Wheat Information Service
Number 78: 13-17 (1994)

II. Articles

Intervarietal polymorphism of heterochromatin in bread wheat, *Triticum aestivum* L.

Ahsan A. Vahidy, Qamar Jahan and A. Mujeeb-Kazi[1]

Department of Genetics, University of Karachi, Karachi-75270, Pakistan

Summary

Polymorphism in banding patterns of somatic chromosomes in *Triticum aestivum* L. cultivars, Jauhar, M-143, Pavon, Sarsabz, Sindh-81, Mexipak, Sonalika, ZA-77 and Chinese Spring was studied by the Giemsa N-banding technique. Sixteen out of twenty one chromosomes exhibited distinct banding patterns, while no bands were observed in 1A and 3D to 6D. The banding polymorphism was observed mainly in the B-genome chromosomes, whereas the D-genome chromosomes revealed maximum consistency. The bands 1BL2.7 and 6BL2.5 reported by earlier workers in Chinese Spring were not observed in our material of the same cultivar, which however, showed the maximum number of bands per haploid genome. Thirteen of these bands were missing in different combinations in other cultivars. Four bands viz., 2BL2.41, 3BL2.41, 7BL2.61 and 2DL1.7 not present in the cv. Chinese Spring, were observed in M-143, Sarsabz, Mexipak; Jauhar, M-143, Pavon, Sarsabz; M-143, Sindh-81, Sarsabz, Mexipak; and Sindh-81, respecitively. On the basis of the banding pattern an homozygous reciprocal translocation involving 5A and 4B chromosomes was observed in a plant belonging to cv. M-143. This led to the formation of 5AL/4BL and 5AS/4BS instead of normal 5A and 4B chromosomes.

Introduction

The N-banding technique has been shown to stain specific heterochromatic regions in many organisms, including cereal crops, such as barley (Islam 1980, Singh and Tsuchiya 1982), rye (Schlegel and Gill 1984) and wheat (Endo and Gill 1983, Gill 1987, Gerlach 1977). It has been employed for detecting translocations between wheat and barley chromosomes, for isolating lines possessing a pair of barley chromosomes substituted for a particular pair of wheat chromosomes (Islam 1980), and for detecting the 1B/1R translocation in many cultivars of wheat (Cai and Liu 1989, Jahan et al. 1990).

The aim of the present study was to explore the extent of band heteromorphy and chromosome polymorphism in several Pakistani wheat cultivars as compared to the check cultivar, Chinese Spring, utilizing Giemsa N-banding technique, and standard chromosome band nomenclature.

Materials and methods

Nine cultivars viz., Jauhar, M-143, Pavon, Sindh-81, Sarsabz, Mexipak, Sonalika, ZA-77 and Chinese Spring of *Triticum aestivum* L. (2n = 6x = 42; AABBDD) were used in the present study. Chinese Spring was included as a check cultivar to make meaningful comparisons.

Seeds were germinated in the petri dishes, lined with moist filter paper, and kept in the dark at room temperature for about 72 hours. Root tips were harvested and transferred to another petri dish for the pretreatment, also kept in dark for 2.5 hours as described by Jahan and Vahidy (1989). The pretreatment solution used was a mixture of 10 mg colchicine, 5 mg 8-Hydroxy-quinoline and 5 drops of DMSO in 20 ml of distilled water. The root tips were fixed in 0.2% acetocarmine and stored for two days in the refrigerator at 4C. The use of 0.2% acetocarmine helped in getting good spread of the chromosomes for N-banding. The cover glasses were removed by liquid nitrogen before further processing. The slides were treated for 10-15 minutes in 45% acetic acid at 60C, air-dried and kept at room temperature for 2-3 days. Treatment of the slides in 2M phosphate buffer was similar to that described by Jahan et al. (1990). Slides were then washed 3-4 times in deionized water and stained in a liquid Giemsa solution, using 10 ml of prepared Giemsa (Fluka No. 48900) in 195 ml of 1/15 M Sorenson's phosphate buffer at pH 6.8 for about 45 minutes at room temperature. The slides were rinsed briefly in 1/15 M Sorenson buffer, air-dried for 2-3 days and mounted in the Canada balsam. From permanent slides at least five cells were selected for photomicrography and karyotyping. The identification of chromosomes was based upon diagnostic banding patterns as elucidated by Endo and Gill (1984) and Gill et al. (1991).

