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ALLELIC VARIABILITY OF CROATIAN WHEAT CULTIVARS AT THE MICROSATELLITE LOCUS XGWM261

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SUMMARY

The plant height of wheat is an important quantitative trait, controlled by several genes with strong effect. However, in worldwide wheat breeding, only several of those genes have been used. Rht8 (Reduced Height Gene) is especially important in agro-climatic conditions of South-East Europe. Because of its close linkage with dwarfing gene Rht8, microsatellite marker gwm261 has been accepted as the diagnostic molecular marker for gene Rht8. In this study, allelic variability at the locus Xgwm261 for 122 Croatian and foreign wheat cultivars by means of microsatellite marker gwm261 was determined. A 192 base pairs allele at the locus Xgwm261 was found for 84 Croatian cultivars. The genetic heritage of Croatian cultivars at the locus Xgwm261 is the consequence of new parental components usage, carriers of short plant and early maturity attributes and the consequent selection of progeny with these traits during breeding process. The results of this research will be helpful in characterization of domestic wheat cultivars, as well as in more accurate selection of parents for hybridization purposes.

Key-words: wheat, Rht8, allelic variability, locus Xgwm261

INTRODUCTION

One of the most important characteristics of breeding modern, high-yielding, hexaploid bread wheat cultivars is the utilization of reduced height genes (Rht) or dwarfing genes, which reduces plant height and simultaneously increases adaptability and grain yield potential (Worland et al., 2001).

The most frequently used height reducing genes with strong effect on plant height shortening are Rht-B1b (Rht1) and Rht-D1b (Rht2), derived from the Japanese cultivar Norin 10. Although the benefits of Rht-B1b and Rht-D1b are apparent in most environments and were the basis of Borlaug's Green revolution in wheat breeding, they fail to combine height reductions with grain yield increases in southern European environments (Worland et al., 2001). Cultivars grown in southern Europe also possess short semi-dwarf stem, if controlled by dwarfing genes derived from Japanese cultivars Akakomugi, and in lower scale from cultivar Saitama 27 (Rht-B1d; Rht1) (Worland and Petrović 1988, Jošt and Jošt, 1989, Worland et al., 1998, Ganeva et al., 2005). In cultivar Akakomugi genetic heritage, gene Rht8 and gene Ppd1 for insensitivity to photoperiod are especially important. Because of its tight linkage, gene Rht8 frequently comes together with gene Ppd1, which reduces plant life cycle and has pleiotropic effect on height shortening (Korzun et al., 1998, Worland et al., 2001). Along with gibberellic acid tests in determination of dwarfing genes presence, the development of different molecular markers technique has allowed their direct detection at DNA level. The microsatellite locus Xgwm261 is tightly linked with locus for Rht8 gene at the chromosome region 2DS (distant only 0.6 cM), thus many authors (Korzun et al., 1998, Röeder et al., 1998, Worland et al., 2001, Ganeva et al., 2005, Zheleva et al.,

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2006, Sip et al., 2010) pointed out that the microsatellite marker gwm261 with 192-bp (base pairs) allele, can be considered as diagnostic for gene Rht8 of hexaploid wheat. The objective of this study was to determine allelic variability at the locus Xgwm261 of Croatian and some foreign wheat cultivars by means of microsatellite marker gwm261.

MATERIAL AND METHODS

A total of 122 hexaploid bread wheat cultivars (*Triticum aestivum* L.), recognized or released in production during the period from 1905 to 2008, were used in the study. Ninety-eight of them were created in Croatia, while 24 were foreign cultivars. Among Croatian cultivars, 74 were created at the Agricultural Institute Osijek (PIO), 17 at the Bc Institute for Breeding and Production of Field Crops d.d., Zagreb (Bc), three at the Faculty of Agronomy of Zagreb University (AFZ), two at the breeding company Jošt sjeme-istraživanja d.o.o., Križevci (JS), one at the Agricultural Centre of Croatia (PCH) and one was introduced by prof. G. Bohutynski (Bohuty.). The experimental part of the study was conducted at the Agriculture and Agri-Food Canada Cereal Research Centre in Winnipeg. Ten seeds of each cultivar were germinated in growth chamber and leaf tissue was harvested from 15 days old seedlings. A total of 1220 separate samples were collected and lyophilized. Extraction of genomic DNA was performed according to Pallotta et al. (2003). DNA was quantified by fluorimetry using Hoechst 33258 stain. Ten genomic DNA samples of each cultivar were genotyped with 12 robust microsatellite markers according to Somers et al. (2005), in order to investigate genetic uniformity within each cultivar. This pre-analysis enabled DNA selection of the prevalent biotype for each cultivar. Afterwards a final analysis was conducted with microsatellite marker gwm261. Table 1 shows the characteristics of microsatellite gwm261 primers pair (Korzun et al., 1998).

