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Triple hybridization with cultivated barley (*Hordeum vulgare* L.)

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Summary. A crossing programme for trispecific hybridization including cultivated barley (*Hordeum vulgare* L.) as the third parent was carried out. The primary hybrids comprised 11 interspecific combinations, each of which had either *H. jubatum* or *H. lechleri* as one of the parents. The second parent represented species closely or distantly related to *H. jubatum* and *H. lechleri.* In trispecific crosses with diploid barley, the seed set was 5.7%. Crosses with tetraploid barley were highly unsuccessful (0.2% seed set). Three lines of diploid barley were used in the crosses, i.e. 'Gull', 'Golden Promise' and 'Vada'. Generally, cv 'Gull' had high crossability in crosses with related species in the primary hybrid. It is suggested that 'Gull' has a genetic factor for crossability not present in cv 'Vada' and cv 'Golden Promise'. One accession of *H. brachyantherum* used in the primary hybrid had a very high crossability (seed set 54.7%) in combination with cv 'Vada' but no viable offspring was produced. In all, two trispecific hybrids were raised, viz. (*H. lechleri* × *H. brevisubulatum*) × 'Gull' ($2n = 27-30$) and (*H. jubatum* × *H. lechleri*) × 'Gull' ($2n = 20-22$). The first combination invariably had a full complement of seven barley chromosomes plus an additional chromosome no. 7, but a varying number of chromosomes (19–22) of the wild-species hybrid. The second combination had a full set of barley chromosomes. The meiotic pairing was low in both combinations.

Key words: Barley – *Hordeum vulgare* – Interspecific hybridization – C-banding – Crossability

Introduction

The difficulty of transferring genes to barley through sexual-wide hybridization has been well documented (Bothmer et al. 1983; Bothmer and Hagberg 1983; Fedak 1985). Primary hybridization comprising cultivated barley (*Hordeum vulgare* L.) and especially polyploid *Hordeum* species is comparatively easy to perform (Bothmer et al. 1983; Fedak 1985; Bothmer and Jacobsen 1989), but backcrossing to barley has been shown to be extremely difficult (Bothmer and Hagberg 1983; Bothmer et al. 1988). Thus, new approaches have to be tried in order to understand the genetic systems regulating incompatibility and recombination. These may include the production of amphiploids, the use of tetraploid lines of barley, complex crosses or triple hybridization, especially at a polyploid level since diploid combinations, except with *H. bulbosum,* yield offspring at a very low frequency only (Bothmer et al. 1983). The present paper reports the results of a programme of triple hybridization with both di- and tetraploid lines of cultivated barley. The material was chosen to represent genetic variation in barley as well as in the wild species. The material of the wild species was mainly chosen from species known to have a certain degree of crossability with barley (Bothmer et al. 1983; Bothmer and Jacobsen 1986).

Materials and methods

The primary hybrids between the wild species were produced in an earlier project involving interspecific crosses in *Hordeum* (Bothmer and Jacobsen 1986). The material represented 11 primary interspecific combinations (Table 1), each of which had either the tetraploid *H. jubatum* L. or the hexaploid *H. lechleri* Steud. as one parent, since these two species show a generally high crossability (Bothmer and Jacobsen 1986). The second parent in the primary hybrid was chosen to represent species closely or more distantly related to *H. jubatum* and *H. lechleri,* respectively (Bothmer and Jacobsen 1986). The distantly related species comprised *H. murinum* L. (4x), *H. secalinum* Schreb. (4x) and *H. capense* Thunb. (4x). Since barley has been demonstrated to be an inferior female parent in interspecific *Hordeum* hybrids (Bothmer et al. 1983), it was throughout used as the male parent, and the primary hybrid was used as the female parent.

