DISTRIBUTION OF ALLELIC VARIANTS OF HEXAPLOID WHEAT GERMPLASM AT XGWM261 AND PPD-D1 LOCUS

Petrović, Sonja; Marić, Sonja; Čupić, Tihomir; Drezner, Georg; Karsai, Ildikó

Source / Izvornik: **Poljoprivreda, 2012, 18, 25 - 29**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:151:639969

Rights / Prava: In copyright / Zaštićeno autorskim pravom.

Download date / Datum preuzimanja: **2025-03-04**

Sveučilište Josipa Jurja Strossmayera u Osijeku
Fakultet agrobiotehničkih znanosti Osijek

Repository / Repozitorij:

Repository of the Faculty of Agrobiotechnical Sciences Osijek - Repository of the Faculty of Agrobiotechnical Sciences Osijek

ISSN 1330-7142
UDK = 631.526.4:633.11

DISTRIBUTION OF ALLELIC VARIANTS OF HEXAPLOID WHEAT GERMPLASM AT *XGWM*261 AND *PPD-D*1 LOCUS

Sonja Petrović [(1)], Sonja Marić [(1)], T. Čupić [(2)], G. Drezner [(2)], Ildikó Karsai [(3)]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

Traits like plant height and response to photoperiod are involved in controlling many quality characteristics that breeders look into the desired genotype. Today, climatic changes in traditional wheat growing regions cause day temperature rising and water supply shortening. Exploitation of various semi-dwarfing (Rht) and photoperiod response (Ppd) genes could help the implementation of wheat breeding programs, especially selection of stabile and stress adaptable varieties targeted for different environments. Microsatellite gwm261 is located on Xgwm261 locus, and it is 0.6 cM distant from Rht8 gene on 2DS chromosome, which can also include Ppd1 gen for insensitivity to photoperiod. PCR screening of Croatian and foreign wheat varieties showed prevalence of 192 bp allele at Xgwm261 locus and photoperiod insensitive allele (Ppd-D1a). The results of this research could be useful for more accurate characterization and selection of Croatian wheat cultivars and foreign ones aiming to cross and create new adaptable varieties.

Key-words: wheat, allelic variability, Xgwm261 locus, Ppd-D1 locus

INTRODUCTION

Wheat production must be increased to meet the overgrowing human population demands. Gregory and George (2011) stated that cereal yields and production had increased 3-fold in the last 50 years and that it would need to continue to increase at the same absolute rate for the next 40 years. Modern breeding relies on stable, high yielding, good quality and short-stemmed wheat varieties. That was and still is the most important goal in most countries for many years. Traits like plant height and response to photoperiod are involved in controlling these quality characteristics that breeders look into the desired genotype. Breakthrough in plant breeding was introduction of semi-dwarfing and dwarfing genes. Reduction in plant height from 150 to 70 cm is itself directly responsible for increases in productivity and resistance to lodging. Those plants are more efficient because of their ability to divert assimilates into production of grain rather than straw and by that dramatically improve the harvest index (Worland and Snape, 2000). Today, climatic changes in traditional wheat growing regions cause day temperature rising and shortening of water supply.

Winter wheat varieties should avoid high summer temperature and heat stress with earlier flowering time and photoperiod insensitivity. Exploitation of various semi-dwarfing (*Rht*) and photoperiod response (*Ppd*) genes could help the implementation of wheat breeding programs, especially selection of stabile and stress adaptable varieties targeted for different environments. Introduction of varieties like Norin 10 and Saitama 27 that carried *Rht* genes (*Rht*-B1b, *Rht*-D1b and *Rht*-B1d) led to significant increase of yield in the first half of 20th century (Worland et al., 2001). These genes are present in the majority of world semi-dwarf wheat varieties and played major role in "Green revolution". Microsatellite gwm261 is located on Xgwm261 (Röder et al., 1998), it is 0.6 cM distant from Rht8 gene on 2DS chromosome, which can also include *Ppd*1 gen for insensitivity to photoperiod (Korzun et al., 1998). Even though few exception regarding the linkage between gwm261 and *Rht*8 had been reported (Ellis et al., 2007), this microsatellite had been very useful in many studies like Worland et al. (2001) on 870 accessions worldwide, Liu et al. (2005) on 408 Chinese breeding lines and 98 CIMMYT, US and European breeding lines, Dvojkovic et al. (2010) on 122 hexaploid wheat accessions (98 Croatian varieties), and

