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Identification of a null allele at the *Wx-B1* locus in some of Iranian bread wheat genotypes

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Behnam Habibi Khaniani, Mohammad Reza Bihamta, Mohammad Esmail Hassani, Godarz Najafian and Farrokh Darvish: Identification of a null allele at the *Wx-B1* locus in some of Iranian bread wheat genotypes

ABSTRACT

Amylose and amylopectin are the two polysaccharides that constitute starch in bread wheat and the enzyme GBSSI (*Granule-bound starch synthase* I), also known as waxy protein, is responsible for amylose synthesis in storage tissues. Bread wheat has three granule-bound starch synthase I (GBSSI) genes (*Wx-A1*, *Wx-B1*, *Wx-D1*). Selection of wheat lines with slightly lower amylose levels is an important goal in wheat breeding programs. Therefore, discriminate of mutations in the wheat waxy genes, which control amylose synthesis, and development DNA marker for identification of null waxy alleles is necessary. In this research, a pair of PCR markers were used to characterize the genetic variability of *waxy* loci in *Wx-B1* locus from 70 Iranian bread wheat cultivars. When this marker was used to evaluate the variability, 67 cultivar showed the 425bp fragment associated with the *Wx-B1* locus (*Wx-B1a* allele) and 3 cultivar showed absence of the 425bp fragment that had the null allele (*Wx-B1b*) in this locus and were classified in groups of partial waxy wheat. The PCR selection method described here, is an easy alternative to the commonly used SDS-PAGE methods for identification of null alleles in waxy loci.

Key words: waxy wheat, *Wx-B1* gene, starch, molecular markers.

Introduction

Starch, which accounts for 65–75% of wheat grain dry weight, is the major constituent of flour and is composed of two types of polymers, amylose and amylopectin. Amylose is an essentially linear α-1,4 glucan and contributes about 20–30% to the total starch, while amylopectin is a branched α-1,4 glucan containing about 5% α-1,6 branch points and constitutes the remaining 70–80% of the total starch. The physical and chemical properties of starch, and consequently the quality of the end products are dependent on the relative amounts of amylose and amylopectin [6]. Waxy protein or granule-bound starch synthase I (GBSSI), is a key enzyme for amylose synthesis in the wheat endosperm [12]. Mutation at the waxy locus that lead to a lack of enzyme activity affect amylose content and result in low-amylose or waxy (amylose-free) mutants [12]. Although waxy mutations were reported in a number of plant species as early as the nineteenth century, only within the last fifteen years have such mutations been identified and studied in hexaploid wheat (*Triticum aestivum* L.). [12]. Bread wheat has three granule-bound starch synthase I (GBSSI) genes (*Wx-A1*, *Wx-B1*, *Wx-D1*), also known as *Waxy* genes [11]. Waxy wheat is deficient in waxy proteins, while partial waxy wheat lacks one or two waxy proteins. Among the partial waxy wheat types, cultivars lacking *Wx-B1* protein are preferable for such noodles, partly due to their low amylose levels [11]. Partial waxy starch is a desirable trait in the development of wheat cultivars suitable for certain types of noodles [5,7]. Several researchers haver reported mutant alleles of the three waxy genes. Yamamori *et al*. [18] found lines lacking each of the three waxy isoproteins and designated these as being due to the *Wx-A1b*, *Wx-B1b* and *Wx-D1b* alleles of the *Wx-A1*, *Wx-B1* and *Wx-D1* genes, respectively. Around the world several wheat collections have been characterized searching for null waxy proteins by 1D or 2D SDS-PAGE [16,4,15] and PCR markers

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[2,1,10,15]. The development of single-locus allele specific markers for waxy null alleles is a desirable goal to find wheat carrying waxy alleles. Recently, genomic DNA sequences of the three wheat waxy genes [9,19] and cDNA sequence of two mutant alleles (*Wx-A1b* and *Wx-D1b*) were characterized [17]. These DNA sequences are useful resources for developing DNA markers. In this study, we used a single –locus allele specific marker for waxy null allele for MAS programs, and the characterization of the genetic variability of the *Wx-B1* locus in Iranian bread wheat cultivars using molecular marker as a tool.

Materials and Methods

Plant materials:

A set of 70 bread wheat cultivars (*Triticum aestivum* L.) from Iran was screened using a molecular marker to assess the variability of *Wx-B1* alleles. Seed stocks were obtained from Seed and Plant Improvement Institude of Iran. The Australian breeding line DHWx12 was used as triple-null control (*Wx-A1b* / *Wx-B1b* / *Wx-D1b*) [13].

DNA extraction:

DNA was extracted from 100 mg of young leaf tissue following the method of Dellaporta *et al*. [3] with modifications.

Primers and PCR conditions:

The primers used to identify mutation in *Wx-B1* locus are listed in Table 1. Primers were used based on Nakamura research [10]. PCR reactions were performed in a total volume of 25µL cotaining 50 ng genomic DNA(3 µl), 12.5µL Master Mix PCR, 10 pmol of each primer(1µL) and 6.5µL ddwater. The PCR cycle consisted of an initial 5-min denaturation at 95^{o}C, followed by 34 cycle of 95^{o}C for 30 sec, 65^{o}C for 30 sec, 72^{o}C fot 2 min, and 1 cycle of 72^{o}C for 7 min.