The diploid lines of cultivated barley comprised: cultivars 'Gull', 'Vada', and 'Golden Promise'; the tetraploid lines 'Haisa

Table 1. Results of interspecific triple crosses including *Hordeum vulgare*

Combination		No. of spikes	No. of flowers	Seed set		Plants
				No.	%	
(*lechleri* × *murinum*)	× *vulg*, 2x	5	125	0	0	
	× *vulg*, 4x	3	79	0	0	
(*lechleri* × *capense*)	× *vulg*, 2x	15	322	0	0	
	× *vulg*, 4x	16	376	0	0	
(*lechleri* × *secalinum*)	× *vulg*, 2x	18	462	0	0	
	× *vulg*, 4x	2	36	0	0	
(*lechleri* × *brevisubulatum*)	× *vulg*, 2x	12	285	1	0.4	1
	× *vulg*, 4x	16	357	1	0.3	0
(*lechleri* × *patagonicum*)	× *vulg*, 4x	3	55	0	0	
(*lechleri* × *procerum*)	× *vulg*, 2x	2	42	0	0	
	× *vulg*, 4x	1	30	0	0	
(*lechleri* × *brachyantherum*, H 325)	× *vulg*, 2x	5	136	0	0	
	× *vulg*, 4x	19	478	0	0	
(*lechleri* × *brachyantherum*, H 253)	× *vulg*, 2x	7	190	104	54.7	0
(*jubatum* × *pubiflorum*)	× *vulg*, 2x	10	242	11	4.3	0
	× *vulg*, 4x	14	338	2	0.6	0
(*jubatum* × *brachyantherum*)	× *vulg*, 2x	10	218	5	2.3	0
	× *vulg*, 4x	10	244	1	0.4	0
(*jubatum* × *lechleri*)	× *vulg*, 2x	9	208	5	2.4	1
	× *vulg*, 4x	1	14	0	0	
Total						
with *vulgare*, 2x		93	2,230	126	5.7	2
with *vulgare*, 4x		85	2,007	4	0.2	0

II, 4x', 'Weihenstephaner Mehltauresistente I', '9208/9' (obtained from Prof. W. Friedt, then at the Institut für Resistenzgenetik, Grünbach, FRG) and 'D8/55' (obtained from Dr. T. Konishi, Barley Germplasm Center, Kurashiki, Japan).

The crosses were performed under controlled conditions in growth chambers with 16 h light, day/night temperatures of 16°/13 °C and a light intensity of 40,000 lx. The methods used for crossing and embryo rescue follow Bothmer et al. (1983), for establishing Giemsa C-banding patterns, Linde-Laursen et al. (1980) and for meiotic analysis, Bothmer et al. (1986).

Results

Crosses

The primary hybrids were more female than male fertile as shown in backcrosses to one of the parents (Bothmer and Jacobsen 1986 and unpublished). They had throughout a low pollen fertility and none set seed spontaneously.

Crosses with tetraploid barley were highly unsuccessful. Only 4 out of 85 attempts resulted in cultivable embryos (seed set: 0.2%, Table 1), none of which germinated. The success rate with diploid barley was significantly higher. However, the results are rather difficult to interpret due to the complexity of the material used in the crosses (see 'Discussion'). The average seed set for the entire material was 5.7% (Table 1), but since different crossing categories were involved, this figure covers a large variation.

A comparison of the crossing results with the three diploid barley varieties 'Vada', 'Gull' and 'Golden Promise' is shown in Table 2. 'Golden Promise' was only used in 12 crosses (7 combinations), none of which resulted in seed. 'Vada' was used with all primary hybrids in Table 1, except with *H. jubatum* × *H. lechleri* and *H. lechleri* × *H. capense*. Thirtyone crosses (6 combinations) gave no seed set. One important exception was with one of the primary, pentaploid *H. lechleri* × *H. brachyantherum* (4x) hybrids. The combination involved two different accessions of the latter species, H 325 from eastern USSR and H 253 from the western USA. Combinations involving H 325 yielded no seeds, whereas those with H 253 had an average seed set of 54.7%. Two reciprocally different primary hybrids were used. The one with H 253 as the female parent of the primary hybrid had a seed set of 61.3%, and the one with H 253 as the male parent had a seed set of 49.5%. 'Gull' used as male gave an average seed set of 2.3%. It gave no seed set with primary hybrids derived from unrelated species, but on an average 3.6% seed set with hybrids derived from related species. The latter combinations gave seed set in 12 out of 26 crossing attempts. Seed set was obtained in all four triple combinations tried, comprising primary hybrids of closely related species. 'Gull' was not crossed with *H. lechleri* × *H. secalinum*, *H. lechleri* × *H. procerum* or *H. lechleri* × *H. brachyantherum* (4x).

