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Original scientific paper

HORDEIN GENE DOSE EFFECTS IN TRIPLOID ENDOSPERM OF BARLEY (*Hordeum vulgare* L.)

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The presence of two maternal chromosome sets in triploid barley endosperm allows the distinction of maternal and paternal hordein bands in an electrophoregram: the maternal bands are stronger due to the higher gene

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dose. In the F_1 generation there are differences between reciprocal crosses and in the F_2 generation all 16 classes that are theoretically possible for a pair of polymorphic loci can be distinguished. This full classification is rarely possible in genetic studies, and allows more accurate estimates of recombination rates. Two hordein gene clusters (*Hor1* and *Hor2*, corresponding to hordein *C* and hordein *B* respectively) were analysed in hybrids obtained by crossing two winter barley cultivars *Partizan* and *HWV-247*. Hordein separation was performed by acid-polyacrylamide gel electrophoresis at pH 3.2 (A-PAGE). A set of most informative bands of B and C hordeins was selected in each cross by two criteria: (1) presence or absence of bands in the parents and (2) signal strength to allow doses scoring. The average genetic distance between *Hor1* and *Hor2* loci was 11 cM. Distances in male and female maps were not significantly different, suggesting a similar recombination rate in male and female meiosis.

Key words: A – PAGE, barley, hordeins, gene dosage effect

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INTRODUCTION

Cereal prolamins (e.g. gliadins in wheat, hordeins in barley, secalins in rye, zeins in maize, etc.) are the main endosperm storage proteins in all cereal grains (SHEWRY *et al.,* 2001). Hordeins, as the main storage protein fraction in barley seeds, which are accumulated exclusively in starch endosperm, accounts for up to half of the total protein in the mature grains. Therefore, they have major influence on grain quality, the quality of malt and beer, and the nutritional value *(*MOLINA-CANO *et al.,* 2001). During the last forty years, electrophoretic polymorphism of hordeins was widely used for genotype identification and for studying its influence on the nutritional value and malting quality (DOLNIK 2006*;* CIFUENTES 2006). Genes controlling the synthesis of the storage proteins are clustered multigene families that are described both on the protein and the DNA level (KANAZIN *et al.,* 1993; SAYANOVA *et al.,* 1993*;* DONG *et al.,* 2009). Although the number of linked loci is rather small, the expression of the storage protein genes is complex and still partially unknown. Hordein genetics is less complex than genetics of gliadins in wheat, because of diploidy in barley versus hexaploidy in wheat. Three homologous chromosomes determine the endosperm genotype. This is the consequence of double fertilisation. The triploid endosperm originates from a female central cell, containing 2n chromosomes, which is the fusion product of two haploid polar nuclei fertilised by a male sperm cell containing n chromosomes (BRIGGS and KNOWLES*,* 1977). Thus, in the triploid endosperm the dosage of the maternal allele is twice the paternal dosage. Therefore resulting dosage effect could be used to determine the genotypic classes for the hordeins in the F_2 generation of barley hybrids.

The expression of hordeins depends on time and tissue and interchromosome interactions (HANSEN *et al.,* 2007). The amount of hordeins is influenced by environmental factors, e.g. availability of nitrogen and sulphur (DUFFUS and COHRANE, 1992) but the composition of hordeins is not (MARCHYLO *et*

al., 1987). Change of the relative intensity of the band staining is a result of the influence of environmental factors, especially the nitrogen supplies (RAHMAN *et al.,* 1983, SIMIC *et al.,* 2008). Apart from having a major influence on grain quality, hordeins are an important tool for cultivar identification (RADOVIĆ, 1995, POMORTSEV *et al.,* 2007). The strong point of identification by hordein patterns is that each hordein provides information about more genes than with any other molecular probe, and that a half of single grain is sufficient for the screen. Nowadays, several systems are applied to characterize hordein composition (SHEWRY *et al.,* 1981; WHITE and COOKE, 1992; KONAREV, 1996, YUEMING *et al.,* 2003; GARCIA-VILLALBA *et al.,* 2006).

Information about the mode of inheritance of the hordein patterns according to dosage effect are presented in several studies (SOZINOV *et al.,* 1979; SHEWRY *et al.,* 1981) and the general conclusion is that the results are often misinterpreted or confused. Such problems can be solved by direct examination of the genetic basis of the dosage effect and the corresponding phenotypic classes in segregating generations. The aim of this study was to examine dosage of endosperm genetic background on hordein pattern, to determine the genotypic classes and mode of inheritance of triploid hordeins in F_1, F_2 and BC generations of barley hybrids. Additional aspects of using the hordein composition in marker assisted selection (MAS) and genotype identification are discussed.

MATERIALS AND METHODS

Winter barley cultivars Partizan and HWV-247 proved to be divergent concerning hordein composition in previous studies (PEROVIC *et al.,* 1998, PEROVIC *et al.,* 2000) and, therefore, were chosen to be crossed. These cultivars and their hybrid progeny and backcrosses were grown in identical conditions in the Center for Small Grains, Kragujevac. The harvest was manually performed at full maturity stage. The number of analyzed grains has been as follows: Partizan x HWV-247 (44 grains), HWV-247 x Partizan (54 grains), F_1 x Partizan (34 grains) and F_1 x HWV-247 (25 grains).

