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Identification of *Haynaldia villosa* chromosomes added to wheat using a sequential C-banding and genomic in situ hybridization technique

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Abstract Genomic in situ hybridization (GISH) offers a convenient and effective method for cytological detection, but can not determine the identity of the chromosomes involved. We integrated C-banding with GISH to identify *Haynaldia villosa* chromosomes in a wheat background. All chromosomes of *H. villosa* showed C-bands, either in telomeric regions or in both telomeric and centromeric regions, which allowed unequivocal identification of each *H. villosa* chromosome. The seven pairs of *H. villosa* chromosomes were differentiated as 1–7 according to their characteristic C-bands. Using a sequential C-banding and GISH technique, we have analyzed somatic cells of F_3 plants from the amphiploid *Triticum aestivum-H. villosa* × 'Yangmai 158' hybrids. Three plants (94009/5-4, 94009/5-8 and 94009/5-9) were shown to contain *H. villosa* chromosome(s). 94009/5-4 (2n = 45) had three *H. villosa* chromosomes (2, 3 and 4); 94009/5-8 (2n = 45) possessed one chromosome 4 and a pair of chromosome 5, and 94009/5-9 (2n = 43) was found to have one chromosome 6 of *H. villosa*. The combination of GISH with C-banding described here provides a direct comparison of the cytological and molecular landmarks. Such a technique is particularly useful for identifying and localizing alien chromatin and DNA sequences in plants.

Key words *Haynaldia villosa* · *Triticum aestivum* · C-banding · Genomic in situ hybridization · Alien chromosome addition

Introduction

Haynaldia villosa (*Dasypyrum villosum* L.), an annual allogamous diploid species (2n = 14) native to the Mediterranean and Caucasus areas, possesses many important agronomic traits such as resistance to powdery mildew (Liu 1988), leaf and stem rust (Hyde 1953), and take-all fungus (*Gaeumannomyces graminis*) (Linde-Laursen et al. 1973) as well as winter hardiness and drought tolerance (Blanco et al. 1983), all of which are attractive for wheat improvement. Many researchers have carried out studies on the crossability of *H. villosa* with species of *Triticum* including common wheat (see review by Friebe et al. 1987), and the chromosomes and genes of *H. villosa* have been introduced into wheat cultivars (Hyde 1953; Driscoll 1983; Liu 1988).

With the introduction of alien chromatin into wheat, convienient and effective detection methods are essential to monitor the behavior of the alien chromosomes. Chromosome-banding techniques are powerful tools for the identification of alien chromosomes and chromosome fragments introgressed into wheat cultivars (Gill and Sears 1988). In the Triticeae, C-banding is the most widely applicable method, and has been used to identify all 21 pairs of chromosomes in common wheat (Endo 1986; Gill et al. 1991); it also permits differentiation of *H. villosa* chromosomes in a wheat background (Friebe et al. 1987). Nevertheless, it is often difficult to interpret chromosome banding patterns, especially when structural changes are involved in complex karyotypes, such as the translocation of small chromosome fragments.

In situ hybridization using repeated DNA sequences as probes offers an alternative approach for cytological detection. Many cloned repeated DNA sequences are available (for a review see McIntyre et al. 1990) and some of them, which are species-specific, have been noted as useful molecular markers for identifying alien chromosomes in interspecific and intergenic hybrids of the Triticeae (Lapitian et al. 1986; McIntyre et al. 1990; Xu and Kasha 1992). However, often only specific regions of chromosomes can be localized, and considerable effort is required to isolate and clone these DNA sequences. Moreover, few repeated DNA sequences have been applicable to in situ hybridization research in *H. villosa* (McIntyre et al. 1988). A more direct approach

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is the use of total genomic DNA as probe, i.e. genomic in situ hybridization (GISH) (Schwarzacher et al. 1989). This technique has been used to discriminate the parental genomes and chromosomes of hybrids in plants (Le et al. 1989; Schwarzacher et al. 1989; Anamthawat-Jonsson et al. 1990; Leitch et al. 1990; Zhong et al. 1991). Unfortunately, the identity of the chromosomes can not be determined when GISH is used alone.

