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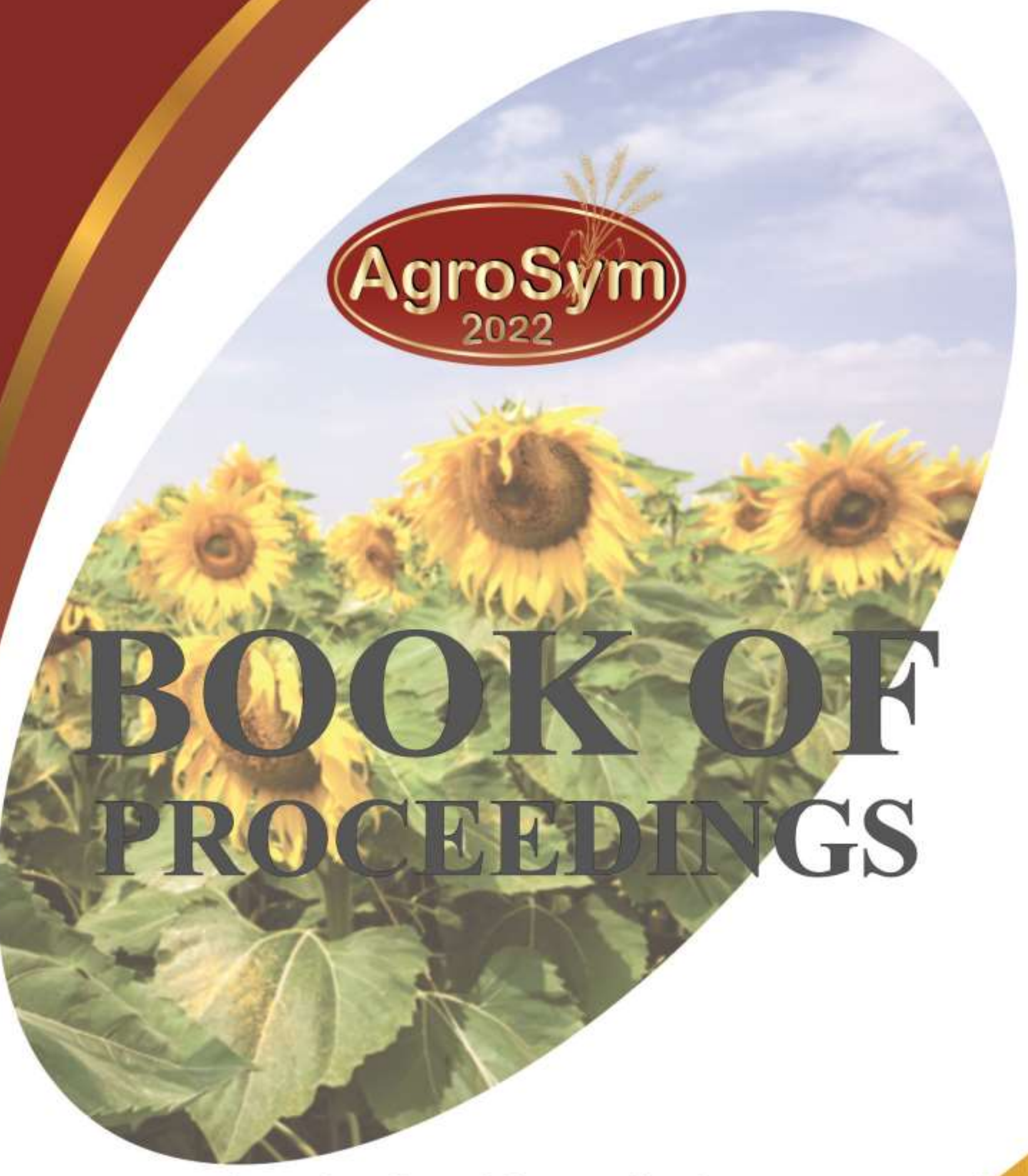

BOOK OF PROCEEDINGS

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VARIABILITY OF GLUTEN PROTEINS IN WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