Table 2. Results of triple crosses with three diploid barley lines as male parents

Combinations[a]	No. of attempts	No. of successful attempts	No. of flowers	Seed set		Plants
				No.	%	
Unrelated × 'Gull'	15	0	332	0	0	0
Related × 'Gull'	26	10	619	22	3.6	2
Unrelated × 'Golden Promise'	6	0	133	0	0	0
Related × 'Golden Promise'	6	0	177	0	0	0
Unrelated × 'Vada'	18	0	344	0	0	0
Related (except H 253 × 'Vada')	13	0	275	0	0	0
Including H 253 × 'Vada'	7	7	190	104	54.7	0

[a] cf. Table 1 and 'Material and methods'

Table 3. Number of alien and *H. vulgare* chromosomes, satellites, NORs, and nucleoli of primary and triple hybrids

Combination	2n	No. of chromosomes		Specific *H. vulgare* chrom.	No. of satellites		Max. no of NORs	Max. no of nucleoli
		alien	*vulg.*		alien	*vulg.*		
HH 566 (*H. lechleri* × *H. brevisubulatum*)	42							
BB 683 [(*H. lechleri* × *H. brevisubulatum*) × *H. vulgare* cv 'Gull']	27–30 (12)	19–22	8	1–7+7		3	6	6[d]
HH 1385 (*H. jubatum* × *H. lechleri*)	34–35 (4)[a]	34–35			5		7	8[b]
BB 566-1 [(*H. jubatum* × *H. lechleri*) × *H. vulgare* cv 'Gull']	21 (4)	14	7	1–7		2	5	5[c]

[a] values in parenthesis are number of cells
[b] incl. one micronucleolus
[c] incl. three smaller nucleoli
[d] incl. three smaller nucleoli

Germination

The germination was very poor (Table 1). Only two plants were raised, viz. BB 683 [(*H. lechleri* × *H. brevisubulatum,* 6x) × 'Gull'] and BB 566 [(*H. jubatum* × *H. lechleri*) × 'Gull']. In the cross (*H. lechleri* × *H. brachyantherum,* 4x) × 'Vada' yielding the very high seed set of 54.7%, the embryos were either lacking or had minute undifferentiated seeds that did not germinate.

Cytogenetics of the triple hybrids

The primary *H. lechleri* × *H. brevisubulatum* (6x) hybrid HH 566 involved in the production of the triple hybrid BB 683 with 'Gull' invariably had 42 chromosomes in its somatic cells. The chromosome number of the somatic cells of BB 683 ranged between 2n = 27–30. C-banding patterns revealed 19–22 chromosomes from the primary hybrid and invariably 8 barley chromosomes comprising a complete set (nos. 1–7) plus an extra chromosome 7 (Table 3, Fig. 1a). All barley chromosomes were identified as derived from the male parent 'Gull'. Chromosome 6 was a banding variant (6D) observed only once before in a 'Gull' derivative (Linde-Laursen and Bothmer 1988). The parental genomes were concentrically arranged with that of 'Gull' closest to the metaphase centre (cf. Linde-Laursen and Bothmer 1988). No more than three chromosomes with nucleolar constrictions were observed. All belonged to *H. vulgare,* indicating nucleolar dominance of the SAT-chromosomes (satellite chromosomes) of this genome. However, up to six NORs (nucleolar organizing regions) and six nucleoli, three larger ones ascribed to *H. vulgare* and three smaller ones ascribed to the primary hybrid, were observed in silver nitrate-stained meta- and interphases, respectively, indicating that the suppression of the activity of the NORs of the SAT-chromosomes of the wild species was incomplete.

Cells at meiosis had 20–30 chromosomes with 28 as the most frequent number. Meiosis was difficult to analyze. One cell was chromosome-doubled with approximately 2n = 56. It showed 9 bivalents, 2 trivalents and 32 univalents. Hypoploid cells with 26 and 27 chromosomes and euploid cells with 2n = 28 had similar pairing, with

