A DNA Fragment Mapped Within the Submicroscopic Deletion of *Ph1*, a Chromosome Pairing Regulator Gene in Polyploid Wheat

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ABSTRACT

Bread wheat is an allohexaploid consisting of three genetically related (homoeologous) genomes. The homoeologous chromosomes are capable of pairing but strict homologous pairing is observed at metaphase 1. The diploid-like pairing is regulated predominantly by *Ph1*, a gene mapped on long arm of chromosome 5B. We report direct evidence that a mutant of the gene (*ph1b*) arose from a submicroscopic deletion. A probe (XksuS1-5) detects the same missing fragment in two independent mutants *ph1b* and *ph1c* and a higher intensity fragment in a duplication of the *Ph1* gene. It is likely that XksuS1-5 lies adjacent to Ph1 on the same chromosome fragment that is deleted in *ph1b* and *ph1c*. XksuS1-5 can be used to tag *Ph1* gene to facilitate incorporation of genetic material from homoeologous genomes of the Triticeae. It may also be a useful marker in cloning *Ph1* gene by chromosome walking.

MORE than 50% of all higher plants, including some of the most important crop plants, are polyploid (AVERETT 1980). Bread wheat (*Triticum aestivum* L. em. Thell.) is an allohexaploid comprised of three homoeologous genomes A, B and D such that an extra dosage (tetrasomy) of a chromosome can compensate for the deficiency (nullisomy) of either of the two homoeologous chromosomes (SEARS 1952). In spite of the homoeology among chromosomes of the different genomes, strict homologous pairing is observed at metaphase I (MI). The diploid-like pairing is under precise genetic control (RILEY and CHAPMAN 1958; SEARS and OKAMOTO 1958). Several genes, both with major and minor effects, regulate chromosome pairing of wheat with the principle control exercised by a locus designated *Ph1* (*Pairing homoeologous*), located on the long arm of chromosome 5B (5BL) (RILEY and CHAPMAN 1958; SEARS 1977). The *Ph1* gene effect is manifested in the hemizygous condition in euhaploid wheat ($2n = 3X = 21$) where only univalents are observed at MI. Haploids nullisomic for 5B ($2n = 20$) have mean pairing of 7.50 univalents, 3.83 bivalents, 1.50 trivalents and occasional higher order associations (RILEY 1960). To the contrary, plants tri-isosomic for 5BL (six doses of *Ph1* gene), show reduced chiasma frequency and more frequent interlocking of bivalents as compared to normal wheat (two doses of *Ph1*) (FELDMAN 1966; YACOBI, MELLO-SAMPYO and FELDMAN 1982).

Several hypotheses have been proposed to explain the mode of action by which the *Ph1* gene regulates chromosome pairing. RILEY (1968) proposed a "two-step" chromosome pairing hypothesis based on the contention that homologous and homoeologous pairing was separated during meiotic prophase I. During the first step (attraction stage) chromosomes could associate irrespective of their homoeology relationship while in the second step only homologous chromosomes could pair precisely. According to the hypothesis, the *Ph1* gene acts by regulating the duration of the first step.

According to another hypothesis, the *Ph1* gene acts by suppressing chromosome association among homoeologous rather than homologous chromosomes during mitotic division preceding meiosis (FELDMAN 1968; YACOBI, LEVANONY and FELDMAN 1985). Based on the observation of the phenocopy effect of *Ph1* and colchicine, AVIVI and FELDMAN (1980) suggested that the *Ph1* gene inhibits homoeologous pairing through the premeiotic mitotic spindle system. In contrast, DRISCOLL, BIELIG and DARVEY (1979) proposed that the *Ph1* gene does not act during premeiotic mitotic stage, but during prophase I of meiosis to resolve the multivalents which are always formed irrespective of the presence of *Ph1* gene. Electron microscopic analysis of synaptonemal complexes at the zygotene/pachytene stage of prophase I confirmed the occurrence of multivalents in the presence of the *Ph1* gene (HOBOLTH 1981; HOLM, WANG and WISCHMANN 1988).