Hordeins were extracted from single grains that were of approximately the same size. The extraction buffer consisted of 70% ethanol, 18% urea and 2% 2-mercaptoethanol according to the modified procedure of DRAPER (1987). Seeds were placed in 1.5 ml tubes, and vigorously mixed with the extraction buffer by metal balls. Samples were then centrifuged for 30 min (5000 rpm). The supernatant was removed into other tubes. Pellets were dissolved in glycerol and loaded to gel with Methyl-green as marker color.

Acid PAGE was conducted on 10% gel (pH = 3.2) fo 4 h at 380 V. The gels were fixed by 10% TCA and then stained overnight by 12% TCA and 0.05% Coomassie brilliant blue R-250 and analysed while they were fresh. The relative mobility (Rm) of the polypeptide bands was determined according to WHITE and COOK (1992) and the relative intensity (Ri) of the stained bands was determined visually (0 – 5 categories) following the procedure of YAN (1996).

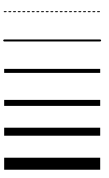
Rm – relative mobility of detected bands (Rm):

$$\mathbf{Rm = (100 \times Dx) / Px}$$

- Dx – distance of the band from the start (mm);
- Px – distance of the basic referent band (100) from the start (mm);

Ri – relative staining intensity of the detected bands (6 classes):

0 – trace
1 (+)
2 (++)
3 (+++)
4 (++++)
5 (+++++)



The percentage of recombination (p) was determined as the number of the recombinant gametes divided by the total number of gametes (n). The standard error for p was calculated as $\sqrt{(p - p^2)/n}$. Map distances in centimorgan were calculated as $-1/2 \log_n (1-2p)$, with standard error $p/(1-2p)$, according to methods described by BAILEY (*1961*).

Theoretical base for genotype classification by dosage effect

The triploid endosperm nucleus in barley is formed by fusion of two polar nuclei from the same meiosis, genetically identical to the egg cell and one sperm cell. Thus, three homologous chromosome sets come together in the endosperm of which two are identical and originate from the female and the third originates from the male parent. Since hordein composition is inherited in the co-dominant manner, both parental compositions are expressed in the F_1 generation (DOLL and BROWN, 1979). Theoretically F_1 plants can form four types of female and four types of male gametes (Figure 1).

Male gametes had *n* chromosomes (haploid), and female gametes had *2n* chromosomes (diploid). The genesis of endosperm begins when paternal spermy (n) fertilises maternal central cell (2n). Central cell is diploid, because it was created by fusion of two nuclei with n chromosomes each. These two nuclei are products of one meiotic cycle and therefore only four types of central cells can be formed. The dosage effect is the consequence of presence of the third chromosome in the expression of the hordein genes. Chromosomal array of two triploid and linked loci in the F_1 and F_2 generations (Figure 2) elucidates this phenomenon.

Differences in reciprocal hybrids are visible in the level of chromosome as identical hereditary units in pairs or single. Sixteen genotype classes can be distinguished when dosage effects are scorable in the F_2 generation. Parental genotypes can be seen in the chromosomal scheme of the F_2 generation, as well as the single and double recombinants. Comparison of the phenotypes and corresponding genotypes of B and C hordeins in the F_2 generation provides whether certain phenotype shown as an electrophoregram originates from the nonrecombinant or recombinant gametes.

	P_1 ♀		P_2 ♂	
	CpCpCpBpBpBp	x	ChChChBhBhBh	
Parental gametes	CpCpBpBp	x	ChBh	Direct
	ChChBhBh	x	CpBp	Reciprocal
F_1 generation	CpCpChBpBpBh			Direct
	ChChCpBhBhBp			Reciprocal

F_2 generation

♀ \ ♂	CpBp	ChBh	CpBh	ChBp
CpCpBpBp	CpCpCpBpBpBp	CpCpChBpBpBh	CpCpCpBpBpBh	CpCpChBpBpBp
ChChBhBh	ChChCpBhBhBp	ChChChBhBhBh	ChChCpBhBhBh	ChChChBhBhBp
CpCpBhBh	CpCpCpBhBhBp	CpCpChBhBhBh	CpCpCpBhBhBh	CpCpChBhBhBp
ChChBpBp	ChChCpBpBpBp	ChChChBpBpBh	ChChCpBpBpBh	ChChChBpBpBp

Back crosses:

F1\ ♀	CpBp
CpCpBpBp	CpCpCpBpBpBp
CpCpBhBh	CpCpCpBhBhBp
ChChBpBp	ChChCpBpBpBp
ChChBhBh	ChChCpBhBhBp

F_1 x Partizan BC1dF_1

F1 \ ♂	ChBh
CpCpBpBp	CpCpChBpBpBh
CpCpBhBh	CpCpChBhBhBh
ChChBpBp	ChChChBpBpBh
ChChBhBh	ChChChBhBhBh

F_1 x Hvw-247 BC1rF_1

Figure 1. Theoretical classes of B and C hordein components in the F_1, F_2 and BC generations of the Partizan x Hvw-247 cross; p denotes hordein alleles of cv. *Partizan* and h denotes hordein alleles of cv. Hvw-247. Parental genotypes of F_2 generation are in gray shaded cells.

