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THEORETICAL AND APPLIED GENETICS

Haploidy from *Hordeum* Interspecific Crosses

Part 3: Trihaploids of *H. arizonicum* and *H. lechleri*

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Summary. *Hordeum arizonicum* (2n=42) and *H. lechleri* (2n=42) were crossed with both *H. bulbosum* (2n=14 or 28) and *H. vulgare* (2n=14 or 28) and progeny plants were obtained through embryoculture. Crosses of *arizonicum* with diploid *bulbosum* invariably resulted in haploids (2n=21) of *arizonicum*, whereas *arizonicum* by tetraploid *bulbosum* or diploid *vulgare* crosses produced both hybrids and haploids of *arizonicum*. The *lechleri* by diploid *bulbosum* or diploid *vulgare* crosses resulted in haploids of *lechleri*, while *lechleri* by tetraploid *bulbosum* resulted in well differentiated embryos which failed to germinate.

Hybrid embryos derived from the haploid producing crosses exhibit chromosome variability, suggesting that chromosome elimination leads to haploid formation.

The results also indicate that the ratio of the parental genomes in the zygote is a critical factor which determines the chromosome elimination or stability in any cross combination. Furthermore, both *arizonicum* and *lechleri* appear to be of similar 'genetic strength' in eliminating *bulbosum* and *vulgare* chromosomes. The possibility of 'stability factors' in overcoming elimination and manipulation towards elimination are discussed.

Key words: Chromosome elimination – Trihaploids – *Hordeum arizonicum* – *H. lechleri*

Introduction

Hybridization between *Hordeum vulgare* and *H. bulbosum* followed by selective elimination of *bulbosum* chromosomes results in haploids of *vulgare* (Kao and Kasha 1969; Kasha and Kao 1970; Lange 1971; Subrahmanyam and Kasha 1973; Bennett et al. 1976). It has been amply demonstrated by comparing data from crosses involving parents of many different ploidy levels that chromosome elimination is dependent on a well defined ratio of parental genomes (Subrahmanyam and Kasha 1973). It has also been revealed that chromosomes 2 and 3 of *vulgare* carry genetic factors controlling elimination of *bulbosum* chromosomes (Barclay et al. 1972; Kasha et al. 1972; Ho and Kasha 1975). The results of Subrahmanyam and Kasha (1973) suggest that *bulbosum* chromosomes also carry factor(s) whose dose ratio to *vulgare* chromosomes 2 and 3 is critical for the elimination process. This unique phenomenon of chromosome elimination has become an important tool in the production of haploids from *Hordeum* interspecific crosses (Subrahmanyam 1977) and from intergeneric crosses of *Triticum aestivum* X *Hordeum bulbosum* (Barclay 1975) *Aegilops crassa* X *H. bulbosum* (Shigenobu and Sakamoto 1977) and *H. vulgare* X *Secale cereale* (Kruse 1967; Fedak 1977).

The objective of this study was to determine the distribution of chromosome elimination leading to haploid formation among *Hordeum* interspecific hybrids and to test the possibility of genome balance in such combinations. This paper presents eight additional *Hordeum* interspecific crosses, five of which produced trihaploids or trihaploids and hybrids.

Material and Methods

The species listed in Table 1 are used in this study. *Hordeum vulgare* and *H. arizonicum* plants were maintained in a glasshouse (25°C ± 2°C) throughout their growth, whereas *H. lechleri* and *H. bulbosum* were vernalised at 10°C with 10 h day length for 2 months. Vernalised clones were repotted into fresh soil mixture. The *lechleri* plants were transferred to a glasshouse, while diploid and tetraploid cytotypes of *bulbosum* were maintained separately in growth chambers to avoid admixture of pollen.

Emasculation, pollination, nutrient solution feeding of cut tillers, gibberellic acid treatment, embryoculture and chromosome number determinations were carried out according to Subrahmanyam (1977). Twelve-day-old embryos from different crosses were cytologically examined following an earlier technique (Subrahmanyam and Kasha 1973). Since chromosomes of different genomes

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and species of *Hordeum* are indistinguishable, the progeny plants were classified as either hybrids or haploids depending on their morphology and chromosome number.

