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N-Banding in *Triticum aestivum* Following Feulgen Hydrolysis

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Summary. Terminal and/or interstitial N-bands were produced on the seven B-genome chromosomes and chromosomes 4 and 7 of the A-genome of *Triticum aestivum* cv. 'Chinese Spring' by a modified BSG technique following a standard Feulgen preparation. The banding was accomplished by modifying the barium hydroxide treatment.

Key words: *Triticum aestivum* – 'Chinese Spring' – Chromosome banding – Hydrolysis – Feulgen

Introduction

The differential production of different Giemsa bands in plants has been amply demonstrated (Vosa 1976; Yen and Filion 1977; Filion and Blakey 1979; Stack et al. 1974). In *Avena* (Yen and Filion 1977) and *Tulipa* (Filion and Blakey 1979) a change in the degree of hydrolysis with HCl in conjunction with a standard BSG technique ($Ba(OH)_2 : 2 \times 55C$) changed the banding pattern from terminal and intercalary under weak hydrolysis to centromeric under strong hydrolysis. The strong hydrolysis treatments were essentially those used for a standard Feulgen staining procedure. There are many characteristics of the Feulgen hydrolysis procedure that are advantageous for chromosome studies but in particular it allows the preparation of complete cells with good chromosome spreads. Chromosome banding can be obtained in *Triticum aestivum* cv. 'Chinese Spring' following Feulgen staining by modifying the BSG technique. The banding pattern obtained is comparable to the N-banding technique in *Triticum* (Gerlach 1977).

Materials and Methods

Plant Material. The stock used was *T. aestivum* L. cv. 'Chinese Spring'.

Mitotic Metaphase Preparations. Seeds were germinated at room temperature (25±2 °C) in a petri dish. Roots were collected when 1–2 cm in length and pretreated in cold H_2O (1 °C) for approximately 24 h then fixed in 3:1 ethanolacetic acid for 1–2 h. Roots were hydrolysed in 1 N HCl at 60 °C for 11 min and stained in Feulgen (0.5 g Fuchsin, 100 mL boiling distilled H_2O, 15 mL 1 N HCl, 1.5 g $K_2S_2O_5$, 0.25 g carbon). The roots were in the Feulgen stain for a minimum of 30′ and a maximum of 90′ while the squashes were being prepared. The slides were subbed with Mayer's egg albumen. The root tips were squashed in 1% aceto-carmine in 45% acetic acid. Cover slips were removed following freezing in dry ice and slides were air-dried for 1 h, placed in 95% ethanol at room temperature for 1 h, briefly airdried and stored in the dessicator at room temperature. Under these conditions it was important for the slides to be aged 10–17 days before BSG technique was applied.

Banding. The slides were placed in 5% $Ba(OH)_2 \cdot 8\,H_2O$ (w/v) for 5 min at room temperature, washed in demineralized water for 20′, rinsed in three 5′ changes of 2×SSC at room temperature, incubated in 2×SSC for 30–60 min at 60 °C, rinsed in Sorenson's 1/15 M phosphate buffer for 5 min, and stained in 2% Giemsa (Gurr's Improved R 66) in Sorenson's 1/15 phosphate buffer for up to 2 h. After staining the slides were rinsed in buffer, air-dried for 2 h and mounted in permount.

Results

Initially a 5 min $Ba(OH)_2$ treatment at 50 °C was used. This treatment normally produced only centric bands on all chromosomes but on rare occasions interstitial and telomere banding was observed. An attempt was made to improve the banding by shortening the hydrolysis by small increments from 11 min at 60 °C. However, shortening the hydrolysis time only resulted in the loss of the centric bands. Shortening the SSC treatment

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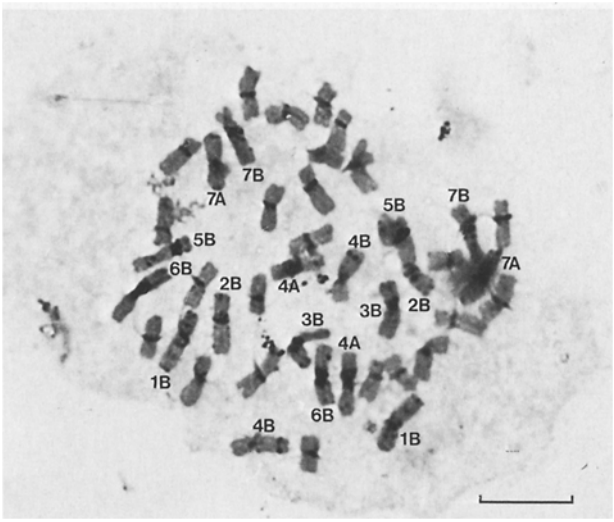

Fig. 1. N-banded root tip metaphase preparation of *Triticum aestivum* cv. 'Chinese Spring'

also did not improve the frequency of banding. When the $Ba(OH)_2$ treatment was carried out at room temperature the banding was obtained consistently. Under these conditions, all three treatments i.e. acid (HCl), base [$Ba(OH)_2$] and salt (2×SSC) were necessary to produce banding. This was true for both barium hydroxide treatments. The other variable studied was the age of the slide following removal of the cover slip. Under the dehydration and storage conditions employed, it was necessary to age the slides for approximately 10 days before the BSG technique yielded good banding results. The banding remained prominent for a further 7 days after which time it deteriorated.

