HYBRIDIZATION OF BARLEY (HORDEUM VULGARE L. EMEND. LAM.) WITH ITS WILD RELATIVES

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ABSTRACT

HYBRIDIZATION OF BARLEY (*HORDEUM VULGARE* L. EMEND. LAM.) WITH ITS WILD RELATIVES

By

Robert Parker Steidl

Hybrids with *Hordeum jubatum* L. (4x) and the *H. jubatum* amphiploids *H. jubatum* X *H. compressum* Griseb. (6x) and *Agropyron sericeum* Hitchc. X *H. jubatum* (8x) were made to transfer to barley (*H. vulgare* L. emend. Lam.) economically useful improvements from *H. jubatum* in these traits: winterhardiness, resistance to the cereal leaf beetle (*Oulema melanopus* L.), tolerance to salty soils and tolerance to wet soil conditions. To transfer these traits, attempts were made to produce an intermating population containing the desired variation from the wild species, expressed in the genetic background of commercial barley. Recurrent selection and intermating could be practiced in this population until transfer of the desired variation was achieved.

Chromosome elimination and cytoplasm from H. jubatum were both instrumental in blocking production of this intermating population by standard crossing methods. Cytoplasm from H. jubatum conferred gross lack of vigor and male sterility to H. vulgare derivative lines. Chromosome elimination greatly reduced the frequency of production of certain genomic constitution hybrids. Chromosome elimination also was shown to be the most logical explanation for the complete loss of wild chromosomes in second H. vulgare backcross progenies of (HJ X HC)(6x) X VV. Thus, it appeared chromosome elimination partially to completely blocked production of certain genomic constitution hybrids and backcross progenies. Problems with the H. jubatum cytoplasm were circumvented by making the hybrids with H. vulgare as female.

A different approach was undertaken, utilizing chromosome elimination in conjunction with callus culture and other techniques to produce the desired parental material for the intermating population. This would either substitute for or be a part of the more traditional backcross method of producing the desired parental material.

This approach involved several intervening steps with the eventual production of a population consisting of plants with different chromosome number and chromosome constitution. Then, hopefully, selection of the desired parental material could be made. If not, the most promising material could again be run through the intervening steps of callusing, subculturing and regenerating large numbers of plants to eventually produce the desired parental material.

All intervening steps were worked out so that production of large numbers of plants by callus culture of one hybrid have already been achieved. Thus, I am confident that by taking advantage of chromosome elimination, techniques including tissue culture can eventually produce the desired parental material.

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By

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TABLE OF CONTENTS

TABLE OF CONTENTS (cont'd.) Page

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Varietal improvement can only be achieved by utilizing useful genetic variability. This variability can be obtained from induced mutations or by introgression within or between biological species. Induced mutations have provided only a relatively minor portion of varietal improvements.

Variability within a crop's biological species is usually readily transferable. Transfer of a single gene into the desired genetic background is most easily achieved by the backcross method of plant breeding. Transfer of quantitatively inherited improvements is much more effectively achieved by recurrent selection and intermating. If the appropriate genetic background is partially fixed by one or two backcrosses, then selection can be mostly limited to the desired trait for transfer.

The basic approach is the same for transfer from related species. That is, transfer of a single gene is usually most easily achieved by the backcross method while

transfer of a quantitatively inherited improvement is usually most successfully achieved by selection in an intermating population containing the desired trait expressed in the crop's genetic background. Plants with the desired chromosomes or translocations from the donor parent can be more easily recognized during the selection phase of cyclic intermating and fertility is generally high enough so the next cycle can be produced.

The greatest difference in transfer of a quantitatively inherited improvement from a related species as opposed to the same species involves problems in obtaining the desired intermating population. These problems include everything from greater sterility and lower recombination in hybrid* and backcross generations to completely unexpected phenomena operating in the material. Cytogenetics can be an extremely useful tool in coming to an understanding of these phenomena and in determining the best approach to deal with them.

Ease of transfer from a related species is determined by the closeness of species relationship and the

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*Throughout this thesis, the term "hybrid" refers to a species hybrid.

ploidy level of the recipient species. Closely related species have greater chromosomal pairing and more similar functions of corresponding genes. Thus recombinant chromosomes will tend to produce changes in a smaller number of gene products and the changes will result in smaller deviations in enzymatic products. Slight deviations in production of enzymatic products for relatively few genes usually result in relatively minor imbalances in gametes and zygotes. Polyploids from closely related species have repeated copies (one copy per chromosome set) of essentially the same gene. Thus, in polyploids from closely related parents chromosome changes such as addition, substitution and recombination will result in smaller changes in production of enzymatic products.

Diploids are generally weakly buffered. The vast majority of genes producing essential enzymes are not repeated throughout the genome. Thus each enzyme and its corresponding gene must perform its function at the correct rate for the biological system. Translocation of a noncorresponding chromosome piece, when fixed, results in essentially four copies of some genes and no copies of other genes. This type of translocation has little chance

of survival because it results in biologically profound changes in the enzymatic rates of all enzymes involved. Transfer of a short and corresponding chromosome piece will hopefully be within the buffering capacity of the diploid species.

Transfer of traits requires production of hybrids and subsequent generations with transfer at some point. Many hybrids between species of the Triticeae (Triticum, Aegilops, Hordeum, Secale, . . .) have been produced. Generally these hybrids were relatively easily obtained. But production of specific combinations can not be effectively predicted, because phenomena such as chromosome elimination, ploidy level and inhibition of fertilization determine ease of production of certain hybrids. Thus certain combinations can be difficult to obtain.

Dr. J. E. Grafius chose to use a H. jubatum-H. compressum amphiploid, which was generously supplied by Dr. A. B. Schooler, as the donating parent of H. jubatum traits to H. vulgare (Schooler, et al., 1966). H. jubatum a perennial tetraploid species, is native to much of the North American continent. H. compressum is a perennial diploid species native to South America.

The main objective of this work was to transfer useful levels of variability from H. jubatum for any of the following characters: winterhardiness, resistance to cereal leaf beetle, tolerance to salty soils, and tolerance to wet soil conditions. H. jubatum has very high levels of useful improvements for all these traits. Although extensive efforts have been made to find good resistance to the cereal leaf beetle within H. vulgare, only moderate levels have been found. Useful levels of variability for salt tolerance do exist, but are only moderate when compared with the tolerance of H. jubatum.

