

## DETERMINATION OF GLUTENIN AND GLIADIN LOCI IN CROATIAN WINTER WHEAT GERMLASM

UDC 664.236 : 633.11(497.5)

*Sonja Marić<sup>1</sup>, Marija Grgić<sup>1</sup>, Sonja Petrović<sup>1</sup>, Ivica Strelec<sup>2</sup>, Sunčica Guberac<sup>1</sup>, Vlado Guberac<sup>1</sup>*

*<sup>1</sup>Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture,  
Kralja P. Svačića 1d, HR-31000 Osijek, Croatia*

*\*Corresponding author: smaric@pfos.hr*

*<sup>2</sup>Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology,  
Franje Kuhača 20, HR-31000 Osijek, Croatia*

### 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, particularly 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 mobility in gel electrophoresis we differentiate  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\omega$  gliadins. Gliadins  $\gamma$ - and  $\omega$ - are mostly encoded at Gli-1 loci (Gli-A1, Gli-B1 and Gli-D1), located on the short ends of homologous group of chromosomes 1, while  $\alpha$ -,  $\beta$ - and some  $\gamma$ - gliadins are encoded at Gli-2 loci (Gli-A2, Gli-B2 and Gli-D2), located on the short ends of homologous 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 controlled 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 because 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  $\omega$ -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

| <b>Nr.</b> | <b>Variety</b>  | <b>Year</b> | <b>Origin</b> | <b>Pedigree</b>                                       |
|------------|-----------------|-------------|---------------|---|
| 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/C1 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                            |

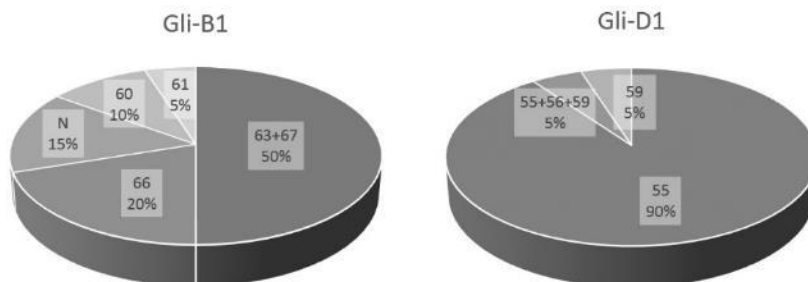
## RESULTS AND DISCUSSION

Analysis of composition and distribution of subunits at  $\omega$  – gliadin loci (Table 2) *Gli*-B1 has showed that combination of subunits 63+67 was the most frequent (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 allele (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  $\omega$ -gliadin subunits in analysed wheat varieties

| Nr. | Variety         | <i>Gli</i> -B1 | <i>Gli</i> -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  $\omega$  – gliadin loci in 50 Croatian wheat varieties and got similar results. They determined that combination of subunits 63+67 is the most frequent 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 necessary 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  $\omega$ -gliadin subunits in analysed wheat varieties

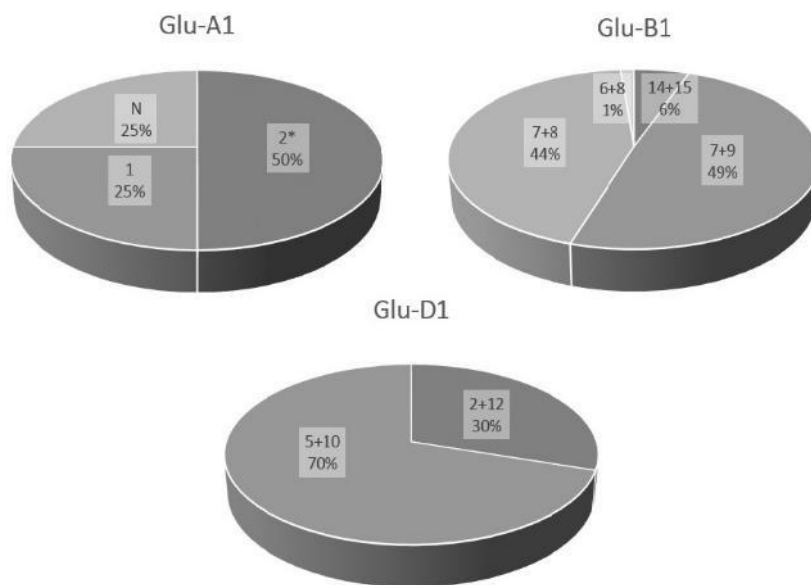
Analysis of Croatian wheat varieties obtained bigger polymorphism at Gli-B1 loci compared to Gli-D1 loci. According to Ruiz *et al.* (2002), gliadin polymorphism is very important because gliadins serve as a genetic markers for genotype identification. Different varieties origins, recombinations and gene mutations can be the reason of high gliadin loci polymorphism (Đukić *et al.*, 2005). Salavati *et al.* (2008b) were comparing genetic polymorphism in Iranian 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 polymorphism of  $\alpha$ - and  $\beta$ -gliadins, controlled by Gli-2 loci, compared to  $\gamma$ - and  $\omega$ - gliadins, controlled 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 superior 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 allele 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  $\omega$  – 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 polymorphism 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.