Results

The seed set and the number of plants established through embryo culture techniques from various crosses are presented in Tables 2 and 3. The variation in per cent seed set is not of significance since the vigour of the parents and environmental conditions at the time of emasculation and pollination appeared to be the critical factors in determining the extent of seed set. No apparent differences were observed in the seed setting ability among different crosses. However, the highest percentage of seed set in each cross combination represents the potential level of success. A greater proportion (44-58%) of embryos from crosses involving *H. arizonicum* gave rise to plants than did those (20-35%) from crosses involving *H. lechleri*. Furthermore, the 49 well differentiated hybrid embryos from the *lechleri* × tetraploid *bulbosum* cross failed to germinate on culturing.

Chromosome numbers and morphologies of different progenies are summarized in table 3 and each combination is dealt with separately.

Table 1. Species of *Hordeum* used in the study

Species	Line designation	Chromosome number	Source
H. arizonicum Covas	ANU 96	42	Dr. D.H.B. Sparrow Wait Agriculture Research Institute University of Adelaide Adelaide, S.A.
H. bulbosum L.	ANU 1	14	
H. bulbosum L.	ANU 3	28	Dr. R. Oram Division of Plant Industry CSIRO Canberra, Australia
H. vulgare L.	Clipper	14	
H. vulgare L.	York	28	Dr. K.J. Kasha Crop Science Department University of Guleph Guleph, Ontario Canada
H. lechleri (Steud.) Schenk	ANU 91	42	Dr. W. Lange Foundation for Agriculture Plant Breeding Wageningen The Netherlands

Table 2. Seed set induced, embryos cultured and the progeny obtained following interspecific hybridisation

Parental species and ploidy levels (X = 7)	No. of florets pollinated	Percentage of seed set		No. of embryos cultured	Percentage of embryos giving plants
		Range	Average		
H. arizonicum 6X by					
(i) *H. bulbosum* 2X	215	0-76.3	46.9	79	44.3
(ii) *H. bulbosum* 4X	258	0-88.9	25.9	52	57.7
(iii) *H. vulgare* 2X	311	0-29.6	15.5	35	48.6
(iv) *H. vulgare* 4X	179	0-16.7	9.5	13	46.2
H. lechleri 6X by					
(i) *H. bulbosum* 2X	796	0-68.4	17.7	78	20.5
(ii) *H. bulbosum* 4X	267	0-53.3	26.6	49	
(iii) *H. vulgare* 2X	119	0-36.8	22.7	14	35.7
(iv) *H. vulgare* 4X	254	–	–	–	–

Table 3. Morphological features, chromosome numbers and types of progeny plants from interspecific crosses

Parental species and ploidy level (X = 7)	No. of plants[a]	Morphology	Chromosome number	Type
H. arizonicum 6X by				
(i) *H. bulbosum* 2X	35	*arizonicum*-like	21	haploids
(ii) *H. bulbosum* 4X	18	*arizonicum*-like	21	haploids
	3	Intermediate	35	hybrids
(iii) *H. vulgare* 2X	13	*arizonicum*-like	21	haploids
	4	Intermediate	28	hybrids
H. lechleri 6X by				
(i) *H. bulbosum* 2X	16	*lechleri*-like	21	haploids
(ii) *H. vulgare* 2X	5	*lechleri*-like	21	haploids

[a] Includes surviving progeny only

Hordeum arizonicum X *H. bulbosum* Cross

The progeny from the crosses involving diploid *bulbosum* invariably had a chromosome number of 2n=21 (Fig. 2) rather than 2n=28, which would be expected in hybrids derived from a hexaploid (2n=6x=42, Fig. 1) and a diploid (2n=2x=14, Fig. 8). On the other hand, the crosses with tetraploid *bulbosum* resulted in 21 mature plants of which eighteen had 21 chromosome complement and three had the expected number (2n=5x=35, Fig. 3). The 21 chromosome plants resembled arizonicum (Figs. 11, 12) whereas the hybrids were more *bulbosum* like vegetatively with a spike morphology (Fig. 13) intermediate between *arizonicum* (Fig. 10) and *bulbosum* (Fig. 14). The 21 chromosome plants reached flowering in 100 days while the hybrids required nearly 8 months to reach flowering.

