Giemsa C-banded karyotypes of *Hordeum secalinum*, *H. capense* and their interspecific hybrids with *H. vulgare*

IB LINDE-LAURSEN¹, ROLAND VON BOTHMER², and NIELS JACOBSEN³

¹ Agricultural Research Department, Risø National Laboratory, Roskilde, Denmark
² Department of Crop Genetics and Breeding, The Swedish University of Agricultural Sciences, Svalöv, Sweden
³ Institute of Botany, The Royal Veterinary and Agricultural University, Copenhagen, Denmark

LINDE-LAURSEN, I., BOTHMER, R. VON, and JACOBSEN, N. 1986. Giemsa C-banded karyotypes of *Hordeum secalinum*, *H. capense* and their interspecific hybrids with *H. vulgare*. -- *Hereditas* 105: 179-185. Lund, Sweden. ISSN 0018-0661. Received December 2, 1985

The European *H. secalinum* (2n=4x=28) and the South African *H. capense* (2n=4x=28) had similar karyotypes with ten pairs of metacentrics, three of submetacentrics, and one of SAT-chromosomes. The C-banded karyotypes of *H. secalinum* from northern Europe were characterized by banding patterns with few bands, those of *H. secalinum* from Spain and *H. capense* by banding patterns with more bands. The bands were mostly small or very small and had no preferential disposition. Intraplant banding pattern polymorphism was observed in North European *H. secalinum*, in agreement with an outbreeding reproductive system. No banding pattern polymorphism was present within plants of *H. secalinum* from Spain and *H. capense*, suggesting self-pollination. In both species banding pattern polymorphism was prevalent among plants. Together with other evidence the fairly similar basic C-banded karyotypes of the two species indicate a rather close relationship.

Ib Linde-Laursen, Agricultural Research Department, Risø National Laboratory, DK-4000 Roskilde, Denmark; Roland von Bothmer, Department of Crop Genetics and Breeding, The Swedish University of Agricultural Sciences, S-268 00 Svalöv, Sweden; and Niels Jacobsen, Institute of Botany, The Royal Veterinary and Agricultural University, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

Material and methods

The plant material used is given in Tables 1 and 2. The material of the two species was derived from seeds collected in nature. The interspecific hybrids were produced as described in BOTHMER et al. (1983). Voucher specimens of the plant material are kept at the Botanical Museum, Copenhagen (C). The plants were grown and their C-banded idioograms constructed as reported in LINDE-LAURSEN et al. (1980). A C-band is here defined as large if it covers more than ten per cent of a chromosome arm. The mean length of the chromosomes and the ratio longest/shortest chromosome of each plant measured are given in Table 1. The number of chromosomes with nucleolus-forming capacity was determined through staining of interphases with AgNO₃ according to LINDE-LAURSEN (1984).

Results

*H. secalinum* Schreb.

This species, which has a European and North African distribution, is generally reported to be a tetraploid (2n=4x=28). Di- and triploids have been reported from Portugal, but these reports are doubt-
Table 1. Chromosome number, maximum number of submetacentrics, SAT-chromosomes, and nucleoli, mean chromosome length, ratio longest/shortest chromosome, amount of constitutive heterochromatin (per cent), and geographic origin of the material of *H. secalinum* and *H. capense* investigated

| Species      | Plant no. | 2n = | Max. number of | Mean chrom. length | Longest/shortest chrom. | Const. het. chromatin per cent | Origin                     |
|--------------|-----------|------|----------------|---------------------|--------------------------|--------------------------------|----------------------------|
|              |           |      | subcentrics    | SAT-chrom. | nucleoli | | | |
| *H. secalinum* | H 192-27  | 28   | 6              | 2        | 2        | 10.9 | 1.6 | 5.4 | Denmark, Jutland, Rømø; coll. |
|              | H 231-25  | 27   | 6              | 2        | 2        | 13.0 | 1.4 | 3.2 | R. von Bothmer, 6.1976        |
|              | H 296-33  | 28   | 6              | 2        | 2        | 10.7 | 1.5 | 8.1 | Sweden, Skåne, Saxän at Landskrona; coll. A. Nilsson, 1975; Spain, 14 km E Huelva; coll. N. Jacobsen no. 563, 26.5.1977 |
|              |           |      |                |          |          | 11.1 | 1.7 | 6.5 | S Africa, prov. Ventersted, Oviston, False Upper Karoo; coll. J. R. Anderson no. 344, 12.1.1972; Lesotho, Donga, Mountain Road, weed; coll. M. Schmitz no. 6595, 2.1976 |

The karyotype normally contains one pair of satellite (SAT) chromosomes (Cauderon and Cauderon 1956; Rajhathy et al. 1964; for literature, see Bothmer and Jacobsen 1979b), but Vosa (1976) has published a C-banded karyotype of a plant from England with two SAT-chromosome pairs.

