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Studies on chromosome elimination in the hybrids between *Hordeum bulbosum and H. vulgare**

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INTRODUCTION

The wild bulbous barley, *Hordeum bulbosum* L., is mainly distributed in the Mediterranean region and South West Asia. This species has two cytotypes, autotetraploid and diploid types (Berg 1936, Morrison 1959) . The most interesting characteristics of *H. bulbosum* are its perennial habit; sprouting from the swellings formed at the basal internode (Leshem 1971, Leshem and Nir 1972, Ofir 1975, Ofir *et al.* 1967) , and its highly outbreeding nature which is eagerly desired for the practical use of heterosis in cultivated barley, *H. vulgare* L. (Beddows 1931, Lundqvist 1962, Smith 1944) . *H. bulbosum* also has such useful traits as resistance to powdery mildew and spot blotch (Hardison 1944, Jones and Pickering 1978, Konzak *et al.* 1951, Liu and Schooler 1963, Prasad *et al.* 1983) . For the introduction of these desirable traits into cultivated barley, a number of interspecific crosses have often been made between them.

The first trial was carried out by Tschermak (1914) using the cross between *H. vulgare* 2X and *H. bulbosum* 4X, but no hybrid plants could be obtained. Konzak *et al.* (1951) first applied the embryo culture technique in the cross between *H. vulgare* 2X and *H. bulbosum* 4X, and obtained a number of hybrid plants. By the aid of embryo culture, various interspecific cross combinations have now become feasible among the diploid and tetraploid strains of the two species.

Davies (1958, 1960) happened to find that the F_1 hybrid between *H. bulbosum* 2X and *H. vulgare* 2X gave rise to a haploid plant ($2n=7$) resembling a cultivar, and supposed that the haploid plant was attributed to the male parthenogenesis (androgenesis) of *H. vulgare* 2X. However, the many interspecific crosses made thereafter have revealed that the haploidization of the hybrid was due to the selective elimination of the *H. bulbosum* chromosomes, and not to the parthenogenesis (Kasha and Kao 1970, Lange 1971a, b, Symko 1969) . This chromosome elimination occurred in almost all of the hybrids between *H. vulgare* 2X and *H. bulbosum* 2X, *H. vulgare* 4X and *H. bulbosum* 4X, and *H. vulgare* 4X and

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H. bulbosum 2X, resulting in haploid or dihaploid of vulgare-like plants regardless of the cross direction. On the other hand, the cross between *H. vulgare* 2X and *H. bulbosum* 4X gave rise to triploid hybrids ($2n=3X=21$) showing no chromosome elimination (Kao and Kasha 1971, Subrahmanyam and Kasha 1973) . The *H. bulbosum* chromosomes have been found to be gradually eliminated from the hybrid embryo in its early developmental stage (Bennett *et al.* 1976, Subrahmanyam and Kasha 1973) .

The genetic control system of the chromosome elimination has been extensively studied. Kao and Kasha (1971) and Subrahmanyam and Kasha (1973) maintained that the chromosome stability in the hybrid depended upon the genomic balance of the parental species, i.e., when the genomic ratio was 2 *bulbosum*: 1 *vulgare*, the chromosomes of the hybrid were stable, but the ratio of 1 *bulbosum*: 1 *vulgare* resulted in the chromosome elimination in the hybrid.

Jensen (1976, 1981) and Pickering and Morgan (1979) found that the rate of chromosome elimination in the hybrid between *H. bulbosum* 2X and *H. vulgare* 2X differed with the genotype of the parental strains of *H. vulgare* 2X. Moreover, Barclay *et al.* (1972) and Ho and Kasha (1975) indicated that the genes located on chromosomes 2 and 3 of *H. vulgare* controlled the chromosome elimination in the hybrid.

The influence of the genotypes of *H. bulbosum* on chromosome elimination was first reported by Fukuyama and Takahashi (1976) using tetraploid strains of both species. A similar influence of *H. bulbosum* genotypes was ascertained in the diploid hybrids (Jensen 1981, Pickering and Morgan 1979, Simpson *et al.* 1980) .

The elimination of the *H. bulbosum* chromosomes was also recognized in the intergeneric hybrids with *Triticum aestivum*, *T. ventricosa*, *Aegilops crassa* and *Ae. triunciaris* (Barclay 1975, Chapman and Miller 1977, Fedak 1983, Miller and Chapman 1976, Shigenobu and Sakamoto 1977, Snape *et al.* 1980) . This kind of cross has been used for haploid breeding in barley and wheat cultivars, and is called the "Bulbosum method" (Ho and Jones 1980, Inagaki 1985a, b, Kasha and Reinbergs 1976, 1979, 1981, Pickering 1980, Reinbergs *et al.* 1976, Snape *et al.* 1980) .

The complete elimination of the *H. bulbosum* chromosomes from the interspecific hybrid with cultivated barley, however, makes it impossible to introduce the desirable characters of *H. bulbosum* into cultivars through the recombination of their homoeologous chromosomes at meiosis. Therefore, the present investigation was done to obtain further insight into the actual situation of chromosome elimination in various crosses between various strains of *H. bulbosum* 4X and *H. vulgare* 4X and 2X.

The tetraploid forms of both species were mainly used in the present study for the following reasons: When *H. bulbosum* 4X was crossed with the pollen of *H. vulgare* 4X, the vigorous F_1 plants could easily be obtained without the need for embryo culture. Second, the F_1 plants are expected to give rise to a highly fertile dihaploid of *H. vulgare* or partially fertile amphidiploid when *H. bulbosum* chromosomes are completely eliminated or retained, respectively. Thus the progenies of those F_1 plants are valuable for genetic investigation.

The present investigation revealed the following: The rate of chromosome elimination markedly differed among the crosses resulting in various F_1 plants, e.g., vulgare-like plants lost all chromosomes of *H. bulbosum* or those retained the chromosomes of both species. The difference in the rate of chromosome elimination was mainly affected by the genotype of the parental strain of *H. bulbosum* 4X. Chromosome elimination occurred in the early developmental stage of the hybrid embryo. In addition, results suggesting the elimination at gametogenesis were obtained. The mechanism of this phenomenon and the possibility of the transfer of the genes of *H. bulbosum* into cultivated barley are discussed.

MATERIALS AND METHODS

Table 1 shows the materials used in the present study. The tetraploid strains of *H. vulgare* and its related species, *H. spontaneum*, had been artificially induced by colchicine treatment from the original diploid strains.

TABLE 1 Materials for the experiments

Species	Strain name	Origin or source
H. bulbosum (4X)	#487	Afghanistan
	S−23	Iran
	S−1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19	Iraq
	Turkey, S−21, 22	Turkey
	1W, #191, S−24, 25, 26, 27, 28, 29	U. S. S. R.
	B. 53	(Unknown)
H. vulgare	H. E. S. Type 15(4X), Hosogara 2(4X), Hosomugi (4X), Shin Ebisu 16 (4X), Wase Gold (4X)	T. Ono (Japan)
	Amsel (2X, 4X), B. 1173/472311 (2X, 4X), Brio (2X, 4X), D 8/55(2X, 4X), Edda(2X, 4X), Firlbeck(2X, 4X), Hatif Boute(4X), Hoffmann 3522(4X), K 1749/13681(2X, 4X), Ky(4X), Missouri(2X, 4X), N. S. Morgenrot(2X, 4X), Stankas früh(2X, 4X), Strengs Franken Ⅲ(2X, 4X), Volla (2X, 4X), Walter(4X), Weihen stephaner M. R. (2X, 4X)	H. Gaul (F. R. Germany)
H. spontaneum	*nigrum* (4X)	T. Ono (Japan)

For intercrossing between these two species, the plants were emasculated 2 to 3 days before anthesis and the spike was immediately covered by a parchment bag for preventing pollen contamination. Two to three days after emasculation, the florets were dusted with the fresh pollen of the male parent. When the strains of *H. bulbosum* 4X were crossed with the pollen of *H. vulgare* 4X, the crossed seeds developed well on the mother plant. While, in the reciprocal cross between *H. vulgare* 4X(♀) × *H. bulbosum* 4X(♂) , and also in the cross between *H. vulgare* 2X(♀) × *H. bulbosum* 4X(♂) , the development of the crossed seeds ceased 10 to 15 days after pollination which was mainly due to endosperm abortion. Then, the hybrid embryo in these cross combinations was picked out from the floret 15 to 20 days after pollination and cultured on the medium as shown in Table 2. Embryos bedded on the medium were incubated in the dark at 20℃. After germination, they were maintained under 14-h light and 10-h dark cycles in the growth chamber kept at 22℃ (light) /17℃ (dark) . At the 2- to 3-leaf stage, the cultured plants were transplanted into the soil.

