Pak. J. Agri. Sci., Vol. 30, No. 2, 1993

N-BANDING KARYOGRAM OF HEXAPLOID WHEAT M30

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In this study on M30, 13 N-banded chromosomes have been identified. The chromosomes 1B to 7B, 2A, 3A, 4A, 5A, 7A and 2D have shown clear dark bands. All the chromosomes of M30 were found to be similar with those of Chinese Spring chromosomes except the chromosome 7B and 2D which showed the deviations to some extent. One extra band was found on the long arm of chromosome 7B. The chromosome 2D possessed one extra cross band on short arm and one extra band on long arm. The test cross results have shown that M30 carry normal 21 bivalents (60%) and there is no evidence of any translocation between chromosomes of hexaploid wheat M30 and Chinese Spring.

INTRODUCTION

Both the N- and C-banding techniques have been widely used for the chromosomes identification in wheat. Wheat chromosomes at metaphase stage of meiosis were first N-banded by Jewel (1979). The extensive use of banding in triticale has been described by Gustafson (1983). Gill and Kimber (1974) by the use of C-bands were able to demonstrate bot translocations between two different wheat chromosomes and also between wheat and rye chromosomes. The banding technique provides a rapid means of detection and characterization of the heterochromatin structure of wheat chromosomes. N-banding strains specialised heterochromatin more intensively (Gill, 1987).

Banding nomenclature is after the system of human chromosomes (ISCN, 1978). Gill and Chen (1987) analysed the paired configuration with N-banding and identified several chromosomes and translocations that distinguish the genome chromosomes of different wheat species. Endo (1986) has described several chromosomes in Aegilops species inducing deletions in monosomic conditions in various wheat chromosomes at a high frequency.

Since last 15 years, attempts have been made to recognise the wheat chromosomes by means of chromosome banding. The heterochromatin diversity has been reported in plants (Cortes and Escalza, 1986) by using different banding techniques in different species (Endo and Gill, 1984). Obviously, this involves studying chromosome structure and banding mechanism (Jack *et al.*, 1986; Matsui *et al.*, 1986) and analysing chromosome evolution and genome relationship among the species (Badaeva *et al.*, 1986).

Chromosome banding pattern is believed to be caused by the extraction of DNA and protein from the euchromatin regions by acids, alkalis, salts, enzymes and other reagents (Pathak and Arrighi, 1973). Endo and Gill (1983) reported an improved N-banding technique that revealed N-bands on 16 chromosomes and these also coincide with observed sites of hybridisation with polypyrimidine tract sequence following longer autoradiograph exposure (Appels, 1982). A more conclusive relationship between N- and C-banded heterochromatin has been demonstrated in rye. Schlegel and Gill (1984) sequentially stained the same

metaphase type chromosomes in a cell acetochromatin/N-banding/C-banding techniques.

The usefulness of this technique for individual chromosome characterisation include physical mapping of genes in relation to cytological landmarks, karyotype description, pairing analysis or for verification of the integrity of various aneuploid stock. It is also useful in analysing the heterochromatin structure of wheat chromosomes.

MATERIALS AND METHODS

The N-banding technique used in the study followed the method of Endo and Gill (1983). The material analysed consists of hexaploid winter wheat M30 originated from USA with collection number 2222 in Federal Republic of Germany.

Germination and pre-treatment: Seeds were got germinated on wet filter paper in petridishes at room temperature. When the primary roots were 1-2 cm long, were pretreated with ice for 24 hours to shorten the chromosomes. Root tips were subsequently preserved in ethanol and acetic acid (3:1).

Slide preparation: The root tips were hydrolysed with 1 N HCl for 1 hour followed by 45% acetic acid for 24 hours. Slides were cleaned and dried. The meristematic part of the root, about 1 mm long, was cut from the root tips on a clean slide in a drop of 45% acetic acid. A cover glass was added and the meristematic tissue was spread by first tapping on the cover glass with a dissecting needle and was ultimately squashed hard, the slide was dried and then placed in 45% acetic acid and kept at 60°C for 10 minutes, again air dried and placed in incubator for 1-2 weeks.

Banding: Dried slides were treated with 0.1 M NaH_2PO_4 (pH 4.2) solution for 120 seconds at 92°C and washed four times in distilled water. Ultimately, the slides were stained in 2% Giemsa stain solution (2 ml stain in 100 ml 1/15 M Sorenson's phosphate buffer pH 6.8) for 40 minutes or until clear and sharp banding pattern appeared. The chromosome identification was carried out by phase contrast analysis. The size of each band was estimated, indicating total length and arm of the respective chromosome. The genomic and homologous relationship of chromosomes were identified according to the classification of Gill and Kimber (1974).

RESULTS AND DISCUSSION

The chromosome banding pattern of M30 was compared with that of Chinese Spring according to the system proposed by Endo and Gill (1983). Through N-banding, total 13 chromosomes (2A, 3A, 4A, 5A, 7A, 1B, 2B, 3B, 4B, 5B, 6B, 7B and 2D) were identified (Fig. 1).

