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MOLECULAR SCREENING OF DOMESTIC GERMPLASM FOR ALLELIC VARIANTS AT THE DWARFING GENE *Rht8* LOCUS IN WHEAT

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In last 7 years, researches revealed the presence of 16 allelic variants at the *Xgwm 261* locus with 165, 174, 180, 192, 194, 196, 197, 198, 200, 201, 202, 204, 205, 207, 210 and 215 base pairs, while nearly 90% of the worldwide wheat germplasm carry one of the alleles with 165bp, 174bp or 192bp. The results of microsatellite screening of 269 wheat varieties and advanced lines from Serbia and Montenegro showed clear selection pressure for the three main fragments at *Rht8* locus, but also trend to expand allelic variability in the locus (in the most recent varieties and advanced lines) avoiding «adaptive uniformity» of the elite germplasm. The presence of 192bp allele as diagnostic of *Rht8* has been confirmed as quite common in wheat genotypes from Serbia and Montenegro, but not at the rate it has been determined in earlier researches. Out of 269 registered wheat varieties and advanced lines 73.6% carry 192bp allele, 14.9% are with 174bp allele, 7% carry 165 fragment and 4.56% novel fragments around 200bp in length. This research revealed that from the beginning of the 1990s wheat breeding objectives in Serbia and Montenegro were

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partially focused towards novel alleles (different from 192bp) at *Xgwm261* locus. Recently registered varieties from Novi Sad have either the 192bp fragment (Cipovka, Simfonija, Balada, Arija, Rapsodija, Helena, Diva, Vila, Astra), 174bp fragment (Sofija, Dragana, Italija, Francuska, Oda) or fragments around 200bp (Sonata, Kantata, Jefimija). In addition, two very promising advanced lines (NS 260/02 and NS 2-4629/1) and some excellent advanced lines frequently used as parents in hybridization (NS 20/96, NS 23/94, NS 85/97 and L152/89) also carry allele around 200bp in size.

Key words: dwarfing genes, microsatellite, Ppd-D1 gene, *Rht8* gene , wheat

INTRODUCTION

In the vast majority of modern wheat varieties height reduction is achieved by the incorporation of one or sometimes both of a pair of semi dwarfing GA-insensitive genes *Rht-B1b* and *Rht-D1b* (WORLAND *et al.*, 2001). Both originate from the old Japanese variety Norin 10 and do not appear to promote the same agronomic benefits of associating a reduction in height with increases in fertility and yield under southern European environment as they do in a majority of wheat growing areas worlwide (WORLAND *et al.*, 1990; WORLAND *et al.*, 1998a).

Breeders from Mediterranean and similar climatic regions had therefore to seek alternative genes to improve plant performance. Pedigree analysis of semi-dwarf wheats from this region shows that dwarfing effects can be traced via Italian varieties (developed by Italian breeders like STRAMPELLI and ORLANDI in early 1930s) to old Japanese variety Akakomughi. *Rht8* acts as a weak allele which reduces plant height by replacing the normal strong height promoting allele with a weaker one, leading to wheat height reduction by approximately 7-8cm in England and former Yugoslavia, with no significant pleiotropic effect on other agronomic characters except for a slight increase in spikelet fertility (WORLAND *et al.*, 1998a; KORZUN *et al.*, 1998).

For many decades, study of the presence and genetic effects of *Rht8* was hampered by difficulties to recognise it in genotypes. In 1998, a tight linkage was found between the wheat microsatellite marker *WMS 261* (marker *Xgwm261*) 0.6 cM distal to dwarfing gene *Rht8* on the short arm of chromosome 2D (KORZUN *et al*, 1998). Researches conducted over last 7 years revealed the presence of 16 allelic variants at the *Xgwm 261* locus with 165, 174, 180, 192, 194, 196, 197, 198, 200, 201, 202, 204, 205, 207, 210 and 215 base pairs. Nearly 90% of the wheat genotypes (varieties) of worldwide origin carried one of the alleles with either 165bp, 174bp or 192bp (KORZUN *et al*, 1998; WORLAND *et al*, 2001; AHMAD and SORRELLS, 2002; SCHMIDT *et al*, 2004). Until recently, varietal screening failed to detect cultivars with *Rht8* outside the southern European and the former SSSR breeding programs. But recent molecular evaluation of Chinese, Japanese and U.S

wheats has revealed the presence of *Rht8* gene in 64% of Chinese, 68% of Japanese and 8% of U.S. accessions (WORLAND *et al.*, 2001; BAI *et al*, 2004).