TABLE 2 Composition of the medium used for embryo culture

Macro nutrients	KNO_3	150mg/ 1
	$Ca(NO_3)_2$	350
	$NaH_2PO_4H_2O$	15
	$MgSO_4 . 7H_2O$	15
Micro nutrients	H_3BO_3	0.5
	$MnSO_4 . 4H_2O$	3
	$ZnSO_4 . 7H_2O$	0.5
	$Na_2MoO_4 . 2H_2O$	0.025
	$CuSO_4 . 5H_2O$	0.025
	$CoCl_2 . 6H_2O$	0.025
	Ferric citrate	10
Vitamins	Thiamine HCl	0.25
	Pyridoxine HCl	0.25
	Inositol	50
	Ca-Pantothenate	0.25
Amino acids	L-Glutamic acid	200
Sucrose		20000
Agar		7000
pH		5.0

The morphological and agronomic characters of the hybrid plants were closely investigated. For the cytological observation, the seminal root-tips were taken from the F_1 plants, pretreated by cold water for 24 hours, and then fixed in ethanol: acetic acid (3 : 1) solution (v/v) . After staining in 2% aceto-carmine solution, the root-tips were squashed in a drop of 45% acetic acid to count the chromosome number. At least 5 cells of a single root-tip were observed.

Time and rate of chromosome elimination in the hybrids were studied in the following way : A strain of *H. vulgare* 4X, D8/55, and two strains of *H. bulbosum* 4X, ♯191 and ♯487, were reciprocally crossed and 40 caryopses from each cross were taken 3, 5, 7, 9, 11 and 13 days after pollination. These caryopses were pretreated by cold water, fixed and stained as was made for the root-tips mentioned above. Then, the embryo was carefully taken out from each caryopsis with a sharp-pointed needle under a dissecting microscope and its chromosome number was counted.

RESULTS

1. *Interspecific Cross between* H. bulbosum *and* H. vulgare

(1) *Crosses between* H. bulbosum 4X *and* H. vulgare 4X

(a) *Seed Setting in the Interspecific Crosses*

H. bulbosum 4X(♀)× *H. vulgare* 4X (♂)

A total of 102 crosses were made between 31 strains of *H. bulbosum* 4X and 15 strains of *H. vulgare* 4X. As shown in Fig.1, seed setting percentages varied from 1% to 71% with the average of 22.7%. Such a wide variation in seed setting seems to be affected by the parental genotypes of both *H. bulbosum* 4X and *H. vulgare* 4X. Crossed seeds developed well on the mother plant, *H. bulbosum* 4X, and were germinated at the rate of 78% on average.

H. vulgare 4X (♀) × *H. bulbosum* 4X (♂)

The crosses were made using a strain of *H. vulgare* 4X, D8/55 as the female parent, and two strains of *H. bulbosum* 4X, ♯191 and ♯487 as the male parents. As shown in Table 3, average seed setting in the two cross combinations was 45.2%, which was 15.6% higher than those of the reciprocal ones of the same cross combination.

TABLE 3 Seed sets in reciprocal crosses between *H. vulgere* 4 X(D8/55) and *H. bulbosum* 4X(♯191 and ♯487)

Cross (♀) × (♂)	Number of florets pollinated	Number of seeds obtained	Seed set (%)
D8/55 × ♯191	2629	1095	41.7
D8/55 × ♯487	3291	1582	48.1
Total	5920	2677	45.2
♯191 × D8/55	1746	450	25.8
♯487 × D8/55	2671	857	32.1
Total	4417	1307	29.6

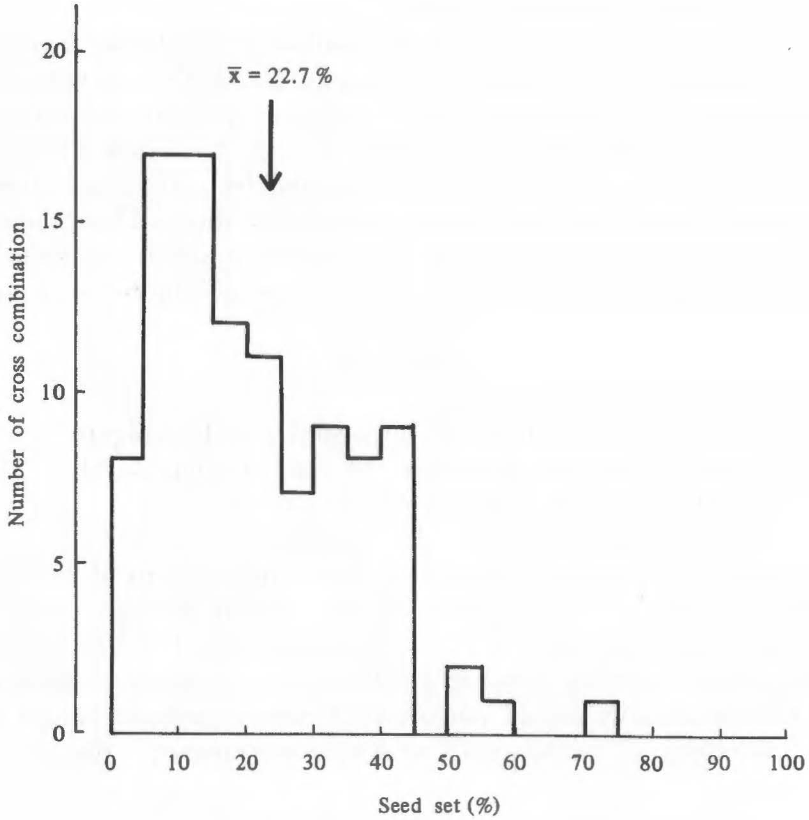

FIG. 1. Various histogram of seed set in a total of 102 cross combinations between *H. bulbosum* 4X (female) and *H. vulgare* 4X (male).

Since the crossed seeds ceased to develop 10 to 15 days after pollination, the embryos were cultured on the medium. According to Table 4, out of 317 embryos cultured, 228 (71.9%) normally germinated both

TABLE 4 Embryo culture of hybrids between *H. vulgare* 4X(D8/55) and *H. bulbosun* 4X(#191 and #487)

Cross (♀) × (♂)	Number of embryos cultured	Number of germinated embryos		
		Shoot + Root	Shoot	Root
D8/55 × #191	83	61 (73.5%)	1 (1.2%)	3 (3.6%)
D8/55 × #487	234	167 (71.4)	12 (5.1)	14 (6.0)
Total	317	228 (71.9)	13 (4.1)	17 (5.4)

shoots and roots, and most of them grew vigorously after transplanting into the soil. On the other hand, 13 (4.1%) and 17 (5.4%) embryos showed abnormalities such as shoot- or root-less germination, respectively, and all of them died before transplanting. The remaining (18.6%) did not germinate at all on the medium.

(b) *Morphological Traits of the F_1 Hybrid*

Since there was no difference in gross morphology of the reciprocal F_1 hybrids between *H. bulbosum* 4X and *H. vulgare* 4X, their description will be given below together.

Though various phenotypes were found among the F_1 hybrids, they were roughly classified into the vulgare-like, intermediate and deformed types.

Vulgare-like type : Plants of this type closely resembled *H. vulgare* 2X or the original diploid cultivar in morphology (Fig. 2). They had no

