THE GENOMES OF AGROPYRON TRACHYCAULUM (LINK) MALTE AND HORDEUM JUBATUM L. AND THEIR HYBRIDIZATION WITH HORDEUM VULGARE L.

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ABSTRACT

THE GENOMES OF AGROPYRON TRACHYCAULUM (LINK) MALTE AND HORDEUM JUBATUM L. AND THEIR HYBRIDIZATION WITH HORDEUM VULGARE L.

by

Rye-Ho Huang

Natural hybrids of Agropyron trachycaulum (Link) Malte with Hordeum jubatum L. were collected from Alaska. The putative parents of these natural hybrids were crossed reciprocally obtaining ten artificial hybrids. Nine of these hybrids resulted with A. trachycaulum as the maternal parent, and one with H. jubatum as the maternal parent. Morphologically, there are no differences among the natural and reciprocal artificial hybrids. Hybrids with Agropyron as the maternal parent show more growth vigor than those with Hordeum as the maternal parent.

Observations of chromosome pairing in pollen mother cell in the natural hybrids show the following averages: 16.38 univalents, 5.44 bivalents, 0.14 trivalents while in the artificial hybrids with Agropyron as the female, averages were 11.51 univalents, 7.22 bivalents, 0.67 trivalents. With H. jubatum as the female the averages were 15.80 univalents,

5.60 bivalents, 0.32 trivalents, and 0.01 quardrivalents. Based on this study, there is one genome in common between A. trachycaulum and H. jubatum, the second genome of H. jubatum has segmental homology with the common genome, and the second A. trachycaulum genome has no homology with the others appearing as seven univalents. The genome formulae were $A_j A_j A'_j A'_j$ and $A_j A_j TT$ for Hordeum jubatum and Agropyron trachycaulum, respectively.

Amphiploids were obtained by colchicine treatment of the natural hybrid by Robert Stiedl. Fertility was restored with seed-set ranging from 0-30 per spike.

Chromosome association in the amphiploid showed significant reduction in univalents, and no increase in the multivalents indicating that the allotetraploid genomes of H. jubatum and A. trachycaulum are partially homologous. Progenies of the amphiploid show variation in morphology and seed-set indicating the possibility that the chromosome abnormality may extend to subsequent generations.

Cultivated barley (Hordeum vulgare L.), diploid and tetraploid, used as pollen parents were crossed with the amphiploid to improve winterhardiness of cultivated barley (for breeding purposes) by transmitting genes from both Agropyron and Hordeum sources. Hybrid plants of the amphiploid X H. vulgare (4x) and H. vulgare (2x) were obtained through embryo culture. The morphology of the hybrid plants approaches H. vulgare more closely for the case where H. vulgare (4x) was used as might be expected. Apomixis, in one case, of a

reduced egg from the amphiploid was found to be morphologically similar to the natural hybrid.

From preliminary cytological observations of pollen mother cells the conclusion was reached that the genome is unbalanced in hybrids with a constitution of $A_jA_jA_j'TV$ whereas the morphological characteristics of the VV genome are predominant over those of $A_jA_j'T$ when present in a constitution of $A_jA_jA_j'TVV$. In the former constitution, three distinct chromosome groups are formed with two spindles resulting in a tripolar division probably based on the presence of three different genomes.

Stainable pollen grains were found only rarely and the hybrid plants were completely sterile. Using H. vulgare as the maternal parent in crosses with the amphiploid gives a promising alternative for restoring H. vulgare chromosomes to its own cytoplasm after recurrent backcrosses to H. vulgare. At the point when fertility is at least partially restored, it is hoped that random mating plus selection will permit transfer of genetic information from the wild species to H. vulgare cultivars.

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INTRODUCTION

The genera Agropyron and Hordeum have been considered wealthy sources for transmitting desirable traits to both barley and wheat. Many substantial achievements have been reported by using Agropyron species to improve wheat disease resistance (Elliott, 1957; Rillo, 1970) and winterhardiness. It should be possible to transfer genes for winterhardiness, disease and insect resistance, drought and salt tolerance etc. to cultivated barley, H. vulgare L.

Barley improvement by interspecific crosses between H. vulgare and one of the wild Hordeum species has been slow due to unknown incompatibility mechanisms from the level of pollination to the level of chromosome elimination in the hybrid embryo development and differentiation.

The combination of the Hordeum and Agropyron genomes presents potential breeding implications for increasing variation and complementary stability in hybridization programs using wild species. Natural and artificial hybrids between A. trachycaulum (Link) Malte and H. jubatum L. were easily obtained. One genome in common has been well documented in a cytogenetic study between these two genera (Boyle and Holmgren, 1954). Partially fertile amphiploids were employed in crosses with H. vulgare in this study. Either genomes of H. jubatum

or A. trachycaulum or genomes from both species might associate with the genome of H. vulgare and transmit desirable characteristics from the wild source to the cultivated barley.

The objectives of this study are:

1) to elucidate the genome relationship between A. trachycaulum and H. jubatum in natural hybrids and artificial hybrids obtained through reciprocal crosses and amphiploid through both cytological and morphological observations,
2) to study the techniques of embryo culture for survival of hybrid plants,
3) to investigate the possibility of transmitting the desired traits from the combined genomes of A. trachycaulum and H. jubatum into the genome of H. vulgare, and
4) to learn how barley can be hybridized with wild species and/or other grains.

LITERATURE REVIEW

I. Intergeneric cross between Agropyron species and Hordeum species.

Spontaneous hybrids of Agropyron species X Hordeum species have been found in several north temperate latitudes since 1872. Hybrid Elymus macounii Vasey was first collected and named by Vasey in 1886. The possibility of hybrids between Agropyron species X Hordeum species was suggested by Stebbins et al. (1946b). In addition, Brink, Cooper and Ausherman (1944) stated that the relative ease with which species in the genera Agropyron, Hordeum, Elymus, Sitanion, Secale, and Hystrix can form "intergeneric" hybrids makes possible the production of F_1 plants which may be morphologically similar yet have different origins. Lepage first made the combined designation as X Agrohordeum macounii (Vasey) Lepage in 1953.

The hybrid between Agropyron trachycaulum (Link) Malte and Hordeum jubatum L. was synthesized by reciprocal crosses by Boyle and Holmgren (1954) proving that at least some entities once identified as Elymus macounii are simply F_1 sterile hybrids between these two genera. Later, similar studies and conclusions were supported by Bowden (1960) and Gross (1960). Gross's studies even produced two different partially fertile octoploid plants (2n=56) by colchicine treatment of sterile tetraploid X Agrohordeum macounii.

The frequent occurence of such intergeneric hybrids and the ease with which they can be produced artificially hinted that more species of these two genera might be involved in possible hybridizations. After typification of Elymus macounii Vasey by Bowden (1960), an intergeneric hybrid was reported by Mitchell and Hodgson (1965) collected in Alaska and reclassified as X Agrohordeum pilosilemma Mitchell and Hodgson. The putative parents were Agropyron sericeum Hitchc. and Hordeum jubatum based on convincing field observations and comparative morphological studies.

II. The genome relationships of Agropyron and Hordeum.

A lot of work has been done on the genome relationships of several Gramineae through their hybrids. Among them Agropyron is one of the most variable genera and is closely related to Triticum. A. trachycaulum is a tetraploid with chromosome number 2n=28 (Myers, 1947; Covas, 1948). Based on cytological observation of its hybrids with Hordeum, Elymus, Sitanium, etc., the genome formula for A. trachycaulum (2n=28) was given as SSXX in which "S" denotes the Agropyron spicatum (Pursh) Scribn. & Smith genome and "X" is a genome of unknown origin (Dewey, 1969, 1968). A trispecies hybrid of Agropyron dasystachyum (Hook) Scribn., A. spicatum and A. trachycaulum had 27 chromosomes, 13 of which were probably contributed by the F_1 of A. spicatum X A. dasystachyum and 14 by tetraploid A. trachycaulum. Complete pairing and multivalent configuration occurred in over half of the cells (Dewey, 1968).

Since the pioneering work by Brink, et al. (1944) on the interspecific and intergeneric hybrids of Hordeum species, H. jubatum has been considered to be an allotetraploid. Based on cytological studies of natural and controlled hybrids between A. trachycaulum and H. jubatum, Boyle and Holmgren (1954) suggested that if the pairing is allosyndetic between chromosomes of H. jubatum and A. trachycaulum, that the genome formulae are AABB and AACC for A. trachycaulum and H. jubatum, respectively. This is also supported by Ashman and Boyle (1955) in their studies of the hybrid and its induced octoploid. By cytological observation of the size differences between the chromosomes of a H. jubatum X Secale cereale L. hybrid, Wagenaar (1959, 1960) concluded that autosyndetic pairing occurred and that H. jubatum was a segmental allotetraploid.

Rajhathy and Morrison (1959) assigned the genome formula AABB to H. jubatum as an allotetraploid from studying hybrids between H. jubatum and other species of the genus. Rajhathy et al. (1964) later agreed with Wagenaar (1959, 1960) that the two genomes of H. jubatum may be partially homologous. Autosyndetic pairing of the Agropyron chromosomes was also found in hybrids of Agropyron species with Secale cereale (Stebbins and Pun, 1958).

Intergeneric hybrids of Agropyron species with Hordeum species were studied by Dewey (1971); he reported that pairing in the Agrohordeum hybrids indicated that H. jubatum and H. brachyantherum Nevski contain a genome in common with Agropyron species, but he did not specify whether the common

genome was the S genome or the other genome. Although Hordeum species do not contain a genome derived from Agropyron, a modified Hordeum genome apparently occurs in Agropyron as well as in Elymus and Sitanion. Earlier, Stebbins and Pun (1953) studied a hybrid between Secale cereale and Agropyron intermedium (Host) Beauv. and suggested that the "genera" Triticum, Aegilops, Agropyron, Secale, Haynaldia and probably Elymus represent a single large genus. In recent genome analysis of Hordeum jubatum with its interspecific hybrids from the aspect of barley breeding, Starks and Tai (1974) designate the genome formula $A_j A_j A'_j A'_j$ as a segmental allotetraploid with partial homology between the two genomes whose pairing may vary under gene influence.

III. Agropyron and Hordeum species as wild sources for breeding.

The wild relatives and progenitors of major cereal crops have been tapped repeatedly for transmitting special attributes which are lacking in the cultivated varieties. Agropyron and Hordeum are closely related to cultivated wheat and barley; some of the wild species are easily crossed to their cultivated relatives but some are not. Agropyron species have been used satisfactorily to improve wheat disease resistance of leaf blotch (Septoria tritici) (Rillo, et al. 1970). Similarly, progeny of the H. vulgare type segregated from hybrids of H. leporinum Link. X H. vulgare have been reported resistant to powdery mildew and possibly to other leaf diseases (Hamilton, et al. 1955). As to drought and saline tolerance,

Agropyron species are among the most capable grasses tested. For example, Forsberg (1953) reported that slender wheatgrass, Agropyron trachycaulum, and tall wheatgrass, A. elongatum (Host.) Beaux, were the most alkaline tolerant of all crops tested. Slender wheatgrass was better suited to drier saline areas than tall wheatgrass.

Barley is the most variable cereal from the point of view of its extreme range of morphological forms and ecological types but it is limited in winterhardiness. It is possible to grow barley economically under a very wide range of soil and climatic conditions. Extending the barley acreage, particularly for a livestock feed, has necessitated the development of a more winterhardy winter barley. Harlan and Shaw (1926) searched for winterhardy barley and found that forms from high altitudes in Asia were considerably more resistant than most other varieties. He emphasized that the genetic constitution for winterhardiness had been much improved through the breeding process. Optimistically, winterhardiness of barley could reach the level displayed in wheat and rye if barley can be crossed with winterhardy species of other genera.

MATERIALS AND METHODS

I. Materials used in the crosses.

The stocks of A. trachycaulum and H. jubatum and their natural hybrids used were collected by Dr. J.E. Grafium from Alaska with the aid of William W. Mitchell and Roscoe Taylor. The amphiploid was derived from natural hybrids by colchicine treatment. Two cultivars and tetraploid line of cultivated H. vulgare (Table 1) were used as pollen sources for crossing with the amphiploid.

