Kragujevac J. Sci. 29 (2007) 139-147. UDC 575.113.2:582.542.11:547.963.1

POLYMORPHISM OF *Gli-A1* ALLELES IN WINTER WHEAT CULTIVARS (*Triticum aestivum* L)

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(Received April 11, 2007)

ABSTRACT. The gliadins composition as well *Gli-A1* allele variation of 25 wheat cultivars was analysed by acid polyacrylamide gel electrophoresis. Electrophoregrams obtained by polyacrylamide gel electrophoresis were used for estimation variability of gliadin components and identification of gliadin blocks. Four gliadin blocks encoded by different alleles at *Gli-A1* locus were apparently expressed and identified. Variability of determined block components indicates that existing polymorphysms of gliadins alleles. Frequency of identified four alleles at *Gli-A1* locus was different and varied from 4% to 44%.

Key words: alleles, gliadin, quality, cultivar, wheat, electrophoresis

INTRODUCTION

TI common wheat, extensive multiple allelism at six gliadin-coding loci was detected by using acid polyacrylamide gel electrophoresis (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). From several to more than 30 alleles were identified for each of six gliadin-coding loci (SOZINOV and POPERELYA, 1980; METAKOVSKY, 1991; KNEZEVIC *et al*., 1993). Gliadin alleles are possible identified from small amount of sample of wheat by analysis of electrophoregrams obtained by

electrophoresis. Genetic variation*et al.* of Russian, French, Yugoslav, Italian, Spanish, common 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). Gliadins have been extensively investigated as a main constituent of grain proteins and important nutritional components. Gliadin molecules have no disulfide bounds and have been divided into four groups α-, β-, γ- and ω-gliadin that are separate by acid polyacrylamide gel electrophoresis. Because of allelic variants of gliadins proteins, may serve as efficient and reliable genetic markers in genetic studies in wheat. In addition some allelic variants of gliadins as well other storage proteins have been shown to influence bread making quality (PAYNE, 1987; METAKOVSKY *et al*., 1990; KNEZEVIC *et al*., 1993; BRANLARD *et al*., 2001; MENKOVSKA *et al*., 2002; JAKUBAUSKIENE and JUODEIKIENE, 2005). Also, in wheat, gliadin proteins have been suggested as linked markers of frost hardiness (SOZINOV and POPERELYA, 1980) heading time (LAFIANDRA *et a*l., 1987), seed size (METAKOVSKY *et al*., 1986), disease resistance (POPERELYA and BABAYANZ, 1978; KNEZEVIC *et a*l., 1995) frost resistance (KNEZEVIC *et al*., 1998). Differences in expressed traits are under the influence one of more alleles encoding storage proteins or other genes located very close to *Gli*-loci at the chromosome (KNEZEVIC, 1996).

The aim of this study was analysis of allele polymorphisms of *Gli-A1* locus in wheat cultivars created in Small Grains Breeding Center of Kragujevac and importance of identified alleles for wheat breeding as well their connection with bread making quality traits.

MATERIALS AND METHODS

Twenty five cultivars of wheat created in Kragujevac's breeding center were studied. At least 20 single kernels were analysed for each cultivar. Gliadins proteins were extracted from single seed wheat meal by 70% ethanole for 30min at 40^0C. Gel electrophoresis was performed in 8.33% polyacrylamide (12.5g acrilamid, 0.62g N,N'-methylenebisacrylamide, 0.15g ascorbin acid, 200µl 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 10µl 3% hydrogen peroxid. Prepared solution was poured in vertically oriented apparatus, where between

glasses plates were formed gels (dimension 150 x 150 x 1.8mm). Sites for applying of samples were formed with special comb, whose cogs were immersed in solution before polymerisation. Amount of gliadin extract (20µl) 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 550V and in 5mM aluminium 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 300ml of fixative, and after that stained in 0.05% ethanol solution of Coomassie Briliant Blue R-250 by adding 250ml 10% threechloroacetic acid. 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 AND DISCUSSION