We have studied one plant from each of two populations from northern Europe and another from Spain (Table 1). Two plants had 2n=28 (Fig. 1a) as expected, whereas one was a hypotetraploid with 2n=27. However, sister plants from all three populations have been observed previously to have 2n=28 (Bothmer and Jacobsen 1979b).

Apart from the missing chromosome of *H. secalinum* H 231-25, the complements of all three plants were similar with ten pairs of metacentrics, three of submetacentrics, and one of satellite (SAT) chromosomes (Fig. 2a, b, c), with satellite lengths about two-thirds of the supporting short arms. The presence of two SAT-chromosomes matched observations of AgNO₃-stained interphases with a maximum of two nucleoli (Table 1; Fig. 1d). The ratio longest/shortest chromosome was nearly the same in all three plants (Table 1). In comparison to the C-banded karyotype of the plant from Spain, H 296-33 (Fig. 2c), the karyotypes of the two North European plants were characterized by a lower level of banding (Fig. 2a, b), also reflected in a lower content of constitutive heterochromatin (Table 1). The C-band patterns of all plants had from zero to eight bands per chromosome with no preferential disposition (Fig. 1a, 2a, b, c), but H 296-33 had about twice as many bands as the other two plants. The bands varied in size from very small to large. Only one chromosome of H 192-27 carried two large

Table 2. Chromosome number and maximum number of SAT-chromosomes and nucleoli of interspecific *Hordeum* hybrids

| Hybrids                      | Plant no. | 2n = | Max. no. of | Origin of parents¹ |
|------------------------------|-----------|------|-------------|---------------------|
|                              |           |      | SAT-chrom.  |                     |
|                              |           |      | nucleoli    |                     |
| *H. secalinum* × *H. vulgare*| HH 2412-3 | 21   | 18-23       | 3                   |
| (H 296-32 × 'Strengs Franken III') | | | | H 296-32: Sister plant of H 296-33 |
| *H. capense* × *H. vulgare*  | HH 1447-1 | 21   | 19+t-22²   | 3                   |
| (H 334 × HP 40)              |           |      | 4³         |                     |

¹ cf. Table 1 ² t=telocentric ³ incl. one micronucleolus
bands. The number of conspicuous and large bands in a karyotype corresponded to the number and size of the chromocentres of interphases (Fig. 1c).

The C-banding patterns of the homologous chromosomes of H 296–33 were homomorphic, whereas those of the North European plants H 192–27 and H 231–25 showed a certain level of banding pattern polymorphism (Fig. 2a, b, c). However, the polymorphism did not interfere with a safe identification of homologues except among the four ± unbanded chromosomes in H 192–27 (Fig. 2a). Banding pattern polymorphism was prevalent among the three karyotypes, preventing identification of homologues beyond the level reached by considering chromosome morphology, i.e., SAT-chromosomes (Fig. 2a, b, c). The banding patterns gave no indication of homoeology among chromosome pairs.

**H. secalinum × H. vulgare**

The plant was derived from the cross of a sister plant of H 296–33 (cf. Table 1) × H. vulgare ('Strengs Franken III') (Table 2). Previous chromosome countings have shown 2n=19–23 (Bothmer et al. 1983, 1985). We studied the chromosomal composition in ten C-banded metaphases with 2n=18–23 (Tables 2, 3; Fig. 1b; Fig. 2A in Bothmer et al. 1985). In all cells C-banding patterns identified seven chromosomes with the complete H. vulgare
Fig. 2 a–c. Idiograms of the chromosomes of *H. secalinum* showing relative sizes and positions of C-bands (solid regions). Broken lines indicate very small bands. a H 192–27
2n=28. b H 231–25 2n=27. c H 296–33 2n=28. Asterisks indicate chromosome (pair) lost in the interspecific hybrid *H. secalinum* × *H. vulgare* HH 2412–3.

In only three of the cells, two with 2n=20 and one with 2n=21, was the quality of the metaphases sufficient for a safe identification of all the remaining chromosomes. The C-banding pattern of each of these matched a similar C-banding pattern of *H. secalinum*, thus giving no indication of intrapopulational banding-pattern polymorphism.

However, a specific *H. secalinum* chromosome (marked with asterisks in Fig. 2c) was missing in the three cells. Further, it was absent from a cell with 2n=22 and one with 2n=23.