II. Method of hybridization.

It is preferable to grow the plants in a cool temperature which will allow the spikes to protrude from the boot before flowering and self-pollination. Plants usually need to be subdivided and replanted for vigorous growth and healthy spikes. A temperature around 15-20°C is satisfactory. The procedure is as follows:

1) Choose a spike a day or two in advance of self-pollination. Cut off top 1/4 of spike and remove bottom 1/4 of spikelets. The tip florets should be removed for better nutrient flow to the basal florets. Insert the tweezers into the basal floret and open the lemma and palea of the floret and remove the young anthers. The emasculated spike then is bagged with aluminum foil. After 1-2 days the bag is removed

Table 1. The designation and source of plants.

Plants	Designation	Source
Agropyron trachycaulum	AT-4	seed from Dr. W.W. Mitchell & R. Taylor, Palmer, Alaska
Hordeum jubatum	HJ-4	seed from Dr. W.W. Mitchell Palmer, Alaska
A. trachycaulum X *H. jubatum* (spontaneous hybrid)	AT X HJ	clone from Dr. W.W. Mitchell, Palmer, Alaska
Amphiploid	$(AT \times HJ)^2$	colchicine treatment obtained by Robert Steidl Dept. of Crop Science, MSU
Hordeum distichum L. Emend Lam. 4x=28 (2 row)	HV-4-2R	Dr. A.B. Schooler N. Dakota State Univ. Fargo, N.D.
Hordeum distichum L. Emend Lam. 2x=14 (2 row)	HV-2-2R	Variety Coho CI 13852
Hordeum vulgare L. 2x=14 (6 row)	HV-2-6R	Variety Larker CI 10648

and the ovule and lodicules expand, opening the lemmas within a short time after exposure to light. Then pollination may be carried out.

2) Crosses are made at early morning in order to obtain fresh pollen. With 1/3 of the lemmas cut from the floret, the anthers fully extrude from the lemma. Pollen may be shed by light tapping of the spike or by clipping the anther and tapping it on the stigma.

3) If the pollen is from the field, the pollen spikes are cut just beneath the flag leaf with the flag leaf base used as a hook to hang then spike upside down in a Coke bottle.

4) For greatest success, pollination is repeated for 2-4 days on the same spike. The pollinated spike is bagged after pollination, with bag removal after one week when no further pollination is possible.

III. Embryo culture.

The young embryos were dissected from the immature seeds, which collapsed within twenty days after pollination, at the stage when the glume appears pale green. The procedure of excising the embryo and transferring to medium was described as follows:

1) The transfer room or chamber with tools is sprayed with 50% commercial bleach prior to use. Tools were sterilized in 75% alcohol and passed through a flame.

2) Harvested spikelets were disinfected by immersing for 5 minutes in a 50% solution of bleach (one part water : one

part commercial bleach) and then trasnferred through three flasks containing distilled water.

3) The embryo was dissected under 180X dissecting microscope with one hand with sharp tweezers holding the seed and the other hand handling a dissecting needle.

4) The excised embryo is carefully oriented with the scutellum facing the surface of the culture medium and the radicle oriented downward. The medium surface should not be broken or the embryo may sink into the medium. The embryo is oriented the same way on a paper towel when liquid media is employed. (Fig. 1)

5) Test tubes with embryos were incubated in the dark at room temperature. When the seedlings have two leaves and visible roots, they should be potted in sterile soil, placed under fluorescent lights for approximately two weeks, and then moved to the greenhouse.

VI. Cytological techniques.

The somatic chromosome numbers of the parents and hybrids were determined by root-tip squash techniques. Young root-tips were pretreated in cold water (0-2°C) overnight, fixed in Farmer's solution, and stored in a refrigerator. The root-tips were rinsed in distilled water, hydrolysed in 1 N HCl at 60°C for 20 minutes, and stained with aceto-carmine.

For meiotic studies, spikes of appropriate stages were fixed early in the morning (approximately 5 A.M.) in Farmer's solution and placed in the refrigerator overnight. Spikes

were stored in 75% alcohol after fixation. Anthers were smeared and stained with aceto-carmine, and chromosome numbers and associations were recorded and photographed for each cell. Pollen stainability was determined using I_2KI.

RESULTS

I. Crosses.

Using A. trachycaulum as the maternal parent in the cross with H. jubatum, eighteen seeds were set, ten seeds germinated and grew vigorously to maturity. In the reciprocal cross using H. jubatum as the maternal parent, more than twenty seeds were obtained; but only one seed germinated, and it grew slowly. The hybrid plants are intermediate morphologically. The plants were subdivided into several clones to stimulate their growth and to build up the population. The amphiploid induced from the natural hybrids by colchicine treatment had 0-30 seeds set per spike. The amphiploid crossed with H. vulgare (2x) and with H. vulgare (4x) produced ten hybrids through embryo culture techniques (Table 2, 3) using two kinds of media (Appendix 1, 2).

Table 2. Seed set from intergeneric crosses between H. jubatum (2n=28) and A. trachycaulum (2n=28).

Plants	No. of Spikes Pollinated	Total No. of Seeds Set	No. of Hybrid Plants Prod.
A. trachycaulum X H. jubatum	20	19	18*
H. jubatum X A. trachycaulum	15	25	1*

*No cultures employed.

Figure 1. Young seedling on paper towel in liquid media.

Table 3. Seed set from Amphiploid crosses with H. vulgare.

Plants	No. of Spikes Pollinated	Total No. of Seeds Set	No. of Plants Produced*
Amphiploid X H. vulgare 4x=28 (2 Row)	34	13	1
Amphiploid X H. vulgare 2x=14 (2 Row)	32	3	1
Amphiploid X H. vulgare 2x=14 (6 Row)	21	31	8

*Embryo culture is necessary.

Table 4. Plants obtained from Amphiploid crosses with H. vulgare using embryo culture techniques.

Media	No. of Embryos Cultured	No. of Plants Obtained	% of Embryos Giving Plants
Liquid	22	6	0.27
Solid	22	5	0.22

Of attempts to germinate three seeds in distilled water; only one seed germinated, but died in a few days.

Table 5. Quantitative characters of the parents and the F_1 hybrids.

Characteristics	AT	HJ	AT X HJ	HJ X AT	Amp.	Amp. X HV
Culm length (cm.)	45 (±4.26)	23 (±1.51)	30 (±2.64)	24 (±2.77)	27 (±3.78)	29 (±2.7)*
Length of flag leaf (cm.)	6.9	4.5	5.8	2.7	6.5	4.0
Length of spike (cm.)	6.5 (±0.77)	3.7 (±0.28)	5.7 (±0.85)	3.5 (±0.68	5.3 (±0.57)	6.1 (±0.61)
No. of spikelets per spike	18	57	27	26	40	28
Awn length (cm.)	0	2.1	0.6	0.4	0.5	0.7
Pubescence	no	short	no	no	short	no
Auricle	short	no	no	no	no	long
Florets/node	6	1	5	4	3	3

AT = Agropyron trachycaulum
HJ = Hordeum jubatum
Amp. = Amphiploid
HV = Hordeum vulgare
*Parenthese indicate standard error of mean

II. Morphological and agronomical characteristics of hybrid plants.

A. Natural hybrid and reciprocal crosses between A. trachycaulum and H. jubatum.

Natural and artificial hybrids of A. trachycaulum (as the maternal, taller parent) crossed with H. jubatum are vigorous, averaging 30 cm. in height, and intermediate between parents (Fig. 2). The leaves of the hybrid are dark green which seems to be a maternal effect from A. trachycaulum. Spike characters showed several intermediate characteristics. For example, H. jubatum has a long-awned glume, A. trachycaulum is awnless, but has a larger glume; the hybrid has a short-awned glume (Fig. 3).

It is difficult to tell which parent was the female in the case of the natural hybrid; however, by means of reciprocal crosses and examination of the artificial hybrids, A. trachycaulum was determined to be the female parent in this study. All of the hybrid pollen was empty, of smaller size and shrunken. No stainable pollen was found. Only one hybrid seed germinated where H. jubatum was the female; this plant is lighter green, with smaller leaves in comparison to the natural hybrid, and the growth rate is much slower (Fig. 4). In contrast to the former hybrid, 18 hybrid plants were obtained from the reciprocal cross, and they closely resembled the natural hybrid.

Figure 2.A. Growth habits of A. trachycaulum X H. jubatum.

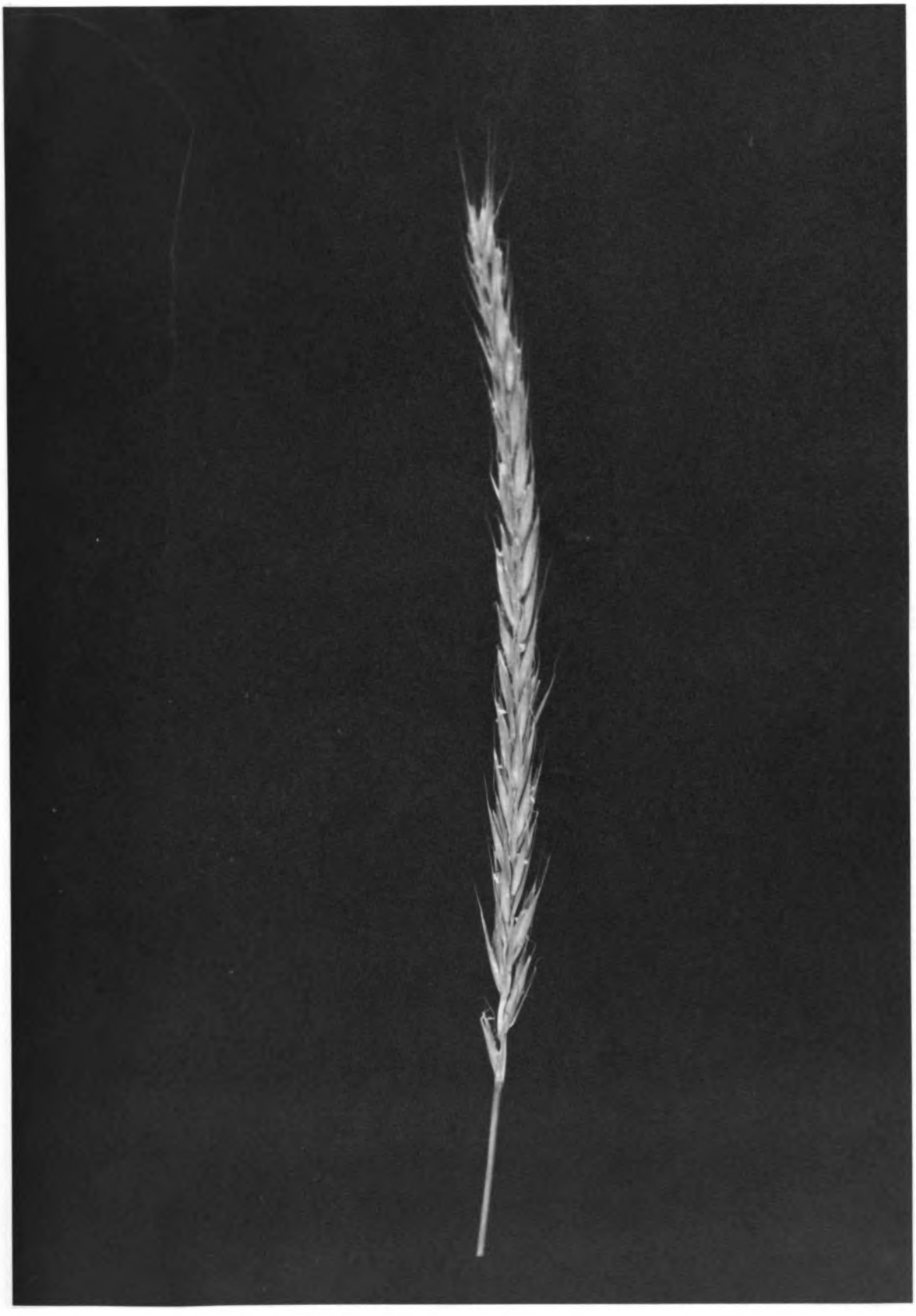

Figure 2.B. Spike of A. trachycaulum X H. jubatum.

