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THEORETICAL AND APPLIED GENETICS

Haploidy from *Hordeum* Interspecific Crosses

Part 2: Dihaploids of *H. brachyantherum* and *H. depressum*

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Summary. Interspecific crosses of *Hordeum brachyantherum* (2n = 28) and *H. depressum* (2n = 28) with *H. bulbosum* (2n = 14 or 28) and *H. vulgare* (2n = 14 or 28) were made. Crosses between *brachyantherum* and diploid *bulbosum* resulted in dihaploids (2n = 14) of *brachyantherum* and hybrids (2n = 21), whilst the crosses of *brachyantherum* by tetraploid *bulbosum* or *vulgare* gave hybrid progeny. Similarly, crosses between *H. depressum* and diploid *bulbosum* resulted in dihaploids (2n = 14) of *depressum* and hybrids (2n = 21), whereas *depressum* by tetraploid *bulbosum* or *vulgare* invariably produced hybrids.

Cytological observations on 12 day old embryos obtained from these crosses revealed chromosome variability down to 14 in crosses with diploid *bulbosum* indicating thereby that chromosome elimination leads to haploid formation. Embryonic cells from the *brachyantherum* by diploid *vulgare* cross also exhibited a certain degree of chromosomal instability as micronuclei.

The results indicate that the ratio of parental genomes in the zygote determines whether haploids or hybrids will be produced in crosses of *brachyantherum* or *depressum* with *bulbosum*. Furthermore, *brachyantherum* appears to be more efficient in eliminating *bulbosum* chromosomes in comparison with *depressum*.

Key words: Chromosome elimination – dihaploids – *Hordeum brachyantherum* – *H. depressum*

Introduction

The identification and comprehensive characterisation of the phenomenon of selective chromosome elimination in hybrids between *Hordeum vulgare* and *H. bulbosum* (Kao and Kasha 1969; Kasha and Kao 1970; Lange 1971a, b; Subrahmanyam and Kasha 1973a) has provided a significant tool for the production of haploids in cultivated barley (Subrahmanyam and Kasha 1973b; Kasha 1974). Chromosome elimination leading to haploid formation from other *Hordeum* interspecific crosses (see Kasha 1974; Subrahmanyam 1977) and from intergeneric crosses of *Triticum aestivum* (6x) × *Hordeum bulbosum* (Barclay 1975, *Aegilops crassa* (6x) × *H. bulbosum* (Shigenobu and Sakamoto 1977), and *H. vulgare* × *Secale cereale* (Kruse 1967; Fedak 1977), indicate a broader distribution of this phenomenon. The cumulative study of the hybrids from the crosses involving *Hordeum vulgare* and *H. bulbosum* at different ploidy levels revealed that the process of chromosome elimination is dependent upon a well defined ratio of parental genomes (Subrahmanyam and Kasha 1973a). It was further demonstrated that this phenomenon is under the control of genetic factors located on *vulgare* chromosomes (Barclay et al. 1972; Kasha et al. 1972; Ho and Kasha 1975) whose dose ratio to *bulbosum* chromosomes is critical to the elimination process (Subrahmanyam and Kasha 1973a).

It was of interest to examine further the distribution of chromosome elimination leading to haploid formation among *Hordeum* interspecific hybrids and to see whether the genome balance is applicable in other combinations. This paper reports eight additional *Hordeum* interspecific crosses, two of which gave dihaploids.

Materials and Methods

The species used in the study are listed in Table 1. *Hordeum vulgare* and *H. depressum* plants were maintained in a glasshouse (25° ± 2°C) throughout their growth period, whereas *H. brachyantherum* and *H. bulbosum* were vernalised at 10°C with 10h day length for 2 months. Vernalised clones were repotted into fresh soil mixture. The *brachyantherum* plants were transferred to a glasshouse, whereas the diploid and tetraploid cytotypes of *bulbosum* were maintained in two different compartments of a growth cabinet to avoid admixture of pollen. Emasculation, polli-

