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Identification of C-banded chromosomes in meiosis of common wheat, *Triticum aestivum* L.

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Summary. The C-banding pattern of nine meiotic chromosomes of common wheat (*Triticum aestivum* L.) as described. In F_1s of crosses between monosomics of 'Chinese Spring' and two Spanish wheat cultivars, univalent chromosomes were used to aid the recognition and analysis of the C-banding pattern for the individual chromosomes. The identification of one chromosome involved in one translocation in 'Chinese Spring' × 'Pané 247' has been made through heterochromatin bands observed in the chromosomes involved in multivalents.

Key words: C-banding – Common wheat – *Triticum aestivum* L. – Translocations – Heterochromatin

Introduction

Reports on karyotype analysis of hexaploid wheat using Giemsa staining methods have been published since 1974 (Gill and Kimber 1974; Gerlach 1977; Jewell 1979; Seal 1982; Armstrong 1982). These methods have improved the identification of individual chromosomes with respect to traditional staining techniques. However, minor observations have been carried out in meiotic chromosomes of wheat with Giemsa staining. Jewell (1979) was able to identify nine bivalents of wheat (1B to 7B, 4A and 7A) using N-banding. A study of the meiotic behavior of 14 wheat pairs (7″A + 7″B) stained with Giemsa in six hexaploid "triticale × *T. aestivum* L." hybrids has been carried out by Jouve et al. (1982). These unidentified pairs were classified in accordance with the pattern of bands and the size and form of the chromosomes.

The identification of chromosomes and arms of chromosomes involved in translocations according to their heterochromatic banding patterns using C-banding method on somatic metaphases is of interest and some attempts have been made to date (Bennett and Smith 1975; Gill and Kimber 1977). The Giemsa staining techniques could facilitate the identification of chromosomes involved in intervarietal translocations analyzing the C-banding pattern of individual chromosomes and chromosomic arms forming part of multivalents at first meiotic metaphase of the hybrids and using 'Chinese Spring' as a reference.

In the present study the feasibility of C-banding to identify chromosomes of hexaploid wheat at mitosis and meiosis is discussed. C-banding pattern, and form of individual univalents and bivalents at first metaphase of the meiosis of PMCs in critical monosomics of the progeny of mono-'Chinese Spring' × 'Pané 247' are used as identifying features. The value of C-banding in identifying chromosomes involved in one reciprocal translocation is also discussed.

Materials and methods

The material studied was the following:

1) Root tips of the Spanish common wheat 'Pané 247' for analysis of somatic chromosomes.
2) Anthers of F_1 "mono$_i$-'Chinese Spring' × 'Pané 247'" ($2n-1=42$), being i = 1A, 2A, 3A, 6A, 7A, 2B, 3B, 6B or 7B.
3) Anthers of F_1 "mono$_i$-'Chinese Spring' × 'Ariana 8'" ($2n-1=41$), being i = 4A, 1B or 5B.

A minimum of 25 pollen mother cells having 20 bivalents + 1 univalent at first metaphase from two anthers for each of the monosomic were examined. Each of the individual univalents and the remaining bivalents in all monosomics were analyzed for its particular C-banding patterns. Thereby the characterization of univalents 7A, 2B, 3B, 6B and 7B of

'Pané 247' and 4A, 1B and 5B of 'Ariana 8' was achieved. The chromosome 4B was identified by its C-banding pattern in 'Chinese Spring' (described by Gill and Kimber (1974) and Seal 1982)).

The C-banding pattern of characterized chromosomes as univalents in critical monosomics was used as a reference in identifying somatic chromosomes and bivalents in the others.

In order to identify the chromosomes involved in a reciprocal translocation, differentiating karyotypes of 'Pané 247' and 'Chinese Spring', critical configurations of n–2 bivalents and one trivalent detected in certain monosomics, and the C-banding pattern of the chromosomes involved in multivalents have been used.

Mitotic metaphase preparations

Seeds were germinated at 22 °C in a Petri dish. When the seminal roots were 1–2 cm in length, they were excised and immersed in a 1-bromonaphthalene saturated solution for 4 h to shorten the chromosomes. The root tips were fixed in acetic acid. Slides were made by squashing the fixed root tips in a drop of acetic acid. Coverslips were removed by CO_2-freezing.

