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Genetic mapping between *Gli-B1* locus and a telomeric C-heterochromatin band in wheat

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Summary. Using C-banding it has been possible to prove that the bread wheat varieties 'Holdfast' and 'Capelle-Desprez' shows an intense band of telomeric heterochromatin on the short arm of chromosome *1B*, while the variety 'Pané-247' presents a very thin band. Gliadin study using pH-acid electrophoresis revealed the existence of differences in the *Gli-B1* locus in the three varieties. Analysis of the progeny of the (P × H) × CD hybrid revealed recombination between the heterochromatin C-band and locus *Gli-B1*, and allowed the genetic distance between the two markers to be calculated as 6.55 ± 3.16 cMorgan. This is the first time the genetic distance from a locus to the chromosome telomere has been directly obtained in wheat. The heterochromatin C-band studied here gives us a cytological marker on chromosome *1B* that can be used as a reference point in the localization of other genes.

Key words: Genetic mapping – Gliadin – C-heterochromatin – *Triticum* – Polyacrylamide gel electrophoresis

Introduction

Some of the endosperm storage proteins in *Triticum aestivum* are coded by genes in chromosome *1B*. Locus *Glu-B1*, which codes for high-molecular-weight glutenin subunits, is found on the long arm, while the *Gli-B1* and *Glu-B3* loci are located on the short arm (Payne 1987). Payne et al. (1984), using a wheat line that presented a satellite deletion on chromosome *1B*, were able to locate the *Gli-B1* locus on the satellite. What is more, in addition to genes for endosperm protein, other genes such as *Lr* (Dyck et al. 1985), *Yr* and *Rg1* (Payne et al. 1986), *Rfe* (Snape et al. 1985), etc., have been located on chromosome *1B*. Linkage relationships among these loci have been established and measured in order to construct a genetic map for chromosome *1B*. Cytological markers such as NOR (Payne et al. 1984), the centromere, and translocation points (Sing and Shepherd 1988 a, b) have also been used, although linkage intensity between two genes does not necessarily correlate well with their physical position on the chromosome.

A cytological marker, consisting of a polymorphic heterochromatin C-band, has been used in this paper to mark the telomere of the short arm of chromosome *1B*, and the genetic distance between this band and locus *Gli-B1* has been calculated.

Materials and methods

Three varieties of bread wheat (*Triticum aestivum*) were used: 'Holdfast' (H), 'Pané-247' (P), and 'Capelle-Desprez' (CD). The first step consisted in obtaining an H × P hybrid, which was then used as female parent and crossed with the variety CD. This testcross was used to score segregation between two linked markers, which were analyzed with C-banding and polyacrylamide gel electrophoresis in acid buffer, pH 3.2 (A-PAGE), as follows.

C-banding

The embryo half of each seed was germinated and the roots were treated with a monobromonaphtalene-saturated solution for one night at 4 °C, fixed in alcohol-acetic acid (3 : 1) for at least 2 months, and then stained following the method of Jouve et al. (1980).

A-PAGE

The gliadins from the endosperm half of each seed were extracted and separated following the method described by Lafiandra and Kasarda (1985), using polyacrylamide gels at 7% and aluminum lactate buffer, pH 3.2. This technique was previously applied to determine the allelic differences on endosperm proteins in the wheat varieties analyzed here (Hueros et al. 1988).

