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Identification of Wheat-Barley Addition Lines with N-Banding of Chromosomes

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Abstract. The seven chromosomes of barley (*Hordeum vulgare*) have been identified individually by their distinctive N-banding pattern. Furthermore all of the barley chromosome N-banding patterns were found to be recognizably different from those exhibited by wheat chromosomes, making it possible to identify individual barley chromosomes when present in a wheat background. N-banding has therefore been used to identify the individual barley chromosomes present in (a) reciprocal wheat-barley F_1 hybrids, including some with abnormal chromosome constitution, and (b) a set of wheat-barley addition lines produced in this laboratory. The value of N-banding for detecting translocations between wheat and barley chromosomes and for isolating lines possessing a pair of barley chromosomes substituted for a particular pair of wheat chromosomes is also demonstrated.

Introduction

Recently we have produced six of the seven possible addition lines having individual pairs of barley chromosomes added to the chromosome complement of hexaploid wheat (Islam et al., 1978). The uniqueness of each of the six lines was established initially from differences in their spike morphology and their isozyme pattern (Hart et al., in preparation) and subsequently by chromosome pairing behaviour at meiosis in progeny derived from intercrosses between the lines. These six different addition lines were arbitrarily designated A, B, C, D, E and F in relation to their serial isolation. Thus the problem remained of relating the barley chromosome present in the addition lines with the standard numbering system assigned to barley chromosomes based on the accepted linkage groups as determined by cytogenetic studies with trisomics and the translocation stocks (Burnham and Hagberg, 1956; Tsuchiya, 1960, 1961; Ramage et al., 1961). The present paper is concerned with the resolution of this problem.

Only three of the seven barley chromosomes can be recognized easily by their morphology in conventional somatic metaphase preparations. Chromo-

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some 5 is the smallest in the complement and the two nucleolar organizer (NO) chromosomes, no. 6 and 7, are readily detected. Linde-Laursen (1975) demonstrated that a C-banding method can be used to identify each of the barley chromosomes and this was confirmed by Noda and Kasha (1976) and Vosa (1976). Although this technique produced good results with barley itself, it is known that all wheat chromosomes also band extensively with C-banding (Gill and Kimber, 1974). Furthermore the centromeric and near centromeric banding that occurs on both the barley chromosomes and on many of the wheat chromosomes makes it difficult to distinguish barley chromosomes in a wheat background. The situation is very different in wheat-rye addition lines where the characteristic telomeric C-banding in most rye chromosomes (Darvey and Gustafson, 1975) facilitates their identification. In addition the C-banding procedure is time consuming. Recently Gerlach (1977) applied to wheat the N-banding method of Funaki et al. (1975), which is simpler and less time consuming to apply than C-banding, and he was able to identify 9 of the 21 chromosomes. The present study was undertaken to find whether N-banding could be used to identify barley chromosomes particularly when they are present in a wheat background.

This paper describes the N-banding patterns of barley chromosomes and the various ways in which N-banding has helped analyse the chromosome constitution of wheat-barley hybrids and addition lines.

Materials and Methods

a) Plant Material Studied. The barley examined with N-banding was material derived from the cultivars Betzes and Shin Ebisu 16. Besides normal disomics, observations were made on some primary trisomics and telo-trisomics of these cultivars (Betzes – chromosomes 1, 2, 3, 4, 6 and 7; Shin Ebisu 16 – chromosomes 2L, 3, 4 and 7). The seeds of these stocks were kindly supplied by Dr. R.T. Ramage and Dr. T. Tsuchiya, respectively.

Also examined were wheat × barley hybrids and derivative material, all of which had been produced in this laboratory. It included (1) reciprocal F_1 hybrids between Betzes and Chinese Spring wheat, (2) six disomic addition lines possessing different pairs of Betzes chromosomes added to the chromosome complement of Chinese Spring wheat (Islam et al., 1978), (3) four lines having different telocentric pairs of Betzes chromosomes added to Chinese Spring wheat, (4) four different near disomic addition lines having one complete barley chromosome plus a telocentric of the same chromosome from which the four above-mentioned ditelosomic lines were isolated.

b) Cytological Procedure. Roots from 2–3 days old seedlings grown at 27° C were treated with 0.05% colchicine for 3–4 h at 27° C. The root tips were then fixed in a freshly prepared mixture of acetic acid/ethanol (1 : 3) and stored at 4° C. Immature spikes chosen for ovary tissue examination were treated in iced water at 2° C for 20–24 h before being placed in the fixative.

