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EVALUATION OF WHEAT *RHT* GENES USING MOLECULAR MARKERS

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Using STSs and SSR markers, three worldwide the most important major height reducing genes, *Rht-B1b*, *Rht-D1b* and *Rht8* were evaluated in this paper. In the analysed set of hexaploid wheat, composed of 172 genotypes originated from more than 20 countries, *Rht-B1b*, *Rht-D1b* and *Rht8* were found in 40%, 22% and 62% of cases, respectively. In genotype groups of domestic and foreign origin, the highest difference in allele frequency was determined in the case of *Rht8,* which was more frequent in domestic genotypes (78, 3%). The *Rht-B1b* was dominantly present in foreign germplasm (57, 6%). Portion of *Rht-D1b* was almost equal with 22, 6% in domestic and 21, 2% in foreign varieties. Obtained results and accepted methodology for detection of these three, the most important *Rht* genes, represent great start point for Marker Assisted

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Selection (MAS) for high yielding wheat genotypes in agro climatic conditions of Serbia and Mediterranean area.

Key word: Rht genes, molecular markers, wheat

INTRODUCTION

In period after Green revolution, plant height in wheat is in great focus of breeding programs. Influence of major *Rht* genes on this quantitative trait and their pleiotropic effect on yield components, make the information about their type and combination very valuable. Development of molecular markers like SSRs and STSs as detection methods for *Rht8, Rht-B1b* and *Rht-D1b*, has provided fast germplasm screening, so made selection much easier and studies about their influence on different traits more accurate.

The aim of this paper was to determine presence of *a* or *b* alleles at *Rht-B1* and *Rht-D1* loci in the set of 172 genotypes, and to revise previous findings of a large number of «null» alleles in microsattelite locus *Xgwm261* for the same genotype set.

MATERIAL AND METHODS

One hundred seventy two varieties and lines of different origin, about 61% of domestic and 39% of germplasm from more then 20 countries, were obtained from Institute of Field and Vegetable Crops, Novi Sad, core collection. Genomic DNA was extracted from seedlings tissue (3 from each variety) using CTAB method (DOYLE & DOYLE, 1990). DNA concentration measurement and purity determination were done with UV-spectrofotometric method (SAMBROOK & RUSSELL, 2001).

Rht-B1b and *Rht-D1b* were detected with PCR based molecular markers (STS) designed to reveal point mutation responsible for difference between "*a"* (tall-wild type) and *"b"* (semi dwarf-mutant type) alleles (ELLIS et al., 2002). Reactions were performed in 20µl total volume in accordance with the original instructions (except 2U Hotstar *Taq* polymerase instead of recommended 1U). Temperature conditions of reactions were slight modified (95ºC instead of 94ºC denaturation step and 15min instead of 5min at 95ºC at initial denaturation step). Reaction for confirmation of *Rht-D1a* presence in material wasn't conducted because of technical problems.

For detection of *Rht8* gene microsatellite marker *Xgwm261* (RÖDER et al., 1998) was used. This locus is closely linked to the *Rht8* (0.6 cM distally). The, point to presence of this The reductor of height in genome is pointed to presence of allele 192bp long. PCR reaction was performed only with genotypes which in earlier investigation (PILIPOVIĆ, 2005) have shown the "null allele" in mentioned microsatellite, with goal to establish facts. Allele type for the rest of material was taken over. Amplification of the *Xgwm261* locus was performed in 20µl volume containing 1x PCR buffer, 1.5mM $MgCl_2$, 25pmol of each primers, 1U *Taq*

Polymerase, 0.2mM of each dNTPs and 100ng genomic DNA. Termocycle conditions were: 94ºC for 3min, 45 cycles: 94ºC for 1min, 55ºC for 1min, 72ºC for 2min, and final elongation on 72ºC for 10min.

PCR products were separated on 2% agarose gels and visualised with 0.02% ethidium bromide (10mg/ml) added directly in agarose solution. In the case of *Xgwm261,* because of lack of precision of agarose method, standard denaturing 6% PAA gel electrophoresis (SAMBROOK & RUSSELL, 2001) and modified silver staining (SANGUINETTI et al., 1994) were used for allele size determination.

RESULTS AND DISCUSSION

The most frequent reductor of height in Novi Sad wheat genetic core collection was *Rht8* gene, present in 62% of material used in this study (Figure 1). In view of material structure in regard to origin (61% domestic germplasm) this was expected. Importance of this *Rht* gene and its linkage with *Ppd-D1* for varieties grown in Mediterranean region is already known and was explained with earlier entry in generative phase and avoidance of extreme high temperatures in the flowering time (WORLAND et al., 1998). In the same collection, but working with 377 genotype, PILIPOVIĆ (2005) found 192bp *Xgwm261* allele in 52,3%. Obvious difference in frequencies can be attributed to a large number of false «null» alleles declared by PILIPOVIĆ (2005) but also to a differences in the material. In domestic germplasm, *Rht8* was found in 78% of genotypes that is in accordance with findings KOBILJSKI et al. (2006). In the group of foreign varieties, *Rht8* frequency was much lower, ~36%, mostly with carriers from Italian, Russian, and selections from surrounding counties (Hungary and Bulgaria) but also in some Japanese, American and Mexican genotypes. The analysis of WORLAND et al. (2001) showed very similar results. High frequency of this gene in material from Southeast Europe (~80%) also reported ZHELEVA et al. (2006). KOBILJSKI et al. (2008) point out to genotypes from South and Northeast of Europe and «wheat belt» in USA where *Rht8,* along with *Ppd-D1,* has a selective advantage compared to other *Rht* genes. LIU et al. (2005) reported presence of 192 bp allele in high percentage in Chinese varieties from Facultative Wheat Zone in China.