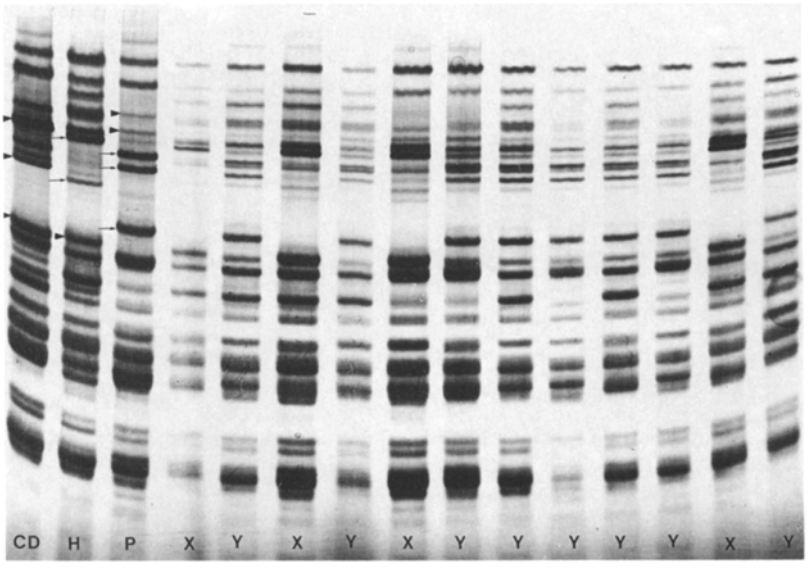

Fig. 1. A-PAGE separation of gliadins from: 'Capelle-Desprez' (CD), 'Holdfast' (H), 'Pané-247' (P), and several segregating plants coming from the testcross (P × H) × CD, carrying the *GLI-B1a* (X) or *GLI-B1b* (Y) locus. Pointed bands were used as markers for locus *Gli-B1a* and *Gli-B1b* present in H and P, respectively. *Arrowheads* point out other bands controlled by *Gli-B1* locus in the three varieties

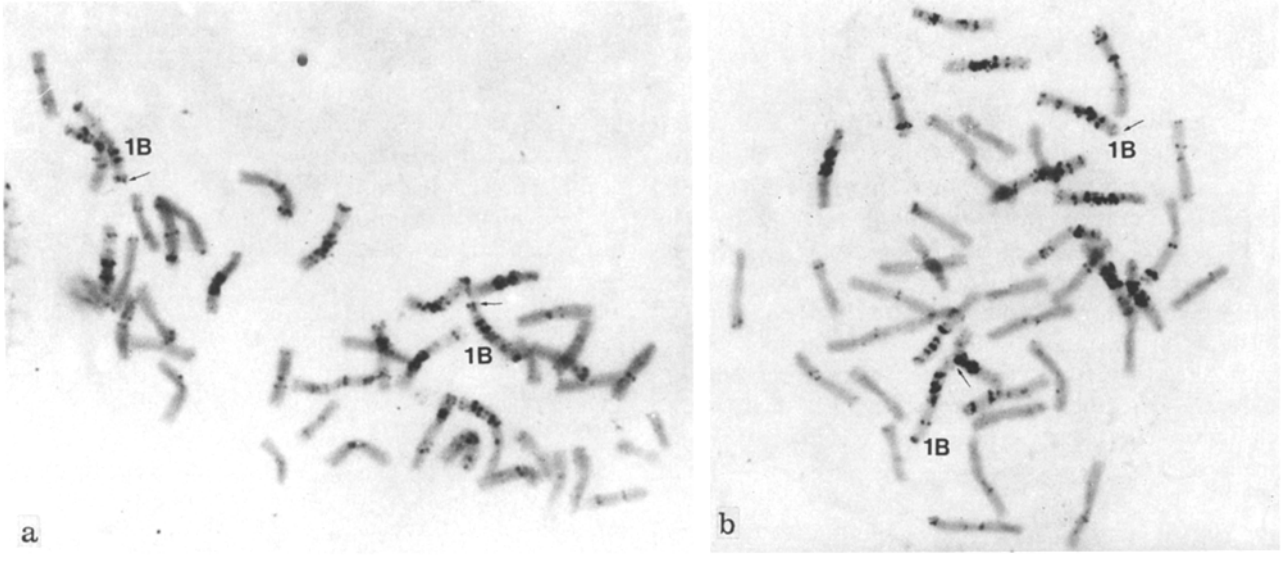

Fig. 2a and b. Microphotographs of C-banded metaphases. **a** 'Holdfast'and **b** 'Pané-247'. Chromosomes *1B* are indicated. *Arrows* point out C-a band and C-b band, respectively