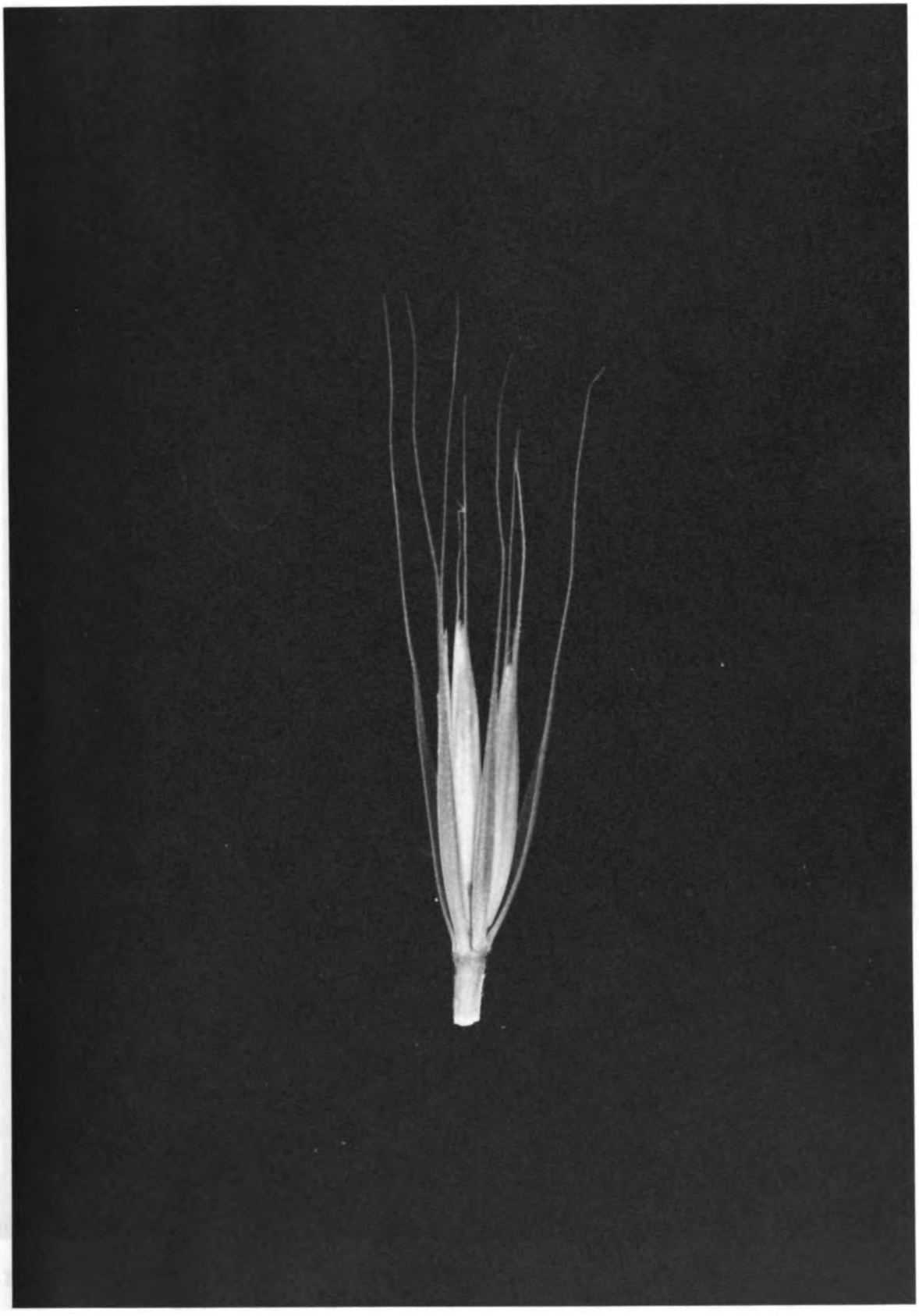

Figure 2.C. Spikelet of A. trachycaulum X H. jubatum.

Figure 3. Spike of A. trachycaulum (right) hybrid (A. trachycaulum X H. jubatum) (center) and H. jubatum (left).

Figure 4.A. Growth habit of H. jubatum X A. trachycaulum.

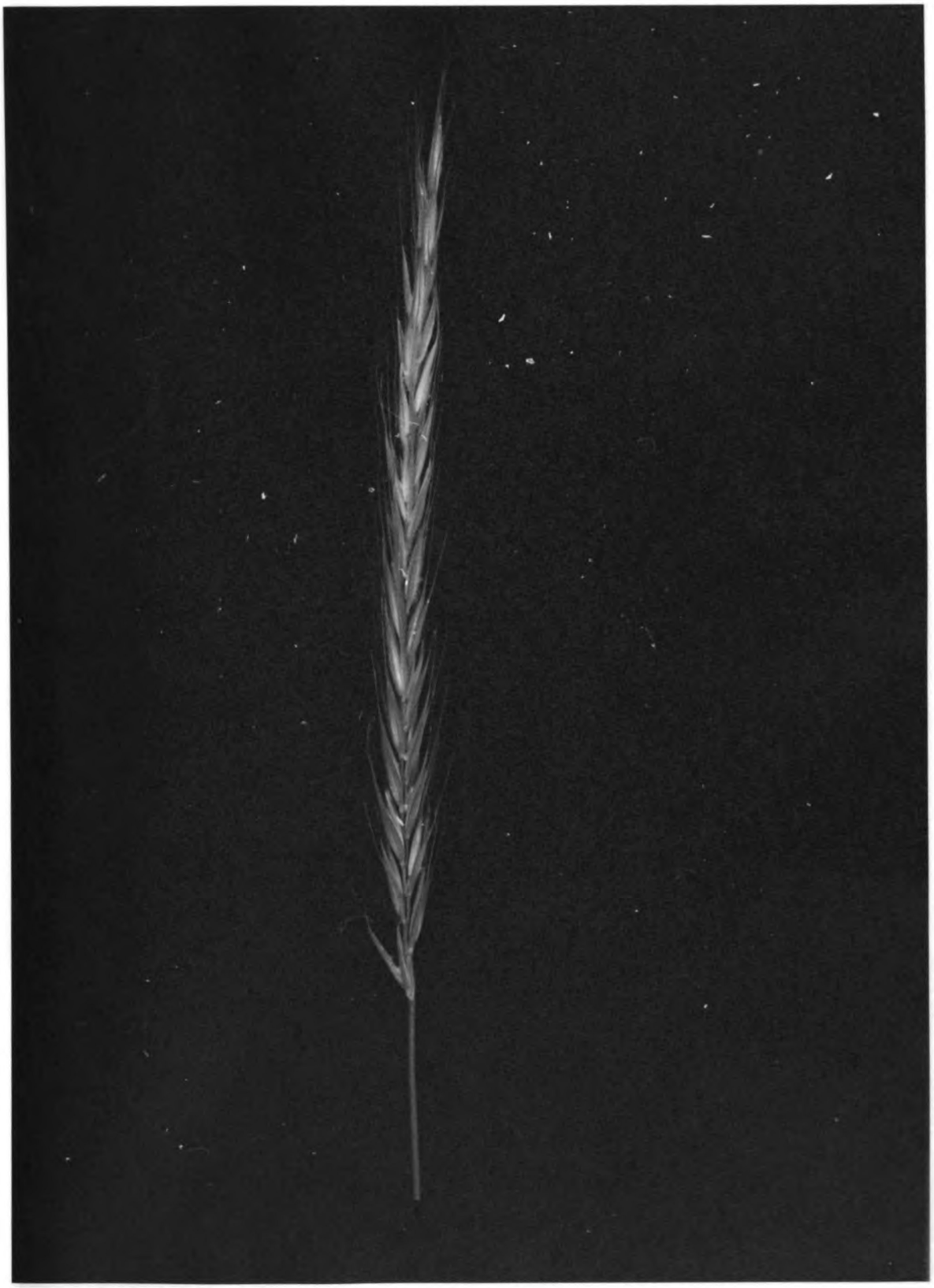

Figure 4.B. Spike of H. jubatum X A. trachycaulum.

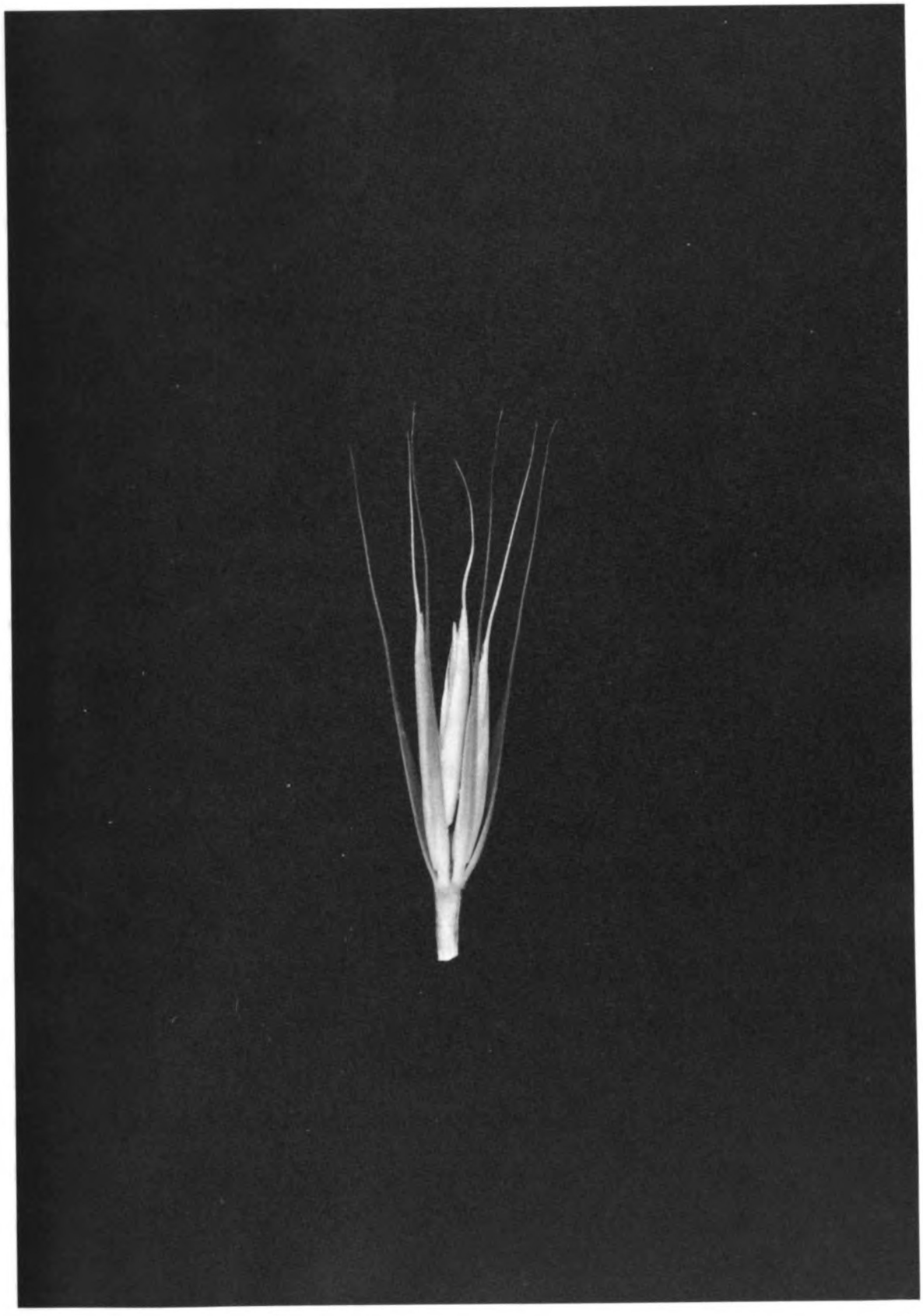

Figure 4.C. Spikelet of H. jubatum X A. trachycaulum.

B. Hybrid plants of amphiploid crosses with H. vulgare.

In most hybrid plants, the tillering stage of the young seedling is sub-critical as compared to embryo germination. The so-called sub-lethal effect due to genetic defects may cause the gradual death of young seedlings although they struggle through germination on artificial media. In the case of a mixoploid or a seed having more than one embryo, they may have severe somatic competition at the tillering stage.