In the present study, a sequential C-banding-GISH procedure was performed on the same cells of *H. villosa-T. aestivum* hybrids, and permitted the unequivocal identification of *H. villosa* chromosomes in a wheat background.

Materials and methods

Plant materials

The materials used included *H. villosa*, the amphiploid *T. aestivum-H. villosa*, the wheat cultivar 'Yangmai 158' and the F_3 individuals derived from the amphiploid *T. aestivum-H. villosa* × 'Yangmai 158'. *H. villosa* and the amphiploid *T. aestivum-H. villosa* were kindly provided by Prof. Li Jingming, College of Biology, Beijing Agricultural University, Beijing.

Chromosome preparation and C-banding

The chromosome preparation and C-banding technique was carried out according to Endo (1986), with the minor modification that the slides prepared were incubated in 45% acetic acid overnight at room temperature (RT) before C-banding treatment.

DNA extraction and labelling

Total genomic DNA used as probe was extracted from young leaves of *H. villosa* following the procedure described by Sharp et al. (1989). After digestion with *Eco*RI, the genomic DNAs were labelled with biotin-14-dATP by nick translation according to the manufacturer's specifications (Gibco BRL).

In situ hybridization and signal detection

Chromosomes were denatured in 70% formamide in 2 × SSC at 70 °C for 2 min, dehydrated through a cold (− 20 °C) ethanol series (70%, 80% and 100% ethanol, 5 min each), and then air-dried completely. Twenty microlitres of a heat-denatured probe mixture (200 ng of labelled *H. villosa* DNA, 50% formamide in 2 × SCC, 10% dextran sulphate and 0.1% SDS) were added to each slide and a plastic coverslip was placed on it. Hybridization was performed at 37 °C overnight in a moisture chamber. After hybridization, the slides were dipped in 2 × SCC at 37 °C for 5 min to float off the coverslips, and washed in the following solutions sequentially: 50% formamide in 2 × SCC for 5 min at 37 °C, 2 × SSC for 5 min at 37 °C, 2 × SSC for 5 min at RT and 1 × PBS (0.0025 M Na_2HPO_4, 0.003 M NaH_2PO_4, 0.13 M NaCl) for 2 min at RT. The sites of in situ hybridization were detected by using the following procedures. The slides were incubated at 37 °C for 1 h in 0.5% (v/v) horseradish peroxidase avidin D (Vector Laboratory) in 1 × PBS containing 5% bovine serum albumin. They were then washed twice (5 min each) in 1 × PBS, one at 37 °C another at RT. In order to amplify the intensity of the signals, the slides were incubated with 5% biotinylated antiavidin D (Vector Laboratory) in 1 × PBS for 1 h at 37 °C. After washing as above, these slides were again incubated in 0.5% horseradish peroxidase avidin D for 1 h at 37 °C. Finally, a solution of 0.05% DAB (diaminobenzidine tetrahydrochloride) and 0.01% hydrogen peroxide (200 µl each) was added for 20 min at RT. The slides were then washed with 1 × PBS for 5 min and counterstained with 5% Wright's solution in 0.03 M disodium hydrogen phosphate and 0.03 M potassium dihydrogen phosphate for 5 min.

Microscopy

C-banded cells with good metaphase spreads were recorded and photographed on Lucky film ASA/100, using an Olympus Vanox microscope. After GISH, the same cells were re-photographed and the results were compared.

Results

C-banding of *H. villosa*

The mitotic metaphase cells of *H. villosa* and the amphiploid *T. aestivum-H. villosa* were analyzed by means of the C-banding technique. The results show that all *H. villosa* chromosomes have clearly visible C-bands (Fig. 1 a), and each chromosome pair can be unequivocally identified and differentiated from wheat chromosomes according to their characteristic C-banding pattern (Fig. 2). A C-banded karyotype and a representive idiogram for *H. villosa* are present in Fig. 1 b, c.

