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Karyotype analysis of regenerated plants from callus cultures of interspecific hybrids of cultivated barley (*Hordeum vulgare* L.)

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Summary. The karyotype of 82 regenerated plants from callus cultures of interspecific hybrids between cultivated barley (*Hordeum vulgare* L.) and seven polyploid wild barley species was examined by C-banding or Feulgen staining. The karyotypic changes observed in 46 plants included aneuploidy, double haploidy, amphidiploidy, deletions, inversions, extra C-bands, and extra euchromatic segments. Apparently, chromosome 5, 6, and 7 of *H. vulgare* were more frequently exposed to elimination or structural change than the other chromosomes of this species. Irradiation of calli seemed to enhance the occurrence of karyotypic variants.

Key words: *H. vulgare* – Interspecific hybrids – Regenerants – Chromosome changes – C-banding

Introduction

Efforts have been made to utilize the variation on agronomic qualities found among wild barley species in the breeding of cultivated barley, but with little success (von Bothmer and Hagberg 1983). Interspecific hybrids with cultivated barley have been produced in several combinations (e.g., von Bothmer and Jacobsen 1986). However, the genome of cultivated barley apparently does not recombine with the genome of the other species in the hybrids (von Bothmer et al. 1983). This necessary recombination may occur if a hybrid tissue goes through a callus phase (Lapitan et al. 1984). Therefore, calli were induced from the interspecific hybrids of cultivated barley and plants were regenerated.

The regenerants were obtained from callus of hybrids between cultivated barley ($2n=14$) and seven wild barley species ($2n=28$ and 42), as well as from a backcross line of cultivated barley. Results on plant regeneration have been reported elsewhere (Jørgensen et al. 1986). The present paper deals with the karyotypic variations observed in 82 regenerated plants analyzed by staining the chromosomes with Giemsa or Feulgen. The variation affecting the *H. vulgare* genome was of principal interest.

Materials and methods

Plant material

The 82 regenerated plants examined karyologically represented nine different interspecific combinations between *H. vulgare* and 7 wild barley species, *H. brachyantherum* Nevski s.l. ($2n=28$), *H. jubatum* L. ($2n=28$), *H. roshevitzii* Bowden ($2n=28$), *H. tetraploidum* Covas ($2n=28$), *H. brevisubulatum* (Trin.) Link ($2n=42$), *H. lechleri* (Steud.) Schenck ($2n=42$), and *H. procerum* Nevski ($2n=42$), and a backcross line, (*H. lechleri* × *H. vulgare*) × *H. vulgare* (Table 1). The hybrids and the backcross line were obtained from R. von Bothmer (The Swedish University of Agricultural Sciences, Svalöv, Sweden) and N. Jacobsen (The Veterinary and Agricultural University, Copenhagen, Denmark). Karyotypes of the hybrids were stable over years but in some cases deviated from the expected (Table 1). Von Bothmer et al. (1983) reported additional information on some of the hybrids.

Plants were regenerated from the calli at monthly intervals. A total of 1,315 plants were grown to maturity, 40 of which originated from irradiated calli. The in vitro culture methods and regeneration frequencies were given in Jørgensen et al. (1986).

Irradiation of calli

Five undifferentiated and three differentiated subcalli from one explant of the hybrid, *H. procerum* × *H. vulgare*, were irradiated with gamma rays from a ^{60}Co source. The radiation doses were 20 Gy (one undifferentiated callus), 30, 50, 100 Gy (one undifferentiated and one differentiated callus per dose), and 150 Gy (one undifferentiated callus). After irradiation, the undifferentiated calli were recultured for 1½ months on a hormone-containing medium in darkness (Jørgensen et al. 1986)

Table 1. Interspecific hybrid and backcross lines with *H. vulgare* from which regenerated plants were obtained through callus culture