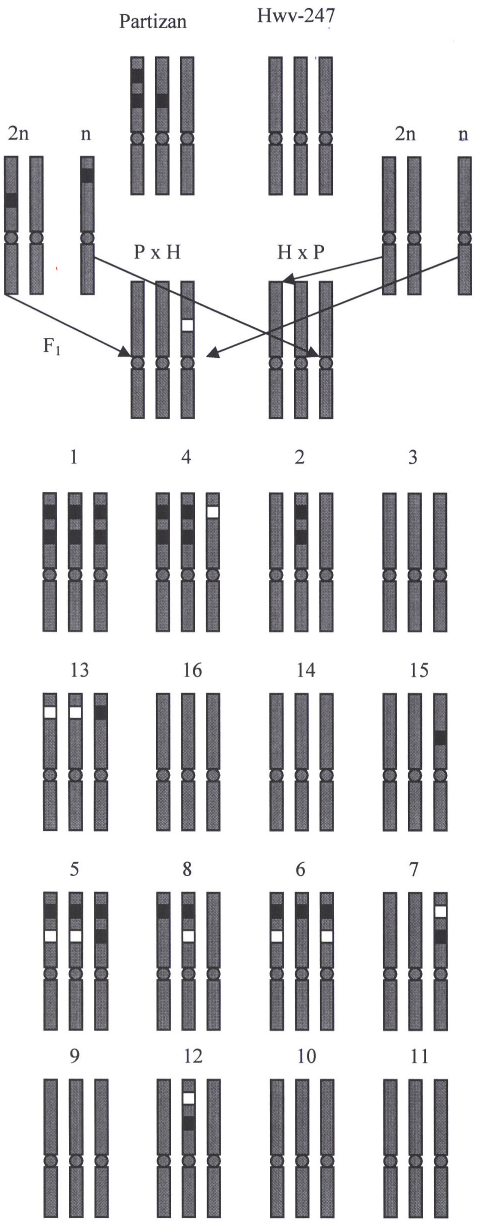


Figure 2. Chromosomal array of gene dosage effect in the F_1 and F_2 generations of the *Partizan* x *Hwv-247* cross. Genotypes designated as 4 and 13 in the F_2 seeds are identical to the phenotypes of the corresponding reciprocal F_1 seeds.

RESULTS

Seven polypeptide bands of the *C* hordein in the *Partizan* cultivar and nine *C* hordein bands in the *Hvw-247* cultivar were detected (Figure 3). Eleven *B* hordein bands in the *Partizan* cultivar and eight *B* hordein bands in the *Hvw-247* cultivar were recorded. All bands originating from both parents were expressed in the F_1 generation which is in agreement with the co-dominant manner of the inheritance of hordein composition. The differences between the reciprocal F_1 hybrids are not related to cytoplasm hereditary factors, but are due to triploid constitution of the endosperm.