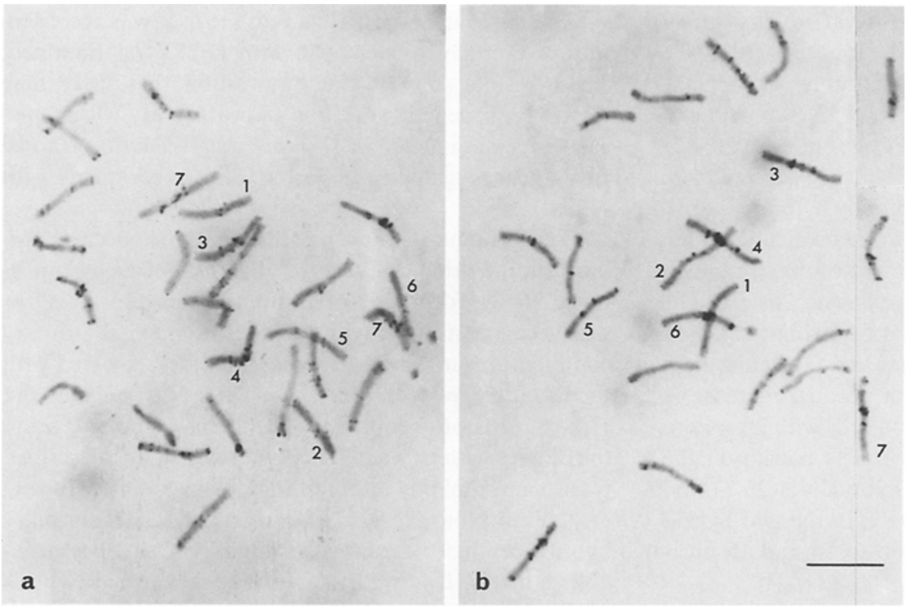

Fig. 1 a and b. Giemsa C-banded chromosomes at somatic metaphase of *Hordeum* hybrids produced through triple hybridization. **a** BB683 [(*H. lechleri* (6x)) × *H. brevisubulatum* (6x)) × *H. vulgare* 'Gull'] 2n = 27; **b** BB566 [(*H. jubatum* (4x) × *H. lechleri* (6x)) × *H. vulgare* 'Gull'] 2n = 21. Numbers (1–7) designate *H. vulgare* chromosomes. *Bar* = 10 μm

Table 4. Meiotic pairing in cells with different chromosome numbers in the triple hybrid BB 683 [(*H. lechleri* × *H. brevisubulatum*) × 'Gull']. Only frequencies with four or more cells have been included. N = number of cells, r = range

2n	N	I	II			Chiasmata/cell
			Total	Rods	Rings	
26	4	20.00	3.00	2.50	0.50	3.50
	r	16–22	2–5	2–5	0–1	2–5
27	4	21.00	3.00	2.00	1.00	4.00
	r	19–25	1–4	0–4	0–2	2–6
28	23	22.00	3.00	2.22	0.78	3.78
	r	18–26	1–5	1–4	0–3	1–8
29	5	20.20	4.40	2.60	1.80	6.20
	r	19–23	3–5	2–4	1–3	4–8

3.50–4.00 chiasmata per cell (Table 4). The hyperploid cells with 2n = 29 showed a significant increase in pairing with a mean of 6.20 chiasmata per cell, due to one additional ring bivalent. Two cells with 2n = 30 analyzed had only 3 chiasmata. The primary *H. lechleri* × *H. brevisubulatum* (6x) hybrid (HH 566) had 19.11 chiasmata per cell with on average 1.41 trivalents and 11.33 bivalents, probably mainly representing autosyndetic pairing of *H. brevisubulatum* chromosomes.

Somatic cells of the primary *H. jubatum* × *H. lechleri* hybrid, HH 1385, the male parent of the triple hybrid, with 'Gull', of BB 566 had 34–35 chromosomes in accordance with its expected pentaploid constitution. BB 566 had 2n = 21 in the cells studied. C-banding patterns indicated that 14 chromosomes derived from the primary hybrid and 7 from barley, representing one full 'Gull' genome (nos. 1–7, Table 3, Fig. 1b). The constituent genomes were concentrically arranged with the *H. vulgare* chromosomes generally closest to the metaphase centre. The nucleolar constrictions of the two *H. vulgare* SAT-chromosomes were expressed in metaphases; however, as in the former triple hybrid, silver nitrate staining revealing up to five NORs in metaphases and five nucleoli in interphases, two large nucleoli ascribed to *H. vulgare* and three smaller ones ascribed to the primary hybrid, this indicated that the nucleolar dominance of the *H. vulgare* SAT-chromosomes was incomplete.