Wild species/hybrid ×	*H. vulgare* 2n = 14	Hybrid	Chromosome no. (2n)	Karyotype[a]
H. brachyantherum, ♀ 2n = 28	'Risø', F_1	HH 657-2	21	not C-banded
	'Welam'	HH 840-2	19–21	not C-banded
H. roshevitzii, ♀ 2n = 28	'Riso', 1508	HH 401-1	20–23	extra w.b.c. present
H. jubatum, ♀ 2n = 28	'Riso', F_1	HH 631-5	17–21	not C-banded
	'Manchuria'	HH 2225-1	19–22	not C-banded
H. tetraploidum, ♀ 2n = 28	'Riso', 1508	HH 2441-1	20+t	H.v.c. 6 lost, H.v.c. 3 lost, an extra H.v.c. 1 present
H. brevisubulatum, ♂ 2n = 42	'Riso', F_1	HH 1-2	27–28	normal hybrid karyotype
H. lechleri, ♀ 2n = 42	'Riso', HP 40	HH 473-3	28–29	two H.v.c. 7 present
	'Vogelsanger Gold'	HH 1284-1	27	H.v.c. 7 lost
	'Vogelsanger Gold'	HH 1284-4	28	normal hybrid karyotype
H. procerum, ♀ 2n = 42	'Riso', F_1	HH 562-6	28	normal hybrid karyotype
(*H. lechleri* × *H. procerum*), ♀ 2n = 42	'Tellus'	Ek 25	29–30	two H.v.c. 7 present; sometimes also an extra w.b.c.
(*H. lechleri* × *H. vulgare*, 'Vogelsanger Gold'), ♀ 2n = 27	'Nery'	Ek 18	28–33	H.v.c. 1–6 present in double dose; H.v.c. 7 present in one dose only

[a] H.v.c. – *H. vulgare* chromosome(s), w.b.c. – chromosome(s) from the wild barley species

before transfer to regenerative conditions. The differentiated calli were transferred to new medium and placed under regenerative conditions immediately after irradiation. Forty plants were obtained from irradiated calli. Karyotypes were studied in six plants.

Chromosome observations

Of the 82 regenerated plants studied, 36 were selected for karyotype analysis because they displayed major morphological changes. The remaining 46 plants were morphologically similar to the original hybrids and were picked out at random. Karyotype observations on regenerated plants were carried out on root-tips and occasionally also on shoot apical meristems.

The chromosome complements of all 82 plants were studied in Feulgen-stained squash preparations. Root-tips were pretreated in 0.1% colchicine for 90 min before staining; 3–16 intact cells per plant (average seven cells) were analyzed.

In the Feulgen-stained preparations, identification of *H. vulgare* SAT-chromosomes 6 and 7 was usually possible because in *H. vulgare* interspecific hybrids, the satellites of the wild species are normally suppressed while the satellites of *H. vulgare* are visible (Jessop and Subrahmanyam 1984; Linde-Laursen et al. 1985). Moreover, the satellite of *H. vulgare* chromosome 6 is somewhat longer than the satellite of chromosome 7; thus discrimination between the two chromosomes can be made. $AgNO_3$ staining of NOR-regions was carried out in one regenerated plant according to Linde-Laursen (1984).

Giemsa C-banding was carried out according to Linde-Laursen et al. (1980). Most of the 33 plants studied by C-banding were picked out on the basis of abnormalities observed in Feulgen-stained cells. All interspecific combinations except *H. tetraploidum* × *H. vulgare* were represented in the C-banded material. Three to 47 cells (average 18 cells) were examined per plant. Our main interest was variation affecting the *H. vulgare* genome. However, numerical variation in the chromosome set of the wild species was also recorded and so were major deletions in their genome, as they were easily observed. The C-banded karyotype of *H. vulgare* is much more well-documented than the C-banding patterns of the wild species. This was also a reason why minor chromosomal rearrangements in wild species chromosomes were very difficult to detect.

Chromosome doubling

Chromosome doubling of 11 regenerated plants was performed by colchicine-treatment according to Jensen (1977).

Pollen fertility

Male fertility in regenerated and colchicine-treated plants was estimated using the pollen grains (100 grains/plant) stained with cotton blue.