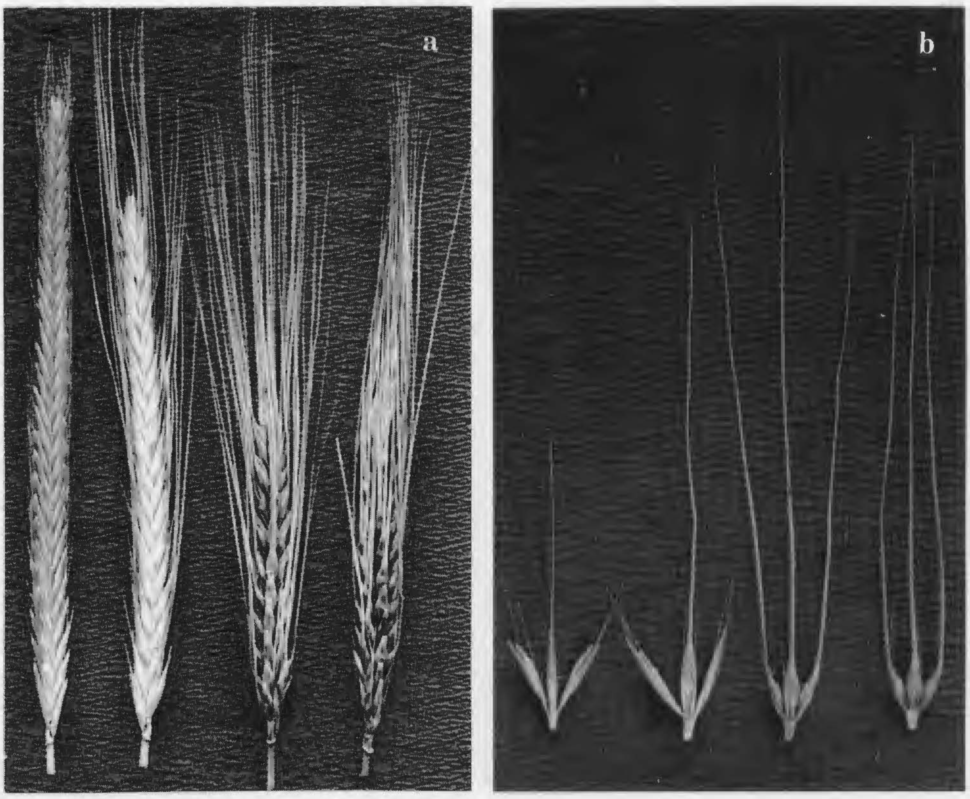

FIG. 2. Spikes(a) and spikelets(b) of *H. vulgare* 4X, *H. bulbosum* 4X and their F_1 hybrids. From left to right, *H. bulbosum* 4X, intermediate F_1, vulgare-like F_1 and *H. vulgare* 4X.

basal internode swelling which was characteristic to *H. bulbosum*. Seed fertility was as high as in the original diploid cultivar. It should be noted here that the vulgare-like plant derived from the cross with *H. bulbosum* 4X as the female parent was accompanied by growth retardation such as later heading, shorter culm and smaller number of tillers as compared with those of the original diploid cultivar having the cytoplasm of *H. vulgare*. This growth retardation was completely recovered when the vulgare-like plant was crossed with *H. vulgare* 2X as the female parent and its cytoplasm had substituted with that of *H. vulgare*. Therefore, such growth retardation is attributed to the cytoplasm of *H. bulbosum*.

Intermediate type : Plants of this type showed morphological features intermediate between the parental species (Fig. 2) . All the plants had the basal internode swelling which was smaller than that of *H. bulbosum* 4X, but was capable of vegetative propagation. Seed fertility varied from 0% to 40%. Among the intermediate type plants, few showed the chimeric condition which bore the tillers with different phenotypes such as intermediate and vulgare-like ones within the same plant.

Deformed type : These plants showed various types of abnormal growth such as extremely repressed growth or chlorophyll abnormalities. All of them died at a seedling stage or before heading.

(c) *Chromosome Number in Root-tips of the* F_1 *Hybrid*

The hybrid plant between tetraploid forms of *H. bulbosum* and *H. vulgare* was expected to have 28 chromosomes in the root-tip, but the F_1 plants had various chromosome numbers ranging from 14 to 28 (Fig. 3) . Table 5 shows the variation in chromosome number and its relationship to the three kinds of phenotypes examined in a total of 768 F_1 plants derived from a number of crosses between *H. bulbosum* 4X (female) and *H. vulgare* 4X (male) . Out of 768 F_1 plants, only 41 (5.3%) had 28 chromosomes as was expected, and 299 F_1 plants (38.9%) had a chromosome number reduced to one half, $2n = 14$. The remaining F_1 plants were aneuploid with various chromosome numbers from 15 to 27, and most of them were found to show mixoploid conditions, e.g., component cells had different chromosome numbers within the same root-tip. Then, for these mixoploids, chromosome numbers from different cells were averaged, and regarded as the average chromosome number. In Table 5, the average chromosome number of the aneuploids was tentatively divided into three groups, $2n = 14$ to 18, $2n = 19$ to 23 and $2n = 24$ to 28. Among the aneuploid F_1 plants, those with an average chromosome number of from 24 to 28 were the most frequent (27.9%) followed by those with that of 19 to 23 (14.2%) , and 14 to 18 (13.7%) .

Next, the interrelationship between chromosome number and the phenotype of the F_1 plants was investigated using the same table.

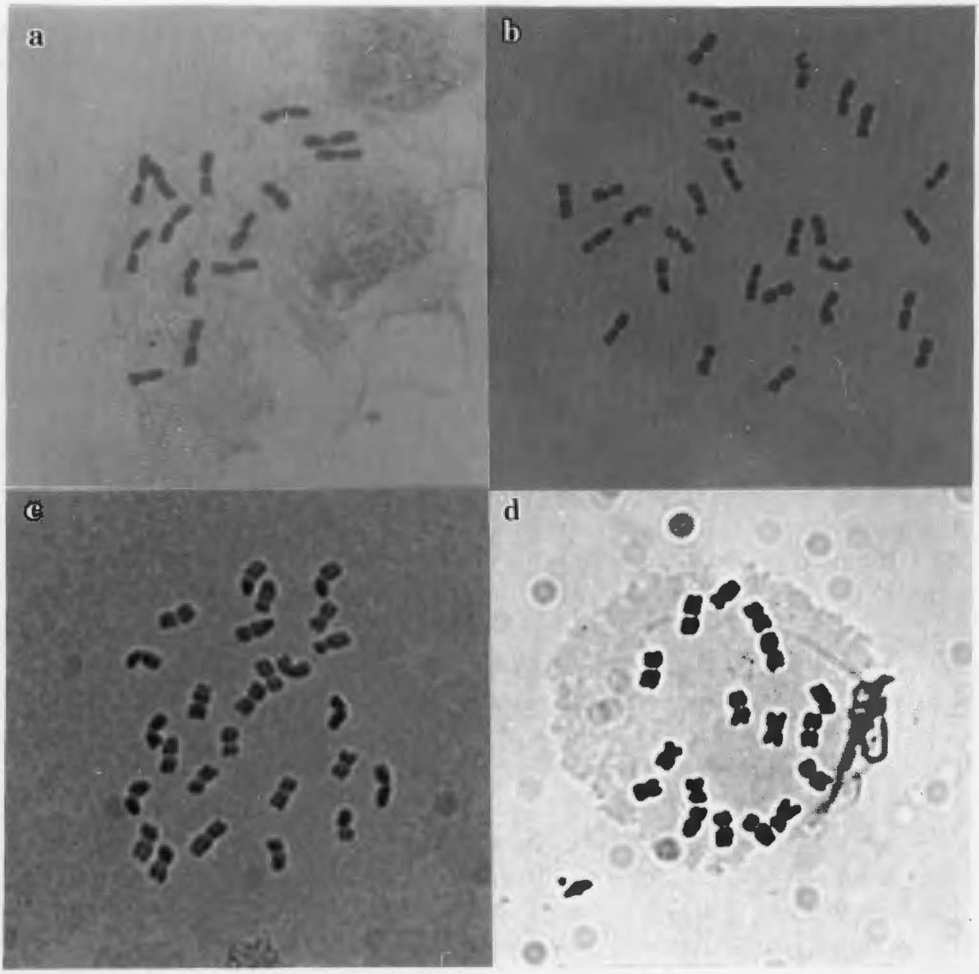

FIG. 3. Various chromosome numbers in root-tip of the hybrid between *H. bulbosum* 4X and *H. vulgare* 4X. a) Dihaploid (2n=14), b) tetraploid (2n=28), c and d) aneuploids (2n=26 and 2n=17).

TABLE 5 Chromosome numbers in root-tip of three different F_1 types between *H. bulbosum* 4X and *H. vulgare* 4X

F_1 type	Eudiploid	Aneuploid			Eutetraploid	Total
	(2n=14)	14~18	19~23	24~28	(2n=28)	
Vulgare-like	291(80.8%)*	50(13.9%)	11(3.1%)	6(1.7%)	2(0.6%)	360
Intermediate						
Non-chimeric	0 (0.0)	19(6.8)	62(22.1)	164(58.6)	35(12.5)	280
Chimeric	1(6.7)	3(20.0)	8(53.3)	1(6.7)	2(13.3)	15
Deformed	7(6.2)	33(29.2)	28(24.8)	43(38.1)	2(1.8)	113
Total	299(38.9)	105(13.7)	109(14.2)	214(27.9)	41(5.3)	768

* : Percentage in parentheses is based on the total number of plants in each F_1 type.