In general, chromosomes of B-genome are the most heterochromatic and bears the highest number of N-bands. One extra band is present on the long arm of the chromosome 7B. The chromosome 2D possess one extra band on the long arm and one on the short arm. Except these two deviations, all the other chromosomes are identical with the N-banded chromosomes of the Chinese Spring. The N-banding results revealed that there are no structural deviations (translocations) between the chromosomes of M30 and the hexaploid wheat cultivars Chinese Spring, established by analysing aneuploid and telocentric (Endo, 1986; Gill, 1987).

For the verification of these results, a test cross between M30 and Chinese Spring was carried out. In this experiment, total 35 pollen mother cells were examined. The chromosomes with 21 bivalents were found to be 60%. It was concluded that the N-banding pattern of M30 chromosomes is

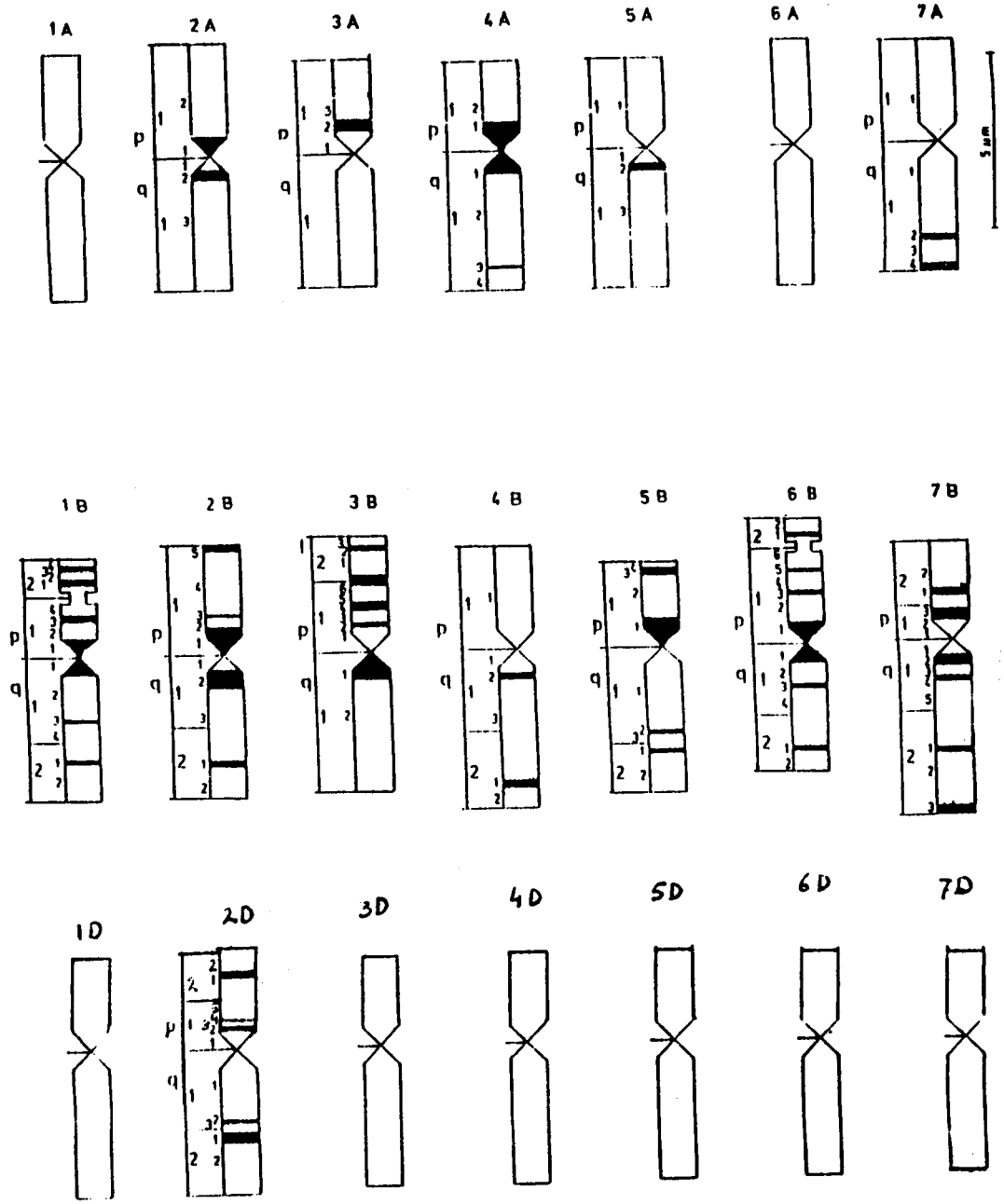

Fig. 1. N-banding karyogram of hexaploid wheat M30 (Ende and Gill, 1983).

identical with those of M30 and Chinese Spring chromosomes.

The small deviations may be due to technical procedure used and the mitotic stages examined. These results are in agreement with those of Gustafson (1983). The cytological analyses is time-consuming and chromosome identification is indirect. The banding technique, for the first time, provided a rapid and direct method of chromosome identification and are now extensively used in plant breeding.

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