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CROSSES BETWEEN *HORDEUM VULGARE* L. AND *H. BULBOSUM* L. I. PRODUCTION, MORPHOLOGY AND MEIOSIS OF HYBRIDS, HAPLOIDS AND DIHAPLOIDS

W. LANGE

Foundation for Agricultural Plant Breeding Wageningen, the Netherlands

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SUMMARY

From crosses between diploid and autotetraploid cytotypes of *Hordeum vulgare* L. (cultivated barley) and *H. bulbosum* L. (bulbous barley grass) diploid, triploid and tetraploid interspecific hybrids were produced. Both directly and after vegetative segregation crosses in either direction also gave rise to haploids and dihaploids resembling *H. vulgare*. The use of embryo culture was necessary. Plant morphology of the hybrids was much like that of *H. bulbosum*, although the hybrid plants were less vigorous. Meiosis in the hybrids was more or less disturbed, and this seemed to be the main cause of the high level of sterility.

INTRODUCTION

For a long time breeders have shown interest in crosses between *Hordeum vulgare* L. and *H. bulbosum* L. Their aim has been to transfer desirable characters from *H. bulbosum* into cultivated barley. Such characters are resistance to diseases, especially powdery mildew, winter hardiness, and more recently features related to the cross-pollinating habit of *H. bulbosum* like large anthers, which are exserted from the flowers at anthesis.

In 1914 VON TSCHERMAK reported unsuccessful crosses between the two species (probably diploid barley and tetraploid *H. bulbosum*). The first hybrid was obtained by KUCKUCK (1934). It was a triploid hybrid originating from the cross *H. vulgare* (2x) × *H. bulbosum* (4x). Several others tried this combination (MENABDE, 1938; RESNIČUK, 1939; SMITH, 1942; VINOGRADOVA, 1946; FREISLEBEN, 1940–1944, reported by LEIN, 1948) but all without success, except two plants which died before flowering.

The first real success was reported by KONZAK et al. (1951) who used embryo culture for raising the young embryos. These investigators obtained 11 viable triploid hybrids from crosses between diploid barley and tetraploid *H. bulbosum*.

DAVIES (1956) tried all eight possible interspecific combinations between diploid and tetraploid cytotypes of both species. (Diploid *H. bulbosum* was discovered and described by LEIN in 1948). Using embryo culture DAVIES produced three diploid hybrids which died before flowering, five triploids hybrid which resembled those of KUCKUCK (1934) and KONZAK et al. (1951), one tetraploid hybrid that died before

flowering and seven plants of the cross *H. bulbosum* (4x) × *H. vulgare* (4x) of which four died before flowering and the remaining three rather closely resembled *H. vulgare*. DAVIES supposed these three to have originated by 'male parthenogenesis' (a term which *in sensu stricto* is difficult to understand). MORRISON and coworkers (1959, 1959) obtained triploid hybrids of the same type as reported by other investigators, the reciprocal cross being unsuccessful. SCHOOLER (1964) crossed the tetraploid cytotypes of the species and obtained diploid and tetraploid progeny, but details of this study have never been published. RAJHATHY (1967) also obtained hybrids from crosses between the tetraploid cytotypes, these hybrids being unstable in chromosome number.

LANGE (1968, 1969) presented the results of extensive studies concerning interspecific crosses between diploid and tetraploid cytotypes of *H. vulgare* and *H. bulbosum*. Because the main part of those papers are in Dutch, this paper will be a summary of part of the results of those studies.

Recently three papers have been published regarding the production of dihaploids and haploids from crosses between the tetraploid cytotypes and between the diploid cytotypes, respectively (KAO and KASHA, 1970; SYMKO, 1969; KASHA and KAO, 1970). These authors presented a hypothesis about elimination of chromosomes which had already been put forward in my papers (LANGE, 1968, 1969) and will be treated in more detail in the next in this series (LANGE, 1971).

MATERIAL, METHODS AND SYMBOLS

The plant material used in the crosses was listed in detail in my previous paper (LANGE, 1969). It consisted of 19 diploid varieties of cultivated barley, 32 specimens of diploid *H. vulgare* of wild or unknown provenance (mainly ssp. *spontaneum*), 16 tetraploid cytotypes of varieties of cultivated barley, 5 specimens of diploid *H. bulbosum* and 37 specimens of tetraploid *H. bulbosum*.

The plants were grown and crossed in the field and bad weather may have influenced the results of crossing. About two weeks after pollination the seedset was checked and the shrivelled and mostly already yellowing hybrid seeds were collected to be prepared for embryo culture. The seeds were surface-sterilized in a solution of calcium hypochlorite and rinsed in sterile water. Then the embryos were dissected out of the caryopses and placed on a nutrient agar in tubes. The nutrient medium consisted of a mixture of inorganic salts (TOMASZEWSKI, 1958 or Difco orchid agar) enriched with casein hydrolysate (0.1%), yeast extract (50 ppm), tryptophane (40 ppm), glutamic acid (500 ppm) and boric acid (0.25 ppm). In the first experiments the sugar concentration was 2%; in later experiments it was raised to 7%. The higher osmotic pressure of the medium was found to be beneficial to the young embryos.