DSc Sonja Petrović, Associate Professor (spetrovic@pfos.hr), Prof.DSc. Sonja Marić, Full Professor – Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Kralja Petra Svačića 1d, Osijek, Croatia (2) DSc Tihomir Čupić, Scientific consultant, Prof.DSc. Georg Drezner, Full Professor - Institute of Agriculture in Osijek, Južno predgrađe 17, Osijek, Croatia (3) DSc Ildiko Karsai, Scientific Consultant, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Brunzvik u.2, Martonvásár, Hungary

Aspland et al. (2012) who reported allelic diversity of the mentioned marker on historical and archaeological wheat varieties from the year 1865 and presented transition from traditional to modern agriculture. Varieties in South eastern part of Europe tend to carry *Rht*8 gene together with *Ppd*-D1 gene, and with relatively short stem and earliness as their common traits allow them to be resistant to lodging and ripen fast before drought begins (Chebotar et al. 2001; Ganeva et al., 2005; Šip et al. 2011). Aims of this research were: (a) to determine allelic variability at locus *Xgwm*261 and (b) to determine existence and diversity of *Ppd*-D1 alleles in 40 winter wheat varieties emphasising Croatian varieties and their importance in wheat breeding and possible use in marker assisted selection (MAS).

MATERIAL AND METHODS

Total of 40 winter bread wheat varieties, registered in Croatia from 1931 to 2008, were used in this study. Varieties originated from Croatia (CR), Austria (AU), France (FR), Italy (IT) and Russia (RU). Twenty plants per genotype were grown in greenhouse during 20 days (20°C; 12h day/12h night), leaves were harvested at three leaf phase. DNA isolation was done by CTAB method (Doyle and Doyle, 1987) modified by Grljušić, (2003). DNA concentration was measured using Thermo Scientific NanoDrop 2000® spectrophotometer, while DNA quality was determined by electrophoresis with standard λ-DNA (Figure 1; number represent varieties in Table 2). Primer sequences of molecular markers used are shown in Table 1. Analysis of microsatellite locus *Xgwm*261 which is 0.6 cM distant from *Rht*8 gene (Korzun et al., 1998) was carried out using microsatellite gwm261 according to Röder et al. (1998). GeneAmp® Thermocycler 9700 was used for PCR reaction (step 1: 5 min at 94°C; step 2: 5 cycles of 45 sek at 95°C, 5 min at 68°C (-2°C by cycle) and 1 min at 72°C; step 3: 5 cycles of 45 sek at 95°C, 2 min at 58°C (-2°C by cycle) and 1 min at 72°C; step 4: 27 cycles of 45 sek at 95°C, 75 sek at 45°C and 1 min at 72°C and final step 5: 10 min at 72°C, after which the products were separated on 6% acrylamide gel using a LI-COR4300 DNA analyser.

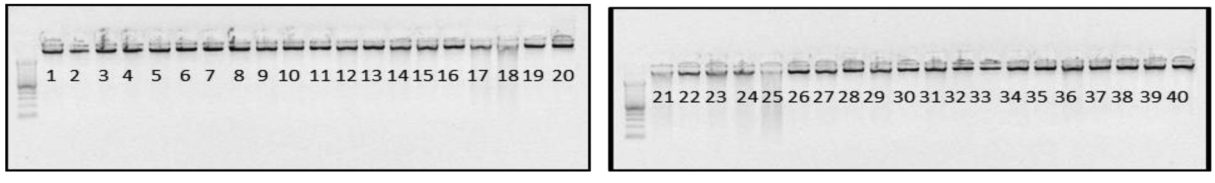

Figure 1. Quality of isolated genomic DNA in comparison with λ-DNA (photo original; S.Petrović)

Slika 1. Kvaliteta izdvojene genomske DNA u usporedbi s λ-DNA (foto original; S.Petrović)

Characterization of photoperiod genotypes at the *Ppd*-D1 locus using allele-specific primers was carried out by Beales et al. (2007). Photoperiod-insensitive allele was labelled as *Ppd*-D1a while *Ppd*-D1b was labelled as photoperiod-sensitive. Allelic variants at the *Ppd*-D1 locus were detected using three gene-specific primers in a multiplex PCR assay, in which the primer pair *Ppd*-D1_F and *Ppd*-D1_R1 produced a 414 bp fragment in genotypes with the photoperiod sensitive allele *Ppd*-D1b, whereas primer pair *Ppd*-D1_F and *Ppd*-D1_R2 produced a 288 bp fragment in those with photoperiod insensitive allele *Ppd*-D1a.