Table 5. Meiotic pairing in cells with different chromosome numbers in the triple hybrid no. BB 566 [(*H. jubatum* × *H. lechleri*) × 'Gull']. Only frequencies with four or more cells have been included. N = number of cells studied, r = range

2n	N	I	II			Chiasmata/cell
			Total	Rods	Rings	
19	7	18.43	0.29	0.29		0.29
	r	17–19	0–1	0–1		0–1
20	11	19.27	0.36	0.09	0.27	0.64
	r	16–20	0–2	0–1	0–1	0–3
21[a]	8	20.14	0.43	0.14	0.29	0.71
	r	17–21	0–2	0–1	0–1	0–3
22	12	20.83	0.58	0.08	0.50	1.08
	r	18–22	0–2	0–1	0–2	0–4
23[b]	6	21.00	1.00	0.40	0.60	1.60
	r	19–23	0–2	0–1	0–2	0–4

[a] One partly chromosome-doubled cell with five ring bivalents (ten chiasmata) excluded

[b] One partly chromosome-doubled cell with four ring bivalents (eight chiasmata) excluded

The meiotic chromosome number varied between 17 and 24 with a mode at 20–22. Metaphase I (MI) was much disturbed with many cells containing fragments, misdivisions and foldbacks. A special phenomenon of increased pairing was found in two cells, one with $2n=21$ and the other with $2n=23$. They probably derived from a 'partial doubling' of what were initially hypoploid cells. The cell with $2n=21$ had five intact ring bivalents, i.e. ten chiasmata, compared with a maximum of one ring bivalent and three chiasmata in other cells with $2n=21$. The cell with $2n=23$ had a maximum of four rings, i.e. eight chiasmata, compared with two rings and four chiasmata in other $2n=23$ cells. There was a gradual increase in mean pairing from 0.29 chiasmata in cells with $2n=19$ to 1.60 in cells with $2n=23$ (Table 5). The pairing in the primary *H. jubatum* × *H. lechleri* hybrid was 25.73 and 22.05 chiasmata per cell, respectively, in the two hybrid families studied. They contained up to 14 and 15 bivalents per cell, respectively.

Discussion

Environmental conditions such as temperature, light and the condition of the parental plants play an important role for the success of wide barley crosses (Pickering 1985). Likewise, genetic factors are of great importance (Simpson et al. 1980). For 'simple hybridization', i.e. crosses between two species, the genetic setup is important, and consequently the degree of heterozygosity of the plants. In an obligate outbreeder like, e.g., *H. bulbosum* with maximum heterozygosity, the genetic constitution of each gamete is unique. In comparison, a more or less obligate inbreeder, such as cultivated barley, normally only forms one type of gámetes. In all species there might exist a large genetic variation for crossability.

Genetically, a triple hybridization is more complicated than a primary one. The primary cross depends on the genetics for crossability and the interaction between gametes of two species. However, when a primary hybrid has been established, also the relationships between the original parents are important. Normally, parental species with closely related homoeologous genomes are more likely to produce fertile (viable) gametes with balanced chromosomal constitutions, than are more distantly related parents, especially those with non-homologous genomes. The last parameter for the triple hybridization is the genetics of crossability and genome relationships of the third, usually the male parent.

The genetic variation in crossability between accessions is evident in the combination (*H. lechleri* × *H. brachyantherum,* 4x) × *H. vulgare.* Two different accessions of *H. brachyantherum* (4x), one from the western USA (H 253) and one from eastern USSR (H 325), were used as the one parent. The other parents in the triple hybridization were accession H 504 of *H. lechleri* and *H. vulgare* cv 'Vada'. With H 325 no seed set was recorded, but an average of over 50% with H 253 was obtained. These results support the supposition that there may exist a large genetic variation in crossability within species. Accession H 253 of *H. brachyantherum* (4x) is to be further investigated in larger crossing experiments with *H. vulgare.*

The genome relationships between the species combined in the primary hybrid is obviously of great importance for the formation of viable gametes and, thus, for the success of a triple hybridization. In the three primary combinations in which *H. lechleri* had been crossed with the distantly related *H. murinum* (4x), *H. secalinum* and *H. capense* (Bothmer et al. 1989), there was no seed set in the crosses with cultivated barley. This might be ascribed to an uneven ploidy level in the primary hybrid. However, uneven ploidy levels (3x and 5x) of related primary hybrids produced a comparatively high frequency of viable gametes. It is well known that pentaploid hybrids may sometimes be semifertile, but especially the high seed set of 4.3% on the triploid hybrid (*H. jubatum* × *H. pubiflorum*) was unexpected. The seed set in the triploid may be the result of an increased production of unreduced gametes. However, since no viable triple hybrids were obtained, this could not be further elucidated.