Gene *Rht-B1b* was found in 40% (Figure1) and *Rht-B1a* in 54% of analysed material. In remaining 6% some other of *Rht-B1* alleles might be present. The *Rht-B1b* had higher frequency in the group of foreign varieties (~58%) than in domestic germplasm (~29%). The most frequent it was in genotypes originated from USA, Mexico, Australia and India. Situation like this is expected because *Norin 10* genes (*Rht-B1b, Rht-D1b*) carriers rarely realize their yield potential in agro ecological conditions of South Europe. The *Rht-D1b* gene was present in ~22% of cases, with small difference between national and varieties of other origin (Figure 1). Relatively high percent of *Norin 10* gene carriers in domestic germplasm can be explained by notable involvement of promising lines in this group. Earlier study revealed that most of Novi Sad varieties in pedigrees have GA-I ancestors so they aren't rare in lines (PETROVIĆ & WORLAND, 1992) neither

in most of spring and minor number of winter wheat varieties (PETROVIĆ et al., 1998).

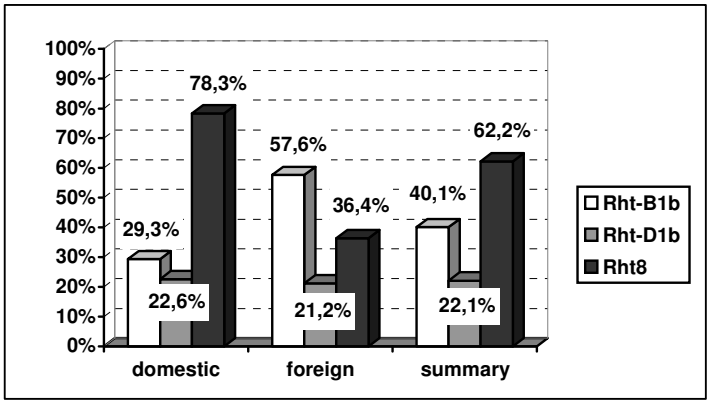

Figure 1. Proportion of *Rht-B1b*, *Rht-D1b* and *Rht8* in domestic and foreign germplasm and their overall frequency in analyzed material

The *Rht-B1b* was also registrated in 5 out of 6 tested genotypes of Croatian descent and few older GA-R domestic varieties (Dina, Jugoslavija, Kolubara, Košava, Biserka, Novosadska rana 2, Balkan). On the other side, according to PETROVIĆ & WORLAND (1992), in Zagreb and Osijek breeding programs, especially in new varieties, only the *Rht-B1d* was found. Observed differences may be consequences of limitations of applied detection method. Because the method is based on SNP (single nucleotide polymorphism), potential explanation for this phenomenon might be unspecific binding of primers in the same locus, and appearance of the same length amplification product even in the absence of tagged sequence (Figure 2). Reliability of method was tested by YANG & LIU (2006) on 430 genotypes, in 2-10 replications. According to molecular and pedigree analysis, successful amplifications using primer pair DF-MR2 (for *Rht-D1b* detection) was very high, with only 0,7% false positive reactions. On the other hand, 237bp product was amplified with BF-MR1 primer pair (*Rht-B1b* detection) in 14,67% cases in cultivars where absence of *Rht-B1b* was expected based on pedigree analysis.

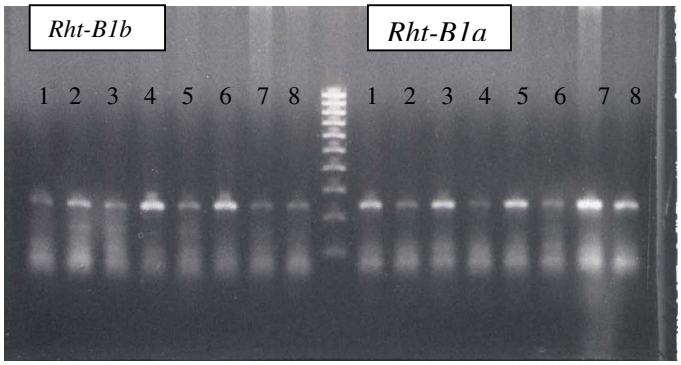

Figure 2. Products of PCR amplification with primer pairs BF-MR1 and BF-WR1 (left and right from 100bp DNA ladder, respectively) for same genotype set

