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The Giemsa C-Banded Karyotype of Rye

(homoeologous/constitutive heterochromatin/chromosomes)

BIKRAM S. GILL AND GORDON KIMBER

Department of Agronomy, University of Missouri, Columbia, Mo. 65201

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ABSTRACT **The chromosomes of rye have been individually identified by their distinctive heterochromatin pattern with Giemsa staining and classified on the basis of their homoeology with wheat chromosomes. The constitutive heterochromatin detected by C-banding has been shown to be identical with the classical heterochromatin as seen in the pachytene of meiosis in rye.**

Recently developed staining techniques that result in differential banding of somatic metaphase chromosomes permit the identification of individual chromosomes and have considerably enhanced cytogenetic studies in mammals (1). With these methods all of the chromosomes have been identified in man, mouse, and many other animal genera; further, in mouse almost all the linkage groups have been correlated with specific chromosomes and chromosome arms (2–8). Unfortunately, the application of these techniques to plant chromosomes has not been particularly successful, although a few reports have appeared (9–14). One of the differential staining techniques, Giemsa C-banding (C = constitutive heterochromatin), which was first applied to animal chromosomes (15–17), involves denaturation-reassociation of DNA, with the highly repetitive DNA reassociating faster and appearing as dark bands. Attempts have been made to identify individual rye chromosomes with conventional staining methods, but the interpretation of the results is difficult (18–20). In this communication, we report a Giemsa staining procedure in rye that can be routinely used and by which the individual chromosomes can be easily identified.

MATERIALS AND METHODS

Actively growing root tips of rye (*Secale cereale* L. var. Imperial) prefixed in monobromonaphthalene for 1–3 hr were then fixed in glacial acetic acid. The root tips were softened for 1–2 hr in a 5% solution of pectinase (EC 3.2.1.15; polygalacturonase) and cellulase (EC 3.2.1.4) to which 2–3 drops of 1 N HCl had been added for each 5 ml of the enzyme solution. Softening by this enzyme solution results in banding, whereas the customary hot hydrolysis of roots with 1 N HCl does not. After application of a cover slip, the cells were separated from each other by tapping the slip. The cover slip was separated from the slide by CO_2 freezing, and the slide was immersed in absolute alcohol for two to three hours and then dried by air blowing.

Denaturation-Renaturation. Dry slides were immersed in a freshly prepared, saturated solution of barium hydroxide for 5 min. After they were washed in three changes of distilled water for a total duration of 10 min, the slides were air dried, incubated in 0.30 M NaCl–0.030 M Na citrate (2) at 60° for 1 hr, washed thoroughly in distilled water, and again air dried. The preparations were stained in Giemsa solution at pH 7 (2) for 1–2 min, washed quickly in water, air dried, stored in xylol over night, and mounted in Canada balsam.

RESULTS

This C-banding technique results in well-defined, sharp, distinct bands in rye chromosomes. The bands differ in size, intensity, and spatial distribution. The terminal bands on the short arms of the chromosomes are generally massive and darkly stained; centromeric bands are of intermediate intensity; and interstitial bands are small and generally faintly stained. Centromeric heterochromatin is universally present. All short arms end in large knobs, while the knobs at the ends of the long arms are variable in size in five cases and absent in two. Interstitial bands are absent in the short arms (except chromosome 1R) and may be present or absent in the long arms. During interphase the rye nucleus shows very characteristic heteropycnotic knobs, the number of which approximates 14 (Fig. 3).

It is possible to identify seven distinct chromosome pairs in the somatic metaphases of rye; however, it is usually not possible to see all seven types in one metaphase cell. This difficulty is due to the cytoplasm taking stain and obscuring the chromosomes it overlies, and thus it is necessary to work with chromosomes that have become isolated from the cytoplasm. In this way a reconstruction of the complete karyotype can be made.