In making root tip and ovary squashes the fixed material was first macerated in 45% acetic acid on microscope slides. After applying the cover glass the preparations were gently heated and then squashed to spread the chromosomes. The cover glass was then removed after freezing with liquid nitrogen. The preparations were dehydrated in 95–100% ethanol, either at room temperature for 2–3 h or left overnight in ethanol at −15° C. Finally, the slides were air dried before applying the N-banding procedure.

c) N-Banding. Gerlach's (1977) procedure for N-banding was followed, with some slight modification. The air-dried slides containing barley material were incubated in 1 M NaH_2PO_4 pH 4.2

for 3–3$^1/_2$ min at 92 ± 1° C. For wheat × barley hybrids and addition lines the treatment time was increased to 4–5 min at 94 ± 1° C. After rinsing in distilled water, the slides were stained for 30–40 min in 7% V/V Gurr's Improved Giemsa R66 in $^1/_{15}$ M Sørensen phosphate buffer (pH 6.8). After rinsing again in distilled water, the slides were air dried and mounted in immersion oil for observations.

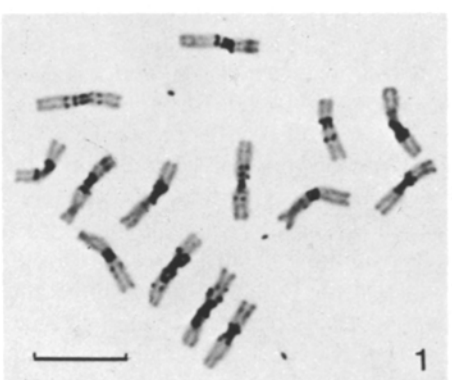
d) Idiogram Construction. To construct an idiogram of barley chromosomes, photomicrographs were taken of 10 well-spread N-banded cells and the lengths of the chromosome arms were measured from the photographs. Following Tuleen (1973), each chromosome arm in the cell was expressed as a percentage of the total sum of the lengths of all the chromosomes in that cell. Using Burnham and Hagburg's (1956) measurement of the long arm of chromosome 6 (62.1 units) as a standard, the length of the long arm of chromosome 6 in the present observations was multiplied by a factor to bring it to 62.1 Measurements of the other chromosome arms were then multiplied by this same factor. The arm lengths and ratios thus determined were used to construct the idiogram.

Results

I. Barley

The N-banded karyotype of Betzes barley is shown in Figure 1. The distinctive banding patterns allow the chromosomes to be arranged in 7 pairs. All 7 barley chromosomes possess centromeric or near centromeric banding and most of the chromosomes also have several interstitial bands.

The identity of the barley chromosomes exhibiting these different banding patterns was determined by examining N-banded preparations of trisomic lines. For example, the N-banded karyotype of Betzes trisomic 3 is shown in Figure 2, and the banding pattern of barley chromosome 3 is identified by its presence in triple dose.



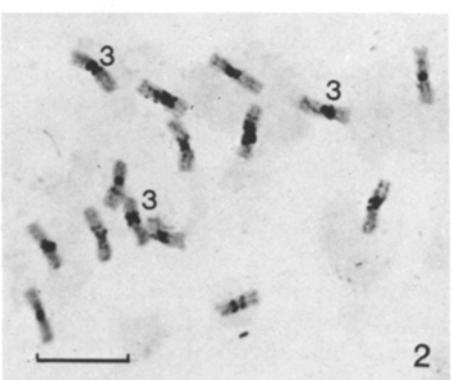


Fig. 1. N-banded mitotic chromosomes of Betzes barley. The bar in all figures indicates 10 μm

Fig. 2. N-banded mitotic chromosomes of Betzes trisomic 3. Chromosome 3 (numbered) can be easily identified