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

Species	Line designation	Chromosome number	Source
H. bulbosum L	ANU 1	14	Dr. D.H.B. Sparrow Wait Agric. Res. Inst. University of Adelaide S.A.
H. bulbosum L	ANU 3	28	Dr. R. Oram, Div. of Plant Industry, CSIRO Canberra, Australia.
H. vulgare L	Clipper	14	„
H. vulgare L	York	28	Dr. K.J. Kasha Crop Science Dept., Univ. of Guelph, Ontario, Canada.
H. brachyantherum Nevski	ANU 36	28	Dr. D.R. Dewey, Utah State Univ. Logan, USA.
H. depressum Scribn, & Smith	ANU 161	28	Dr. R.A. Finch Plant Breeding Institute, Cambridge, England.

nation, detached tiller feeding, gibberellic acid treatment, embryoculture and chromosome number determinations were done according to Subrahmanyam (1977). Twelve-day-old embryos from different crosses were cytologically examined following an earlier technique (Subrahmanyam and Kasha 1973a). The progeny from each cross were classified as either haploids or hybrids, depending on their morphology and chromosome number as the chromosomes of different species of *Hordeum* are indistinguishable.

Results

The seed set, embryos cultured and progeny obtained from various crosses are presented in Table 2. The percentage of seed set in each cross was quite variable depending on the vigour of the parents and the environmental conditions at the time of pollination. Nonetheless, there were differences in seed settings between different crosses. For instance, crosses of both *brachyantherum* and *depressum* with *bulbosum* resulted in higher seed sets than with *vulgare*. The proportion of embryos giving rise to progeny plants were also different for different crosses varying from zero in *brachyantherum* by tetraploid *vulgare* cross to 75% in *depressum* by diploid *vulgare* cross.

Morphological features and chromosome numbers of different surviving progenies from various crosses are summarised in Table 3 and are dealt with separately.

Hordeum brachyantherum x *H. bulbosum Cross*

The crosses with diploid *bulbosum* resulted in a total of 21 mature plants of which nineteen had a chromosome number of 2n = 14 (Fig. 1b) instead of a hybrid number (2n = 3x = 21) expected between a tetraploid *brachyantherum* (2n = 4x = 28, Fig. 1a) and a diploid *bulbosum* (2n = 2x = 14, Fig. 1d). The remaining two plants showed 2n = 21 (Fig. 1c). The spikes (Fig. 2b) and morphology of the 14 chromosome progeny resembled *brachyantherum* (Fig. 2a), whereas the 21 chromosome plants were intermediate (Fig. 2c) between *brachyantherum* (Fig. 2a) and *bulbosum* (Fig. 2e).

All the plants obtained from the cross involving tetraploid *bulbosum* were hybrids with the expected chromo-

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

Cross combination and ploidy levels (x = 7)	Number of florets pollinated	Percentage of seed set		Number of embryos cultured	Percentage of embryos giving plants
		Average	Range		
H. brachyantherum 4x by					
(i) *H. bulbosum* 2x	558	18.1	0-73.3	73	28.8
(ii) *H. bulbosum* 4x	214	25.7	0-88.2	33	9.1
(iii) *H. vulgare* 2x	213	18.3	6.5-68.0	15	13.3
(iv) *H. vulgare* 4x	128	3.9	0-10.3	4	
H. depressum 4x by					
(v) *H. bulbosum* 2x	285	40.4	3.6-81.8	114	50.9
(vi) *H. bulbosum* 4x	429	27.0	0-67.9	105	9.5
(vii) *H. vulgare* 2x	77	20.8	4.6-41.4	16	75.0
(viii) *H. vulgare* 4x	357	12.6	0-18.5	39	12.8

