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MOLECULAR CHARACTERISATION OF GLUTENIN ALLELES AT THE Glu-D1 LOCUS

ABSTRACT: It is well known that the composition of high-molecular-weight (HMW) glutenin subunits impacts the bread making quality. The HMW subunits 1Dx5-1Dy10 are typically associated with high dough strength and good bread making quality, contrary to 1Dx2-1Dy12 subunits. Bread wheat cultivars from Institute of Field and Vegetable Crops in Novi Sad have been screened for the alleles present at Glu-D1 locus using traditional SDS PAG electrophoresis method and a new PCR based approach. The Glu-D1 locus was screened for two main x-type alleles which code for HMW glutenins 2 and 5, and two main y-type alleles which code for HMW glutenin subunits 10 and 12. Among the analyzed cultivars, 55.6% expressed the presence of 1Dx5 and 1Dy10 alleles at the Glu-D1 locus. These results confirmed that by using marker-assisted selection (MAS) it is possible to identify genotypes with alleles for good bread making quality, which could be successfully used in wheat breeding programs.

KEY WORDS: Glu-D1 locus, marker-assisted selection, PCR, SDS PAG electrophoresis, wheat

INTRODUCTION

In hexaploid wheat, Triticum aestivum, the genes controlling the synthesis of high-molecular-weight glutenins are located on the long arms of the chromosomes 1A, 1B and 1D at the loci Glu-A1, Glu-B1 and Glu-D1, respectively. Har ber d et al. (1987) proved that there are two closely linked genes at each of Glu-1 loci, one gene controlling the synthesis of x-type HMW glutenin subunits, another controlling the synthesis of y-type subunits. All glutenin genes are organized with a large central region containing repeat short motifs coding for the same amino acid flanked by unique N- and C-terminus domains (De Busto s et al., 2000).

The properties of wheat dough have been related to the allelic combination of the genes coding for the high-molecular-weight (HMW) subunits of
glutenin. Payne (1987) demonstrated that bread-making quality is associated with the presence of HMW subunits x5+y10 at Glu-D1 locus providing good quality and x2+y12 providing poor quality.

Traditionally, HMW glutenins had been analyzed using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the protein bands representing subunits have been numbered according to their mobility on the gel (Payne, 1987). More recently, D'Ovidio et al. (1994), Ahmad (2000), De Bustos et al. (2001) have demonstrated the usefulness of PCR-based analysis for distinguishing between cultivars with different HMW subunits.

The aim of this paper was molecular characterization of glutenin alleles at the Glu-D1 locus of selected bread wheat cultivars using SDS PAGE electrophoresis and new specific PCR based primers, in order to identify wheat genotypes carrying glutenin allelic combinations related with good bread making quality.

MATERIAL AND METHODS

Material. Eighteen bread wheat cultivars from Institute of Field and Vegetable Crops in Novi Sad were used in this study. Wheat cultivars Jugoslavija (x5+y10) and Baranjka (x2+y12) served as controls for HMW composition analysis.

Polyacrylamide gel electrophoresis. Extraction of proteins and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) was done on 10% gel, in Tris-glycine buffer (pH 8.3) according to the procedure described by Vapa and Savic (1988).

DNA isolation. Total genomic DNA was isolated from wheat grains according to Plaschke et al. (1995). The DNA concentrations were determined spectrophotometrically.

PCR analysis. PCR analysis was performed according to Ahmad (2000). The 50-μl amplification reaction mixture contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl2, 300 mM dNTP (deoxyribonucleotide), 250 ng of each primer, 1U of Taq DNA polymerase and 50 ng genomic DNA. Primers used for identifying 2 vs. 5 x-type allele, designated as P1 and P2, and primer pair applied for identification of 10y vs. 12 y-type allele, designated as P3 and P4, were published in Ahmad (2000). Amplifications were performed in an Eppendorf Mastercycler programmed at 94°C for 5 minutes, followed by 45 cycles at 94°C for 1 min, 63°C for 1 min and 72°C for 1 min. After 45 cycles, the extension temperature was kept at 72°C for 10 min. PCR products were analyzed on 2% agarose gel containing 0.5 μg/ml ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

During SDS PAG electrophoresis, proteins are separated into their subunits according to molecular weight. HMW glutenin subunits coded by the
Glu-1 loci are grouped in the upper part of the gel. In the examined cultivars, only subunits 5+10 and 2+12 controlled by the genes at the Glu-D1 locus were determined (Figure 1). The subunits 5+10 were more frequent as a result of Novi Sad wheat breeding programs favoring the parents carrying these subunits (Vapa et al., 1995, 1997; Vapa and Obreht, 2000).

![Image](image1)

Figure 1. SDS PAGE of the analyzed wheat cultivars (K1 — Jugoslavija, K2 — Baranjka, 1 — Alfa, 2 — Anastasija, 9 — Dična, 10 — Draga and 11 — Dragana)

When primer combinations P1 and P2 were used, 450 bp amplification products were visible on the gel in ten out of the eighteen analyzed genotypes, indicating the presence of Dsx5 alleles. In the other eight cultivars amplifica-

![Image](image2)

Figure 2. Separation of amplification products on agarose gel (2%) using the P1/P2 primer set (M — 100 bp marker 5, 6 and 7 are cultivars Bečejka, Cipovka and Dejana)
tion products were detected indicating that allele 2 at *Glu-D1* locus was not present (Figure 2).

