after electrophoretic mobility (1 stands for Bx6; 2 for Bx13 and Bx20; 3 for Bx7^{oe} and Bx14; 4 for Bx7 and Bx7, 5 for Bx22, 6 for Bx17). N represents the null allele from NS 100 cultivar (1B/1R)

All non-Bx7 genotypes (Bx6, Bx13, Bx14, Bx20, and Bx22) possessed two PCR products (Fig. 1). Cultivars that possessed HMW GS 13+16 or 20, had PCR products somewhat longer than 'Red River' ($Bx7^{oe}$). Cultivars with subunits 6+8 had much larger PCR products than reference 'Red River'. Cultivars expressing HMW GS 22 produced two PCR bands (about 730 and 670 bp in length). Differentiation of genotypes with *Glu-B1* HMW GS 7 (*Glu-B1*a allele) and subunit 14+15 was not possible by simply comparing the mobility of PCR products because their length was the same or very similar to products of 'Red River'. SCHWARZ *et al.* (2004) developed a PCR-based marker for negative selection of Bx6 HMW GS, but this marker was not able to differentiate Bx7 and Bx17 genotypes. Concerning the application of this marker in conventional marker-assisted selection, the two different PCR assays to screen predominant *Glu-B1* alleles in hexaploid wheat are indispensable. Results obtained here suggest that applying one PCR assay the genotypes expressing Bx6, Bx22, Bx17, Bx7* and $Bx7^{oe}$ could be clearly distinguished, while genotypes with Bx13 and Bx20 could be differentiated only on PAA gels. One of the main advantages of these findings could be a wide-ranging use of Bx primers for a simple and rapid screening of *Glu-B1* allelic variability especially in South-eastern European breeding programs known for their diverse germplasm.

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REFERENCES

AHMAD, M. (2000): Molecular marker-assisted selection of HMW glutenin alleles related to wheat bread quality by PCR-generated DNA markers. Theor. Appl. Genet. *101*: 892-896.

BUTOW, B.J., W. MA, K. R. GALE, G. B. CORNISH, L. RAMPLING, O. LARROQUE, M. K. MORELL, and F. BEKES (2003): Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. Theor. Appl. Genet. *107*: 1524-1532.

DeBUSTOS, A., P. RUBIO, and N. JOUVE (2000): Molecular characterisation of the inactive allele of the gene *Glu-A1* and the development of a set of AS-PCR markers for HMW glutenins of wheat. Theor. Appl. Genet. *100*: 1085-1094.

MA, W., W. ZHANG, and K. R. GALE (2003): Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica *134*: 51-60.

MARCHYLO, B.A., O. M. LUKOW, and J. E. KRUGER (1992): Quantitative variation in high-molecular-weight glutenin subunit 7 in some Canadian Wheats. J. Cereal Sci. *5*: 29-37.

PLASCHKE, J., M. W. GANAL, and M. S. RODER (1995): Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet. *91*: 1001-1007.

SANGUINETTI, C.J., E. DIAS NETO, and A. J. G. SIMPSON (1994): Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. Biotechniques *17*: 915-919.

SCHWARZ G., F. G. FELSENSTEIN, and G. WENZEL (2004): Development and validation of a PCR-based marker assay for negative selection of the HMW glutenin allele *GluB1-1d* (Bx-6) in wheat. Theor. Appl. Genet.*109*: 1064-1069.

VAPA Lj., S. DENČIĆ, E. ŠOLTES-RAK, S. KEVREŠAN (1995): Genetic variation in *Glu-1* loci and bread-making quality in wheat. Cereal Res. Commun. *23*: 161-166.

IDENTIFIKACIJA *Glu-B1* ALELA SORTI PŠENICE PRIMENOM PCR

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Izvod

Zahvaljujući jedinstvenim karakteristikama polimernog proteina - glutena, brašno hlebne pšenice ima sposobnost razvoja viskoznih i elastičnih osobina testa. Kvalitativnim karakteristikama glutena u najvećem procentu doprinose proteinski produkti *Glu-1* lokusa, od kojih lokus *Glu-B1* pokazuje najveću alelnu varijabilnost. Alelna kompozicija *Glu-B1* lokusa dvadesetosam genotipova heksaploidne pšenice je analizirana primenom PCR-zasnovanih markera sa ciljem ocene preciznosti i fleksibilosti metode u svetlu njene implementacije u programe marker asistirane selekcije pšenice. Na osnovu analize veličine produkata amplifikacije, utvrdjeno je da od 4 genotipa nosilaca 7+8 subjedinice, samo sorta Centurk poseduje jaču ekspresiju subjedinice 1Bx7. Pored već poznatih literaturnih nalaza o mogućnosti razlikovanja i alela *Glu-B1i,* odgovornog za sintezu HMW gluteninske subjedinice 17+18, elektroforetskim razdvajanjem na 6% nedenaturišućem PAA gelu, na osnovu svoje mobilnosti mogli su se razlikovati i aleli *Glu-B1d* (subjedinica 6+8), *Glu-B1f* (subjedinica 13+16), *Glu-B1e* (subjedinica 20) i *Glu-B1k* (subjedinica 22). Prezentovani rezultati otvaraju mogućnost za efikasnu primenu ovog tipa markera u proučavanju polimorfnosti *Glu-B1* lokusa u germ plazmi heksaploidne pšenice.

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