Among 360 vulgare-like F_1 plants, 291 or 80.8 % were found to be 2n = 14, and the remaining showed an aneuploidy condition with an average chromosome number ranging from 14 to 28. Eutetraploids (2n=28) showing a vulgare-like feature were very rare (0.6%) .

Among the F_1 plants classified as the intermediate type, the aneuploids with 24 to 28 average chromosome numbers were most frequent (58.6%) followed by those with 19 to 23 chromosomes (22.1%) . The frequency of eutetraploids was only 12.5%. No plant having 14 chromosomes was found among the intermediate type plants. Only one exception was a chimeric plant with the tillers of intermediate and vulgare-like types. The roots showing 2n = 14 seemed to have been taken from the part of vulgare-like tillers of the chimeric plant.

Most of the deformed F_1 plants had a chromosome number of 2n=24 to 28. Eudiploids and eutetraploids were very rare (6.2% and 1.8%, respectively) .

Judging from the phenotype and chromosome number of the F_1 plants, the vulgare-like F_1 plants were considered to have lost all their chromosomes of *H. bulbosum* and had only the *H. vulgare* genomes, while most of the intermediate and deformed F_1 plants had retained all or a part of the chromosomes from both parental species, though the origin of some lost chromosomes was not clear.

(2) *Crosses between* H. vulgare 2X *and* H. bulbosum 4X

(a) *Seed Setting*

A strain of *H. vulgare* 2X, D8/55, was pollinated with two strains of *H. bulbosum* 4X, #191 and #487. As shown in Table 6, seed setting percentages in the two crosses were 68.2% and 80.2%, respectively. Crossablity in the triploid cross was apparently higher than that of the tetraploid cross stated before.

TABLE 6 Seed sets in the crosses between *H. vulgare* 2X(D8/55) and *H. bulbosum* 4X(#191 and #487)

Cross (♀) × (♂)	Number of florets pollinated	Number of seeds obtained	Seed set (%)
D8/55 × #191 (2X) (4X)	1280	873	68.2
D8/55 × #487 (2X) (4X)	748	600	80.2

Hybrid embryos of the triploid cross were cultured on the medium 10 to 14 days after pollination. Table 7 shows the results of embryo culture. In the cross of D8/55(2X) ×#191(4X) , 416 (63.3%) of 657 embryos

TABLE 7 Embryo culture of F_1 hybrids between *H. vulgare* 2 X(D8/55) and *H. bulbosun* 4 X(#191 and #487)

Cross	Number of embryos	Number of embryos germinated		
(♀) × (♂)	cultured	Shoot + Root	Shoot	Root
D 8 /55 × #191	657	416	83	19
(2 X) (4 X)		(63.3%)*	(12.6%)	(2.9%)
D 8 /55 × #487	437	164	90	10
(2 X) (4 X)		(37.5)	(20.6)	(2.3)

* : Percentage in parentheses is based on the number of embryos cultured.

normally germinated on the medium, and among the remaining embryos, 83 (12.6%) and 19 (2.9%) developed only shoots and roots, respectively. In another cross, D8/55(2X) × #487(4X), the rate of germination was 37.5% which was much lower than that obtained for D8/55(2X) × #191 (4X), and the frequency of the embryos that developed only shoots was relatively high (20.6%).

(b) *Morphology of the Hybrid*

The F_1 plants closely resembled *H. bulbosum* 4X in plant growth type, head type and the basal internode swelling, although the size of swelling was much smaller than that of *H. bulbosum* 4X. Seeds of these F_1 plants were completely sterile. The remaining F_1 plants died at their seedling stage, or failed to reach heading even if they survived this stage.

(c) *Chromosome Number of the Hybrid*

The chromosome numbers of a total of 124 F_1 plants derived from two cross combinations were examined (Table 8). In both crosses, the triploid chromosome number, 2n = 21, was most frequent (60% or more). In

TABLE 8 Chromosome numbers of F_1 hybrids between *H. vulgare* 2X(D8/55) and *H. bulbosum* 4X(#191 and #487)

Cross	No. of plants with chromosome numbers indicated						Total
(♀) × (♂)	14	19	20	21*	21**	22	
D 8 /55 × #191	1	3	7	48	18	2	79
(2 X) (4 X)	(1.3%)	(3.8%)	(8.9%)	(60.8%)	(22.8%)	(2.5%)	
D 8 /55 × #487	0	1	2	30	12	0	45
(2 X) (4 X)	(0.0)	(2.2)	(4.4)	(66.7)	(26.7)	(0.0)	

* : Eutriploid.

** : Plants with 21 chromosomes on average, but showing mixoploidy.

addition, as was already seen in the tetraploid hybrids, aneuploids with various chromosome numbers from 14 to 22 were observed. Among them, those with an average chromosome number of $2n=21$ were the most frequent(22.8% and 26.7%, respectively). Only one F_1 plant of $2n = 14$ was found in the cross of D8/55(2X) × #191(4X) , but it died at the seedling stage.

These results indicate that the F_1 hybrid between *H. vulgare* 2X and *H. bulbosum* 4X was highly stable in chromosome number as compared with the tetraploid hybrid between *H. bulbosum* 4X and *H. vulgare* 4X.

2. *Genetic Control of Chromosome Elimination in the Hybrid*

(1) *Variation in Frequency of vulgare-like Dihaploid among Cross Combinations*

As stated before, various phenotypes appeared in different frequencies in the F_1 generation of the cross between *H. vulgare* 4X and *H. bulbosum* 4X. Among them, the vulgare-like dihaploid ($2n = 14$) was regarded to have resulted from complete elimination of the whole set of *H. bulbosum*

TABLE 9 Frequencies of the three different F_1 plant types produced from the crosses of various 4X strains of *H. bulbosum* with a 4X strain Shin Ebisu 16 of *H. vulgare*

H. bulbosum 4 X strain (♀)	Frequencies of 3 types indicated				Number of plants
	Vulgare-like	Intermediate		Deformed	
		Non-chimeric	Chimeric		
S- 5	67 (87.0%)	0 (0.0%)	2 (2.6%)	8 (10.4%)	77
S- 4	24 (75.0)	2 (6.3)	0 (0.0)	6 (18.8)	32
S-17	16 (72.7)	0 (0.0)	0 (0.0)	6 (27.3)	22
S-29	8 (72.7)	1 (9.1)	0 (0.0)	2 (18.2)	11
S-27	50 (70.4)	0 (0.0)	2 (2.8)	19 (26.8)	71
S-19	25 (67.6)	4 (10.8)	0 (0.0)	8 (21.6)	37
S-26	11 (64.7)	1 (5.9)	1 (5.9)	4 (23.5)	17
S-21	16 (64.0)	3 (12.0)	0 (0.0)	6 (24.0)	25
#191	79 (57.7)	15 (10.9)	5 (3.6)	38 (27.7)	137
S-14	6 (54.5)	3 (27.3)	0 (0.0)	2 (18.2)	11
S- 7	7 (53.8)	3 (23.1)	2 (15.4)	1 (7.7)	13
S-23	31 (47.7)	19 (29.2)	2 (3.1)	13 (20.0)	65
S-18	25 (46.3)	4 (7.4)	1 (1.9)	24 (44.4)	54
S- 3	29 (44.6)	20 (30.8)	6 (9.2)	10 (15.4)	65
#487	36 (13.7)	136 (51.7)	6 (2.3)	85 (32.3)	263
S-22	4 (12.1)	10 (30.3)	1 (3.0)	18 (54.5)	33
S-11	1 (10.0)	7 (70.0)	0 (0.0)	2 (20.0)	10
S-15	2 (5.1)	28 (71.8)	0 (0.0)	9 (23.1)	39

chromosomes, while the other two types had retained at least some or all of the chromosomes of *H. bulbosum*. Therefore, the rate of elimination of the whole chromosomes of *H. bulbosum* can be estimated by the frequency of vulgare-like dihaploids.

First, 18 different strains of *H. bulbosum* 4X were commonly crossed with a strain of *H. vulgare* 4X, Shin Ebisu 16, and the frequencies of the three types of plants appearing in the following generation were investigated.

As shown in Table 9, vulgare-like dihaploid frequencies markedly differed among these cross combinations ranging from 5.1% to 87.0%, and the variation of dihaploid frequencies was almost continuous. Similarly, a marked difference in dihaploid frequency was observed in another series of crosses of 4 strains of *H. bulbosum* 4X with a single strain of *H. vulgare* 4X, Hosomugi (Table 10.)