Results

Morphological variation

A broad spectrum of morphological variation was observed among the 1,315 regenerated plants. Slender or dwarfish plants were observed along with plants more vigorous in all parts. Some regenerants looked more like cultivated barley than the primary hybrid, others more like the wild species. Abnormal characters such as excess

Table 2. Numerical chromosome changes in plants regenerated from interspecific *Hordeum* hybrids

Hybrid	Regenerated plant[a]	Chromosome no.	Karyotype variation[b]	Morphology[c]
Aneuploids				
Loss of *H. vulgare* chromosomes				
HH 1284-4	197-041	27	H.v.c. 5 lost	n
	-042	27 (26)	H.v.c. 5 lost	d
	199-240	27	H.v.c. 7 lost	d
HH 562-6	787-609	26+t–27	H.v.c. 7 lost	n
Ek 25-2	411-975	30–31	the extra H.v.c. 7 lost	d
Ek 18	942-848	27 (28)	one H.v.c. 3 lost	n
	944-750	29 (28–31)	one H.v.c. 2 lost	n
	1107-707	30 (28–31)	one H.v.c. 5 lost	n
	-709	29–30	one H.v.c. 3 and 5 lost	n
Loss of chromosomes from the wild species				
HH 1-2	933-358	24 (23)	4–5 w.b.c. lost	d
HH 1284-4	304-647	25 (26)	2–3 w.b.c. lost	d
HH 562-6	204-B-040	25+t	2 w.b.c. lost	d
	-B-245	25 (26)	2–3 w.b.c. lost	d
	787-725	27	1 w.b.c. lost	n
Addition of chromosomes from the wild species				
HH 657-2	216-930	22	1 w.b.c. gained	n
Ek 25-2	411-975	30–31	1–2 w.b.c. gained	d
Loss of unidentified chromosomes				
HH 657-2	349-578	20	1 chromosome lost	n
HH 1284-4	295-841	24	4 chromosomes lost	d
	304-006	26+t	1 chromosome lost	d
	306-792	26+t	1 chromosome lost	d
HH 562-6	204-B-515	25	3 chromosomes lost, only one SAT-chromosome observed	d
	-B-798	26	2 chromosomes lost, only one SAT-chromosome	d
	787-607	26	2 chromosomes lost	d
Addition of unidentified chromosomes				
HH 657-2	292-526	22	1 chromosome gained	n
HH 840-2	960-332	23–24	2–3 chromosomes gained	d
HH 631-5	910-594	22	1 chromosome gained	n
	910-254	22	1 chromosome gained	n
Doubled haploids of the wild species				
HH 1284-1	79-590	40–42	doubled haploids of *H. lechleri*	*H. lechleri*-like
	-593	40–41		
	-594	42		
HH 1284-4	199-142	43		
	304-652	41–42		
	307-552	42		
HH 562-6	787-610	41+t	doubled haploid of *H. procerum*	*H. procerum*-like
Amphidiploids				
HH 631-5	912-255	37 (38–39)	two *H. vulgare* genomes plus 23–25 w.b.c.	d
	914-811	36	apparently chromosome doubled	d
	-812	34-36		
HH 1284-4	199-047	52-54		
	199-514	46+t–54+t		
	-628	52–54	two *H. vulgare* genomes plus 38–40 w.b.c.	d
	-630	42–49	apparently chromosome doubled	d

[a] Figure to the left of the dash gives the explant number, and that to the right of the dash the plant number, B – regenerated plant from irradiated callus

[b] H.v.c. – *H. vulgare* chromosome(s), w.b.c. – chromsome(s) from wild barley species

[c] n – normal morphology, d – deviating morphology

Table 3. Structural chromosome changes in plants regenerated from interspecific *Hordeum* hybrids

Hybrid	Regenerated plant[a]	Chromosome no. (2*n*)	Karyotype variation[b]	Morphology[c]
Deletions in *H. vulgare* chromosomes				
HH 473-3	215-489	29+t/28+t	deletion in long arm of one of the two H.v.c. 7	n
HH 1284-4	304-006	26+t	deletion in H.v.c. 61	d
	306-792	26+t	deletion in H.v.c. 71	d
Deletions in wild species chromosomes				
HH 1284-4	300-898	27+t (28+t)	deletion in w.b.c.	n
HH 562-6	204-B-040	25+t	deletion in w.b.c.	d
	204-198	27+t	deletion in w.b.c.	d
	787-610	41+t	deletion in the long arm of satellite w.b.c.	*H. procerum*-like
Deletion in unidentified chromosome				
HH 1284-4	199-514	46+t-54+t	one arm lost in unidentified chromosome	d
Inversion				
HH 401-1	201-432	22	inversion in distal part of H.v.c. 7s	d
Extra bands or segments				
HH 1284-4	300-810	28	H.v.c. 61 with two extra bands	n
Ek 18	942-848	27 (28)	satellite on H.v.c. 6 ('Vogelsanger Gold') with extra band	n
	1107-841	27 (28)	extra euchromatic segement between the NOR and the distal band of H.v.c. 6s ('Nery')	d