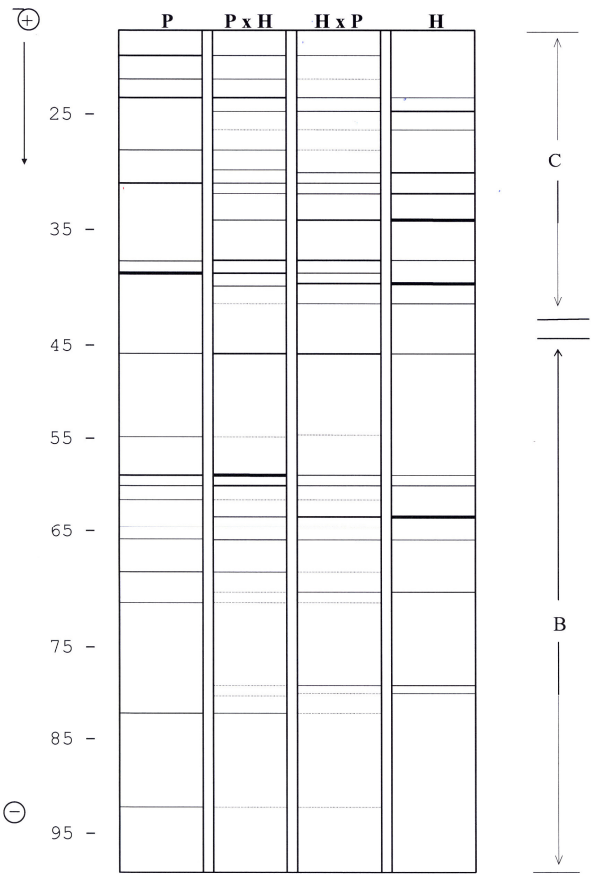


Figure 3. Hordein electrophoregrams of the Partizan x Hvw-247 F_1 hybrid

Since *B* and *C* hordeins are expressed as a group of six to ten polypeptide bands, an alternative scoring method could be proposed. Thus, scoring can be simplified by selecting the most informative band (marker band) for each group of linked bands. The marker band should have a high staining intensity and no other band in the same relative mobility area in another parental cultivar should be present. The dosage effect can be evaluated by comparing the relative intensity of the marker bands in the different phenotypes. Sixteen genotypes of different dosage effect combinations are possible in the F_2 generation. In the case of *C* hordeins, band of the relative mobility 20 and the relative intensity 3 for the *Partizan* cultivar and, in the case of B hordeins, band of the relative mobility 63 and the relative intensity 4 for the *Hwv-247* cultivar were selected as markers. Different staining intensities of the bands belonging to the direct and the reciprocal F_1 hybrids corresponded with the different polypeptide amount in the given band. The *C* marker band in the *Partizan* x *Hvw-247* F_1 hybrid has the relative staining intensity 2 because it is determined by the two alleles in cv. *Partizan* and by the one allele in the *Hvw-247*. In the *Partizan* x *Hwv-247* F_1 hybrid progeny the staining intensity was 2 for the marker bend (two alleles), and in the *Hwv-247* x *Partizan* F_1 hybrid the staining intensity was 1 (one allele). In the *Partizan* x *Hwv-247* F_1 hybrid the *Partizan* cultivar was the maternal component. This hybrid progeny expressed the staining intensity 2, because its band was determined by two alleles originated from *Partizan* and by one non-functional allele originated from *Hwv-247*. Reciprocal F_1 hybrid *Hwv-247* x *Partizan* had *C* marker band with the staining intensity 1, e.g. the synthesis of this band was controlled by one allele belonging to the father *Partizan* cultivar. The same scoring behaviour of the *B* marker band was recorded. Relative intensities were 3 and 2, in direct and reciprocal cross, respectively.

There were no recombinations between the *B* or *C* hordein components in the F_2 generation. This is in agreement with the results previously achieved by SALL (1990) and HOSSAIN and SPARROW (1991). Recombinations within *C* and *B* hordein components (*Hor1* and *Hor2* loci) are mostly recorded in mutant genotypes which were exposed to mutagenic treatment or in exotic genotypes, while there were not any in the hybrid progenies of the commercial varieties (SHEWRY, 1992). The *B* and *C* allele ratio in the F_2 generation is presented in Table 1.

The distribution of four phenotypic classes in monohybrid inheritance of the *B* and *C* hordein bands was studied. The recorded phenotypic classes and the corresponding genotypic formulae for the *C* hordeins are:

1. *Partizan* - *CpCpCp*
2. Strong *Partizan* weak *Hvw-247* - *CpCpCh*
3. Strong *Hvw-247* weak *Partizan* - *ChChCp*
4. *Hvw-247* - *ChChCh*

Table 1. – B and C hordein alleles ratio in the F_2 and the BC generations of the hybrid Partizan x Hvw-247

Hybrid	Hordein	Class Obs.No.		χ^2	p >
		1.	10		
	C	2.	10	1.09	0.70
Partizan		3.	14		
x		4.	10		
HVW-247		1.	9		
(direct)	B	2.	11	0.72	0.80
		3.	11		
		4.	13		
		1.	16		
	C	2.	16	2.00	0.50
HVW-247		3.	12		
x		4.	10		
Partizan		1.	16		
(reciprocal)	B	2.	15	1.26	0.70
		3.	11		
		4.	12		
		1.	26		
	C	2.	26	1.10	0.70
		3.	27		
Total		4.	19		
(dir + rec)		1.	25		
	B	2.	26	0.37	0.90
		3.	22		
		4.	25		

The dosage effect could be noticed in the given genotypic formulae in the triploidly determined traits. This effect was also recorded in the case of the seed storage proteins in wheat (BURNOUF and BOURIQUET, 1983, VAPA 1985), maize (SOAVE *et al.,* 1981), rice (KUMAMARU *et al.,* 1989) and other species. Obviously, in the case of co-dominant inheritance of the triploidly determined traits, more phenotypes are expressed than in the case of the dominant-recessive inheritance. The distribution of the four phenotypic classes was analysed by the χ^2 test and it was found that the experimental ratio was not significantly different from the expected ratio of the 1:1:1:1. Fourteen non parental phenotypes were found in the F_2 generation. Similar results have been reported by PEROVIC *et al.* (2000), where 12 classes were found in a set of analysed F_2 populations.

The two out of 14 phenotypes were combinations of the parental gametes, but were different because of the opposite direction of the dosage effect expression.

The frequencies of the *Partizan* x *Hvw-247* F_2 phenotypes are shown in Table 2. Double recombinants were not formed because of the close linkage between the two loci, and the small population size. All recombinant individuals were single. Fourteen seeds of the recombinant genotype were present in the direct hybrid *Partizan* x *Hvw-247* while six such seeds were found in the reciprocal hybrid *Hvw-247* x *Partizan*. Nine recombinations occurred in the male gamete synthesis and eleven in the female gamete synthesis.