Secondary aspects of this study deal with various problems and techniques arising from attempts to transfer improvements from H. jubatum.

LITERATURE REVIEW

Transfer of genes is the major objective of this study, therefore it is important that we understand the most important factors controlling ease of transfer. These factors are ploidy level of the recipient parent and closeness of species relationship. Their importance to gene transfer can be better understood by thinking of their effect on the buffering capacity of a species (Harlan, 1966). Closely related species generally have reasonable levels of pairing and corresponding genes on the chromosomes perform the same function at essentially the same rate. Thus, transfer in closely related species generally involves a very small translocation which performs essentially the same functions at the same rates.

When the parents of a polyploid are closely related, there are repeated copies (one copy per chromosome set) of essentially the same gene. Thus polyploids from closely related parents are much more buffered against biologically important changes in enzymatic rates.

Hexaploid wheat is a beautiful example of the greater buffering capacity which polyploidy confers. Comparison of hexaploid wheat, Triticum aestivum, with its diploid progenitors T. monococcum (AA), Aegilops speltoides (BB), and A. squarrosa (DD) and its intermediate progenitor, tetraploid T. turgidum (AABB) provides an opportunity to compare the buffering capacity of polyploid levels of this polyploid series.

All members of this series have only bivalent formation due to genetic suppression of homoeologous pairing. Suppression or removal of 5BL (gene causing the greatest suppression of homoeologous pairing) allows considerable multivalent formation at the tetraploid and hexaploid levels (Riley and Kempanna, 1963). Species crosses at the diploid level when 5BL is either not present or suppressed have considerable bivalent formation. Based on pairing data, these species are relatively closely related.

Chromosomes of these three genomes (AA, BB, and DD) can be grouped into seven sets of three chromosome pairs. The degree to which one of the three homoeologous chromosome pairs can compensate for another pair of the

homoeologous group also indicates the degree of chromosome relation. If considerable compensation occurs, then the homoeologous chromosomes have largely the same genetic content.

Lilienfeld (1951) showed substitution did not occur at the diploid level between the A and B genomes. Substitution lines at the tetraploid level were difficult to obtain and there was good reason to assume many of the substitution chromosomes were actually recombinant chromosomes (Sears, 1969).

At the hexaploid level there was enough buffering capacity that the compensation potential of all 42 possible homoeologous substitution lines (e.g. 1A tetrasomic, 1D nullisomic) could be observed (Sears, 1966). Compensation was measured by comparing the substitution line with its corresponding nullisomic line. Compensation ranged from some restoration of vigor but no restoration of fertility, to near normal size, vigor and fertility of all six homoeologous substitution lines of group III.

Although pairing configurations showed these species were fairly closely related, they diverged to

the extent that considerable buffering was needed to allow reasonably easy chromosome substitution.

A more dramatic expression of the substitution potential of hexaploid wheat was demonstrated by the numerous intergeneric substitution lines. Disomic substitutions of rye homoeologous groups 1, 2, 3, 5, and 6 chromosomes were made for part or all of their corresponding homoeologous chromosomes (Sears, 1968; O'Mara, 1947; Riley, 1965; Lee, et al., 1969; Mettin, et al., 1973). Compensation was generally good. The commercial varieties 'Saladin,' 'Orlando,' 'St 2153/63,' 'Zorba' and 'Salzmunder' are 1R/1B substitution lines (Zeller, 1973). 1R imparts high resistance to rust and moderate resistance to powdery mildew.

Disomic substitution of Agropyron elongatum homoeologous chromosome groups 1, 3, 4, 5, 6 and 7 have been produced (Sharma and Knott, 1966; Larson and Atkinson, 1972; and Dvorak and Sosulski, 1974). Compensation was very good. Disomic substitution lines with several other species have been produced.

Monosomic and disomic addition or substitution lines are very useful in transferring traits because

they provide continued opportunity for producing recombinant chromosomes. Most of the early transfers also utilized irradiation to break chromosomes. Transfers utilizing irradiation include leaf rust resistance from Aegilops umbellulata (Sears, 1956), stem rust resistance from Agropyron elongatum (Knott, 1961), leaf rust resistance from Agropyron elongatum (Sharma and Knott, 1966), leaf rust and powdery mildew resistance from rye (Driscoll and Jensen, 1963), leaf rust resistance from Agropyron intermedium Wienhues, 1966), and leaf rust resistance from rye (Mukade, et al., 1970). All of these are well documented.

Transfer has also been achieved from rye by natural translocations (Driscoll and Sears, 1963; and Mettin, et al., 1973). These translocations involve 4A/2R and 1B/1R. Natural breakage and transfer is the most likely explanation for both of these lines, because wheat and rye chromosomes do not appear to pair even with 5BL suppressed (Riley and Kimber, 1966). Furthermore, most of the chromosomal translocations in the literature appear to be of this type. Larsen (1973) showed that of the twenty reported chromosomal translocations in wheat only three involved homoeologous chromosomes. Thus stable interspecific addition and

substitution lines can be very useful in obtaining the desired transfers even without any appreciable pairing.

Recently, transfer from species with some pairing affinity with wheat has been very successfully achieved by interfering with the genetic control over homoeologous pairing. Riley (1968) used genetic suppression of 5BL to transfer yellow rust resistance of Aegilops comosa to wheat. Athwal and Kimber (1972) showed genetic suppression of 5BL could be used to transfer traits from Aegilops umbellulata. Sears (1973) used nullisomic 5B plants to transfer leaf rust resistance from Agropyron elongatum chromosomes to their homoeologous wheat chromosomes. Many 3Ag/3D and 7Ag/7D transfer lines were produced. For both translocation types, male transmission was excellent for some lines. Pairing of 3Ag with 3D and of 7Ag with 7D was about 30% and a corresponding percentage of the progenies had translocations.

These results are so spectacular that it would seem reasonable to conclude transfer from many other more closely related species could be just as easily achieved by utilizing similar methods.

Hexaploid wheat's highly buffered genetic system can handle considerable genetic manipulation. Thus transfer from species like rye, with essentially no pairing homology, is possible. The weak buffering of diploids is demonstrated by the failure of fairly extensive attempts to transfer traits from wheat to rye (Deorikar, 1963).