Meiotic metaphase preparations

One anther from each flower was used to check the meiotic stage. The remaining two anthers were fixed and stored in 3 : 1 ethanol-acetic acid. Slides were made by squashing the fixed anthers according with the above mentioned procedure.

C-banding

The staining technique used was previously reported by Jouve et al. (1980).

Results

Both the mitotic and meiotic C-banding pattern of the chromosomes 4A, 7A and 1B to 7B are shown in Fig. 1.

An unambiguous characterization of remaining chromosomes was not reliable because of the absence of distinctive bands, mainly in the bivalents. These chromosomes showed pericentromeric bands that were generally small and faintly stained (Fig. 2 a).

The C-banding pattern of the wheat chromosomes can be described as follows:

4A: This submetacentric chromosome was identified as a univalent in 'Ariana 8' and exhibits large pericentromeric dark bands of heterochromatin and a small telomeric band in its large arm. The C-banding pattern of the chromosome 4A of 'Ariana 8' was found to be identical to that published by Gill and Kimber (1974) and Seal (1982) for 'Chinese Spring'. The terminal band of the large arm of 'Pané 247' is smaller

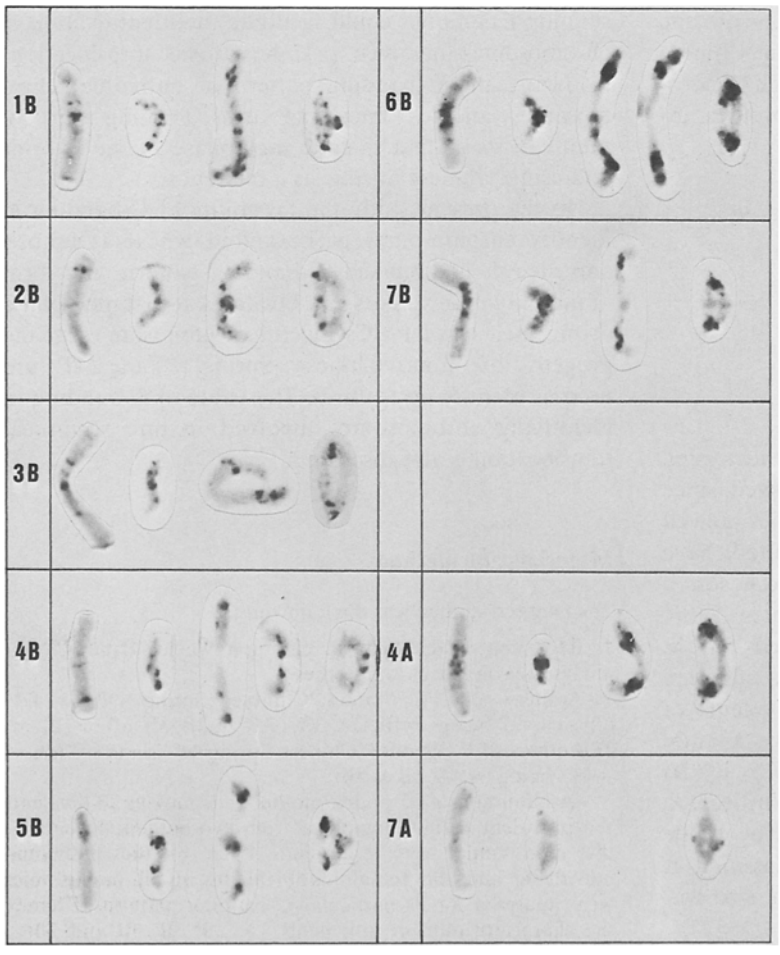

Fig. 1. Comparison of nine somatic and meiotic (univalents, open bivalents and ring bivalents) chromosomes of wheat