This paper reports the results on microsatellite screening of 269 wheat varieties and advanced lines from Serbia and Montenegro for allelic variants at the *Rht8* dwarfing gene locus in wheat.

MATERIAL AND METHODS

A diverse array of 269 wheat varieties and advanced lines listed in Table 1. were chosen for microsatellite screening. The seed stocks were obtained from Institute of Field and Vegetable Crops germplasm collection.

The method used for determining the allelic variant at the *Xgwm261* microsatellite locus are fully described by KORZUN *et al.* (1998). For molecular analysis, DNA was extracted from five grains using procedures described by PLASCHKE *et. al* (1995). Polymerase chain reactions (PCR) were perfomed in a volume of 25 ml in Eppendorf Gradient thermocycler. The reaction mixture contained 400 n*M* of each primer, 160 m*M* of each deoxynucleotide, 1,5 m*M* $MgCl_2$, 1 U *Taq* polymerase, 1 X PCR pufer (Eppendorf, 10 X Taq Buffer with 15 mM Mg^{2+}) and 150-200 ng template DNA. The 45 cycles were performed with 1 min at 94°C, 1 min at 55°C, 2 min at 72°C and the final extension of 10 min at 72°C. From each PCR amplification 5 ml was mixed with the 2 ml loading buffer (formamide-containing dye) and after denaturation for 2 min at 94°C, 5 ml of mix were loaded onto sequencing gels of 6% polyacrylamid and 8% urea (0.4 mm thickness, 40 cm height and 33 cm width). Electrophoresis was performed at constant 1900V, 50mA, 80W and maintaining a gel surface temperature of 45-50°C. The samples were screened by silver staining as described by Sanguinetti et al.(1994) and DNA fragments were scored by comparation with DNA standard (PCR 100 bp Low Ladder, Sigma).

RESULTS AND DISSCUSION

Microsatellite analysis of 269 domestic wheat cultivars has revealed that 198 genotypes carry 192bp allelic variant at the *Xgwm261* locus, followed by 40 genotypes with 174bp, 19 with 165 fragment and 12 with fragments of different sizes which could not be joined to either one of the three most frequent alleles (Table 1).

The previously determined alleles (WORLAND *et al.*, 1998a, 1998b; AHMAD and SORRELLS, 2002; PESTSOVA and RÖDER, 2002) have been confirmed for the following subset of genotypes from this research: 192bp fragment - Biserka, Duga, Dugoklasa, Jarka, Jugoslavija, Kolubara, Košava, NSR 2, Partizanka, Partizanka niska, Raduša, Rana niska, Sava and Sremica; 174bp fragment - Balkan; 165bp fragment - Dugoklasa.

The presence of the 192bp allele as diagnostic of *Rht8* has been confirmed to be quite common in the wheat genotypes from Serbia and

Montenegro (198 genotypes), while the presence of 174bp, 165bp and fragments around 200bp have also been detected but at a much lower rate compared to 192bp allele (Table 1). From these results it can be concluded that breeders from Serbia and Montenegro are looking for certain allelic variability at *Rht8* locus avoiding «adaptive uniformity» of the elite germplasm. This is understandable, since in countries like SCG, which are geographically and/or climatically diverse, a range of environmental effects are present in light of global climate change, so wheat breeders have to maintain broader germplasm variability in order to make their varieties better adapted to such environment.

Since the 192bp allele corresponds to a height-reducing phenotype of *Rht8* and 174bp allele correlates with a neutral phenotype (WORLAND *et al.*, 1998b), it is clear, at least for the 40 genotypes from this group, that wheat breeders from SCG tend to increase plant height. The preference for taller plants may be caused by drier growing conditions, a shorter growing season or other environmental factors as stated by AHMAD and SORRELLS (2002). Additional decrease in plant height by selecting for the 192bp fragment (and, if the linkage is not broken, for *Ppd-D1* gene) together with *Rht-B1b, Rht-D1b* or other dwarfing genes could become a disadvantage since it may produce a phenotype too short and too early to achieve adequate yield in SCG wheat growing areas.

Out of the 269 examined wheat genotypes, 19 carry the 165bp allelic variant at *Xgwm261* locus. Majority of the genotypes from this group are either spring or winter wheats of which at least one parent was from Mexico, Australia or China (Table 1). It is well known that excelent results have been obtained in wheat breeding programmes worlwide by incorporation of «spring» alleles into «winter» wheat background resulting in increased yield potential. In this respect, the winter genotypes from this group deserves additional atention from SCG wheat breeders.

