

## USES OF N-BANDING FOR GENETIC AND CYTOLOGICAL STUDIES OF WHEAT, *TRITICUM AESTIVUM* L.

David C. JEWELL and A. MUJEEB-KAZI

*CIMMYT, Londres 40, Apdo. Postal 6-641, Delg. Cuauhtemoc 06600 Mexico, D.F., Mexico*

### SUMMARY

This paper reports on a preliminary study of the use of N-banding to detect homologous chromosome exchange in wheat and to observe the segregation of homologues. An  $F_2$  population was used in order to study the chromosomal products of the  $F_1$  meiosis.

Chromosomal recombinants were observed in the long arm of chromosome 3B. The results were consistent with the theory that most chromosomal recombination occurs distally to the centromere.

The segregation of 1B/1R and 1B was observed to be random. However, the segregation of the two parental 3B chromosomes significantly differed from random. It is necessary to establish whether random segregation takes place before drawing conclusions as to the agronomic advantage of one homologue with respect to the other.

Other potential uses of N-banding in wheat are also discussed in the paper.

### INTRODUCTION

Polymorphisms in the size, intensity and pattern of chromosome bands produced by differential staining techniques are known to exist in both the plant and animal kingdoms. Iordansky *et al.* (1978) used C-banding and observed a high level of morphological diversity among the chromosomes of triticales and polyploid wheats which they attributed to intensive introgression. Zurabishvili *et al.* (1978) also noted considerable variation in the total amount of heterochromatin identified by C-banding.

Seal (1982) described and compared the wheat chromosomes in 7 triticales and 7 wheat genotypes using C-banding. Considerable variation in the size, total number and total length of the C-bands was observed. Iordansky *et al.* (1978) proposed that the wheat chromosomes be placed into two groups, "constant" and "variable;" the division, however, was deemed unnecessary by Seal (1982).

Polymorphisms in the banding patterns of 9 N-banded wheat chromosomes have also been observed (Jewell 1978, 1983). The variation observed in the N-banding patterns of wheat chromosomes has not led to confusion in chromosome identification.

To date, chromosome banding patterns have been shown to be heritable and reproducible. Furthermore, the bands are not influenced by hybridization or cytoplasmic differences.

The fact that chromosome bands are inherited as Mendelian units and exhibit polymorphisms led Linde-Laursen (1979, 1981) and Linde-Laursen *et al.* (1982) to propose the

use of C-bands in barley as chromosome markers for pedigree analysis and as descriptors for varieties. The C-banding patterns of barley chromosomes were, in the main, inherited as complete units. However, some possible recombinations in the distal portions of the chromosomes were noted. Linde-Laursen (1981) also observed large differences between the genetic and physical distances which supported the generally accepted theory of restricted chiasma formation and crossing over in the proximal parts of the barley chromosomes.

Jewell (1980) detected a possible cross-over product between two homologous chromosomes that exhibited different polymorphisms. Seal (1982) stated that C-banding may be useful in determining the location of cross-over events in wheat and may permit a comparison of the genetic maps with physical dimensions.

A preliminary study was therefore designed in order to use N-bands to cytologically demonstrate homologous chromosome exchange and to observe chromosome segregation in a random  $F_2$  population of wheat. The  $F_1$  parents were chosen because they differed in N-band polymorphisms for chromosome 3B and were respectively homozygous for a 1B/1R translocation and for the normal 1B chromosome.

## MATERIALS AND METHODS

The N-banding technique used has been fully described (Jewell 1981) and was performed using 2M buffer (Jewell and Mujeeb-Kazi 1982). The chromosomes present in a random sample of  $F_2$  plants from the cross between two hexaploid wheats were N-banded. *Triticum aestivum* L. variety Chinese Spring and *T. aestivum* L. variety Veery "S", and the reciprocal cross was selfed to generate the  $F_2$  population.

The individual plants were scored for the presence of the 1B/1R chromosome (1BL/1RS) from Veery or the 1B chromosome from Chinese Spring. The 3B chromosomes were also observed and scored with respect to parental origin or recombination events. The segregation of these chromosomes was checked for divergence from the expected 1:2:1 ratio using the chi-square test and for a 1 : 1 chromosome frequency.

