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Identification of a chromosome-specific probe that maps within the *Ph1* deletions in common and durum wheat

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Abstract The *Ph1* (pairing homoeologous) gene is the major factor that determines the diploid-like chromosome behavior of polyploid wheat. This gene, which is located on the long arm of chromosome 5B (5BL), suppresses homoeologous pairing at meiosis while allowing exclusive homologous pairing. In an effort to tag the specific chromosomal region where this gene is located, we have previously microdissected chromosome arm 5BL from bread wheat and produced a plasmid library by random PCR amplification and cloning. In this work we isolated from this library a 5BL-specific probe, WPG90, and mapped it within the interstitial deleted chromosome fragments carrying *Ph1* in common and durum wheat. A PCR assay of *Ph1* based on WPG90 was developed that allows an easy identification of homozygous genotypes deficient for this gene.

Key words Chromosome-specific DNA sequence · *Ph1* gene · Wheat

Introduction

The gene system that determines the cytological diploid-like meiotic behavior of polyploid wheat has long been a subject for intensive cytogenetic studies (for reviews see Riley 1960, 1966; Sears 1976; Feldman 1993). The major gene in this system is *Ph1* (pairing homoeologous), which is located on the long arm of chromosome 5B (5BL) (Okamoto 1957) in hexaploid and tetraploid wheat.

By X-irradiating pollen of the common wheat cultivar 'Chinese Spring' (CS), Sears (1977) induced a recessive mutation in the *Ph1* locus, designated *ph1b*, which allows homoeologous pairing in common wheat and a high level of pairing in interspecific and intergeneric hybrids. The *ph1b* mutation, resulting from a submicroscopic interstitial deletion that includes the *Ph1* locus (Sears 1977; Gill and Gill 1991), is located near the middle of chromosome arm 5BL, about 1.0 centiMorgan (cM) (Sears 1984) to several centiMorgans (Gill et al. 1993) from the centromere. Another high-pairing mutation, *ph1c*, was induced by X-irradiation of the durum cultivar 'Cappelli' (Giorgi 1978). This mutation is a microscopic deletion in the middle of the 5BL arm that includes the *Ph1* locus (Dvorak et al. 1984; Jampates and Dvorak 1986).

We report here the characterization of a sequence, WPG90, that has been found to be missing in both hexaploid and tetraploid plants homozygous for *ph1b* and *ph1c*, respectively.

Materials and methods

Source of the probe

We have previously microdissected chromosome arm 5BL from the common wheat cultivar 'Chinese Spring' (CS) and produced a plasmid library in pGEM-T (Promega) by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) amplification and cloning (Vega et al. 1994; B. Liu et al., in preparation). A 279-bp random clone, WPG90, was isolated. For preparation of the probe, the plasmid insert was amplified by PCR, purified with a PCR purification kit (Wizard, Promega, USA), and radiolabeled (Feinberg and Vogelstein 1984).

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Results and discussion

Chromosomal location of WPG90

When hybridized to a blot carrying *Eco*RI-digested DNA from cvs CS and 'Cappelli', the WPG90 probe revealed a major polymorphic band ranging from 7.6 kb in CS to 5.4 kb in 'Cappelli' (Fig. 1). This band was missing from DNA of plants deficient for chromosome 5B, i.e. N5BT5D, but present in DNA of plants deficient for the homoeologous chromosomes 5A, i.e. N5AT5B (Fig. 1) and 5D (data not shown). Considering that this band was present in DT5BL, we concluded that WPG90 is 5BL-specific. Moreover, being absent from DNA of both *ph1b* and *ph1c* mutants (Fig. 1), WPG90 detects a 5BL-specific fragment that maps within the *Ph1* deletions of the two mutants. WPG90 also recognizes two minor bands in CS (only one band in *T. turgidum* var '*durum*') that have not yet been assigned to specific chromosomes.

