DNA Markers for High Molecular Weight Glutenin Subunits 5+10 Used in Wheat and Triticale Breeding

Miroslava VINTEROVÁ[1], Jan BEDNÁŘ[1], Ivana JEŽÍŠKOVÁ[1] and Petr MARTINEK[2]

[1]Faculty of Agronomy, Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic; [2]Agricultural Research Institute Kroměříž, Ltd., Kroměříž, Czech Republic

Abstract: Prediction of flour breadmaking quality was verified using DNA markers in seven genotypes of winter wheat (*T. aestivum* L., 2*n* = 6*x* = 42, AABBDD) of different quality classes, four genotypes of triticale (× *Triticosecale* Wittmack, 2*n* = 6*x* = 42, AABBRR), and selected progenies of triticale Presto with the T1R.1D$_{5+10}$-2 translocation. DNA isolated from fresh leaves (the stage of the first true leaf) was used to detect the *Glu D1 5+10* allele based on the SPLAT protocol according to D'Ovidio and Anderson (1994). The presence of the *Glu D1 5+10* allele was verified using a product of 450 bp size. It was detected in the wheat genotypes Athlet, Brea, Bruneta, Iris, Lívia, Mona, Sida, and in all analysed progenies derived from the Presto triticale sample with the T1R.1D$_{5+10}$-2 translocation. Effects are discussed of other loci on the final breadmaking quality in the wheat varieties Athlet, Lívia, and Mona with *Glu D1 5+10* and a lower grain breadmaking quality.

Keywords: triticale; wheat; SPLAT; *Glu D1 5+10*; translocation; T1R.1D

The use of molecular markers significantly supplements various classic methods for genetic analyses. Mostly protein (isoenzyme) molecular markers were used earlier, whereas DNA markers are increasingly applied at present. The most important method of current molecular biology is PCR (Polymerase Chain Reaction). Most methods for the study and detection of DNA markers are derived from the standard PCR. The results of the detection of DNA polymorphism can be successfully used similarly to HMW glutenin subunits for the prediction of wheat breadmaking quality. It can also be used in triticale (× *Triticosecale* Wittmack) that was man-made as an allohexaploid hybrid derived from the cross of wheat (*Triticum* spp.) and rye (*Secale cereale*). In recent years, an increasing interest in triticale has been recorded among growers and breeders. The assortment of the triticale registered varieties comprises hexaploid forms (2*n* = 6*x* = 42, AABBRR) that carry wheat A and B genomes, and rye R genome. In contrast with wheat, the triticale grain is not acceptable for breadmaking purposes because triticale contains chromosomes R instead of chromosomes D. The presence of several genes on chromosomes R (particularly 1R and 6R) causes worse viscoelastic properties of dough, and thus a lower breadmaking quality of flour in comparison with wheat. An important positive effect on the breadmaking quality in wheat is assigned to the *Glu D1 5+10* allele (on chromosome 1DL) that is considered as a marker conferring a good breadmaking quality. This allele is present in most of wheat varieties with elite (E) breadmaking quality. The absence of the *Glu D1 5+10* allele and the presence of some secalin alleles on chromosomes 1R and 6R are the reasons why the common triticale varieties cannot be used for breadmaking purposes (Woś *et al*. 2002).

Unsatisfactory quality parameters of triticale can be improved by transferring the *Glu D1 5+10* allele into triticale using the translocation of chromosome 1R with 1D. Such a type of translocation has been developed at the University of California. Two types of translocations designated T1R.1D$_{5+10}$-1 and T1R.1D$_{5+10}$-2 have been described that differ from each other by the length of the segment

Supported by the Grant Agency of the Czech Republic, Project No. 521/03/0113.

transferred from chromosome 1D (Lukaszewski 1994, 1998).

The objective of this work was to detect the *Glu D1 5+10* allele in wheat genotypes with different grain quality, in triticale standard varieties and lines, and to verify the presence of T1R.1D$_{5+10}$-2 translocation (a shorter transferred segment from chromosome 1D) in the selected triticale accessions.

MATERIAL AND METHODS

The seed of the analysed genotypes was obtained from the collection of genetic resources at the Agricultural Research Institute Kroměříž, Ltd. (Table 1).

The analysis of DNA polymorphism was carried out in seven wheat (*T. aestivum* L.) varieties with different grain quality, two German triticale varieties, and two breeding lines and four progenies of triticale derived from the Presto variety sample containing the 1R.1D$_{5+10}$-2 translocation. The original sample of Presto triticale with this translocation was provided by Prof. Adam J. Lukaszewski in 2000.