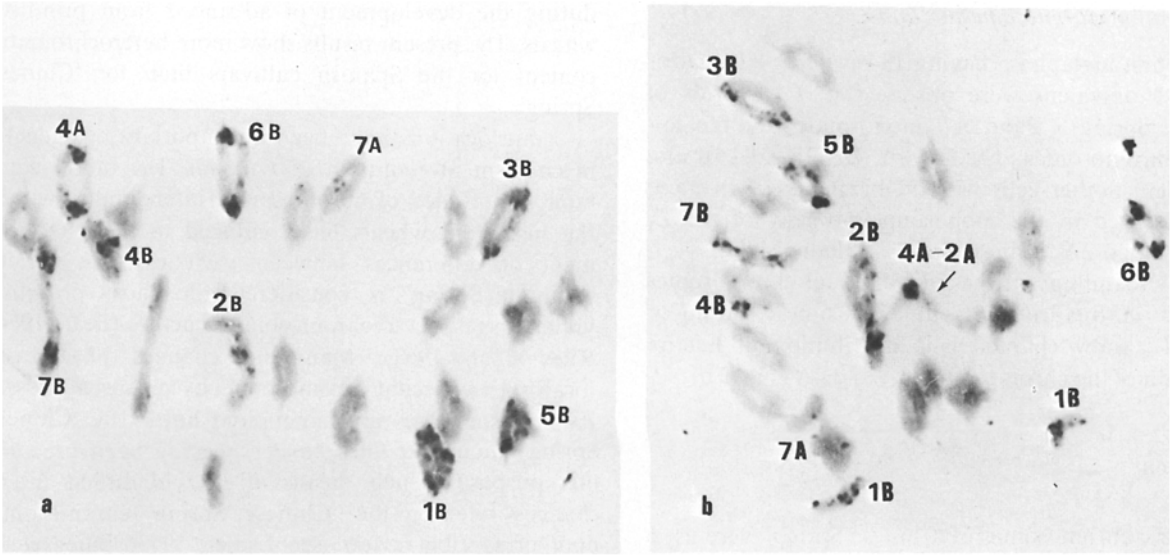

Fig. 2, a, b. C-banded metaphase plates of mono-5A'Chinese Spring'×'Pané 247': **a** – C-banded identified chromosomes as bivalent are indicated; **b** – *arrows* indicates 2A–4A quadrivalent

than in 'Ariana 8'. The bivalent 4A in 'Chinese Spring' ×'Pané 247' shows an assimetrical telomeric pattern of bands in the large arms.

7A: This chromosome is large and metacentric and shows one dark telomeric band in one arm in 'Pané 247'. 'Chinese Spring' shows bands of telomeric heterochromatin in both arms. Ring bivalent 7A in 'Chinese Spring'×'Pané 247' exhibits unbalanced bands in telomeric regions. This chromosome was not observed as an open bivalent in the metaphase cells analyzed.

1B: This submetacentric chromosome has more heterochromatin and more dispersed C-bands than any other in the karyotype. It presents a secondary constriction. There is no variation between the C-banding pattern of the univalent 1B of 'Ariana 8' and the one from the bivalent 1B observed in the hybrid mono-1B 'Chinese Spring'×'Pané 247'. The bivalent is easily distinguished from others because of the presence of a obvious telomeric band in its large arm.

2B: The chromosome 2B is submetacentric. It has a band close to the centromere in the short arm. A second band in a terminal position in the same arm is exhibited. A faint terminal band in the large arm not exhibited in 'Chinese Spring', was rarely visible in some bivalents.

3B: Chromosome 3B is submetacentric. The univalent 3B of 'Pané 247' exhibits one centromeric dark band and one intercalary band in each arm. A short band in the telomeric region of the short arm is also observed. Mitotic chromosomes 3B show additional intercalary faint bands. The intercalary band of the short arm was absent in 'Chinese Spring', thus allowing for the identification and characterization of the bivalent in the hybrids between 'Chinese Spring' and 'Pané 247'.

4B: This submetacentric chromosome exhibits one intensely stained subterminal band in its large arm in all cases and one faint intercalary band in the centromeric region. This chromosome showed the same C-banding pattern in 'Chinese Spring' and 'Pané 247'.

5B: This subtelocentric chromosome has been analyzed as a univalent in 'Ariana 8'. It shows an intensely stained region around the centromere and one intercalary band in its large arm. The size of the intercalary region between genotypes was variable being lower in 'Pané 247' than in 'Ariana 8'. Bivalent 5B shows differences in size and intensity of centromeric bands in hybrids of 'Chinese Spring'×'Pané 247' being smallest in 'Chinese Spring'. Less obvious heterochromatic differences between centromeric regions in bivalents 5B of 'Chinese Spring'×'Ariana 8' were observed.