[a] Figure to the left of the dash gives the explant number, and that to the right of the dash the plant number, B – regenerated plant from irradiated callus
[b] H.v.c. – *H. vulgare* chromosome(s), w.b.c. – chromosome(s) from wild barley species
[c] n – normal morphology, d – deviating morphology

hairiness, supernumerary or deformed spikes, loss of violet pigmentation (pigment being a characteristic of some of the wild barley species and their hybrids), and adventitious aerial shoots were noticed. Sixty-nine major morphological variants (other than albinos) were found and possibly more variants could have been identified with morphometric methods. Albinos occurred frequently (Jørgensen et al. 1986).

Karyotype variation

Many regenerated plants were aneusomatic with normal cells found together with hyper- or hypoploid cells (Tables 2 and 3). Also polyploid cells with the doubled chromosome number were often found. In characterizing variant plants, this pattern of mixoploidy had to be considered. Numerical variants were established as such only if the majority of cells analyzed had a deviating chromosome number.

Of the 82 plants examined, 46 exhibited chromosome variation compared to their donor line; 4 of them were obtained from irradiated calli. A total of 53 numerical or structural chromosome changes were observed in the 46 plants. Six plants carried both a numerical and a structural change, and 1 plant had two different numerical changes. Thirty of the karyotype variants deviated morphologically from their donor hybrid, the remaining 16 chromosomal variants apparently showing no conspicuous morphological changes. Four plants exhibited major morphological abnormalities; however, no chromosomal basis of these aberrations could be found. Only 1 of the 4 plants was C-banded.

The results from the C-banding and Feulgen staining of regenerated plants are given in Tables 2 and 3 for the numerical and structural chromosome variation, respectively. The chromosomal abnormalities observed included aneuploidy, double haploidy, amphidiploidy, deletions, inversions, and extra heterochromatic or euchromatic segments.

The age of callus has been claimed to influence the amount of variation encountered among the regenerated plants (e.g., review by Larkin and Scowcroft 1981). Figure 1 illustrates the relationship between the occurrence of karyotypic/morphological variants and callus age. Variation was found already after the first subculture (callus age = 2 months) and the percentage of variants apparently increased with increasing callus age.

The heterogeneity of a callus subculture was reflected by the fact that plants with chromosome aberrations were regenerated together with normal plants from the same callus.

In the irradiation experiments, the calli of *H. procerum* × *H. vulgare* which received 100 and 150 Gy turned brownish a few weeks after irradiation and did

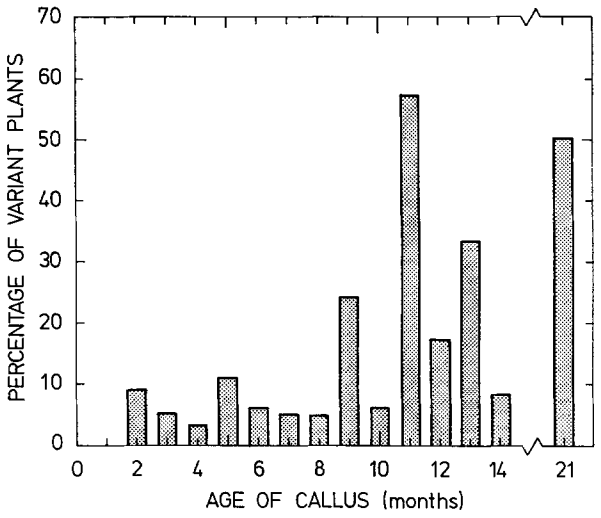

Fig. 1. Correlation between duration of the callus culture and percentage of variant plants (karyotypic plus morphological variants)

not regenerate plants. A single plant regenerated from the calli which received 50 Gy died before the potting stage. The calli which received 20 and 30 Gy were subdivided and produced 31 and 9 plants, respectively. Six of the former and 2 of the latter were found to differ morphologically from the primary hybrid; they were slender, dwarfish, or looked like haploids of the wild species. Two of the 6 plants from irradiated calli examined had the expected chromosome number and 4 were hypoploids. C-banding of two hypoploids showed that they had lost chromosomes from the wild species, and 1 of them further carried a deletion in a wild barley chromosome. Unirradiated subcalli from the same explant of *H. procerum* × *H. vulgare* regenerated 55 plants. Only 1 of them deviated morphologically from the primary hybrid. Karyological examination revealed a deletion in a *H. procerum* chromosome.