Employing this porcedure it was possible to study good Feulgen karyotypes and then obtain additional information from chromosome banding (Fig. 1). Eighteen chromosomes (9 pairs) were banded. It was immediately apparent that the chromosomes banded were the same 18 chromosomes that are banded by the N-banding technique (Gerlach 1977). The most immediate observation which indicated this was that both pairs of satellite bearing chromosomes were banded and they could be identified by the banding pattern defined by Gerlach (1977) as 1B or 6B. Similarly, the remaining 5 homologues of the B genome could be identified. Chromosome 5B and 4B were particularly easy to identify as was chromosome 7A of the A genome. Chromosomes 4A, 2B, 3B, and 7B were the most difficult to identify. Distinguishing between all of these chromosomes was not possible in all cells. However, with a good Feulgen preparation which offered a number of complete cells with well spread chromosomes, a cell could always be found which contained most or all of these chromosomes with their unique combination of diagnostic bands.

The banding procedure worked well on chromosomes of various lengths from long prometaphase to extremely shortened metaphase chromosomes. The longer chromosomes showed the individual bands more clearly but the diagnostic pattern was still evident on the shortest. Overcontracting of the chromosomes obscures the satellites. While it was not necessary, the presence of the satellites was an additional diagnostic feature which particularly aided in the identification of chromosome 6B.

Discussion

This banding procedure perhaps does not offer better banding patterns than either the C-banding procedure (Gill and Kimber 1974) or the N-banding procedure (Gerlach 1977). However, it does yield well-defined, diagnostic bands which allow the identification of the 7 chromosomes of the B-genome as well as chromosomes 4A and 7A of the A genome. Its particular advantage is that it employs the normal hydrolysis treatment used to make a Feulgen stained preparation. Thus, cells with the complete chromosome complement, well-spread, can be easily obtained. In addition, information about chromosome morphology from the Feulgen preparation can be obtained prior to banding. The advantages of using one preparation to obtain a chromosome count, a Feulgen karyotype, and a C-banded karyotype are obvious.

Some nomenclatural difficulty is encountered in naming this banding procedure. Bands are normally defined as C-bands, G-bands, N-bands, etc. on the basis of the technique used to produce them. The banding pattern produced by this procedure is essentially the same as the N-banding procedure defined by Gerlach (1977) in that it bands only the B-genome plus 4A and 7A of the A genome. In addition, the banding patterns obtained on these chromosomes are more comparable to the N-banding pattern than to the C-banding pattern described by Gill and Kimber (1974). Therefore because of the banding pattern we propose to define this as an N-banding pattern even though it is produced by a modified C-band procedure.

Previous evidence has amply demonstrated that there are different types of C-bands or heterochromatin which probably differ in their lability to acid hydrolysis. In *Avena* (Yen and Filion 1977) and *Tulipa* (Filion and Blakey 1979) a long acid hydrolysis such as would be required for optimal Feulgen staining produced only centric bands. The production of terminal and interstitial bands was obtained only when considerably shorter hydrolysis times were employed. Similarly, with

T. aestivum cv. 'Chinese Spring' centric bands were obtained (rarely interstial banding) when hydrolysis was for 11 min in 1N HCl at 60 °C followed by treatment with 5% $Ba(OH)_2 \cdot 8\,H_2O$ at 50 °C for 5 min. In this instance, however, decreasing the $Ba(OH)_2 \cdot 8\,H_2O$ treatment by lowering the temperature (room temperature for 5') resulted in the production of interstitial and terminal bands on nine pairs of chromosomes of *T. aestivum* cv. 'Chinese Spring'. However, it cannot be assumed that the procedure would result in the production of banding following normal Feulgen hydrolysis in other species, since it produced only centric band on five of the A-genome chromosomes and all seven of the D-genome chromosomes.

C-banding in mammalian tissues involves the extraction of DNA from non C-banded regions (Comings 1978). Homquist (1979) has shown that under normal C-banding procedures the DNA is depurinated by acid hydrolysis, denatured by alkali treatment and depolymerized and extracted by the hot salt. In our case, it may be that the banded regions of the B-genome and two A-genome chromosomes are more resistant to or not as accessible for denaturation as other regions and therefore the DNA is not extracted by the hot salt following the shortened alkali treatment. Limited attempts to shorten the alkali treatment still further by reducing the time of treatment at room temperature did not result in the production of any additional bands on *T. aestivum* cv. 'Chinese Spring'. In fact there appeared to be some deterioration in the banding on the A and B genome chromosomes. However, it may still be possible to obtain additional bands by further modification of the banding treatment. Under normal Feulgen acid hydrolysis some denaturation, depolymerization, and extraction of DNA occurs. It is possible that this has already destroyed the banded regions of the other chromosomes but since heterochromatin is more resistant to depurination than euchromatin (for discussion see Holmquist 1979) and since under our conditions the omission of any one of the three steps resulted in uniformly Giemsa stained chromosomes, it seems unlikely. Therefore if the banding pattern produced is the result of the differential extraction of DNA, it is possible that the normal Feulgen acid hydrolysis has not destroyed any banded regions and additional bands might be obtained by further modifications to the subsequent banding treatments. However, evidence linking chromosome banding to differential DNA extraction is not conclusive in plants (Gendel and Fosket 1979; Kongsuwan and Smyth 1978; Limin and Dvorak 1976). It is interesting to note that a satellite DNA which has been isolated in wheat is principally localized at the N-banded sites (Gerlach et al. 1978; Gerlach and Peacock 1980). Therefore, some properties of this DNA repeated sequence and/or its interaction with chromosomal protein may allow it to resist extraction following the full hydrolysis and shortened denaturation procedure.

The importance of the N-banding procedure with respect to studies concerning the evolution of the B-genome have been discussed (Gerlach 1977). It follows that the present procedure for producing N-banding should contribute greatly to this investigation.

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