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
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 \textit{a} or \textit{b} alleles at \textit{Rht-}\textit{B1} and \textit{Rht-D1} loci in the set of 172 genotypes, and to revise previous findings of a large number of «null» alleles in microsatellite locus \textit{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 than 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 (SAMBUS & RUSSELL, 2001).

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

For detection of \textit{Rht8} gene microsatellite marker \textit{Xgwm261} (RODER et al., 1998) was used. This locus is closely linked to the \textit{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 (PILIPOVIC, 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 \textit{Xgwm261} locus was performed in 20\textmu l volume containing 1x PCR buffer, 1.5mM MgCl\textsubscript{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% (Figure 1) 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 registered 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.
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.
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 Xgwm261192 (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, Xgwm261192 is not always diagnostic for the Rht8 and it is now required additional evidence of his presence, such as a pedigree analysis or height 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.

Received January 18th, 2008
Accepted March 4th, 2008

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EVALUACIJA RHT GENA PŠENICE KORIŠČENJEM MOLEKULARNIH MARKERA

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Izvod

Koristeći STSs i SSR markere, u ovom radu izvršena je evaluacija tri, na svetskom nivou najznačajnijih 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 najuobičajenija 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 grope 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 asistirana selekciju (MAS) u oplemenjivanju visokoprinosnih genotipova pšenice u agroklimatskim uslovima Srbije i područja Mediterana.