Gluten proteins are formed from proteins of flour, gliadin and glutenins which in contact with water, begin to interact through the formation of chemical bonds. The aim of this study is identification of encoding genes polymorphisms of gliadin and glutenins in 10 bread wheat genotypes. For analysis used 30 seeds of 10 wheat genotypes for extraction of gliadins by 70% ethanole, and glutenins by 10% β-mercaptoethanol.The gliadins were separated by acid page electrophoresis (pH=3.1) on 8.33% polyacrylamide gel, while glutenins were separated by SDS-PAGE (pH-8.6) on 11.8% gel. Electrophoregrams were used for determining *Gli-1* and *Gli-2* alleles. The three alleles (***a, b, m***) at the *Gli-A1*, four alleles (***b, g, l, k***) at the *Gli-B1*, five alleles (***a, b, f, g, k***) at the *Gli-D1*, five alleles (***b, e, f, g, k***) at the *Gli-A2*, four alleles (***b, h, j, p***) at the *Gli-B2* and three alleles (***a, b, r***) at the *Gli-D2* locus were identified. For high molecular weight glutenin subunists (HMWGS) the three alleles (***a, b, c***) at the *Glu-A1*, three alleles (***b, c, d***) at the *Glu-B1* and two alleles (***a, d***) at the *Glu-D1* were identified. Gluten proteins varied according to composition alleles encoding gliadin and glutenins in analyzed wheat genotypes what related with established polymorphisms of each gliadin and glutenin loci.

Keywords: *wheat, gliadin, glutenin, allele, polymorphism quality.*

Introduction

Gluten is complex group of proteins consisting gliadins and glutenins, approximately in equal amount (Wrigley et al., 200). Gliadin and glutenins are deposited in endosperm of grain which, are important in determining quality of flour, dough and bread (Knezevic et al. 2017). Hydrated gliadin and glutenins interact through the formation of chemical bonds and begin to stick to each other and forms a very extensible, elastic structure that is responsible for the gas-holding ability of bread dough. and determines the viscoelasticity, strength, resilience and stretchability of the dough (Menkovska et al., 2002; Shewry, 2007; Torbica et al., 2007). Gliadins are a heterogeneous group of proteins which contain different type of polypeptide molecules (α-, β-, γ- and ω-gliadins), globular conformation, with intra disulfide bonds single chains (Bietz, 1997) and most of them have molecular mass (16kDa to 50kDa). Gliadin are encoded by genes located on the short arm of 1. and 6. group of A, B and D chromosomes (Sozinov and Poperelya, 1980) i.e. loci *Gli A*1, *Gli B*1, *Gli D*1, *Gli A*2, *Gli B*2 and *Gli D*2 respectively, which characterized families of multiple alleles (Metakovsky, 1991;

Metakovsky et al, 2018).. Polymorphisms of gliadin alleles in Russian, French, Yugoslav, Italian, Spanish, wheat cultivars were established (Metakovsky et al, 1991; 1994; 1997; 2000). The glutenins contain two types of polypeptides, one type with low molecular weight 20kDa to 50kDa (LMW GS) and shorter, and another with high molecular weight 50kDa to 200kDa (HMW GS) or more. The glutenin proteins characterize intermolecular disulfide bonds between polypeptide. The HMW-GSs are encoded by three loci, *Glu-A1*, *Glu-B1*, *Glu-D1*, located on long arm of chromosomes (Payne et al., 1987; Knežević et al., 1993), and LMW-GSs are encoded by genes located on the short arm of *Glu-A*3, *Glu-B*3, *Glu-D*3.
The aim of this study was identification (i) alleles at *Gli-1*, *Gl-2* loci encoding gliadin proteins (ii) *Glu-1* loci encoding high-molecular weight (iii) determination variability of gliadin allele composition and (iv) determination variability of glutenins allele composition in analyzed wheat genotypes.

Material and methods

The 10 genetically divergent wheat genotypes (G-3626-1, G-3618-2, G-3606-4, G-3636-3, G-3627-1, G-3621-1, G-36-6-5, G-3607-5, G-3606-6, G-3632-1) were included for analysis variability of gliadin and glutenins on the base of identification encoding gene alleles.
At least 30 single seeds were used for extratction gliadin proteins in 70% ethanole at room temperature for one hour. After that samples centrifuged at at 5000 rpm for 20 min. For separation of gliadins used acid PAG electrophoresis method developed by Novoselskaya et al. (1983). Gliadin extract (20 µl) were loaded on the gel 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) Electrophoresis was performed during 2.5 to 3 hours, in electric field under constant voltage from 550 V and in 5 mM aluminum lactate buffer. The separated gliadin bands were stained in 0.05% ethanol solution of Coomassie Briliant Blue R250 by adding 250 ml 10% threechloroacetic acid (TCA) and after that gels photographed. Gels and photographs were used for determination of gliadin blocks alleles according to method Metakovsky (1991).
For gluttenin extraction used residue of the same kernel sample, which treated by 120 mM Tris-HCl, pH=6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol) and boiled for 5 min. The sample were centrifuged at 12000 rpm for 10 min. Protein resolved by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) Laemmli, (1970) with 11.8% gel and electrophoresed at 20mA for 2h. Gels were stained by Commassie Briliant blue dye resloved in 10% TCA and 250ml methanols. After staining, the electrophoregrams are used for analysis and determining HMW-GS and identification of *Glu-1* alleles (Payne and Lawrence, 1983).