Table 1. Primer sequences and annealing temperatures for detecting alleles at *Xgwm*261 and *Ppd*-D1 loci in wheat

Tablica 1. Parovi početnica i temperatura nalijeganja početnica za identifikaciju alela na lokusima Xgwm261 i Ppd-D1u pšenice

Locus *Lokus*	Molecular marker *Molekularni marker*	Primer sequence (5-3') *Sekvenca početnica (5'-3')*	Annealing temperature (°C) *Temperatura nalijeganja početnica (°C)*
*Xgwm*261	gwm261	CTCCTGTACGCCTAAGGC CTCGCGCTACTAGCCATTG	55
Ppd-D1	Ppd-D1_F	ACGCCTCCCACTACACTG	54
	Ppd-D1_R1	TTGGTTCAAACAGAGAGC	
	Ppd-D1_R2	CACTGGTGGTAGCTGAGATT	

PCR was performed in Thermocycling conditions with an initial denaturation at 94°C for 2 min, followed by 40 cycles of 30' at 94°C, 30 s at 52°C,1 min at 72°C, and a final extension step at 72°C for 5 min. Amplified PCR fragments were separated on 1.2% agarose gel at 200 V for 30 min, stained with ethidium bromide, and visualized using UV light.

RESULTS AND DISCUSSION

Microsatellite analysis of 40 wheat varieties indicated that these varieties carry four different allelic variants on locus X*gwm*261 (Figure 2, Table 2). For total of 40 wheat genotypes, 28 were classified as the 192 bp, 6 as the 174 bp, 4 as the 165 bp and 2 as 196 bp. From 40 tested varieties 31 had *Ppd*-D1*a* allele and nine had *Ppd*-D1*b* allele

(Table 1). Among 192 bp classified varieties are Libellula, Bezostaja and Zlatna dolina, and 174 bp variety Soissons which is in accordance to results by Worland et al. (1998) and Dvojković et al. (2010). The above mentioned allele size (192 bp) is diagnostic allele for *Rht*8 and *Ppd*1 whose background can be correlated from Japanese variety Akakomughi, introduced in wheat breeding program by Nazareno Strampeli, and from Russian varieties Bezostaja, Kavkaz and Aurora (Worland et al., 1998; Borojević and Borojević, 2005; Zheleva et al., 2006). These Italian and Russian varieties are in background pedigrees of many Croatian genotypes with identified allele size of 192 bp. The majority of breeders in southeast part of Europe selected precisely these adapted genotypes with *Rht*8/*Ppd*1, which had traits like short stem and earliness and therefore higher spike productivity.

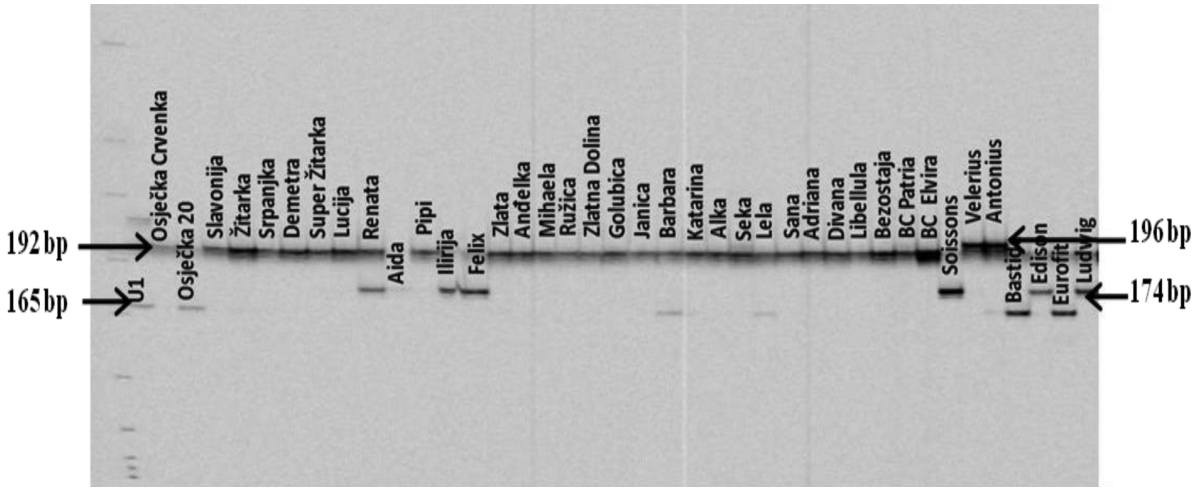

Figure 2. Distribution of alleles at *Xgwm*261 locus for 40 winter wheat varieties