Numerical chromosome changes

Twenty-seven aneuploids, 7 doubled haploids, and 7 amphidiploids comprised the 41 numerical chromosome changes observed (Table 2).

Aneuploids. Loss of *H. vulgare* chromosomes was found in nine regenerants from combinations with *H. lechleri* or *H. procerum*. Plants from callus of the primary hybrids had lost either chromosome 5 or 7. No extra *H. vulgare* chromosomes were observed in any of the regenerated plants.

Loss as well as gain of chromosomes from the wild species were registered in 7 plants from combinations with *H. brachyantherum, H. brevisubulatum, H. lechleri,* and *H. procerum*. A regenerant had lost four to five chromosomes from *H. brevisubulatum,* the maximum number of wild species chromosomes missing (Fig. 2a). This plant was obtained from the only combination with cytoplasm from cultivated barley. The numerical change in seven hypoploids and four hyperploids was not specifically identified.

Dihaploids. Seven doubled haploids were reared from calli of hybrids with *H. lechleri* or *H. procerum*. Two of the regenerants were verified as doubled haploids by C-banding; the other 5 were identified on the basis of chromosome number and morphology. The doubled haploid condition was observed in cells from shoot apical meristems as well as in root meristems. Despite the two homologous chromosome sets present, the plants remained sterile.

Amphidiploids. Seven amphidiploids were regenerated from combinations with *H. jubatum* and *H. lechleri*. Amphidiploidy was confirmed by C-banding of one plant from each combination (Fig. 2b). These amphidiploids were not fertile. Analysis of meiosis showed regular bivalent formation, but at later stages of meiosis laggards and micronuclei were observed (Fig. 2c).

Eleven regenerated plants were treated with colchicine, but only 1 showed the doubled chromosome number in all cells. This plant was from a *H. jubatum* × *H. vulgare* combination, the very same combination which gave rise to 3 of the spontaneous amphidiploids. Like these, it was sterile. Male fertility of all colchicine-treated plants was low (0%–8%).

Structural chromosome changes

Twelve structural chromosome changes were found; they included eight deletions, one inversion, and three structural perturbations of an unsolved nature (Table 3).

Deletions. Deletions in *H. vulgare* chromosomes were found in 3 plants from two combinations with *H. lechleri* (Fig. 2d). The deletions were found in the satellite chromosomes only and comprised 4/5 or more of the long arm of chromosome 6 or 7. Deletions in the chromosomes of the wild species were found in 4 regenerants from hybrids with *H. lechleri* and *H. procerum*. A chromosome arm was lost in an amphidiploid which was not C-banded.

Invasion. One plant from the *H. roshevitzii* × *H. vulgare* combination carried an inversion in *H. vulgare* chromosome 7 (Fig. 2e). The breakpoints were localized in the telocentric part of the short arm and in the NOR. This was confirmed by the presence of two NORs in this segment observed in silver nitrate-stained preparations.