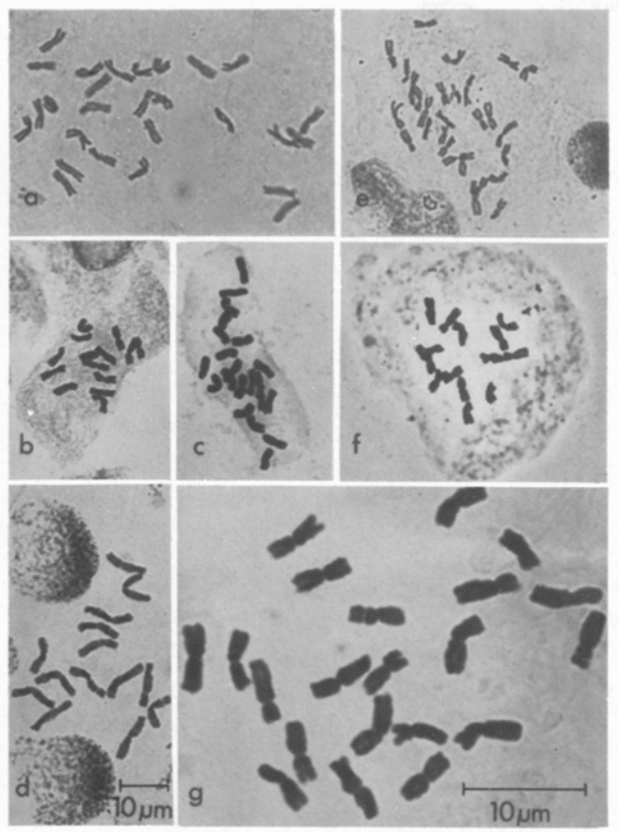

Fig. 1a-g. Somatic chromosomes of parents and progeny from various crosses. **a** *H. brachyantherum* (28), **b** Haploid *brachyantherum* (14) from *brachyantherum* by diploid *bulbosum* cross, **c** Hybrid (21) between *brachyantherum* and diploid *bulbosum*, **d** *H. bulbosum* (14), **e** *H. depressum* (28), **f** Haploid *depressum* (14) from *depressum* by diploid *bulbosum*, **g** Hybrid (21) between *H. depressum* and diploid *bulbosum*

some complement (2n = 4x = 28). The spike (Fig. 2d) and vegetative characters were *bulbosum*-like.

All the progeny between these species required vernalisation to flower.

Hordeum brachyantherum x *H. vulgare Cross*

All the progeny from crosses with diploid *vulgare* were intermediate between the two parents and had 21 chromosomes, but died before reaching maturity. No progeny was obtained from crosses involving tetraploid *vulgare.*

Hordeum depressum x *H. bulbosum Cross*

Eight out of the 41 surviving progeny from the crosses with diploid *bulbosum* had a chromosome number of 2n = 14 (Fig. 1f) with a morphology (Fig. 3a) similar to *depressum* (Fig. 3e). The other 33 plants had a chromosome complement of 2n = 3x = 21 (Fig. 1g) with an intermediate morphology (Fig. 3b) expected of a hybrid between a tetraploid *depressum* (Fig. 1e) and a diploid *bulbosum* (Fig. 1d). The haploids reached flowering in 110 days while the hybrids flowered by this stage.

All the progeny from crosses with tetraploid *bulbosum* were mostly *bulbosum*-like with intermediate spikes (Fig. 3c) and had an expected chromosome number of 2n = 4x = 28. These tetraploid hybrids required over 6 months to reach flowering.

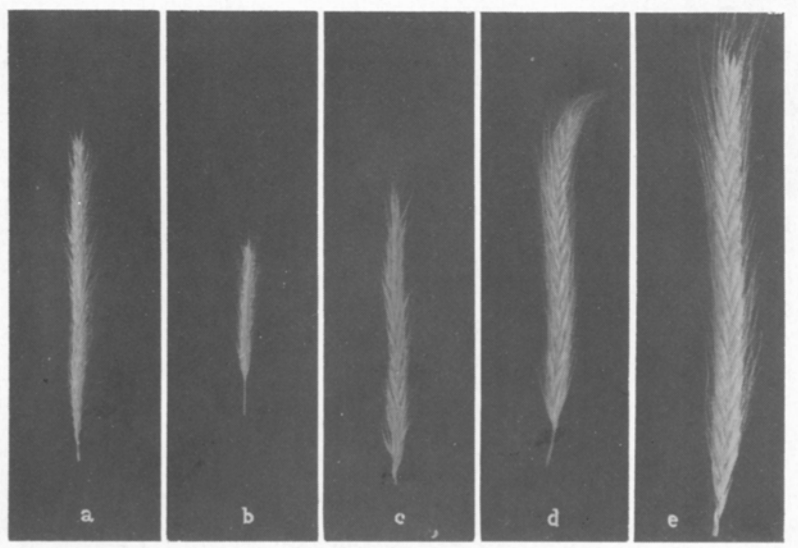

Fig. 2a-e. Spikes of parents and progeny from crosses involving *H. brachyantherum*. **a** *H. brachyantherum*, **b** Haploid *brachyantherum*, **c** Hybrid between *H. brachyantherum* and diploid *bulbosum*, **d** Hybrid between *H. brachyantherum* and tetraploid *bulbosum*, **e** *H. bulbosum* (embedded in plastic resin prior to photographing)