Attempts to transfer traits to H. vulgare is another good example of fairly extensive efforts to transfer traits to a diploid species. No properly verified examples of transfer to H. vulgare exist in the literature. The only possible examples of transfer are from H. murinum (Hamilton, et al., 1955) and from H. bulbosum (Schooler, 1964). Transfer of improvements in any one of several traits to H. vulgare could result in substantial improvement in overall productivity of the crop.

Many of the previous attempts to transfer improvements to H. vulgare have been made with H. jubatum as the donor parent. The main objective of these previous workers was to transfer improved levels of winterhardiness from H. jubatum. H. jubatum has excellent winterhardiness. Transfer of winterhardiness would result in

a large increase in overall productivity of this crop, because a winter hardy barley can utilize considerably more of the environmental resources than spring barley. Improvements in winterhardiness within H. vulgare have plateaued and little progress has been achieved in the last 10 years. (Personal communication, Dr. J. E. Grafius)

The H. jubatum-H. vulgare cross was first made by Quincke (1940). Since then several researchers--Morrison, et al. (1959), Vinogradova (1946, cited in Smith, 1951, and Price, 1968), and Kerber (unpublished)--have successfully repeated this cross, but none have gotten beyond the high levels of F_1 hybrid sterility. Cytogenetic results of Kerber's cross were cited by Wagenaar (1960).

My crosses all have H. jubatum or a H. jubatum amphiploid as one of the parents. H. jubatum, like wheat, is a member of a polyploid series (Rajhathy and Morrison, 1961). Relatively little work has been done on this polyploid series. When using polyploids either as the recipient or donor species, the possibility of genetic control over homoeologous pairing must be seriously considered. Murry (1975) found essentially no pairing in the hybrid

from the cross diploid H. vulgare X H. jubatum. This could be caused by either genetic control over pairing, no homology, or physiological problems inherent in this hybrid.

Some data exist on the pairing homology of the species in the H. jubatum amphiploids I used. Starks and Tai (1974) found H. compressum has some homology with H. jubatum. H. jubatum also has some homology with one of the genomes of Agropyron sericeum (Murry, 1975). The only species reported to have some pairing with the biological species H. vulgare is H. bulbosum (Kasha and Sadasivaiah, 1971).

MATERIALS AND METHODS

I. PLANT MATERIALS

The stock of the Hordeum jubatum X H. compressum amphiploid was obatained from Dr. A. B. Schooler. The natural hybrids Agropyron trachycaulum X H. jubatum and A. sericeum X H. jubatum were collected in Alaska by Dr. J. E. Grafius with help from Dr. W. W. Mitchell and Dr. R. Taylor of the Alaska Experiment Station Staff (Table 1).

II. METHODS OF HYBRIDIZATION

Good growing conditions must be maintained. Hybrids are much more successfully produced from vigorously growing parental material. Frequent repotting and high fertility levels are very helpful in maintaining good vigor. The most vigorous growth was obtained with daytime temperatures between 15C and 29C. Hybrid material in H. jubatum cytoplasm required warmer conditions (generally between 22C and 29C) for most vigorous growth.

TABLE 1. The designation and source of plants.

PLANT	DESIGNATION	SOURCE
Hordeum jubatum X *H. compressum* amphiploid	(HJ X HC)(6x)	Selfed seed from Dr. A. B. Schooler, North Dakota State U., Fargo, N.D.
Agropyron trachycaulum X *H. jubatum* (spontaneous hybrid)	AT X HJ	Clone from Dr. W. W. Mitchell, Palmer, Alaska
A. sericeum X *H. jubatum* (spontaneous hybrid)	AS X HJ	Clone from Dr. W. W. Mitchell, Palmer, Alaska
Hordeum jubatum	HJ	Seed from Dr. W. W. Mitchell and Dr. R. Taylor, Palmer, Alaska
Hordeum vulgare (tetraploid)	VVVV	A. B. Schooler, North Dakota State U., Fargo, North Dakota
Hordeum vulgare (diploid)	VV	Several varieties, bulks and populations from Dr. J. E. Grafius, Michigan State University, East Lansing, Michigan

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In some crosses temperatures above 26C were detrimental to good hybrid seed development. When using H. vulgare as the male parent, night temperatures of 15C or below were necessary to allow the spike to protrude from the boot before the pollen was shed.

Procedure Used When Female Is Male Sterile

1. Healthy heads of the female parent with some open florets were selected. Florets were clipped, removing 1/3 to 1/2 of the lemma and palea. Several heads were then grouped together and loosely wrapped with aluminum foil. If possible, the flag leaves were kept outside the aluminum foil; otherwise, the flag leaves were removed. When necessary, the head wrapped in aluminum foil was supported with an iron rod.

2(a). When H. vulgare was used as the male parent, spikes with undehisced yellow anthers were selected. Florets were clipped, removing 1/3 of the lemma and palea, and crosses made by the approach method (Curtis and Croy, 1958) or the anthers were allowed to fully extrude and pollen was disseminated by gently tapping the spike of the male parent above the female.

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2(b). When wild species were used as the male parent, spikes with freshly extruded anthers were gently tapped above the female. This required luck in guessing when anther extrusion would occur. Clipping of florets to cause anther extrusion met with little success. Abundant fresh pollen was obtained by cutting spikes with a few extruded anthers and storing with stems in water at 5C for two days. After storage, spikes were transferred to the greenhouse in a cold (5C) box. Rapid anther extrusion occurred after removal of spikes from the box.

3. Greatest success was achieved when plants were pollinated with massive amounts of pollen for two or three days. Good success in producing hybrid or backcross seed required much higher pollen loads than species selfing.

Procedures Used When the Female Was Male Fertile

1. Healthy spikes, which were to be used as the female, with yellow-green anthers were selected. The older the spike, the greater the chance of selfing, but emasculation and drying out caused much more injury to younger spikes. Spikes with pollen shed during emasculation or having several obvious selfed seeds were usually

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discarded. This precaution and the obvious morphological differences between interspecific hybrid and selfed seed helped avoid mistaking a selfed seed for a hybrid seed during embryo transfer.

