SHORT COMMUNICATION

AN ACETOCARMINE-GIEMSA STAINING OF RYE CHROMOSOMES

NOBORU NAKATA, YOSHIMASA YASUMURO AND MUTSUO Sasaki

Faculty of Agriculture, Tottori University, Tottori 680

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Various techniques for Giemsa C-bandng have been reported since Pardue and Gall (1970) found that constitutive heterochromatin can be stained specifically by Giemsa solution. Sarma and Natarajan (1973) and Merker (1973) showed first that C-bandng technique is available for characterization of each pair of rye chromosomes and for identification of the chromosome complement of rye in the Triticale genome. Gill and Kimber (1974a), Verma and Rees (1974), Vosa (1974) and Weimarck (1975) showed a C-bandng pattern of rye chromosome complement. However, the chromosomes treated with a Giemsa staining result in deformation in the shapes which makes it difficult to recognize morphological characteristics of individual rye chromosomes. In order to make a C-bandng pattern coincide with karyotype in rye, Gill and Kimber (1974b), Darvey and Gustafson (1975) and Gustafson et al. (1976) used a series of addition of individual rye chromosomes to wheat as a material, and de Vries and Sybenga (1976) a series of telocentric substitution lines of rye. We devised a method of acetocarmine succeeded by Giemsa staining in order to obtain both complete shapes and C-bands of the same chromosomes in a single cell. Using this acetocarmine-Giemsa staining to compare the shapes and bands of rye chromosomes in the same root-tip cells, we were able to identify more precisely each rye chromosome. This method is also available for PMC’s.

This staining method is described as following steps.
1) Treat 7 to 15 mm long root-tips with 0° to 1°C cold distilled water for 20 h and then fix with 1:3 (v/v) acetic alcohol.
2) After making slide preparations by acetocarmine squashing and taking photographs of good cells, remove cover glasses by using dry ice method (Conger and Fairchild 1963).
3) Place the slides in 99% ethyl alcohol for 15 min and air-dry for at least one day. Acetocarmine stain of the chromosomes disappears in ethyl alcohol and it has no adverse influences on following Giemsa staining.
4) Put in 5% solution of barium hydroxide for 15 min at 50° ±2°C and rinse in tap water.
5) Place in 2×SSC (0.3M NaCl+0.03M Na₃C₆H₅O₇) for 50 min at 50°±2°C and rinse in tap water.

6) Stain with Giemsa solution for 3 to 20 min. This stain can be made easily from diluting 3 ml of commercial Giemsa solution for blood stain with 50 ml of Sörensen's phosphate buffer (pH 6.8).

7) Rinse in tap water, air-dry thoroughly and mount in Canada balsam diluted with xylene.

This C-banding technique is a modification of Sumner's method (1972) to be applied for rye chromosome.

This acetocarmine-Giemsa staining method is useful for identification of rye chromosomes in Triticale and for karyotype study in genus Secale and other plants.

Fig. 1 shows a result of this staining method applied to an inbred rye strain, IR 18, developed at our Laboratory. Combining the data of the chromosome morphology and C-banding obtained from Figs. 1a and 1b, we were able to draw the idiogram showing characteristics of the chromosome complement of IR 18 as shown in Fig. 2. Identification of the homoeologous group for each chromosome of IR 18 was based on the results of the study on homoeologous relationships of rye chromosomes to those of wheat obtained by Jenkins (1966), Sears (1968), Koller and Zeller (1976) and many workers, and the results of the study on the relation between C-banding of the rye chromosomes and their homoeologous groups obtained by Gill and Kimber (1974b), Darvey and Gustafson (1975), Gustafson et al. (1976) and de Vries and Sybenga (1976).

Fig. 1. Metaphase in a root-tip cell of inbred rye (2n=14, Tottori Univ. stock IR 18).

1a: Acetocarmine staining. The numbers indicate presumed homoeologous group numbers. 1b: The same cell stained successively by Giemsa solution. ×1470.
Characteristics of each chromosome of IR 18 shown in Fig. 2 are briefly described as follows:

1R: Submetacentric chromosome with the largest satellite, having a weak band at the end of the short arm. Since other strains had prominent bands on satellite and both ends of the arms, there must be variation in C-banding pattern among strains of Secale cereale.

2R: The longest metacentric chromosome having a prominent terminal band on each of the arms.

3R: Metacentric chromosome having a terminal band on each of the arms. However, most of other strains have a prominent band at the end of the long arm.

4/7R: Submetacentric chromosome with small satellite having a band and having a weak band at the end of the long arm.

5R: Subterminal chromosome characterized by secondary constriction in the long arm, satellite, and a double band at the end of the long arm.

6R: Subterminal chromosome characterized by satellite with a band and two interstitial bands on the long arm.

7/4R: Submetacentric chromosome having a terminal band at each of the arms. Discrimination between 3R and 7/4R was very difficult in this strain.

LITERATURE CITED


Vosa, C. G., 1974 The basic karyotype of rye (*Secale cereale*) analysed with Giemsa and fluorescence methods. Heredity 33: 403-408.

Weimarck, A., 1975 Heterochromatin polymorphism in the rye karyotype as detected by the Giemsa C-banding technique. Hereditas 79: 293-300.