The plants for DNA isolation were taken at the stage of the first true leaf. Hydroponic cultivation was performed under the light regime of 12 h light and 12 h dark, and at the temperature of 20°C. To detect the *Glu D1 5+10* allele, DNA isolated from 100 mg of fresh green leaves (an average sample of 20 plants) was used. DNA was isolated using a DNasy Plant Mini Kit (Qiagen Firm) and separated by means of horizontal electrophoresis on agarose gel stained with ethidium bromide. After the evaluation of the quality of the isolated DNA, it was employed for the detection of the *Glu D1 5+10* allele using PCR SPLAT technique according to D'Ovidio and Anderson (1994), and modified by Vejl (1998). The primers JEDL 11 (5') GCC TAG CAA CCT TCA CAA TC and JEDL 12 (5') GAA ACC TGC TGC GGA CAA G were applied for annealing (Jedličková *et al*. 2002).

SPLAT was carried out in a TECHNE PROGENE thermocycler under the following temperature regime: denaturation (94°C, 60 s), annealing (63°C, 45 s), elongation (72°C, 30 s); 30 cycles in total. After finishing the SPLAT, DNA was separated by horizontal electrophoresis in agarose gel.

RESULTS AND DISCUSSION

Based on the analyses of the results obtained by means of the SPLAT protocol, the *Glu D1 5+10* allele was detected in five wheat genotypes and in

Table 1. Wheat and triticale genotypes analysed

Variety (line)	Year of registration in the CR	Country of origin	Breadmaking quality
Wheat			
Athlet	1996	DEU	C
Brea	1996	CZE	E
Bruneta	1996	CZE	B
Iris	1983	SVK	–
Lívia	1991	SVK	C
Mona	1994	CZE	B
Sida	1993	CZE	B
Triticale			
Babbor		DEU	–
Binova		DEU	–
SG-U 204	Breeding lines of the Selgen, Co. Ltd.		
SG-U 214			
Presto No. 1	Selected progenies derived from the sample of the Presto variety with the T1R.1D$_{5+10}$-2 translocation		
Presto No. 3			
Presto No. 4			
Presto No. 5			

CZE – Czech Republic, SVK – Slovak Republic, DEU – Germany

all progenies of the translocated hexaploid triticale (Figure 1 and Table 2).

The winter wheat varieties Iris (quality close to C class), Sida (B), and triticale genotypes without translocations do not possess the *Glu D1 5+10* allele, and therefore they do not possess a good breadmaking quality. By contrast, some wheat varieties with the *Glu D1 5+10* locus are characterised by a low grain quality. Such varieties are, for example, Athlet and Lívia; although they contain the *Glu D1 5+10* allele, they have a low quality on the level of C class – unacceptable quality for bread making. A similar case is with the variety Mona which is included in B class – bread quality. In these varieties, the positive effect of the *Glu D1 5+10* allele is suppressed by the gliadin allele *Gld B1 3* that was transferred into wheat by T1BL.1RS translocation. The presence of individual gliadin and glutenin alleles in all varieties registered in the Czech Republic was studied by Šašek *et al*. (2000) who also characterised their effects on the grain quality by means of glutenin scores. It was found that *Gld B1 3* participated in a considerable decrease in the breadmaking quality, and it was a marker of low grain quality. The variety Mona with high glutenin score carries the *Glu D1 5+10* and simultaneously the *Gld B1 3* allele whose interaction leads to lower breadmaking quality.

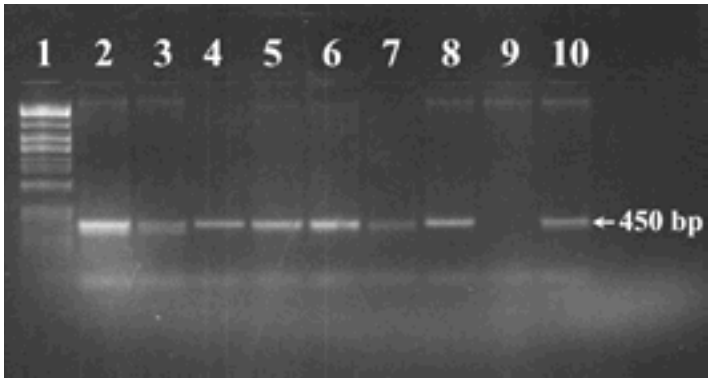
Table 2. Detection of the *Glu D1 5+10* allele in the analysed genotypes of wheat and triticale

Wheat		Triticale	
Name	*Glu D1 5+10*	Name	*Glu D1 5+10*
Athlet	+	Babbor	–
Brea	+	Binova	–
Bruneta	+	SG-U 204	–
Iris	–	SG-U 214	–
Lívia	+	Presto No. 1	+
Mona	+	Presto No. 3	+
Sida	–	Presto No. 4	+
		Presto No. 5	+

The variety Sida is characteristic of the presence of the *Glu D1 2+12* allele (marker of lower breadmaking quality) and the *GLD B1 3* allele (Šašek *et al*. 2000).