Results and discussion

Sixteen out of twenty one chromosomes exhibited distinct N-banding patterns. No bands were observed in chromosomes 1A, 3D, 4D, 5D and 6D. N-banding polymorphism was observed mainly in the B-genome chromosomes in all cultivars. The chromosomes 3A, 1B, 5B, 1D and 7D exhibited maximum banding pattern consistency. The overall banding pattern among cultivars was similar to that of Chinese Spring, though some differences described in Table 1 were observed.

The chromosome 2A consistently had faint proximal 2AL1.3 and terminal 2AL1.5 bands in the long arm and a prominent proximal 2AS1.3 band in the short arm. The chromosomes 2A and 3A can often be confused with each other and could be misidentified as both have a similar sized bands in the proximal region of the short arm (Endo and Gill 1984). The terminal band 2AL1.5 of Chinese Spring was not observed in Jauhar, Sindh-81 and Sarsabz cultivars. Proximal band 4AL1.3 was not observed in Jauhar, M-143, Pavon and Sarsabz. The terminal band 4AL2.7 was not observed in any cultivar except Sonalika and Chinese Spring (Table 1). The proximal band 5AL1.3 was darker in M-143 as compared to the other cultivars.

A reciprocal translocation was observed in M-143 cultivar. Instead of normal 5A and 4B chromosomes 5AL/4BL and 5AS/4BS translocated chromosomes were observed (Fig. 1). Gill and Kimber (1977) observed a translocation in 4A (later renamed as 4B) and the 6B chromosomes of cultivar Thatcher. The characteristic twin bands, 6AL1.3 and 6AL1.5 were not observed in Pavon and Sindh-81 (Table 1). Endo

and Gill (1984) also did not observe these bands in pure lines and substitution lines of four wheat cultivars. The terminal band 7AS1.5 was missing in Sarsabz and ZA-77, while the terminal band 7AL1.7 was not observed in Jauhar.
The cultivars M-143, Sarsabz and Mexipak exhibited an additional band 2BL2.41, not observed in other cultivars including Chinese Spring. The terminal band 3BS2.7 was missing in Jauhar and Sarsabz. The band 3BL2.41 observed in Jauhar, M-143, Pavon and Sarsabz was absent in other cultivars. Jewell and Mujeeb-Kazi (1983) reported maximum differences in N-banding patterns of 3B chromosome in Chinese Spring and Veery. Proximal band 4BS1.7 was not observed in M-143, Sindh-81, Sarsabz and Mexipak, while the proximal band 4BL1.5 was missing in M-143 and Pavon. The terminal band 6BS3.6 of the satellite was not observed in Mexipak, Sonalika and ZA-77, while in the rest of cultivars this band was very faint. The terminal bands 1BL2.7 and 6BL2.5 observed in Chinese Spring by Gill et al. (1991) were not found in our material of the same cultivar. An extra terminal band 7BL2.61 was observed clearly in M-143, Sindh-81, Sarsabz (Fig. 2) and faintly in Mexipak, while it was not observed in rest of the cultivars. Chen et al. (1988) reported the differences in N-banding patterns of B genome in three endemic hexaploid wheats from western China.

Chromosomes 1D and 7D showed characteristic bands 1DS1.3 and 7DS1.5, respectively, in the interstitial regions, while the chromosome 2D exhibited bands 2DS1.3 and 2DL1.7. The terminal band 2DL1.7 was observed in cv. Sindh-81 only (Table 1). According to Endo and Gill (1984), chromosomes 3A, 5A, 2D and 7D showed the same banding patterns in their four cultivars as compared to the Chinese Spring. The bands reported earlier as 1BL2.7 and 6BL2.5 in cv. Chinese Spring were not observed in our material. Four bands viz., 2BL2.41, 3BL2.41, 7BL2.61 and 2DL1.7 not observed in the cv. Chinese Spring, were observed in some of the local cultivars (Table 1). Endo (1986) reported bands in all chromosomes except 1A of Chinese Spring by the C-banding and found the similar banding patterns in fourteen chromosomes as those found in the N-banded karyotype.

An intervarietal polymorphism of heterochromatin distribution was confirmed by the banding technique which may be used to study the pedigree, chromosomal aberrations like deletion, duplication, translocation and to differentiate among cultivars. Through DNA composition the heterogeneity of heterochromatin can be revealed further as specific DNA sequences are involved in the composition of heterochromatin (Gerlach and Peacock 1980). The differential DNA sequences and DNA protein composition may account for heterochromatic heterogeneity as observed by banding procedures.

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