6B: This submetacentric chromosome exhibits large pericentromeric dark bands of heterochromatin and one small intercalary band in its large arm. 6B chromosome has a secondary constriction in its short arm. The satellite region was clearly distinguished from both arms in the bivalents because of its strongly condensed chromatin. This circumstance permits a clear distinction of bivalent 6B from 4A and 7B.

7B: This submetacentric chromosome shows pericentromeric large bands dispersed in somatic chromosomes and bivalents and aggregated in univalents. A fine intercalary band is shown in the large arm.

Translocation chromosomes of 'Pané 247'

Cells at first metaphase having 18 bivalents + 1 quadrivalent + 1 univalent were observed in F_1 hybrids of 'Chinese Spring' × 'Pané 247' monosomics for the following chromosomes: 1A, 3A, 6A, 7A, 2B, 3B, 6B and 7B. Pollen mother cells with 19 bivalents + 1 trivalent were observed in the monosomic progeny of the F_1 hybrid "mono-2A 'Chinese Spring' × 'Pané 247'".

The C-banding pattern of individual chromosomes involved in quadrivalents in F_1 'Chinese Spring' × 'Pané 247' show characteristic distribution of heterochromatin of the chromosomes 2A and 4A (Fig. 2 b).

Discussion

Telocentric chromosomes of 'Chinese Spring' were used to aid the identification and characterization of individual chromosomes by Giemsa staining techniques (C-banding: Gill and Kimber 1974; N-banding Gerlach 1977). The ideogram constructed depicting the C-bands positions in chromosomes of 'Chinese Spring' has been subsequently used as reference (Jewell 1979; Seal 1982; Armstrong 1982).

Giemsa C-banding applied to meiotic chromosomes of monosomics permits a clear distinction of the bands in critical univalents and bivalents. Thus, the pattern of C-bands and size and form of chromosomes, observed as univalents, leads to the unequivocal recognition of nine individual chromosomes.

The C-banding pattern of univalent chromosomes was used as a reference to identify the corresponding somatic chromosomes. There is a characteristic C-banding pattern for the individual chromosomes in somatic metaphases in 'Pané 247'. The C-banding pattern observed in 'Pané 247' coincides well with the distribution of heterochromatin in corresponding chromosomes of the genomes A and B studied in other cultivars by Seal (1982). Minor variations observed in intercalary or telomeric bands are in agreement with little variation in the distribution and content of heterochromatin between homologous chromosomes of different cultivars observed by Seal (1982). Although it is possible to identify without ambiguity chromosomes 1B, 4B, 5B, 6B and 7A, others, such as 4A and 7B, 2B and 3B, showed no clear distinctive C-bands. These chromosomes were easily distinguished in critical monosomics in which the univalent and bivalent condition was used as cytogenetical markers.

Intervarietal variation in content and distribution of heterochromatin in bivalents of hybrids between 'Chinese Spring' and 'Pané 247' and 'Ariana 8' is in disagreement with the results of Iordansky et al. (1978). They suggested a progressive loss of heterochromatin during the development of advanced from primitive wheats. The present results show more heterochromatin content for the Spanish cultivars than for 'Chinese Spring'.

Translocations have been an important cytogenetic mechanism in evolution of *Triticum*. The rather common importance of chromosomal interchanges within the hexaploid wheats has conduced to the establishment of differences between varieties. The variety 'Chinese Spring' is considered the most primitive variety in respect to chromosome structure (Sears 1954; Riley et al. 1967). Many interchanges have been localized to specific chromosomes by means of crosses to monosomic or other aneuploid lines. The 'Chinese Spring' aneuploid lines have especially been used for this purpose, which means all the identified interchanges refer to the 'Chinese Spring' chromosome numbering (for review, see Larsen 1973; Baier et al. 1974; Vega and Lacadena 1983). Nearly 50% of identified interchanges involved some of the nine pairs of chromosomes here identified by its C-banding pattern. A combination of Giemsa banding technique and classical cytogenetic analysis at meiosis of hybrids (nine C-banded identified chromosomes) and critical monosomics (remaining 12 chromosomes) would be of value in order to verify the identification of individual chromosomes involved in intervarietal interchanges.

Our results on C-banding meiotic analysis of wheat chromosomes open good perspectives for the application of this techniques in studying the role of heterochromatin in meiotic pairing, formation and maintenance of chiasmata, univalent shifts, identification of structural changes, etc. Work on these aspects are in progress.

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