## RESULTS AND DISCUSSION

The  $F_1$  (Veery/Chinese Spring and its reciprocal cross) was, as expected, heterozygous for the parental 3B chromosomes and for the translocation chromosome 1B/1R and 1B. The two parental 3B chromosomes could be differentiated on the basis of the N-band variation in both the short and long arms (see Figs. 1 and 2). Parental origin was assigned by comparison with the parental karyotype.

The selfing of the  $F_1$  and the scoring of the  $F_2$  plants provided a direct method of observing the segregation of the chromosomes, as the chromosomes of the  $F_2$  plants are equal to the product of the  $F_1$  gametes. The idiograms of possible recombinants that could be cytologically detected using N-bands are presented in Fig. 1. The N-banding patterns allow for the detection of recombination resulting from a break between bands 1 and 2 on the short arm (region A) and between bands 2 and 4 on the long arm (region B). The N-banding patterns also provide for the detection of an exchange between the first band

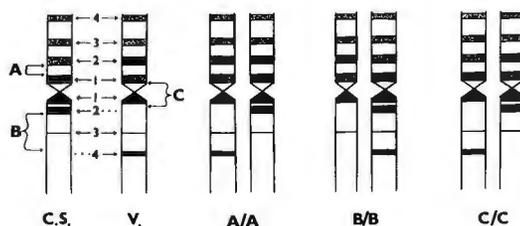


Fig. 1. CS and V are the idiograms of the N-banded Chinese Spring and Veery 3B chromosomes. A/A, B/B and C/C represent the expected changes in the parental idiograms resulting from a reciprocal single cross-over event in regions A, B and C respectively.

on the short arm and the second band on the long arm (region C).

The reciprocal products of an exchange between bands 2 and 4 on the long arm were both observed in the  $F_2$  plants (see Fig. 2). The cross-over frequency between bands 2 and 4 was calculated to be 7.5 percent which is proposed to be equal to 7.5 chromosome map units. In this instance this relates to a physical distance of about one-third of the long arm of chromosome 3B.

No recombination products were observed as the result of cross-overs between bands 1 and 2 on the short arm of chromosome 3B. No chromosome patterns resulting from the exchange of arms or cross overs between the first band on the short arm and the second band on the long arm of chromosome 3B were observed. This is consistent with the theory of restricted chiasma formation and crossing over in the proximal parts of chromosomes. Considerable variation of the N-banding pattern exists in wheat for chromosome 3B (see Fig. 3). Thus, with further analysis of selected crosses, it should be possible to cytologically map the whole of chromosome 3B. As wheat has, on average, approximately 1.6 chiasmata

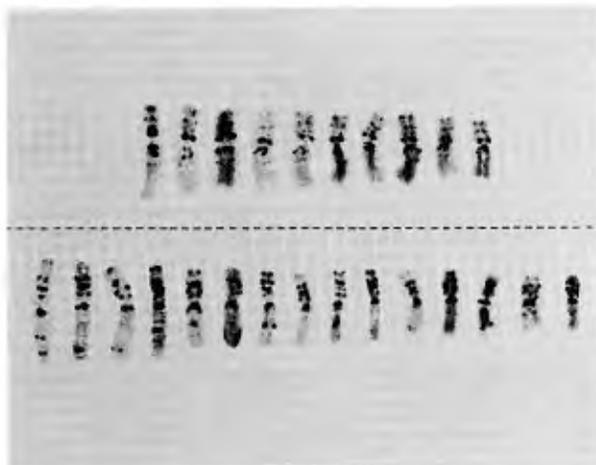


Fig. 2. Variations observed in N-banding patterns of chromosome 3B with respect to the Chinese Spring banding pattern consistency (top line). The bottom line represents polymorphisms observed in 15 different triticales and wheat varieties.