Table 2, – Frequency of the F_2 phenotypes in the Partizan x Hvw-247 cross

f / m	CpBp	ChBh	CpBh	ChBp	Hybrid
	6	7	3	0	direct
CpCpBpBp	15	14	0	1	reciprocal
	21	21	3	1	total
	9	8	2	1	direct
ChChBhBh	10	9	2	0	reciprocal
	19	17	4	1	total
	1	3	0	0	direct
CpCpBhBh	1	1	0	0	reciprocal
	2	4	0	0	total
	3	1	0	0	direct
ChChBpBp	0	1	0	0	reciprocal
	3	2	0	0	total

Percent of recombination and the map distance between *Hor1* and *Hor2* loci were calculated based on the phenotype frequencies in the F_2 generation (Table 3). The distance between *Hor1* and *Hor2* loci in the studied population was 11.4 $\pm$ 2.71 cM. The distances between parental components were 10.15 $\pm$3.58cM for male individuals and 12.71 $\pm$ 4.11 cM for female (maternal) individuals.

Table 3. Percent of recombination and the map distance between Hor1 and Hor2 loci in the F2 and the BC generations of the Partizan x Hvw-247 cross

Hybrid	Level of estimation	Recombinations (%)	Distance (cM)
Partizan x HVW-247 (total)	gametes	0.1020 $\pm$ 0.022	11.41 $\pm$ 2.71
	m	0.0918 $\pm$ 0.029	10.15 $\pm$ 3.58
	f	0.1122 $\pm$ 0.032	12.71 $\pm$ 4.11
Partizan x HVW-247 (direct)	gametes	0.1591 $\pm$ 0.039	19.15 $\pm$ 4.40
	m	0.1364 $\pm$ 0.052	15.93 $\pm$7.11
	f	0.1818 $\pm$ 0.058	22.59 $\pm$ 9.13
HVW-247 x Partizan (reciprocal)	gametes	0.0556 $\pm$ 0.022	5.89 $\pm$ 2.47
	m	0.0556 $\pm$ 0.031	5.89 $\pm$ 3.50
	f	0.0556 $\pm$ 0.031	5.89 $\pm$ 3.50

Backcross analysis

Beside the F_1 and the F_2 generations in the *Partizan* x *Hvw-247* hybrid, the inheritance of the hordein composition was studied in the recurrent cross generations, denoted as the BC1dF1 and the BC1rF1. BC1dF1 generation was the progeny of the (*Partizan* x *Hvw-247*) x *Partizan* direct cross, while the BC1rF1 was the progeny of the (*Hvw-247* x *Partizan*) x *Hvw-247* reciprocal cross.

Four distinct genotypes (and phenotypes) were distinguished in each recurrent generation, making a total of eight. The possibility of the recognition of the larger number of phenotypes than in the dominant – recessive cases is due to the dosage effect in co-dominantly inherited *B* and *C* hordeins. When analyzing each group of hordeins and the monohybrid inheritance in the backcross generations, the experimental segregation ratios were compared with the expected ones by χ^2 test (Table 4). On the basis of the χ^2 values, the ratio of the phenotype classes of both major hordein groups was 1:1 in the backcross generations, which indicated that the hordein composition was co-dominantly inherited. It was possible to distinguish two phenotype classes in each genotype in the monohybrids and four phenotype classes in each genotype in the dihybrids due to the gene dosage effect. Two genotypes were identical to the parents in the BC generation (shaded) and the others were recombinants (Table 5).

Table 4. Phenotypic frequencies in the BC1dF1 and the BC1rF1 generation in the Partizan x Hvw-247 hybrid

Hybrid	Hordeins	E	χ^2	p >
	C	16	0.12	0.70
BC1dF1		18		
	B	16	0.12	0.70
		18		
	C	14	0.36	0.50
BC2F1		11		
	B	14	0.36	0.50
		11		

Table 5. Phenotypic frequencies in the BC1dF1 and the BC1rF1 generation in the Partizan x Hvw-247 hybrid

Hybrid	$BC1dF_1$	$BC1rF_1$
f / m	CpBp	ChBh
CpCp BpBp	14	13
CpCp BhBh	2	1
ChCh BpBp	2	1
ChCh BhBh	16	10

The genotype frequency data were used to calculate the percent of recombination and the distance between the two hordein loci (Table 6). Since 50% of gametes in the backcross is identical in genotype composition calculation of the percent of recombination based on gametes was not preferred to using genotypes, as in the case of the F_2 generation. But its information had the same value (four gametes and four genotypes were present in each cross combination in the co-dominant inheritance in dihybrids). The distance between *Hor1* and *Hor2* loci, calculated on the basis of the backcross *Partizan* x *Hvw-247* hybrid progeny, was 13.41 $\pm$ 7.20 cM in the BC1dF1 and 8.72 $\pm$ 6.40 cM in the BC1rF1.

