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DETERMINATION OF GLUTENIN AND GLIADIN LOCI IN CROATIAN WINTER WHEAT GERMPLASM

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ABSTRACT

Aim of the study was to examine and determine distribution of glutenin (Glu-A1, Glu-B1 and Glu-D1) and gliadin (Gli-B1 and Gli-D1) loci in twenty Croatian winter wheat varieties using SDS PAGE. Highest frequency at Glu-A1 loci was recorded for subunit 2* (50 %). At Glu-B1 loci subunit 7+9 was dominant with frequency of 45 % while the subunit 7+8 was at second place with 40 %. At these loci lowest frequency (5%) had subunit 14+15. Subunit 5+10 prevailed at Glu-D1 with frequency of 70 %. At Gli-B1 loci we determined prevalence of subunits 63+67 combination with a frequency of 50 %, while the lowest prevalence had subunit 61 with a frequency of 5 %. Subunits 60, 66 and null allele (N) were also present. At Gli-D1 locus, the most common subunit was 55 with a frequency of 90 %, combination of subunits 55 + 56 + 59 and the subunit 59 were also present with frequency of 5 %.

Keywords: winter wheat, germplasm, glutenin loci, gliadin loci

INTRODUCTION

Rapid increase of human population has led to the increased demand for producing high yielding crops able to face those demands. Since wheat is primarily a nutrition crop, creating a stabile, high-yielding and high-quality wheat is a main goal of wheat breeders. Wheat quality, particulary its protein content, is related to the protein composition of the grain since the most important proteins are found in the grain endosperm. It is well known that flour quality depends on the composition and quantity of wheat protein gluten. This protein consists of two major fractions, gliadins and glutenins, which account for about 80% of total grain proteins (Xu *et al*., 2007). Composition of these reserve proteins has a great influence on flour and bread quality and is also associated with other important traits of wheat (Dimitrijević and Petrović, 2008).

Gliadins are mostly monomeric proteins with a molecular weight of 28 000 – 55 000 kDa, soluble in 70-90 % ethanol (Wieser, 2007). Based on their mobilty in gel electrophoresis we differentiate α, β, γ and ω gliadins. Gliadins γ- and ω- are mostly encoded at Gli-1 loci (Gli-A1, Gli-B1 and Gli-D1), located on the short ends of homologus group of chromosomes 1, while α-, β- and some γ- gliadins are encoded at Gli-2 loci (Gli-A2, Gli-B2 and Gli-D2), located on the short ends of homologus group of chromosomes 6 (Salavati *et al*., 2008a). Combination of different alleles at these loci provides a great genetic diversity among varieties. There are also some smaller gliadins loci, Gli-3, Gli-5 and Gli-6 that are controled with a few smaller gliadins blocks and can be used to differ two identical genotypes (Metakovsky and Branlard, 1998). Gliadin alleles at Gli-1 loci can serve as a genetic markers for noodle quality beacaus they are closely linked to Glu-3 alleles (Zhang *et al*., 2002).

Glutenins are divided into two groups: high molecular weight glutenins (HMW) and low molecular weight glutenins (LMW). Glutenins are larger molecules consisting of disulfide-bonded subunits (Dimitrijević and Petrović, 2008). HMW subunits are encoded with genes located on the long arm of 1A, 1B and 1D chromosomes at Glu-A1, Glu-B1 and Glu-D1 loci. LMW subunits are encoded with genes located on the short arm of group 1 chromosome at the Glu-A3, Glu-B3 and Glu-D3 loci (Zhang *et al*., 2002). All varieties of hexaploid bread wheat have six HMW subunits, two at each chromosome (1A, 1B and 1D); one high molecular weight subunit type x and one low molecular weight type y (Shewry *et al*., 2001). Number and composition of HMW glutenin subunits has a great influence on bread making quality, dough strength and elasticity (Horvat *et al*., 2006). According to Zhang *et al*. (2002) alleles Glu-A1b (Ax2*) and Glu-D1d (Dx5 + Dy10) are responsible for premium dough quality. HMW glutenin subunits account for approximately 10% of gluten, while LMW account for approximately 20 % of it (Wieser, 2007).

Aim of this study was to examine and determine distribution of glutenin (Glu-A1, Glu-B1 and Glu-D1) and gliadin (Gli-B1 and Gli-D1) loci in twenty Croatian winter wheat varieties using SDS PAGE.