In the current study, hybrid plants also showed significantly poor growth rates even after the tillering stage. In the embryo culture hybrids, some young seedlings died at the 2-3 leaf stage either in culture media or just after being transplanted to sterile soil. Similar cases were shown by Dr. A.B. Schooler for his hybrid plants.[2] Sublethality of young seedlings has been documented by Morrison, et al. (1963) in interspecific hybrids of H. murinum L. X H. vulgare, H. californicum Covas & Stebbins X H. vulgare, and H. californicum X H. bulbosum L.; and by Davies (1960) in interspecific hybrids H. vulgare X H. bulbosum.

Figure 5 shows one of the amphiploid crosses with H. vulgare and hybrid designated as AHV 442-2 (Fig. 6) photographed at full-bloom stage in August 1974 in the greenhouse. The plants were about 29 cm. tall at this time, of a dark green color inherited from the A. trachycaulum

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[2]Presentation at 9th Barley Malting Conference at Milwaukee, Oct. 6-10, 1974.

Figure 5.A. Growth habit of amphiploid derived from natural hybrid by colchicine treatment.

Figure 5.B. Spike of amphiploid derived from natural hybrid by colchicine treatment.

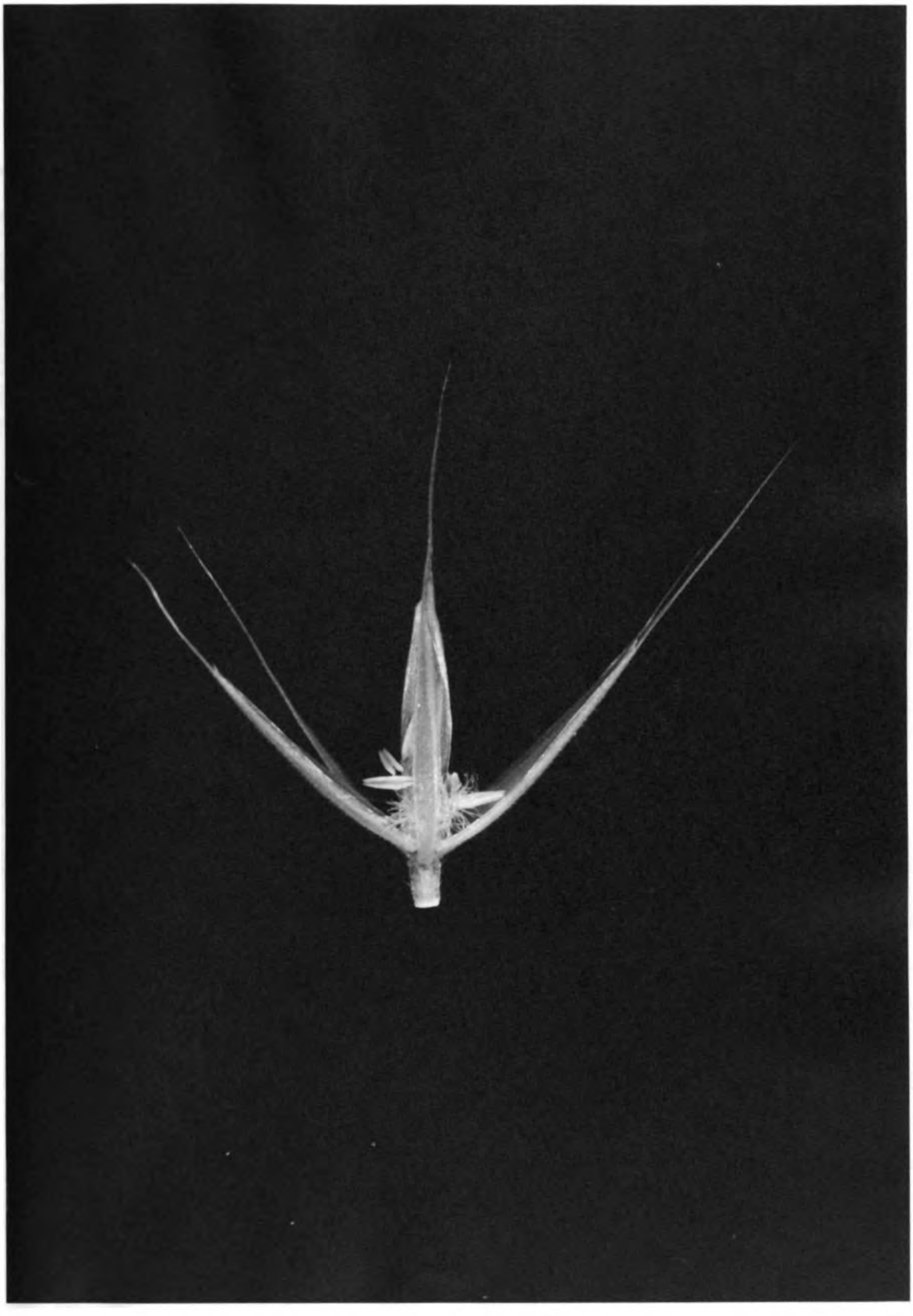

Figure 5.C. Spikelet of amphiploid derived from natural hybrid by colchicine treatment.

Figure 6.A. Growth habit of hybrid of amphiploid crossed with H. vulgare (2x) designated as AHV 442-2.

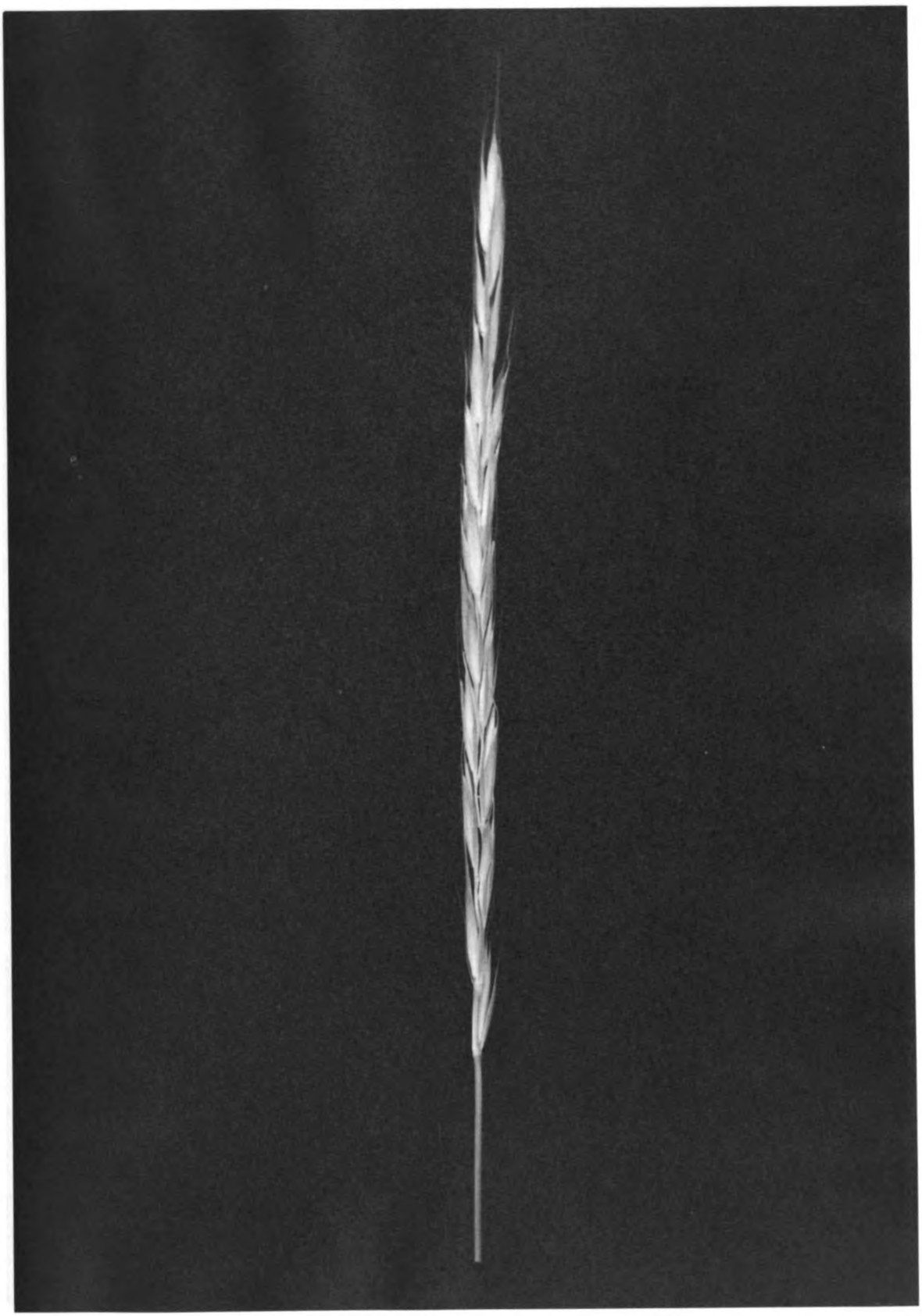

Figure 6.B. Spike of hybrid of amphiploid crossed with H. vulgare (2x) designated as AHV 442-2.

Figure 6.C. Spikelet of hybrid of amphiploid crossed with H. vulgare (2x) designated as AHV 442-2.

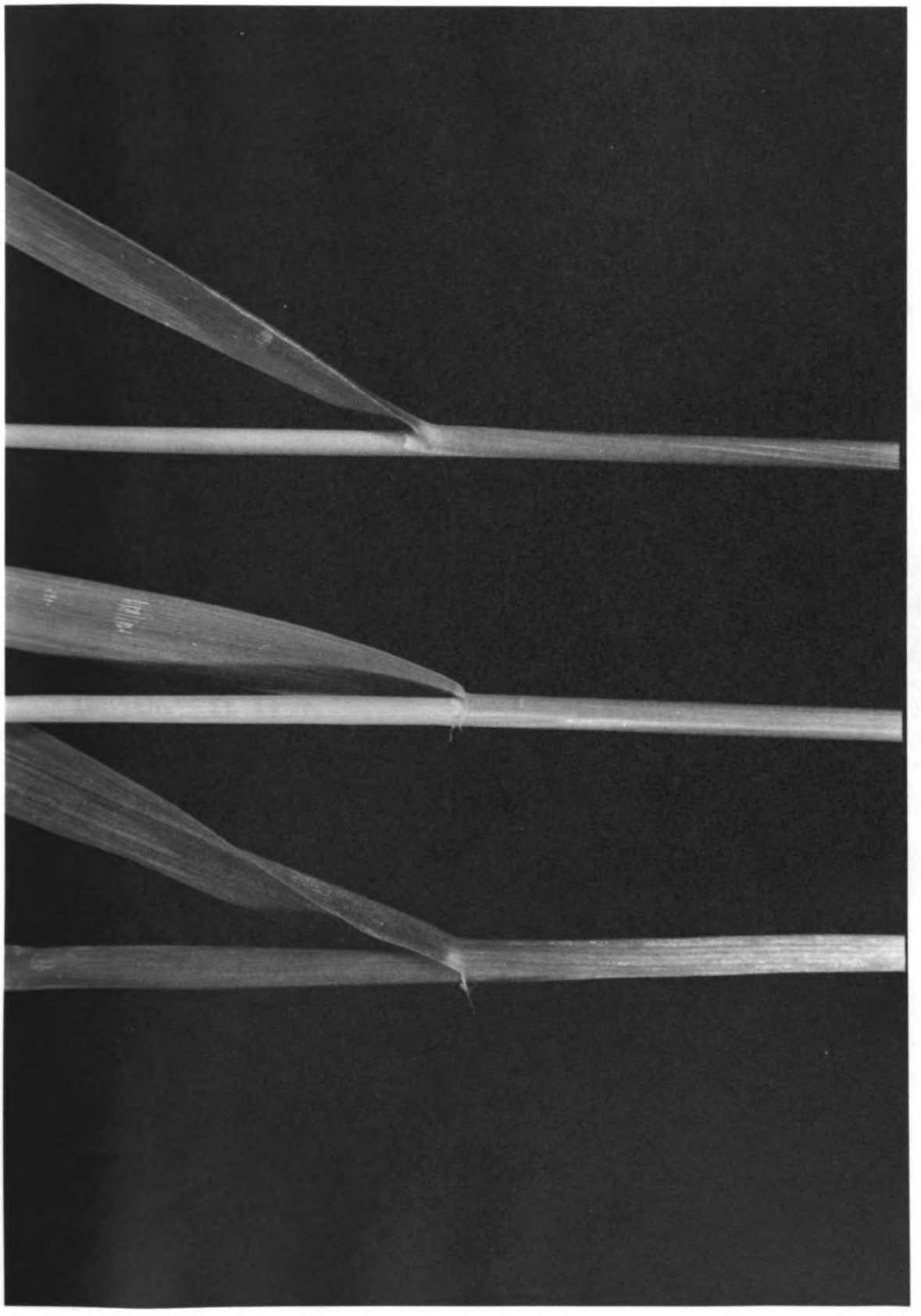

Figure 6.D. Auricle of hybrid of amphiploid crossed with H. vulgare (2x) designated as AHV 442-2.

parent. Although the plant flowered freely and grew satisfactorily, no seeds were set. Stainable pollen was rarely found in the anther (Fig. 37).

