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Molecular marker-based detection of *Ph1b* mutation to increase homoeologous pairing in wheat (*Triticum aestivum*)*

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Modern wheat cultivars belong primarily to two allopolyploid species: hexaploid bread wheat [*Triticum aestivum* L. ($2n = 6x = 42$; AABBDD)] composed of three related genomes (A, B and D) and tetraploid durum wheat [*Triticum turgidum* L. ($2n = 4x = 28$; AABB)] with two related genome (A and B). Chromosome pairing in polyploidy bread and durum wheat occurs only between homologous chromosomes resulting in strictly diploid-like pairing and disomic inheritance. The homologous pairing of polyploidy wheat is controlled by *Ph1* (*pairing homologous*) locus located on long arm of chromosome 5B which suppresses pairing and recombination between homoeologous chromosomes (Riley and Chapman 1958). Such a *Ph1* enforced pairing was essential for the evolution of these wheats (Jauhar 2003). Cytogenetic manipulation of this pairing is also of great interest from standpoint of alien gene transfer into wheat (Sears 1977, Giorgi 1978)

Wild relatives of wheat are rich sources of genes resistance to pathogens and insect pests, and improved agronomic performance and those can be transferred trough wide hybridization. Cytogenetic manipulations for suppressing the *Ph1* pairing regulation would be necessary to bring about the desired chromosome pairing and hence alien gene transfers into cultivated wheat (Jauhar 2006). The methods of circumventing the *Ph1* system include: (i) using substitution lines lacking chromosome 5B and hence *Ph1*, (ii) suppressing the activity of the *Ph1* by crossing polyploidy cereal crops with appropriate genotypes of wild donors that partially or fully inactivate the regulatory genes as some genotypes of *Aegilops speltoides* harbor the Ph^I gene (*Ph* inhibitor) which suppresses the effect of *Ph1* gene and permits homoeologous chromosome pairing (Chen *et al.* 1994), and (iii) using the *ph1bph1b* mutant of wheat as female parent in crosses with the wild donor species (Jahaur 2006).

Cytological analysis at meiotic metaphase I is routinely used to score homoeologous chromosome pairing in the plants. Several methods have been developed to replace the time and labour-consuming cytological analysis. The PCR–based methods have been reported to identify *Ph1* locus (Gill and Gill 1996, Segal *et al.* 1997, Qu *et al.* 1998, Wang *et al.* 2002). In the present study, PCR-based marker was used to transfer the recessive mutation in *Ph1* locus (*Ph1b*) from bread wheat cultivar Chinese Spring to Indian bread and durum wheat cultivars.

Chinese spring (CS, *Ph1*), CS *ph1b* mutant, CS homoeologous group 5 nullisome-tetrasomic lines, elite bread wheat cultivars (DBW 16 and PBW 502) and durum wheat cultivar ((HI 8498) were used in this study during 2010. F_2 populations of 154 individuals derived from the crosses of DBW 16 × CS *ph1b*, PBW502 × CS *ph1b* and HI 8498 × CS *ph1b* were studied for co-segregation of PCR marker with *Ph1* locus. Total genomic DNA was isolated from leaves of three weeks old seedlings by CTAB method (Doyle and Doyle 1990). The primers, PC3 9 forward: 5´-TAACGCCAGGGCATACTC-3´ and PC3 9 reverse: 5´-CTGCAGGAGGCGCTGGAA-3´ were used in a plus/minus PCR assay to detect the Ph1 locus (Qu *et al.* 1998). For marker analysis, a polymerase chain reaction (PCR) was performed in a 25 µl volume containing 100 ng of genomic DNA, 2.5 µl of 10 × PCR buffer, 200 µM of each dNTP, 0.2 µM of each primer, 1.5 mM $MgCl_2$ and 1.0 unit of *Taq* DNA polymerase in (Bio-Rad). The thermocycling program consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of 45 sec at 94°C, 45 sec at 56°C, 60 sec at 72°C and a final cycle of 5 min at 72°C in MJ Mini™ Personal Thermal Cycler (Bio-Rad). The amplified product were separated by electrophoresis on 2% (w/v) agarose gel and stained with ethidium bromide.

*Short note

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Chromosome pairing at meiotic metaphase I (MI) was investigated by the acetocarmine squash method. The spikes were fixed in Carnoy's solution (six parts 95% ethanol to three parts chloroform to one part glacial acetic acid) containing a pinch of ferric chloride for a better colour contrast. After three days, the spikes were transferred to 70% ethanol and stored at 4°C for futher analysis. The immature anthers were squashed in a drop of acetocarmine stain (2% carmine powder in 45% acetic acid). The meiotic metaphase I of pollen mother cells was examined for chromosome pairing in different crosses involving *Ph* mutant and normal cultivars. Photographs were taken with inbuilt digital camera of Trinocular Olympus microscope using 100×15 X size lenses.

The PCR-based SCAR marker linked to *Ph1* locus was used to identify presence and absence of *Ph1* locus (Qu *et al.* 1998). Initially, the PCR analysis was carried out on Chinese Spring (CS), CS *ph1b* mutant, nullisomic-tetrasomic lines of CS (N5AT5B, N5DT5B and N5BT5A), hexaploid wheat (DBW 16 and PBW 502) and tetraploid wheat cultivar (HI 8498). At an annealing temperature of 56°C, the expected specific single band of ~210 bp was present in CS, N5AT5B, N5DT5B, DBW 16, PBW 502 and HI 8498, whereas the expected band was absent in CS *ph1b* mutant and nullisomic 5B line (N5BT5A). This indicated that the *Ph* locus is present on 5B chromosome of wheat and absence of amplified fragment indicates *ph1b* homozygous plants at *Ph1* locus.