Independent mutants of *Ph1* gene, *ph1b* in hexaploid wheat and *ph1c* in tetraploid wheat, have been recovered using X-ray irradiation. Both mutants may be interstitial deletions in the 5BL arm (SEARS 1977; GIORGI and CUOZZO 1980; GIORGI and BARBERA 1981). We report that the *ph1b* mutation arose from a submicroscopic, interstitial deletion and have identified a DNA fragment that maps within the deleted region, both in *ph1b* and *ph1c*.

MATERIALS AND METHODS

Genetic/aneuploid stocks: The two mutants of *Ph1* gene, used in the study, have been recovered independently using X-ray irradiation. A mutant (*ph1b*) in hexaploid wheat cultivar 'Chinese Spring' (CS) was obtained by irradiating seeds (SEARS 1977). The mutant was predicted to be an interstitial deletion 1 cM from the centromere (SEARS 1977). Another mutant of *Ph1* gene (*ph1c*), also an interstitial deletion, was recovered in tetraploid wheat cultivar 'Capelli' (GIORGI and BARBERA 1981). From the same experiment, GIORGI and BARBERA (1981) also recovered a plant possessing a duplication of an interstitial region of chromosome 5BL. The duplicated chromosome region in this line encompasses the *Ph1* gene. These three mutant lines were used to identify DNA fragment cosegregating with the *Ph1* gene. Wheat group 5 nullisomic-tetrasomic and long arm ditelosomic lines of wheat cultivar 'CS' (SEARS 1954) were used to map DNA fragments to their respective arms of group 5 chromosomes.

DNA analysis: All the DNA analysis procedures used during the study have been described (GILL *et al.* 1991). Since the clones used were generated from a genomic library of *Aegilops squarrosa* L. (the D-genome progenitor species of wheat), the DNA filters were washed at low stringency (1 × SSPE, 0.5% sodium dodecyl sulfate (SDS) at 37°) after hybridization.

RESULTS AND DISCUSSION

SOUTHERN (1975) analysis of restriction digested genomic DNA of normal 'CS,' *ph1b* mutant, and the group 5 nullisomic-tetrasomic and long arm ditelosomic chromosome stocks was performed using wheat homoeologous group 5 specific DNA clones (GILL *et al.* 1991). A DNA probe (XksuS1-5), from a genomic library of *A. squarrosa*, was identified which detected a single fragment for each of the long arms of chromosomes 5A, 5B and 5D (Figure 1). The chromosome 5B specific fragment, which was present in 'CS,' was missing in *ph1b* mutant. No differences were observed between 'CS' and *ph1b* by similar analysis using 11 other wheat chromosome group 5 long arm specific probes (data not shown), indicating presence of a deletion in the mutant chromosome 5BL.

To rule out the possibility of a deletion, independent of *ph1b* mutant, we used the *ph1c* mutant. The mutation is a deletion of a part of the 5BL chromosome region and may have originated as a consequence of unequal crossing over (DVORÁK, CHEN and GIORGI 1984). A duplication line for the same chromosome region was also recovered. The duplication line was later confirmed to possess four doses of *Ph1* gene on chromosome 5B (JAMPATES and DVORÁK 1986). Evidence from Southern analysis of the deletion and the duplication lines with the XksuS1-5 probe supported the above results. A chromosome 5BL-specific fragment was missing in the deletion line (*ph1c*) and was present at twice the intensity in the duplication line (Figure 2).

Similar morphology and the banding patterns (GILL and KIMBER 1974) of mitotic metaphase chromosome 5B of normal 'CS' and of *ph1b* mutant (Figure 3) suggest the deletion to be submicroscopic (less than 0.2 μm). We predict that the deletion encompasses less than 14 million base pairs of DNA as 1 μm length