TABLE 10 Frequencies of three F_1 plant types in the crosses of four 4X strains of *H. bulbosum* with a 4X strain (Hosomugi) of *H. vulgare*

H. bulbosum	Frequencies of 3 types indicated				Number of
	Vulgare-like	Intermediate		Deformed	
4 X strain(♀)		Non-chimeric	Chimeric		plants
#191	74 (61.2%)	9 (7.4%)	8 (6.6%)	30 (24.8%)	121
S- 7	3 (25.0)	6 (50.0)	1 (8.3)	2 (16.7)	12
S- 6	4 (15.4)	9 (34.6)	0 (0.0)	13 (50.0)	26
#487	10 (12.7)	48 (60.8)	3 (3.8)	18 (22.8)	79

Next, the influence of the parental strains of *H. vulgare* 4X on the rate of chromosome elimination was tested using two sets of crosses. The first one consisted of the crosses of 18 different strains of *H. vulgare* 4X and *H. spontaneum* 4X with a strain of *H. bulbosum* 4X, #487 which was known to give as low a dihaploid frequency as 13.7% when crossed with Shin Ebisu 16 (c.f. Table 9) .The second set consisted of the crosses of 8 different strains of *H. vulgare* 4X and *H. spontaneum* 4X with a single strain of *H. bulbosum* 4X, #191 showing a high dihaploid frequency (57.7%) in the cross with Shin Ebisu 16. According to Tables 11 and 12, dihaploid frequencies varied from 0% to 30.8% and from 57.1% to 88.9%, respectively.

The range of variation in those two sets of crosses, 30.8% and 31.8%, was narrower than that among the parental strains of *H. bulbosum* 4X stated above.

It was concluded from these results that, although the rate of chromosome elimination was affected by the factor(s) involved in both

TABLE 11 Effects of different 4 X strains of *H. vulgare* on the frequency of the vulgare-like F$_1$ plants resulting from the crosses with a 4X strain (#487) of *H. bulbosum*

H. vulgare 4 X strain (♂)	Number of plants with phenotypes indicated		Total
	Vulgare - like	Others*	
H. spontaneum (4 X)	4 (30.8%)	9 (69.2%)	13
Hosogara 2 (4 X)	7 (25.0)	21 (75.0)	28
Wase Gold (4 X)	10 (22.2)	35 (77.8)	45
D 8/55 (4 X)	11 (17.7)	51 (82.3)	62
Edda (4 X)	9 (15.3)	50 (84.7)	59
H. E. S. (4 X)	11 (14.1)	67 (85.9)	78
B 1173 (4 X)	3 (13.6)	19 (86.4)	22
Hatif Boute (4 X)	2 (13.3)	13 (86.7)	15
Hosomugi (4 X)	10 (12.7)	69 (87.3)	79
Firlbeck (4 X)	3 (12.5)	21 (87.5)	24
Shin Ebisu 16 (4 X)	30 (12.2)	217 (87.9)	247
St. Franken (4 X)	2 (8.7)	21 (91.3)	23
Missouri (4 X)	2 (8.7)	21 (91.3)	23
K 1749 (4 X)	1 (6.3)	15 (93.7)	16
Volla (4 X)	1 (4.8)	20 (95.2)	21
Ky (4 X)	0 (0.0)	43 (100.0)	43
N. S. Morgenrot (4 X)	0 (0.0)	18 (100.0)	18
Brio (4 X)	0 (0.0)	15 (100.0)	15
Total	106 (12.8)	725 (87.2)	831

* : Others include intermediate and deformed F$_1$ plants.

TABLE 12 Effects of 9 different 4 X strains of *H. vulgare* on the frequency of the vulgare-like F$_1$ plants resulting from the crosses with a 4X strain, #191, of *H. bulbosum*

H. vulgare 4 X strain (♂)	Number of plants with types indicated		Total number of plants
	Vulgare - like	Others*	
H. E. S. (4 X)	16 (88.9%)	2 (11.1%)	18
H. spontaneum (4 X)	11 (84.6)	2 (15.4)	13
D 8/55 (4 X)	18 (78.3)	5 (21.7)	23
Firlbeck (4 X)	26 (70.3)	11 (29.7)	37
Volla (4 X)	7 (70.0)	3 (30.0)	10
Shin Ebisu 16 (4 X)	65 (67.0)	32 (33.0)	97
Wase Gold (4 X)	24 (66.7)	12 (33.3)	36
Hosomugi (4 X)	74 (61.2)	47 (38.8)	121
Hosogara 2 (4 X)	12 (57.1)	9 (42.9)	21
Total	253 (67.3)	123 (32.7)	376

* : Others include intermediate and deformed F$_1$ plants.

H. bulbosum and *H. vulgare*, the influence of *H. bulbosum* was much larger than that of *H. vulgare*.

For comparison, Table 13 shows the variation of dihaploid frequency among 31 strains of *H. bulbosum* 4X, including #191 and #487, when crossed with various strains of *H. vulgare* 4X. In this table, the data were combined in the crosses between each *H. bulbosum* 4X strain and some

TABLE 13 Variation of genetic effects of various 4X strains of *H. bulbosum* on segregating vulgare-like plants in their F_1 hybrids when crossed with 4X strains of *H. vulgare*

H. bulbosum 4X	Frequencies of 3 types indicated			Total number
strain(♀)	Vulgare-like	Intermediate	Deformed	of plants
S-28	10(90.9%)	0(0.0%)	1(9.1%)	11
S- 5	67(87.0)	2(2.6)	8(10.4)	77
S-24	6(85.7)	0(0.0)	1(14.3)	7
1 W	23(85.2)	2(7.4)	2(7.4)	27
B. 53	17(81.0)	0(0.0)	4(19.0)	21
S- 8	28(80.0)	0(0.0)	7(20.0)	35
S- 4	24(75.0)	2(6.3)	6(18.8)	32
S-16	11(73.3)	0(0.0)	4(26.7)	15
S-17	20(71.4)	1(3.6)	7(25.0)	28
S-26	15(71.4)	2(9.5)	4(19.0)	21
S-27	50(70.4)	2(2.8)	19(26.8)	71
S-19	25(67.6)	4(10.8)	8(21.6)	37
S-21	22(66.7)	4(12.1)	7(21.2)	33
#191	280(63.9)	45(10.3)	113(25.8)	438
S- 9	31(63.3)	6(12.2)	12(24.5)	49
S- 1	25(59.5)	7(16.7)	10(23.8)	42
S-29	19(59.4)	9(28.1)	4(12.5)	32
S-14	27(51.9)	13(25.0)	12(23.1)	52
S-12	9(50.0)	7(38.9)	2(11.1)	18
S-23	31(47.7)	21(32.3)	13(20.0)	65
Turkey	7(46.7)	4(26.7)	4(26.7)	15
S-18	25(46.3)	5(9.3)	24(44.4)	54
S- 7	10(40.0)	12(48.0)	3(12.0)	25
S-10	7(38.9)	7(38.9)	4(22.2)	18
S- 3	30(37.5)	35(43.8)	15(18.8)	80
S- 2	3(27.3)	0(0.0)	8(72.7)	11
S-11	8(19.5)	25(61.0)	8(19.5)	41
S- 6	5(13.2)	11(35.5)	15(48.4)	31
#487	112(13.2)	484(57.1)	251(29.6)	847
S-22	4(11.4)	11(31.4)	20(57.1)	35
S-15	2(3.8)	35(67.3)	15(28.8)	52
Total	953(41.1)	756(32.6)	611(26.3)	2320

different strains of *H. vulgare* 4X because of the minor effect on the rate of chromosome elimination among *H. vulgare* strains. Among these *H. bulbosum* 4X strains, S-28 showed the highest dihaploid frequency, 90.9%, and S-15 the lowest, 3.8%. It should be noted here that the variation among *H. bulbosum* strains was almost continuous.

(2) *Cytoplasmic Effect on Chromosome Elimination*

As stated before, the difference in the rate of chromosome elimination among the parental strains of *H. bulbosum* was quite clear. However, since all crosses were made using *H. bulbosum* as the female parent, it was necessary to determine whether the factor(s) affecting chromosome elimination was involved in the nucleus or in the cytoplasm of *H. bulbosum*. Two kinds of experiments were made for this purpose.

First, a strain of *H. vulgare* 4X, D8/55, was reciprocally crossed with two strains of *H. bulbosum* 4X, #191 and #487, and their hybrids were raised by the aid of embryo culture. As shown in Table 14, no appreciable differences in the frequency of vulgare-like dihaploids were found between reciprocal crosses. This indicates that the cytoplasms of both *H. bulbosum* and *H. vulgare* have little effect on the rate of chromosome elimination.

