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POLYMORPHISM OF *Gli-B1* ALLELES IN 25 KRAGUJEVAC'S WHEAT CULTIVARS (*Triticum aestivum* L)

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ABSTRACT: Gliadins composition of 25 wheat cultivars was analyzed by acid polyacrylamide gel electrophoresis. Electrophoregrams obtained by polyacrylamide gel electrophoresis were used for estimation variability of gliadin components and identification of gliadin alleles. Alleles at *Gli-B1* locus were identified on the base of gliadin block components by compare electrophoregrams of investigated cultivars with electrophoregrams of check cultivars. Variability of determined block components indicates that existing high polymorphysms of gliadins alleles. Six alleles at *Gli-B1* locus were identified in analyzed wheat cultivars. Frequency of alleles was different and varied from 4% to 44.

INTRODUCTION

The gliadin proteins of wheat grain endosperm are controlled by genes located on the short arm of the 1st and 6th groups of homologous chromosomes. At each locus were established multiple allelism (Metakovsky, 1991). The genes controlling individual gliadin bands at each chromosome have been shown to segregate as a unit (block) Sozinov and Poperelya (1980). Gliadins have been extensively investigated as a main constituent of grain proteins and important nutritional components. Gliadin molecules, smaller than glutenins 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. The extensive studies of gliadin allelism because of their linkage with biological traits of wheat were carried out (Payne, 1987; Metakovsky *et al.*, 1990; Knežević *et al.*, 1993; Menkovska *et al.*, 2002). In wheat, gliadin proteins have been suggested as linked markers of frost hardiness (Sozinov and Poperelya, 1980) heading time (Lafiandra *et al.*, 1987), seed size (Metakovsky *et al.*, 1986), disease resistance (Poperelya and Babayanz, 1978; Knežević *et al.*, 1994) frost resistance (Dimitrijević. 1997; Knežević *et al.*, 1998). Also, linkage between alleles at the Gli-A1 locus and bread making quality were reported by many investigations (Payne, 1987; Metakovsky *et al.*, 1990; Knežević *et al.*, 1993). 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 (Knežević, 1996).

This paper provides analysis of allele polymorphisms of *Gli-B1* locus in wheat cultivars created in Small Grains Breeding Center of Kragujevac and importance of identified alleles for wheat breeding and their connection to bread making quality traits.

MATERIALS AND METHODS

Grain samples of wheat cultivars were obtained from wheat breeding Center Kragujevac. All 25 cultivars created in this selection center. 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. 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

The focus of study was analysis of gliadin allele polymorphisms at the *Gli-B1* locus on the short arm of 1B chromosome. In study of 25 Kragujevac's wheat cultivars, the allelic variation at the *Gli-B1* locus was established. Determination of alleles carried out by using electrophoresis and six alleles (***b, e, f, g, k, l***) at the *Gli-B1* locus were identified (Table 1). At the *Gli-B1* locus were identified six alleles in Australian wheat (Metakovsky *et al.*, 1990), seven alleles in Yugoslav wheat cultivars (Metakovsky *et al.*, 1991), 16 alleles in Russian wheat cultivars (Metakovsky, 1991). By previous investigations of 57 Yugolsav wheat cultivars were identified 5 different alleles at the *Gli-B1* locus (Metakovsky *et al.*, 1991); while in analysis of 91 Yugoslav cultivars were identified seven different alleles at *Gli-B1* locus (Knežević, 1992). In the analysis of 10 Kragujevac's wheat cultivars (Knežević, 1992) were identified only 3 alleles (***b, l, f***) at the *Gli-B1* locus, while in cultivars originated from selection Center Novi Sad were identified five alleles.

Table 1. Identified alleles at the ***Gli-B1*** locus in Kragujevac's wheat cultivars

Gli-B1 alleles	Wheat cultivars
b	Lazarica, Toplica, Takovčanka, Bujna, KG-56 S, Ljubičevka, KG-58, KG-56, Zastava, KG-78, Oplenka
l	Ana Morava, Srbijanka, Šumadija, KG-100, Matica, Studenica, Ravanica
f	Orašanka, Lepenica, Vizija
k	Kosmajka, Gružanka
e	Morava
g	KG-75

In analyzed Kragujevac's wheat cultivars, the identified alleles encoding gliadin block that including four-seven different components. Several blocks have one or two similar bands in assessment of their relative mobility and color intensity on the gel (Fig. 1). Similar block of gliadins according to block phenotype we can group into three subgroups. We found similarity among block encoded by ***b, e, f, g*** alleles, and remain two gliadin block encoded by ***k*** and ***l*** allele were different. Identified gliadin block encoded by ***Gli-B1b, Gli-B1e, Gli-B1f, Gli-B1g*** consists components in β-, γ- region while block encoded by ***Gli-B1k*** consists components at the γ- and ω-gliadin region and block controlled by ***Gli-B1l*** have components in β-, γ- gliadin region.

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. The earlier investigation showed at least three distinct groups of ***Gli-B1*** encoded blocks and that some blocks belong to one group can originate from once another through single mutation events (Metakovsky, 1991). However, none of the blocks could originate through intralocus recombination between members of the three different families of blocks.