Table 1. Characteristics of microsatellite marker gwm261 primers pair

Tablica 1. Svojstva para početnica mikrosatelitnog biljega gwm261

Marker *Biljeg*	Chromosome location *Kromosomska lokacija*	Primer sequence (5'→3') *Sekvenca početnice (5'→3')*		Motif *Motiv*	T_a(ºC)
		Forward/*Početnica 1*	Reverse/*Početnica 2*		
gwm261	2DS	CTCCCTGTACGCCTAAGGC	CTCGCGCTACTAGCCATTG	$(CT)_{21}$	55

T_a (ºC); primer annealing temperature

T_a (ºC); temperatura nalijeganja početnice

Amplification conditions were as described in Somers et al. (2004) and McCartney et al. (2004). Reaction mix contained (1) amplification components (Applied Biosystems, Foster City, CA, USA), (2) gwm261 primers pair (Invitrogen, Carlsbad, CA, USA) and (3) genomic DNA (Table 2).

Table 2. Concentration and volume of polimerase chain reaction (PCR) mix components

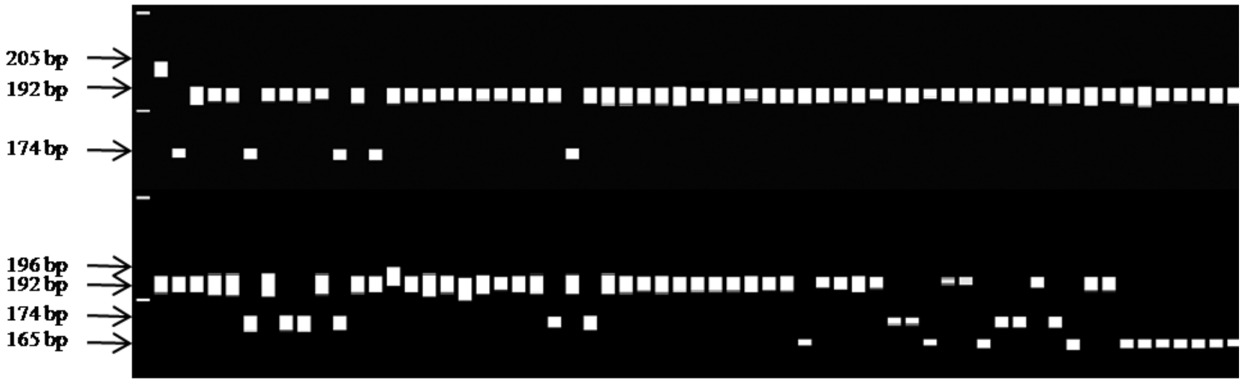
Tablica 2. Koncentracija i volumen sastavnica smjese za lančanu reakciju polimerazom (PCR)

Reaction mix/ *Reakcijska smjesa*		Concentration/*Koncentracija*		Volume per amplification (μl) *Volumen po amplifikaciji (μl)*
		Stock/*Ishodišna*	Working/*Radna*	
1	PCR buffer/*PCR pufer*	10 X	1 X	1.00
	$MgCl_2$	25 mM	1.50 mM	0.60
	dNTP	10 mM	0.20 mM	0.80
	M13 primer/*M13 početnica*	10 pmol μl^{-1}	1.80 pmol	0.18
	Taq polymerase/*Taq polimeraza*	20 U μl^{-1}	0.50 U	0.025
	Demineraliz.H_2O/*Demineraliz.H_2O*	-	-	2.00
2	Forward primer/*Početnica 1*	1 pmol μl^{-1}	0.20 pmol	0.20
	Reverse primer/*Početnica 2*	10 pmol μl^{-1}	2 pmol	0.20
3	Genomic DNA/*Genomska DNA*	> 50 ng μl^{-1}	10 ng μl^{-1}	5.00 μl
				∑= 10.005 μl

Polymerase chain reaction was conducted using MJ Research Dyad Disciple Thermocycler (Bio-Rad, Hercules, CA, USA). In order to enable discrimination among amplification products, M13 primer (CACGACGTTGTAAAACGAC) fluorescently labeled with 6-FAM, HEX, NED (Applied Biosystems, Foster City, CA, USA) was added to the 5' end of the forward primer during primer synthesis (Schuelke, 2000). Fluorescent capillary electrophoresis was performed using 16 capillary ABI Prism 3100 Genetic Analyzer and internal molecular weight standard Gene-scan 500-ROX (Applied Biosystems, Foster City, CA, SAD). Chromatograms collected by fluorescent capillary electrophoresis were converted to gel-like images, which were used for measuring microsatellite allele sizes using computer software Genographer (http://www.hordeum.oscs.montana.edu/genographer).