Table 6. Percent of recombination and map distances between Hor 1 and Hor 2 loci in the Partizan x Hvw-247 BC generation

Hybrid	Level of estimation	Recombinations (%)	Distances (cM)
$BC1dF_1$	Genotypes	0.1176 ± 0.065	13.41 ± 7.20
$BC1rF_1$	Genotypes	0.0800 ± 0.054	8.72 ± 6.40

DISCUSSION

The hordein composition in the cultivars studied was determined by two complex loci (*Hor1* and *Hor2*) which are closely linked. Complex composition of hordein loci in several linked genes is without introns, and is different in the length of the coding sequences *(*SAIANOVA *et al.* 1994). In the expression of the hordein composition in the hybrid progeny, the gene dosage effect is recorded and it is the consequence of the triploid constitution of the barley seed endosperm. The values of the distance between two major hordein loci varied in the investigated hybrid progeny and were in the interval of 7.82 – 13.41 cM. These results were similar to those of the other researchers (SHEWRY *et al.,* 1978*,* 1980b; BLAKE *et al.,* 1983; POMORTSEV *et al.,* 1985*;* SALL *et al.,* 1991). The experimental value of the distance was in agreement with the molecular maps of barley (KLEINHOFS *et al.,* 1993; LANGRIDGE *et al.,* 1995).

Additional aspects of using the hordein composition in marker assisted selection (MAS) and genotype identification could be mentioned:

1. Co-dominant inheritance of the hordein composition enables more precise calculation of distance between two loci than it is possible in the case of morphological markers (dominantly inherited). Estimation of the recombinant chromosomes percent enables to determine the distance between two loci in both parental genotypes. This advantage of co-dominant markers (biochemical and molecular) is the basis for their successful use in plant breeding.
2. Hordein composition could be efficient marker of traits whose loci are situated on the short arm of the *1H* chromosome. For example, seven *M1*

loci are responsible for resistance of barley to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) (WEI *et al.,* 1999). Presence or absence of some hordein groups or components could also indicate technological quality of the barley grain.

3. Acid PAGE is a cheap complementary technique, which provides precise identification of genotypes, and allows legal protection of breeder's rights.
4. Considering the large numbers of bands, each coded by one or more different genes in the cluster, barley storage protein is still a marker system which differentiates the largest number of genotypes in germ-plasm identification.

CONCLUSION

Hordeins, as the main storage proteins in barley grain, were analyzed by acid PAGE in order to determine their mode of inheritance in the F_1, F_2 and BC *Partizan* x *Hvw-247* hybrid progenies. Polypeptide bands originating from both parents were expressed in the F_1 generation and allowed scoring of gene dosage. Two allele forms of the multiple hordein loci created four phenotype classes in the F_2 and it was possible to distinguish heterozygotes due to differential expression of the dosage effect. Therefore, all genotypes in the F_2 generation were distinguishable, too. The observed ratio of the allele groups was not significantly different from the expected 1:1:1:1 ratio. No recombination was recorded within *B* and *C* hordein patterns. Scoring of hordein phenotypes in the F_2 grains was considerably easier by previous choice of marker bands. Gene dosage effect was investigated by comparative evaluation of the relative intensity of the marker bands, leading in easily and more reliably determination of genotypes. The distance between *Hor1* and *Hor2* loci in the studied population were 10.15 $\pm$3.58cM for male individuals and 12.71 $\pm$ 4.11 cM for female (maternal) individuals, while the distances of 13.41 $\pm$ 7.20, 8.72 $\pm$ 6.40 and 11.41 $\pm$ 2.71 cM were recorded at the BC1dF1, BC1rF1 and F_2 generation, respectively.

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REFERENCES

BAILEY, N.T.J. (1961): Introduction to the mathematical theory of linkage. Oxford University Press.

BLAKE, T.K., S.E. ULLRICH, and R.A. NILAN (1983): Mapping of the *Hor-3* Locus Encoding *D* Hordein in Barley. Theoretical and Applied Genetics, *63*: 367-371.

BURNOUF, T. and R. BOURIQUET (1983): Inheritance of Glutenin Subunits in F_1 Seeds of Reciprocal Crosses Between European Hexaploid Wheat Cultivars. Theoretical and Applied Genetics, *64*: 103–107.

BRIGGS, F. N. and P.F. KNOWLES (1977): Introduction to plant breeding. Reinhold Publishing corporation. Printed in the USA. pp. 426.

CIFUENTES, A. (2006): Recent advances in the application of capillary electromigration methods for food analysis. Electrophoresis, *27* (1): 283-303.

DOLL, H. and A.H.D. BROWN (1979): Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*H. vulgare*) barley. Can. J. Genet. Cytol. *21*: 391–404.

DOLNIK, V. (2006): Capillary electrophoresis of proteins 2003-2005. Electrophoresis, *27* (1): 126-141.

DONG, K., C.Y. HAO, A.L. WANG, M.H. CAI, and Y.M. YAN (2009): Characterization of *HMW* Glutenin Subunits in Bread and Tetraploid Wheats by Reversed-Phase High-Performance Liquid Chromatography. Cereal Research Communications, *37* (1): 65-73.

DRAPER, S. R. (1987): ISTA Variety Committee Report of the working group for Biochemical Tests for Cultivar Identification 1983-1986. Seed Sci. & Technol., *15*: 431-434.

DUFFUS, C.M. and M.P. COCHRANE (1992): Grain structure and composition. In: „Barley: genetics, biochemistry, molecular biology and Biotechnology" (P.R. Shewry, ed.), CAB International, Wallingford, pp. 291-317.

