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POLYMORPHISM OF *Gli-D1* ALLELES OF KRAGUJEVAC'S WINTER WHEAT CULTIVARS (*Triticum aestivum* L.)

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Composition of gliadins encoded by *Gli-D1* allele as well polymorphisms of *Gli-D1* allele investigated in 25 wheat cultivars by using acid polyacrylamide gel electrophoresis. Electrophoregrams obtained by polyacrylamide gel electrophoresis were used for estimation variability of gliadin components and identification of gliadin blocks. Five gliadin blocks encoded by different alleles at *Gli-D1* locus were apparently expressed and identified. Gliadin blocks differed according to number of components and their molecular mass. Variability of determined block components indicates that existing polymorphysms of gliadins alleles. Frequency of identified 5 alleles at *Gli-D1* locus was in ratio from 4% to 52%. The highest frequency of *b* allele and the of *g* allele was found.

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INTRODUCTION

Gliadins represent a heterogeneous mixture of proteins which are soluble in aqueous alcohol and α-, β-, γ- and ω-gliadins can be distinguished. Together with glutenins, gliadins play important role for bread making quality. Large differences established in bread making quality among wheat cultivars even in case when protein contents of cultivars are similar (ZHU and KHAN, 2004). For bread wheat are identified multiple allelism at six gliadin-coding (METAKOVSKY, 1991). The gliadins encoded by 3 *Gli-1* and 3 *Gli-2* loci the short arm of the 1. and 6. group homologous chromosomes in three genomes (ABD). Genes which encoding ω- and many of γ-gliadins are tightly clustered at three homologous loci *Gli-A1*, *Gli-B1* and *Gli-D1*, while the α-, β- and some γ-gliadins are encoded by tightly clustered at three homologous loci *Gli-A2*, *Gli-B2* and *Gli-D2* loci. D-genome has high contribution to bread quality. As we know due to absence of D-genome durum wheat is less used for bread production than *Tr. aestivum*, while in Ethiopia both bread and durum wheat are primarily used for traditional bread production (TAREKEGNE and LABUSCHAGNE, 2005). From several to more than 30 alleles were identified per each of six gliadin-coding loci (METAKOVSKY, 1991; KNEZEVIC et al., 2007). Gliadin alleles are possible identified from small amount of sample of wheat by analysis of electrophoregrams obtained by electrophoresis. Genetic variation of Russian, French, Yugoslav, Italian, Spanish, wheat germplasm was studied by analysis of allelic variation at *Gli-1* and *Gli-2* loci (METAKOVSKY et al, 1991; KNEZEVIC, 1992; METAKOVSKY et al., 1994; METAKOVSKY et al., 1997; Metakovsky et al., 2000). Because of allelic variants of gliadins proteins, may serve as efficient and reliable genetic markers in genetic studies in wheat. Some allelic variants of gliadins as well other storage proteins have been shown to influence bread making quality (Payne, 1987; METAKOVSKY et al., 1990; BRANLARD et al., 2001; MENKOVSKA et al., 2002; He et al., 2005; JAKUBAUSKIENE and JUODEIKIENE, 2005) which also, have been suggested as linked markers of frost hardiness, heading time, seed size, disease resistance (SOZINOV and POPERELYA, 1980; LAFIANDRA et al., 1987; METAKOVSKY et al., 1986; KNEZEVIC et al., 1995; TANAKA et al., 2003). Those differences in expressed traits could be influenced by one of more alleles encoding storage proteins or other genes located very close to *Gli*-loci at the chromosome (KNEZEVIC, 1996).

The purpose of this study was to provide allele polymorphisms of *Gli-D1* locus in wheat cultivars created in Small Grains Breeding Center of Kragujevac which can use in breeding program to generate new lines with good bread making quality and adaptability.

MATERIALS AND METHODS

Twenty five cultivars of wheat created in Kragujevac's breeding center were investigated. At least 20 single kernels were analyzed for each cultivar.