TABLE 14 Frequencies of three F_1 plant types in the reciprocal crosses between two 4X strains (#191 and #487) of *H. bulbosum* and a 4X strain (D8/55) of *H. vulgare*

Cross (♀)×(♂)	Frequencies of 3 types indicated Vulgare -like	 Intermediate	 Deformed	Total number of plants
#191×D8/55(4X)	42(63.6%)	13(19.7%)	11(16.7%)	66
D8/55(4X)×#191	38(63.3)	2(3.3)	20(33.3)	60
#487×D8/55(4X)	56(32.7)	37(21.6)	78(45.6)	171
D8/55(4X)×#487	30(19.9)	30(19.9)	91(60.3)	151

In the second experiment, two strains of *H. bulbosum* 4X, #191 and #487,were reciprocally crossed, and each of the hybrid plants was crossed with two strains of *H. vulgare* 4X, Shin Ebisu 16 and Missouri. Fig. 4 shows the variation and mean of dihaploid frequency in the crosses between #191, #487 or their hybrids and Shin Ebisu 16 or Missouri. A strain #191 showed a variation in dihaploid frequency of from 63.6% to 75.0% with an average of 67.0% in the crosses with *H. vulgare* 4X, whereas another strain, #487, that of from 6.7% to 20.8% with an average of 12.1%.

When 8 clones derived from the *H. bulbosum* hybrid, #191 (♀) × #487 (♂) ,were crossed with Shin Ebisu 16 (4X) and Missouri (4X) , a total of 207 F_1 hybrids were obtained, in which dihaploid frequencies continuously varied from 14.3% to 87.5% with a mean of 51.2%. Eleven clones of another reciprocal hybrids, #487 (♀)× #191 (♂) , gave a

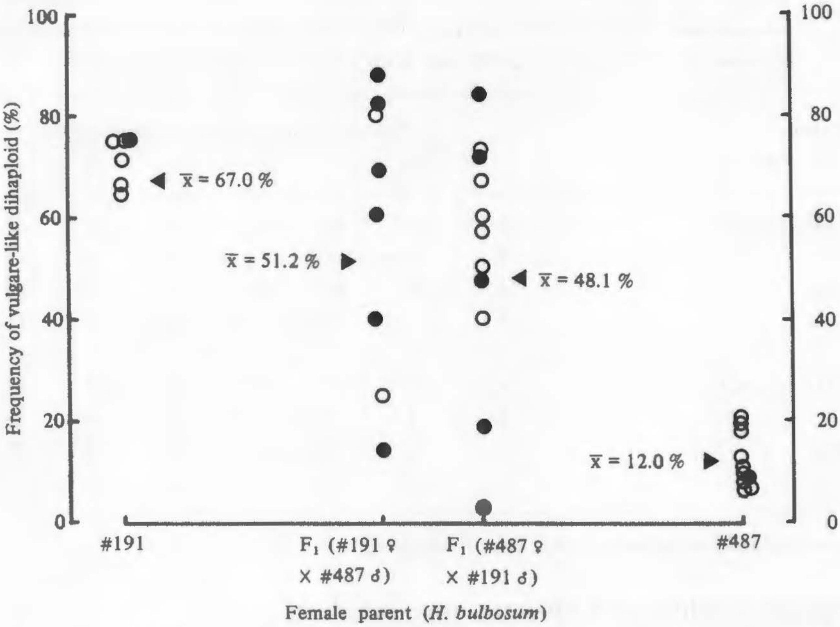

FIG. 4. Frequency distribution of vulgare-like dihaploid F$_1$ plants in the crosses between two 4X strains of *H. bulbosum* (♯191 and ♯487) or their reciprocal hybrids and two 4X strains of *H. vulgare* (Shin Ebisu 16, open circle and Missouri, closed circle).

total of 210 F$_1$ hybrids showing the dihaploid frequencies of from 3.3% to 82.8% with a mean of 48.1%, which was quite similar with the variation and mean obtained from the crosses mentioned above.

These results indicate that the difference in dihaploid frequency between *H. bulbosum* 4X strains, ♯191 and ♯487, is not due to their cytoplasm, but due to the nuclear gene(s), and the continuous variation of dihaploid frequency among the parental clones of the *H. bulbosum* hybrids suggests that the nuclear gene(s) is of a minor or polygenic nature, and not of a major genic nature.

3. *Time and Situation of Chromosome Elimination*

Two strains of *H. bulbosum* 4X, ♯191 and ♯487, were reciprocally crossed with a strain of *H. vulgare* 4X, D8/55. Although 40 caryopses of each cross were taken 3, 5, 7, 9, 11 and 13 days after pollination for cytological studies to know the time and situation of chromosome elimination, the chromosome number of only 23 to 37 embryos could be counted because the embryo could not be taken out from the caryopsis or because of unsuccessful fertilization (Table 15). The average number of countable cells varied from 4.1 to 21.9, being slightly higher at a later sampling date. Almost all of the embryos in these hybrids showed a

TABLE 15 Numbers of embryos (a) and cells per embryo (b) used for the examination of chromosome numbers at six different times after pollination in the reciprocal hybrids between *H. bulbosum* 4X (#191 and #487) and *H. vulgare* 4X (D8/55)

Cross		Sampling time (days after pollination)					
(♀) × (♂)		3	5	7	9	11	13
#191×D8/55(4X)	a*	26	33	35	37	31	32
	b	5.4	7.3	8.2	21.9	9.0	6.2
Reciprocal	a	25	35	31	31	32	36
	b	4.3	9.1	9.2	14.4	11.2	11.0
#487×D8/55(4X)	a	29	25	29	30	33	23
	b	4.9	6.8	5.3	16.9	10.4	18.3
Reciprocal	a	23	28	29	35	32	33
	b	4.1	9.3	5.0	8.8	11.6	14.3

* : 40 caryposes were taken on each day of sampling.

mixoploid condition in chromosome number.

Fig. 5 shows the change in average chromosome number of the hybrid embryos in the four crosses for a period of 13 days after pollination. Generally speaking, four kinds of hybrids showed a more or less sigmoidal change in chromosome number with the advance in sampling time. In the hybrid between #191 and D8/55, the average chromosome number was 24-25 on the third day, but during the following two days (3-5 days) the chromosome number was abruptly reduced, the rate of chromosome elimination per day being as large as 4.5. From 5 to 9 days after pollination, chromosome elimination became slow, and no appreciable change in chromosome number was recognized thereafter.

A similar trend of chromosome elimination was observed in the hybrids between #487 and D8/55 although the rate of elimination was much lower than that observed for the hybrid between #191 and D8/55. The fluctuation in chromosome number after 9 days may be due to a sampling error. These results indicate that the chromosome elimination gradually occurred in the early developmental stage of the hybrid embryo and the maximum rate of elimination was seen 3 to 5 days after pollination. The lack of any notable difference in the rate and situation of chromosome elimination between reciprocal hybrids means that the cytoplasm of both *H. bulbosum* and *H. vulgare* have little influence on this phenomenon.

Next, the vulgare-like dihaploid frequency was studied in the F_2 progenies of the intermediate type F_1 plants which was partially fertile as stated before. As shown in Table 16, 6 F_2 populations derived from the crosses between *H. bulbosum* 4X strain #191 and *H. vulgare* 4X showed the dihaploid frequency of 91.8% on average, which was 25% higher than the value found in the F_2 generation. Also, 12 F_2 populations from the crosses with another strain of *H. bulbosum* 4X, ♯487, gave a much higher dihaploid frequency than in the F_1 generation. Thus, a marked increase in dihaploid frequency was noticed in the F_2 generation.