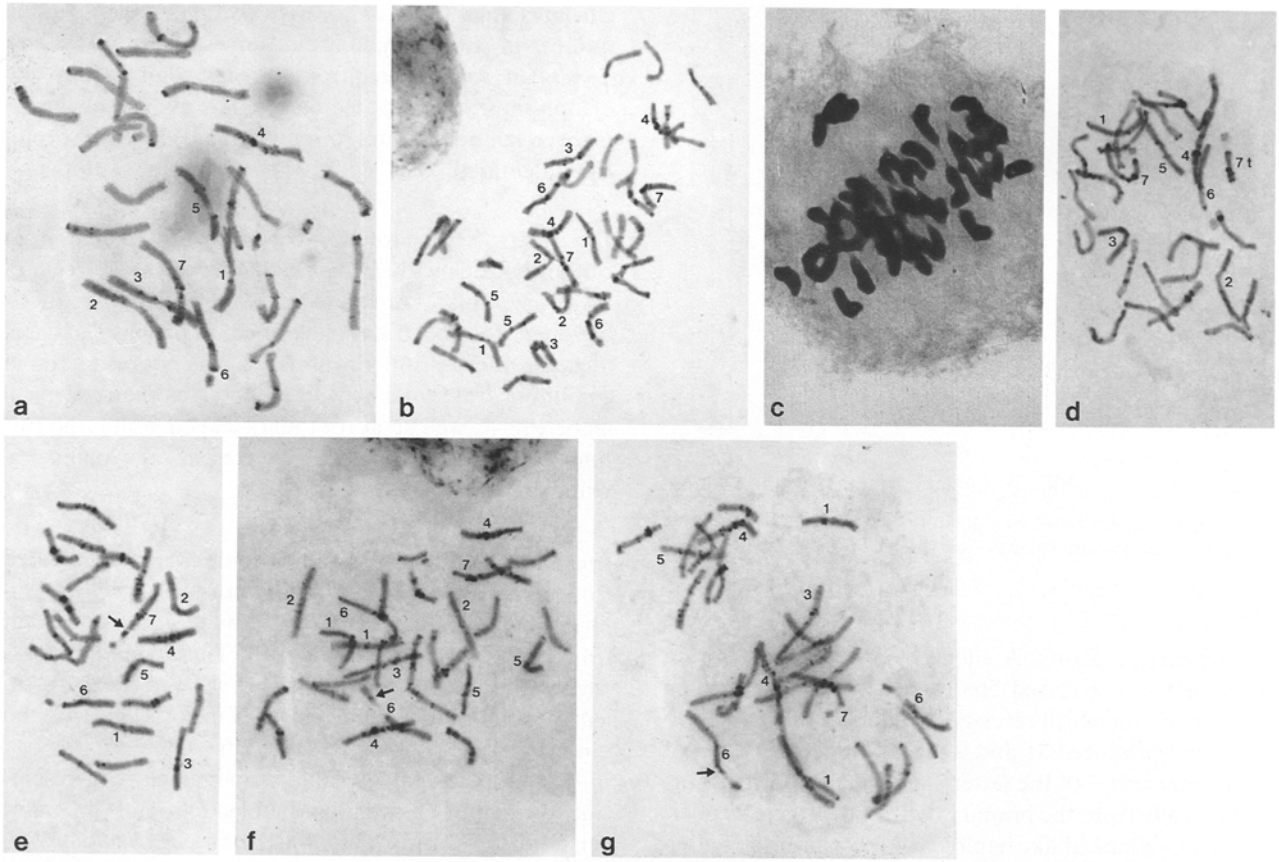

Fig. 2a–g. Chromosome variation in plants regenerated from callus of interspecific hybrids of cultivated barley. *H. vulgare* chromosomes 1–7 are indicated. **a** C-banded karyotype of a hypoploid (933-358, $2n=24$) from *H. vulgare* × *H. brevisubulatum* ($2n=28$). **b** C-banded karyotype of an amphidiploid (912-255, $2n=37$) from *H. jubatum* × *H. vulgare* ($2n=17-21$). **c** Meiosis of the amphidiploid from b ($1_I-16_{II}-1_{IV}$). **d** Deletion in *H. vulgare* chromosome 7 in plant (215-489) from callus of *H. lechleri* × *H. vulgare* ($2n=28-29$). **e** Inversion in *H. vulgare* chromosome 7 in plant (201-432) from callus of *H. roshevitzii* × *H. vulgare* ($2n=20-23$). **f** Extra band on satellite of chromosome 6 from *H. vulgare* in plant from callus of (*H. lechleri* × *H. vulgare*) × *H. vulgare* ($2n=28-33$). **g** Extra euchromatin segment on *H. vulgare* chromosome 6 in plant (1107-841) from callus of (*H. lechleri* × *H. vulgare*) × *H. vulgare* ($2n=28-33$)