The genetic variation in crossability among the barley lines used as male parents in triple hybridizations is also important for seed set. At the diploid level, the three varieties 'Vada', 'Gull' and 'Golden Promise' were used. 'Golden Promise' did not set any seeds and neither did 'Vada', except in the crosses with the primary hybrid (*H. lechleri* × *H. brachyantherum*) containing accession H 253. 'Gull' had throughout a much higher seed set. It was crossed with six primary hybrids. Of the 41 atempts, 12 were successful (i.e. attempts in which seed set was registered). The 12 attempts represented 4 combinations, all with closely related species in the primary hybrid (female parent), indicating that 'Gull' has a general good crossability (compatibility). In spite of their variation in crossability, the pedigrees of the three diploid barley varieties show that they are closely related. 'Gull' is an old Swedish selection of a landrace from the island of Gotland (released 1913), and both 'Vada' and 'Golden Promise' have 'Gull' in their pedigree. 'Vada' (released 1956) is a cross between 'Gull' and the barley line *H. distichum* var. *laevigatum,* and 'Golden Promise' (released 1965) has 'Gull' as a grandparent. It thus seems that the more primitive cv 'Gull' has retained some characteristics of crossability from the landrace background, and that these have been unconsciously selected against during the breeding process. One theory is that there is a greater chance for finding genes for high crossability in older, more primitive barley material, including perhaps also *H. vulgare* ssp. *spontaneum.* This is to be tested in further studies.

Pickering and Hayes (1976) in an extensive study found that the barley varieties 'Luke', 'Vada', 'Universe' and 'Georgie' and the line *H. distichum* var. *laevigatum* gave low seed set (average < 30%) after pollination with several genotypes of *H. bulbosum.* In contrast, the varieties 'Vogue', 'Paladin', 'Lud' and 'Porthos' gave consistently high seed set (> 80%). The varieties in the first category all derived from crosses having *H. distichum* var. *laevigatum* in their ancestry. The authors (Pickering and Hayes 1976) concluded that a single dominant gene controlling low seed set was present in var. *laevigatum,* which then had been transmitted to the other varieties. The variety 'Lud' had obviously not obtained the low seed set factor despite having 'Vada' in its pedigree. The crossability gene is probably located on barley chromosome no. 7 (Pickering 1983). The genetic variation for incompatibility is also substantial in the male parent, *H. bulbosum* (Pickering 1980; Pickering and Morgan 1985; Craig and Fedak 1985).

Thomas and Pickering (1985) found that 'Vada' was the most successful *H. vulgare* in crosses with *H. chilense.* The highest seed set obtained was 87.7%, and all 'Vada' crosses had bigger and better-differentiated embryos than crosses with other varieties. These data are in contrast to the high incompatibility level in 'Vada' × *H. bulbosum* combinations (Pickering and Hayes 1976), and in the triple crosses including 'Vada' of the present study. However, in a later study Pickering and Rennie (1989) showed that there exists substantial genetic variation for crossability also in *H. bulbosum.*

Crosses between barley and wheat have been tried to an increasing extent. Fedak (1980) found a very high seed set in *H. vulgare* cv 'Betzes' × *Triticum aestivum* cv 'Chinese Spring'. Fedak and Jui (1982) concluded that 'Chinese Spring' carries genes for crossability with 'Betzes'. Barley functions considerably better as the female than as the male parent in combinations with wheat. For example, the cross 'Betzes' × 'Chinese Spring' had a seed set of 48.9% (Fedak 1980), but the reciprocal one had only 1.3% (Islam et al. 1981). These data contrast the interspecific *Hordeum* crosses, in which barley is always inferior as the female parent (Bothmer et al. 1983; Bothmer and Jacobsen 1986).

In crosses between three tetraploid lines of cultivated barley as female parent and diploid rye, the seed set varied between 23.6% and 77.6% (Pickering and Thomas 1979). It was thus considerably higher than in our triple crosses with tetraploid barleys. In crosses between diploid barley and rye, the seed set was 42.3%–95.9% (Thomas and Pickering 1979). With 'Vada' the seed set was 76.7%. Clauss and Kunert (1981) obtained from 0% up to 57.5% embryos with seven different *Hordeum* taxa and different di- and tetraploid lines of rye as male parents. It is evident that cultivated barley functions better and should be used as the male parent in interspecific *Hordeum* crosses, but as the female parent in most intergeneric crosses.