The embryos were cultured in the dark at 25°C. As soon as the plantlets had reached a length of 1–2 cm the tubes were transfered to a glasshouse. One or two weeks later the young seedlings were planted in soil and kept in the glasshouse until the plants were vigorous enough to be planted out in the field.

Chromosome numbers were checked in root-tip squashes using the Feulgen staining technique, after pretreatment with 8-hydroxy-quinoline (TJIO and LEVAN, 1950) and fixation in acetic-alcohol (1:3). Meiotic preparations were made by the haematoxylin squash technique of WITTMANN (1965). The quality of the pollen was studied by

counting the number of normal looking grains that stained dark red in a mixture of 100 ml lactophenol and 8 ml acid fuchsine (1%) in water, (SASS, 1964). About 500 pollen grains per plant were scored.

In this paper the parental species and the hybrids will be denoted by genome symbols: VV = *H. vulgare* (2x), BBBB = *H. bulbosum* (4x), VBB = triploid hybrid between these two species using VV as female parent, BBV = same triploid hybrid from the reciprocal cross, etc.

RESULTS OF THE CROSSES

In Table 1 a summary of the crosses is presented. The number of florets pollinated was not counted, but this figure can be roughly estimated by multiplying the number of spikes crossed by 20, when the female parent was *H. vulgare* and 25 when it was *H. bulbosum*. The number of embryos cultured was much lower than the seedset. Many seeds were very small and the embryos too badly developed to be worth culturing.

In six of the eight possible interspecific combinations, hybrids were produced. Only one combination yielded relatively many hybrids (VV × BBBB→ VBB). These were the same type of triploid hybrids as have been mentioned several times before in the literature (KUCKUCK, 1934; KONZAK et al., 1951; DAVIES, 1956; MORRISON et al., 1959; KAO and KASHA, 1970). This cross combination also produced one haploid plant. All other successful combinations yielded only a few hybrids, but especially in those combinations where the parents had the same number of chromosomes (viz: 2x × 2x and 4x × 4x), haploid and dihaploid plants were formed. All haploids and dihaploids resembled *H. vulgare* regardless of the direction of the cross. This phenomenon is also reported by DAVIES (1956), KAO and KASHA (1970), SYMKO (1969) and KASHA and KAO (1970).

The karyotypes of the parental species have been studied (LANGE, 1964) and subsequently used in the establishment of the genomic character of derivatives of interspecific crosses.

Table 1. Summary of crosses and results of crossing.

Cross combination	Number			Character and number of progeny
	crosses (spikes)	embryo cultures	plants	
VV × BB	487	450	3	VB (2) ,V (1)
BB × VV	325	143	6	BV (4) ,V (2)
VV × BBBB	391	801	146	VBB (145) ,V (1)
BBBB × VV	328	90	12	BBV (12)
VVVV × BB	93	16	0	
BB × VVVV	50	10	0	
VVVV × BBBB	441	328	26	VVBB (11) ,VV (15)
BBBB × VVVV	228	50	8	BBVV (5) ,VV (3)

The three plants which resulted from the combination VV × BB were not viable, and all died before heading. Their genomic character was established in studies of chromosome morphology. The progeny of the reciprocal cross BB × VV consisted of four viable hybrids. The hybrids were perennial but had to be maintained in the glasshouse. The two haploids that were produced from this cross combination died before heading, but their genomic constitution was also established from their chromosome morphology.

The combination VV × BBBB yielded many vigorous triploid hybrids. The plants were maintained in the field for many years but many of them eventually lost their viability and died. The haploid plant which originated from this cross combination was very vital. It was kept alive in the glasshouse for two years. The triploid hybrids of the reciprocal combination BBBB × VV were very vigorous as well.

The eleven hybrids which originated from the cross VVVV × BBBB behaved in a characteristic way. Most of them showed chromosomal instability in that within one plant, chromosome numbers ranging from 14 to 29 could be observed. The euploid and near euploid numbers predominated. Six plants died before heading. Three of them were unstable. The other three died so early that only few cells could be studied. The remaining five hybrids had a longer lifetime, although they had to be maintained in the glasshouse. All the plants showed chromosomal instability in different degrees. In three plants the instability was expressed in plant morphology as well. On these plants, shoots developed which completely or partly resembled *H. vulgare*. When only a part of the shoot resembled *H. vulgare* this was always the upper part. The cross combination VVVV × BBBB also produced 15 dihaploid plants having two genomes of *H. vulgare*. Nine plants died before heading, and the other six plants resembled the female parent in all respects.

The hybrid progeny of the reciprocal cross combination, BBBB × VVVV, showed the same type of chromosomal instability. Three hybrids showed their instability in plant morphology as well, for again shoots resembling *H. vulgare* were produced. In two hybrids the part resembling *H. vulgare* was so large that the whole plant behaved more or less as an annual. Another hybrid seemed to be stable, but a chromosomal check showed that it had changed from a tetraploid hybrid into a diploid hybrid. The change from tetraploid to diploid happened during the first year after the production of the plant. The cross combination BBBB × VVVV also gave rise to three dihaploid plants, which had two genomes of *H. vulgare* in the cytoplasm of *H. bulbosum*. The plants resembled *H. vulgare* in many respects. Some differences will be mentioned later.