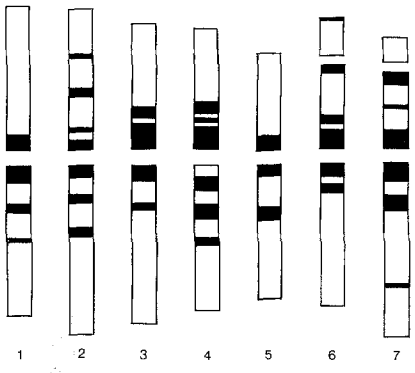


Fig. 3. Idiogram of the N-banded mitotic chromosomes of Betzes barley

The banding pattern exhibited by barley chromosomes is shown in Figure 3 and described below. Chromosome 1 has medium centromeric bands in both arms and two interstitial bands in the long arm. Chromosome 2 has small centromeric bands in both arms with 3 interstitial bands in the short arm and 2 interstitial bands in the long arm. The banding intensity in this chromosome is light. Chromosome 3 has a large centromeric band, accompanied by one small proximal band in the short arm and a small centromeric band and a diffuse interstitial band in the long arm. Chromosome 4 is the most heavily banded of all the barley chromosomes. One large centromeric band and 2 small proximal bands are present in the short arm. No centromeric band is evident in the long arm, but 3 interstitial bands are present. Chromosome 5 possesses small centromeric bands in both arms and a very conspicuous interstitial band in the long arm. Both chromosomes 6 and 7 possess centromeric bands in both arms and a band in the NO region of the short arm. The band in the NO region of chromosome 7 is, however, more pronounced than the corresponding band in chromosome 6. In addition one proximal band is present in both arms of chromosome 6. Chromosome 7 also possesses a distinctive interstitial band in the long arm and a faint band in distal position in each arm.

The bands show some variation in intensity between slides and between cells on the same slide. Sometimes faint interstitial bands observed in less condensed chromosomes do not show up in a more condensed stage. Not clearly evident in some of the preparations were the most distal band in each arm of chromosome 2, the telomeric band in the short arm of chromosome 6 and the distal band in the long arm of chromosome 7.

The banding pattern of Shin Ebisu 16 was similar to that of Betzes. Minor differences were present in the interstitial band in the long arm of chromosome 3, which is more pronounced in Shin Ebisu 16 than the diffuse band in that region in Betzes. Additionally the distal band in the long arm of chromosome 4 of Betzes was absent from Shin Ebisu 16. Similar small intervarietal differences in banding were observed by Linde-Laursen (1975, 1978a) and Noda and Kasha (1976, 1978) in C-banded preparations of barley. However, Vosa (1976) reported even larger differences between barley cultivars.

From N-banded preparations of telotrisomic 2L of Shin Ebisu 16, the chro-

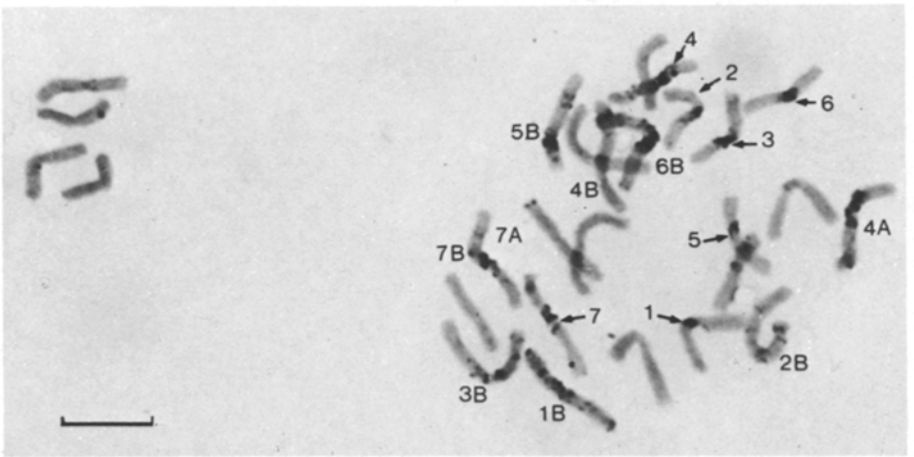


Fig. 4. N-banded somatic chromosomes of a 28-chromosome barley × wheat F_1 hybrid (one chromosome is outside the frame). The barley chromosomes are numbered 1 to 7 and the banded wheat chromosomes are designated 1B, 2B etc.

mosome arm present was identified and the validity of the arm designation was checked. Telotrisomic 2L agreed with the long arm of chromosome 2, as was also reported by Linde-Laursen (1978b) from C-banded preparations.