MATERIALS AND METHODS

The study included 20 Croatian varieties of hexaploid bread wheat (*Triticum aestivum* L. spp. *vulgare*). Varieties were selected based on the year of release, importance in agricultural production and growing area (Table 1).

Distribution of ω-gliadin subunits at Gli-B1 and Gli-D1 loci, as well as the distribution of HMW glutenin subunits at Glu-A1, Glu-B1 and Glu-D1 loci, was analysed using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE, Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis) according to the BSA guide for wheat electrophoresis (Bundessortenamt, 2007). Vertical electrophoresis system Amersham Biosciences, SE 600 Ruby® was used, with gel dimensions 18 x 16 cm. Analysis

were conducted in Laboratory for plant genetics and biotechnology at Faculty of Agriculture and in Laboratory for biochemistry at Faculty of food technology in Osijek.

Table 1. Year of release, origin and pedigree of analysed wheat varieties

Nr.	Variety	Year	Origin	Pedigree
1.	U1	1936.	PIO	Carlotta Strampeli/Marquis
2.	Sirban Prolifik	1905.	Bohutinsky	-
3.	Zlatna Dolina	1971.	Bc institut	Zg 414-57/Leonardo
4.	Perla	1997.	AG	-
5.	BC Patria	1994.	Bc institut	Odesskaya-51/Zg-IPK-8210/2/ GK-32-82
6.	Fiesta	1998.	AG	By 87-83/Osk.3.68/2-85
7.	Gabi	1999	AG	Srpanjka/GK 32-82
8.	Kalista	2005.	AG	Divana/Soissons
9.	Matea	2005.	AG	Soissons/Perla
10.	AFZG Karla	2010.	AGRZG	SVK/VID/OBR
11.	Sana	1983.	AGRZG	Mura/Cl 14123/Bc-2413/72
12.	Mihelca	1996.	Bc institut	ZG 1325/78/SO-1065
13.	Aura	1997.	Bc institut	434 K-4CM/7903-93-1
14.	Cerera	1993.	Jošt	NE-7060-76-Y-342/VG-19
15.	Divana	1995.	Jošt	Favorit/5/Cirpiz/4/J.Kwang/2/Atlas66/Co mac./3/Velvet
16.	Žitarka	1985.	PIO	Osk. 6.30-20/Slavonka/3/Eph. M68/Osk.154 -19/ Kavkaz
17.	Srpanjka	1989.	PIO	Osk. 4.50-1-77/Zg 2696
18.	Golubica	1998.	PIO	Slavonija/Gemini
19.	Aida	2006.	PIO	Srpanjka/Rialto
20.	Ilirija	2008.	PIO	Osk.-14-294-16-95/Soissons

RESULTS AND DISCUSSION

Analysis of composition and distribution of subunits at ω – gliadin loci (Table 2) *Gli*-B1 has showed that combination of subunits 63+67 was the most freuqent (50 %), present in 10 wheat varieties. The second was subunit 66, with frequency of 20 %, present in four wheat varieties (Kalista, Mihelca, Aura and Ilirija). Null allel (N) had frequency of 15% and was present in three wheat varieties (Sirban Prolifik, Fiesta and Aida). Subunit 60 was determined in two wheat varieties (Cerera and Divana), with frequency of 10 %. Subunit 61 was present in one wheat variety (AFZG Karla), with frequency of 5 %. Highest frequency at *Gli*-D1 loci had subunit 55 (90 %), present in 18 wheat varieties. Subunit 55+56+59 was determined in wheat variety Mihelca with frequency of 5 %. Subunit 59 was also present in one wheat variety (Aura), with frequency of 5 % (Fig. 1).

Table 2. Distribution of ω-gliadin subunits in analysed wheat varieties

Nr.	Variety	*Gli*-B1	*Gli*-D1
1.	U1	63+67	55
2.	Sirban Prolifik	N	55
3.	Zlatna Dolina	63+67	55
4.	Perla	63+67	55
5.	BC Patria	63+67	55
6.	Fiesta	N	55
7.	Gabi	63+67	55
8.	Kalista	66	55
9.	Matea	63+67	55
10.	AFZG Karla	61	55
11.	Sana	63+67	55
12	Mihelca	66	55+56+59
13.	Aura	66	59
14.	Cerera	60	55
15	Divana	60	55
16.	Žitarka	63+67	55
17.	Srpanjka	63+67	55
18	Golubica	63+67	55
19.	Aida	N	55
20.	Ilirija	66	55

Rukavina *et al*. (2012a) analysed ω – gliadin loci in 50 Croatian wheat varieties and got similiar results. They determined that combination of subunits 63+67 is the most freuqent one (64 %) at Gli-B1 loci, and subunit 55 is the most frequent (94 %) at Gli-D1 loci. They also claim that in the future it will be neccesary to establish a connection between gliadin loci and biological traits since gliadins have an important role as a genetic markers of wheat.