In cells with more than 20 chromosomes and thus with more than one homologue of one or more *H. secalinum* chromosomes it could be ascertained that the multiplication had affected different *H. secalinum* chromosomes. No cell contained more than two chromosomes with clearly visible nucleolar constrictions (Table 2). These were always *H. vulgare* chromosomes 6 and 7. However, the observation of a faint secondary constriction in a third chromosome later identified as the *H. secalinum* SAT-chromosome (cf. Fig. 2c) was missing in the three cells. Further, it was absent from a cell with 2n=22 and one with 2n=23.

In both plants the chromosome pairs were identified by banding patterns and chromosome morphology. However, banding pattern polymorphism prevented a safe identification of homologues between plants beyond the level reached by the use of chromosome morphology, i.e., the SAT-chromosomes and the smallest pair of submetacentrics. Comparisons of banding patterns gave no indication of homoeology among chromosome pairs.

**H. capense** × **H. vulgare**

The plant was derived from the cross *H. capense* H 334 × *H. vulgare* (line HP 40) (Table 2). Previous chromosome countings found 2n=20 and 21.
Fig. 3 a–e. C-banded somatic metaphase chromosomes and silver nitrate-stained interphase nuclei showing nucleoli of *H. cupense* 2n=28 (a, d) and the interspecific hybrid *H. cupense* × *H. vulgare* (cell with 19 chromosomes and 1 telocentric (marked with "t")) (b, e) and Giemsa-stained interphase nucleus showing chromocentres of *H. cupense* (c). a and c H 335. b and e HH 1447–1. d H 334. In Fig. 3b each *H. vulgare* chromosome is marked by its chromosome number (cf. LINDE-LAURSEN 1981). Bars = 10 μm.

The chromosomal composition was studied in three C-banded metaphases with 2n=19+t, 21 and 22 (Tables 2, 3; Fig. 3b). In the three cells C-banding patterns identified seven chromosomes with *H. vulgare* chromosomes 1–7. The remainder of the chromosomes could be identified with their homologues in H 334 (cf. Table 1; Fig. 4a). The cells with 2n=21 and 22 had the full *H. capense* genome, and the cell with 2n=22 had an extra, long *H. capense* metacentric. The cell with 2n=19+t had lost a different *H. capense* metacentric and the long arm of a third *H. capense* metacentric.

Most metaphases observed contained three SAT-chromosomes (Table 2). In these, the nucleolar constriction of *H. vulgare* chromosome 6 was the one most frequently observed and most clearly expressed. The observation of three SAT-chromosomes agrees with the observation of up to three standard nucleoli, one always smaller than the other two, in many interphases (Fig. 3e). However, one cell in addition had a micronucleolus.

In the few cells examined the chromosomes of the *H. vulgare* genome were situated closer to the metaphase centre than the *H. capense* chromosomes (cf. Fig. 3b).
Discussion

The two tetraploid *Hordeum* species, *H. secalinum* and *H. capense*, showed great similarity in chromosome morphology and a fair degree of similarity in C-banding patterns. In combination with observations of very high pairing in PMCs of a *H. secalinum* × *H. capense* hybrid (0.10 + 10.3011 (6.60) + 3.7011 (6.40) + 0.4011 + 0.88 + 0.50; 22.40 xtal/cell) compared to other tetraploid cross-combinations comprising one of the two species (Bothmer and Jacobsen 1979b) and isoenzyme patterns (Jørgensen 1986), this indicates a close relationship between the two species, and closer than previously assumed (Bothmer and Jacobsen 1979b).

The variation in C-banding patterns between the plants from northern Europe and Spain was larger than normally observed within a cytotype of a *Hordeum* taxon (Vosa 1976; Linde-Laursen 1981; Linde-Laursen and Bothmer 1984a, 1986a). Another differentiation of the plants from northern Europe and that from Spain was the difference in banding pattern polymorphism. Both plants from northern Europe showed intraplant banding pattern polymorphism in agreement with an outbreeding reproductive system (Bothmer and Jacobsen 1979b), whereas no banding pattern polymorphism was observed in the material from Spain, suggesting self-pollination of plants of the population. The hypothesis is supported by a high seed-setting upon isolation of the plants. Further, a rather pronounced differentiation in morphology (Bothmer and Jacobsen, 1979b) and biochemical characters (Jørgensen unpubl.) has been observed between materials from the two areas. No banding pattern polymorphism was observed within the two *H. capense* plants; this supports the theory that the species is mainly self-pollinating (Bothmer and Jacobsen 1979b). The high level of banding pattern polymorphism among plants of different populations of the two species agrees with observations in all other *Hordeum* species previously examined (Vosa 1976; Linde-Laursen et al. 1980, 1986; Linde-Laursen 1981; Linde-Laursen and Bothmer 1984a). Vosa (1976) reported a C-banded *H. secalinum* karyotype deviating from those studied by us with respect to (1) chromosome morphology as it had two SAT-chromosome pairs, and (2) C-banding patterns, as it had conspicuous centromeric bands, and telomeric bands of different sizes were found in at least one arm of all chromosomes. One of the SAT-chromosome pairs carried long satellites. This pair is very similar in morphology to a SAT-chromosome pair found in all North American and at least some South American *Hordeum* species (Linde-Laursen et al. 1986; Linde-Laursen and Bothmer 1986a). Vosa's (1976) plant was drawn from a population from England, whereas our plants were from populations from mainland Europe. The differences are of such a magnitude that the problem needs investigation.