Preliminary observations of wheat (*Triticum aestivum*) chromosomes have shown that they appear not to have the characteristic dense band in the terminal position on the short arm. This allows the recognition of individual rye chromosomes when they are added to wheat. Consequently, the seven disomic additions of Imperial rye chromosomes to wheat were examined at somatic metaphase for C-banding. In each case it was possible to identify the rye chromosome, and in all cases to positively relate the banding pattern on the added chromosome to that of one of the rye chromosomes in rye cells. Thus, not only is it possible to recognize C-banding patterns unique to each of the seven chromosomes of rye, but all of them may be related to the homoeologous groups already established in wheat (21).

Fig. 1 shows the seven chromosomes, as stained in rye root tips, arranged according to the homoeologous classification.

The individual chromosomes can be characterized as follows:

1R: Submedian. The telomeric knobs are large and slightly unequal in size. The long arm possesses one small, interstitial band. In the short arm, there is a large interstitial band.

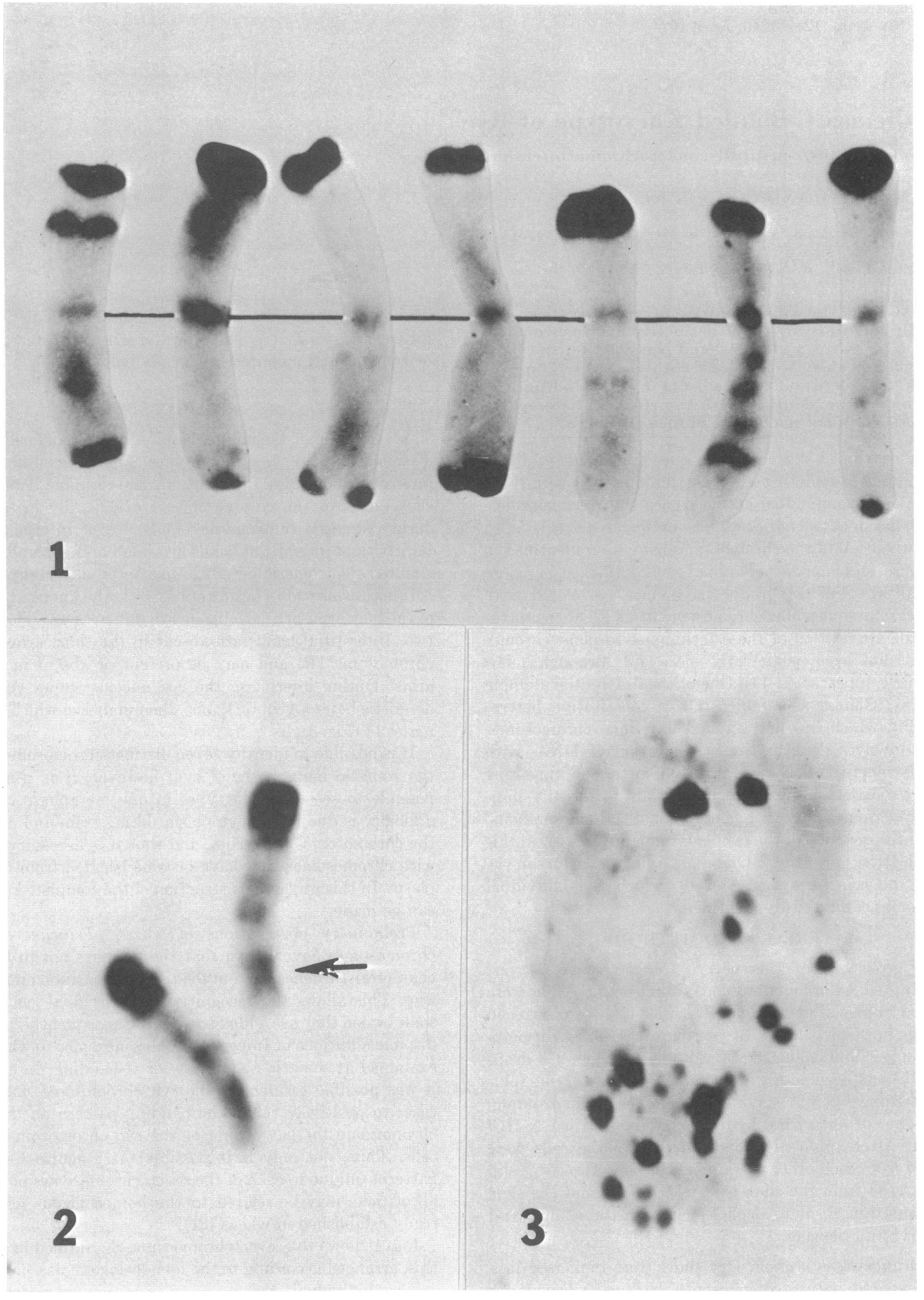

FIG. 1. Giemsa C-banding of rye chromosomes. The seven chromosomes are, from *left* to *right*, 1R to 7R.

FIG. 2. Giemsa C-banding of two B-chromosomes in a somatic cell of rye. The *arrow* denotes the centromeric position.

FIG. 3. Heteropycnotic regions stained by Giemsa in rye somatic-interphase nucleus.

2R: Submedian. The terminal knob is large on the short arm and considerably smaller in the long arm. The long arm has one interstitial band near the terminal knob.

3R: Submedian. The terminal knob is large in the short arm and of medium size in the long arm. A small interstitial band is present in the proximal portion of the long arm. This band did not show in the wheat–rye addition line.

4R: Submedian. Terminal knobs large and of equal size.

5R: Submedian. The short arm has a large knob. The long arm is knobless but has one interstitial band.

6R: Submedian. The short arm has a large terminal knob. The long arm has no such knob but possesses a series of four intercalary bands, the distal one of which is the most prominent. In the wheat–rye addition lines, the long arm of 6R ends in a minute telomere.

7R: Submedian. The terminal knob is large on the short arm and small on the long arm. In the long arm one interstitial band is always present.