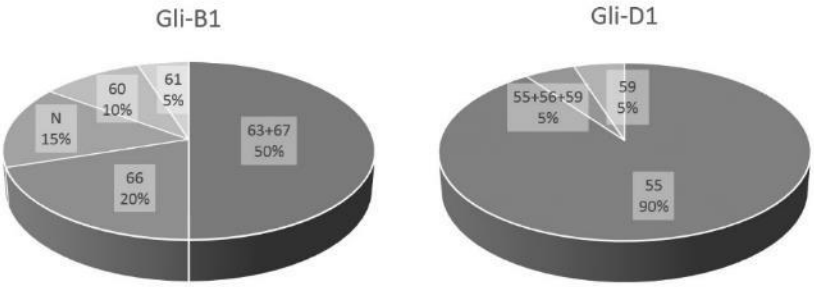

Figure 1. Frequency of ω-gliadin subunits in analysed wheat varieties

Analysis of Croatian wheat varieties obtained bigger polymorhism at Gli-B1 loci compared to Gli-D1 loci. According to Ruiz *et al*. (2002), gliadin polymorphism is very important because gliadins serve as a genetic markers for genotype identification. Different varieties origins, recombinations and gene mutations can be the reason of high gliadin loci polymorphism (Đukić *et al*., 2005). Salavati *et al*. (2008b) were comparing genetic polymorphism in Iranien wheat varieties and concluded that Gli-2 loci show higher genetic variability compared to Gli-1 loci. Ruiz *et al*. (2002) came to the same conclusion when investigating Spanish wheat varieties. Different results were obtained by Zaefizadeh *et al*. (2010.) who investigated gliadins and genetic variability of Iranian and Azerbaijan durum wheat varieties. They found a smaller polimorphism of α- and β-gliadins, controled by Gli-2 loci, compared to γ- and ω- gliadins, controled mostly by Gli-1 loci. Tanaka *et al*. (2003) confirmed the same with their results.

Analysis of composition and distribution of HMW glutenin subunits (Table 3) showed that the most frequent subunit at Glu-A1 loci was subunit 2* with frequency of 50 %, present in 10 wheat varieties. Subunit 1 had a frequency of 25%, and was present in five wheat varieties (Sirban Prolifik, BC Patria, Gabi, Srpanjka and Aida). Null allele was also found in five wheat varieties (Perla, Fiesta, Sana, Žitarka and Golubica) with frequency of 25%. At Glu-B1 loci combination of subunits 7+9 had the highest frequency (45 %), and at the second place was combination of subunits 7+8 with frequency of 40 %. Combination of subunits 6+8 was present in two wheat varieties (Zlatna Dolina and Sana), with frequency of 10 %. The lowest frequency at this loci had combination of subunits 14+15

(5 %), present in one wheat variety (U1). At Glu-D1 loci the most prevalent combination of subunits was 5+10, present in 14 wheat varieties, with frequency of 70 %. Combination of subunits 2+12 was present in six wheat varieties, with frequency of 30 % (Fig. 2). Similiar results were obtained by Rukavina *et al*. (2012b) who investigated 50 Croatian varieties of hexaploid winter wheat. They determined that the most frequent subunit at Glu-A1 loci was subunit 2* (56 %), at Glu-B1 loci subunit 7+8 (40%) and at Glu-D1 loci subunit 5+10 (68 %). The average number of alleles per locus was 4.33.