RESULTS AND DISCUSSION

After chromatogram conversion in gel-like images (Figure 1), allele sizes of amplification products for 122 wheat cultivars at locus Xgwm261 were determined and expressed in base pairs (bp) (Table 3).



bp-base pairs number/*bp - broj parova baza*

Figure 1. Allelic variability at locus Xgwm261 for 122 wheat cultivars; gel-like images presented in computer software Genographer

Slika 1. Alelna varijabilnost na lokusu Xgwm261 za 122 kultivara pšenice - slike nalik gelu prikazane u računalnom programu Genographer

The 192-bp allele at locus Xgwm261 was determined in 84 out of 98 Croatian cultivars, in Italian cultivars San Pastore, Libellula and Gemini, as well as in cultivars Bezostaja, Renan, Gobe, Othalom and Pesma. The main source of the 192-bp allele, diagnostic for gene Rht8, as well as the origin of the Ppd1 gene in Croatian cultivars, according to the pedigree data (not presented) can be traced to the Italian cultivars Ardito, Villa Gloria and Damiano Chiesa. The genes Rht8 and Ppd1 were introduced in those cultivars from the Japanese wheat Akakomugi in the 1920s by the Italian wheat breeder Nazareno Strampelli (Worland et al., 1998, Jošt and Cox, 1989, Jošt and Jošt, 1989). Additionally, the Russian cultivars Bezostaja, Kavkaz and Aurora can also be traced to Strampelli's cultivar Ardito via the Argentinian wheat Klein 33 (Worland et al., 1998).

Table 3. Allelic variability at locus Xgwm261 for 122 wheat cultivars

Tablica 3. Alelna varijabilnost na lokusu Xgwm261 za 122 kultivara pšenice

No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*	No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*	No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*
1	Zl.Dolina	Bc	192	42	Demetra	PIO	192	83	Tonka	PIO	192
2	Bistra	Bc	190	43	Fortuna	PIO	192	84	Vila	PIO	192
3	S. Zlatna	Bc	192	44	Podravina	PIO	192	85	Elvira	PIO	192
4	Sana	Bc	192	45	Danica	PIO	192	86	Suzana	PIO	192
5	Marija	Bc	192	46	Feniks	PIO	192	87	Osk. 236-01	PIO	192
6	Adriana	Bc	192	47	Neretva	PIO	192	88	Ficko	PIO	174
7	Rugvica	Bc	174	48	Ruža	PIO	192	89	Zlata	PIO	192
8	Tina	Bc	192	49	Snaša	PIO	192	90	Aida	PIO	174
9	Mihelca	Bc	174	50	Maja	PIO	192	91	Osk. 266-03	PIO	174
10	Zdenka	Bc	192	51	Inga	PIO	192	92	Pipi	PIO	192