Results and discussion

Gliadin alleles variability encoding gliadin proteins. The sudy of gliadin allele composition at *Gli-A1* and *Gli-A2* loci showed differences among the analyzed wheat genotypes. In ten wheat genotypes were identified 24 alleles at six *Gli*-loci, three of them (***a, b, m***) at *Gli-A1*, four alleles (***b, g, l, k***) at *Gli-B1*, five alleles (***a, b, f, g, k***) at *Gli-D1*, five alleles (***b, e, f, g, k***) at *Gli-A2*, four alleles (***b, h, j, p***) at *Gli-B2* and three alleles (***a, b, r***) at *Gli-D2* locus (table 1).
In analysis in some genotypes identified heterozygosity of some gene loci. The two different alleles at two loci identified in the genotype G-3606-4 at the locus *Gli-D1* (***b***+***g***), at *Gli-A2* (***g***+***e***) and in genotype G-3627-1 at the locus *Gli-A1* (***m***+***a***), at *Gli-D1* (***b***+***a***). Also, in two genotypes identified two different alleles at one locus and with the genotype G-3607-5 at the locus *Gli-A2* (***k***+***g***) and in genotype G-3606-6 at the locus *Gli-D2* (***a***+***b***) table 1.

The hetrozigosity indicates that wheat genotypes are not genetically homogenized for specified loci, which requires further selection in order to achieve genetic homozygosity of specified loci. The gliadin allele polymorphisms of each ***Gli-1*** and ***Gli-2*** loci was established in numerous invetigation of wheat varieties (Knežević et al., 2006; 2007; 2008; Knezevic et al., 2017, Metakovsky et al., 2018; 2021; Utebayev et al., 2019).

Table1. Gliadin and glutenin allele of winter wheat genotypes

Genotype	Gli- alleles						High molecular weight glutenin subunits			Glu-1 alleles			Glu-1 quality score
	A1	B1	D1	A2	B2	D2	1AL	1BL	1DL	**A1**	**B1**	**D1**	
G-3626-1	a	b	a	k	b	a	2*	7+9	5+10	**b**	**c**	**d**	**9**
G-3618-2	m	l	k	b	?	a	2*	7+8	5+10	**b**	**b**	**d**	**10**
G-3606-4	a	k	b+g	g+e	h	a	N	7+9	2+12	**c**	**c**	**a**	**5**
G-3636-3	b	l	a	k	j	b	N	6+8	2+12	**c**	**d**	**a**	**4**
G-3627-1	m+a	b	b+a	b	p	r	1	7+9	5+10	**a**	**c**	**d**	**9**
G-3621-1	a	l	b	f	h	a	2*	7+9	5+10	**b**	**c**	**d**	**9**
G-3606-5	b	g	b	g	b	b	2*	7+8	5+10	**b**	**b**	**d**	**10**
G-3607-5	b	b	f	k+g	b	b	N	7+9	2+12	**c**	**c**	**a**	**5**
G-3606-6	b	k	g	e	h	a+b	N	6+8	2+12	**c**	**d**	**a**	**4**
G-3632-1	a	l	a	f	b	b	1	7+9	5+10	**a**	**c**	**d**	**9**