In 14% of examined genotypes none of the major height reductor genes were found (Figure 3). One of three analysed *Rht* was present in 49% material, with *Rht8* as the most frequent (31%). *Rht-D1b* as the only height reductor was found in small number of domestic lines. Two of three *Rht* genes were observed in 34% varieties and lines, with *Rht-B1b+Rht8* as the highest incidence combination (20%). Similar combining GA-I and *Rht8* was reported by GANEVA et al. (2005) in Bulgarian varieties, especially in modern material. Combination *Rht-B1b+Rht-D1b+Rht8* were detected in 3% of genotypes. If it is taken into account actual somewhat lower incidence of *Rht-B1b* as a consequence of imprecision of the applied method, this value should be taken with reserve, particularly in this group because phenotype doesn't always fits to molecular establish status.

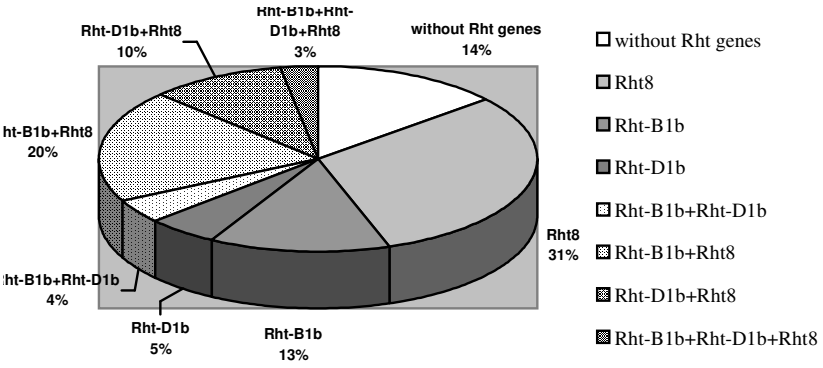

Figure 3. Portion of different combinations of *Rht* genes in wheat material

The most recent research of ELLIS et al. (2007) showed that presence of 192 bp allele in *Xgwm261* microsatellite locus is not always associated with *Rht8* gene and reduction of height. It was found that Norin 10 (obtained from four different sources) carries a $Xgwm261_{192}$ (100% of nucleotide identity when compared with Akakomughi 192 bp allele), instead of 174 bp allele found by WORLAND et al. (1998). The source of this height neutral allele linked with *Rht-B1b* and *Rht-D1b* were cultivars Pitic 62 and Siete Cerros, important parents in CIMMYT and many other breeding programs around the world. As a consequence, $Xgwm261_{192}$ is not always diagnostic for the *Rht8* and it is now required additional evidence of his presence, such as a pedigree analysis or hight reducing effect on 2DS chromosome. In the light of this new facts, the results of this work requests further investigations on the same material before making any firm conclusions.

CONCLUSIONS

The analysis of presence of three the most exploited reduced height genes within Novi Sad genetic core collection using molecular markers, indicate that the most frequent in this material was *Rht8,* alone or in combination with *Rht-B1b* and *Rht-D1b* (especially in domestic germplasm). *Rht-B1b* carriers were more usually present among foreign then domestic genotypes, while *Rht-D1b* had almost equally, but much lower incidence in both groups. Relatively high proportion of genotypes with all three analysed *Rht* genes quite possibly was result of appearance of false positive results on the presence of either *Norin 10* or *Rht8* genes as a consequence of imprecision of the applied method.

Questions about reliability of used methods for detection of these three genes have arisen, so more attention in future investigations and additional work on this task will be required.

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EVALUACIJA *RHT* GENA PŠENICE KORIŠĆENJEM MOLEKULARNIH MARKERA

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Izvod

Koristeći STSs i SSR markere, u ovom radu izvršena je evaluacija tri, na svetskom nivou najznačajnija major gena reduktora visine, *Rht-B1b*, *Rht-D1b* i *Rht8*. U grupi od 172 genotipa heksaploidne pšenice, poreklom iz više od 20 zemalja, *Rht-B1b*, *Rht-D1b* i *Rht8* geni nađeni su u 40%, 22% i 62% slučajeva, respektivno. U grupama genotipova domaćeg i stranog porekla najuočljivija razlika u frekvencijama karakterističnih alelnih formi utvrđene su u slučaju *Rht8*, koji se pokazao kao češći kod domaćih genotipova (78,3%), i *Rht-B1b* koji je dominirao stranom germplazmom (57,6%). Zastupljenost *Rht-D1b* bila je gotovo ujednačena u obe grupe sa 22,6% (domaći genotipovi) i 21,2% (strane sorte). Dobijeni rezultati i usvojena metodologija za detekciju ova tri *Rht* gena od izuzetnog značaja, predstavljaju odličnu polaznu tačku za marker asistiranu selekciju (MAS) u oplemenjivanju visokoprinosnih genotipova pšenice u agroklimatskim uslovima Srbije i područja Mediterana.

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