With primer sets P3 and P4, specific 576 bp fragment characterized Dy10 allele and it was present in ten cultivars. The other eight cultivars expressed 612 bp amplification product showing the presence of Dy12 allele at the analyzed locus (Figure 3).

![Image](image.png)

Figure 3. Separation of amplification products on agarose gel (2%) using the P3/P4 primer set (M — 100 bp marker and 5 and 6 are cultivars Bečejka and Cipovka)

Identification of HMW glutenin alleles at *Glu-D1* locus of the 18 selected bread wheat cultivars revealed that 10 cultivars possessed 1Dx5 and 1Dy10 alleles at the *Glu-D1* locus, while 8 cultivars possessed 1Dx2 and 1Dy12 alleles at the analyzed locus (Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Cultivar</th>
<th>1Dx2 (none)</th>
<th>1Dx5 (450 bp)</th>
<th>1Dy10 (576 bp)</th>
<th>1Dy12 (612 bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alfa</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>2.</td>
<td>Anastasija</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Bajka</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>4.</td>
<td>Balkan</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>5.</td>
<td>Bečejka</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Cipovka</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>7.</td>
<td>Dejana</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>8.</td>
<td>Delta</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 1. Glutenin alleles presence at *Glu-D1* locus in the selected bread wheat cultivars
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>9.</td>
<td>Dična</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Draga</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Dragana</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Evropa</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Evropa 90</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Fortuna</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Italija</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Ivanka</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Jarebica</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Jarka</td>
<td>+</td>
<td></td>
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</tr>
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</table>

Bread wheat cultivars typically possess Dx5+Dy10 or Dx2+Dy12 allelic combinations at the *Glu-D1* locus. In wheat cultivars of different origin, allelic combinations Dx5+Dy12 and Dx2+Dy10 were reported as well (Payne, 1987, Margiotta et al., 1993). Since HMW subunits x5+y10 at *Glu-D1* locus provide good quality and subunits x2+y12 provide poor quality, simultaneous marker-assisted selection for both alleles is crucial in wheat breeding. 1Dx2 and 1Dx5 alleles have a high degree of homology, similarly to 1Dy10 and 1Dy12 alleles (Anderson et al., 1989). Selected site-specific primers proved to be efficient in distinguishing 1Dy10 and 1Dy12 (Ahamad, 2000).

In this research each set of allele-specific primers amplified a single product, 450 bp in size, in the case of 1Dx5 allele, and specific 576 bp and 612 bp products, in the case of 1Dy10 and 1Dy12 alleles, respectively. Ten of the eighteen analyzed genotypes possessed Dx5+Dy10 allelic combination, representing potentially good bread making quality genotypes that can be used in wheat breeding programs. In order to completely qualify these genotypes as cultivars with good bread making quality characteristics, it is necessary to apply the same approach of marker-assisted selection and define the HMW glutenin allelic compositions at *Glu-A1* and *Glu-B1* loci.

The development of PCR-generated DNA markers in the concept of marker-assisted selection represents the approach that can help to avoid misleading interpretation of the results obtained by SDS-PAGE analysis and can help to identify genotypes for specific purposes in wheat breeding programs. Simplicity and speed make the new PCR-generated DNA markers a valid alternative to standard SDS PAGE method.

**CONCLUSION**

Eighteen bread wheat cultivars developed at Institute of Field and Vegetable Crops in Novi Sad were screened for the alleles present at Glu-D1 locus using SDS PAGE and PCR-based approach. The *Glu-D1* locus was analyzed for two main x-type alleles which code for HMW glutenins 2 and 5, and two main y-type alleles which code for 10 and 12 HMW glutenin subunits. Among the analyzed cultivars, 55.6% expressed the presence of 1Dx5 and 1Dy10 alleles at *Glu-D1* locus. They are potentially good bread making quality genotypes, which can be used as parents in wheat breeding programs.
Diagnostic PCR system with primers specific for nearly identical alleles represents a useful new tool in marker-assisted selection, enabling identification and selection of a single allele, instead of the polypeptide gene product.

ACKNOWLEDGEMENTS

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REFERENCES


МOLEКУЛARНА КАРАТЕРИЗАЦИЈА ГЛУТЕНИНСКИХ АЛЕЛА Glu-D1 ЛОКУСА

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Резиме

Познато је да композиција субјединица глутенина велике молекулске масе утиче на квалитет хлеба. Најзначајнија је чињеница да, насупрот субјединицима 1Dx2-1Dy12, субјединици 1Dx5-1Dy10 успостављају добр уластишности теста и доприносе квалитету хлеба. У раду су анализирани алели Glu-D1 локуса сорти пшенице Института за ратарство и повтарство у Новом Саду. Примењена је стандардна натријум додеци сулфат полиакриламид гел електрофореза (SDS PAGE) и молекуларна анализа базирана на ланчаној реакцији полимеразе (PCR). У локусу Glu-D1 пронађена су два основна типа алела 2 и 5, као и два основна типа у алела 10 и 12. Утврђено је да 55,6% сорти поседује 1Dx5 и 1Dy10 алеле у Glu-D1 локусу. Ови генотипови представљају потенцијално добре родитеље у оплемењивању пшенице на добар квалитет хлеба. Резултати селекције помоћу молекуларних маркеров могу помоћи идентификацији генотипова за специфичну примену у програмима оплемењивања пшенице, при чему се представља у PCR анализама појединчених алела, а не протеинских продуката гена, добијених SDS PAGE методом.