A DNA Fragment Mapped Within the Submicroscopic Deletion of Phl, 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 I. The diploid-like pairing is regulated predominantly by Phl, a gene mapped on long arm of chromosome 5B. We report direct evidence that a mutant of the gene (phlb) arose from a submicroscopic deletion. A probe (XksuS1-5) detects the same missing fragment in two independent mutants phlb and phlc and a higher intensity fragment in a duplication of the Phl gene. It is likely that XksuS1-5 lies adjacent to Phl on the same chromosome fragment that is deleted in phlb and phlc. XksuS1-5 can be used to tag Phl gene to facilitate incorporation of genetic material from homoeologous genomes of the Triticeae. It may also be a useful marker in cloning Phl 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 Phl (Pairing homoeologous), located on the long arm of chromosome 5B (5BL) (Riley and Chapman 1958; Sears and Okamoto 1958). The Phl 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 1969). To the contrary, plants tri-isosomic for 5BL (six doses of Phl gene), show reduced chiasma frequency and more frequent interlocking of bivalents as compared to normal wheat (two doses of Phl) (Feldman 1966; Yacobi, Mello-Sampyo and Feldman 1982).

Several hypotheses have been proposed to explain the mode of action by which the Phl gene regulates chromosome pairing. Riley (1968) proposed a "two-step" chromosome pairing hypothesis based on the conention 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 Phl gene acts by regulating the duration of the first step.

According to another hypothesis, the Phl 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 Phl and colchicine, Avivi and Feldman (1980) suggested that the Phl gene inhibits homoeologous pairing through the premeiotic mitotic spindle system. In contrast, Driscoll, Bieleg and Darvey (1979) proposed that the Phl 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 Phl 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 Phl gene (Hobolth 1981; Holm, Wang and Wischmann 1988).

Independent mutants of Phl gene, phlb in hexaploid wheat and phlc in tetraploid wheat, have been recovered using X-ray irradiation. Both mutants may be interstitial deletions in the 5BL arm (Sears 1977; Giorghi and Cuozzo 1980; Giorghi and Barbera 1981). We report that the phlb mutation arose from a submicroscopic, interstitial deletion and have identified a DNA fragment that maps within the deleted region, both in phlb and phlc.
MATERIALS AND METHODS

Genetic/aneuploid stocks: The two mutants of Phl gene, used in the study, have been recovered independently using X-ray irradiation. A mutant (phlb) 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 Phl gene (phlc), 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 Phl gene. These three mutant lines were used to identify DNA fragment cosegregating with the Phl 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 X SSPE, 0.5% sodium dodecyl sulfate (SDS) at 37°C) after hybridization.

RESULTS AND DISCUSSION

SOUTHERN (1975) analysis of restriction digested genomic DNA of normal 'CS,' phlb 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 phlb mutant. No differences were observed between 'CS' and phlb 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 phlb mutant, we used the phlc 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 Phl 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 (phlc) 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 phlb 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.
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 Phl 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 Phl 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 phl-induced chromosome pairing is a key step in the transfer of desirable traits from alien genera into wheat. The use of phl mutant in such introgression experiments has been difficult in part due to the difficulty in scoring 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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LITERATURE CITED


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