Gliadins proteins were extracted from single seed wheat meal by 70% ethanole for 30 min at 40 ^{0}C. Gel electrophoresis was performed in 8.33% polyacrylamide (12.5 g acrilamid, 0.62 g N,N'-methylenebisacrylamide, 0.15 g ascorbin acid, 200 µl 10% ferosulfate heptahydrate, diluted in 150 ml Al-lactate buffer pH=3.1) according to method developed by NOVOSELSKAYA et al. (1983). Polymerisation of gel was initiated by 10 µl 3% hydrogen peroxid. Prepared solution was poured in vertically oriented apparatus, where between glasses plates were formed gels (dimension 150 x 150 x 1.8 mm). Sites for applying of samples were formed with special comb, whose cogs were immersed in solution before polymerisation. Amount of gliadin extract (20 µl) were loaded on the gel by micropipette. Fractionation of the gliadin molecules was performed during 2.5 to 3 hours, in electric field under constant voltage from 550 V and in 5 mM aluminum lactate buffer. At the begining of analisys, temperature of electrophoretic buffer was 10°C, while at the end was 25-30°C.

After performed electrophoresis, gels were immersed 15 minutes in 300 ml of fixative, and after that stained in 0.05% ethanol solution of Coomassie Briliant Blue R-250 by adding 250 ml 10% threechloroacetic acid (TCA). Staining was carried out during night. Next day, solution of stain was poured off. Gels were washed in water and photographed. Photographs are used for determination of gliadin blocks alleles.

RESULTS

Investigation of gliadin alleles at the *Gli-D1* locus cultivars was shown differences among 25 analyzed wheat cultivars. The focus of this investigation was analysis of polymorphisms at *Gli-D1* locus, based on short arm of 1D chromosome. The allelic variation at the *Gli-D1* locus was established. Five different alleles (*a, b, f, g,* and *k*) were determined at *Gli-D1* locus (Table 1). Four from 25 wheat cultivars carried *Gli-D1a* allele, 13 cultivars *b* allele, 3 – *f* allele, one *g* allele and 4 cultivars *k* allele. In numerous studies reported that each allele of gliadins has specific connection to biological traits of wheat and could use as a markers for some quality traits (METAKOVSKY et al., 1997; MENKOVSKA et al., 2002), agronomic traits and environmental adaptation (METAKOVSKY and BRANLARD, 1998; RAM et al., 2005). Genetic polymorphism of gliadins has been studied in different Countries, because of cultivar identification. In Australian wheat at the *Gli-D1* locus were identified 5 alleles (METAKOVSKY et al., 1990), 6 alleles in Yugoslav wheat cultivars (KNEZEVIC, 1992), 11 alleles in Russian wheat cultivars (METAKOVSKY, 1991), 10 alleles in Spanish cultivars (METAKOVSKY et al., 2000), and 7 allele in Czech winter wheat cultivars (BRADOVA and SASEK, 2005). By previous investigations of 57 Yugolsav wheat cultivars were identified 5 different alleles at the *Gli-D1* locus (METAKOVSKY et al., 1991).

Also, by analysis of 10 Kragujevac's wheat cultivars were identified only 3 alleles (*a, b, k*) at the *Gli-A1* locus, while in cultivars originated from selection Center Novi Sad were identified 5 alleles (*a, b, f, g, k*) KNEZEVIC (1992).

Table 1. Identified alleles at the Gli-D1 locus in Kragujevac's wheat cultivars

Gli-D1 *alleles*	Wheat cultivars	Frequency (%)
a	Studenica, Ravanica, Bujna, Lepenica	16
b	Šumadija, Gružanka*[k], Zastava, KG-56, KG-58, KG-78*[g], Ljubičevka, Srbijanka, Takovčanka, KG-56 S, Toplica, Vizija, Ana Morava	52
f	Oplenka, Lazarica, Matica,	12
g	Orašanka	4
k	KG-75*[g], Kosmajka*[f], Morava, KG-100	16

*Cultivars with heterozygous *Gli-D1* locus -heterogeneous cultivars

In this investigation in 4 wheat cultivars were identified two alleles at the *Gli-D1* locus what indicated that that those cultivars were heterozygous for this locus (Table 1). It mean that locus *Gli-D1* was heterozygous 0,16 for analyzed cultivars.

Genetic study of gliadin electrophoregram and identification of gliadin alleles provides method for estimation of genotypes. Numerous studies of gliadin alleles carried out for evaluation of their correlation with bread making quality, yield, some physiological traits (METAKOVSKY et al., 1991; KNEZEVIC et al., 1998a; THIS et al., 2001; GIANIBELLI et al., 2001; Menkovska et al., 2002; DJUKIC et al., 2007). Enormous gliadin polymorphisms make gliadin alleles much more suitable for wheat genotype identification and distinction than other polymorphic protein alleles.