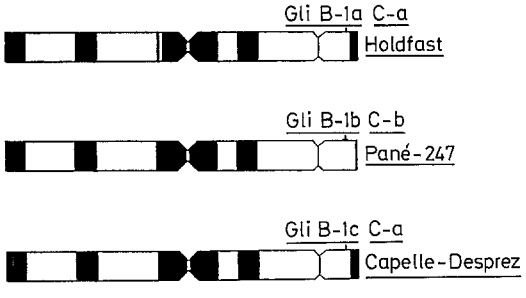

Fig. 3. Scheme of chromosome *1B* of the wheat varieties 'Holdfast' 'Pané-247' and 'Capelle-Desprez.' The intervarietal differences for both markers, *Gli-B1* and the telomeric heterochromatin, are indicated

Results

The varieties studied – H, P, and CD – presented different alleles on locus *Gli-B1*, and these were designated as *Gli-B1a* for H, *Gli-B1b* for P, and *Gli-B1c* for CD. Figure 1 shows the bands that marked the *Gli-B1* locus on each of the three varieties, when fractionated in A-PAGE. The bands used to discriminate between *Gli B-1a* and *Gli B-1b* are marked by arrows.

The satellite of chromosome *1B* showed differences in the C-band pattern among the varieties. The telomeric C-band was heavy in H (Fig. 2a) and CD varieties, and was designated *C-a*. In P the telomeric C-band was

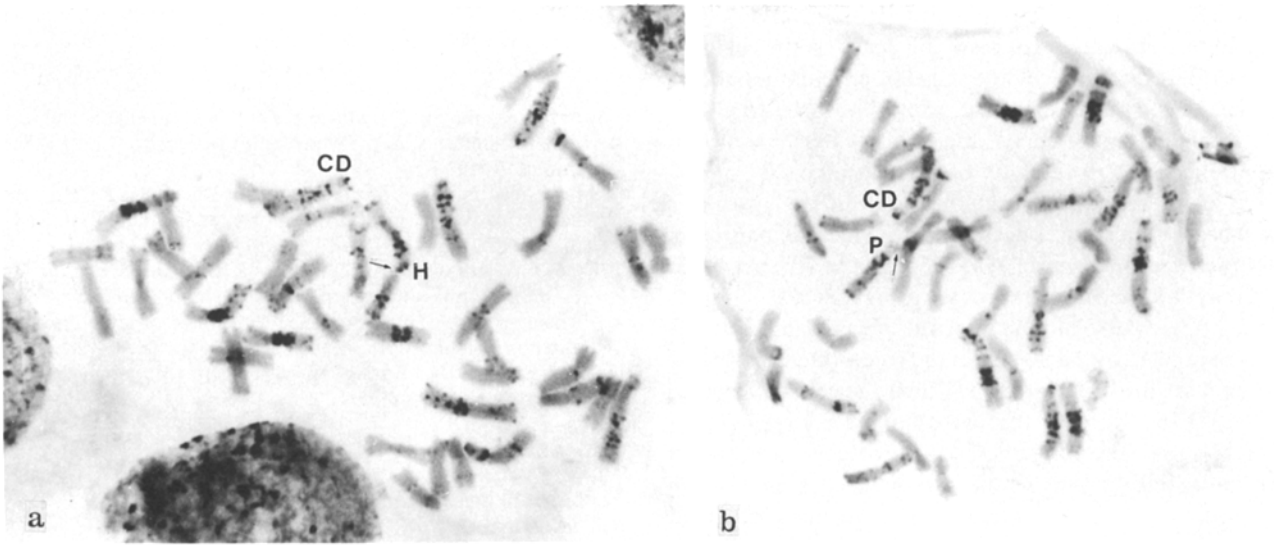

Fig. 4. Microphotographs of C-banded metaphases of trihybrids. **a** Cell containing C-a band (*arrow*) of chromosome *1B* from H and chromosome *1B* of CD. **b** Cell containing C-b band (*arrow*) of chromosome *1B* from P and chromosome *1B* of CD

Table 1. Results from A-PAGE and C-banding of the trihybrids analyzed

	Gli-B1a and C-a band	*Gli-B1b* and C-b band	*Gli-B1a* and C-b band	*Gli-B1b* and C-a band
No. of seeds	24	33	3	1

so weak as to be almost invisible (Fig. 2b), and was designated *C-b*. Figure 3 represents chromosome *1B* of each variety with indication of the markers *Gli B-1* and C-band used in this work.