No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*	No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*	No. *Br.*	Cultivar *Kultivar*	Origin *Podrijetlo*	Xgwm 261*
11	Liberta	Bc	192	52	Elza	PIO	192	93	Felix	PIO	174
12	Aura	Bc	192	53	Joza	PIO	192	94	Osk. 189-04	PIO	192
13	Lana	Bc	192	54	Manda	PIO	192	95	Osk. 244-04	PIO	192
14	Nina	Bc	192	55	Eva	PIO	192	96	Osk. 293-04	PIO	196
15	Prima	Bc	192	56	S. Žitarka	PIO	192	97	Ružica	PIO	192
16	BC Elvira	Bc	192	57	Barbara	PIO	192	98	Osk. 201-05	PIO	192
17	BC Antea	Bc	192	58	Monika	PIO	192	99	San Pastore	ITA	192
18	S. Prolific	Bohuty.	205	59	Kata	PIO	192	100	Libellula	ITA	192
19	Sivka	AFZ	192	60	Klara	PIO	192	101	Bezostaja	RUS	192
20	Kuna	AFZ	192	61	Sofija	PIO	192	102	Gemini	ITA	192
21	Magdalen	AFZ	192	62	Edita	PIO	192	103	Soissons	FRA.	174
22	Dukat	PCH	192	63	Golubica	PIO	192	104	Rialto	GB	174
23	Divana	JS	192	64	Jasna	PIO	192	105	Flori 2	HUN	165
24	Talia	JS	165	65	Julija	PIO	192	106	Othalom	HUN	192
25	U1	PIO	165	66	Martina	PIO	192	107	Gobe	HUN	192
26	Dubrava	PIO	192	67	Hana	PIO	192	108	Arina	SUI	165
27	Tena	PIO	192	68	Panonka	PIO	192	109	Justus	SUI	174
28	Os. Crven.	PIO	192	69	Ema	PIO	192	110	Ludwig	AUT	174
29	Osječka 20	PIO	165	70	Lucija	PIO	192	111	Renan	FRA	192
30	Osječanka	PIO	192	71	Panonija	PIO	192	112	Bussard	GER	174
31	Krušarka	PIO	192	72	Nevena	PIO	192	113	Victo	USA	165
32	Osječanka 2	PIO	192	73	Željka	PIO	192	114	Pesma	SRB	192
33	Nada	PIO	165	74	Teuta	PIO	192	115	Pobeda	SRB	165
34	Slavonija	PIO	192	75	Kiki	PIO	192	116	AC Barrie	CAN	165
35	Ratarka	PIO	165	76	Ševa	PIO	192	117	AC Elsa	CAN	165
36	Poljarka	PIO	192	77	Petra	PIO	192	118	AC Majestic	CAN	165
37	Žitarka	PIO	192	78	Senka	PIO	192	119	BW 346	CAN	165
38	Njivka	PIO	192	79	Zrnka	PIO	192	120	Grandin	USA	165
39	Ana	PIO	192	80	Alka	PIO	192	121	Thatcher	USA	165
40	Srpanjka	PIO	192	81	Janica	PIO	192	122	Marquis	CAN	165
41	Aljmašanka	PIO	192	82	Kleopatra	PIO	192	-	-	-	-

*Allele lengths at locus Xgwm261, expressed in base pairs number

**Alelne dužine na lokusu Xgwm261, izražene u broju parova baza*

Italian cultivars like Libellula and San Pastore, and Russian cultivars like Bezostaja and Kavkaz, later become major progenitors in Croatian wheat cultivars improvement. A high prevalence of the 192-bp allele at the locus Xgwm261 also was found in other southern European countries (Italy, Romania, Bulgaria, Hungary, Serbia), as well as in Chinese and Japanese cultivars (Worland et al., 1998 and 2001, Ganeva et al., 2005, Liu et al., 2005, Zheleva et al., 2006). According to Worland et al. (1998), low frequency of the Rht-B1b (Rht1) and Rht-D1b (Rht2) genes in relation to gene Rht8 in southern European countries is probably connected with the fact that under certain circumstances, where warm temperatures occur around the time of meiosis, interactions between these dwarfing genes and the environment can cause fertility reductions and loss of yield advantages. Many authors (Korzun et al., 1998, Worland et al., 1998 and 2001, Ahmad and Sorrells, 2002, Ganeva et al., 2005) pointed out that gene Rht8 reduces plant height by around eight centimeters, while gene Ppd1 for insensitivity to photoperiod additionally reduces height by around 10 centimeters. The height reduction associated with Ppd1 would be a pleiotropic effect of the gene accelerating ear emergence time and reducing the plants life cycle by about a week; Ppd1 influences many characters including a shortening of height due to a reduction in the number of vegetative primordia (Worland et al., 2001). These effects of genes Rht8 and Ppd1 together play a vital role in the increased adaptability of cultivars to high temperatures in southern Europe, which may occur either in the time of ear emergence or in late grain filling period. Martinić (1971, 1976) stated that absent or weak photoperiod reaction is a frequent characteristic of cultivars with wide adaptability in many successful wheat breeding programs worldwide, and that because of the prevalence of that trait, probably exist strong correlation with economically important characters, especially with grain yield. The selection of this highly desirable allele in breeding process, and consequently its highest frequency in investigated Croatian cultivars, caused low values of Polimorfic Information Content (PIC=0.266) and gene diversity (H_E=0.276) of marker gwm261 (data not shown). The importance of the genes Rht8 and Ppd1, as well as maybe of some other Rht genes, possibly is the most evident in the case of cultivar Srpanjka, the earliest and shortest Croatian cultivar. According to Agricultural Institute Osijek multi-seasonal comparative trials results on the average, the heading date of cultivar Srpanjka is the first of May, while plant height (to the ear base) is 64 centimeters. As one of the best yielding varieties, cultivar Srpanjka is prevalent in Croatian wheat production (around 30% of wheat acreage; http://zsr.hr). A 174-bp allele at the locus Xgwm261 was found for certain cultivars from north-western Europe (Rialto, Soissons, Bussard, Ludwig and Justus) and several Agricultural Institute Osijek cultivars (Ficko, Aida, Felix and Osk. 266-03), as well as for two cultivars of Bc Institute for Breeding and Production of Field Crops d.d., Zagreb (Mihelca and Rugvica). The origin of the 174-bp allele in cultivars Ficko, Aida and Osk. 266-03 genetics background is probably from cultivars Rialto and Soissons in their parental base. The 165-bp allele found in cultivars Talia, Osječka Šišulja (U1), Osječka 20, Nada and Ratarka, can be traced to ancestral basis partially originated from USA and Canada. Those findings are supported by the fact that all Canadian and USA cultivars included in this study had the 165-bp allele, being in accordance with previous results of Worland et al. (1998). Korzun et al. (1998) and Worland et al. (2001) in research about adaptive significance of different allelic variants at the locus Xgwm261 showed that in relation to 192-bp allele, alleles with 165 and 174-bp at the locus Xgwm261 have stronger impact on height promoting than height shortening and pointed out that these allelic variants often comes in the cases where cultivars already possess genes Rht-B1b and Rht-D1b which together with Rht8 and pleiotropic effect of Ppd1 could produce a phenotype too short. The complexity of the locus Xgwm261 with 165, 174 and 192-bp alleles is emphasized by the discovery of many rare or unique alleles (180, 184, 194, 195, 196, 197, 198, 200, 201, 202, 203, 204, 205, 210, 212, 215, 216 and 251-bp), reported in studies of Worland et al (1998, and 2001), Manifesto and Suarez (2002), Ahmad and Sorells (2002), Chebotar et al. (2001) and Liu et al. (2005). The unique 196-bp allele in Osk. 293-04 was probably derived from Argentinian parent, while the 190 bp allele found in cultivar Bistra can be related to the parental cultivar Zeka. The old cultivar Sirban Prolific, created before introduction of Rht genes either from cultivar Akakomugi, Norin 10 or Saitama 27, carries the uniqe 205-bp allele.