Analysis of gliadin alleles at the *Gli-A1* locus in 25 wheat cultivars were shown differences among cultivars. The focus of this investigation was analysis of polymorphisms at *Gli-A1* locus, based on short arm of 1A chromosome. By analysis allelic variation at the *Gli-A1* locus was established. Four different alleles (***a, b, c, f***) were determined at at *Gli-A1* locus (Table 1). Ten from 25 wheat cultivars carried *Gli-A1a* allele, seven alleles ***b,*** one allele ***c,*** and seven allele ***f.*** 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). Because of importance of gliadin alleles, the genetic polymorphysm of gliadins has been studied in different Countries. In Australian wheat at the *Gli-A1* locus were identified 6 alleles (Metakovsky et al., 1990), 6 alleles in Yugoslav wheat cultivars (Knezevic, 1992), 15 alleles in Russian wheat cultivars (Metakovsky, 1991), and 11 alleles in Spanish cultivars (METAKOVSKY *et a*l., 2000). By previous investigations of 57 Yugolsav wheat cultivars were identified 5 different alleles at the *Gli-A1* locus (METAKOVSKY *et al*., 1991).

Table 1. Identified alleles at the ***Gli-A1*** locus in Kragujevac's wheat cultivars

Gli-B1 alleles	**Wheat cultivars**	**Frequency (%)**
a	KG-75, Šumadija, Kosmajka, Gružanka, Morava, Lazarica, KG-100, Toplica, Matica, Bujna	40
b	Zastava*[a], Oplenka, Lepenica, Studenica, Ravanica, Takovčanka, Vizija	28
c	KG-78*[b,f,o]	4
f	KG-56, Orašanka, KG-58, Ljubičevka*[b], Srbijanka, KG-56 S, Ana Morava*[a]	28

Also, by analysis of European *Triticum turgidum* and *Triticum durum* were identified 6 alleles at *Gli-A1* locus. In the analysis of 10 Kragujevac's wheat cultivars (KNEZEVIC, 1992) were identified only 3 alleles (***b, c, f***) at the *Gli-A1* locus, while in cultivars originated from selection Center Novi Sad were identified 4 alleles (***a, b, c, f***).

In this investigation in 4 wheat cultivars were identified two or three alleles at the *Gli-A1* locus what indicated that that those cultivars were heterogenous for this locus (Table 1).

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; DIMITRIJEVIC *et al.,* 1998; KNEZEVIC, 1994; KNEZEVIC *et al*., 1998; THIS *et a*l., 2001; GIANIBELLI *et al*., 2001; MENKOVSKA *et al*., 2002; DJUKIC *et al*., 2005; DJUKIC *et al*., 2007). Enormous gliadin polymorphism makes gliadin alleles much more suitable for wheat genotype identification and distinction than other polymorphic protein alleles.

In analyzed Kragujevac's wheat cultivars, the identified alleles encoding gliadin block that including 2-4 different components (Fig. 1). Block encoded by allele ***a*** and ***c***, consists two bands, one in γ-, and other in β- region of gliadin spectra. In both blocks in β- region expressed components with same colour intensity and mobility. In γ- region components of both blocks are intensive collored, while components of blocks encoded by ***Gli-A1c*** are faster than bands encoded by ***Gli-A1a***. Gliadin blocks encoded by ***Gli-A1b*** consist two components, too. Components in β- region have same relative mobility with previous two blocks but with less colour intensity. Component in γ- region is faster than components of gliadin block ***Gli-A1c*** but more intensive colour. For block

encoding by ***Gli-A1f*** are characteristic 3 components, one in ω-region, one in γ-region with less colour intensity and one in β- region, with more colour intensity.