Morphology of the hybrids

In general the morphology of the hybrids (excluding haploids, dihaploids and shoots with changed morphology due to chromosomal instability) was much like that of *H. bulbosum*, although the diploid and tetraploid hybrids looked somewhat more intermediate than the triploid hybrids. Big differences existed between plants of the same genomic constitution. These differences were probably due to the wide range of parental genotypes used in the crosses. No morphological differences could be detected between plants of the same genomic constitution originating from reciprocal crosses. The plants were not as tall or as vigorous as *H. bulbosum*. They exhibited a perennial

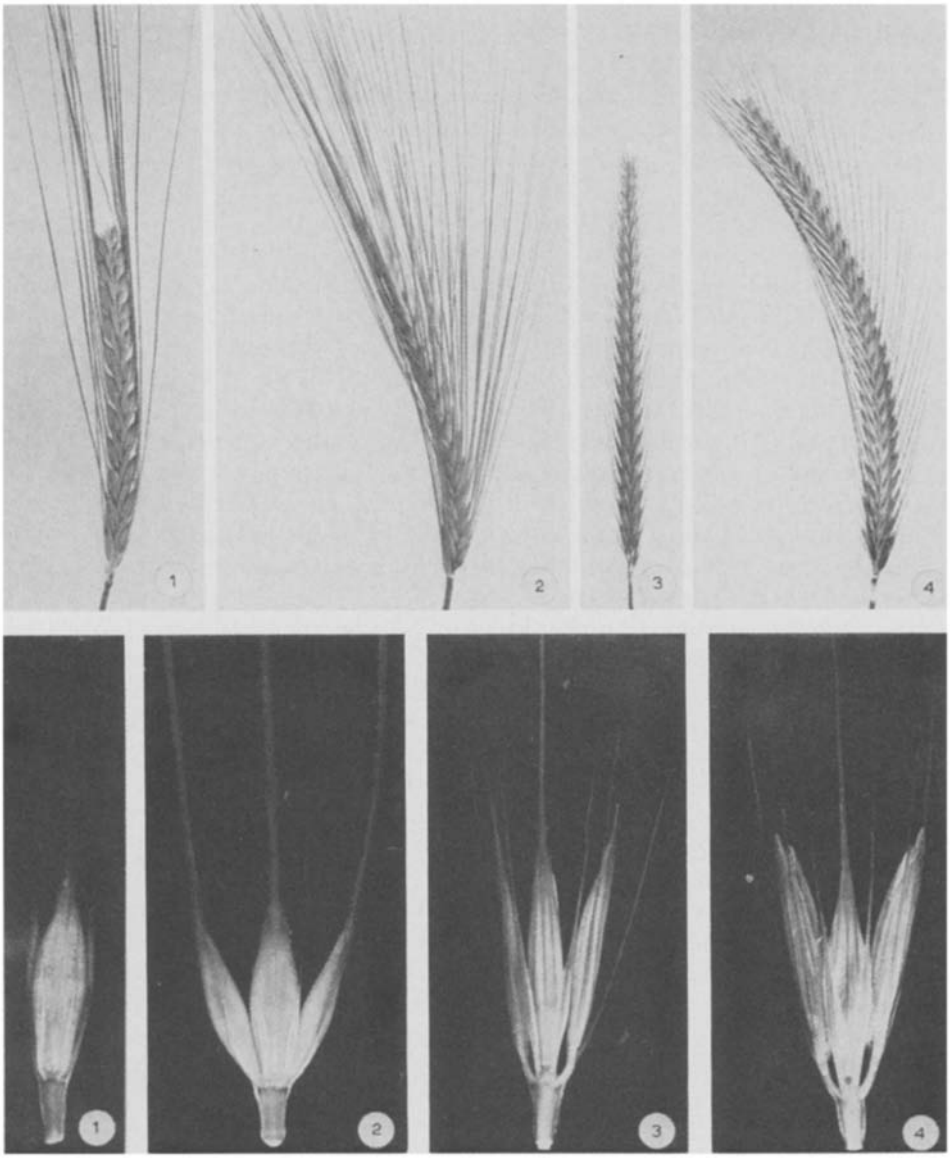

Fig. 1. Spikes and triplets of spikelets of parental species (spikes × 0.4, triplets × 3).
1. *H. vulgare* (2x), two-rowed; 2. *H. vulgare* (2x), four-rowed; 3. *H. bulbosum* (2x); 4. *H. bulbosum* (4x).