Extra bands or segments. From combinations with *H. lechleri,* 2 regenerated plants carried extra C-bands (Fig. 2f) and 1 regenerant revealed an extra euchromatic segment (Fig. 2g). These structural changes were all found in chromosome 6 of *H. vulgare.*

Fertility of regenerated plants

The fertility of the primary hybrids has been shown to be very low (von Bothmer and Hagberg 1983). Ten regenerated plants showed no improved pollen fertility compared to the primary hybrid. However, seeds were obtained from a few regenerated plants: a plant from *H. lechleri* × *H. vulgare* callus (HH 1284-4) with hybrid morphology and a chromosome number of $2n=28$ produced two seeds which germinated. The chromosome number of the resultant plants was $2n=42$. They looked like the wild barley parent and were fertile. Two regenerants derived from *H. roshevitzii* × *H. vulgare* and one from *H. brachyantherum* × *H. vulgare* (HH 657-2) produced germless seeds.

Discussion

Of the 1,315 regenerated plants, only 82 were examined karyologically. Forty-six had deviating chromosomal constitutions. In addition, 35 regenerants with major morphological deviations were observed, the karyological basis of which was not studied. This gives a percentage of variants of over 6%. Karp et al. (1987) and Gaponenko et al. (1988) found that regenerants of cultivated barley showed very little variation, though much variation was present in calli of this species (Gaponenko et al. 1988; Karp et al. 1987; Scheunert et al. 1978; Singh 1986;

Mohanty et al. 1986). In diploids like cultivated barley, the variation present in calli is apparently selected against during differentiation (Vasil 1986). Polyploids are probably more tolerant to chromosomal aberrations than are diploids (Karp and Bright 1985; Karp et al. 1987), an observation that corresponds well with the results of our study. With prolonged in vitro culture the frequency of variants increased (Fig. 1); this is in agreement with previous reports on other species, e.g., tobacco (Barbier and Dulieu 1980).

Aneuploidy was by far the most common type of chromosomal change observed in the regenerated plants and hypoploidy seemed to occur twice as often as hyperploidy. Loss of *H. vulgare* chromosomes probably takes place in a non-random way. Finch (1983) reported that in the combination *H. marinum* × *H. vulgare* (translocation line Tuleen 346), the satellite chromosomes of *H. vulgare* were eliminated before the other chromosomes of this species. In agreement with this, Linde-Laursen and von Bothmer (1986, 1988) found that in offspring from the cross *H. lechleri* × *H. vulgare,* the satellite chromosomes (6 and 7) plus chromosomes 1 and 5 of *H. vulgare* were lost more often than chromosomes 2, 3, and 4. Table 2 shows that 4 regenerants from *H. lechleri* × *H. vulgare* and *H. procerum* × *H. vulgare* had lost either chromosome 5 or chromosome 7, which corresponds well with the above-mentioned reports; *H. procerum* is considered a close relative of *H. lechleri* (Jørgensen 1986). In regenerated plants from the backcross line, elimination of *H. vulgare* chromosomes seemed less selective (Table 2). Remarkably, the structural chromosome changes in the *H. vulgare* genome observed in this study also seemed to affect either chromosome 6 or 7. Why chromosomes 6 and 7 differ in this way from the rest of the *H. vulgare* genome is unknown. Chromosomes 6 and 7 carry much repetitive DNA, e.g., the rDNA found in the NOR. The repetitive sequences are known to occur in the heterochromatic regions. In tissue cultures of *Crepis capillaris,* Sacristan (1971) reported that chromosome breakage was associated with heterochromatic regions. In tissue culture of oats (*Avena sativa* L.), Johnson et al. (1987) reported late replication of heterochromatin around primary and secondary constrictions, and mentioned this as a possible reason why chromosome breaks are more frequent in these regions. Also, in induced translocation lines of barley, the majority of breakpoints were found at chromosome constrictions (Hagberg 1966; Linde-Laursen 1988).