In crosses between *Hordeum* species as the female parent and different species of *Secale* as the male parents, the seed set varied between 53.0% and 97.4%, highest with the tetraploid *H. tetraploidum* (= *H. parodii,* 4x, Gupta and Fedak 1987a, b). However, the production of embryos was considerably lower, viz. 10.8%–76.2% (Gupta and Fedak 1987a, b) and 0%–15.0% (Clauss and Kunert 1981). Wojchiechowska (1984) obtained embryos with a frequency of 0.4%–29.4% in crosses between ten cultivars of barley and rye species.

In triple hybrids including *H. vulgare, H. jubatum,* and di- and tetraploid rye lines, Clauss and Kunert (1981) and Wojciechowska (1984) found variation in embryo formation. The highest frequencies, 16.3%–32.3%, were found in the cross barley (six varieties) × (*H. jubatum* × *Secale cereale,* 4x cv 'Biale Tetra'). A comparable range (2.1%–31.3%) was obtained in a similar cross, but including the diploid rye 'Prolific'. Clauss and Kunert (1981) used *H. jubatum* × *H. vulgare* as the female parent in the triple cross and obtained considerably lower embryo formation (0.1%–0.4%).

The C-banding patterns of the triple hybrids BB 683 [(*H. lechleri* × *H. brevisubulatum,* 6x) × 'Gull'] and BB 566 [(*H. jubatum* × *H. lechleri*) × 'Gull'] revealed that the latter combination invariably had one complete set of seven chromosomes of barley, and that BB 683 invariably had one complete set of barley chromosomes plus an additional chromosome no. 7. Compared to the other barley chromosomes, an additional copy of chromosome 7 seems to be the more frequent one in both primary and complex interspecific *Hordeum* hybrids (Bothmer et al. 1988; Linde-Laursen and Bothmer 1988, and unpublished results), suggesting that this situation is better tolerated than the addition of other chromosomes. Besides, chromosome 7 may get duplicated more often than the others. The cause of the duplication may be chromosomal instability in interspecific hybrids, resulting in elimination and/or duplication of chromosomes at early zygotic divisions (Linde-Laursen and Bothmer 1988). The chromosomal constitutions of BB 683 and BB 566 indicate that both contain chromosomes of wild *Hordeum* corresponding to three and two full genomes, respectively. As the chromosomes derived from the primary hybrids were not identified to species, it is not known whether they represent pure or mixed complements. However, the low pairing at meiosis of both hybrids was at levels corresponding with those of polyhaploids of *H. lechleri* and *H. jubatum,* respectively (Bothmer and Subrahmanyam 1988). Meiosis of especially BB 566 (5x × 2x) was, as expected, rather unbalanced with mitotic and meiotic chromosome numbers of $2n = 19-23$. The low pairing showed a gradual increase in numbers of chiasmata per cell correlated with the increase in chromosome numbers (0.29–1.60), although the increase in pairing is much lower than expected.

An interesting observation is the extraordinarily high pairing in a few cells, with four or five ring bivalents. The pairing deviated so markedly from the pattern observed in the other cells that these cells probably have a different background. Due to the unbalanced nature of this hybrid as seen in the varying chromosome number and other chromosomal aberrations (micronuclei, fragments, laggards, etc.), we suspect that the cells with the increased pairing had been 'partly duplicated'), i.e. they may have arisen through duplication of the whole chromosome set in cells with rather low chromosome numbers (16–19) followed by elimination of a number of chromosomes through successive cell cycles prior to meiosis. Cells with doubled chromosome numbers are not uncommon in interspecific hybrids of *Hordeum* (Finch and Bennett 1980; Bothmer et al. 1983, 1988).

The concentric arrangement of the chromosomes of the different genomes in somatic cells with the genome of *H. vulgare* generally closest to the metaphase center and the nucleolar dominance of *H. vulgare* SAT-chromosomes match previous observations in interspecific and intergeneric *Hordeum* hybrids. Likewise, the variation in chromosome numbers of such hybrids due to the loss or gain of one or two chromosomes of the alien parent was noted earlier (e.g. Bothmer et al. 1988; Linde-Laursen and Bothmer 1988).

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