## REFERENCES

- Bundessortenamt (2007): Guidelines for the conduct of electrophoresis tests for distinctness, uniformity and stability of wheat.
- Dimitrijević M., Petrović S. (2008): Effect of HMW glutenin allelic variation on wheat. In: 43rd Croatian and 3rd International Symposium on Agriculture, February 18 – 21, Opatija, Croatia, pp. 286-289.
- Đukić N., Matić G., Konjević R. (2005): Biochemical analysis of gliadins of wheat *Triticum durum*, *Kragujevac Journal of Science*, 27, 131-138.
- Horvat D., Drezner G., Šimić G., Dvojković K. (2006): Determination of wheat storage proteins by RP-HPLC method, *Agriculture*, 12 (2), 42-47.
- Horvat, D., Kurtanjek, Ž., Drezner, G., Šimić, G., Magdić, D. (2009): Effect of HMW glutenin subunits on wheat quality attributes, *Food Technol. Biotechnol.* 47(3), 253-259.
- Metakovsky E. V., Branlard G. (1998): Genetic diversity of French wheat germplasm based on gliadin alleles, *Theoretical and Applied Genetics*, 96, 209-218. doi: 10.1007/s001220050729.
- Ruiz M., Rodriguez-Quijano M., Metakovsky E. V., Vazquez J. F., Carillo J. M. (2002): Polymorphism, variation and genetic identity of Spanish common wheat germplasm based on gliadin alleles, *Field Crops Research*, 79, 185-196. doi:10.1016/S0378-4290(02)00139-9.
- Rukavina I., Marić S., Guberac V., Petrović S., Čupić T., Tepper C. (2012a): Gliadin loci composition analysis of croatian hexaploid wheat germplasm, *Seed Science Journal*, 29 (3-4), 91-100.
- Rukavina I., Marić S., Guberac V., Petrović S., Čupić T., Tepper C. (2012b): Glutenin loci variability of croatian wheat germplasm, *Agriculture*, 18(2), 30-35.
- Salavati A., Boushehri A. A. S. N., Hassani M. E., Yazdi – Samadi B. (2008a): Evaluation of Iranian bread wheats by storage proteins „gliadins“. In: 11th International Wheat Genetics Symposium, 24 – 29 August 2008, Brisbane, QLD, Australia, pp 283-288.
- Salavati A., Sameri H., Boushehri A. A. S. N., Yazdi-Samadi B. (2008b): Evaluation of Genetic Diversity in Iranian Landrace Wheat *Triticum aestivum* L. by Using Gliadin Alleles, *Asian Journal of Plant Sciences*, 7 (5), 440-446.
- Shewry P. R., Popineau Y., Lafiandra D., Belton P. (2001): Wheat glutenin subunits and dough elasticity: findings of the Eurowheat project, *Trends in Food Science & Technology*, 11(12), 433-441. doi:10.1016/S0924-2244(01)00035-8.



Tanaka H., Tomita M., Tsujimoto H., Yasumuro Y. (2003.): Limited but specific variations of seed storage proteins in Japanese common wheat (*Triticum aestivum* L.), *Euphytica*, 132, 167-174. doi: 10.1023/A:1024638616507.

Wieser H. (2007): Chemistry of gluten proteins, *Food Microbiology*, 24 (2), 115-119. doi:10.1016/j.fm.2006.07.004.

Xu J., Bietz A. J., Carriere C. J. (2007): Viscoelastic properties of wheat gliadin and glutenin suspensions. *Food Chemistry*, 101(3): 1025-1030. doi:10.1016/j.foodchem.2006.02.057.

Zaefizadeh M., Jamaati-e-Somarin S., Ojaghi J., Seyedi S. M., Zabihi-e-Mahmoodabad R., Ochi M. (2010): Genetic diversity for gliadin patterns of durum wheat landraces in the Northwest of Iran and Azerbaijan. *Pesquisa Agropecuária Brasileira*, Brasília, 45(11): 1425-1432. <http://dx.doi.org/10.1590/S0100-204X2010001200013>.

Zhang W., Gianibelli M. C., Ma W., Rampling L., Gale K. R. (2002): Identification of SNPs and development of allele-specific PCR markers for  $\gamma$ -gliadin alleles in *Triticum aestivum*. *Theoretical and Applied Genetics*, 107(1): 130-138. doi: 10.1007/s00122-003-1223-2.