Table 1: Primer sequences to identify *waxy* null allele in *Wx-B1* locus

Names	Sequences	Reference
BDFL	5´-CTGGCCTGCTACCTCAAGAGCAACT-3´	(Nakamura *et al*. 2002)
BRD	5´-CTGACGTCCATGCCGTTGACGA-3´	

Analysis of PCR products:

A 7µl aliquot of the PCR mixture were resolved in 2% agarose gels, stained with Gel Red and visualized with UV light.

Results and Discussion

Differentiation of normal and mutant alleles of the Wx-B1 locus:

Primers BDFL and BRD were used to detect mutations in the *Wx-B1* locus amplified PCR products. Since the mutation in the *Wx-B1* gene appears to result in the deletion of most or all of the coding region [17], no PCR product was produced from the *Wx-B1b* allele. The size of the fragment from the *Wx-B1b* normal allele (425 bp) was clearly distinguishable from PCR products originating from the *Wx-D1* (497 bp) and *Wx-A1* (455 bp) genes. With primers BDFL and BRD can not identified the difference between waxy and normal alleles in both *Wx-A1* and *Wx-B1* locus, Because the amplified fragment sizes was similar for both waxy and normal alleles (455 bp for *Wx-A1* and 497 bp for *Wx-D1* locus). In Figure 1, the larger fragment (497bp) belongs to D genome, the intermediate (455bp) to the A genome and the smaller one (425bp) to the B genome. When this marker was used to evaluate the variability in Iranian bread wheat germplasm, 67 cultivar (95.7%) showed the 425bp fragment associated with the *Wx-B1* locus (*Wx-B1a* allele) (Figure 1 lines 3,4,5,6,8 and 9), and 3 cultivar (4.2%) showed absence of the 425bp fragment (Figure 1 lines 2 and 7) that had the null allele (*Wx-B1b*) in this locus, Therefore, cultivars "karaj3", "Navid" and "Az-Landrace 8" in *Wx-B1* locus had waxy null allele and were classified in groups of partial waxy wheat.

The marker reported here clearly discriminates the normal allele (*Wx-B1a*) and the null allele (*Wx-B1b*) coming from DHWx12. The observed difference for the bands representing these two alleles results from the deletion in the null allele and is consistent with the report of Nakamura *et al*. [10]. The mutant allele of *Wx-B1* gene in DWHx12 is from a landrace, "Tammin" [20]. The importance of the identification of new forms of waxy protein is related to a reduction in the amylose content found in genotypes carrying these mutations (null mutations). In this work, we have detected one allele (*Wx-B1b*) with a deleterious effect in protein function. The *Wx-B1b* allele was detected in 4.2% of wheat cultivars. These cultivars carrying partial waxy starch can be an attractive target in the development of local adapted cultivars, suitable for certain specialties like dry white chinese noodles [7].

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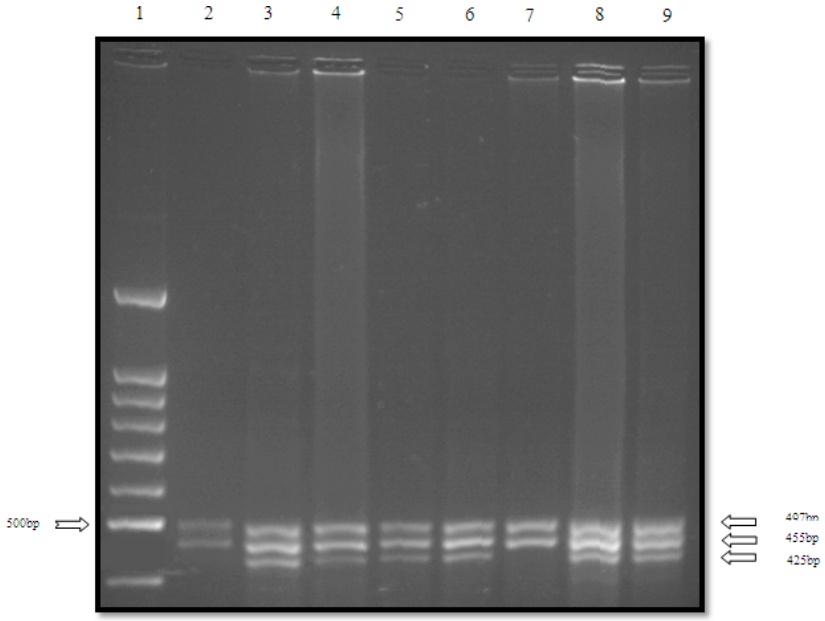

Fig. 1: PCR with primers BDFL and BRD to distinguish the wild type (*Wx-B1a*) and null allele of *Wx-B1* gene. Lanes from left to right:100-bp DNA ladder, breeding lines/cultivars DHWx12 (with null *Wx-B1* allele), Cross shahi, Kaveh, Alborz, Karaj2, Karaj3, Kc-1200 and Sorkh tokhm.

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