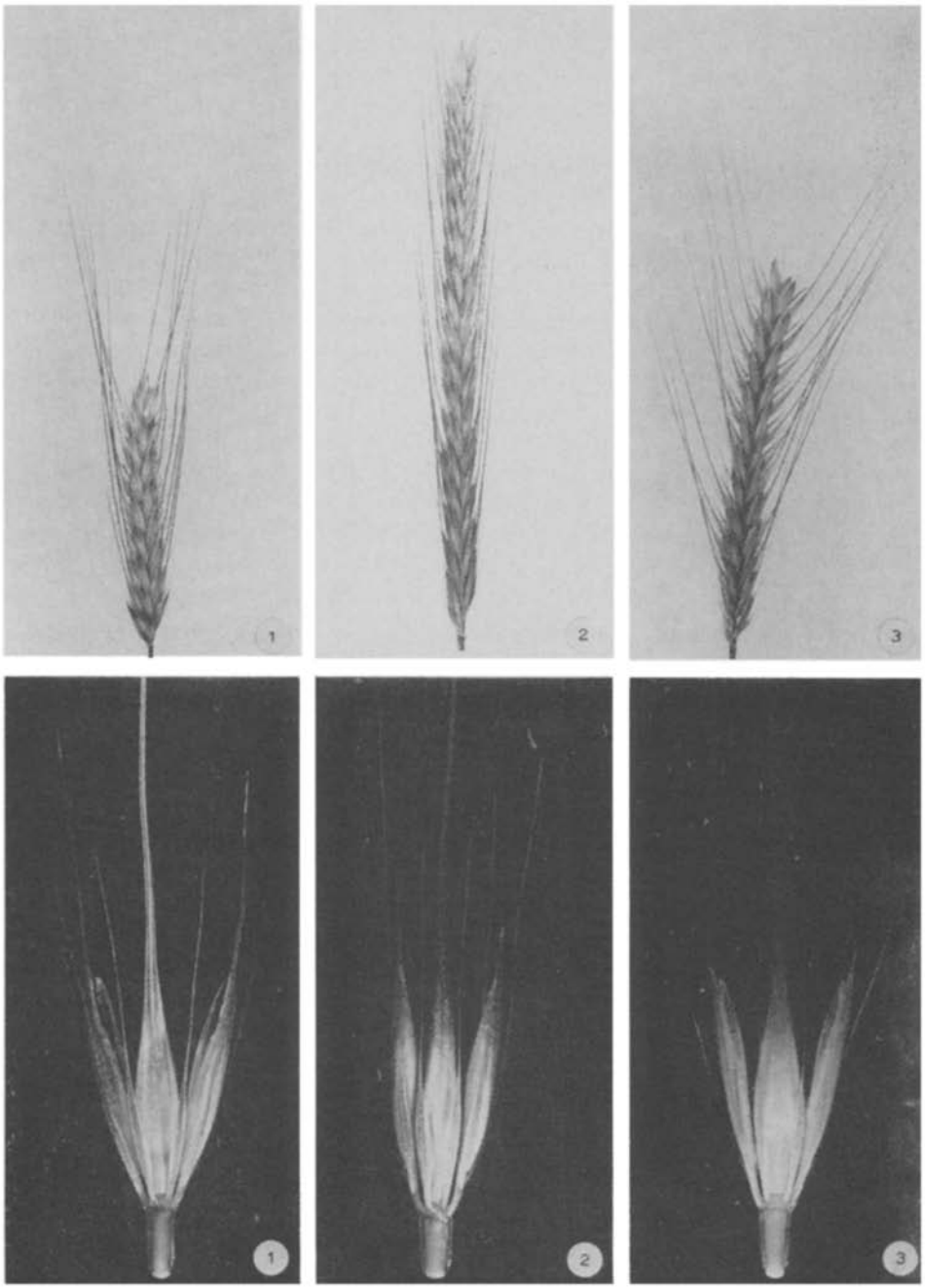

Fig. 2. Spikes and triplets of spikelets of hybrids (spikes × 0.6, triplets × 3). 1. diploid hybrid (BV); 2. triploid hybrid (VBB); 3. tetraploid hybrid (VVBB).

habit, which seemed to be reduced by their lower viability. The leaves and in several plants the leave-sheaths as well had few or many long, white hairs. The bulbs on the stem base, which are typical of *H. bulbosum*, were not or scarcely formed in diploid and tetraploid hybrids. The triploids had a bulb which was about half the size of that of *H. bulbosum*.

The spikes and triplets of spikelets of parental species and hybrids are shown in Fig. 1 and 2. The rachis of the hybrids was not as brittle as in *H. bulbosum*, and in some plants there was hardly any brittleness. As in *H. bulbosum*, the lateral spikelets of the hybrids had a short pedicel, and the inner and outer glumes of these spikelets were different. Lemmas and paleas of all spikelets were much narrower than those of *H. vulgare*, but never as narrow as those of *H. bulbosum*. The lateral spikelets had no awn or a short awn. The awn of the central spikelet was twice as long as that of *H. bulbosum*, and nearly half as long as that of *H. vulgare*. As in *H. bulbosum*, all flowers of the hybrids had female and male organs, but in the flowers of the lateral spikelets the female organs were very much reduced. The anthers were about the same length as those in *H. vulgare* or somewhat longer. They were never as large as in *H. bulbosum*. The hybrids were highly sterile: exceptionally a small and shrivelled seed was formed, probably a result of spontaneous pollination or selfing. Controlled selfing, crosses or backcrosses very seldom gave rise to seed formation. The plants obtained from these seeds were studied, and the results will be presented in another paper.

Morphology of a haploid, of dihaploids and of changed shoots

The only haploid plant which could be studied in detail originated from the cross VV $\times$ BBBB. It had one set of chromosomes of *H. vulgare* in the cytoplasm of the same species. This plant looked very much like barley, but was only about two-thirds of the size of a normal barley plant. The haploid was completely sterile and experiments to double its chromosome number were unsuccessful. Dihaploid plants originated from reciprocal crosses between the autotetraploid cytotypes of the species. All had two sets of chromosomes of *H. vulgare*, some plants in the cytoplasm of the same species, others in the cytoplasm of *H. bulbosum*. Morphologically the plants looked very much like *H. vulgare*. Only small differences could be detected between the dihaploids with cytoplasm of *H. vulgare* and the true species.