CONCLUSION

The 192-bp allele at the locus Xgwm261 was found in 84 of a total of 98 Croatian wheat cultivars studied. These findings indicate the presence of gene Rht8 and probably gene Ppd1 in most Croatian cultivars. The genetic heritage of Croatian cultivars at the locus Xgwm261 is the consequence of usage of new parental components, carriers of short plant and early maturity attributes and the consequent selection of progeny with these traits during breeding process. The results of this study, as well as analysis of possible presence and frequency of genes Rht-B1b (Rht1), Rht-D1b (Rht2) and Rht-B1d (Rht1-Saitama 27) at DNA level, will be helpful in characterization of domestic wheat cultivars, as well as in more accurate selection of parents for hybridization purposes.

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ALELNA VARIJABILNOST HRVATSKIH KULTIVARA PŠENICE NA MIKROSATELITNOM LOKUSU XGWM261

SAŽETAK

Visina biljke pšenice važno je kvantitativno svojstvo kontrolirano s više gena jakog učinka. Međutim, u oplemenjivanju pšenice u svijetu, najčešće se koristi samo nekoliko takvih gena. U agroklimatskim uvjetima jugoistoka Europe posebno je važan gen Rht8 (Reduced Height Gene). Mikrosatelit gwm261 je, zbog svoje bliske vezanosti s lokusom za gen Rht8, prihvaćen kao dijagnostički molekularni biljeg gena Rht8. U ovome istraživanju je pomoću mikrosatelita gwm261 utvrđena alelna varijabilnost na lokusu Xgwm261 za 122 hrvatska i strana kultivara pšenice. Alel sa 192 para baza na lokusu Xgwm261 utvrđen je za 84 hrvatska kultivara. Genetsko naslijeđe hrvatskih kultivara na lokusu Xgwm261 posljedica je korištenja novih roditelja, nositelja svojstava niže stabljike i ranozrelosti te odabira potomstva s tim svojstvima tijekom oplemenjivačkoga procesa. Rezultati istraživanja pomoći će u karakterizaciji domaćih kultivara pšenice, kao i u preciznijem odabiru roditelja u svrhu križanja.

Ključne riječi: pšenica, Rht8, alelna varijabilnost, lokus Xgwm261

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