GARCIA-VILLALBA, R., S. CORTACERO-RAMIREZ, A. SEGURA-CARRETERO, and A. FERNANDEZ-GUTIERREZ (2006) Free-zone capillary electrophoresis analysis of hordein patterns at different stages of barley malting. Journal of Agricultural and Food Chemistry, *54* (18): 6713-6718.

HANSEN, M., M. LANGE, C. FRIIS, G. DIONISIO, P.B. HOLM, and E. VINCZE (2007) Antisense-mediated suppression of *C*-hordein biosynthesis in the barley grain results in correlated changes in the transcriptome, protein profile, and amino acid composition. Journal of Experimental Botany, *58* (14): 3987-3995.

HOSSAIN, M.A. and D.H.B. SPARROW (1991): Resistance to powdery mildew (*Erysiphe graminis* f.sp. *hordei*) in the barley cultivar *Galleon*. II. Chromosomal location and linkage with hordein protein genes. Euphytica, Vol. *52* (1): 11-17.

KANAZIN, V., E. ANANIEV, and T. BLAKE (1993): Variability among members of the *Hor 2* multigene family. Genome, *36*: 397-403.

KLEINHOFS, A., A. KILIAN, M.A. SAGHAI MAROOF, R.M. BIYASHEV, P. HAYES, F.Q. CHEN, N. LAPITAN, A. FENWIK, T.K. BLAKE, E. ANANIEV, L. DAHLEN, D KUDRNA, J. BOLLINGER, S.J. KNAPP, B. LIU, M. HEUN, J.D. FRANCKOWIAK, D. HOFFMAN, R. SKADSEN and B.J. STEFFENSON (1993): A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. TAG, *86*: 705-712.

KONAREV, V.G. (1996): Molecular biological aspects of applied botany, genetics and plant breeding. In: Theoretical basis of plant breeding (Ed Konarev, V.G.). Volume 1. VIR, Sankt-Petersburg, p. 227.

KUMAMARU, T., H. SATOH, T. OMURA and M. OGAWA (1989): Mutants for rice storage proteins. IV. Maternally inherited mutants for storage proteins of protein bodies in the starchy endosperm. Heredity, *64*: 9-15.

LANDGRIDGE, P., A. KARAKOUSIS, N. COLLINS, J. KRETSCHMER and S. MANNING (1995): A consensus linkage map of barley. Molecular breeding *1*: 389-395.

MARCHYLO, B. A. (1987): Barley cultivar identification by *SDS* gradient page analysis of hordein. Can. J. Plant Sci., *67*: 927–944.

MOLINA-CANO, J.L., J.P. POLO, A. SOPENA, J. VOLTAS, A.M. PEREZ-VENDREL, and I. ROMAGOSA (2001): Relationships between barley hordeins and malting quality in a mutant of cv. *Triumph*. I. Genotype by environment interaction of hordein content. Journal of Cereal Science, *34* (3): 285-294.

PEROVIC D., Y. YAN, S. PRODANOVIC, M. VRACAREVIC, and D. ZORIC (1998): Characterization of spring barley cultivars by hordein seed storage protein analysis. Rachis, ICARDA, *17* (1-2): 1-3.

PEROVIC, D., S. PRODANOVIC, Y. YUEMING, G. SURLAN-MOMIROVIC, M. VRACAREVIC, M. MILOVANOVIC, D. ZORIC, , and D. SMILDE, (2000): Hordein gene dose effects in triploid endosperm allow full classification of F_2 genotypes. Proceedings of the 8th International Barley Genetics Symposium Vol. III, pp. 204-206.

POMORTSEV A.A., V.P. NETSVETAEV, and A.A. SOZINOV (1985): Polymorphism of cultured barley (*Hordeum vulgare*) for hordeins. Genetika. *21* (4): 629-639.

POMORTSEV, A.A., S.P. MARTYNOV, and E.V. LYALINA (2007): Hordein locus polymorphism of cultivated barley (*Hordeum vulgare* L.) in Turkey. Russian Journal of Genetics, *43* (11): 1294-1300.

RADOVIC, D. (1995): Geneticka analiza rezervnih i funkcionalnih proteina jecma. Magistarska teza, Poljoprivredni fakultet, Novi Sad.

RAHMAN, S., P.R. SHEWRY, B.G. FORDE, M. KREIS and B.J. MIFLIN (1983): Nutritional control of storage proteins synthesis in developing grain of barley (*Hordeum vulgare* L.), Planta, *159*: 366-372.

SAIANOVA, O.V., S.L. MEHEDOV, L.G. ZELNIN, T.A. HOHLOV and V. ANANIEV (1993): Nucleotide organization of the *C* hordein genes from barley. Genetika, *29* (7): 1070-1079.

SAIANOVA, O.V., S.L. MEHEDOV, E.M. BURKITBAEV, N.I. SHCHIKOVA and E.V. ANANIEV (1994): Structural organization of *C*-hordein genes from barley. Genetika, *30* (6): 749-755.

SALL, T. (1990): Genetic control of recombination in barley. II. Variation in linkage between marker genes. Hereditas, *112*: 171-178.

SALL, T. (1991): Genetic control of recombination in barley. III. Recombination between the hordein loci in three different genotypes. Hereditas, 115: 13-16.