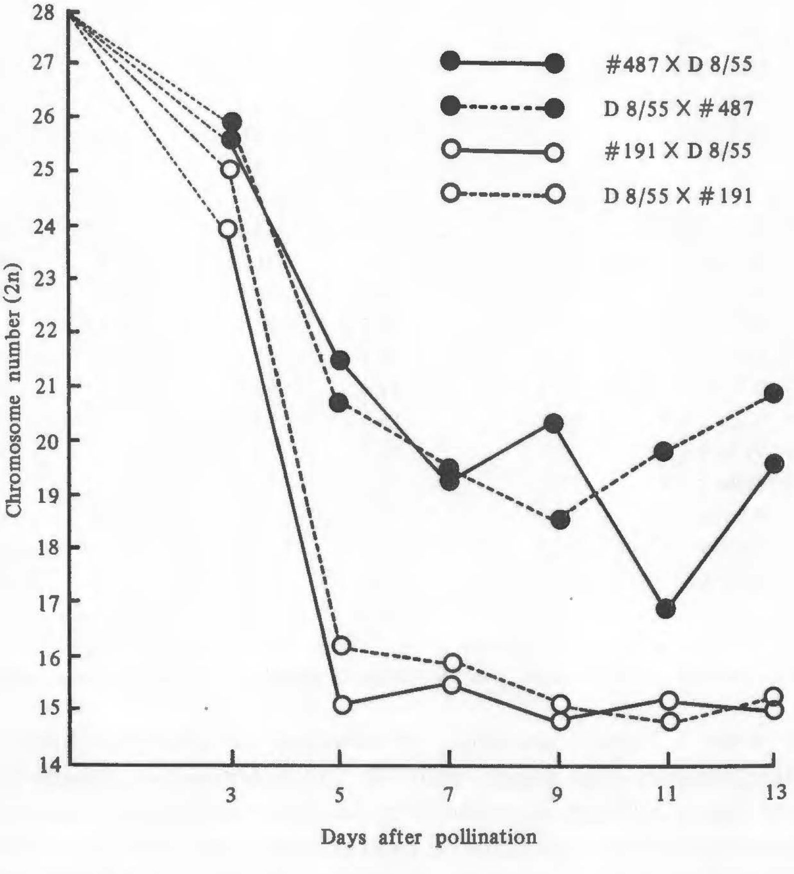

FIG. 5. Average chromosome numbers in the reciprocal hybrids between *H. bulbosum* 4X (♯191 or ♯487) and *H. vulgare* 4X (D8/55) at six different dates after pollination.

DISCUSSION

First, the chromosome elimination in the hybrids between *H. bulbosum* and *H. vulgare* is discussed.

Judging from the present investigation, the crosses between *H. vulgare* 2X and *H. bulbosum* 4X give rise to a stable triploid hybrid (2n= 21) , while those between *H. vulgare* 4X and *H. bulbosum* 4X produce

TABLE 16 Segregation of three plant types in the progenies of the intermediate F_1 plants obtained from the crosses between *H. bulbosum* 4X and *H. vulgare* 4X

H. bulbosum 4X *H. vulgare* * 4X	Chromosome No. in F_1 (2n)	Frequencies of 3 types indicated			No. of plants
		Vulgare-like	Intermediate	Deformed	
#191 × SE (4X)	-	100.0%	0.0%	0.0%	9
〃 × HG (4X)	25.5	100.0	0.0	0.0	8
〃 × HG (4X)	-	100.0	0.0	0.0	8
〃 × HM (4X)	-	100.0	0.0	0.0	21
〃 × HM (4X)	-	90.5	7.1	2.4	42
〃 × HM (4X)	-	60.0	10.0	30.0	10
Mean		91.8	4.1	4.1	
#487 × SE (4X)	26.0	70.0	10.0	20.0	10
〃 × SE (4X)	28.0	25.0	41.7	33.3	12
〃 × SE (4X)	25.2	52.4	33.3	14.3	21
〃 × SE (4X)	26.8	66.7	22.2	11.1	9
〃 × SE (4X)	26.6	40.0	40.0	20.0	5
〃 × B 1173 (4X)	22.9	61.9	23.8	14.3	21
〃 × Volla (4X)	24.7	20.0	53.3	26.7	30
〃 × Volla (4X)	-	83.3	8.3	8.3	12
〃 × Volla (4X)	19.0	43.8	37.5	18.8	16
〃 × D8/55 (4X)	23.0	75.0	0.0	25.0	12
〃 × D8/55 (4X)	23.0	84.6	7.7	7.7	13
〃 × Ky (4X)	25.7	9.1	72.7	18.2	11
Mean		50.0	31.4	18.6	

* : SE, HG and HM are abbreviated from Shin Ebisu 16, Hosogara 2 and Hosomugi, respectively.

hybrids with a highly unstable chromosome number resulting in the production vulgare-like plants with 14 chromosomes, or intermediate or deformed plants with an aneuploid chromosome number of from 15 to 27. The appearance of a true tetraploid hybrid (2n = 28) was very rare. The chromosomal stability of these hybrids differed markedly with the genotype of the parental strains of *H. bulbosum* 4X, but only slightly with that of the *H. vulgare* 4X strains.

A close investigation of the morphology and chromosome number in somatic cells of the hybrids between *H. vulgare* 4X and *H. bulbosum* 4X revealed that the eliminated chromosomes were those of *H. bulbosum*. Namely, the F_1 plants classified into the vulgare-like type had no morphological characteristics of *H. bulbosum* and were highly fertile, most of them (80% or more) showing a chromosome number reduced by half, 2n=14. A few of the vulgare-like F_1 plants had 15 or more chromosomes in their seminal root-tips, but their similarity in morphology and seed fertility with dihaploid vulgare-like F_1 plants suggests that one or more chromosomes of *H. bulbosum* retained in the seminal root-tip of these hybrids were eliminated at a later developmental stage, or, if remaining, the cells with these chromosomes had a disadvantage in cell division and/or development as compared with the cells having 14 chromosomes of *H. vulgare* existing together in a chimeric condition, and consequently they disappered from the developing tissues of the hybrid.

All the F_1 plants classified into the intermediate type were similar in gross morphology, but most of them were aneuploids with 15 to 27 chromosomes, and very few were a true tetraploid hybrid ($2n = 28$) . Decrease in chromosome number from the tetraploid level seems to be due to the elimination of *H. bulbosum* chromosomes, because these intermediate F_1 plants frequently segregated vulgare-like dihaploids in their F_2 progenies.

Since the karyotypes of *H. bulbosum* and *H. vulgare* were quite similar, the karyological discrimination between them is very hard (Kasha and Sadasivaiah 1971) . However, Finch(1983) used a specific tester line of translocation of *H. vulgare* 2X, Tuleen 346, which made distinguishable each chromosome of *H. vulgare* from that of *H. bulbosum* (Finch and Bennett 1982) , and he produced cytological evidence showing that the eliminated chromosomes in the diploid hybrids were derived from *H. bulbosum.*

Also, it has been well recognized that the chromosomes of *H. bulbosum* are preferentially lost in the hybrids between *H. bulbosum* and several species of the genus *Hordeum,* i.e., *H. arizonicum, H. brachyantherum, H. depressum, H. jubatum, H. lechleri, H. parodii* and *H. procerum* (Isram and Sparrow 1974, Rajhathy and Symko 1974, Subrahmanyam 1977, 1979, 1980) . Moreover, the elimination of *H. bulbosum* chromosomes has also been reported in the intergeneric hybrids with *Triticum aestivum, T. ventricosa, Aegilops crassa* and *Ae. triunciaris* (Barclay 1975, Chapman and Miller 1977, Fedak 1983, Miller and Chapman 1976, Shigenobu and Sakamoto 1977, Snape *et al.* 1980) .

Such preferential elimination of the chromosomes is well known in the somatic hybrid cells of two kinds of mammals (Handmaker 1973, O'Brien and Nash 1982) .

Based on these facts, I propose the following hypothesis; within the F_1 hybrid cells involving *H. vulgare* and *H. bulbosum* genomes altogether, the chromosomes of *H. vulgare* are always stronger in ability to survive in the cells with only a little difference between genotypes, while the chromosomes of *H. bulbosum* are relatively weak. Consequently, all or a part of the *H. bulbosum* chromosomes are preferentially lost from the F_1 cells. It seems probable that the above stated situation occurs when the ratio of genomes of two parental species is 1:1, but two doses of the *H. bulbosum* genomes exert stronger surviving ability in the cell coexisting with a single dose of *H. vulgare* genome, resulting in a highly stable triploid hybrid. It must be mentioned here that there are considerable differences among the genotypes of *H. bulbosum* strains in ability of chromosome survival compared to *H. vulgare*, and these differences are attributed to the polygenes but not the small number of major genes.

Subrahmanyam (1977, 1979, 1980) made a number of crosses between *Hordeum* species including *H. bulbosum*, and established a hierarchy in the chromosome elimination among these species according to the production of either (poly)-haploid or hybrids in the F_1 generation. This conception agrees with the above stated proposal except that there is wide genotypic variation in the rate of chromosome elimination within the parental species.

Most interesting is the nature or function of the difference in surviving ability or hierarchy at the time of chromosome elimination. This problem may directly relate to the mechanism of chromosome elimination. Unfortunately, however, the mechanism of this phenomenon could not be clarified here. Hypotheses for the mechanism proposed so far are asynchrony of mitotic cell cycle time (Subrahmanyam and Kasha 1973) , or of mitotic rhythms (Lange 1971b) , cleavage of *H. bulbosum* chromosomes by endonuclease (Davies 1974) , and spindle or centriole abnormalities caused by the disturbance of protein synthesis (Bennett *et al.* 1976, Noda 1984) . Among them, the first hypothesis seems to be unlikely because the two strains of *H. bulbosum* 4X, ♯191 and ♯487, formed a stable tetraploid hybrid although they markedly differed in the rate of chromosome elimination when crossed with *H. vulgare* 4X. If the difference in chromosome elimination between ♯191 and ♯487 is caused by the difference in cell cycle time with *H. vulgare* 4X, the cross between ♯191 and ♯487 will also produce a hypotetraploid F_1.