By contrast the cytoplasm of *H. bulbosum* did have some influence on plant development, though it did not introduce gross morphological differences. (Data about this influence were obtained mainly through studies of the progenies of the original alloplasmic plants). In some alloplasmic dihaploids the tillering capacity was increased, but an abnormally high number of tillers remained vegetative, so that these plants had fewer spikes per plant than the true species. The length of the alloplasmic plants was reduced considerably. Their development in the spring was slow, which made them later in flowering and seed ripening. The size of the spike and the fertility of the flowers were somewhat reduced, and so fewer grains per spike were formed. The thousand grain weight was also somewhat reduced. These differences remained more or less constant during the next two generations and preliminary results have shown that they can be eliminated by using the alloplasmic dihaploids as male parents in crosses with barley, thus proving that the differences were introduced by the alien cytoplasm.

The morphology of changed shoots which originated on tetraploid hybrids is

Fig. 3. Spikes, triplets of spikelets and culm of tetraploid hybrids which showed morphological changes during their development. 1. spike which resembles *H. vulgare* (× 0.5); 2. triplet of spikelets of this spike (× 3); 3. triplet of spikelets of other spike on the same plant (× 3); 4. spike which resembles *H. vulgare* of other plant (× 0.8); 5. culm of this spike, note dense hairiness which is a hybrid character.

difficult to describe, because each change observed happened in a different way and at a different time. In general, a change happened after a period of slow growth of the hybrid plants. When growth started again, one or more of the developing shoots had a changed morphology or changed its morphology during further development. The change could be observed through the development of thicker stems and broader leaves, through reduced or zero growth of hairs on leaves and leave-sheaths, and most clearly through spike morphology and sometimes fertility. Spike morphology very much resembled that of normal *H. vulgare* (see Fig. 3), although sometimes differences could be observed, mostly as parts of the spikes developing differently. Six of ten tetraploid hybrids showed this type of change. Three of these hybrids had the cyto-

plasm of *H. vulgare*, the others the cytoplasm of *H. bulbosum*. Four plants showed the change in the first year after their production and never again. The other two plants changed two and three years after their production. When the whole or nearly the whole plant was involved in the change the plant became annual. When only part of the plant changed, the unchanged part retained its perennial habit. In five of six cases the change in morphology resulted also in restored fertility, although its level was much reduced. The progeny of these changed shoots will be described elsewhere, together with the progeny of the dihaploids, especially the alloplasmic dihaploids.

Meiosis in the hybrids

Diploid, triploid and tetraploid hybrids all showed a meiosis which was more or less disturbed. In general three types of disturbance could be distinguished: (1) there were many aneuploid cells, especially in the diploid and tetraploid hybrids, (2) there were different types of cell inclusions, most of them probably extranuclear, predominantly in the aneuploid cells and (3) chromosome association in early meiotic stages without formation of chiasmata, resulting partly in desynapsis and partly in MI configurations in which the chromosomes were stuck together with so-called pseudochiasmata. Sometimes other disturbances could be observed as well, like reduced synchronization of meiosis and a characteristic orientation of the spindle as a half moon in one half of the cell instead of in the centre of the cell.

The MI configurations of the hybrids and the parental species are summarized in Table 2 (see also Fig. 4). Only the euploid cells of the hybrids are mentioned. The aneuploid cells will be treated in the next paper of this series. In the diploid hybrids there were 5.78 bivalents per cell and even some multivalents, but many of the bivalents and probably all multivalents seemed to have resulted from pseudochiasmata. All types of disturbance mentioned above were found in these hybrids, although one of the plants studied was more irregular than the other. First anaphase in the diploid

Table 2. Association of chromosomes at metaphase I (MI) in hybrids (euploid cells) and parental species.
I = univalent, II = bivalent, III = trivalent and IV = quadrivalent.
Bound arms per cell refers to arms which were connected with another arm by chiasmata or pseudochiasmata.

Genome symbol	Cytoplasm	Number		Configurations per cell				Bound arms per cell
		plants	cells	I	II	III	IV	
BV	*bulb.*	2	189	2.21	5.78	0.05	0.02	18.12
VBB	*vulg.*	17	725	6.49	6.41	0.51	0.03	26.27
BBV	*bulb.*	1	90	6.47	6.47	0.53	–	26.68
VVBB	*vulg.*	1	4	2.50	11.00	0.50	0.50	44.75
BBVV	*bulb.*	1	62	0.42	11.02	0.19	1.24	49.00
VV	*vulg.*	several	477	<0.01	7.00	–	–	27.40
VVVV	*vulg.*	several	99	0.28	6.61	0.20	3.47	51.58
BB	*bulb.*	several	1560	<0.01	7.00	–	–	27.32
BBBB	*bulb.*	several	160	0.17	5.90	0.14	3.90	52.59