In *H. villosa*, a C-banding polymorphism has been observed for some chromosomes including numbers 1, 3, 5 and 6, which are shown in Fig. 1 d.

In addition, a pair of chromosomes 1 R of rye, which replaced the chromosome 1 B of wheat, were identified in the amphiploid *T. aestivum-H. villosa* (Fig. 2). The chromosome 1 R derived from the wheat parent used to produce the amphiploid (Li Jingming, personal communication).

Sequential C-banding and GISH analysis

Mitotic cells of F_3 individuals from the amphiploid *T. aestivum-H. villosa* × 'Yangmai 158' hybrids were analyzed by the C-banding-GISH technique. Three of the plants were found to contain a *H. villosa* chromosome(s). These plants were numbered as 94009/5–4, 94009/5–8 and 94009/5–9 respectively.

Figure 3 illustrates a cell of 94009/5–4 treated by the sequential C-banding-GISH procedure using genomic DNA of *H. villosa* as a probe. Strong in situ hybridization signals were distributed over the entire length of the three *H. villosa* chromosomes, whereas few cross-hybridization sites were observed in wheat chromosomes (Fig. 3 b). Two of the three labelled chromosomes were identified as numbers 2 and 3, respectively, of *H. villosa* according to their C-banding patterns (Fig. 3 a); the other one, which showed faint C-bands in this cell, due to the light staining, could be identified as chromosome 4 of *H. villosa* since the characteristic C-bands of this chromosome were clearly observed in other cells analyzed.