Slika 2. Distribucija alela na lokusu Xgwm261 za 40 sorata ozime pšenice

Many authors (Korzun et al., 1998; Worland et al., 1998; Chebotar et al., 2001; Zhang et al., 2006), state that 192bp allele at *Rht*8 gene is correlated with decreased plant height by 7 to 10 cm, while *Ppd*1 gene have pleiotropic effect on earliness and maturity for about 8 days and also decrease plant height for 10 cm more. These results are in concordance with previous study by Petrović (2011) where Srpanjka, Mihaela and Zlata had plant height of 65 cm and belonged to

Table 2. Distribution of alleles at *Xgwm261* and *Ppd*-D1 locus in 40 winter wheat varieties

Tablica 2. Distribucija alela na lokusima Xgwm261 i Ppd-D1 u 40 sorata ozime pšenice

No. *Br.*	Variety *Sorta*	Origin *Podrijetlo*	*Xgwm261* alelles *Aleli na lokusu Xgwm261*	*Ppd*-D1 alleles *Aleli na lokusu Ppd-D1*	No. *Br.*	Variety *Sorta*	Origin *Podrijetlo*	*Xgwm261* alelles *Aleli na lokusu Xgwm261*	*Ppd*-D1 alleles *Aleli na lokusu Ppd-D1*
1	U1	CR	165 bp	b	21	Janica	CR	192 bp	a
2	Os.crvenka	CR	192 bp	a	22	Barbara	CR	192 bp	a
3	Osjecka 20	CR	165 bp	a	23	Katarina	CR	192 bp	a
4	Slavonija	CR	192 bp	a	24	Alka	CR	192 bp	a
5	Zitarka	CR	192 bp	a	25	Seka	CR	192 bp	a
6	Srpanjka	CR	192 bp	a	26	Lela	CR	192 bp	a
7	Demetra	CR	192 bp	a	27	Sana	CR	192 bp	a
8	Su. zitarka	CR	192 bp	a	28	Adriana	CR	192 bp	a
9	Lucija	CR	192 bp	a	29	Divana	CR	192 bp	a
10	Renata	CR	192 bp	a	30	Libellula	IT	192 bp	a
11	Aida	CR	174 bp	b	31	Bezostaja	RU	192 bp	a
12	Pipi	CR	192 bp	a	32	BC Patria	CR	192 bp	a
13	Ilirija	CR	174 bp	a	33	BC Elvira	CR	192 bp	a
14	Felix	CR	174 bp	b	34	Soissons	FR	174 bp	a
15	Zlata	CR	192 bp	a	35	Valerius	AU	196 bp	b
16	Anđelka	CR	192 bp	a	36	Antonius	AU	196 bp	b
17	Mihaela	CR	192 bp	a	37	Bastide	FR	165 bp	b
18	Ružica	CR	192 bp	a	38	Edison	AU	174 bp	b
19	Zl.dolina	CR	192 bp	a	39	Eurofit	AU	165 bp	b
20	Golubica	CR	192 bp	a	40	Ludwig	AU	174 bp	b

the group of very early varieties according to morpho-agronomical traits.

Only three Croatian varieties (Ilirija, Aida and Felix) had 174bp allele. This allele is associated with photoperiodic sensitive gene (*ppd*-D1) prevalent in French, German and varieties from the United Kingdom, originated and introduced in breeding programs from Japanese variety Norin 10 (Worland et al., 1998; Zheleva et al., 2006). According to recent studies later heading varieties that carry *Xgwm*261 alleles174-bp and 165-bp, often in combination with *Ppd*-D1b, could guarantee broader adaptability, which is highly desirable for changeable weather conditions (Šip et al., 2010). Nevertheless in this study, varieties Aida and Felix are early varieties (Petrovic, 2011) but that carry 174 bp and Ppd-D1b. Contrary to Worland et al. (2001) these varieties do not fit in the characterization that the presence of *Ppd*-D1a contribute to height reduction through accelerating ear emergence time and reducing the life cycle by about 7 days. In study by Drezner et al. (2010) wheat varieties Aida and Felix showed significantly higher yield during two vegetation years on three different locations, with addition of high quality traits of variety Felix. This could be explained with pedigree and genetic background of these varieties, as well as possible interactions with other maturity genes. Presence of the mentioned alleles in Aida and Felix could favour broader adaptability which can be implemented in crosses and future breeding programs. Wheat varieties: U1, Osječka 20, Bastide and Eurofit had height-promoting allele 165 bp which is related with results by Dvojković et al. (2010). Old variety U1 and Austrian variety Eurofit are tall with average plant height over 100 cm which is consistent with results by Korzun et al. (1998) who established that 165 bp allele had height increasing effect, while Worland et al. (1998) determined his origin from, also Japanese variety, Saitama 27 and its prevalence in CIMMYT germplasm.