III. Cytological studies.

Meiotic chromosome behavior of A. trachycaulum, H. jubatum, spontaneous and synthetic reciprocal-crosses of A. trachycaulum and H. jubatum, amphiploid, and amphiploid X H. vulgare is summarized in Table 6.

The following is a general description of cytological observation:

A. A. trachycaulum

The somatic chromosome number of A. trachycaulum from the root-tip cells was counted as 2n=28 (Fig. 7). Most of the chromosomes of A. trachycaulum are almost the same length. It is difficult to identify each individual chromosome just by chromosome size and/or centromere position, but two pairs of satellited chromosomes were observed (Fig. 7 arrow). At metaphase I, fourteen ring bivalents were consistently observed (Fig. 8).

B. H. jubatum

The somatic chromosome number was 2n=28 (Fig. 9). The length of the chromosome varied more than those of A. trachycaulum. Fourteen bivalents were consistently observed in metaphase I (Table 6).

C. Spontaneous hybrid of Agrohordeum macounii

Twenty-eight chromosomes were observed in the root-tip cells (Fig.10). Metaphase I was the main stage used for

the study of chromosome associations (Fig. 12). Loosely paired trivalents were observed in six cells, only one cell had two trivalents, and the mean number of trivalents per cell was 0.14. Eighty-four percent of the fifty cells observed had tightly associated bivalents. The bivalents ranged from 4-7 per cell with a mean of 5.44 per cell. The range of univalents was from 7-14. A minimum of seven univalents, presumably representing the non-homologous A. trachycaulum genome, appeared in every cell observed. The other seven univalents appear either randomly distributed or attached to bivalents to form the loosely paired trivalents.

As to abnormalities in meiosis, one to several pairs of homologous chromosomes were excluded from the major group at the early zygotene stage (Fig. 11). Metaphase II as observed normally follows irregular cell division giving rise to the microspore development (Fig. 13, 14). Non-stainable pollen of different size resulted from meiotic abnormality and irregular cytokinesis. The anther was shrunken and non-dehiscent, the plant was completely sterile and no seeds were set.

D. Synthetic hybrid of A. trachycaulum X H. jubatum

Univalents ranged from 8-18 with a mean of 11.51 per cell. At diakinesis, seven univalents were isolated from the major group and formed bivalent-like configurations (Fig. 15). Probably, these seven univalents lagged at anaphase I and divided precociously at that time (Fig. 16).

The number of bivalents ranged from 5-10 with a mean of 7.22 per cell. A range of 0-3 trivalents was observed in eight cells. From 0-7 laggards were observed at metaphase II, and consequently, micronuclei also ranging from 0-7 were found in 97% of the quartats (Fig. 17, 18).

E. Synthetic hybrid of H. jubatum X A. trachycaulum

Inconsistent chromosome condensation and different size chromosome groups were found at early zygotene stage (Fig. 19). Connections between chromosomes were observed at metaphase I, these might have arisen from early separation of multivalents or from sticky chromosomes (Fig. 20). Univalents ranged from 7-22, averaging 15.80 per cell. A range of 0-2 trivalents were observed in four cells with a mean of 0.01. Loosely coiled chromosomes were observed in telophase I (Fig. 21). Micronuclei also ranged from 0-7 in 95% of the quartets (Fig. 22).

F. Amphiploid

The chromosome number of the amphiploid was 2n=56 (Fig. 25). Two nuclei were observed at pachytene stage (Fig. 23). Quadrivalents ranged from 0-1 cells with an average of 0.05 per cell, while trivalents ranged from in 0-2 cells with an average of 0.71 per cell (Fig. 24). Univalents ranged from 0-6 with an average of 2.36 per cell. Compared to the hybrids, the number of univalents per cell and the number of micronuclei which ranged from 1-3 per quartet, decreased in the amphiploid (Fig. 26). Fertility was partially restored in the amphiploid with 45% stainable pollen grains and 0-30 seeds set per spike.

G. Amphiploid X H. vulgare (2x)

Cytological observations were carried out on only one of the hybrid plants, AHV-442-2. Since the amphiploid had a chromosome number 2n=56 and the H. vulgare had 2n=14, the chromosome number of this hybrid is expected to be 2n=35. However, meiotic counts varied from 17 to 35 (Table 7). Unequal chromosome segregation was observed in metaphase I (Fig. 27) and in anaphase I (Fig. 28). The chromosome bridge formed irregular cytokinesis is evident in this observation (Fig. 28). Presumably, the presence of H. vulgare chromosomes in this hybrid contributes to the phenomenon of tripolar division (Tai 1970) and irregular spindle formation and location after metaphase I (Fig. 30). Several chromosomes in different sized microspores were excluded from the major group (Fig. 31, 32, 33). Stainable pollen grains were rarely observed within stained anthers (Fig. 34). Normally, the anthers do not dehisce, and the plant sets no seed.

Table 6. Chromosome association at metaphase I in *Agropyron trachycaulum*, *Hordeum jubatum*, spontaneous and artificial hybrids, Amphiploid and Amphiploid X *Hordeum vulgare* (2x).

Plants		Chromosome Association I	II	III	IV	No. of Cells
A. trachycaulum (2n=28)	Range	0	14	0	0	100
	Mean	0	14.0	0	0	
H. jubatum (2n=28)	Range	0	14	0	0	100*
	Mean	0	14.0	0	0	
spontaneous hybrid (2n=28)	Range	7-24	2-8	0-2	0	50
	Mean	16.38	5.44	0.14	0	
A. trachycaulum X *H. jubatum* (2n=28)	Range	7-18	5-10	0-3	0	31
	Mean	11.51	7.22	0.67	0	
H. jubatum X *A. trachycaulum* (2n=28)	Range	7-22	3-7	0-2	0-1	96
	Mean	15.80	5.60	0.32	0.01	
Amphiploid (2n=56)	Range	0-6	22-28	0-2	0-1	71
	Mean	2.36	25.63	0.71	0.05	
Amphiploid X *H. vulgare* (2n=35)	Range	0-9	**	**	**	50
	Mean					

*From Gil Stark's data.
**Due to chromosome variation as follows on Table 7.

Table 7. Chromosome variation observed from P.M.C. in AHV 442-2.

Chromosome Number	No. of Cells
35	1
34	1
33	1
32	9
31	8
28	6
26	4
25	5
22	5
19	4
17	6
	50

Table 8. Comparison of micronuclei per quartete in natural hybrid, artificial hybrid, and amphiploid. (Parentheses indicate percent.)

No. of Micronuclei Per Quartete								Total
0	1	2	3	4	5	6	7	
Natural hybrid								
12 (4.27)	70 (24.91)	75 (26.69)	68 (24.19)	33 (11.74)	12 (4.21)	9 (3.2)	2 (0.7)	281
Artificial hybrid								
12 (2.79)	43 (9.81)	61 (13.92)	81 (18.49)	102 (23.28)	82 (18.72)	38 (8.67)	19 (4.33)	438
Amphiploid								
101 (34.12)	116 (39.18)	58 (19.59)	21 (7.09)	0	0	0	0	296

Plate I

Figure 7. Somatic chromosomes in root-tip metaphase I of A. trachycaulum showing two pairs of satellited chromosomes (arrow) 2n = 28.

Figure 8. Metaphase I in A. trachycaulum showing 14 bivalents.

Figure 9. Somatic chromosome in root-tip cells of H. jubatum, 2n = 28.

Figure 10. Somatic chromosome in root-tip cell of natural hybrid of A. trachycaulum X H. jubatum 2n = 28.

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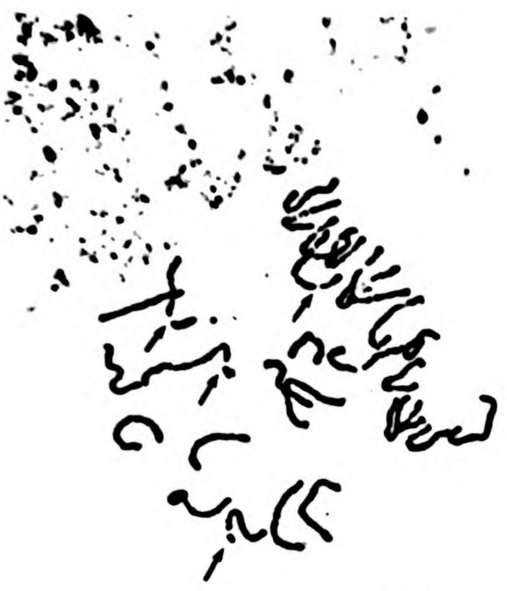

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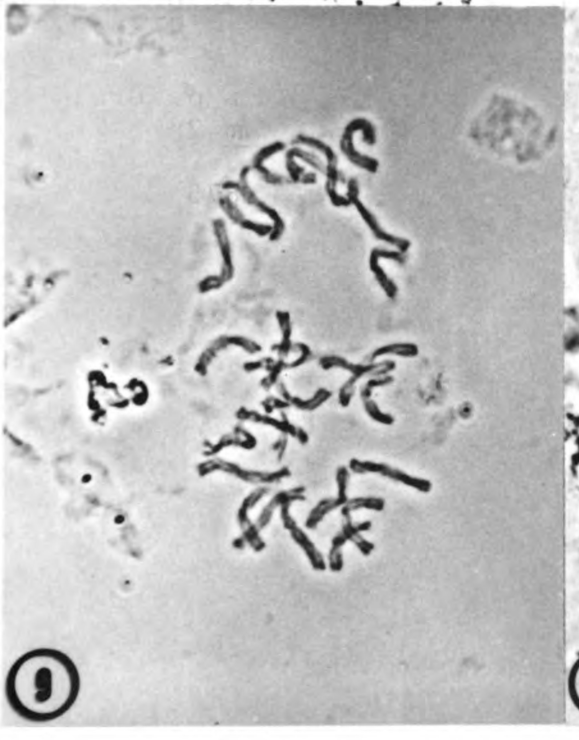

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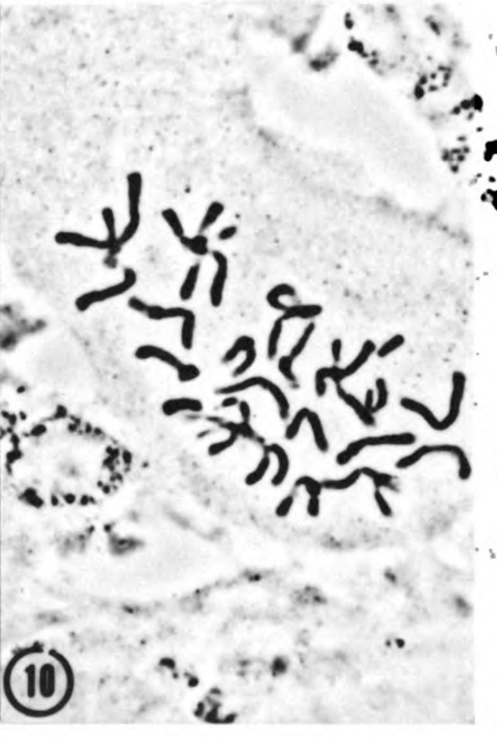

(10)

Plate II Spontaneous hybrid.