In all the species crosses, selfed seed had plenty of starch deposition before endosperm collapse occurred in hybrid seed. At this time, hybrid seeds usually took on a shrunken or thinner appearance from reduced starch deposition. Hybrid seed with H. vulgare as the female remained plump with a watery sac-like endosperm with little or no starch deposition.

2. Each floret was gently opened by holding the tip of the palea with the thumb and using tweezers to pull open the lemma. Anthers were then removed. Usually the bottom and top spikelets were too immature and were removed. In material with more than one floret per spikelet, e.g. (AS X HJ)(8x), all florets past the second were removed.

3. Crosses were made when the female stigma was feathery (Figure 1B). Sometimes this was the same day as emasculation. Florets either opened on their own or were gently opened before pollination.

FIGURE 1.--B2.2 spike, crossing technique, B2.2 seed and flat with plants.

A. B2.2 plant with approximately 40% self-fertility

B. Emasculated spike of (HJ X HC)(6x) with open receptive florets

C. Crossed spikes wrapped in aluminum foil

D. B2.2 seed

E. Flat of colchicine treated 69-101

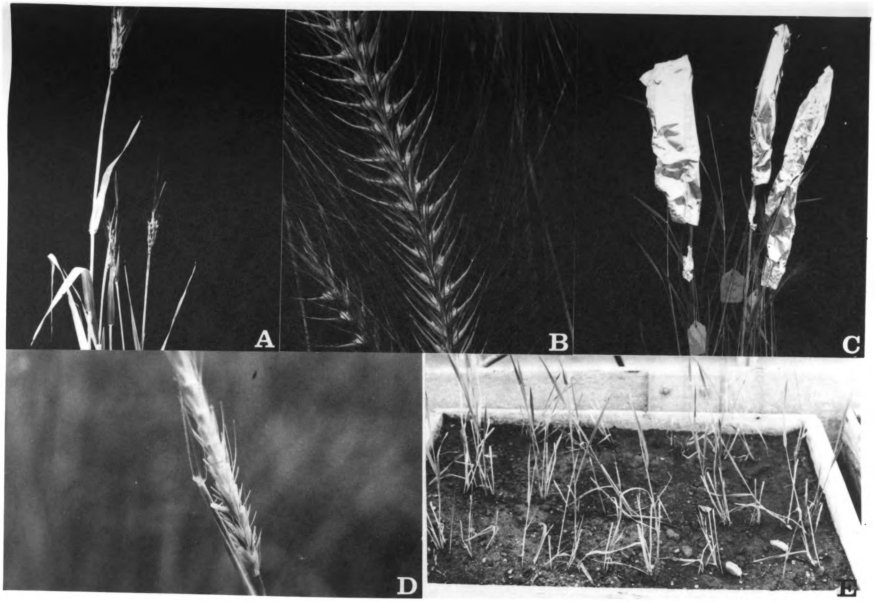

Figure 1

III.

III. EMBRYO CULTURE MEDIA

Embryo culture should allow normal embryonic growth and differentiation; then normal germination should occur. Normal development and germination are two separate stages. At present, the optimum media for normal development of the two stages is quite different. I had no particular success with transferring embryos to a second medium to optimize development in both stages. Thus the best results were obtained by using a wick medium system* for well differentiated embryos and Norstog's Medium II (Norstog, 1973) for poorly differentiated embryos (See Tables A and B of the appendix for a complete listing of chemicals in these media.) Embryos near completion of development (having a well-developed scutellum) were very successfully grown on a wick medium (Figure 2D), while embryos with poor development could only be successfully grown to mature plants on Norstog's Medium II. Even Norstog's medium resulted in a low production of mature plants from poorly differentiated embryos. No mature plants were produced by embryos lacking in differentiation (proembryos). Both media were moderately successful

*Wick medium system is hereafter referred to as wick medium.

FIGURE 2.--Embryo culture.

A. Chung Lee's material on wick medium with cotton stopper wrapped in aluminum foil.

B. Chung Lee's transfer technique; 1/2 caryopsis placed on wick medium.

C. Seedling of [(HJ X HC)(6x) X HJ] X VVVV near death.

D. B2.2 seedling on wick medium.

E. HJ X VV seedling with tillers, auricles and roots on agar medium.

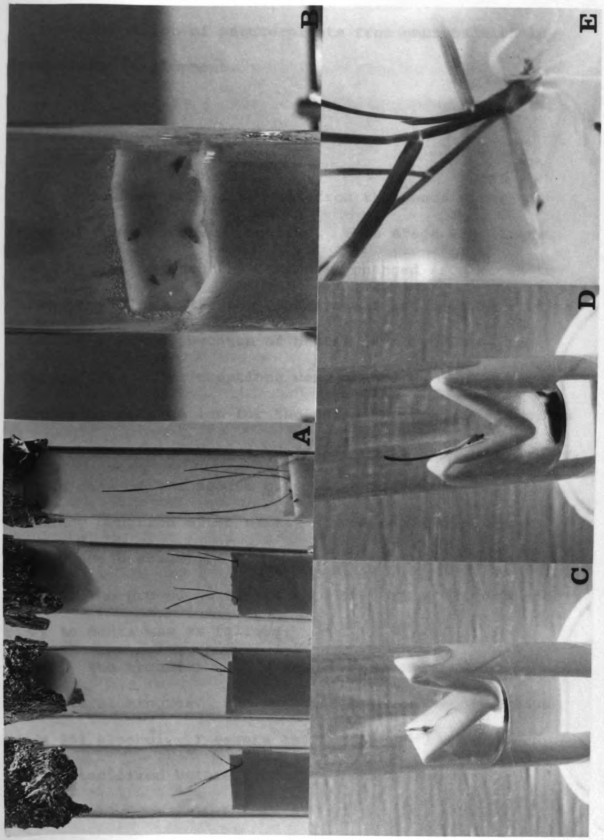

Figure 2

in the production of mature plants from embryos with intermediate development.

IV. EMBRYO CULTURE

Embryos were dissected from the seeds after collapse of the endosperm occurred. This stage was determined by noting when the caryopsis changed from green to white or yellow. The best success was achieved by waiting until this stage, because of better development of the embryo. The only exceptions were caused by dormancy and chromosome elimination for the second H. vulgare backcross of (HJ X HC)(6x) X VV and VV X (AS X HJ)(8x), respectively. In the case of the second H. vulgare backcross, embryo dormancy occurred after normal starch deposition began (early dough stage).