Hordeum arizonicum X *H. vulgare* Cross

The cross involving diploid *vulgare* resulted in two kinds of progeny. Thirteen plants contained 21 chromosomes and were morphologically similar to *arizonicum* (Fig. 16). Four plants were vegetatively *arizonicum* like with an intermediate spike morphology (Fig. 17) and the chromosome number of 2n=4x=28 (Fig. 4) expected of a hybrid between a hexaploid (Fig. 1) and a diploid (Fig. 5). The 21 chromosome plants also reached flowering in 100 days while the hybrids required over 4 months to reach flowering.

Progeny from the cross involving tetraploid *vulgare* died before reaching maturity and prior to chromosome number determinations.

Hordeum lechleri X *H. bulbosum* Cross

All the plants obtained from the cross involving diploid *bulbosum* had a chromosome number of 2n=21 (Fig. 9) instead of the hybrid number (2n=4x=28) expected in hybrids between a hexaploid (2n=6x=42, Fig. 7) and a diploid (2n=2x=14, Fig. 8). Moreover, vegetatively these plants were similar to those of *H. lechleri* and have not reached flowering even after 7 months.

From the crosses with tetraploid *bulbosum*, no progeny plants were obtained on culturing the very well developed and differentiated embryos.

Hordeum lechleri X *H. vulgare* Cross

Five plants were obtained from crosses involving diploid *vulgare*. All these plants had a chromosome number of 2n=21 (Fig. 6) rather than the 2n=28 which would be expected in hybrids between a hexaploid (2n=6x=42, Fig. 7) and a diploid (2n=2x=14, Fig. 5). These 21 chromosome plants were similar to *lechleri* and had not reached flowering, similar to the 21 chromosome plants obtained from the *lechleri* by diploid *bulbosum* cross.

No progeny was obtained from crosses involving tetraploid *vulgare*.

Chromosome Variation in Hybrid Embryos

The proportion of cells with different chromosome numbers in 12 day-old embryos from different crosses could not be determined because of the technical difficulties in getting chromosome spreading. The observation on the few countable metaphase cells in various cross combinations revealed chromosome variation in embryos from six of the eight crosses. In crosses between *arizonicum* or *lechleri* and diploid *bulbosum* only haploid (2n=21) to hyperhaploid complements were observed, whereas crosses between *arizonicum* or *lechleri* with tetraploid *bulbosum* or diploid *vulgare* in addition revealed hybrid (35 or 28) to near hybrid complements. In hybrid embryos of