This primer pair was further used to screen 154 F_2 plants of three different crosses involving *Ph* mutant line; CS *ph1b* mutant × PBW 502, CS *ph1b* mutant × DBW 16, CS *ph1b* mutant × HI 8498. Out of 58 F_2 plants from cross of CS *ph1b* mutant × PBW 502, 43 plants showed the presence of desired PCR band of ~210 bp size (Fig 1). Similarly, 49 and 28 F_2 plants from crosses CS *ph1b* mutant × DBW 16, CS *ph1b* mutant × HI 8498 respectively, showed the presence of desired PCR band. The remaining plants showed the absence of PCR product. One hundred twenty F_2 plants amplified PCR fragment of ~210 bp size, indicating the presence of *Ph1* locus (Table 1). The remaining 34 plants showed the absence of PCR fragment, indicating mutant homozygous plants at *Ph1* locus (*ph1b* mutant). The genotypes showing the presence of PCR fragment include both dominant homozygous and heterozygous plants because the PCR-based marker used in the present study was a dominant type of marker. The chi-square goodness-of-fit test showed that in three crosses marker segregated as a single gene in Mendelian ratio of 3:1 (Table 1).

Table 1 Screening of F_2 populations of different crosses with SCAR marker associated with *Ph1* locus and χ^2 analysis for goodness-of-fit test

Cross	No. of plants	Molecular marker		χ2	*P*
		+	–		
PBW502 x CS *ph1b*	58	43	15	0.879	0.30
DBW 16 x CS *ph1b*	60	49	11	0.233	0.50
HI 8498 x CS *ph1b*	36	28	8	0.700	0.30

+, Marker present; –, marker absent

To validate the accuracy of genotype scoring by the SCAR primer, chromosome pairing at meiotic metaphase I (MI) of pollen mother cells was investigated by the acetocarmine squash method. The types of chromosome pairing were counted from different PMCs for each individual. F_2 populations were scored at meiotic metaphase I for univalent, rod and ring bivalent, and higher order association (tri-, quadri-, penta-, and hexavalent). Plants which appeared to be dominant homozygous (*Ph1*/*Ph1*) during PCR assay formed predominantly 21 bivalents at MI (Fig. 2a). In contrast, plants which were diagnosed by PCR as having *ph1b/ph1b* genotype formed both bivalents and higher-order (multivalent) chromosome associations (Fig 2b)). Comparison of univalent frequency and chromosome pairing in the three crosses indicated significant reduction of univalent frequency and increase in higher association chromosomes in the absence of *Ph1* locus. This SCAR marker was also used to identify the *Ph1b* genotypes from a segregating population between cultivated winter wheat cultivar Nogada 95 and CS *Ph1b* mutant (Wang *et al.* 2000). Therefore the result of this study suggests that the marker-assisted indirect selection for the *Ph1b* locus using PC3-9 SCAR marker concurs with meiotic

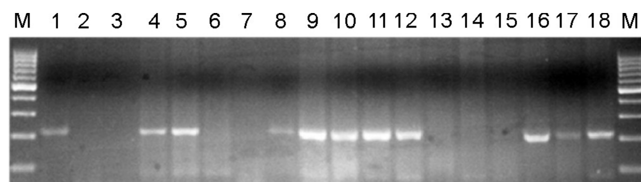

Fig 1 PCR amplification of genomic DNA of parents and F_2 population of PBW502 × Chinese Spring (*Ph1b*) with marker linked to *Ph1* locus. Lane M- 100 bp DNA ladder, 1- PBW 502, 2- Chinese Spring (*Ph1b*), 3 to 18- F_2 plants

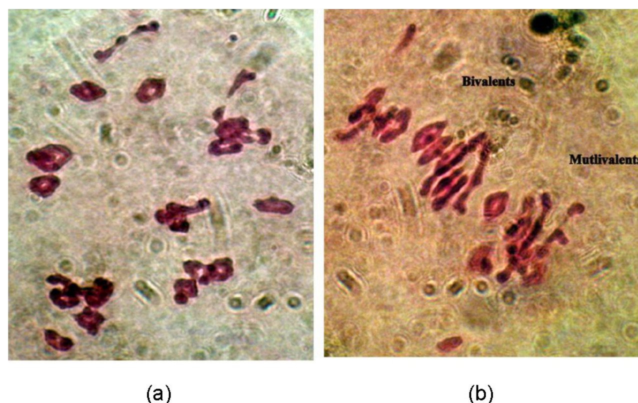

Fig 2 Chromosome pairing at meiotic metaphase I. (*a*) PBW 502 (*b*) F_2 of Chinese Spring (*Ph1b*) x PBW502.

analysis and can be used for alien gene transfer in Indian wheat genotypes having *Ph1b* locus.

SUMMARY

Despite the close relationship between three genomes of wheat, meiosis in wheat is characterized by formation of bivalents only. The homologous pairing of polyploidy wheat is controlled by *Ph1* (*pairing homologous*) locus located on long arm of chromosome 5B which suppresses pairing and recombination between homoeologous chromosomes. However, similar genes have also been identified in *T. turgidum* and *T. timopheevii* with lesser impact. Cytogenetic manipulations for suppressing the *Ph1* pairing regulation would be necessary to bring about the desired chromosome pairing and hence alien gene transfers into cultivated wheat. In the present study, PCR based marker was used to transfer the recessive mutation in *Ph1* locus (*Ph1b*) from bread wheat cultivar Chinese Spring to Indian bread and durum wheat cultivars. The availability of *Ph1b* locus in Indian wheat genetic background will help in transfer of alien genes of economic and agronomic importance

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