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Chromosome elimination in *Hordeum vulgare* × *H. bulbosum* hybrids

1. Comparisons of stable and unstable amphidiploids

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Summary. Both diploid and tetraploid hybrids of *Hordeum vulgare* × *H. bulbosum* exhibit chromosome instability during plant development and generally lose the *H. bulbosum* chromosomes by the next generation. However an amphidiploid involving the cultivar 'Vada' was found to retain the *bulbosum* chromosomes through a sexual cycle. Comparisons were made between this stable amphidiploid and a second, unstable amphidiploid involving the cultivar 'Emir'. At meiosis there was a wide range of chromosome numbers in the 'Emir' amphidiploid but only a narrow range in the 'Vada' amphidiploid. Degraded chromosomes were commonly found in the PMCs of the 'Emir' amphidiploid but none was found in the stable 'Vada' amphidiploid. The two amphidiploids were reciprocally crossed and the chromosome numbers of the progeny checked. The *H. bulbosum* chromosomes had been retained in many of the progeny and both triploid and tetraploid hybrids were found. It is proposed that a gene(s) exists in 'Vada' capable of preventing the elimination of *bulbosum* chromosomes from *H. vulgare* × *H. bulbosum* hybrids.

Key words: *Hordeum* – Amphidiploids – Chromosome elimination – Chromosome stability – Gene balance

Introduction

The cross between *Hordeum vulgare* and *H. bulbosum* is widely used in the production of haploid *H. vulgare*. Usually the *H. bulbosum* chromosomes are selectively eliminated during embryogenesis (Lange 1971a; Subrahmanyam and Kasha 1973; Bennett et al. 1976). However in some embryos elimination does not take place and hybrid plants are produced.

Similarly when tetraploid *H. vulgare* and tetraploid *H. bulbosum* are crossed most of the progeny are dihaploid *H. vulgare* following the elimination of the *bulbosum* chromosomes. But tetraploid hybrids with the genomic constitution VVBB (V = *vulgare*, B = *bulbosum*) are also produced.

The production of hybrids vs haploids is influenced by both parental genotypes and environment (Pickering 1980). Under certain conditions the progeny may consist of more than 70% hybrid plants from some genotype combinations of diploids.

When chromosome elimination occurs in the developing embryo it is rapid with 0–3 (and sometimes as many as 7) chromosomes per cell eliminated at each mitotic division (Bennett et al. 1976). However when elimination does not take place at this stage and hybrid plants are produced, elimination of the *H. bulbosum* chromosomes is slow and erratic in the somatic tissue. Elimination may occur in some parts of the plant and not in others. Subrahmanyam and Kasha (1973), Ho and Kasha (1975) and Fukuyama and Takahashi (1976) found that parts of plants reverted to *H. vulgare* while other parts retained their hybrid morphology. Humphreys (1978) found different rates of chromosome elimination in different tillers of the same plant. Elimination is sufficiently slow in hybrid tissue that many *bulbosum* chromosomes are retained until meiosis where a range of chromosomes per PMC is found (Lange 1971a; Kasha and Sadasivaiah 1971). The rate of elimination from somatic hybrid tissue is dependent on tissue type: Noda and Kasha (1981) found more cells with micronuclei in spike primordia than in root and leaf meristems. Humphreys (1978) found higher rates of chromosome elimination at higher temperatures and also found genotypic differences.

Lange (1971a) and Kasha and Sadasivaiah (1971) found that triploid hybrids with one genome of *vulgare* and two of *bulbosum* were more stable than either the diploid or tetraploid hybrids. Barclay et al. (1972) and Ho and Kasha (1975) have shown that this stable balance is dependent on chromosomes 2 and 3 of *H. vulgare*. Lange and Jochemsen (1976) obtained few seed from triploid VBB hybrids. Those which germinated produced perennial plants morphologically like interspecific hybrids or similar to *H. bulbosum*. Most of the progeny they obtained from tetraploid hybrids were *vulgare*-

like but a few had hybrid morphology and variable chromosome numbers.