Baum (1978) referred the *H. brachyantherum* populations found in Newfoundland and Labrador to *H. secalinum*. However, comparisons of morphological, biochemical, and cytological characters in material of the two species and *Hordeum* material from Newfoundland clearly identifies the latter with *H. brachyantherum* Nevski (Linde-Laursen et al. 1986; Jørgensen 1986, and unpubl.).

The chromosomal composition of the *H. secalinum* × *H. vulgare* and *H. capense* × *H. vulgare* hybrids indicated that both contained a complete *H. vulgare* genome in all cells examined, whether euploid or aneuploid. This corresponds with observations in *H. vulgare* × *H. brevisubulatum* ssp. turke-

**Table 3. Number of chromosomes in root tip cells of *H. secalinum* × *H. vulgare* and *H. capense* × *H. vulgare* hybrids**

| Hybrid    | No. of cells | 2n=  |
|-----------|--------------|------|
| HH2412-3  | 52           | 18   |
| HH1447-1  | 15           | 9α   |

1 Two cells with 19 + telocentric *H. capense* chromosome
staniculum, 6x-, H. roshevitzii, 4x × H. vulgare-, and H. jubatum × H. vulgare hybrids (Linde-Laursen and Jensen 1984). In hybrids of all five combinations aneuploidy was caused by the loss or gain of non-H. vulgare chromosomes. Another picture was observed in H. lechleri × H. vulgare-, H. arizonicum × H. vulgare-, and H. tetraploidum × H. vulgare hybrids, in which the loss or acquisition of chromosomes could affect both parental genomes (Linde-Laursen and Bothmer 1986a, b). The two cross-combinations H. lechleri × H. vulgare and H. arizonicum × H. vulgare are known to produce haploids of the non-H. vulgare parent through somatic elimination of H. vulgare genome (Rajhathy and Symko 1974; Subrahmanyan 1980), whereas no haploids of H. tetraploidum have been obtained as yet from the latter combination (Bothmer et al. 1983, 1985). The difference between the two groups of hybrids with respect to the preservation of the H. vulgare genome suggests inequalities in the interaction of parental genomes. The mechanism may be similar to that observed in H. marinum × H. vulgare- and H. vulgare × H. bulbosum hybrids by Finch (1983) and in a H. vulgare × Psathyrostachys fragilis hybrid by Linde-Laursen and Bothmer (1984b).

The arrangement of the parental genomes in the present hybrids with the H. vulgare genome closest to the metaphase centre agrees with previous observations of a concentric arrangement of genomes in other interspecific and intergeneric hybrids (see Bennett 1984; Linde-Laursen and Jensen 1984; Linde-Laursen et al. 1986; Linde-Laursen and Bothmer 1986a). As in these hybrids, the concentric arrangement was accompanied by a more or less pronounced suppression of the activity of the nucleolar organizers of the outside genome.

Acknowledgments. — The skilful technical assistance of Mrs. Elly Ibsen is greatly appreciated.