The procedure of excising the embryo and transferring to media was as follows:

1. The transfer hood was turned on and allowed to run for several minutes. The transfer area was sponged down with 95% alcohol. Tweezers and dissecting needles were flame-sterilized before transfer of each embryo.

2. Seeds were surface sterilized by immersing for 2 minutes in a 20% Clorox solution. When possible, the lemma and palea were removed before sterilization. If not, they were removed in the first step of dissection of the embryo.

3. The embryo was dissected out under a dissecting microscope with a tweezer in one hand and a dissecting needle in the other. The embryo is at the basal end of the seed on the lemma side. Thus the most effective method of dissection was to hold the distal half of the seed in the tweezers with the lemma side facing up and carefully slit the lateral side of the seed, starting in the middle and working toward the basal end. The embryo would usually then pop out. If it did not, it could be easily found just below the seed coat at the basal tip of the seed on the lemma side. The location of the embryo and its distinctive appearance make even very small embryos easy to find after a little practice.

4. The excised embryo was then transferred to the culture medium. This required flaming the culture bottle mouth after removing the foam stopper and again just before reinserting the stopper. To transfer onto wick

medium required careful orientation of the embryo with the scutellum facing the surface of the filter paper and the radicle oriented downward. Failure to orient the radicle correctly can be corrected later, but incorrect orientation of the scutellum is fatal. On agar media, either the embryo was placed on the medium surface and oriented with the scutellum facing down or the embryo was submerged slightly and orientation ignored.

V. CHEMICAL TREATMENT OF PLANT MATERIAL

Colchicine Treatment

Due to the high levels of hybrid sterility, use of chromosome doubling agents is common in interspecific crossing programs. For doubling purposes I chose to use nine different colchicine treatments. Treatments one and two were the method Wellensiek (1947) used and a modification of it, respectively. (1) Near single-tiller divisions were alternately immersed (dipped) in a .05% colchicine solution during daytime and in water at night for four consecutive days; and (2) several consecutive daily sprayings of leaves of vigorously growing clones were

done using a .05% colchicine solution with 4% DMSO (dimethyl sulfoxide, a surfactant).

Treatments three through nine were all modifications of Pope and Love's (1952) treatment. In these treatments washed tiller divisions were dipped in .5% colchicine. Plants were wounded by making a short slit in the leaf base near the meristem with a dissecting needle. These treatments were:

	TREATMENT	TIME
(3)	nonwounded root dip	6 hours
(4)	wounded root dip	6 hours
(5)	nonwounded root dip	12 hours
(6)	wounded root dip	12 hours
(7)	horizontal treatment on paper towels with roots protected from colchicine	7 hours
(8)	wounded horizontal treatment	7 hours
(9)	inverted plant dip with roots protected from drying with wet paper towels	12 hours

After colchicine treatment, divisions were planted in flats at the rate of approximately 200 per flat in three or four rows per flat (Figure 1E). Many treatments resulted in 50% mortality. A few hybrid seedlings were

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also colchicine treated using Pope and Love's method. The wick embryo culture medium was poured off at the two leaf stage and .05% colchicine solution added to the test tube. Seedlings were treated for 8 hours, the colchicine solution was carefully washed off, and the seedlings transferred to soil.

ε-Amino Caprioic Acid (EACA) Treatment

EACA is a relatively cheap, highly water-soluble animal-effective immunosuppressant. Bates, et al., (1974) concluded EACA appeared to be the most effective of four immunosuppressants tested at blocking a hypothetically similar immuno-system in plants. The immunosuppressants were injected daily into the leaf enclosing the spike starting before meiosis and ending with emasculation. In a personal communication, Dr. Bates suggested a modification for narrow leaf barley material. This modification, which I used, involved spraying spikes with 1 mg/ml EACA with .1% X-77 (a surfactant) from before meiosis to near transfer time. The surfactant was used to insure adequate uptake of the immunosuppressant.

Gibberellic Acid Treatment

Gibberellic acid (GA_3) treatment of the maternal plants during seed development was started by Larter and Enns (1960). Their objective was to maintain more normal post-fertilization development of the seed tissues of hybrids. They treated tetraploid H. vulgare crossed with diploid H. vulgare by clipping the flag leaf tip and placing the flag leaf in a shell vial with 100 ppm GA. Treatment was continuous from time of emasculation to embryo transfer. This treatment produced near-normal development of the endosperm and embryo, while untreated seeds were shrunken and had much smaller embryos.

Kruse (1967, 1969, 1973) found this method time consuming and not particularly effective. He placed one drop of 75 ppm GA on the stigma of each floret for each of the two days directly following pollination. Using this technique, he had remarkable success in producing interspecific and intergeneric hybrids. I modified this technique, because I found it extremely tedious for large numbers of crosses. Spikes were immersed once daily in 75 ppm gibberellic acid for a few seconds the next one or two days after pollination.

RESULTS AND DISCUSSION

The enormous phenotypic plasticity of weeds (Harlan, 1975) is very similar to the plasticity in the "weed" X cultivated barley crosses. Many of these plants vary from an extremely robust and large to a very small plant. Thus phenotypic criteria used in describing these plants must be sharply limited. I have, however, found these criteria useful: overall plant type and vigor in several environments, vigor in cooler environments, presence and relative size of auricles, relative size of lemmas and overall head type.

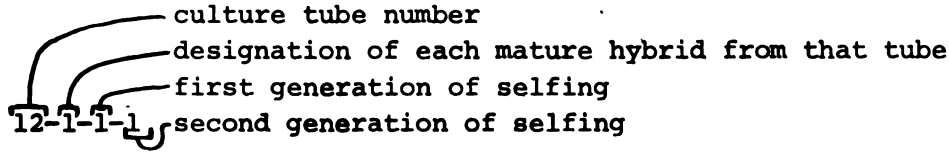
I. CROSSES MADE BY CHUNG LEE

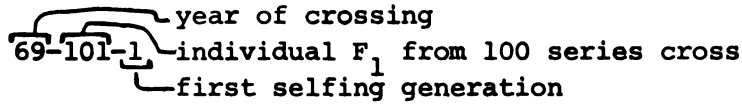
Chung Lee, a former graduate student of Dr. J. E. Grafius, crossed the H. brachyantherum X H. depressum amphiploid with diploid H. vulgare in the spring of 1969. No further work was done with this cross. In the spring of 1969 he also crossed the H. jubatum X H. compressum amphiploid with diploid H. vulgare (Table 2). His embryo

TABLE 2. Crosses made by Chung Lee.