The failure of *bulbosum* chromosomes to be retained in the amphidiploid through a sexual cycle was confirmed by Pickering (1979). However an amphidiploid obtained after colchicine treatment from a cross between *H. vulgare* cv. 'Vada' (2n = 2x = 14) and *H. bulbosum* (stock S1) (2n = 2x = 14) retained the *bulbosum* chromosomes through a sexual cycle (Pickering, loc. cit.; Pickering 1980). Since this time more amphidiploids have been produced between 'Vada' and other stocks of *H. bulbosum* which have also retained the *bulbosum* chromosomes into the next generation (Pickering and Thomas, unpublished data).

In this paper we describe the behaviour of the amphiploid between 'Vada' and *H. bulbosum* (S1) in comparison with a second amphidiploid between *H. vulgare* cv. 'Emir' and *H. bulbosum* (S1).

Materials and methods

The hybrids were produced using techniques similar to those described by Pickering (1980). Crosses were made in a heated glasshouse with 16 h daylength (natural daylight supplemented with high pressure mercury fluorescent bulbs). After conventional emasculation the florets of *H. vulgare* were pollinated with *H. bulbosum* using a small camel hair brush. One day after pollination a solution of gibberellic acid (75 mg/l GA_3) + Tween 20 wetting agent (0.25%) was sprayed onto individual florets and the spikes covered with brown paper bags. About 14 days later the embryos were excised from the caryopses and cultured in wide-mouth glass tubes containing 7 ml of a modified Gamborg B5 medium (Gamborg et al. 1968) with 2% w/v sucrose and 7 g/l agar, the 2,4-D being omitted. The embryos were incubated in darkness at 22 °C until germination started when the culture vials were transferred to a 16 h daylength environment (12,000 lux – warm white fluorescent tubes) at room temperature (22 ± 2 °C). At a height of approximately 5–6 cm the seedlings were transferred to John Innes No. 3 compost in 10 cm pots and the hybrids (VB) identified by their pubescent leaf sheaths.

When tillering was established the plants were removed from the compost, their roots cut back to 1 cm and incisions made at each shoot base. The plants were immersed for 5 h in a solution of 0.1% colchicine + 2% dimethyl sulphoxide, then washed in tap water for 2–3 min and repotted in 15 cm pots using a similar compost. They have since been maintained vegetatively in the glasshouse.

For cytological studies actively growing root-tips were removed from plants growing in pots or from seedlings germinated on moist filter paper.

After pretreatment in distilled water at 1 °C for 24 h and fixation in ethanol : acetic acid (3 : 1), they were stained by the Feulgen method and squashed in 1% aceto-carmine.

Five cells only were chromosome counted from the two primary hybrids. In the progeny, where possible three root-tips from each genotype were counted.

For meiotic studies inflorescences were taken before emergence, fixed in Carnoy (6 : 3 : 1) solution and anthers squashed in 1% aceto-carmine.

The plant material has been coded as follows:

H. vulgare cv. 'Vada' (2x) × *H. bulbosum* S1 (2x)	– Vada VB1[1] (2n = 28)
H. vulgare cv. 'Emir' (2x) × *H. bulbosum* S1 (2x)	– Emir VB10[1] (2n = 28)
selfed progeny of Vada VB1	– VB1/n
selfed progeny of Emir VB10	– VB10/n
Emir VB10 (♀) × Vada VB1 (♂)	– VB14/n
Vada VB1 (♀) × Emir VB10 (♂)	– VB15/n

Results

The chromosome number in root-tips of both Emir VB10 and Vada VB1 was 28. Meiosis in both amphidiploids showed a range of chromosome number; 14 to 30 in Emir VB10 with a mean of 23.73 and 24 to 30 in Vada VB1 with a mean of 27.25 (Fig. 1). Chromosome pairing was similar to that previously reported for *H. vulgare* × *H. bulbosum* amphidiploids (Lange 1971b; Kasha and Sadasivaiah 1971) and no significant differences could be found between the two amphidiploids in any aspect of chromosome pairing.

A striking difference between the two plants was the presence of degraded chromosomes in Emir VB10 PMCs (Fig. 2a, b); 57% of PMCs contained either degraded chromosomes or chromosome fragments while none were found in Vada VB1 (Fig. 1).

In Emir VB10 first anaphase was not regular. Laggards were common (Fig. 2c) and there were signs of more than two spindle poles (Fig. 2d). Frequently at the tetrad stage polyads with various numbers of segments of different sizes were found and micronuclei were commonplace (Fig. 2e, f).