Literature cited

BAUM, B. R. 1978. The status of Hordeum brachyantherum in eastern Canada, with related discussions. — Can. J. Bot. 56: 107–109

BENNETT, M. D. 1984. Nuclear architecture and its manipulation. — In Gene Manipulation in Plant Improvement. Proc. 16th Studier Genetics Symp., Columbia 1984 (Ed. J. P. Gustafson), Plenum Publ. Corp., p. 469–502

BOTHMER, R. VON, FLINK, J., JACOBSEN, N., KOTIMÄKI, M. and LANDSTRÖM, T. 1983. Interspecific hybridization with cultivated barley (Hordeum vulgare L.). — Hereditas 99: 219–244

BOTHMER, R. VON, FLINK, J., and LINDE-LAURSEN, I. 1985. Development and meiosis of three interspecific hybrids with cultivated barley (Hordeum vulgare L.). — Z. Pflanzenzücht. 96: 107–114

BOTHMER, R. VON and JACOBSEN, N. 1979a. Biosystematic investigations in the genus Hordeum. — In Induced Variability in Plant Breeding (Ed. C. Broerjties), Pudoc, Wageningen, p. 220–231

BOTHMER, R. VON and JACOBSEN, N. 1979b. A taxonomic revision of Hordeum secalinum and H. capense. — Bot. Tidsskr. 74: 223–235

CAUDERON, Y. et A. 1956. Etude de l'hybride entre Hordeum bulbosum L. et H. secalinum Schreb. — An. Amel. Pl. 6: 307–317

FINCH, R. A. 1983. Tissue-specific elimination of alternative whole parental genomes in one barley hybrid. — Chromosoma 88: 386–393

JORGENSEN, R. B. 1986. Relationships in the barley genus (Hordeum). An electrophoretic examination of proteins. — Hereditas 104: 273–291

LINDE-LAURSEN, I. 1981. Giemsa banding patterns of the chromosomes of cultivated and wild barleys. — In barley Genetics IV. Proc. 4th Int. Barley Genet. Symp., Edinburgh 1984 (Eds. M. J. C. ASHER, R. P. ELLIS, A. M. HAYTER and R. N. H. WHITEHOUSE), Edinburgh Univ. Press, Edinburgh, p. 786–795

LINDE-LAURSEN, I. 1984. Nucleolus organizer polymorphism in barley, Hordeum vulgare L. — Hereditas 100: 33–43

LINDE-LAURSEN, I. and BOTHMER, R. VON 1984a. Giemsa C-banded karyotypes of two subspecies of Hordeum brevisubulatum from China. — Pl. Syst. Evol. 145: 259–267

LINDE-LAURSEN, I. and BOTHMER, R. VON 1984b. Somatic cell cytology of the chromosome eliminating, intergeneric hybrid Hordeum vulgare × Psathyrostachys fragilis. — Can. J. Genet. Cytol. 26: 436–444

LINDE-LAURSEN, I. and BOTHMER, R. VON 1986a. Giemsa C-banding in two polyploid, South American Hordeum species, H. tetraploidum and H. lechleri, and their aneuploid hybrids with H. vulgare. — Hereditas 105: 000–000

LINDE-LAURSEN, I. and BOTHMER, R. VON 1986b. Preferential loss and gain of specific Hordeum vulgare chromosomes in hybrids with three alien species? — In Genetic Manipulation in Plant Breeding. Proc. EUCARPIA Symp., Berlin 1985 (Eds. W. HORN, C. J. JENSEN, W. OENENBACH and O. SCHIEDER), Walter de Gruyter, Berlin & New York (in press)

LINDE-LAURSEN, I., BOTHMER, R. VON and JACOBSEN, N. 1980. Giemsa C-banding in Asiatic taxa of Hordeum section Stenostachys with notes on chromosome morphology. — Hereditas 93: 235–254

LINDE-LAURSEN, I., BOTHMER, R. VON and JACOBSEN, N. 1986. Giemsa C-banded karyotypes of Hordeum taxa from North America. — Can. J. Genet. Cytol. 28: 42–62

LINDE-LAURSEN, I. and JENSEN, J. 1984. Separate location of parental chromosomes in squashed metaphases of hybrids between Hordeum vulgare L. and four polyploid, alien species. — Hereditas 100: 67–73

RAJHATHY, T. and SYMKO, S. 1974. High frequency of haploids from crosses of Hordeum lechleri (6x) and H. vulgare (2x) and H. jubatum (4x) and H. bulbosum (2x). — Can. J. Genet. Cytol. 16: 468–472

RAJHATHY, T., MORRISON, J. W. and SYMKO, S. 1964. Interspecific and intergeneric hybrids in Hordeum. — In Barley Genetics I (Eds. S. BROOKHUIZEN, G. DANTUMA, H. LAMBERTS and W. LANGE), Pudoc, Wageningen, p. 195–212

SUBRAHMANYAM, N. C. 1980. Haploidy from Hordeum intergeneric crosses. Part 3: Trihaploids of H. arizonicum and H. lechleri. — Theor. Appl. Genet. 56: 257–263

VOSA, C. G. 1976. Chromosome banding patterns in cultivated and wild barleys (Hordeum spp.). — Heredity 37: 395–403