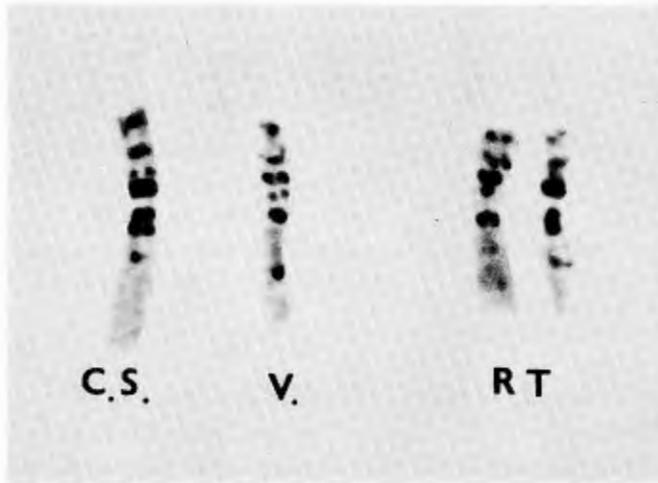


Fig. 3. Chromosome 3B N-banding patterns of *T. aestivum* varieties, Chinese Spring (C.S.) and Veery (V.), and the reciprocal recombination products (RT) from a single cross-over event in the long arm of this chromosome.

for 3BL (Sallee and Kimber 1978), it can reasonably be expected that the total cytological map distance of 3BL is about 75 chromosome map units. Only 7.5 chromosome map units were observed in the proximal half of 3BL, thus most (about 90%) of the expected recombinations for the long arm of 3B must occur in the distal half of this chromosome.

The fact that the reciprocal arm exchange for chromosome 3B is less than 1% is consistent with the theory that chromosome arms do not segregate independently and that the position of the centromere should be ignored in genetic mapping studies across the centromere.

The plants containing recombinant chromosomes of 3B were removed from the total of plants scored and the segregation of the parental 3B chromosomes examined (see Table 1). Using the chi-square test, the observed segregation ratio was found to be significantly different to the expected 1:2:1 ratio at the 10 % level. The Veery chromosome 3B was present significantly more often than expected (at the 5% level) and this suggested that it had

Table 1. Observed and expected frequencies of chromosome complements of chromosome 3B and 1B/1R and 1B in the F<sub>2</sub> population of Chinese Spring/Veery.

	1	2	1
	Homozygous for the Veery chromosome	Heterozygous for the Veery and Chinese Spring chromosome	Homozygous for the Chinese Spring chromosome
Chromosome 3B:			
Observed	22	37	9
Expected	17	34	17
Chromosome 1B/1R and 1B:			
Observed	12	47	21
Expected	20	40	20

a genetic advantage for transmission to the F<sub>2</sub> progeny as compared to the Chinese Spring 3B chromosome. The mechanism of this preferential transmission requires further study.

Veery is known to be homozygous for the translocation chromosome 1B/1R (Merker 1982; Mujeeb-Kazi 1982) and, as the 1B/1R chromosome is easily distinguished from the 1B chromosome, the segregation of these chromosomes was also studied by scoring the F<sub>2</sub> karyotypes (see Table 1). In this case, no significant deviation from the expected 1:2:1 ratio was observed at the 10% level. It thus appears that the transmission frequency of the translocation chromosome 1B/1R is random with respect to chromosome 1B of Chinese Spring.

The random segregation of a chromosome needs to be established before conclusions can be drawn as to agronomic advantage. If the parental chromosomes in an F<sub>1</sub> hybrid do not segregate randomly, this may lead to false conclusions about the relative agronomic value of the parental chromosome drawn on the basis of its presence in a majority of the advanced generation breeding material.

Chromosome banding in wheat has been successfully used for chromosome identification and to identify translocations (Gill and Kimber 1977; Jewell 1978, 1983; Seal 1982). N-banding can be used in wheat in the same way as C-banding for barley (Linde-Laursen *et al.* 1982) and wheat (Seal 1982). It can be used to study pedigrees and chromosome inheritance, and as a source of varietal descriptors. Using selected crosses, the distance between the bands can be mapped by cross-over frequency and hence the chiasma frequency in discrete physical sections and total length of a chromosome can be estimated. N-banding can also be used as a teaching aid to cytologically demonstrate recombination and to study chromosome segregation. If the F<sub>1</sub> is backcrossed reciprocally, the two BC<sub>1</sub> progenies can be studied to detect preferential chromosome transmission, or the lack of it, in both the male and female gametes.

Chromosome banding studies should continue to contribute significantly to the understanding of wheat cytology and genetics.

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