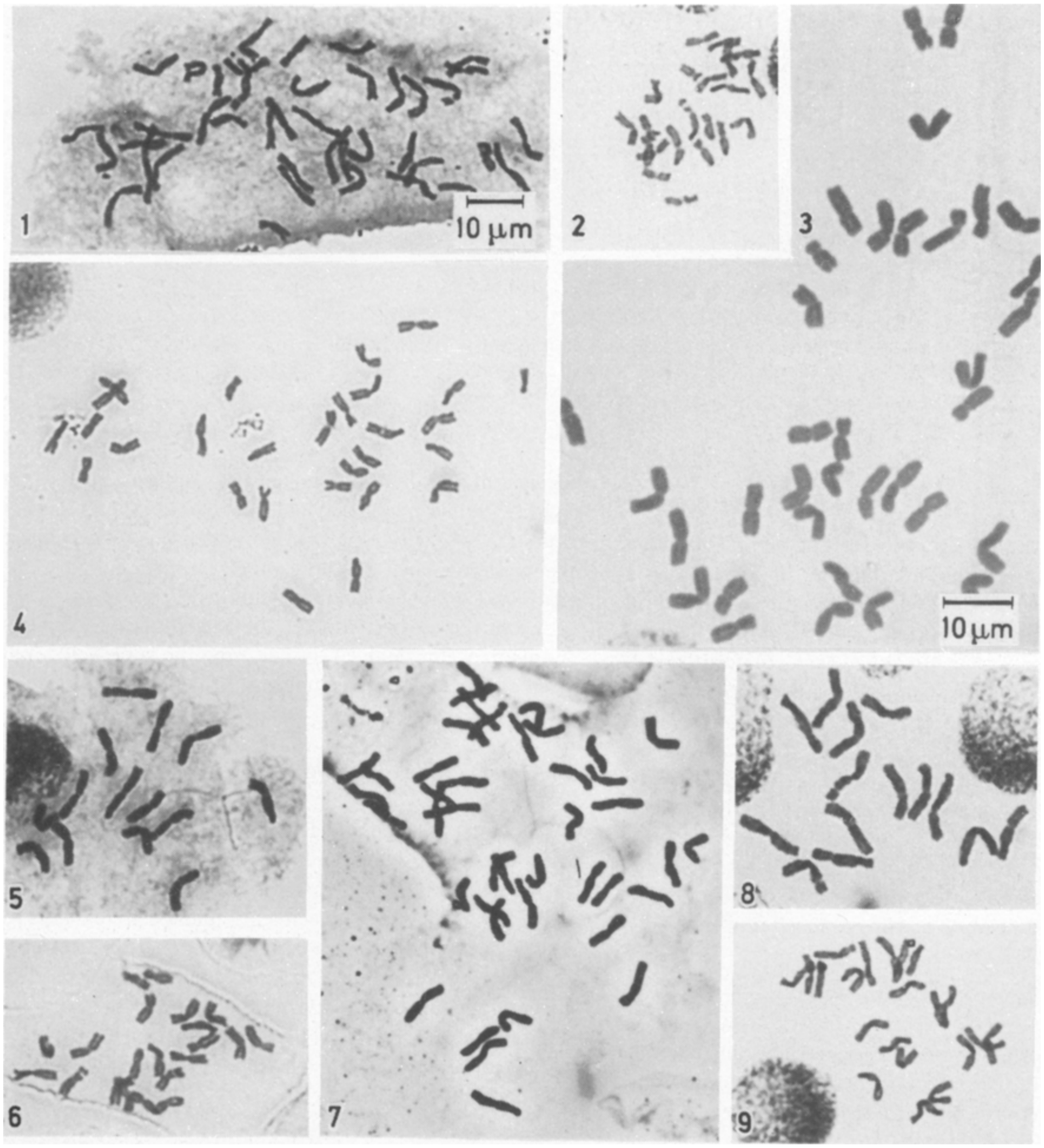

Plate I, Figs. 1-9. Somatic chromosomes of parents and progeny from various crosses. 1 *H. arizonicum* (42); 2 Haploid *arizonicum* (21) from *arizonicum* by diploid *bulbosum* cross; 3 Hybrid (35) between *arizonicum* and tetraploid *bulbosum*; 4 Hybrid (28) between *arizonicum* and *vulgare*; 5 *H. vulgare* (14); 6 Haploid *lechleri* (21) from *lechleri* by *vulgare* cross; 7 *H. lechleri* (42); 8 *H. bulbosum* (14); 9 Haploid *lechleri* (21) from *lechleri* by diploid *bulbosum* cross

arizonicum by tetraploid *vulgare*, there was no evidence of chromosome instability.

Discussion

The present results reveal that five out of the eight crosses gave rise to trihaploids of one of the parents. The observations on chromosomal variability in the embryonic cells for the corresponding crosses are consistent with progeny produced (Table 3). The chromosomal variability in embryos from *arizonicum* by *bulbosum* (2x) or *vulgare* (2x) crosses was similar to that observed by Islam and Sparrow (1974). The presence of chromosome numbers down to 21 in the embryonic cells expected to contain 28 chromosomes, and the morphological similarities of the progeny to one of the parents (*arizonicum* or *lechleri*) from crosses of *arizonicum* (6x) by *bulbosum* (2x), are indicative of selective elimination of *bulbosum* chromosomes leading to haploid formation as in *vulgare*-*bulbosum* hybrids (Sub-