PARENTAGE	HYBRID DESIGNATION	DATE CROSS MADE	PEDIGREE DESCRIPTION
(HJ X HC)(6x)X CI 12528	12-1*	March 1969	69-101**
	12-3		69-102
	12-4		69-103
(HJ X HC)(6x)X CI 12518	11-2	March 1969	69-104
	11-3		69-105
(HJ X HC)(6x)X CI 12518	14-3	March 1969	69-106
(HJ X HC)(6x)X Manchuria	16-1	March 1969	69-107
(HJ X HC)(6x)X Manchuria	18-1	March 1969	69-108
(HJ X HC)(6x)X CI 12518	10-1	March 1969	69-109
(HJ X HC)(6x)X CI 12518	26-1	March 1969	69-110
(HJ X HC)(6x)X Paragon	12-1/B_1	Dec. 1969	69-101-B1.2*** (abbrev.: B1.2

*12-1 stands for a particular mature F_1 plant





** 69-101-1

*** B1.2 is a simplified notation used by Mather and Jinks (1971) to denote a first backcross to parent two (BC_1P_2)

culture techniques are shown in Figure 2A,B. At that time he only cut the seed in half and didn't dissect out the embryo. This method is not particularly reliable and only produces mature plants from very well-developed embryos.

He was successful in backcrossing one of these hybrids, 69-101, to diploid H. vulgare. This clone, 69-101-B1.2 (abbreviated B1.2), is morphologically and cytologically similar to the "pentaploid" hybrid, 74-405, from (HJ X HC)(6x) X VVVV (Figure 3E and Table 8), which leads to speculation that B1.2 is the progeny from a normal H. vulgare pollen grain and either an unreduced egg or a doubled sector of 69-101.

Some of the F_1 hybrids produced selfed seed. In many cases, subsequent generations were essentially completely fertile. I grew plants of 69-101-1, 69-101-1-1, 69-101-1-1-1, 69-102-1, 69-105-1, 69-106-1, 69-106-1-1, 69-107-1, 69-107-1-1, 69-109-1, and 69-109-1-1.

Only 69-101-1 had any morphological differences from the H. jubatum-H. compressum amphiploid (Figure 3B). This plant was also extremely different cytologically. 69-101-1 had a highly variable chromosome number at metaphase I of meiosis and multipolar cell divisions in meiosis. Starks

FIGURE 3.--Spike morphology (left to right)

A. H. compressum

H. jubatum

(HJ X HC)(6x)

(HJ X HC)(6x) X HJ

B. 69-101-1

69-101-B1.2 (B1.2)

Spike and tiller with auricles of VV X (HJ X HC)(6x) hybrid

69-101

VV spike and tiller with clasping auricles

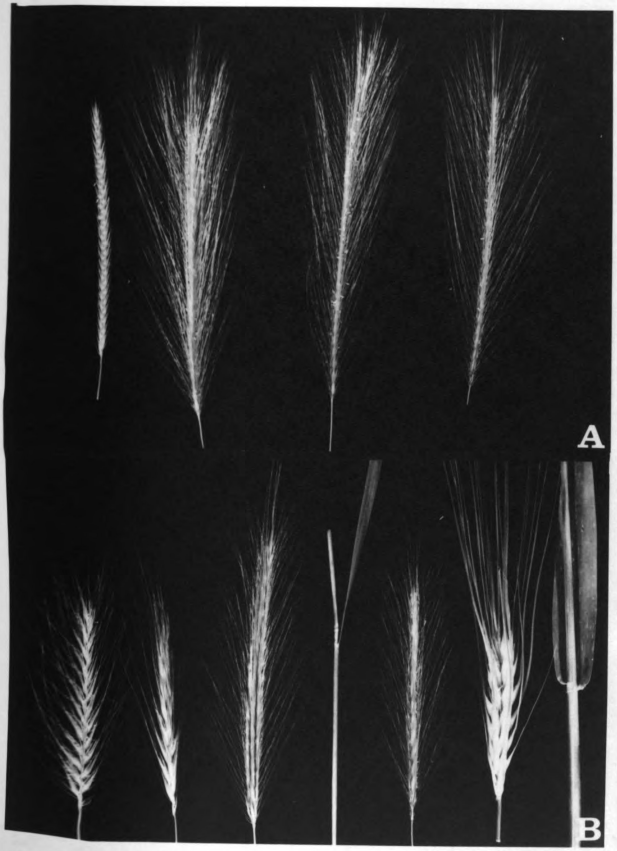


Figure 3 A,B

FIGURE 3. Spike morphology (cont'd.)

C. 69-101

69-101-B1.2 (B1.2)