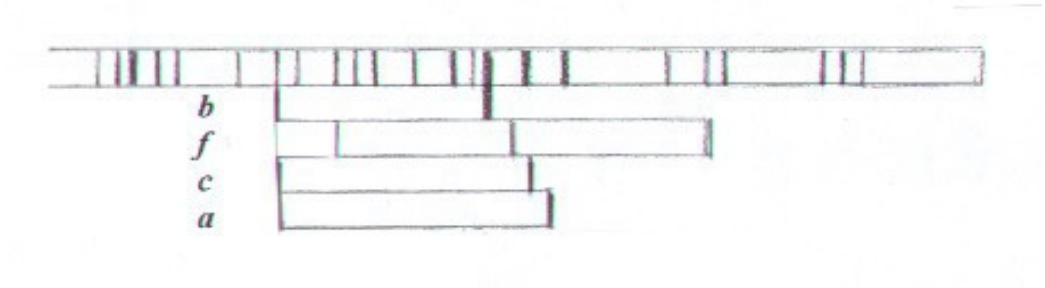

Figure 1. Identified gliadin block components encoded by designed *Gli-A1* alleles

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-A1* was different. The most frequent was *Gli-A1a* (40,0%) and the least frequency *Gli-A1c* (4,0%) (Table 2). In Australian wheat cultivars frequency of alleles at the *Gli-A1* locus was in ratio from 3.2% to 32% METAKOVSKY *et al*., (1990). By investigation of Russian cultivars the highest frequency was found for *Gli-A1f* over the 50% depends of region (METAKOVSKY and KOPUS, 1991). In earlier investigation of Yugoslav wheat cultivars the highest frequency had *Gli-A1b* (40.4%) and the lowest *Gli-A1f* (9,6%) KNEZEVIC *et al*. (1998a). By analysis of 10 Kragujevac's wheat cultivars the highest frequency showed *Gli-A1b* (50.0%) KNEZEVIC (1992) while in Yugoslav wheat cultivars the most frequent alleles was *Gli-A1b* (55,6%) and the least frequency had *Gli-A1a* (11,1%). In Spanish cultivars the highest frequency had *Gli-A1o* (30,0%) while the lowest frequency (1,0%) had several alleles ***b, d, g, r*** and ***t*** at the *Gli-A1* locus.

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 is in their contribution to a higher plant adaptability. It has been shown that *Gli-A1f* 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-A1g, 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-A1b Gli-A1f* with high frequency had positive effect to low temperature resistance (KNEZEVIC *et al*., 1998). Besides this allele positive influence to low temperature resistance was found for *Gli-B1**b,** Gli-A2**b***, *Gli-D1**b,** Gli-B2**h*** and *Gli-D2**b*** in Yugoslav wheat cultivars (KNEZEVIC *et al*., 1998; KNEZEVIC *et al*., 2006). The established connection between alleles and resistance to low temperature could not be use as reliable marker but these alleles indicating indirect influence.

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 (LAWRENCE *et al*., 1988; METAKOVSKY *et al*., 2000; BRANLARD *et al*., 2001; 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 value and *Gli-A1f* was established in Yugoslav wheat cultivars (KNEZEVIC *et al*., 1993). Also, positive connection of *Gli-A1a*, *Gli-A1b* with high value of loaf volume, was confirmed in the same investigation (KNEZEVIC, 1992). The positive connection between dough resistance and *Gli-A2**e,*** allele as well as dough elasticity and *Gli-D2**b*** 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-A1* locus in analyzed wheat cultivars created in selection centre in Kragujevac. By analysis of 25 wheat cultivars were identified 4 *Gli-A1* alleles (*a, b, c, f).* Frequency of identified alleles variate from 4% (*Gli-A1c)* to 40% (*Gli-A1a)*. The high frequency (28%). were found for *Gli-A1b* and *Gli-A1f.* Alleles with high frequency could indicate their favourable adaptive and selection value and could be results of limited genetic variability for crossing or direct selection of desirable traits, for example: yield, particular yield components, harvest index, disease resistance, series traits of technological quality, physiological 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 *Gli-A1a* can use for crossing in the aim of improvement of technological quality, and another cultivars that possess *Gli-A1b* and *Gli-A1f* can use for disease resistance improvement.

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