II. Wheat-Barley Hybrids

The F_1 wheat-barley hybrids could only be obtained from cultured embryos (Islam et al., 1975) and only a limited number of root tips were therefore available. Hence, dividing ovarian tissue was used as a source of somatic metaphase chromosomes.

From N-banded preparations (Fig. 4) of the F_1 barley × wheat hybrids all the 7 barley chromosomes (arrowed) were easily identified and distinguished from the 9 N-banded wheat chromosomes. These hybrids had 28 chromosomes, whereas the reciprocal F_1 wheat × barley hybrids possessed an array of chromosome numbers varying from 21 to 36 in different plants (Islam et al., 1978). The identity of these chromosomes could not be determined from conventional cytological studies, nor could their constitution be resolved by test crosses to wheat since no viable seeds were obtained. However, with N-banding of one such F_1 hybrid with 33 somatic chromosomes it was possible to establish that it possessed duplicate members of wheat chromosome 1B and barley chromosomes 1, 2, 4, 5, 6 and 7. This hybrid was also deficient for wheat chromosome 4B. Although N-banding can be used to trace all barley chromosomes, only the 7 chromosomes of the B-genome and 2 chromosomes of the A-genome of wheat, can be distinguished with this procedure (Gerlach, 1977).

The value of N-banding was also revealed when establishing the identity

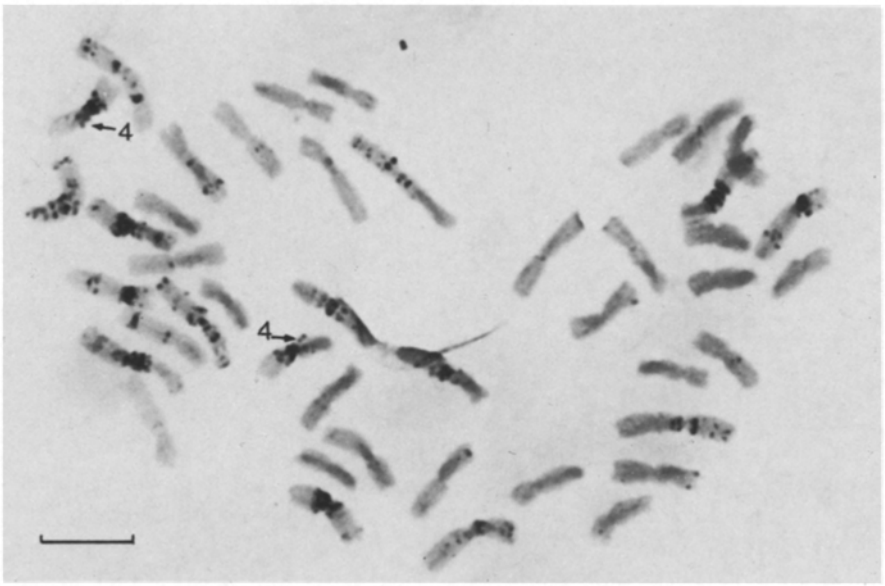


Fig. 5. N-banded somatic chromosomes of wheat-barley addition line A. Barley chromosome 4 is arrowed

of the precise chromosome pair present in each of the 6 disomic wheat-barley addition lines. It was possible to identify the barley chromosomes by relating their banding patterns to that of the trisomics of barley. The addition lines previously designated with letters as A, B, C, D, E and F were found to possess standard barley chromosomes 4, 7, 6, 1, 2 and 3, respectively. An N-banded cell of an addition line having chromosome 4 of barley is shown in Figure 5, where it can be easily identified from the 18 N-banded wheat chromosomes. It was also possible with N-banding to establish the arms involved in 4 ditelosomic addition lines. This was achieved by banding the 42 + 2t chromosome ditelosomic along with the 43 + 1t chromosome near disomic additions. The latter plants were helpful in confirming which chromosome arm was present in the ditelosomic additions because the telo was present together with the complete barley chromosome.

Finally it was found that N-banding could be used to detect translocations involving wheat and barley chromosomes. One such translocation line was recovered in the course of the production of the addition lines. From gel electrophoresis of prolamin proteins it was known only that the translocation line possessed most of the barley prolamins and thus involved part of chromosome 5 of barley. With N-banding it was established that the short arm of chromosome 5 of barley was involved in a translocation with an unknown wheat chromosome which does not band with this technique.