Selfed progeny of B2.2

B3.2

D. VV X (HJ X HC)(6x)

Haploid H. vulgare spike from VV X HJ progeny

Normal spike of same progeny from VV X HJ

HJ X VV progeny

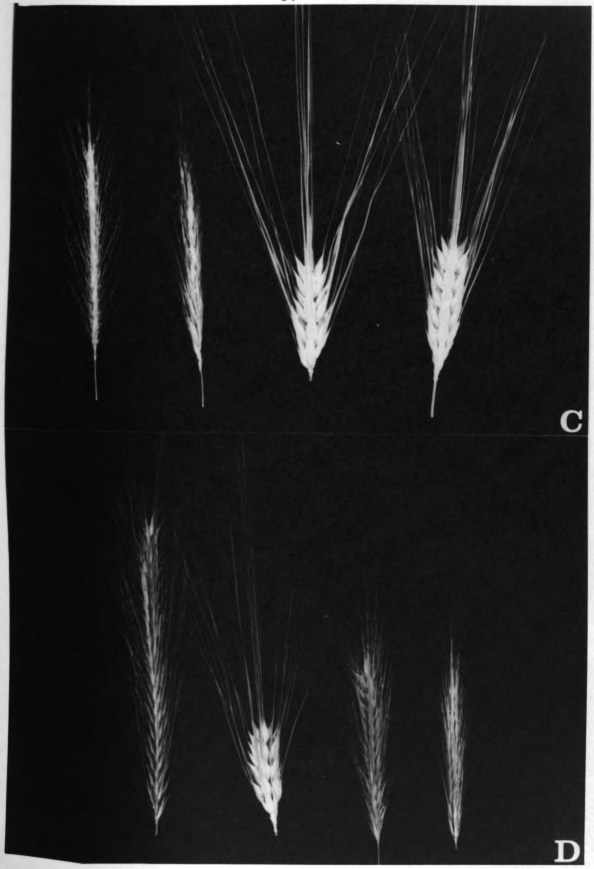


Figure 3 C,D

FIGURE 3. Spike morphology (cont'd.)

E. Polyhaploid from (HJ X HC)(6x) X VVVV

Intermediate progeny from (HJ X HC)(6x) X VVVV

Pentaploid from (HJ X HC(6x) X VVVV

Bl.2

F. (HJ X HC)(6x)

VVVV spike and tiller with clasping auricles

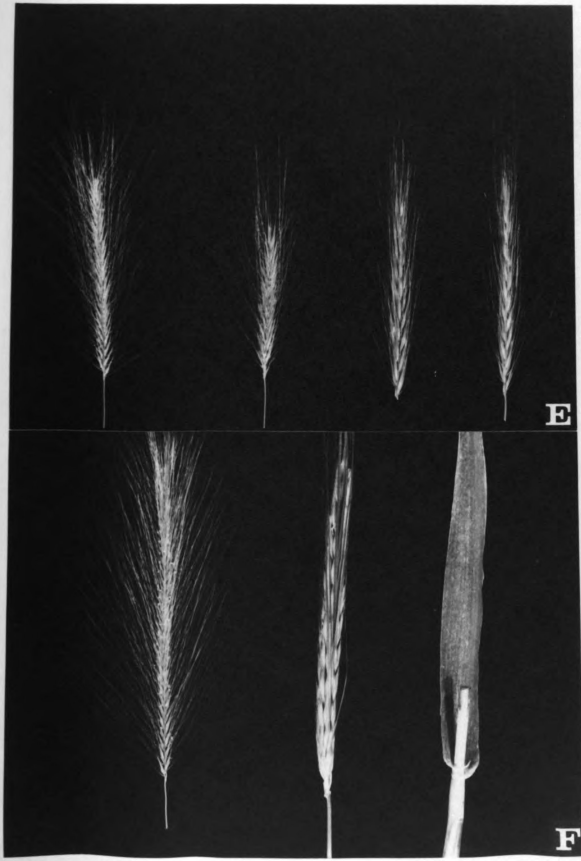


Figure 3 E,F

FIGURE 3. Spike morphology (cont'd.)

G. Agropyron sericeum

H. jubatum

(AS X HJ)

(AS X HJ)(8x)

H. VV

VV X (AS X HJ)(8x)

(AS X HJ)(8x) X VVVV

VVVV

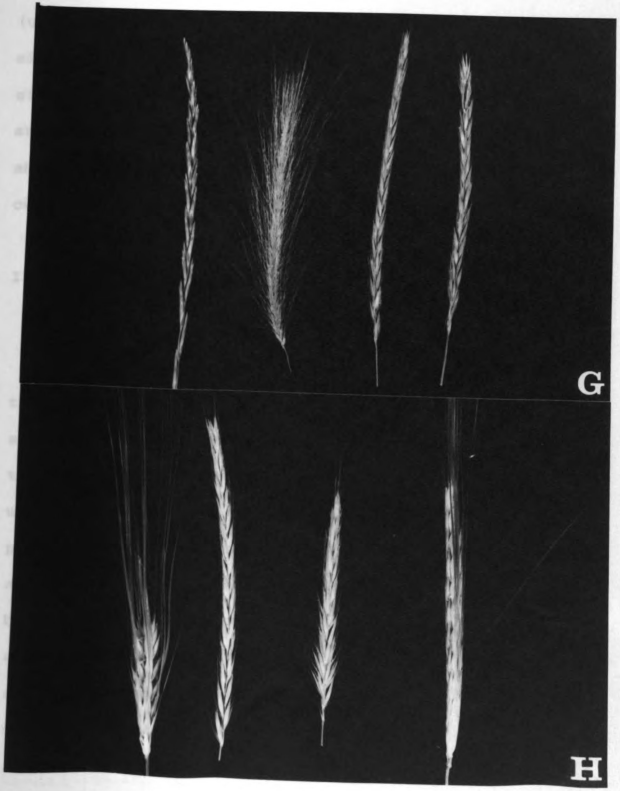


Figure 3 G,H

(unpublished) did not find multipolar cell divisions in either 69-101 or B1.2 although both were studied extensively. The other hybrid selfs could easily be contaminant selfed progenies from the H. jubatum-H. compressum amphiploid. This amphiploid and H. jubatum often produce contaminant plants in greenhouse pots and field clumps.

II. SEQUENCE AND OBJECTIVES OF CROSSES AND COLCHICINE TREATMENT

This section contains a sequential description of the crossing and colchicine treatment program. At present, several of these crosses are essentially worthless for transferring traits to H. vulgare, but their production was useful in coming to a much better understanding of the problems involved in making these transfers. Problems such as lack of good pairing and hybrid sterility are expected barriers to transfer of traits in this type of material. I also found H. jubatum cytoplasm imparted gross lack of vigor and cytoplasmic sterility to H. vulgare derivatives. Chromosome elimination also made certain combinations difficult and relatively useless in a standard backcross intermating program.

Kasha (1974) listed several criteria as evidence for chromosome elimination in interspecific or intergeneric crosses: 1) genome balance in hybrids; 2) high seed set, but seeds were small and usually non-viable; 3) chromosome instability of hybrid leading to mosaics, chimeral sectors and variable chromosome number in pollen mother cells; and 4) production of hybrids and haploids.

In this section and preceding sections I have attempted to describe my results so that they might be of help in interpreting other crossing results.