Table 3. Distribution of HMW glutenin subunits in analysed wheat varieties

Nr.	Variety	*Glu*-A1	*Glu*-B1	*Glu*-D1
1.	U1	2*	14+15	2+12
2.	Sirban Prolifik	1	7+9	2+12
3.	Zlatna Dolina	2*	6+8	2+12
4.	Perla	N	7+8	5+10
5.	BC Patria	1	7+9	5+10
6.	Fiesta	N	7+9	5+10
7.	Gabi	1	7+9	5+10
8.	Kalista	2*	7+8	5+10
9.	Matea	2*	7+8	5+10
10.	AFZG Karla	2*	7+8	5+10
11.	Sana	N	6+8	2+12
12.	Mihelca	2*	7+9	5+10
13.	Aura	2*	7+8	5+10
14.	Cerera	2*	7+9	5+10
15.	Divana	2*	7+9	5+10
16.	Žitarka	N	7+8	2+12
17.	Srpanjka	1	7+9	5+10
18.	Golubica	N	7+9	2+12
19.	Aida	1	7+8	5+10
20.	Ilirija	2*	7+8	5+10

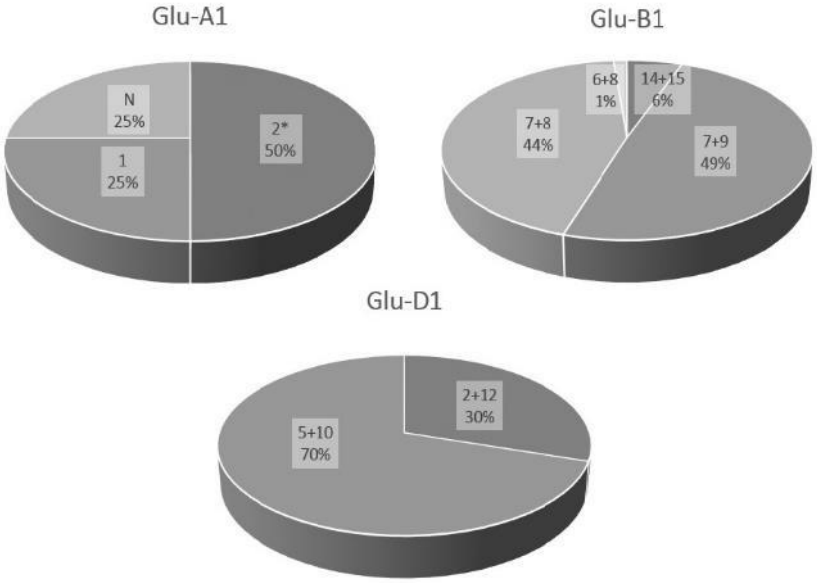

Figure 2. Frequency of HMW glutenin subunits in analysed wheat varieties

Dimitrijević and Petrović (2008) investigated genetic variability of Glu-A1, Glu-B1 and Glu-D1 loci of hexaploid wheat. They concluded that allelic variability at Glu-A1 has an influence on rheological properties of wheat, where subunit 2* had the most optimal effect. Allelic variability at Glu-B1 loci had the smallest influence on quality. The biggest influence on phenotypic variability had allelic variability at Glu-D1 loci. Same authors claim that some subunit combinations can serve as an orientation phenotypic marker for selection of superiror genotypes in early generations of breeding process. According to Horvat *et al*. (2009) different combinations of HMW-GS alleles have a different influence on bread making quality. Subunits 1 and 2* at Glu-A1, 7+9 and 17+18 at Glu-B1 and 5+10 at Glu-D1 loci are related with higher dough strength and bread volume, while null allel at Glu-A1, subunit combination 6+8 at Glu-B1 and subunit combination 2+12 at Glu-D1 loci are connected with negative effects on bread making quality. The high frequency of 2*, 7+9 and 5+10 subunits at gliadin loci of analysed wheat varieties is probably result of breeding for bread making quality in wheat.

CONCLUSIONS

The analysis of 20 Croatian wheat varieties on composition of ω – gliadin loci determined that at Gli-B1 loci the most frequent subunit combination was 63+67 (50 %) and at Gli-D1

loci subunit 55 (90 %). Also, bigger polymorhism was obtained at Gli-B1 loci compared to Gli-D1 loci. Analysis of HMW glutenin loci determined that at Glu-A1 loci the most frequent subunit was 2* (50%), at Glu-B1 loci subunit combination 7+9 (49 %) and at Glu-D1 loci subunit combination 5+10 (90 %). The high frequency of this subunits is probably result of breeding for bread making quality in wheat, since these subunits are connected with premium quality of wheat. Genetic variability of glutenin and gliadin loci is very important for future breeding programs and selection of wheat varieties for special purposes. Also, these loci have an important role and potential as genetic markers of wheat.

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