The banding of B chromosomes was studied in *Secale cereale* var. Prolific. The short arm is wholly heterochromatic. The long arm possesses three intercalary bands, one being very close to the terminal knob. The terminal knob in the long arm is large, as in most of the A chromosomes (Fig. 2).

DISCUSSION

The C-banding of rye chromosomes is easily accomplished and provides a unique diagnostic tool. The recognition of the individual chromosomes allows the investigation of various phenomena such as intervarietal variation and evolutionary patterns in the genus.

The similarity of the banding pattern of the A and B chromosomes must indicate a basic similarity in structure and perhaps also in function. This would corroborate the recent findings of Chilton and McCarthy (22), who concluded that the DNA of B chromosomes is closely related to that of A chromosomes.

The dark bands in the somatic chromosomes detected by Giemsa staining represent constitutive heterochromatin (15–17). Detailed maps of pachytene chromosomes of rye exist (18), in which the distribution of heterochromatin may be compared to that of the constitutive heterochromatin as now revealed. There is considerable agreement. Large knobs are present at pachytene at the end of the short arms of all of the chromosomes, and only five of the seven long arms show terminal heterochromatin, as with the Giemsa staining. Centromeric heterochromatin is present in all the pachytene chromosomes and corresponds to the C-banding associated with the centromere in somatic chromosomes. Chromosomes I, II, and V of Lima-de-Faria (18) are very similar to chromosomes 4R, 3R, and 1R, respectively. Chromosomes III and IV are similar to 2R and 7R, and VI and VII are similar to 5R and 6R.

In studies in *Vicia faba*, *Scilla sibirica* and *Tulbaghia* (9, 14), in *Trillium grandiflorum*, *Fritillaria* and *Crepis* (11), and in *Trillium kamtschaticum* (13), it was found that heterochromatin detected by cold treatment was Giemsa positive. Hsu (23) demonstrated in *Drosophila* that classical heterochromatin is identical to the Giemsa C-banding.

Sarma and Natarajan (24) have also described Giemsa staining of heterochromatic regions in rye chromosomes. However, they did not observe either the centromeric or interstitial bands that we have described. It is most probable that this difference can be attributed to small differences in the respective techniques and, additionally, to the different rye variety (Petkus) used by Sarma and Natarajan.

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