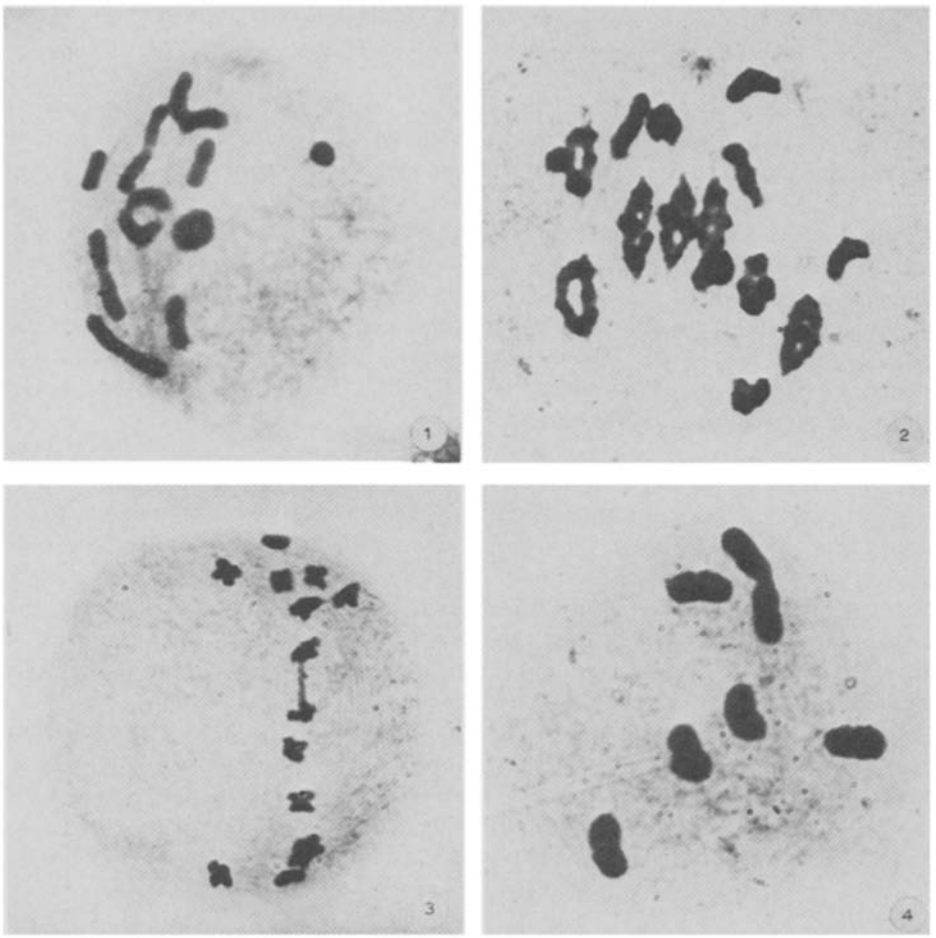

Fig. 4. Meiosis in hybrids and in haploid. (1, 2 and 4 × 1400, 3 × 950).
1. M I in diploid hybrid: 5 I + 4 II + 2 inclusions; 2. M I in triploid hybrid: 7 I + 7 II; 3. A I in diploid hybrid; 4. M I in haploid *H. vulgare*: 5 I + 1 II.

hybrids was highly irregular, with unequal distribution of the chromosomes at the poles, lagging chromosomes, precocious division of lagging chromosomes and also with bridges between chromosomes or chromatids. Consequently the second meiotic division was very irregular, resulting in abnormal tetrads and pollen grains. In 200 tetrads of the two diploid hybrids 754 micronuclei and 52 micro pollen grains were found, one plant being more disturbed than the other. Pollen grain stainability was 0%.

Chromosome association in the triploid hybrids was near to the theoretical expectation of 7I + 7II, but sometimes multivalents occurred. In general this meiosis was much more normal than the one in the other hybrids, and disturbances were due mainly to triploidy. There were only a few aneuploid cells, almost no inclusions and only a low number of pseudochiasmata. At first anaphase many univalents lagged at

the equator and divided precociously. In 570 tetrads of seven plants 3902 micronuclei and 478 micro pollen grains were found. Mean pollen grain stainability in six plants was 2.7% with a range of 0–6.6%.

Meiosis in the tetraploid hybrids was highly disturbed. Of three plants studied, one had only hypoploid cells, another had predominantly hypoploid cells and the third had mainly euploid cells. Because each chromosome in the euploid cells had a nearly or completely homologous partner, one would expect nearly normal chromosome association. Instead of this, only about eleven bivalents were formed per cell and many of these were rod bivalents or had probably resulted from pseudochiasmata. On the other hand multivalents were formed. All mentioned types of disturbance were observed in different degrees. First anaphase and second division were irregular as well, resulting in 745 micronuclei and 146 micro pollen grains in 200 tetrads. Pollen grain stainability in three plants was 60.2%, 15.1% and 1.3%, but even the plant with about 60% good pollen was highly sterile.

Meiosis in a haploid and in dihaploids

Table 3 summarizes the chromosome associations at MI in one haploid plant and several types of dihaploids, including one shoot with changed morphology and the progeny of such shoots. In the haploid, some chromosome association was observed but the bivalents did not look normal (Fig. 4) and could have resulted from pseudochiasmata. Another abnormality were the aneuploid cells and cells with inclusions. Owing to haploidy meiosis was very irregular, resulting in abnormal tetrads often consisting of only micro pollen grains.