SHEWRY, P.R., H.M. PRATT, R.A. FINCH and B.J. MIFLIN (1978): Genetic Anaylisis of Hordein Polypeptides from Single Seeds of Barley. Heredity *40*: 463-466.

SHEWRY, P.R., A. J. FAULKS, S. PARMAR and B. J. MIFLIN (1980)a: Hordein polypeptide pattern in relation to malting quality and the varietal identification of malted barley grain. J. Inst. Brew., *86*: 138-141.

SHEWRY, P.R., A. J. FAULKS, R.A. PICKERING, I.T. JONES, R.A. FINCH and B.J. MIFLIN (1980)b: The Genetic Analysis of Barley Storage Protein. Heredity *44*: 383-389.

SHEWRY, P.R., E. J.-L. LEW and D. KASARDA (1981): Structural homology of storage proteins coded by the *Hor-1* locus of barley (*Hordeum vulgare* L.). Planta, *153*: 246-253.

SHEWRY, P.R. (1992): Barley seed proteins. In: Barley chemistry and technology, (J. MacGregor, R. Bhatty, eds.) AACC St. Paul Minnesota, USA, pp. 131-197.

SHEWRY, P.R., A.S. TATHAM, and N.G. HALFORD (2001): Nutritional control of storage protein synthesis in developing grain of wheat and barley. Plant Growth Regulation, *34* (1): 105-111.

SIMIC, G., R. SUDAR, A. LALIC, Z. JURKOVIC, D. HORVAT, and D. BABIC (2007): Relationship between hordein proteins and malt quality in barley cultivars grown in Croatia. Cereal Research Communications, *35* (3): 1487-1496.

SOAVE, C., R. REGGIANI, N.D. FONZO and F. SALAMINI (1981): Clustering of genes for 20 kD zein subunits in the short arm of maize chromosome *7*. Genetics, 97: 363-377.

SOZINOV, A. A., V. P. NETSVETAEV, E. M. GRIGORYAN and I. S. OBRAZTSOV (1978): Mapping of *Hrd* locuses in barley (*Hordeum vulgare* L. Emed.Vav. et Bacht.). Genetika (Moskow) *14* (9): 1610-1619.

VAPA, LJ. (1985): Nasledjivanje Glijadina kod Mutantnih i *Lr* Linija Psenice. Genetika, *17* (2): 91-96.

WEI, F., K.G. WERNER, S.M. MORROLL, J. KURTH, L. MAO, R. WING, D. LEISTER, P.S. LEFERT and R. WISE (1999): The *Mla* (powdery Mildew) Resistance Cluster is Associated with Three *NBS-LRR* gene Families and Suppressed within a 240-kb DNA Interval on Chromosome *5S* (*1HS*) of Barley. Genetics, *153*: 1929-1948.

WHITE, J. and R.J. COOKE (1992): A standard classification system for identification of barley varietis by electrophoresis. Seed Sci. & Technol., *20*: 663-676.

YAN, Y.M., Y. JIANG, J.Z.YU, M.H. CAI, Y.K. HU, and D. PEROVIC (2003): Characterization of seed hordeins and varietal identification in three barley species by high-performance capillary electrophoresis. Cereal Research Communications, *31* (3-4): 323-330.

YAN, Y. (1996): Nasleđivanje komponenata glijadina u zrnu F_1, F_2 i BC generacija hibrida pšenice. Doktorska disertacija, Poljoprivredni fakultet Zemun, Beograd, p. 163.

EFEKTI DOZE GENA KOD HORDEINA U TRIPLOIDNOM ENDOSPERMU JEČMA (*Hordeum vulgare* L.)

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I z v o d

Prisustvo dva majčinska seta hromozoma u triploidnom endospermu zrna ješma omogućuje razlikovanje hordeinskih traka u elektroforegramu koje potiču od majke i oca: majčinske komponente su jače ispoljene usled dejstva efekta doze gena. U F_1 generaciji vidljive su razlike kod recipročnog ukrstanja, a u F_2 generaciji je moguce razlikovati svih 16 teoretski očekivanih klasa za par polimorfnih lokusa. Ova potpuna klasifikacija redak je fenomen u genetičkim studijama te zbog toga omogućuje precizniju procenu rekombinacija. Dve grupe hordeinskih gena, *Hor1* i *Hor2* koji kodiraju *C* I *B* hordeine, su analizirane kod hibrida nastalih ukrštanjem dve ozime sorte ječma *Partizan* i *HWV-247*. Separacija hordeina je izvršena primenom kisele poliakrilamidne gel elektriforeze pri pH 3.2 (A-PAGE). Set najinformativnijih hordeinskih trajka za *B* i *C* hordeine je odabran na osnovu sledećih kriterijuma: (1) prisustvo ili odsustvo trake kod roditelja i (2) jačina signala koja omogućava ocenu efekta doze gena. Prosečna genetička distance između *Hor1* i *Hor2* lokusa bila je 11 cM. Genetičke distance kod roditeljskih komponenti nisu se statistički značajno razlikovale što sugeriše sličnu distribuciju rekombinacija u obe mejoze.

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