Occasionally in interspecific crosses with *H. vulgare,* the genome of cultivated barley is eliminated, giving rise to F_1 plants which are haploids of the wild species (von Bothmer and Jacobsen 1986 and unpublished). In this study no haploid but doubled haploid plants of the wild species were obtained among plants regenerated from calli of hybrids with *H. lechleri* and *H. procerum.* The double haploids may be more competitive than the haploids, in analogy with the regenerated plants from anther culture of, e.g., cultivated barley that produces more double haploids than haploids (e.g., Wenzel and Foroughi-Wehr 1984). Possibly the double haploid chromosome constitution of the wild barley regenerants was achieved by elimination of the *H. vulgare* chromosomes from spontaneously doubled cells in the hybrid callus. A puzzling characteristic of all the callus-derived double haploids was the complete seed sterility, though *H. lechleri* and *H. procerum* normally self-pollinate. Contrasting with this is the high fertility of the two double haploids of *H. lechleri* obtained from the seeds of a hybrid-like regenerated plant from a *H. lechleri* × *H. vulgare* callus. The double haploid condition was also confirmed in cells from apical meristems. Therefore, germ mother cells should also be double haploid, and some seed setting expected. Similarly, amphidiploid regenerants were sterile. Their inability to go through a normal meiosis was demonstrated (Fig. 2c). The sterility of double haploids and amphidiploids could result from variation induced during callus culture, however, the colchicine doubled regenerant was also sterile.

The three deletions and the inversion in the *H. vulgare* genome had the chromosomal breakpoints localized in transition zones between hetero- and euchromatin or in the heterochromatic NOR. In hybrids between wheat and rye regenerated from tissue culture of hybrid callus, Lapitan et al. (1984) reported that 12 of 13 breakpoints were located in heterochromatin; also Johnson et al. (1987), in an oat plant derived from tissue culture, found the breakpoints in heterochromatin. However, in a C-banded preparation it is difficult to distinguish between heterochromatin regions and transition zones. In induced translocation lines of barley, Linde-Laursen (1988) found no breaks in C-bands. He suggested that breaks most often occurred in interband regions, possibly at heterochromatin/euchromatin junctions, as found also in cells of *Vicia faba* (Rieger et al. 1977; Döbel et al. 1978).

In the hybrid calli, translocations might take place between the genome of *H. vulgare* and that of the wild species. One plant with an extra segment on one of the *H. vulgare* chromosomes was found. However, the segment was not characterized by any C-bands and it was not large enough to be identified as a deletion in one of the other chromosomes; therefore, its origin was not determined. The extra C-bands found in two plants could have originated in several ways, translocation being one of the least probable explanations. It is more likely that euchromatic regions can become heterochromatic under specific conditions. In conclusion, with the Giemsa-staining only large structural changes or changes found in chromosome parts characterized by obvious bands would be visible. This situation was perhaps reflected in

the disproportion in number between structural changes (12) and numerical changes (41) observed.

The utility of variant plants from callus cultures of *Hordeum* hybrids is diminished, as many of these variants are aneusomatic and therefore may not carry the desired aberrations in all cells. If regeneration takes place from a single cell in the callus, e.g., by embryogenesis, only one and the same chromosome number would normally be found in the somatic tissues of the regenerated plants. Alternatively, if plants originate from a group of callus cells, they could reveal the heterogeneity in chromosome number among cell clusters. Even if the regenerant has a single cell origin, extensive backcrossing to *H. vulgare* would be necessary, as all plants regenerated from the polyploid combinations carry many chromosomes from the wild species. These crosses would be hampered by the low fertility of the regenerants. Previously one of the hybrids, *H. jubatum* × *H. vulgare*, has been reported to produce haploids of *H. vulgare* from callus of hybrid tissue (Orton 1980). The diploid combinations, *H. vulgare* × *H. bulbosum* (2×) and *H. vulgare* × *Psathyrostachys fragilis* (2×), which can produce haploids of *H. vulgare* from hybrid tissue by elimination of wild species chromosomes, had a low regenerative ability (Jørgensen et al. 1986) and were not included in this study.

The karyological study of 82 regenerated plants reared from calli of interspecific hybrids between cultivated and wild barley species showed that variants are quite common. Some of the regenerated plants, e.g., amphidiploids, plants with extra C-bands or chromosome segments, or those showing elimination of wild barley chromosomes might serve as breeding material. The usefulness of these materials, though, is rendered difficult because of their low fertility. However, the variants elucidate the consequences of in vitro culture on specific chromosomes in different genotypic or cytoplasmic combinations.

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