Meiosis was more regular in Vada VB1 than in Emir VB10 and normal dyads formed at the end of AI. However in Fig. 1 a small third cell with one chromosome dividing at AII is shown; such aberrations were infrequent. At the tetrad stage less than a quarter of the tetrads had micronuclei.

There was a difference in pollen grain size between Vada VB1 and Emir VB10. In Emir VB10 there was a

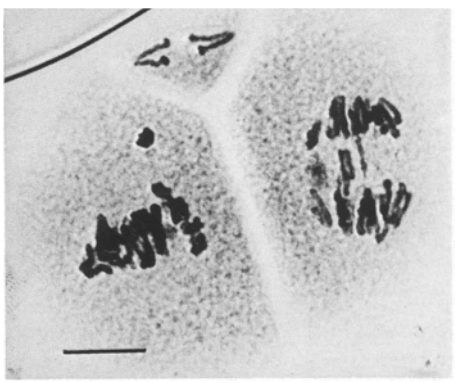

Fig. 1. Second anaphase in Vada VB1. The left hand cell contains a micronucleus; a single chromosome undergoes normal division in a third cell. Bar represents 10 μm

1 Chromosomes doubled by colchicine treatment

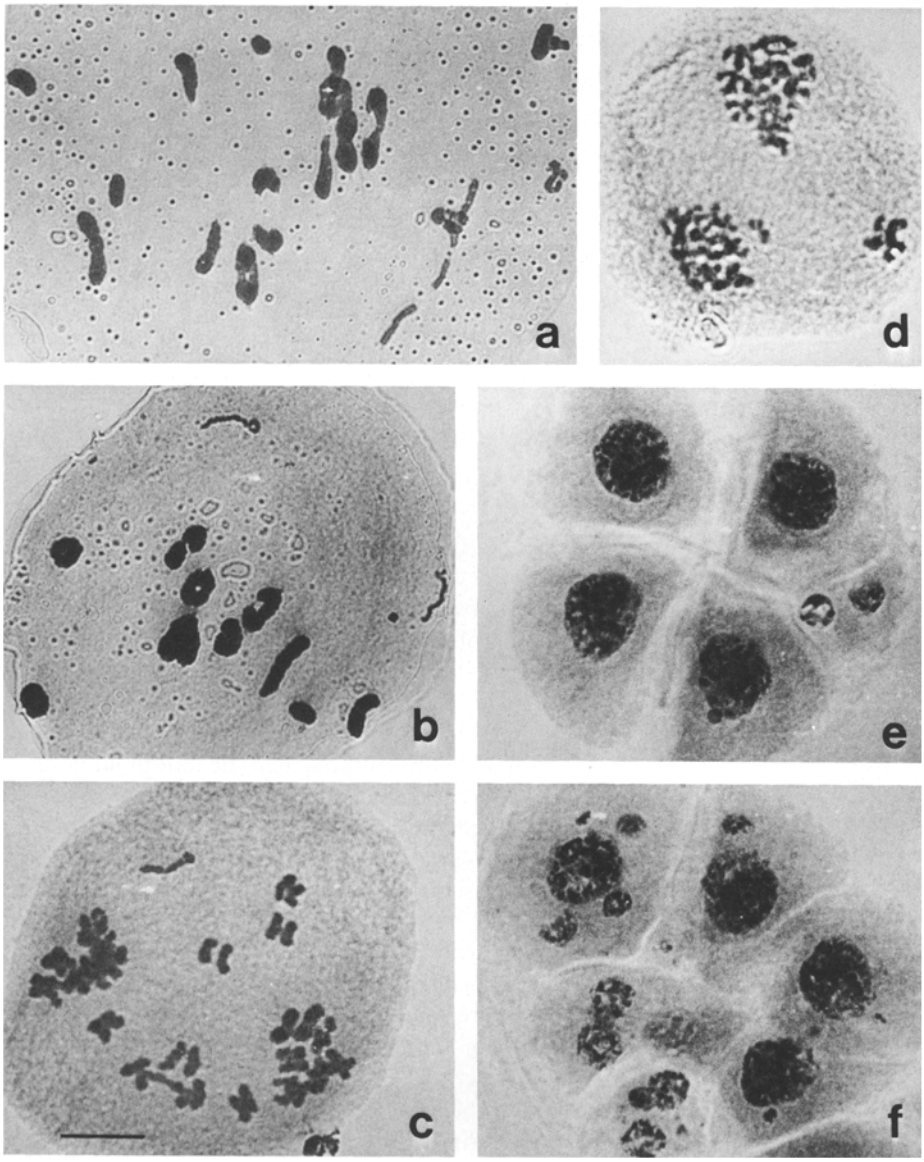

Fig. 2a – f. Meiosis in Emir VB10. **a, b** degraded chromosomes at 1st metaphase; **c** 1st anaphase with laggards, bridge and a degraded chromosome; **d** tri-polar 1st anaphase; **e, f** polyads. Bar represents 10 μm

wide range of sizes; measurements were made of 100 cells:

Diam. in arbitrary units	12	11	10	9	8	7	6	5	4	3	total
Number of grains		1	12	36	17	7	3	1	3	11	9	100

Vada VB1 pollen grains could be classified into two distinct groups, 325 (97%) were 9–10 arbitrary units in diameter and the remaining 10 were 3–4 units in diameter.

Chromosome counts were made at first pollen grain mitosis in two inflorescences of Emir VB10 and one inflorescence of Vada VB1 (Table 1). In the first inflorescence of Emir VB10 there appears to have been little or no chromosome elimination while in the second the majority of cells had only 7 chromosomes. There was no elimination at meiosis leading up to the formation of the pollen in the Vada VB1 amphiploid.

Pollen grains with one or two pores were present in both hybrids. However, in Emir VB10 only 8% of grains were biporate while in Vada VB1 38% were biporate. The small grains in both hybrids were either uniporate or non-porate.