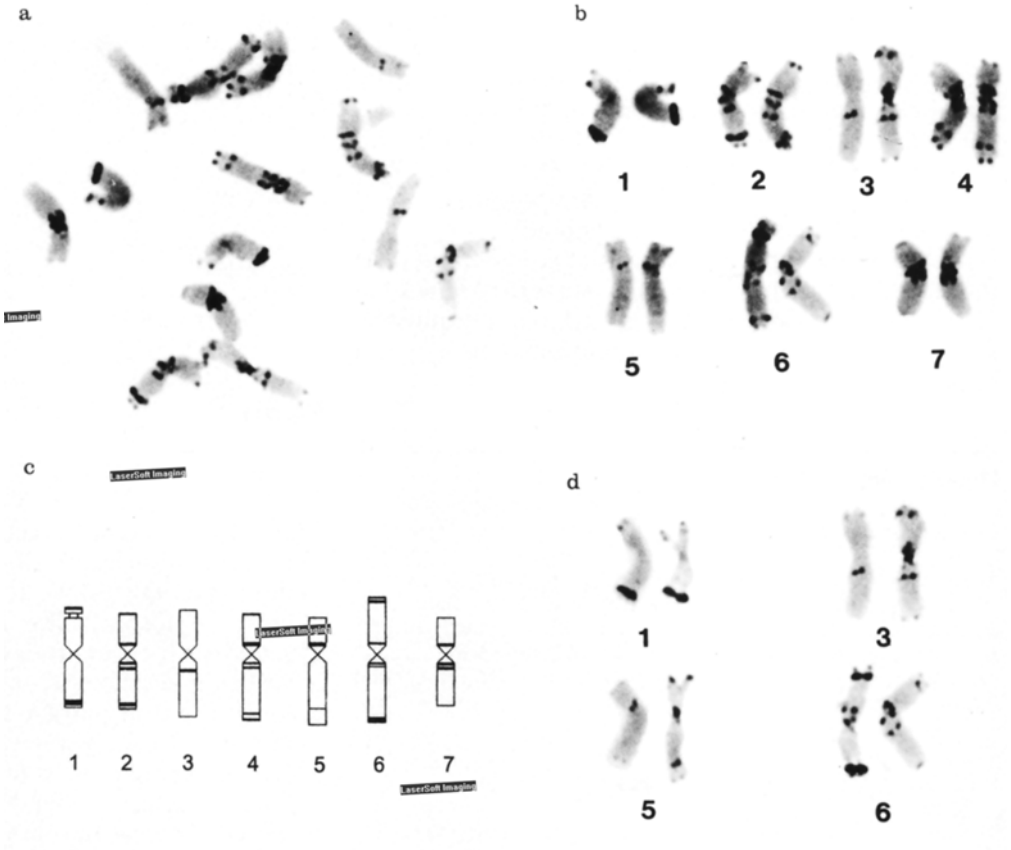

Fig. 1 **a** C-banded mitotic metaphase cell of *H. villosa*. **b** C-banded karyotype. **c** Idiogram. **d** C-banding polymorphisms of chromosomes 1, 3, 5 and 6

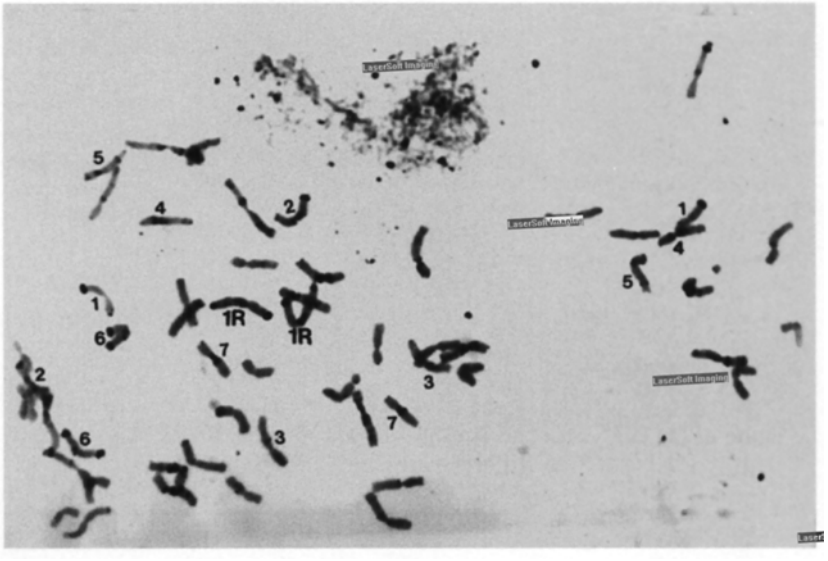

Fig. 2 C-banded mitotic metaphase cell of the amphiploid *T. aestivum-H. villosa*. The chromosome 1 R of rye and the *H. villosa* chromosomes are labelled

Three *H. villosa* chromosomes were also detected in 94009/5–8 with a chromosome number of 2n = 45. These consist of one number 4 and two number 5 chromosomes.

Forty-three chromosomes were observed in 94009/5–9. GISH showed that one *H. villosa* chromosome was present, which was identified as number 6 based on its corresponding C-bands. One cell of 94009/5–9 produced following the sequential treatments of C-banding and GISH is illustrated in Fig. 3 c, d. In this plant, chromosome 1 R, which was present in the amphiploid *T. aestivum-H. villosa*, was also detected (Fig. 3 c).