The molecular screening has shown the presence of certain selection presure for specific fragments, but also suggesting there is a recent trend in wheat breeding programmes in Serbia and Montenegro of introduction of novel *Xgwm261* locus alleles (beside 3 main ones) into elite germplasm and varieties. Recently registered varieties from Novi Sad have either the 192bp fragment (Cipovka, Simfonija, Balada, Arija, Rapsodija, Helena, Diva, Vila, Astra), 174bp fragment (Sofija, Dragana, Italija, Francuska, Oda) or fragments around 200bp (Sonata, Kantata, Jefimija). In addition, two very promising advanced lines (NS 260/02 and NS 2-4629/1) and some excellent advanced lines frequently used as parents in hybridization (NS 20/96, NS 23/94, NS 85/97 and L152/89) also carry allele around 200bp in size. From these data it can be concluded that phenotypic selection for improved varieties in SCG recently implies promotion of novel fragments at Wgwm261 locus. The reasons for this are not yet clear, but we presume the research which is on going now will revealed the proper answers.

Table 1. - The presence of allelic variants at Rht8 dwarfing gene locus in varieties and advanced lines from SCG

192 bp (*Rht 8*)	NS 0.683	NS 69/97	174 bp	165 bp
	NS 0.694	NS 69/98		
Alfa	NS 0.733	NS 7/93	Balkan	Delta
Anastasija	NS 1/92	NS 71/92	Dragana	Dugoklasa
Arija	NS 1/93	NS 73/97	Evropa	Kraljevica
Astra	NS 1/98	NS 732	Evropa 90	L-1/91
Bajka	NS 112/92	NS 736	Francuska	L-154/89
Bečejka	NS 114/98	NS 75/01	Italija	L-183/90
Beograđanka	NS 116/95	NS 77/95	Kratka	Nevesinjka
Biserka	NS 118/01	NS 79/00	Novosadska 100	NS 10/94
Bujna	NS 12/77	NS 79/90	Novosadska 6001	NS 119/95
Balada	NS 124/95	NS 82/00	Novosadska 6389	NS 32/99
Cipovka	NS 125/98	NS 90/96	NS 109/96	NS 39/93
Danica	NS 13/93	NS 900	NS 156/98	NS 39/97
Dejana	NS 133/96	NS 92/97	NS 158/01	NS 54-52
Dična	NS 135/90	NS 96/97	NS 18/00 alb	NS 56/90
Diva	NS 14-33	NS 974/1	NS 20/00	NS 74/95
Dina	NS 152/98	NS2-2874/2	NS 34/91	NS 76/95
Draga	NS 152/01	NS2-3218F	NS 36/91	NS 83/92
Duga	NS 163/98	NS2-3827/1	NS 36/94	NSJP 471
Fortuna	NS 17/93	NS2-4523/3	NS 36/98	Venera
Helena	NS 171/96	NS2-4558	NS 37/90	
Inđija 89	NS 173/98	NS3-2503	NS 40/96	~ 200 bp
Ivanka	NS 18/93	NS3-2062/1	NS 42/96	
Jarebica	NS 197/98	NSP 16	NS 44/95	NS 20/96
Jarka	NS 22/92	NSP 187	NS 46/98	NS 23/94
Jugoslavija	NS 22/93	NSP 192	NS 56/97	NS 260/02
KG 100	NS 25/93	NSP 52	NS 625	Jefimija
KG 56	NS 261/02	NSP 54	NS 66/92	Kantata
Kolubara	NS 29/94	NSR 2	NS 67/01	L-152/89
Kompas	NS 3/00	NSR 5	NS 68/01	NS 602
Košava	NS 3/90	Panonija	NS 76/01	NS 85/97
Kosovka	NS 30/00	Partizanka	NS 8/95	NS2-4629/1
Košuta	NS 30/95	Partizanka niska	NS 81/01	NSP 40
Kraljevica	NS 33/90	PKB Krupna	NS 9/93	Sonata
Kremna	NS 35/00	Pobeda	NS 90/92	Maksima
L-165/94	NS 38/00	Prima	NS 97/95	
L-1A/91	NS 38/93	Proteinka	NSP 199	
L-63/89	NS 322	Prva	NSP 88	
L-64/89	NS 4/93	Raduša	Oda	
L-69/92	NS 40/00	Rana niska	Pesma	
L-74/92	NS 40/94	Rapsodija	Sofija	
Lasta	NS 42/00	Renesansa		
Lepenica	NS 45/00	Rodna		
Lira	NS 46/90	Rusija		
Ljiljana	NS 46/96	Sara		
Majevica	NS 46/98	Sava		
Matica	NS 48/93	Simfonija		
Milena	NS 5/92	Simonida		
Milica	NS 51-11	Slavija		
Mina	NS 54/01	Sloga		
Nizija	NS 55-25	Sonja		
Novosadska Crvena	NS 55-30	Srećа		
Novosadska 5804	NS 559	Sremica		
Novosadska 6002	NS 56/91	Sremka		
Novosadska 6238	NS 56-11	Stamena		
Novosadska 6439	NS 57/00	Stepa		
Novosadska 6864	NS 57/92	Sutjeska		
Nova Jadranka	NS 58-97	Takovčanka		
NS 0.1079	NS 59/91	Tera		
NS 0.1080	NS 60/01	Tiha		
NS 0.1081	NS 603	Viktorija		
NS 0.1084	NS 63-24	Vila		
NS 0.1085	NS 64/91	Zlatka		
NS 0.1202	NS 68/97	Zvezda		
NS 0.32	NS 69/93			
NS 0.58	NS 69/96			