A total of 484 progeny obtained from selfing the Emir VB10 were scored for morphological characters and disease resistance: 454 (93.8%) were *vulgare* like and 30 (6.2%) had some *H. bulbosum* characters such as hairy basal leaf sheaths at the seedling stage, but these also resembled *H. vulgare* as mature plants. Of the 674

Table 1. Pollen grain mitosis in Emir VB10 and Vada VB1

No. of cells	Chromosome No.								
	16	15	14	13	12	9	8	7	Total
Emir VB10a		1	9		1				11
Emir VB10b		1		1	2	1	2	11	18
Vada VB1	2	2	7						11

Table 2. Chromosome numbers in seedling root-tips of selfed progeny of Vada VB1

Plant No.	Mean	Range	No. of cells
VB1/28[a]	21.31	9 – 25	36
VB1/29	14	–	25
VB1/30 root 1	23.92	17 – 26	25
VB1/30 roots 2 + 3	14	–	8
VB1/32	24.84	18 – 27	25
VB1/33	24.84	16 – 29	25
VB1/34	26.6	21 – 29	25

[a] 44% of cells contained a long dicentric chromosome, 8% contained a telocentric chromosome

Table 3. Chromosome numbers of progeny from Emir VB10 × Vada VB1 (VB14/n) and from Vada VB1 × Emir VB10 (VB15/n)

Plant No.	Mean	Range	No. of cells
VB14/1 root 1	14	–	10
VB14/1 root 2	20.2	14 – 21	25
VB14/2	14.1	13 – 16	10
VB14/3	14	–	10
VB14/4	18.88	16 – 20	17
VB15/1	23.78	19 – 27	23
VB15/2	14	–	8
VB15/3	14.79	14 – 17	19
VB15/4	14	–	8
VB15/5	26.12	19 – 28	25

selfed seeds harvested from Vada VB1 only 95 (14.1%) were *vulgare* like while 579 (85.9%) had hybrid morphology.

Nine seedlings from Emir VB10 were chromosome counted and all had a constant number of 14. They resembled diploid *H. vulgare* in every respect. The chromosome numbers of six of the progeny of Vada VB1 are presented in Table 2. Only one of the six plants was entirely diploid but two roots of another plant (VB1/30) gave a constant count of 14 chromosomes. The other root of this plant and the roots of the other four plants examined had a range of chromosome numbers with means between triploid and tetraploid.