Discussion

Friebe et al. (1987) reported the C-banding pattern of *H. villosa* and designated the seven pairs of C-banded *H. villosa* chromosomes as A–G, respectively, which

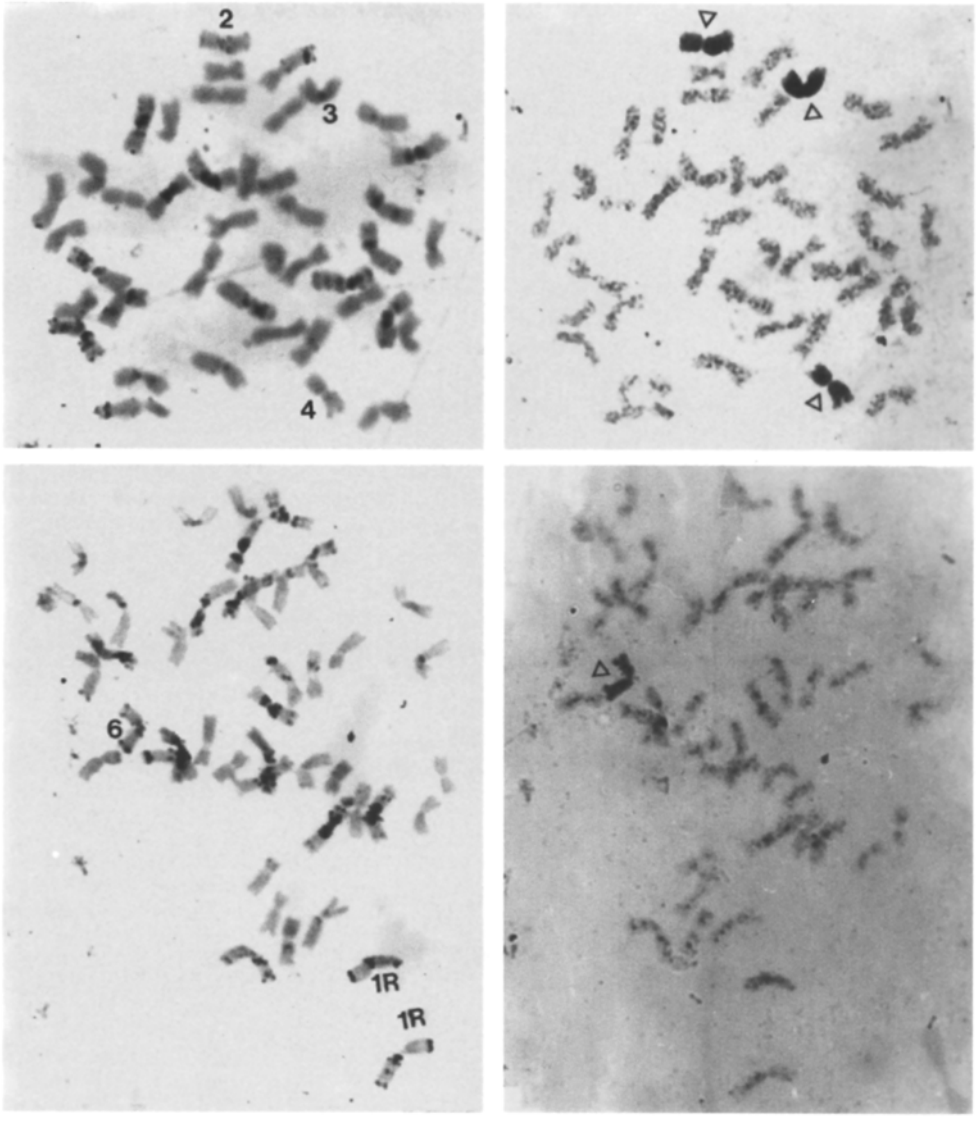

Fig. 3a–d Sequential C-banding-GISH analysis of wheat-*Haynaldia villosa* addition lines. **a** A C-banded cell of 94009/5–4 with chromosomes 2, 3 and 4 of *H. villosa*. **b** The same cell after GISH using biotin-labelled *H. villosa* DNA as a probe. **c** A C-banded cell of 94009/5–9 with chromosome 6 of *H. villosa*. **d** The same cell after GISH

corresponded to those (1H–7H) belonging to the addition lines of E.R.Sears. However, they also showed that a similarity was evident between chromosomes B (2H) and F (6H), which are sometimes difficult to separate (Friebe et al. 1987). In the present study, all *H. villosa* chromosomes could be differentiated according to their characteristic C-banding patterns, and the idiogram given in Fig. 1 d is more detailed than the one for the C-banded mitotic chromosomes of *H. villosa* described by Friebe et al. (1987), which may in part be a reflection of the greater resolution achieved with the C-banding procedure used here.