Glutenin alleles variability encoding high-molecular glutenin proteins However, in those ten wheat genotypes were identified eight alleles at the *Glu-1* loci, three of them (***a, b, c***) at the *Glu-A1*, three (***b, c, d***) at the *Glu-B1* and two alleles (***a, d***) at the *Glu-D1* locus (table1).
The relationship between *Glu-1* alleles of the HMWG subunits and the bread-making quality was determined (Payne, 1987; Lafiandra, et al., 1987;Metakovsky et al., 1990). For each allele at the three *Glu-1* loci, assigned mark for contribution to quality score in assesing bread making quality. The highest mark 4 determined for alleles ***d*** at *Glu-D1*, while mark 3 is for alleles ***a, b,*** at the *Glu-A1*, as well for ***b, i, f*** at the *Glu-B1*. Mark 2 determined for alleles ***a,*** at the *Glu-D1* and ***c***, at the *Glu-B1*, while the lowest mark 1 determined for alleles ***c, Glu-A1,*** and ***a, d, e,*** at the ***Glu-B1*** (Payne and Lawrence 1983).
In our study the highest *Glu*-1 quality score varied between 4 and 10. The highest value of *Glu*-1 quality score established in two genotypes G-3618-2 and G-3606-5, while the lowest in G-3636-3 and G-3606-6 (table 1).
Frequency of identified alleles at Gli-1, Gli-2 and Glu-1. The frequency of identified gliadin alleles was different. At the ***Gli-A1*** locus the highest frequency computed for two alleles ***a, b*** (40.0%), while the lowest had allele ***m*** (20%). At the ***Gli-B1*** locus the highest frequency had allele ***l*** (40.0%), lower had alleles ***b*** (30%) and ***k,*** (20%), and the lowest had allele ***g*** (10%). At the ***Gli-D1*** locus the most frequent was allele ***b*** (40.0%), lower frequency had allele ***a*** (30%)**,** and the lowest frequency had alleles ***f, g, k*** (10%). At the ***Gli-A2*** locus the most frequent was allele ***k*** (30.0%), while three alleles ***b, f, g*** had frequency (20.0%) and the lowest frequency had allele ***e*** (10%). At the ***Gli-B2*** locus the most frequent was allele ***b*** (40.0%), while the lowest and equal frequency had alleles ***j, p*** (10%). At the ***Gli-D2*** locus the most frequent was allele ***a*** (50.0%) and the lowest frequency had allele ***r*** (10%), while high frequent was allele ***b*** (40%) table 2.
The frequency of glutenin alleles varied at all three loci. At the ***Glu-A1*** locus the highest and equal frequency found for alleles ***b, c*** (40.0%), while the lowest had alleles ***a*** (20%). At the ***Glu-B1*** locus the most frequent was allele ***c*** (60.0%), while the lowest and equal frequency

had alleles ***b, d*** (20.0%). At the ***Glu-D1*** locus the highest frequency had allele ***d*** (60.0%), while the lowest frequency had alleles ***d*** (40%) table 2.
The different frequency of the *Gli-1*, *Gli-2* and *Glu-1* allele may be the result of a directed selection of the genotype according to some desirable component of quality and yield or adaptability to biotic and abiotic factors, which also indicates the associability of the identified gliadin alleles with desirable traits as for example: frost, resistance, resistance to diseases, grain hardness, flour and dough quality, lipid composition and starch properties etc. In some cases, high allele frequency is results of using parent varieties that carry low genetic variability at certain loci. Differences in allele frequencies are interpreted in a similar way in other studies (Knezevic et al., 1998; 2017; Lookhart et al., 2001; This et al., 2001).

Table 2. Frequency of alleles at *Gli-1*,*Gli-2* and *Glu-1* loci

Gliadin alleles												Glutenin aleles					
Gli-A1		*Gli-B1*		*Gli-D1*		*Gli-A2*		*Gli-B2*		*Gli-D-2*		*Glu-A1*		*Glu-B1*		*Glu-D1*	
Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%	Alel	%
a	40	*b*	30	*a*	30	*b*	20	*b*	40	*a*	50	*a*	20	*b*	20	*a*	40
b	40	*g*	10	*b*	40	*e*	10	*h*	30	*b*	40	*b*	40	*c*	60	*d*	60
m	20	*k*	20	*f*	10	*f*	20	*j*	10	*r*	10	*c*	40	*d*	20		
		l	40	*g*	10	*g*	20	*p*	10								
				k	10	*k*	30	?	10								

Conclusion

The variability of gluten proteins, based on identified alleles at gliadin and glutenin loci. The polymorphism of each *Gli-1*, *Gli-2* and *Glu-1* locus was identified. A different number of alleles were identified at each locus. In ten wheat genotypes, at the six gliadin loci were identified 24 different alleles, while at three *Glu-1* loci were identified eight different alleles. The highest polymorphisms were established at the *Gli-D1* and *Gli-A2* locus, on which five different alleles were identified in analyzed ten wheat genotypes. Frequency of identified gliadin alleles varied between 10 and 50% and for glutenin alleles between 20 and 60%. The most frequent alleles are *Gli-A1a, Gli-B1l, Gli-D1b, Gli-A2k, Gli-B2b, Gli-D2a*, *Glu-A1b, Glu-B1c* and *Glu-D1d*. Composition of gliadin and glutenin alleles was different and specific for each wheat genotype and can be used as reliable marker for quality traits in breding program considering.

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