Table 3 shows the chromosome number of seedlings obtained by pollinating Emir VB10 with Vada VB1 (VB14/n) and by pollinating Vada VB1 with Emir VB10 (VB15/n). Two of the VB14 plants were diploid, a third was partly diploid and partly an irregular hypo-triploid and the fourth was entirely an irregular hypotriploid, i.e. they had a range of chromosome numbers less than 21. Two of the five VB15 plants were diploid a third had a range of 14–17 and the other two ranged from 19–27 and 19–28 chromosomes respectively.

Discussion

The amphidiploid Emir VB10 behaved much as the tetraploid hybrids described by Lange and Jochemsen (1976). The overwhelming majority of the progeny from selfing had reverted to diploid *H. vulgare* the *H. bulbosum* chromosomes having been eliminated sometime between first metaphase of meiosis and the germination of the seedlings. However the behaviour of Vada VB1 was quite different. Here 86% of the selfed progeny had retained *bulbosum* characteristics and chromosome counts confirmed that in many cases the chromosome number approached the tetraploid level.

With a mean of 27.25 chromosomes in PMCs of Vada VB1, and a range of 24–30, chromosome elimination during plant development has been negligible or even non-existent, with merely an increase in range. There was a highly significant difference ($P < 0.001$) between the chromosome numbers of the two amphidiploids at meiosis and PMCs with as few as 14 chromosomes were found in Emir VB10. But although the elimination in Emir VB10 was highly significant there were still cells of 28–30 and the modal class was 27. One cannot therefore compare the elimination of chromosomes during plant development with that found during embryogenesis where the entire *bulbosum* genome may be eliminated in a few cell divisions.

Meiosis

The differences that exist between the amphidiploids did not manifest themselves in chromosome pairing; the behaviour of the two hybrids was very similar with large cell to cell variation within plants and even between cells with the same chromosome number.

In Emir VB10 57% of PMCs contained degraded chromosomes or chromosome fragments. In different cells the degraded chromosomes appeared to be at different stages of breakdown. Chromosomes that appeared to have a fuzzy outline were interpreted as to be at an early stage of degradation. In other cells there were chromosomes in which chromatin loss had advanced to the point that the cells contained thread-like

structures, that were interpreted as remnants of chromosomes. Lange (1971a, b) described "inclusions" in PMCs of diploid hybrids which he believed were of nuclear origin. From his photograph (1971a, Fig. 3c, p. 187) these appear to be what we describe here as degraded chromosomes.

The gradual breakdown of the entire chromosomes could be explained by the action of endonuclease on specific chromosomes as was hypothesised by Davies (1974). Subrahmanyam et al. (1976) treated root-tip cells of *H. vulgare* and *H. bulbosum* with a site-specific endonuclease preparation and observed a progressive degradation of chromosomes. They found differences in effect on the chromosomes of the two species, but the endonuclease used in their experiment had a more severe effect on *H. vulgare* chromosomes than those of *H. bulbosum.* They did however consider the morphologies of degraded nuclei and micronuclei after enzyme treatment to be similar to those found in *H. vulgare* × *H. bulbosum* hybrids.

No degraded chromosomes were found in Vada VB1. If degradation was the result of endonuclease action then clearly the enzyme was not present, or at least not active, in the PMCs of Vada VB1. As Vada VB1 retained its *bulbosum* chromosomes into the subsequent generation and Emir VB10 did not, degraded chromosomes appear to be related to chromosome elimination, though whether they are the major cause of chromosome elimination is unknown. There were insufficient degraded chromosomes present in most cells to account for the number of chromosomes lost. If however it was an ongoing process the degraded chromosomes would represent only those chromosomes in the process of being lost. No degraded chromosomes were found in cells with 28 or more chromosomes.

Another difference between the hybrids was the regularity of first anaphase. In Emir VB10, AI frequently resulted in two large daughter cells together with one or more smaller cells. These underwent a second division and led to polyads with segments of widely differing sizes and micronuclei were also commonly seen providing another major source of chromosome loss. AI in Vada VB1 usually resulted in two equal sized daughter cells which underwent normal second division – producing regular tetrads.