Next, the time and situation of chromosome elimination in the hybrid will be considered. The present investigation revealed that the chromosome elimination in the hybrids between *H. bulbosum* 4X and *H. vulgare* 4X occurred at an early stage of the embryogenesis of from 3 to 9 days after pollination. The maximum rate was observed on the 3rd to 5th day. These results were quite similar with those obtained from the diploid hybrids between *H. bulbosum* 2X and *H. vulgare* 2X (Bennett *et al.* 1976, Subrahmanyam and Kasha 1973) .

Regarding the time of chromosome elimination, another interesting results were obtained from the progeny test of the intermediate F_1 plants. The dihaploid frequency in the F_2 progenies was much higher than in the F_1 generation. This increase in the F_2 progenies may be explained by the following: Since the intermediate F_1 plants showed hypotetraploid chromosome number in their somatic cells, their gametes would be consisted of hypodiploid chromosome number and they would produce hypotetraploid F_2 zygotes. It seems that the chromosomes of these hypotetraploid F_2 progenies would be much highly unstable at their embryogenesis as compared with true-tetraploid condition in the F_1 generation, and lead to much higher occurrence of dihaploid plants. However, in this case remains the problem whether such hypodiploid gametes are able to accomplish fertilization or not. The average seed set of the intermediate F_1 plants was as high as 12.2%.

Another possibility of higher production of dihaploids in the F_2 generation is that the gametes with diploid chromosome number derived from the intermediate F_1 plants in mixoploid condition would be selectively fertilized and produced true-tetraploid embryos, where the chromosome elimination would occur in the same rate in the F_1 generation. In addition, the intermediate F_1 plants would produce haploid gamete with only *H. vulgare* chromosomes through the *H. bulbosum* chromosome elimination in the gametogenesis. As a matter of fact, the meiosis of the diploid and tetraploid hybrids between *H. bulbosum* and *H. vulgare* have frequently brought about abnormalities such as laggards or bridge formation, or multi-polar cell division (Lange 1971b, Subrahmanyam and Kasha 1973, Thomas and Pickering 1983a, b). It is very likely that these abnormalities result in chromosome elimination in gametogenesis and the formation of gametes with no *H. bulbosum* chromosomes.

Moreover, Noda and Kasha (1981 a, b) reported the instability of the chromosomes in the spike primordium (3 mm in length) of the diploid and triploid hybrids between *H. bulbosum* and *H. vulgare.*

Judging from these facts, at least three different growing stages can be enumerated as the time of chromosome elimination; embryogenesis, spike primordium formation and gametogenesis. Why chromosome elimination frequently occurs at these stages is uncertain, but the solution of this problem will give a new clue to this phenomenon. Noda and Takayama (1986) discussed the relationship between the physiological activity of the cells and the rate of chromosome elimination and supposed that a higher physiological activity results in a higher rate of chromosome elimination.

It should be noted here that the difference in the rate of chromosome elimination among the genotypes of the parental *H. bulbosum* strains was similarly exhibited in both embryogenesis and gametogenesis, which suggests that the elimination in both stages is affected by common genetic factors.

Finally, the possibility of the transfer of the desirable traits involved in *H. bulbosum* into cultivated barley will be considered. Crosses between *H.*

bulbosum and *H. vulgare* were originally made to introduce the traits of *H. bulbosum* into cultivated barley. However, the finding of the chromosome elimination in their hybrids diverted the attention of the barley workers to the production of haploid barley.

A few attempts to transfer the *H. bulbosum* characters have been made using a diploid hybrid (Jones and Pickering 1978, Lange and Jochemsen 1976a, b), but most of them were not successful, probably because the materials used gave too low a frequency of diploid hybrids to allow the exchange of the genes through crossing-over between homoeologous chromosomes of the parental species.

I used the tetraploid cytotypes of both species, and could easily obtain a number of vigorous F_1 hybrids. Among them, the intermediate plants with both chromosomes of *H. bulbosum* and *H. vulgare* were partially fertile, because the genomic constitution of these hybrids was partly amphidiploid of the parental species. Since the genome relationship between *H. bulbosum* and *H. vulgare* is considerably close (Kasha and Sadasivaiah 1971), the crossing-over and recombination between homoeologous chromosomes of both species can be expected in meiosis of the intermediate F_1 plants or their progenies. And also, since the intermediate hybrids bore the basal internode swelling capable of vegetative propagation, the seeds of the F_1 plants or clones will be available for the transfer of the *H. bulbosum* traits as long as they survive.

SUMMARY

The present investigation was made to elucidate the situation and genetic control of chromosome elimination occurring in the interspecific hybrid between *H. bulbosum* and *H. vulgare*. The results are summarized as follows:

1. Seed setting for a total of 102 cross combinations between *H. bulbosum* 4X (♀) × *H. vulgare* 4X (♂) was 22.7% on average. The reciprocal cross showed somewhat higher seed setting, 45.2%, on average. The seed settings of the two crosses between *H. vulgare* 2X (♀) × *H. bulbosum* 4X (♂) were 68.2% and 80.2%, which were considerably higher than that of the crosses between tetraploid forms.
2. The hybrid embryos of the crosses between *H. vulgare* 4X (♀) × *H. bulbosum* 4X (♂) and between *H. vulgare* 2X (♀) × *H. bulbosum* 4X (♂) should be cultured on media because of endosperm abortion, while those of the crosses between *H. bulbosum* 4X (♀) × *H. vulgare* 4X (♂) developed satisfactorily on the mother plant without embryo culture.
3. The F_1 hybrids between *H. bulbosum* 4X and *H. vulgare* 4X could

be classified into three types based on morphology and chromosome number in root-tips. The first was a vulgare-like type which was highly fertile and closely resembled the *H. vulgare* 2X cultivar in morphology. The chromosome number was reduced to 2n=14, which was caused by the elimination of *H. bulbosum* chromosomes. The second type had intermediate morphology between the parents and partially fertile. Chromosome numbers varied from 15 to 28, most of which showed a mixoploid condition. The third type was a deformed type with various abnormalities in growth and the plants died at seedling stage. Their chromosome numbers were similar to those of the intermediate type.

4. The hybrids between *H. vulgare* 2X and *H. bulbosum* 4X gave rise to stable triploid F_1 plants (2n=21). They resembled *H. bulbosum* 4X but were completely sterile.

5. The rate of chromosome elimination was compared among various crosses between *H. bulbosum* 4X and *H. vulgare* 4X using a frequency of vulgare-like dihaploid in F_1 generation. Dihaploid frequencies differed markedly among the genotypes of the parental *H. bulbosum* 4X strains. On the other hand, the genotypes of *H. vulgare* 4X had only a slight influence. Cytoplasmic factors involved in both species hardly affected chromosome elimination.

6. The nuclear genes for chromosome elimination involved in *H. bulbosum* seemed to be of minor or polygenic nature but not of a major genic nature.

7. The preferential elimination of *H. bulbosum* chromosomes was explained by the following hypothesis:

The surviving ability of chromosomes in the hybrid cell differs in the parental species, *H. vulgare* chromosomes being always stronger than the *H. bulbosum* chromosomes. Consequently, *H. vulgare* chromosomes are retained, but all or some chromosomes of *H. bulbosum* are preferentially lost from the hybrid cell. Also, there are considerable variations in surviving ability among the genotypes of *H. bulbosum*. Such a difference in surviving ability seems to be influential when the parental genome ratio is 1:1 in the hybrid, but two doses of *H. bulbosum* genome exert equivalent surviving ability against a dose of *H. vulgare* genome and the cross between *H. bulbosum* 4X and *H. vulgare* 2X produced a stable triploid hybrid.

8. Elimination of *H. bulbosum* chromosomes was recognized in an early developmental stage of the hybrid embryo and also elimination in gametogenesis was suggested from the progeny test of the intermediate F_1 hybrid.

9. The intermediate type F_1 between *H. bulbosum* 4X and *H. vulgare* 4X was suggested to be valuable for the transfer of *H. bulbosum* genes into

cultivated barley because they were partially fertile amphidiploids involving chromosomes of both species with a considerably close relationship.

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