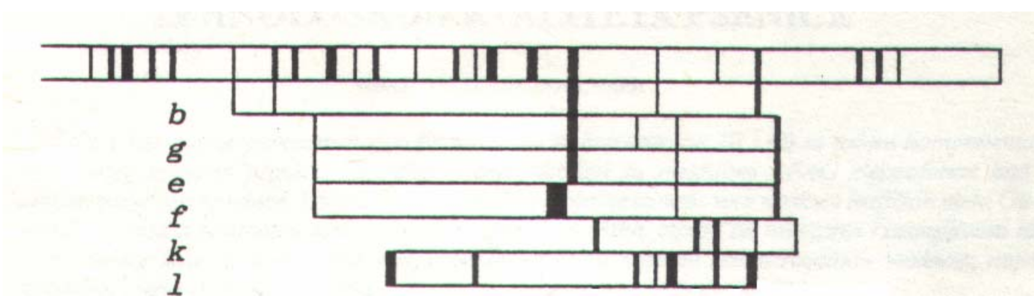

Figure 1. Identified gliadin block components encoded by designed *Gli-B1* alleles

Frequency of *Gli-B1* was different. The most frequent was *Gli-B1b* (44.0%) and the least frequency *Gli-B1e* (4.0%) and *Gli-B1g* (4.0%) (Tab. 2). In Australian wheat cultivars frequency of alleles at the *Gli-B1* locus was in ratio from 3.et al.2% (*Gli-B1d*) to 32% (*Gli-B1j*) Metakovsky *et al.*, (1990). By investigation of Russian cultivars the highest frequency was found for (*Gli-B1b* and *Gli-Bc*) over the 45% depends of region (Metakovsky and Kopus, 1991). In earlier investigation of Yugoslav wheat cultivars the highest frequency had *Gli-B1l* (34.0%), *Gli-B1b* (29.0%) Metakovsky *et al.* (1991). By analysis of ten Kragujevac's wheat cultivars the highest frequency showed *Gli-B1b* (55.0%) Knežević (1992) while in Yugoslav wheat cultivars the most frequent alleles was *Gli-B1b* (42.9%) and the least frequency had *Gli-B1f* (3.3%).

Table 2. Frequency of identified *Gli-B1* alleles in analyzed Kragujevac's wheat cultivars

Gli-B1 **alleles**	***b***	***l***	***f***	***k***	***e***	***g***
Frequency (%)	44	28	12	8	4	4

Genetic study of gliadin electrophoregram and identification of gliadin alleles provides method for assessment of genotypes. A lot of studies of gliadin alleles carried out for evaluation of their correlation with bread making quality, yield, some physiological traits (Metakovsky *et al*., 1991; Dimitrijević *et al*., 1998; Knežević, 1994; Knežević *et al*., 1998; This *et al*., 2001; Gianibelli *et al*., 2001; Menkovska *et al.,* 2002; Đukić *et al*., 2005). Enormous gliadin polymorphism makes gliadin alleles much more suitable for wheat genotype identification and distinction than other polymorphic protein alleles.

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. The value of frequent alleles may be in their contribution to higher plant adaptability. It has been shown that *Gli-B1e* with high frequency in Russian cultivars represent. Positive correlation between sedimentation value and *Gli-B1b* was established in Yugoslav wheat cultivars (Knežević *et al*., 1993). Also, in the same investigation were found that *Gli-B1b* positive connection with high value of loaf volume, dough resistance. 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; Knežević *et al.,* 1993). However, besides influence of alleles at *Gli-1* and *Gli-2* loci to those two dough properties there are influences of alleles of HMW and LMW glutenins (Lawrence *et al*., 1988) as well as gliadin/glutenin ratio (Reddy and Appels, 1990; He *et al*., 2002; Yan *et al*., 2004).

The high frequency *Gli-B2l* in Yugoslav wheat cultivas that is found in this investigation of Kragujevac's wheat cultivars represent results of favored in selection presumably because of its linkage to some disease resistant genes (Poperelya and Babayantz, 1978). In Russian wheat cultivars were found different influence of alleles at *Gli-1* and *Gli-2* loci to frost resistance. Alleles *Gli-A1m, Gli-1g, Gli-A2f, Gli-B2o* and *Gli-D2e* showed high influence to frost resistance (Sozinov and Poperelya, 1984).

By investigation of Yugoslav wheat cultivars positive connection of *Gli-B1**b*** with leaf rust resistance is established. However, in the same study were established the highest positive connection of *Gli-B2**h*** and *Gli-D2**g*** with leaf rust resistance (Knežević *et al*., 1995). In another investigation was found that the most frequent allele *Gli-B1b* had positive effect to low temperature resistance (Knežević *et al*., 1998) as well combination of *Gli-B1**b*** and *Gli-D1**b*** (Dimitrijević, 1997; Knežević *et al*., 1998). Besides this allele positive influence to low temperature resistance was found for *Gli-A2**b***, *Gli-D1**b**,* *Gli-B2**h*** and *Gli-D2**b*** in Yugoslav wheat cultivars (Knežević *et al*., 1998). The established connection between alleles and resistance to low temperature could not be use as reliable marker but these alleles indicating indirect influence.

CONCLUSION

Gliadins of 25 winter wheat cultivars were analyzed by method of electrophoresis on polyacrylamide gel, and gliadins blocks and their encoding gliadin alleles were identified. This investigation showed existence of allele polymorphisms of *Gli-B1* locus in analyzed wheat cultivars created in selection center in Kragujevac. By analysis of 25 wheat cultivars six *Gli-B1* alleles (*b, l, f, k, e, g)*were identified. Frequency of identified alleles variate from 4% (*Gli-B1g* and *Gli-B1e)* to 44% (*Gli-B1b)*. The high frequency showed *Gli-B1l* (28%). Alleles with high frequency could indicate their favorable 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. Identified alleles considering their connection with technological quality traits (sedimentation of proteins, loaf volume and dough properties), adaptive values have importance for further breeding process and creation of advanced wheat cultivars. In the breeding program is a very important incorporate gene controlling desirable characters. From this study it was clear that the incorporation of a single gene into a plant was likely to result in the desired phenotype. The wheat cultivars carried *Gli-B1b* can use for crossing in the aim of improvement of technological quality, and another cultivars that possess *Gli-B1l* can use by crossing for increasing disease resistance.

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