CONCLUSION

Distinctive features of allelic distribution at *Xgwm*261 and *Ppd*-D1 loci in winter wheat germplasm were revealed. PCR screening of Croatian and foreign wheat varieties showed prevalence of gwm261_192 bp allele and photoperiod insensitive allele (*Ppd*-D1*a*). The results of this research should be helpful to breeders for more accurate characterization and selection of Croatian and foreign wheat varieties in order to optimize the choice of parents for crossings and selection strategy in different environments. Further analysis should be made broadening the study to more *Rht* and *Ppd* genes, combining it with their effect on agronomic traits.

ACKNOWLEDGEMENT

The research work is a part of the research project 079-0730718-0268 financed by the MZOS.

REFERENCES

1. Aspland , L., Leino, M.W., Hagenblad, J. (2012): Allelic variation at the *Rht*8 locus in 19th century wheat collection. The Scientific World Journal, doi:10.1100/2012/385610
2. Beales, J., Turner, A., Griffits, S., Snape, J.W., Laurie, D.A. (2007): A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 115(5): 721-733.
3. Borojević, K., Borojević, K. (2005): Historic role of the variety Akakomughi in Sothern and Central European wheat breeding programs. Breeding Science 55: 253-256.
4. Chebotar, S.V., Korzun, V.N., Sivolap, Y.M. (2001): Allele distribution at locus WMS261 marking the dwarfing gene *Rht*8 in common wheat cultivars of Southern Ukraine. Russian Journal of Genetics 37(8): 894-898.
5. Doyle, J.J., Doyle, J.L. (1987): A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19: 11-15.
6. Drezner, G., Dvojković, K., Guberac, V., Marić, S., Novoselović, D., Horvat, D,. Španić, V., Šimenić, J., Primorac, J. (2010): Novi genotipovi pšenice – procjena uroda i kakvoće u više okolina. Zbornik radova 45. hrvatskog i 5. međunarodnog simpozija agronoma, Opatija, Hrvatska, 15. -19. veljače 2010: 399-40.
7. Dvojković, K., Šatović, Z., Drezner, G., Somers, D.J., Lalić, A., Novoselović, D., Horvat, D., Marić, S., Šarčević, H. (2010): Allelic variability of Croatian wheat cultivars at the microsatellite locus *Xgwm*261. Poljoprivreda 16(1): 32-37.
8. Ellis, H.M., Bonnett, D.G., Rebetzke, G.J. (2007): A 192 bp allele at the Xgwm261 locus is not always associated with the Rht8 dwarfing gene in wheat (*Triticum aestivum*), Euphytica 157 (1-2): 209-214.
9. Ganeva, G., Korzun, V., Landjeva, S., Tsenov, :, Atanasova, M. (2005): Identification, distribution and effects on agronomic traits of the semi-dwarfing Rht alleles in Bulgarian common wheat varieties. Euphytica, 145: 305-315.
10. Grljušić, S. (2003): Genetska varijabilnost kultivara crvene djeteline (*Trifolium pratense* L.) nakon selekcije u brdsko-planinskim uvjetima. Doktorska disertacija, Sveučilište u Zagrebu, Agronomski fakultet, Zagreb, Hrvatska
11. Gregory, P.J., George, T.S. (2011): Feeding nine billion: the change to sustainable crop production. J Exp. Bot. 62(15): 5233-5239
12. Korzun, V., Röder, M.S., Ganal, M.W., Worland, A.J., Law, C.N. (1998): Genetic analysis of the dwarfing gene (*Rht*8) in wheat. Part I. Molecular mapping of *Rht*8 on the short arm of chromosome 2D of bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 96: 1104-1109.
13. Liu,Y., Liu, D., Zhang, H., Wang, J., Sun, J., Guo, X., Zhang, A. (2005): Allelic variation, sequence determination and microsatellite screening at the XGWM261 locus in Chinese hexaploid wheat (*Triticum aestivum*) varieties. Euphytica 145 (1-2): 103-112.
14. Petrović, S. (2011): Genetska različitost gerplazme ozime krušne pšenice (*Triticum aestivum* L. ssp. *vul-*

gare). Doktorska disertacija, Sveučilište Josipa Jurja Strossmayera u Osijeku, Osijek, Hrvatska