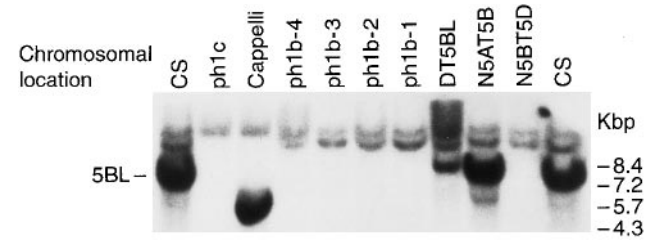

Fig. 1 Southern analysis using the chromosome arm 5BL-specific probe WPG90 derived from a 5BL chromosome-arm library. This probe was hybridized to *Eco*RI-digested DNA of the following lines: *CS* the standard hexaploid wheat cultivar 'Chinese Spring', *N5BT5D* nullisomic 5B-tetrasomic 5D line of CS, *N5AT5B* nullisomic 5A-tetrasomic 5B line of CS, *DT5BL* ditelosomic 5BL line of CS, *ph1b -1* to *-4*, four different mutant individual plants of CS deficient for *Ph1*, Cappelli the tetraploid wheat *T. turgidum* var '*durum*' cv 'Cappelli', *ph1c* a mutant line of *T. turgidum* var '*durum*' cv 'Cappelli' deficient for *Ph1*.

Distribution of WPG90 in wheat and related species

The occurrence of this sequence was studied in a representative sample of hexaploid and tetraploid wheats of diverse origin and in their diploid progenitors, and in other *Gramineae* species (data not shown). WPG90 was detected in all species studied including rye, wheatgrass, barley, and even rice. Fragment length polymorphism was noticed in most species at all ploidy levels. Considering all data, we conclude that WPG90 is an ancient sequence that appeared very early in the evolution of the Gramineae.

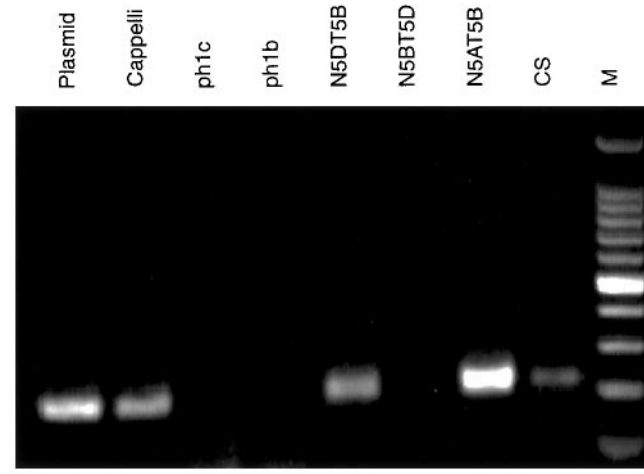

Fig. 2. PCR amplification of genomic DNA from the plant materials of Fig. 1 with WPG90- specific primers. *M* 100-bp DNA ladder

A PCR assay of *Ph1* based on WPG90

Several probes that map to the *Ph1* deletions have recently been isolated (Gill and Gill 1991; Clarke et al. 1992; Gill et al. 1993; Dunford et al. 1995). Hybridization of these probes to wheat genomic DNA revealed several bands, of which only some are due to a fragment(s) in the *Ph1* deletions; the other bands are due to fragments on the homoeologous chromosomes 5A and 5D as well as on non-homoeologous chromosomes. WPG90, however, is *Ph1*-specific, revealing a single major band when hybridized to *Eco*RI-digested genomic DNA of common and durum wheat.

Evidently, a screening assay based on PCR incorporates many advantages. The feasibility of this approach to select *Ph1* mutants was recently demonstrated (Gill and Gill 1996). We therefore sequenced WPG90 and designed a PCR protocol to amplify this target in wheat genomic DNA. The 50-µl reaction mixture included 200 ng of DNA, 120 ng of forward (5′-TAATTAGAAAGAATCTAATACGTTAGT) and reverse (5′-TTTCTGAGATCTGTTAAGTTTAATTTT) primers, 0.2 m*M* dNTPs, 1.5 m*M* $MgCl_2$, 1 × *Taq* polymerase buffer, and 2 units of *Taq* DNA polymerase (Promega). The temperature conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min. The amplified products were fractionated on a 2% agarose gel and visualized by ethidium bromide staining and UV irradiation (Fig. 2). A unique band of the expected size (202 bp) was observed in CS, N5AT5B, N5DT5B, cv 'Cappelli' and a positive control of plasmid DNA; no band was detected when DNA from N5BT5D, *ph1b* and *ph1c* was amplified. Hence, in view of the specific mapping of WPG90 to the *Ph1* deletions, this probe can be utilized as a marker for *Ph1* in wheat breeding, allowing easy identification of homozygous genotypes deficient for this gene. It could also be useful in molecular approaches aiming to isolate the *Ph1* gene.

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