Figure 11. Early zygotene stage showing a couple of chromosomes which were excluded from the major group.

Figure 12. Metaphase I in natural hybrid showing 6 bivalents (two ring and four rod) and one loosely paired trivalent.

Figure 13. Metaphase II in natural hybrid.

Figure 14. Irregular cell division in microspore.

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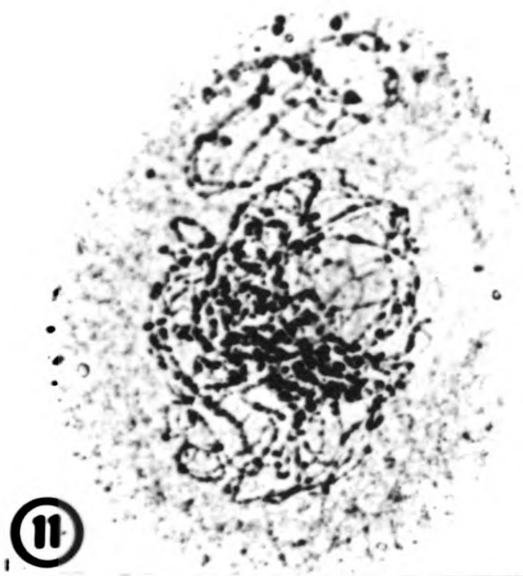

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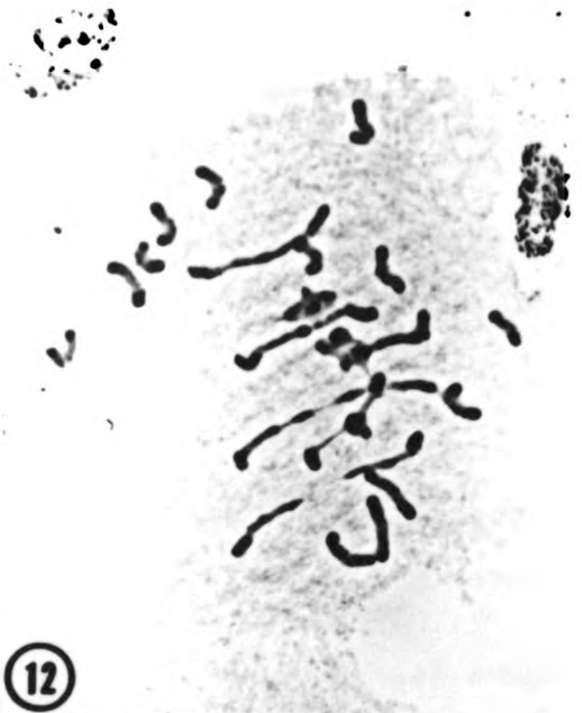

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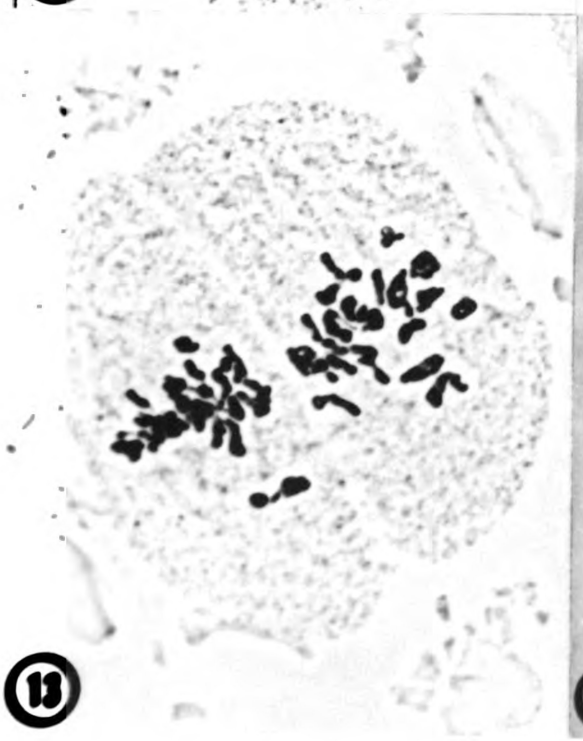

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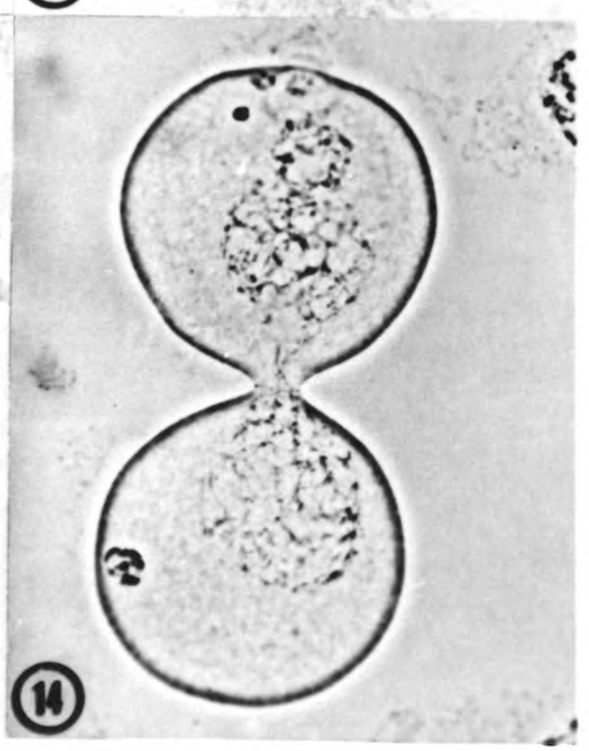

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Plate III Synthetic hybrid A. trachycaulum X H. jubatum

Figure 15. Diakinesis in the hybrid showing seven univalents spread out earlier from major group.

Figure 16. Late telophase I in hybrid showing six chromatids separated at first division.

Figure 17. Two micronuclei after first division in hybrid.

Figure 18. Tetrads having micronuclei in hybrid.

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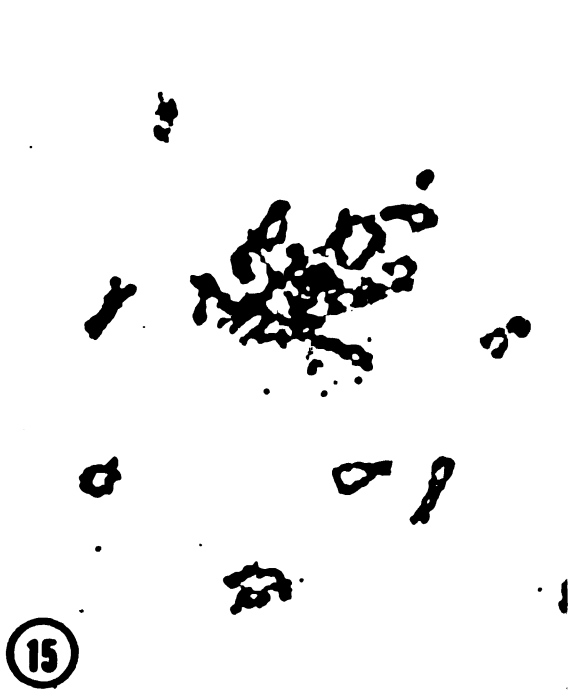

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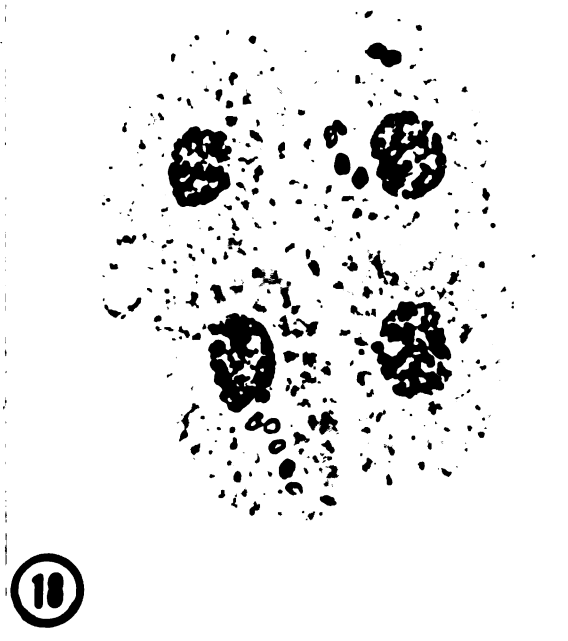

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Plate IV *H. jubatum* X *A. trachycaulum*

Figure 19. Different size and condensation of the chromosome group in prophase I in hybrid.

Figure 20. Admixture chromosome association and seven univalents at metaphase I in hybrid.

Figure 21. Decoiled chromosome at late telophase I in hybrid.

Figure 22. Tetrads having micronuclei in hybrid.

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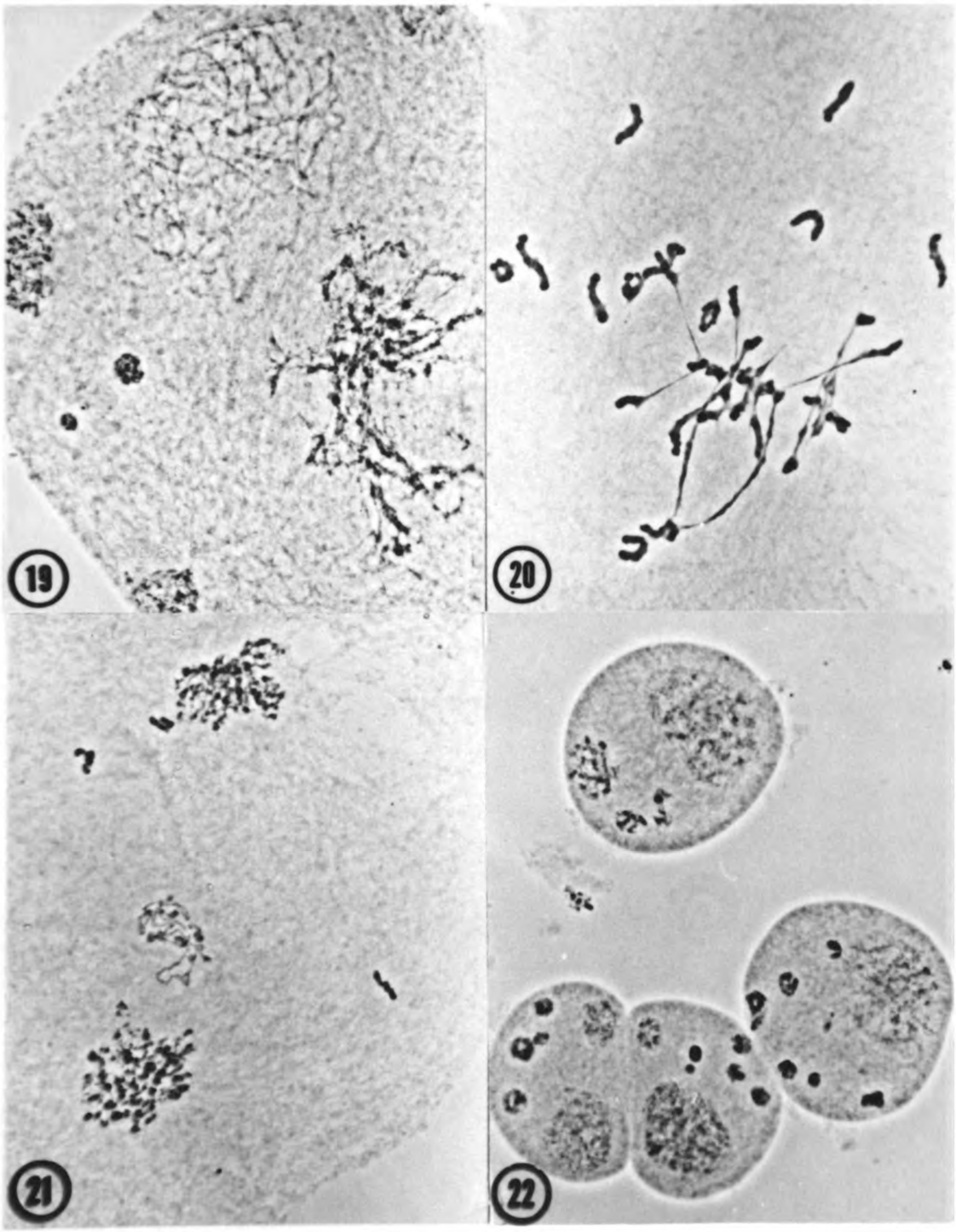
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Plate V Amphiploids

Figure 23. Pachytene stage showing two nuclei in amphiploid.