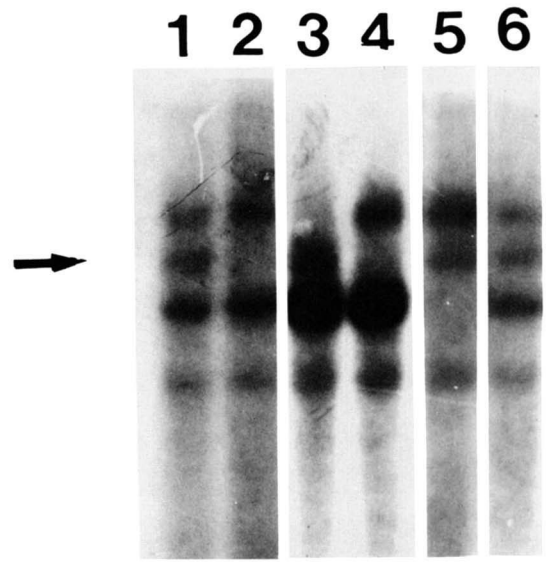

FIGURE 1.—Fifteen micrograms of *Hin*dIII-digested genomic plant DNA were separated on 0.8% agarose gel, transferred to MSI membrane, and hybridized with ^{32}P-labeled XksuS1-5 probe. The lanes are: 1, Chinese Spring (CS); 2, *ph1b* mutant 'CS'; 3, nullisomic 5A-tetrasomic 5B (NT5A(5B)); 4, NT5B(5D); 5, NT5D(5A); and 6, ditelosomic 5BL. Hybridization was performed in 50% formamide, 6 × SSPE, and 0.5% SDS; and the filter was washed at low stringency (1 × SSPE, 0.5% SDS at 37°). The probe is derived from a *Pst1* genomic DNA library of *A. squarrosa*. The missing chromosome 5BL-specific fragment of 'CS,' in *ph1b* is indicated by the arrow.

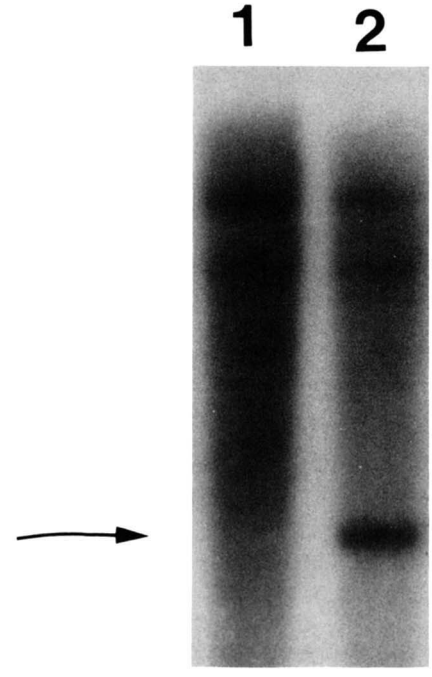

FIGURE 2.—Fifteen micrograms of *Eco*RI-digested genomic DNA of: 1, 'Capelli' *ph1c* mutant, and 2, 'Capelli' *Ph1* duplication line, probed with XksuS1-5 as described in Figure 1. The chromosome 5BL-specific fragment present in the duplication line is missing in the *ph1c* mutant (arrow).

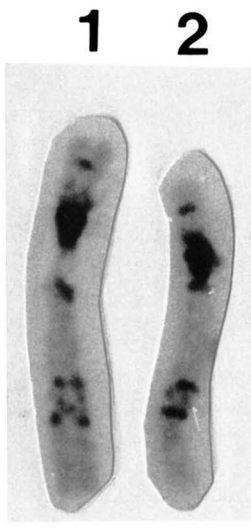

FIGURE 3.—The N-banded (ENDO and GILL 1984) root tip metaphase chromosome 5B of 'CS' and *ph1b* mutant. Note the general similarity of chromosome morphology and banding pattern indicating the deletion to be submicroscopic.

of metaphase chromosome of wheat corresponds to about 70 million base pairs of DNA (estimated from genomic DNA content of 16 billion base pairs divided by 250 μm, total length of the chromosome complement of wheat).

Cloning genes with unknown products is difficult particularly in the crop plants with large genomes. However, cloning of the *Ph1* gene should be feasible by "reverse genetics" (ORKIN 1986) since the gene has already been bracketed by a small deletion. We intend to make a contiguous map of the deleted region and then "walk" toward the gene. The identification of the *Ph1* gene sequence would allow thorough analysis of the various postulated mechanisms of mode of action of the gene.

One immediate application of our finding is in chromosome engineering experiments where *ph1*-induced chromosome pairing is a key step in the transfer of desirable traits from alien genera into wheat. The use of *ph1* mutant in such introgression experiments has been difficult in part due to the difficulty in scoring for the mutant. The use of XksuS1-5 should make gene scoring more accurate and efficient as relatively large populations can be screened rapidly in order to find the desirable genotype.

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