In analyzed Kragujevac's wheat cultivars, the 5 identified alleles encoding gliadin blocks that including 3-6 different components (Fig. 1). Components of those blocks positioned in ω- and γ- region of gliadin spectra. For all blocks is characteristic presence of intensity stained bands in slow γ-region. On the base of components in ω-region is possible identified similarity among *Gli-D1b* and *Gli-D1a, Gli-D1f, Gli-D1k* while block encoded by allele *Gli-D1g* are different from those two groups. Block encoded by *Gli-D1b* has five components (3 in ω-region and 2 γ- region). Block *Gli-D1f* characterize 4 components, 2 bands with similar color intensity in ω-region and 2 in γ-region. This block is different from *Gli-D1b* according to the slowest band which not present in *Gli-D1f*. The block *Gli-D1a* comprises 4 bands. Two bands in ω-region and one band in γ- region are identical to bands of *Gli-D1f*. Fourth component is positioned in γ- region which is little bit faster than fourth component of *Gli-D1f*. Gliadin block encoded by *Gli-D1k* consist three components (2 in ω-region and 1 in γ- region) which are the same as in previously described blocks. One component in γ- region is missing in γ- region. Gliadin block encoded by *Gli-D1g* consist 6 components (4 in ω-region and 2 γ-region). This block is recognizable according to components in ω-region.

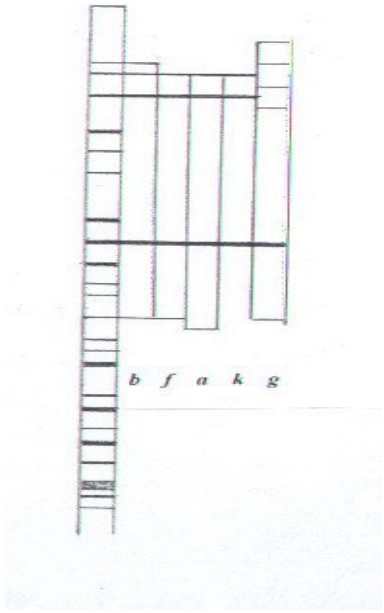

Figure 1. Identified gliadin block components encoded by designed *Gli-D1* alleles

The similarity of gliadin block and identified polymorphisms could be results of mutation of common precursor. These different gliadin components controlled by one gliadin coding locus had included into one block are subject of natural mutation process in different degree or different selection value.

Frequency of *Gli-D1* alleles was different. The most frequent was Gli-*D1b* (52.0%) and the least frequency *Gli-D1g (*4.0%) Table 1. In Australian wheat cultivars frequency of alleles at the *Gli-D1* locus was in ratio from 3.2% to 21.2% METAKOVSKY et al., (1990). By investigation of Russian cultivars the highest frequency was found for *Gli-D1a* and *Gli-D1g over* the 50% depends of region (METAKOVSKY and KOPUS, 1991). The most frequent (70.2%) allele of analyzed 80 Czech wheat cultivars was Gli-D1b (BRADOVA and ŠAŠEK, 2005) In earlier investigation of Yugoslav wheat cultivars created in breeding Center in Novi Sad, the highest frequency had *Gli-D1b* (55.8%) and the least *Gli-D1g* (1.9%) KNEZEVIC et al. et al. (1998a). By analysis of 10 Kragujevac's wheat cultivars the highest frequency showed *Gli-D1b* (66.7%) while the least frequency had *Gli-D1k (*11.1%) KNEZEVIC (1992). By analysis of 57 Yugoslav wheat cultivars the most frequent alleles was *Gli-D1b* (38.5%) and the least frequency had *Gli-D1g* (1.7%)

METAKOVSKY et al. (1991). In Spanish cultivars the highest frequency had *Gli-D1b* (46.0%) while even the less frequency (1.0%) had two alleles (**h, r**) at the *Gli-D1* locus (METAKOVSKY et al., 2000).