15. Röder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M.H., Leroy, P., Ganal, M.W. (1998): A microsatellite map of wheat. Genetics 149: 2007-2023.
16. Šíp, V., Chrpova, J., Žojfajová, A., Pánková, K., Užík, M., Snape, J.W (2010): Effects of specific *Rht* and *Ppd* alleles on agronomic traits in winter wheat cultivars grown in middle Europe. Euphytica 172: 221-233.
17. Šíp, V., Chrpova, J., Žojfajová, A., Milec, Z., Pánková, K., Snape, J.W. (2011): Evidence of selective changes in winter wheat in middle-European environments reflected by allelic diversity at loci affecting plant height and photoperiodic response. J Agri. Sci. 149:313-326.
18. Worland, A.J., Korzun, V., Röder, M., Ganal, M.W., Law, C.N. (1998): Genetic analysis of the dwarfing gene (*Rht*8) in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht*8 locus of wheat as revealed by microsatellite screening. Theor. Appl. Genet. 96: 1110-1120.
19. Worland A.J., Sayers, E.J., Korzun, V. (2001): Allelic variation at the dwarfing gene Rht8 locus and its significance in international breeding programs. Euphytica 119: 155-159.
20. Worland, T., Snape, J.W. (2000): Genetic basis of worldwide wheat varietal improvement. In: Bonjean AP, Angus WJ (ed.) World wheat book. Lavoisier publishing, Paris, France, 59-100.
21. Zhang, X., Yang, S., Zhou, Y., He, Z., Xia, X. (2006): Distribution of the Rht-B1b, Rht-D1b and Rht8 reduced height genes in autumn-sown Chinese wheat detected by molecular markers. Euphytica 152: 109-116.
22. Zheleva, D., Todorovska E., Jacquemin, M., Atanassov, A., Christov, N., Panayotov, I., Tsenov, N. (2006): Allele distribution at microsatellite locus Xgwm261 marking the dwarfing gene Rht8 in hexaploid wheat from Bulgarian and Belgium gene bank collections and its application in breeding programs. Biotechnol. And Biotechnol. Eq. 20(2): 45-56.

DISTRIBUCIJA ALELNIH VARIJANTI GERMPLAZME HEKSAPLOIDNE PŠENICE NA LOKUSIMA XGWM261 I PPD-D1

SAŽETAK

Velik broj svojstava koje oplemenjivači traže u željenim genotipovima kontrolirana su od strane gena koji utječu na visinu biljke i osjetljivost na fotoperiod. Danas promjena klimatskih prilika u tradicionalnim uvjetima uzgoja uzrokuju visoke temperature i sušu. Korištenje različitih Rht (Reduced Height Gene) te gena odgovornih za osjetljivost na fotoperiod (Ppd) mogu biti od velike pomoći u oplemenjivačkim programima pšenice, posebice u selekciji genotipova otpornih na stres koji će biti prilagođeni različitim okolinama. Mikrosatelit gwm261 je smješten na lokusu Xgwm261 koji je 0,6 cM udaljen od Rht8 gena na 2DS kromosomu te koji može uključivati i Ppd1 gen za neosjetljivost na fotoperiod. Rezultati dobiveni na temelju PCR reakcije pokazuju da u hrvatskoj germplazmi prevladava alel sa 192 parova baza, na lokusu Xgwm261 te alel za neosjetljivost na fotoperiod (Ppd-D1a). Rezultati ovoga istraživanja mogu biti korisni za preciznije vrednovanje i odabir hrvatskih i stranih sorata kao roditelja u križanjima radi stvaranja boljih adaptabilnih sorata.

Ključne riječi: pšenica, alelna varijabilnost, lokus Xgwm261, lokus Ppd-D1

(Received on 2 October 2012; accepted on 29 November 2012 - *Primljeno 02. listopada 2012.; prihvaćeno 29. studenoga 2012.*)