Figure 24. Metaphase I in amphiploid showing one trivalents (arrow).

Figure 25. Anaphase II in amphiploid showing chromosome bridge (arrow).

Figure 26. Tetrad having micronuclei in amphiploid.

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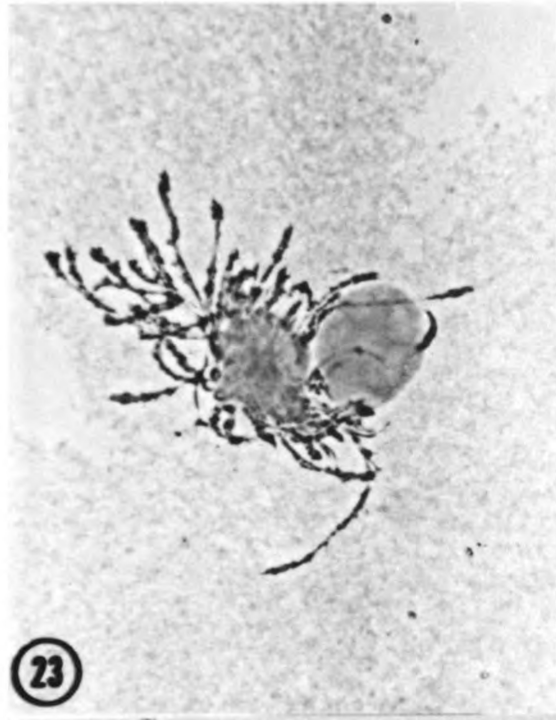
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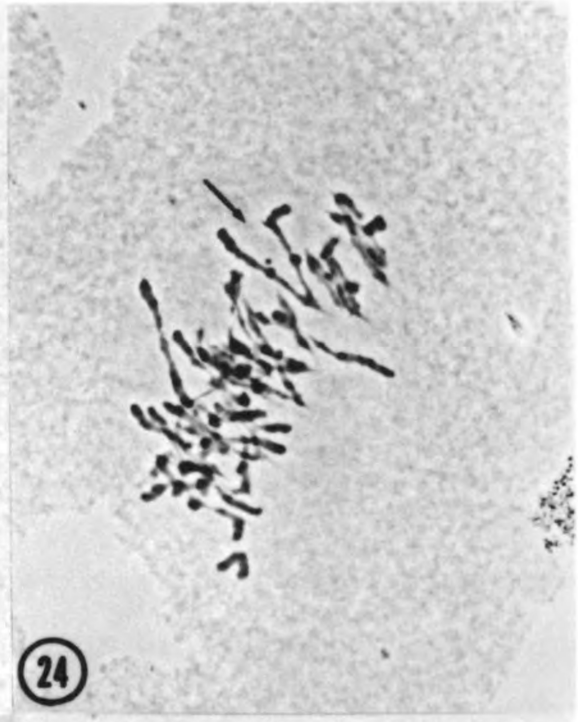
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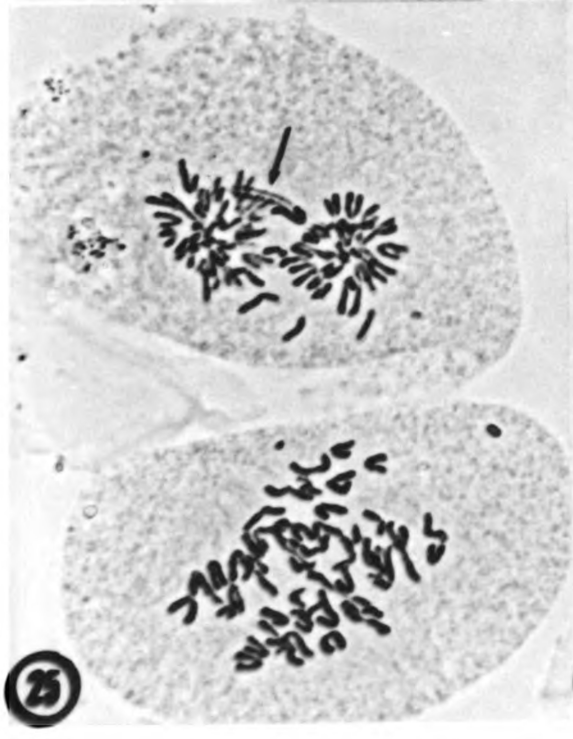
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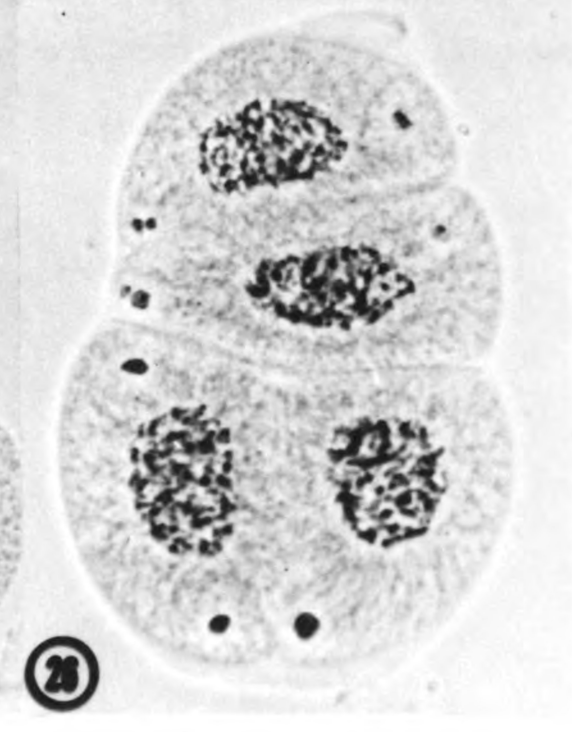
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Plate VI AHV 442-2

Figure 27. Unequal segregation at metaphase I.

Figure 28. Unequal segregation at Anaphase I.

Figure 29. Irregular cytokinesis with chromosome bridge.

Figure 30. Three distinct groups with two spindles at metaphase I.

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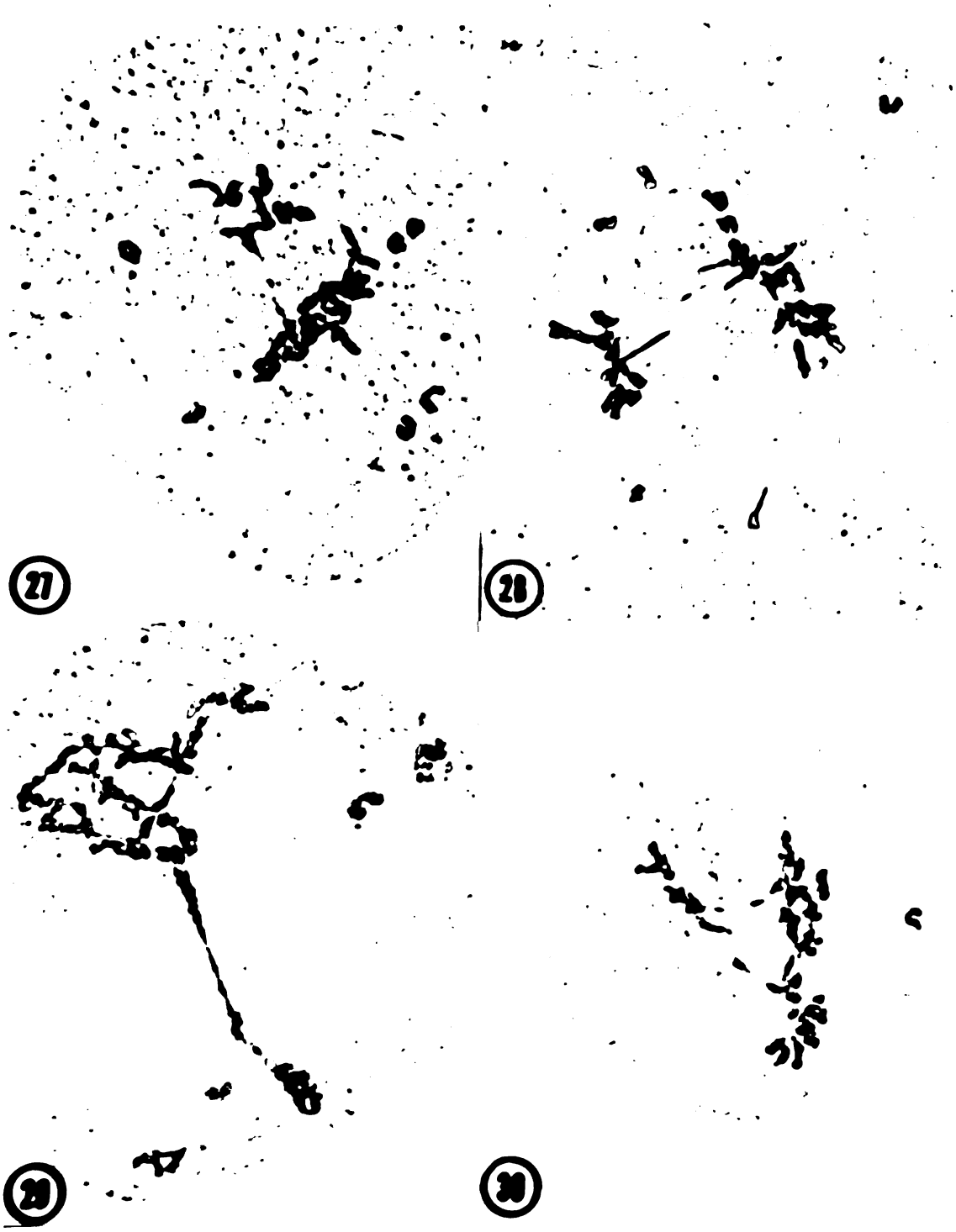
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Plate VII AHV 442-2

Figure 31. Irregular cell division with four chromosomes in one cell.

Figure 32. Microspore with three chromosomes.

Figure 33. Multipolar cell division having more than four tetrads and an irregular chromosome number in microspore.

Figure 34. Rare stainable pollen in anther tissue.