Nearly all dihaploids had meiosis and pollen grain stainability as in the normal diploid *H. vulgare*. The only exception was one shoot of a tetraploid hybrid just after its morphologically visible change to a barley-like shoot. Here chromosome association was reduced, resulting in some univalents and more rod bivalents. In this shoot seven tetrads of a hundred showed abnormalities, there being fifteen micronuclei and

Table 3. Association of chromosomes at metaphase I (MI) in haploid and dihaploid plants which resembled *H. vulgare*, including shoot with changed morphology and progeny of such shoots. For explanation see table 2.

Plant status	Cyto-plasm	Number		Configurations per cell			Bound arms per cell
		plants	cells	I	II (rod)	II (ring)	
Haploid	*vulg.*	1	135	6.51	0.21	0.04	0.58
Dihaploid	*vulg.*	6	981	0.02	0.34	6.65	27.28
Dihaploid[1]	*vulg.*	1	202	0.30	1.78	5.07	23.84
Dihaploid[2]	*vulg.*	1	102	–	0.30	6.70	27.39
Dihaploid	*bulb.*	3	642	–	0.33	6.67	27.33
Dihaploid[2]	*bulb.*	2	212	–	0.42	6.58	27.16

[1] from shoot with changed morphology, shortly after change.
[2] from progeny of shoots with changed morphology.

three micro pollen grains. No data are available on pollen grain stainability of shoots with changed morphology.

DISCUSSION AND CONCLUSIONS

In general it can be concluded that the production of interspecific hybrids from crosses between *H. vulgare* and *H. bulbosum* is a difficult task. With embryo culture it has been possible to obtain viable hybrids from several cross combinations, but with the exception of triploid hybrids (VBB), the number of hybrids was very low. The crossing barrier can be divided into three phases: (1) reduced seedset, (2) abnormal development of caryopses resulting in seed abortion, and (3) reduced viability of the hybrids, which started in the hybrid embryos (LANGE, 1969). The most complete set of data about seedset were presented by DAVIES (1956), but the number of parental genotypes used by him was rather low. In general seedset was higher when *H. vulgare* was used as the female parent, and can be influenced by environmental conditions and parental genotypes (KUCKUCK, 1934; KONZAK et al., 1951). The cause of the reduced seedset is not known and has never been studied, because it was not a major difficulty in these interspecific crosses.

The main part of the crossing barrier consisted of abnormal seed development resulting in seed abortion. From extensive studies of microtome preparations (LANGE, 1969), it was concluded that disturbances occurred in endosperm and embryo development. The disturbances in the endosperm development were probably the main cause of seed abortion. The development was very poor and stopped at an early stage, leading to shrivelling of the seed and starvation of the embryo. Before this starvation, however, disturbances were also observed in the embryo. These were partly the same as the disturbances in the endosperm, and probably accelerated embryo abortion or reduced viability of the embryo when it was taken in toculture. These abnormalities in embryo development were probably a main cause of the rather low percentage of success of the embryo culture. Many embryos seemed to have a good early development on the culture medium, but died at a later stage of development. This phenomenon was also observed by KONZAK et al. (1951) and by DAVIES (1956, 1960), but these authors explained it by the unnatural conditions in which the embryo was placed and by possible damage during the preparation procedure.

The morphology of the diploid, triploid and tetraploid hybrids was more or less the same, although the triploids had a better vitality than the others. The description of triploid hybrids by KUCKUCK (1934), KONZAK et al. (1951), MORRISON and RAJHATHY (1959) and DAVIES (1956, 1960) fits the triploids obtained in this study very well. The general appearance of the hybrids was like that of *H. bulbosum* but the plants were much smaller, less vital and highly sterile. Some of the characters, however, were intermediate between the parents. It was not possible to detect any systematic difference between plants with the same genomic constitution but derived from reciprocal crosses. If these differences existed they were masked by the variability within the groups of hybrids with the same parental origin. The crossfertilizing character of *H. bulbosum*, c.q. its enormous heterozygosity, was the main source of this variability.

The morphology of the haploid and dihaploid plants was so much like that of *H. vulgare* that unchanged genomes from this parental species must have been trans-

mitted into this special type of progeny. In some plants the cytoplasm originated from *H. bulbosum*, and indeed this alien cytoplasm had an influence on plant development, which could be eliminated by crossing these plants with normal barley, using them as the male parent (LANGE, unpubl.). Several hypotheses for the origin of haploid and dihaploid plants from these interspecific crosses can be put forward: (1) semigamy (TURCOTTE and FEASTER, 1967) – penetration of a male gamete into the egg cell, followed by vegetative segregation of paternal and maternal nuclei in early stages of embryo development, after failure of gametic fusion; (2) parthenogenesis – development of the embryo from an unfertilized egg; (3) gynogenesis and androgenesis – development of the embryo from the egg (gynogenesis), or the male gamete (androgenesis), only, after the male gamete has entered the egg cell without achieving fertilization; (4) chromosome elimination – normal fertilization and fusion of male and female gametes, followed by gradual elimination of the chromosomes of one of the parents.