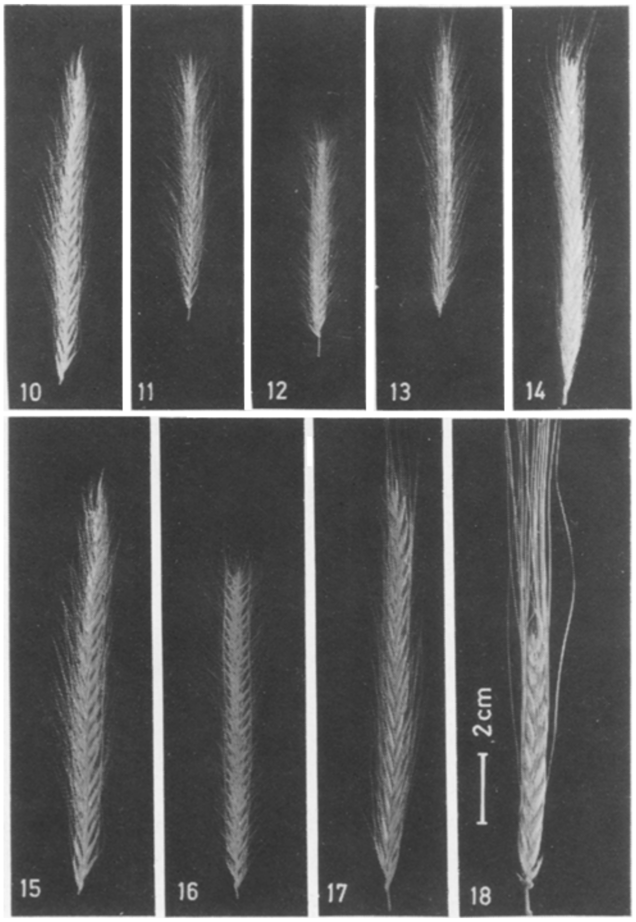

Plate II, Figs. 10-18. Spikes of parents and progeny from crosses involving *H. arizonicum* embedded in plastic resin prior to photographing. **10** *H. arizonicum*; **11** Haploid *arizonicum* from *arizonicum* by diploid *bulbosum* cross; **12** Haploid *arizonicum* from *arizonicum* by tetraploid *bulbosum* cross; **13** Hybrid between *H. arizonicum* and tetraploid *bulbosum*; **14** *H. bulbosum*; **15** *H. arizonicum*; **16** Haploid *arizonicum* from *arizonicum* by diploid *vulgare* cross; **17** *H. arizonicum-vulgare* hybrid; **18** *H. vulgare*

rahmanyam and Kasha 1973; Bennett et al. 1976). Occurrence of haploids resembling *arizonicum* and hybrids from the crosses *arizonicum* by tetraploid *bulbosum* or diploid *vulgare*, and the apparent chromosome instability in the hybrid embryos from each of the corresponding crosses, also suggest that elimination of *bulbosum* or *vulgare* chromosomes leads to haploid formation in the respective crosses. Similarly it is inferred that the elimination of *vulgare* chromosomes leads to the formation of haploids of *lechleri* following hybridization between *lechleri* (6x) and vulgare (2x). While the progeny from this cross consisted of only haploids of *lechleri*, the embryonic cells exhibited hybrid (2n=28) to near hybrid chromosome numbers. These cytological observations are consistent with earlier reports that both haploids of *lechleri* and hybrids were obtained from *lechleri* by *vulgare* crosses (Rajhathy and Symko 1974). The absence of any hybrids in the present report is probably due to the small number of progeny obtained.

The failure to obtain any plants from the well differentiated hybrid embryos from the *lechleri* (6x) by tetraploid *bulbosum* cross is probably due to early dormancy or some other disturbance.

Genome Balance

Production of haploids only from *arizonicum* by diploid *bulbosum*, hybrids and haploids from *arizonicum* by tetraploid *bulbosum*, and similarly haploids from *lechleri* by diploid *bulbosum*, and only hybrids (2n=35) or near hybrid chromosome numbers in embryos from *lechleri* by tetraploid *bulbosum* crosses, strongly suggest that a balance between the ratio of the parental genomes in each hybrid determines whether predominantly haploid or hybrid progeny are produced. For example, a ratio of 3 *arizonicum* (arz) genomes to 1 *bulbosum* (blb) genome leads to the elimination of *bulbosum* genome and subsequent formation of *arizonicum* haploids, whereas the 3 arz: 2 blb genomic ratio results in some hybrids. Similarly a ratio of 3 *lechleri* (lch): 1 blb genomes gives haploids of *lechleri*, while the 3 lch: 2 blb results in embryos with hybrid chromosome number. It is well documented in *vulgare-bulbosum* crosses (Subrahmanyam and Kasha 1973), and other interspecific cross combinations in *Hordeum* (Subrahmanyam 1977, 1978, 1979), that stable hybrids are obtained by increasing the *bulbosum* genomes. The formation of haploids from *arizonicum* by diploid *vulgare* crosses and the absence of chromosome instability in embryonic cells from the *arizonicum* by tetraploid *vulgare* cross also indicates that a genome balance effect may be operative in the elimination or retention of *vulgare* chromosomes in hybridizations with *arizonicum*. It is thus likely that crosses of tetraploid *vulgare* by *lechleri* (6x) result in stable hybrids unless physiological disturbances, such as dormancy etc., interfere with the germination of hybrid embryos.