Genetic maps have been constructed largely through the use of backcross generations. The intervarietal cross used in this work has the following constitution for the gene and heterochromatin markers: (*Gli-B1a C-a*/ *Gli-B1b C-b*) × *Gli-B1c C-a*, and can be used as a test-cross to estimate the map distance between the markers, based on the recombination value in the female gametogenesis.

In the search for the phenotypic classes that emerge in both *Gli-B1* and the telomeric C-band, the segregation in 61 (H × P) × CD offspring was studied (Tab. 1). Since the endosperm has a triploid nature, the proteins from the variety used as the pollinator, CD, had a 50% relative staining intensity with respect to the H or P proteins. Karyologically, they presented one chromosome *1B* from CD, and another chromosome *1B* that could present the telomeric C-band from H *C-a* (Fig. 4a) or else the *C-b* band and correspond to P (Fig. 4b).

The results of recombination that took place between both the linked marker gene *Gli-B1* and the heterochromatin C-band on the short arm *1B* are given in Table 1. From these data we have calculated the genetic distance between locus *Gli-B1* and the telomere of the short arm *1B* by the maximum likelihood method; this distance was 6.55 ± 3.16 cMorgan.

Discussion

To construct a cytological map of wheat chromosomes, different cytological markers such as NOR (Payne et al. 1984), the centromere (Sing and Shepherd 1988a, b), or heterochromatin bands (Linde-Laursen 1982; Dvořák and Appels 1986) have been previously used. Dvořák and Chen (1984) used the telomere indirectly when calculating the distance between locus *Gli-B2* and the telomere of the short arm of chromosome *6B*, by subtracting the genetic distance between two markers and the centromere from the total arm length, which they estimated from the recombination value.

It has been shown that in some species, the chiasma distribution is not random, and that the recombination frequency is very low in the regions close to the centromere and increases distally. Linde-Laursen (1982) found a very low recombination rate in the regions closest to the centromere in barley. In wheat, Payne et al. (1984) found that *NOR-1* and *Glu-B1*, although on different chromosome arms, were 22 cMorgan apart, while *NOR-1* and *Gli-B1*, found on the satellite of chromosome *1B*, where 46 cMorgan apart (Snape et al. 1985). Similar results were obtained by Dvořák and Appels (1986) in chromosome *6B* when they calculated the map distance between *NOR-2* and the centromere and between *NOR-2* and *Gli-B2*. These findings indicate that recombination preferentially takes place in the distal part of the chromosomes in wheat. Thus, the telomere could serve better

than the centromere or other interstitial markers as a useful reference point for mapping genes on the chromosomes. The available telomeric heterochromatin band to mark the end of the short arm of chromosome *1B* allows us to achieve this objective, and also gives us an additional marker for a new region of chromosome *1B*.

The intervarietal hybrids between different varieties of wheat often show a decrease in the meiotic pairing in MI (Schlegel and Mettin 1981; Mettin and Kimber 1983; Ferrer et al. 1986). In chromosome *6B* of wheat, Dvořák and Appels (1986) found that the lower pairing at MI was paralleled by a decrease in the recombination rate. If this occurs in chromosome *1B*, then the genetic distance (6.55 ± 3.16 cMorgan) that we obtained between *Gli-B1* and the telomere of the short arm of chromosome *1B* probably underestimates the real distance.

Moreover, the recombination frequencies obtained using either translocation or telosomic lines may not always coincide (Payne et al. 1982; Sing and Shepherd 1988 b), since chiasma formation can be altered when chromosomes with a single arm or translocated segments are used. This is why the use of complete chromosomes is preferable. Chromosomes *1B* used in this study present no visible translocations nor deletions, and therefore the distortion or the recombination frequency should be minimal.

Since chiasma distribution in wheat is not random, genetic and cytological maps that attempt to locate genes along the chromosome are clearly in contradiction. The development of an effective methodology for in situ hybridization of single-copy genes will permit the genes to be precisely located at specific sites on the chromosomes. The possibility of comparing this information with the resulting genetic maps will help solve their contradictions.

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