Fig. 3a-g. Spikes of parents and progeny from crosses involving *H. depressum*. **a** Haploid *H. depressum*, **b** Hybrid between *H. depressum* and diploid *bulbosum*, **c** Hybrid between *H. depressum* and tetraploid *bulbosum*, **d** *H. bulbosum*, **e** *H. depressum*, **f** *H. depressum – vulgare* hybrid, **g** *H. vulgare* (embedded in plastic resin prior to photographing)

Hordeum depressum × *H. vulgare Cross*

The cross involving diploid *vulgare* resulted in plants with hybrid chromosome number and of intermediate morphology but with mostly *vulgare*-like spikes (Fig. 3f). These hybrids flowered in 80 days. The progeny from the cross with tetraploid *vulgare* died before reaching maturity or before chromosome number could be determined.

Chromosome Variation in Hybrid Embryos

Chromosome instability was evident in embryos from the crosses *brachyantherum* by diploid *bulbosum* and *depressum* by diploid *bulbosum*. The minimum number of chromosomes in each embryo examined was 14, which is the gametic number for both *brachyantherum* and *depressum*. Furthermore, no single embryo showed cells with a uniform chromosome number of 14. Micronuclei in interphase cells and occasional chromosome fragments in dividing cells of embryos from crosses of *brachyantherum* or *depressum* with diploid *bulbosum* were also evident. The embryonic cells from *brachyantherum* by diploid *vulgare* cross showed 21 chromosomes in the few countable cells. Nevertheless, up to 10 per cent of the interphase cells exhibited micronuclei.

Discussion

The results reveal that two out of the eight crosses produced haploids of one of the two parents. Chromosomal variation in the embryonic cells for the corresponding crosses are consistent with the results (Table 3). The presence of chromosome numbers varying down to 14 in cells from hybrid embryos expected to have 21 chromosomes and the morphological resemblance of the haploid progeny to one of the parents (*brachyantherum* or *depressum*) in the crosses of *brachyantherum* (4x) by *bulbosum* (2x) and *depressum* (4x) by *bulbosum* (2x) strongly suggest that selective elimination of *bulbosum* chromosomes leads to haploid formation. The phenomenon of chromosome elimination is known in seventeen other *Hordeum* interspecific hybrids (Subrahmanyam 1977 and unpublished). Thus, a total of nineteen *Hordeum* interspecific cross combinations exhibit chromosome elimination.

Formation of mostly haploids from *brachyantherum* by diploid *bulbosum*, only hybrids from *brachyantherum* by tetraploid *bulbosum*, and haploids of *depressum* and hybrids from *depressum* by diploid *bulbosum*, and only hybrids from *depressum* by tetraploid *bulbosum* substantiates earlier proposals (Kasha and Kao 1970; Subrahmanyam and Kasha 1973; Subrahmanyam 1977) that a balance or ratio of the parental genomes in each hybrid determines whether the resulting progeny are haploids or hybrids. For instance, a ratio of 2 *brachyantherum* (bra) genomes to 1 *bulbosum* (blb) genome results in mostly haploids of *brachyantherum*, whilst the 2 bra:2 blb genomic ratio results in hybrids. Similarly, a ratio of 2

Table 3. Morphological features, chromosome numbers and types of progency from the interspecific crosses