A great attention was paid to the study of the importance of the individual glutenin and gliadin alleles in relation to the grain quality and, based on protein electrophoresis, their effects on the quality of a viscoelastic storage protein complex was investigated to a certain extent. DNA markers can be used for the detection of the loci examined during the breeding process.

In the analysed varieties and new breeding lines of triticale, the result corresponds with the genome structure (AABBRR). The absence of D genome in common triticale varieties (Babbor and Binova) and breeding lines (SG-U 204 and SG-U 214) excludes the presence of the *Glu D1 5+10* allele, and thus of course, a good breadmaking quality in this crop. Similar conclusions drawn from the detection of protein polymorphism using the SDS-PAGE technique in wheat were also reported by other authors (Šašek & Černý 1998, Šašek *et al*. 2000). The molecular analysis confirmed that all analysed progenies derived from the Presto triticale with the T1R.1D$_{5+10}$-2 translocation contained *Glu D1 5+10.* Thus, the existence of the corresponding translocated segment of wheat chromosome 1D was confirmed indirectly in these progenies. Furthermore, the results show that the original sample obtained from the USA was obviously homogeneous enough. Significant findings were achieved for further experimental work. The triticale accessions with the T1R.1D$_{5+10}$-2 translocation are used for hybridisation with important triticale varieties in



1 – size marker; 2 – Mona; 3 – Lívia; 4 – Presto No. 3; 5 – Presto No. 5; 6 – Presto No. 1; 7 – Presto No. 4; 8 – Bruneta; 9 – SG-U-214; 10 – Athlet

Figure 1. Electrophoretic analysis of SPLAT amplified products of the molecular marker conferring *Glu D1 5+10* allele

order to develop new breeding materials prospective for an improved breadmaking quality.

Conclusion

The marker of good breadmaking quality is the *D1 5+10* glutenin allelic locus. It was detected by a SPLAT technique (D'Ovidio & Anderson 1994). The product in agarose gel is of 450 bp size. This DNA marker confirmed the presence of *Glu D1 5+10* allele in the varieties Athlet, Brea, Bruneta, Lívia, and Mona. The *Glu D1 5+10* allele was detected in all analysed progenies derived from the sample of the Presto variety with the T1R.1D$_{5+10}$-2 translocation. The results obtained justify the utilisation of the SPLAT protocol of the marker for the detection of the *Glu D1 5+10* allele in wheat and triticale.

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Received for publication March 6, 2003
Accepted after corrections June 3, 2003

Abstrakt

Vinterová M., Bednář J., Ježíšková I., Martinek P. (2003): **DNA markery pekařské jakosti pšenice a tritikale**. Czech J. Genet. Plant Breed., **39**: 69–72.

Predikce pekařské kvality mouky byla ověřována pomocí DNA markerů u sedmi genotypů ozimé pšenice (*T. aestivum* L., 2*n* = 6*x* = 42, AABBDD) různé třídy pekařské jakosti, 4 genotypů tritikale (× *Triticosecale* Wittmack, 2*n* = 6*x* = 42, AABBRR) a vybraných potomstev tritikale Presto s translokací T1R.1D$_{5+10}$-2. DNA izolovaná z čerstvých listů (fáze 1. pravého listu) byla použita pro detekci alel *Glu D1 5+10* podle protokolu SPLAT (D'Ovidio & Anderson 1994). Výskyt alel *Glu D1 5+10* je verifikován produktem o velikosti 450 bp. Byl detekován u genotypů pšenice Athlet, Brea, Bruneta, Iris, Lívia, Mona, Sida a všech analyzovaných potomstev odvozených ze vzorku tritikale Presto s translokací T1R.1D$_{5+10}$-2. U odrůd pšenice Athlet, Livia a Mona s výskytem *Glu D1 5+10* a nižší pekařskou kvalitou zrna je diskutováno o vlivu jiných lokusů na konečnou pekařskou jakost.

Klíčová slova: tritikale; pšenice; SPLAT; *Glu D1 5+10*; translokace; T1R.1D

Corresponding author:

Ing. Miroslava Vinterová, Ústav botaniky a fyziologie rostlin, Agronomická fakulta, Mendelova zemědělská a lesnická univerzita v Brně, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 136 067, fax: + 420 545 133 011, e-mail: vinter@mendelu.cz