Comparing our results with those of Friebe et al. (1987), we discovered a general C-band similarity between six chromosomes, namely, 1 and 1H, 2 and 2H, 4 and 4H, 5 and 5H, 6 and 6H, 7 and 7H, with some bands stained darker than those of Friebe et al. (1987). Small variations observed for the presence and absence of some bands between these pairs of chromosomes may be due to the differential sensitivity of the heterochromatic bands to the different C-banding procedures, or the stains used (Seal and Bennett 1982). In the case of chromosome 3 and 3H, a marked difference was observed, and in our case a quite different C-banding pattern was discovered between the two members of this chromosome in a single cell of *H. villosa* (Fig. 1 a, b). This may be caused by differential heterochromatinization among homologous chromosomes of the allogamous species *H. villosa*.

In humans, various combinations of chromosome banding techniques such as G-, Q- and R-banding with in situ hybridization have been reported (see review by McNeil et al. 1991), and the simultaneous visualization of bands and DNA sequences in specific chromosome regions was also successfully achieved by such a combination (Bhatt et al. 1988), allowing direct and precise localization of DNA sequences (genes) in a specific chromosome area. However, in plants, although both chromosome banding and in situ hybridization have been applied to chromosome analysis, few reports have

been published on the sequential analysis of the same cell preparations with these two techniques (Hutchinson and Seal 1983; Jiang and Gill 1983).

Hutchinson and Seal (1983) reported a sequential ISH (using radioactive probe)-C-banding analysis on rye chromosomes, but the resolution of the subsequent C-banding was reduced compared with that obtained without prior ISH according to the authors' illustrations. Jiang and Gill (1993) also showed that sequential ISH/GISH-N-banding gave poor results, though the resolution of the sequential N-banding-ISH/GISH was excellent. In our laboratory, we tried a GISH-C-band ing procedure, and found that C-banding gave uninformative results due to the poor resolution obtained (data not shown). It is not known why the resolution of C- or N-banding is reduced after ISH/GISH. Probably, the ISH protocol results in considerable damage to chromosome structure, which in turn affects the subsequent banding results.

Our observations showed that C-banded *H. villosa* chromosomes in a wheat background were always labelled heavily when C-banding-GISH was performed, and contrasted strongly to the wheat chromosomes (Fig. 3 b, d). This indicates that the prior banding procedure has little deleterious effect on the subsequent GISH. Jiang and Gill (1993) obtained even better ISH and GISH contrast using modified N-/C-banding-ISH/GISH procedures compared with standard ISH and GISH (without prior banding) using the same probes and the same materials.

In some cells of the plants analyzed above, we have found that while the *H. villosa* chromosomes were deeply labelled and kept their form after the C-banding-GISH procedure, the unlabelled wheat chromosomes became faint. This is most likely due to the inevitable loss of DNA during the in situ hybridization treatment (Leitch and Heslop-Harrision 1992), whereas this DNA loss from the *H. villosa* chromosomes would be compensated by the labelled genomic DNA, so making the labelled *H. villosa* chromosomes appear intact. Even when chromosome outlines are not clear following GISH, chromosome analysis can be conducted since the corresponding C-banded chromosomes are available and can be compared with their counterparts subjected to GISH, thus providing one advantage for the sequential C-banding and GISH technique.

Using the sequential C-banding-GISH technique, we have demonstrated that individual chromosomes of *H. villosa* can be easily identified and quantitatively measured in a wheat background. This technique should be widely applicable for detecting and identifying small alien chromosome fragments in complex karyotypes.

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