In the literature several cases of the development of barley-like plants from crosses between *H. vulgare* and *H. bulbosum* have been mentioned. DAVIES (1956, 1958) obtained three such plants, which he explained by male parthenogenesis. SCHOOLER's (1964) results might have been of the same nature, but he presented no details and explanations. RAJHATHY (1967) mentioned the origin of barley-like plants from 4x × 4x crosses. SYMKO (1969) obtained many haploids from 2x × 2x crosses. Some were intermediate in early stages of their development, others were barley-like right from the beginning. SYMKO concluded that these haploids originated either through male parthenogenesis or through chromosome elimination. KASHA and KAO (1970) reported haploids from 2x × 2x crosses and came to the conclusion that they originated through elimination of chromosomes in early embryonic stages. Finally, KAO and KASHA (1970) found dihaploids from 4x × 4x crosses which again were most probably formed after chromosome elimination.

From all these data and from those presented here, viz: the vegetative segregation observed in tetraploid hybrids and resulting predominantly in dihaploid barley-like shoots, it can be concluded that the hypothesis of gradual chromosome elimination provides the best explanation for most observations. In the next paper of this series (LANGE, 1971) more evidence in favour of this hypothesis will be presented. One thing that remains difficult to understand is the difference in frequency of events that may occur after hybridizing *H. vulgare* and *H. bulbosum*. Accepting the hypothesis that formation of haploids and dihaploids, as well as the chromosomal instability all are the result of chromosome elimination, one can conclude only that this elimination is controlled by genes or by environmental conditions or both. Different authors used different materials and probably worked under different conditions. These differences might have influenced the time chromosome elimination started and the efficiency of the process.

Meiosis in the hybrids is characterized by several types of disturbance, viz: aneuploidy, which is of pre-meiotic origin and will not be treated in this paper, low chiasma frequency, and formation of pseudochiasmata. In diploid hybrids two probably homeologous sets of chromosomes are together. One might expect that pairing occurs between homeologous chromosomes (homeologous allosyndesis). Some pairing may also take place between non-homologous chromosomes of the same species (non-

homologous autosyndesis) as in haploid *H. vulgare* (TOMETORP, 1939; CLAVIER and CAUDERON, 1951; this study, Table 3), but allosyndetic pairing between non-homeologous chromosomes seems unlikely. In general the pairing in diploid hybrids was rather good but at metaphase I some of it was no longer visible owing to desynapsis and another part was visible only through the pseudochiasmata. Multivalents can be explained by a combination of homeologous allosyndesis and non-homologous autosyndesis (two allosyndetic pairs connected to each other by autosyndetic association).

In the triploid hybrids meiosis was more regular and more like the theoretical expectation. The data presented here are in agreement with data from the literature (KONZAK et al., 1951; MORRISON and RAJHATHY, 1959; DAVIES 1956, 1960), and in general most bivalents must presumably have been the result of homologous autosyndesis of *H. bulbosum* (the tetraploid cytotypes of this species behaved like a typical autotetraploid) and most univalents must have been *H. vulgare* chromosomes. All authors explained trivalents by homeologous allosyndesis and quadrivalents by an interchange in the *H. bulbosum* complement. The last part of this explanation, however, is very unlikely, because in *H. bulbosum* (4x), itself, no configuration of more than four chromosomes was found. By looking at the maxmuim number of associated chromosomes that occurred in the triploid hybrids, one can easily find another explanation. Several cells were observed with more than seven bivalents, which indicates that non-homologous autosyndesis occurred in the *H. vulgare* complement. If this non-homologous autosyndesis were combined with homeologous allosyndesis a quadrivalent could result. By looking at the minimum number of associated chromosomes that occurred another conclusion can be drawn. Several cells had more than seven univalents (up to eleven univalents were found per cell), which indicates that chromosome association in the *H. bulbosum* complement was not as strong as expected. This could be explained by assuming less homology in *H. bulbosum* than might be expected from its autotetraploid nature, but it is more likely that association of *H. bulbosum* chromosomes was reduced by the influence of the *H. vulgare* chromosomes. This phenomenon was also found in the tetraploid hybrids and in other interspecific combinations in the genus *Hordeum* (WAGENAAR, 1960; RAJHATHY, 1967).

In the tetraploid hybrids it was unlikely that non-homologous autosyndesis occurred because each chromosome had a homologous partner. Bivalents must have resulted from homologous autosyndesis and multivalents most likely from a combination of homologous autosyndesis and homeologous allosyndesis. The occurrence of univalents in many cells was most probably caused by an influence of the chromosomes of *H. vulgare* on association in the *H. bulbosum* complement, as is supposed by RAJHATHY (1967).

In conclusion it can be said that in hybrids between *H. vulgare* and *H. bulbosum* three types of chromosome pairing can occur: homeologous allosyndesis, homologous autosyndesis and non-homologous autosyndesis. It seems likely that normal chiasmata were formed predominantly in homologous, autosyndetic combinations, while the other types of combinations were made visible mainly by the so-called pseudochiasmata. Since it was not possible to distinguish for each association by which type of connexion it was formed, no more exact statement can be made. In the literature at least two hypotheses for the origin of this type of pseudochiasma can be found: (1) matrix connexions (WALTERS, 1954; WAGENAAR, 1959, 1960) and (2) heterochro-

matic fusion (RILEY and CHAPMAN, 1957). The reduced frequency of normal chiasmata probably occurred predominantly in the *H. bulbosum* complement and seems to have been caused by the chromosomes of *H. vulgare*. This influence was probably genic as in other material (STEBBINS, 1958; WAGENAAR, 1959, 1960; RAJHATHY, 1967).

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