Manipulation Towards Elimination

Islam and Sparrow (1974) predicted from their cytological observations on hybrid embryos from *H. arizonicum* by diploid *bulbosum* crosses that chromosome elimination would occur in crosses with *jubatum*, on the basis that the hexaploid species *H. arizonicum* and *H. lechleri* are considered to have *H. jubatum* genomes in common (Rajhathy et al. 1963). The formation of *jubatum* haploids following hybridization with diploid *bulbosum* (Rajhathy and Symko 1974) agrees well with this prediction. However, *jubatum* by *vulgare* crosses result in hybrids (Rajhathy et al. 1963) and not in haploids of *jubatum*. An alternative interpretation is that the third genome in *arizonicum* or *lechleri* in combination with the *jubatum* genomes reaches a dosage equivalent to the genetic factors (eliminating factors) required to initiate elimination of *vulgare* chromosomes. The elimination of *vulgare* chromosomes from hybrid embryos from crosses between an experimental amphihexaploid of *jubatum-compressum* and *vulgare* (Orton and Tai 1977), and the formation of hybrids only in crosses of *vulgare* with either *jubatum* or *compressum* (Rajhathy et al. 1963), collectively demonstrate the above principle. In other words, the number of *compressum* genomes had been increased from 2 in the *jubatum* (Starks and Tai 1974) to 4 in the amphihexaploid of *jubatum-compressum* (Orton and Tai 1977). This is clearly an experimental manipulation of genome balance towards chromosome elimination in the hybrids between the amphiploid and *vulgare*. This manipulation is similar to the use of tetraploid *vulgare* instead of a diploid in crosses with tetraploid *bulbosum* to bring about elimination of *bulbosum* genomes.

Genetic Control of Chromosome Elimination and Stability

It was demonstrated that chromosomes 2 and 3 of *vulgare* carry genetic factors controlling the elimination of *bulbosum* chromosomes (Ho and Kasha 1975). It is proposed that such 'eliminating factors' (Subrahmanyam 1977) are widespread among *Hordeum* species. Although there are experimental leads (Subrahmanyam and Kasha 1973), suggesting the presence of stability factors on the *bulbosum* chromosomes to overcome or offset the 'eliminating factors', it has not been possible to assign 'stability factors' on specific chromosome(s) of *bulbosum* because of the lack of cytogenetic stocks of this species. It should now be possible to use trisomics of *vulgare* to identify specific chromosomes carrying 'stability factors' to overcome elimination of *vulgare* chromosomes in crosses with *arizonicum* or *lechleri*.

Mechanism of Chromosome Elimination

The present results and earlier reports (Subrahmanyam 1977, 1978, 1979) together reveal that a total of nineteen *Hordeum* interspecific cross combinations exhibit chromosome elimination. From the widespread distribution of this unique phenomenon among *Hordeum* interspecific hybrids, and the generality of a genome balance controlling elimination or stability, it is conceivable that the mechanism of elimination is similar to that of *vulgare-bulbosum* hybrids. Although several hypotheses have been proposed (Kasha 1974), spindle abnormalities (Handmaker 1973) and/or a 'modification-restriction system' (Davies 1974) appear to be operative. Bennett et al. (1976) emphasized the failure of chromosomes to initiate or complete either congression at metaphase or migration to the poles at anaphase as the cause of elimination. Treatment of *bulbosum* and *vulgare* root-tip cells with bacterial restriction endonucleases resulted in chromosome degradation (Subrahmanyam et al. 1976), which is similar to the occasionally observed degradation of chromosomes in *vulgare-bulbosum* hybrid cells (Subrahmanyam and Kasha 1973). Whether the chromosomes that fail to congress or migrate are degraded, or a selective endonuclease activity in interspecific hybrids leads to the failure of specific chromosomes to congress or migrate during cell division, remains to be studied.