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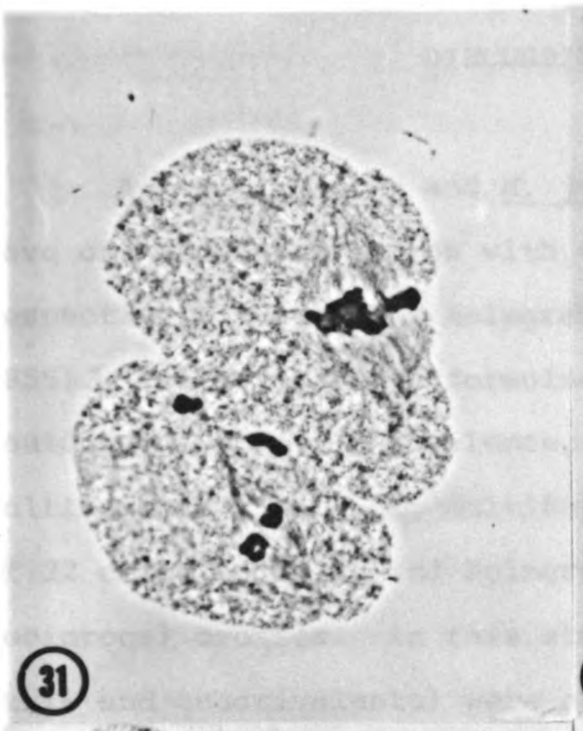
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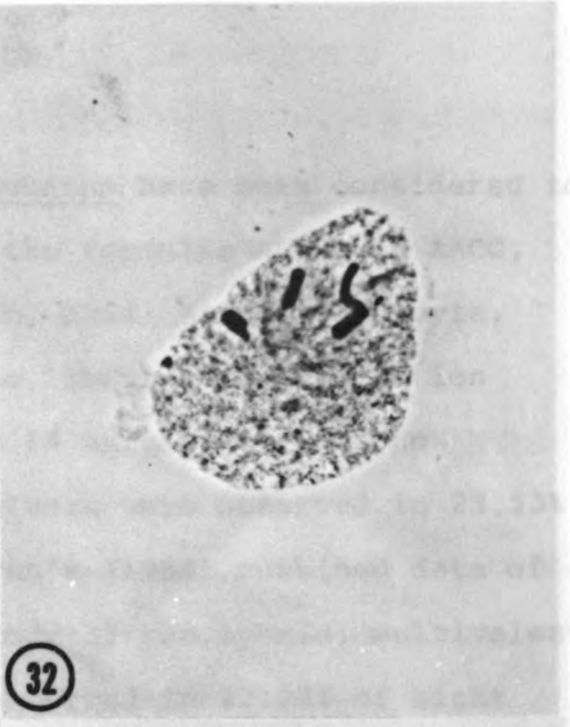
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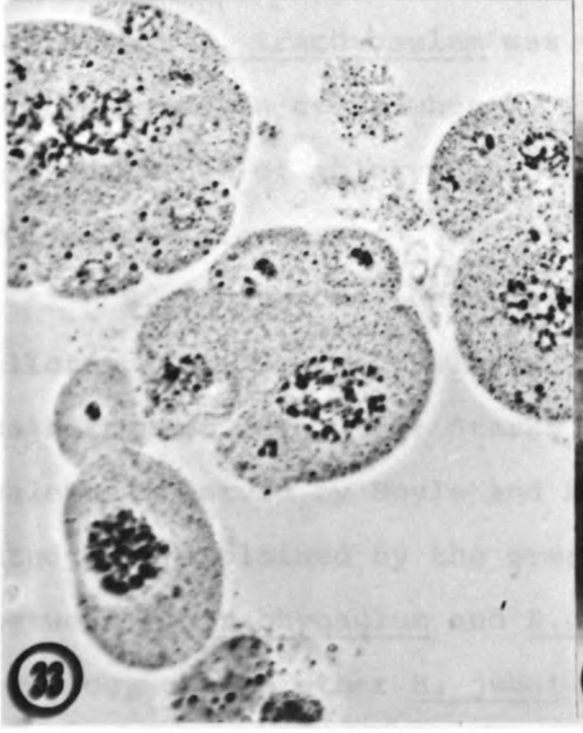
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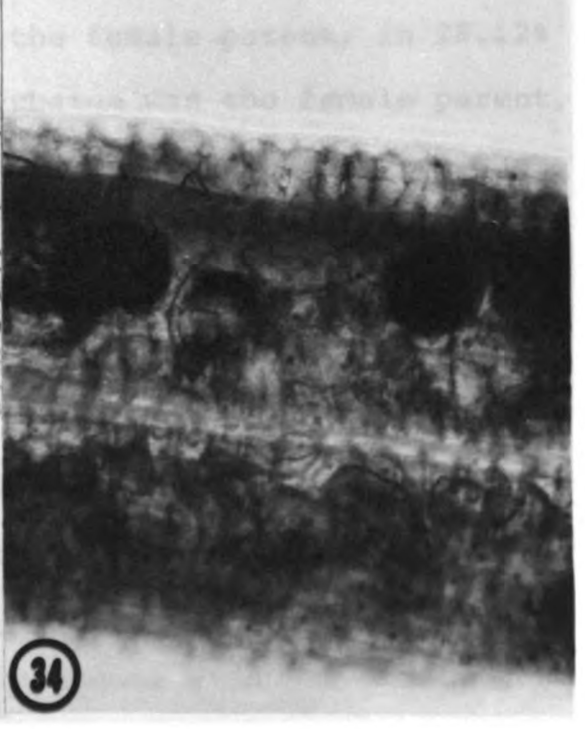
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DISCUSSION

A. trachycaulum and H. jubatum have been considered to have one genome in common with the formulae AABB and AACC, respectively (Boyle and Holmgren, 1954; Ashman and Boyle, 1955). Following these formulae, chromosome association would range from 1-7 bivalents, 14 univalents with no multivalents. In fact, multivalents were observed in 23.53% of 22 cells in Boyle and Holmgren's (1954) combined data of reciprocal crosses. In this study of the hybrid, multivalents (tri- and quadrivalents) were observed in 22.22% of eight cells when A. trachycaulum was the female parent, in 28.12% of twenty-seven cells when H. jubatum was the female parent, and in 27.55% of thirty-five cells in combined data of the reciprocal crosses.

H. jubatum has been considered to be a segmental allotetraploid by several authors (Wagenaar, 1959, 1960; Rajhathy, et al., 1964; Starks and Tai, 1974). The multivalents observed by Boyle and Holmgren (1954) and in this study are explained by the presence of a common genome between A. trachycaulum and H. jubatum and by the segmental homology of the other H. jubatum genome with the common genomes. The remaining A. trachycaulum genome has no homology with the other three genomes and consistently shows a minimum of seven

univalents in the hybrids. Therefore, the formulae, AABB and AACC given by Boyle and Holmgren (1954) were ruled out; and the formulae, $A_jA_jA'_jA'_j$ and A_jA_jTT, were assigned in this study for H. jubatum and A. trachycaulum respectively. If trivalents are formed by the $A_jA_jA'_j$ association and range from 1-7, the corresponding range of univalents is from 7-13. Quadrivalents may be formed by $A_jA_jA'_j$ with a range of 1-3 and univalents, 8-12. In special cases, $A_jA'_j$ of 1-7 bivalents and 14-27 univalents may occur. Starks and Tai (1974) suggested the genome formula, $A_jA_jA'_jA'_j$ for H. jubatum, and proposed that chromosome pairing at a single dosage, $A_jA'_j$ in the hybrids shows homologous pairing with approximately seven bivalents observed, while at double dosage, $A_jA_jA'_jA'_j$ shows homologous pairing with seven bivalents consistently observed in H. jubatum and no increase in the number of multivalents in the amphiploid.

In addition, autosyndetic pairing of the Agropyron chromosomes was found in hybrids of Agropyron species with Secale cereale (Stebbins and Pun, 1953), and in intergeneric hybrids of Agropyron species with Hordeum species, a modified Hordeum genome apparently occurs in Agropyron as well as in Elymus and Sitanion (Dewey, 1971). A. trachycaulum may possibly be segmentally homologous with the formula $A'_tA'_tA''_tA''_t$ instead of a typical allotetraploid AACC, because more than seven bivalents were observed in both spontaneous and artificial hybrids. Each $A_jA'_j$ and $A'_tA''_t$ homologous pairing under single dosage contributed more than seven bivalents in both the spontaneous

and artificial hybrids. A_t' is believed to be partially homologous with A_jA_j' of H. jubatum, while A_t'' is not. Capital "A" and its apostrophes refer to partial homology of the genomes whereas the subscripts (j) and (t) represent where the genomes are found and do not signify the origin of the chromosomes. When the chromosome number is doubled as found in the amphiploid, complete genomes are formed in terms of gene dosage. Bivalent associations are expected to prevail over multivalent ones under double gene dosage influences. But multivalents rarely resulted if autosynthetic pairing occured between A_jA_j' or $A_t'A_t''$ instead of allosyndetic pairing between A_jA_j' and A_t' and ranged from 0-3 per cell in both this and Boyle and Holmgren's study.

In the reciprocal crosses between A. trachycaulum and H. jubatum, morphological differences were observed. The hybrid of H. jubatum as female parent showed a much slower growth rate, was a relatively paler green, was shorter in height, and had a smaller flag leaf and spike length. Based on these observations supplemented with cytological observation of chromosome behavior, A. trachycaulum was assumed to be the female parent of the natural hybrid, Agrohordeum macounii collected from Alaska.

The amphiploid has been successfully crossed with both diploid and tetraploid types of cultivated H. vulgare. However, from the viewpoint of crop breeding, it is still too early to compare the relative ease and potentiality of combining A. trachycaulum and H. jubatum genomes with the single genome of H. vulgare versus a direct cross of H. vulgare X H. jubatum.

Superficially, combined genomes of two wild sources should contribute more variability and complementary stability through a backcross to the recurrent H. vulgare. In point of fact, we have been unable to obtain these backcrosses. On the evolutionary dimension, cultivated H. vulgare might represent an opposite evolutionary current from its wild species. Inserting the related third genome might neutralize incompatible factors which upset the genome balance between H. vulgare and its wild relatives and prevent the driving out of the wild chromosomes too rapidly. For example, chromosome elimination at certain stages occurred after fertilization in the hybrid of H. vulgare crossed with its wild relative, H. bulbosum, and abruptly resulted in haploid H. vulgare progeny (Lao & Kasha 1969, Kasha & Kao 1970, Ho 1973). An analogous case was also reported in the genus, Nicotiana (Gupta & Gupta, 1973). Based on cytological observation, three distinct groups of chromosomes with two or more spindle formations occurred. It is evident that H. vulgare characteristics were also introduced in the hybrid growth habit. Because of taxonomic classification, the A. trachycaulum chromosomes would be expected to be driven out before H. jubatum in any backcross to H. vulgare. The manipulation required to gradually substitute A. trachycaulum chromosomes and/or H. jubatum chromosomes into cultivated H. vulgare may be considerable. For instance, Triticale is a newly synthesized amphiploid with a constitution of AABBRR resulting from the cross of durum wheat and rye. By means of the new Giemsa staining technique for individual chromosome

identification, it was found that two pairs of the Secale chromosomes were substituted for two pairs of the D genome from common wheat (Gustafson and Zillinsky, 1973).

On the other hand, it is possible to shorten the time for breeding improvement if translocation(s) between the H. vulgare genome and its wild relatives can be induced by irradiation before the wild genomes are driven out. Perhaps, this would provide for the release of the desirable gene from its unfavorable linkage.

In addition, cytoplasmic effect should not be neglected. The chromosomes in the nucleus can be replaced through a backcrossing, but the cytoplasm remains more or less constant. It has been our experience in this laboratory that H. jubatum cytoplasm induces male sterility in (H. jubatum X H. compressum) X H. vulgare[2]. For this reason using H. vulgare as female in a cross with the amphiploid will provide a harmonious cytoplasm for the H. vulgare chromosomes after several recurrent backcrosses to H. vulgare.[2]

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[2]Personal communication with Dr. J.E. Grafius.

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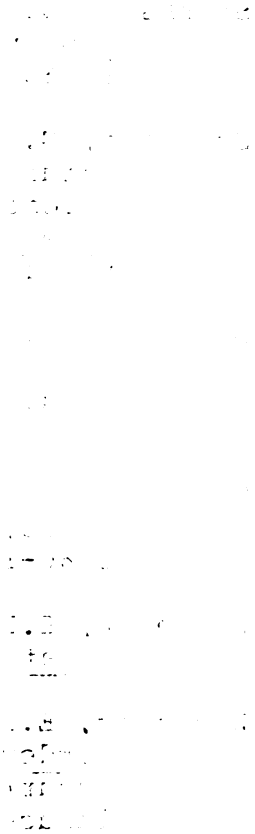

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APPENDIX

APPENDIX I

Liquid media for embryo culture.

Ingredients	Amount (gm/litre)
Na_2SO_4	.8
$CaNO_3 \cdot 4H_2O$	.58
$MgSO_4 \cdot 7H_2O$	.33
KNO_3	.08
KCl	.005
$NaH_2PO_4 \cdot H_2O$	.038
Micronutrients:	1000x
$MnSO_4$	.45
$ZnSO_4$	.6
H_3BO_3	.00375
KI	.03
Glycine	3.0
Thiamine HCl	.1
Calcium pantothenlate	2.5
$CaSO_4 \cdot 5H_2O$	.025
$NaMoO_4$	.025
$CoCl_2 \cdot 6H_2O$	.025

1. The major solution plus 1 ml. micronutrients, 10 ml. iron and 20 gms. sucrose.
2. Adjust the PH to 4.5 for young embryos and 5.4 for old embryos.