Cross combination (ploidy levels x = 7)	No. of plants	Morphology	Chromosome number	Type
H. brachyantherum 4x by				
(i) *H. bulbosum* 2x	19	*brachyantherum*-like	14	haploids
	2	intermediate	21	hybrids
(ii) *H. bulbosum* 4x	3[a]	*bulbosum*-like	28	hybrids
(iii) *H. vulgare* 2x	2[a]	intermediate	21	hybrids
H. depressum 4x by				
(i) *H. bulbosum* 2x	4	*depressum*-like	14	haploids
	4[b]	*depressum*-like	14	haploids
	33	intermediate	21	hybrids
(ii) *H. bulbosum* 4x	10	mostly *bulbosum*-like with intermediate spikes	28	hybrids
(iii) *H. vulgare* 2x	12	intermediate mostly *vulgare*-like spikes	21	hybrids

[a] Died before reaching maturity

[b] Initially looked like hybrids and later became *depressum*-like in over 8 months time

depressum (dep):1 blb genomes gives *depressum* haploids and hybrids, while the 2 dep:2 blb ratio produces exclusively hybrids.

Production of haploids of *jubatum* following hybridizations with diploid *bulbosum* (Rajhathy and Symko 1974) and of hybrids in crosses of *jubatum* with tetraploid *bulbosum* (Wagenaar 1960) is similar to the results from *brachyantherum* by *bulbosum* crosses. This similarity of *jubatum* and *brachyantherum* in eliminating *bulbosum* chromosomes is not surprising considering the conspecific nature of *brachyantherum* and *jubatum* (Rajhathy et al. 1963; Starks and Tai 1974).

Although only hybrids were obtained from crosses between *brachyantherum* and *vulgare*, the presence of micronuclei in embryonic cells is an indication of a certain degree of chromosomal instability. *Hordeum compressum* (2x), one of the progenitors of *jubatum*, forms hybrids with *H. vulgare* (2x) (Rajhathy et al. 1963). Increasing the number of *compressum* genomes, as in the amphiploid *jubatum-compressum* (6x), and crossing with *vulgare*, resulted in elimination of *vulgare* chromosomes (Orton and Tai 1977). This is clearly a manipulation of genomic ratio to achieve new combinations leading to chromosome elimination. The successful production of *Hordeum* interspecific hybrids at specific ploidy levels (Raghathy et al. 1963) is indicative of the importance of genome balance to obtain stable hybrids. Thus, one could predict that crosses between octaploids of *brachyantherum* or *jubatum* and *vulgare* may exhibit elimination of *vulgare* chromosomes.

Over 90% of the progeny (Table 3) obtained from the crosses between *brachyantherum* and diploid *bulbosum* were haploids of *brachyantherum*, whereas only 20% of the progeny were *depressum* haploids from *depressum* by diploid *bulbosum* cross. These differences in the frequencies of haploids can be attributed to the differences in their 'genetic strength' in eliminating *bulbosum* chromosomes. In the haploid producing crosses of *procerum* (6x) and *parodii* (6x) with *bulbosum, procerum* displayed a stronger genetic mechanism than *parodii*, since only *procerum* forms haploids when crossed with tetraploid *bulbosum* by eliminating the two *bulbosum* genomes (Subrahmanyam 1977). There are differences in the proportions of haploids (among the progenies obtained) from hybridizations of *procerum* or *arizonicum* (6x) with tetraploid *bulbosum* or diploid *vulgare* (Subrahmanyam 1977, 1978). For instance, the progenies from the crosses involving *procerum* with *bulbosum* (4x) or *vulgare* (2x) consisted of 22% or 42% *procerum* haploids, respectively (Subrahmanyam 1977), whereas *arizonicum* (6x), on crossing with the same genotypes of *bulbosum* (4x) or *vulgare* (2x), gave progenies with 85% or 76% *arizonicum* haploids, respectively (Subrahmanyam 1978). Thus, *arizonicum* has a greater 'potential' than *procerum* in eliminating *bulbosum* or *vulgare* chromosomes and *procerum*, in turn, is stronger than *brachyantherum* or *de-*

pressum in eliminating *bulbosum* chromosomes. Further experimentation is needed to determine whether these differences are manifested through the rate and/or magnitude of chromosome elimination.

The distribution of selective chromosome elimination and the similarities in the genetic basis (genome balance) for this phenomenon in a wide range of *Hordeum* interspecific hybrids led me to suggest that the genetic control and the mechanism of elimination is similar to that for *vulgare-bulbosum* hybrids, as discussed earlier (Subrahmanyam 1977).

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