The high frequency of allele could be results of the pedigree effects during breeding process or selection plants according to trait concepts. The most frequent allele should have some definite value, since it has succeeded in competition with many other alleles during the breeding process. It could be evaluate that this allele is linked to genes influencing agronomical important traits in certain environmental conditions (LAGRAIN et al, 2005; RAM et al., 2005). The value of frequent alleles my be in their contribution to a higher plant adaptability. It has been shown that *Gli-D1f* with high frequency in Russian cultivars expressed high adaptability in Omsk, Donska and Volga region (METAKOVSKY and KOPUS, 1991). In Russian wheat cultivars were found different influence of alleles at *Gli-1* and *Gli-2* loci to frost resistance. Alleles *Gli-A1m, Gli-D1g, Gli-A2f,* Gli-*B2o* and *Gli-D2e* showed high influence to frost resistance (SOZINOV and POPERELYA, 1984). In another investigation was found that allele *Gli-D1b Gli-D1f* with high frequency had positive effect to low temperature resistance (KNEZEVIC et al., 1998b), while BRADOVA and ŠAŠEK (2005) suggested Gli-*D1g* as a marker of frost hardiness. Besides this allele positive influence to low temperature resistance was found for *Gli-B1b, Gli-A2b*, *Gli-D1b, Gli-B2h* and *Gli-D2b* in Yugoslav wheat cultivars (KNEZEVIC et al., 1998b). The established connection between alleles and resistance to low temperature could not be use as reliable marker but these alleles indicating indirect influence (KNEZEVIC et al., 2006).

The high values of technological traits are under the influence of numerous alleles from different *Gli-1, Gli-2, Glu-1* and *Glu-3* loci (METAKOVSKY et al., 2000; OAK et al., 2006; DJUKIC et al., 2007) as well as gliadin/glutenin ratio (REDDY and APPELS, 1990; HE et al., 2002; YAN et al., 2004). Positive correlation between sedimentation Zeleny value and *Gli-D1b* was established in Yugoslav wheat cultivars. Also, positive connection of *Gli-D1a*, *Gli-D1b* with high value of loaf volume and dough extensibility, while alleles *b* and ***k*** showed positive connection with resistance of dough extensibility (KNEZEVIC et al., 1993). At least two *Gli-D1* alleles (*a* and *j*) were positively associated to dough strength (BRANLARD et al., 2001). Except those alleles the positive connection between dough resistance and *Gli-A2e,* allele as well as dough elasticity and *Gli-D2b* allele, were established by investigation of Australian and Yugoslav wheat cultivars (METAKOVSKY et al., 1990; KNEZEVIC et al., 1993).

CONCLUSION

This investigation showed allele polymorphisms of *Gli-D1* locus in analyzed wheat cultivars created in selection centre in Kragujevac. By analysis of 25 wheat cultivars were identified 5 *Gli-D1* alleles (*a, b, f, g, k).* Frequency of identified allelic variation from 4% (*Gli-D1g)* to 52% (*Gli-D1b).* The high

frequency (52%). were found for *Gli-D1b.* Alleles with high frequency could indicate their favorable adaptive and selection value, connection with biological traits. However high frequency could be results of limited genetic variability for crossing or directed selection of desirable genetic, morphological, physiological, technological quality traits. Established connections among gliadin alleles and biological traits are very important for the breeding practice and incorporation of a single gene into a plant for creation desired phenotype. The wheat cultivars carried *a, b,* and *k* can use for crossing in the aim of improvement of technological quality. Gliadins represent efficient and reliable genetic markers in wheat genetic study.

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POLIMORFNOST *Gli-D1* LOKUSA KOD KRAGUJEVAČKIH OZIMIH SORTI PŠENICE (*Triticum aestivum* L)

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I z v o d

U radu je izučavana kompozicija glijadina koji su pod kontrolom *Gli-D1*alela, kao i polimorfnost alela *Gli-D1* lokusa kod 25 sorti pšenice stvorenih u Centru za strna žita Kragujevac. Glijadini su razdvajani metodom elektroforeze na poliakrilamidnom gelu. Dobijeni elektroforegrami su korišćeni za ocenu varijabilnosti glijadinskih komponenti i identifikaciju glijadinskih blokova. U izučavanjima je identifikovano 5 alela (*a, b, f, g, k*) na *Gli-D1* lokusu. Glijadinski blokovi su se razlikovali prema broju komponenti i njihovim molekulskim masama. Ustanovljena varijabilnost blokova komponenti glijadina je pokazala postojanje polimorfnosti glijadinskih alela *Gli-D1* lokusa. Frekfencija identifikovanih alela je bila različita i nalazi se u rasponu od 4% do 52%. Najveću učestalost je imao alel *Gli-D1b* a najmanju alel *Gli-D1g*

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