Finally, in the majority of the papers published in last 30 years in general and 7 years in particular, it was suggested that besides plant height *Rht8* gene is effecting no other agronomic trait except slight increase in spikelet fertility (KORZUN *et al*, 1998), so we can presume the positive effect on many agronomicaly important traits in SCG wheat varieties and advanced lines is actualy obtained by *Ppd-D1* gene. Since, *Ppd-D1* gene is reducing wheat height as a direct pleiotropic effect of earlier flowering (shorter life cycle) it seems that different agronomic performance of the germplasm with different allelic variants at *Rht8* locus in this research could be rather joint effect of *Ppd-D1* and *Rht8* than the effect of *Rht8* gene alone. Previous findings presumed that in majority of the SCG and Southern European wheats the linkage between *Rht8* and *Ppd-D1* has been preserved but findings of novel fragments from this research are very strong indication that the linkage has been broken.

Unfortunately, in this moment, we are unable to evaluate on molecular basis 2DS wheat chromosome segment which carry either *Rht8* and/or *Ppd-D1* gene, and than look for possible association of particular alleles to phenotypic performance. But in regard to present interest of wheat scientiests on Ppd genes issues it is very likely that in very near future we will be able to disect the particular segment harbouring both *Rht8* and Ppd-D1 gene and to gain additional knowledge in this respect.

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MOLEKULARNA ANALIZA ALELNIH VARIJANTI U LOKUSU *Rht8* GENA DOMAĆE GERMPLAZME PŠENICE

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I z v o d

U poslednjih 7 godina istraživači su utvrdili postojanje 16 alelnih varijanti u *Xgwm 261* lokusu i to sa: 165, 174, 180, 192, 194, 196, 197, 198, 200, 201, 202, 204, 205, 207, 210 i 215 baznih parova, pri čemu skoro 90% svetske germplazme pšenice poseduje jedan od alela sa 165bp, 174bp ili 192bp. Rezultati analize sa mikrosatelitima 269 sorti i linija pšenice poreklom iz Srbije i Crne Gore (SCG) pokazuju snažan selekcioni pritisak za tri glavne alelne varijante u lokusu *Rht8* gena, ali i trend proširenja alelne varijabilnosti u ovom lokusu (kod najnovijih sorti i linija) izbegavajući «adaptivnu uniformnost» elitne germplazme. Prisusutvo alela sa 192bp allele koji je "dijagnostički" za *Rht8* gen je potvrđeno kao vrlo frekventno kod genotipova pšenice poreklom iz SCG, ali ne u frekvenciji koja je ranijim istraživanjima utvrđena. Od 269 ispitivanih sorti i linija 73.6% ima 192bp alel, 14.9% je sa 174bp alelom, 7% poseduje fragment od 165bp dok 4.56% sorti i linija sadrži nove fragmente veličine oko 200bp. Ovim istraživanjem je utvrđeno da su od početka 1990-tih godina ciljevi oplemenjivanja pšenice u SCG delimično orijentisani ka introdukciji novih alela (različitih od 192bp) u lokusu prajmera *Xgwm261*. Najnovije priznate sorte iz Novog sada imaju ili fragmente sa 192bp (Cipovka, Simfonija, Balada, Arija, Rapsodija, Helena, Diva, Vila, Astra), 174bp (Sofija, Dragana, Italija, Francuska, Oda) ili oko 200bp (Sonata, Kantata, Jefimija). Nadalje, dve veoma perspektivne linije (NS 260/02 and NS 2-4629/1) i nekoliko odličnih i često korišćenih linija za ukrštanja (NS 20/96, NS 23/94, NS 85/97 and L152/89) takođe poseduju alele veličine oko 200bp.

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