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Literature

Barclay, I.R. (1975): High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256, 410-411

Barclay, I.R.; Shepherd, K.W.; Sparrow, D.H.B. (1972): Chromosome elimination in *Hordeum vulgare-Hordeum bulbosum* hybrids. Biennial Report (1970-71) Waite Agric. Res. Inst., Univ. of Adelaide, S. Australia, pp. 39-40

Bennett, M.D.; Finch, R.A.; Barclay, I.R. (1976): The time rate and mechanism of chromosome elimination in *Hordeum* hybrids. Chromosoma 54, 175-200

Davies, D.R. (1974): Chromosome elimination in interspecific hybrids. Heredity 32, 267-270

Fedak, G. (1977): Haploids from barley X rye crosses. Canad. J. Genet. Cytol. 19, 15-19

Handmaker, S.D. (1973): Hybridization of eukaryotic cells. Ann. Rev. Microbiol. 27, 189-204

Ho, K.M.; Kasha, K.J. (1975): Genetic control of chromosome elimination during haploid formation in barley. Genetics 81, 263-275

Islam, R.; Sparrow, D.H.B. (1974): Chromosome elimination in interspecific hybrids involving *Hordeum arizonicum* (6x) X *H. vulgare* (2x) Barley Genet. Newsl. 4, 36-37

Kao, K.N.; Kasha, K.J. (1969): Haploidy from interspecific crosses with tetraploid barley. Barley Genetics 2, 82-87

Kasha, K.J. (1974): Haploids in higher plants – Advances and potential. pp. 10-14, Proc. of the First Int. Symp., Univ. of Guelph

Kasha, K.J.; Kao, K.N. (1970): High frequency haploid production in barley (*Hordeum vulgare* L.) Nature 225, 874-876

Kasha, K.J.; Reinbergs, E.W.A.; Subrahmanyam, N.C.; Ho, K.M. (1972): Barley haploid studies. Barley Genet. Newsl. 2, 36-41

Kruse, A. (1967): Intergeneric hybrids between *Hordeum vulgare* L. ssp. *distichum* (v. Pallas 2n=14) and *Secale cereale* L. (v. Petkus 2n=14) Kal. Vet. Landb. Arsskr. pp. 82-92

Lange, W. (1971a): Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. 1: Production, morphology and meiosis of hybrids, haploids and dihaploids. Euphytica 20, 14-29

Lange, W. (1971b): Crosses between *Hordeum vulgare* L. and *H. bulbosum* L. 2: Elimination of chromosomes in hybrid tissues. Euphytica 20, 181-194

Orton, T.J.; Tai, W. (1977): Chromosome elimination in a complex hybrid of the genus *Hordeum*. Canad. J. Bot. 55, 3023-3033

Rajhathy, T.; Symko, S. (1974): High frequency of haploids fom crosses of *Hordeum lechleri* (6x) × *H. vulgare* (2x) and *H. jubatum* (4x) × *H. bulbosum* (2x). Can. J. Genet. Cytol. 16, 468-472

Rajhathy, T.; Morrison, J.W.; Symko, S. (1963): Interspecific and intergeneric hybrids in *Hordeum*. Barley Genetics 1, 195-213

Shigenobu, T.; Sakamoto, S. (1977): Production of a polyhaploid plant of *Aegilops crassa* (6x) pollinated by *Hordeum bulbosum*. Jap. J. Genet. 52, 397-401

Starks, G.D.; Tai, W. (1974): Genome analysis of *Hordeum jubatum* and *H. compressum*. Canad. J. Genet. Cytol. 16, 663-668

Subrahmanyam, N.C. (1977): Haploidy from *Hordeum* interspecific crosses. I. Polyhaploids of *H. parodii* and *H. procerum*. Theor. Appl. Genet. 49, 209-217

Subrahmanyam, N C. (1978): Haploids and hybrids following interspecific crosses. Barley Genet. Newsl. 8, 97-99

Subrahmanyam, N.C. (1979): Haploidy from *Hordeum* interspecific crosses. 2: Dihaploids of *H. brachyantherum* and *H. depressum*. Theor. Appl. Genet. 55, 139-144

Subrahmanyam, N.C.; Gould, A.R.; Doy, C.H. (1976): Cleavage of plant chromosomes by restriction endonucleases. Plant Sci. Lett. 6, 203-208

Subrahmanyam, N.C.; Kasha, K.J. (1973): Selective chromosomal elimination during haploid formation in barley following interspecific hybridization. Chromosoma 42, 111-125

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