Discussion

Gerlach (1977) noted some correspondence between his N-banded preparations and the C-banding preparations of Chinese Spring wheat published by Gill

and Kimber (1974). In the present study although there was some agreement between N-banding of barley and C-banding of earlier studies, the correspondence was far from exact. Linde-Laursen (1975) reported the absence of centromeric bands from both arms of chromosome 2 and from the long arm of chromosome 3. Noda and Kasha (1976, 1978) also failed to observe any centromeric band in the long arm of chromosome 5. In the present observations centromeric bands were observed in both arms of all the barley chromosomes except for the long arm of chromosome 4. Moreover, the band position, number and thickness of bands in chromosome 2, 4 and 6 differed to some extent from the C-banded preparations of Linde-Laursen (1975) and Noda and Kasha (1978). The present N-banding pattern did not agree with the C-banding pattern observed by Vosa (1976). From my results and also from those of Linde-Laursen (1975, 1978a) and Noda and Kasha (1976, 1978) it appears that Vosa's designation of chromosome 6 and 7 should be interchanged.

From the analysis of multiple translocation stocks of barley, Tuleen (1973) and Künzel (1975) raised doubts about the correspondence between the numbers assigned to the linkage groups and the standard karyotype. Similarly Noda and Kasha (1978) and Linde-Laursen (1978b), using C-banded preparations, also found inconsistency between the numbering of the standard karyotype and the trisomics. The present results for the trisomic lines of Betzes and Shin Ebisu 16 are in agreement with those of Noda and Kasha (1978) who found that the extra chromosome in trisomic 1 is not the longest chromosome of the barley complement but corresponds in length and arm ratio to chromosome 3 of the standard karyotype, whereas chromosome 2 is the longest barley chromosome. Also I agree with Noda and Kasha (1978) that chromosome 3 (trisomic 3) probably corresponds to chromosome 1 of the standard karyotype. That is, when the arm ratios of chromosome 1 and 2 observed by Tjio and Hagberg (1951) were compared with chromosome 3 of Tuleen (1973), Noda and Kasha (1978) and those in the present study, chromosome 3 with a more dissimilar arm ratio was found to match better with chromosome 1 of the standard karyotype. In my work trisomes 4, 6 and 7, however, matched well with the corresponding chromosome of the standard karyotype, whereas trisomic 5 was not available for analysis. As the trisomic numbering is more generally accepted in the literature and because the genetic linkage groups are based on it, the barley chromosomes involved in the addition lines were identified and numbered according to the chromosome involved in the trisomics irrespective of the karyotype numbering.

The merits of N-banding were very evident when attempting to resolve the chromosome constitution of the various hybrid stocks. Because of the distinctive banding patterns of individual barley chromosomes, and the lack of correspondence with any wheat chromosomes, they were easily identified in the F_1 barley × wheat hybrids. N-banding was even more useful when determining the chromosome constitution and hence the probable origin of the unusual F_1 hybrids obtained when wheat was used as the female parent. These abnormal hybrids probably arose following mitotic disturbances during early zygotic divisions of the F_1 hybrid embryos (Islam et al., in preparation). N-banding of one of these F_1 hybrids with 33 somatic chromosomes clearly indicated a duplication of some wheat and barley chromosomes and deficiencies of some wheat

chromosomes. This technique was also of great value for identifying the barley chromosome involved in the disomic wheat-barley addition lines. From isozyme studies, 6 different barley isozymes were located in 4 different addition lines but the identity of the respective barley chromosome was not known. Similarly it was difficult to recognise the barley chromosome arm involved in the ditelosomic addition lines from somatic metaphase preparations, because of the lack of any distinctive features apart from the nucleolar organizers on chromosomes 6 and 7. With N-banding, the individual barley chromosome and the arm involved could be identified. The technique will also be useful for identifying translocations involving the 9 wheat chromosomes (the ones that band with this technique) and any barley chromosome. With translocations involving the other 12 wheat chromosomes only the barley chromosome will be known. One such line involving the short arm of chromosome 5 of barley has already been identified.

The N-banding method is currently being applied to isolate lines where individual pairs of barley chromosomes are substituted for specific wheat chromosome pairs. Already a putative substitution of chromosome 4 of barley for 4A of wheat has been detected in progeny from a double monosomic stock, using isozyme studies and it was possible to confirm the presence of chromosome 4 of barley and the absence of 4A of wheat in this plant by N-banding.

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