Gametes

The differences in anaphase separations were reflected in the size of pollen grains, with a wide range of grain sizes in Emir VB10 while most grains in Vada VB1 were large and presumably 2x. The chromosome counts made at first pollen grain mitosis illustrate that elimination takes place in Emir VB10, at least in some anthers, between first meiotic division and this stage. The grains which were chromosome counted were all larger and it is likely that the smaller grains had fewer than 7 chromosomes.

Again there were differences between the hybrids in the proportions of uniporate and bi-porate grains. Dover (1974) found pore numbers to be related to the potential number of spindle poles, though that potential number of spindles may not necessarily be formed. Tai (1970) proposed that each genome had its own spindle organiser; the number of pores could therefore reflect the number of genomes present.

Chromosome counts of first pollen grain mitosis in Emir VB10 showed there were both 1x and 2x grains. But as 94% of the selfed progeny were diploid *H. vulgare,* either further chromosome elimination must have taken place before or after fertilization, or the haploid *"vulgare"* pollen had a selective advantage in fertilization. When Emir VB10 pollen was used to fertilize Vada VB1 some of the progeny (VB15/1 and VB15/5) had tetraploid or near tetraploid cells. Some of the functional pollen from Emir VB10 must therefore have been 2x. So when Emir VB10 was selfed, elimination must have taken place after fertilization, most likely during embryogenesis, to produce the *H. vulgare* progeny in the numbers found.

When the amphidiploids were crossed using Emir VB10 as female and Vada VB1 as male, no plants with more than 21 chromosomes were found (VB14/n). It is likely therefore that in this small number of successful crosses all the Emir VB10 egg cells involved were haploid *H. vulgare* the *bulbosum* chromosomes having been eliminated before fertilization. For elimination of the *bulbosum* chromosomes to have occurred after fertilization it would involve eliminating one set of *bulbosum* chromosomes but not the other which must be regarded as very unlikely.

When Emir VB10 was selfed haploid *vulgare,* or possibly hybrid, egg cells were fertilized by either haploid or hybrid pollen. When hybrid gametes were involved the *bulbosum* chromosomes were eliminated during embryogenesis. But when a hybrid gamete from Vada VB1 was involved the *bulbosum* chromosomes were not always eliminated during embryogenesis. This applied even when only one set of 'Vada' with one set of 'Emir' chromosomes were present as in the VB14/n and VB15/n plants. When hybrid (2x) gametes from both Vada VB1 and Emir VB10 were involved hypotetraploids with the presumed genomic constitution $V^{Va}V^{Em}BB\pm$ were produced (VB15/1 and VB15/5). When a hybrid (2x) gamete from Vada VB1 and a haploid *vulgare* gamete from Emir VB10 were involved hypotriploids with the presumed genomic constitution $V^{Va}V^{Em}B\pm$ (VB15/3) or $V^{Em}V^{Va}B\pm$ (VB14/1 and VB14/4) were produced.

Conclusion

It has been possible with one set of chromosomes from 'Vada' to produce triploid hybrids with two *vulgare* and

one *bulbosum* genomes; a combination which had been thought impossible. Lange (1971 b) produced hybrids with various combinations of *vulgare* and *bulbosum* genomes but failed to obtain VVB triploids by crossing tetraploid *H. vulgare* and diploid *H. bulbosum.* Kasha and Sadasivaiah (1971) obtained only diploid *H. vulgare* progeny from this cross combination. Recently however, triploid hybrids with the genomic constitution VVB were reported by Szigat and Pohler (1982) from crosses between diploid *H. vulgare* and stable BBVV amphidiploids.

From the hybrids produced so far at this Institute it appears that a gene(s) in 'Vada' is dominant over the elimination genes present in other *H. vulgare* cultivars and that when the full haploid set of chromosomes from 'Vada' is present with another *vulgare* genome ('Emir' in this case), *bulbosum* chromosomes may be retained during embryogenesis.

Lange (1971 a) and Kasha and Sadasivaiah (1971) found that when there were two *bulbosum* genomes to one *vulgare* genome, *bulbosum* chromosomes were not eliminated. It may be that the gene(s) in Vada has a similar effect to those in *H. bulbosum* in preventing elimination. In which case the gene balance in $V^{Va}V^{Em}B$ triploids may be similar to that in VBB triploids previously reported.

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