In Michigan, the greenhouse crossing season begins in September and lasts through June. Many of the potential parents (species and hybrids) do not produce good heads until November. By June, greenhouse conditions are too hot for most hybrid seeds to develop properly. Summer greenhouse use is mostly restricted to maintaining stocks in as good condition as possible.

My crossing program began with making the second H. vulgare backcross of (HJ X HC)(6x) X VV. Our objective was to produce parental material for an intermating population containing the desired genetic variation. Then cyclic intermating and selection would be practiced with the hope

of achieving the desired recombination and ultimately a commercial variety with the desired improvements. Although this cross was made during the first two greenhouse seasons (1972-73 and 1973-74), it failed to produce the desired parental material (Tables 3 and 4). Figure 3A to 3H shows spike morphology of progeny and parents of all crosses.

During January to March of the first crossing season, clones of AT X HJ and AS X HJ were divided to tiller divisions and were colchicine treated (Table 5). The objective was to produce higher level amphiploids as potential donor parents to H. vulgare. Amphiploids of both hybrids were obtained. An extensive attempt to double (HJ X HC)(6x) X VV was also begun. The objective was to produce an amphiploid parent which, when crossed with diploid H. vulgare would produce progeny with two sets of H. vulgare chromosomes. It was hoped these hybrids would be more fertile and could produce the parental material for an intermating population. Attempts to double this cross were terminated in late 1974 after 10,000 tiller divisions were treated without success.

In May of the first greenhouse crossing season, another cross--VVVV X (HJ X HC)(6x)--was attempted with

TABLE 3. Designation of all crosses attempted and mature progenies produced.

CROSS DESIGNATION	GENOME DESIGNATION	NUMBER OF MATURE PROGENY	PLANT PEDIGREE DESIGNATION
(HJ X HC)(6x) X VV	AA'A" V	10	69-101 to 110
	AA'A" V	12	74-111 to 122*
VV X (HJ X HC)(6x)	VAA'A"	14	75-151 to 164
69-101 X VV	vulgare backcross AA'A"VV	1	69-101-B1.2 (Abbrev. B1.2)
(69-101 X VV) X VV	VV	12	69-101-B2.2
HJ X VV	AA'V	9	74-201 to 209
VV X HJ	VAA'	11	75-251 to 261
(HJ X HC)(6x) X HJ	AAA' A' A"	12	73-301 to 312
(HJ X HC)(6x) X HC	AA' A" A"	0	---
(HJ X HC)(6x) X VVVV	AA'A" (polyhaploids)	3	74-401 to 403
	intermediate progeny	1	74-404
	AA' A" VV (pentaploid)	1	74-405

TABLE 3 (cont'd.)

VVVV X (HJ X HC)(6x)	VVAA'A"	0	---
73-304 X VVVV	---	0	---
HJ X VVVV	AA'VV	0	---
(AS X HJ)(8x) X VVVV	BA*AA'VV	1	74-501
VV X (AS X HJ)(8x)	VBA*AA'	1	75-851
(AS X HJ)(8x) X HJ	BA*AAA'A'	0	---

*74-122 74 is the year the cross was made
122 is the 22nd F_1 from this particular cross

Numbers 01-49 of every one hundred series are designated for hybrids with non-H. vulgare cytoplasm, while 51-99 are reserved for hybrids with H. vulgare cytoplasm.

TABLE 4. Order in which crosses were made.

CROSS	TIME	PROGENY
Bl.2 X VV	Nov. 1972-June 1973	6
VVVV X (HJ X HC)(6x)	May 1973	0
(HJ X HC)(6x) X HJ	June 1973	12
(HJ X HC)(6x) X HC	June 1973	0
Bl.2 X VV	Nov. 1973-June 1974	6
(HJ X HC)(6x) X VVVV	Feb.-April 1974	5
[(HJ X HC)(6x) X HJ] X VVVV	Feb.-April 1974	0
(HJ X HC)(6x) X VV	Feb.-April 1974	6
(AS X HJ)(8x) X HJ	April 1974	0
(AS X HJ)(8x) X VVVV	April 1974	1
HJ X VVVV	Summer 1974	0
HJ X VV	Summer & Fall 1974	9
(HJ X HC)(6x) X VV	Summer & Fall 1974	6
VV X HJ	Oct. 1974 to Feb. 1975	11
VV X (HJ X HC)(6x)	Feb.-April 1975	14
VV X (AS X HJ)(8x)	March-May 1975	1
[VV X (HJ X HC)(6x)] X VV	Aug. 1975 to March 1976	0

TABLE 5. Colchicine treatment.

MATERIAL TREATED	TREATMENT PERIOD	NUMBER OF TILLERS TREATED	AMPHIPLOIDS PRODUCED
(AS X HJ)	January-March 1973	1000	30
(AT X HJ)	January-March 1973	1000	3
(HJ X HC)(6x) X VV	Summer 1973-Nov. 1974	10,000	0

the hope that two sets of H. vulgare chromosomes and H. vulgare cytoplasm would greatly increase fertility of the hybrid and backcross progeny. It was hoped this would provide a better chance to form an intermating population containing the desired variation. Attempts to produce this cross were stopped after the first six heads failed to show signs of floret closure, stigmatic change from a receptive appearance, or development of seeds.

In June of the first crossing season another cross --(HJ X HC)(6x) X HJ--was successfully made. Progeny of this cross were used by Starks and Tai (1974) to better elucidate the genomic relationship of H. jubatum and H. compressum.

At that time, six heads of the H. jubatum X H. compressum amphiploid were also backcrossed to H. compressum for the same objective. Although the amphiploid was massively pollinated, no signs of floret closure, stigmatic change, or seed development were observed.

During February, March, and April of 1974, (HJ X HC)(6x) X VVVV, 73-304 X VVVV and (HJ X HC)(6x) X VV were attempted. The first two crosses were made with the hope that two sets of chromosomes from H. vulgare would greatly increase the fertility of the hybrid and backcross progenies.

True hybrids and progenies with partial and complete elimination of the chromosomes of H. vulgare were obtained from (HJ X HC)(6x) X VVVV. Seedlings of 73-304 X VVVV died before transfer to soil, probably due to extreme chromosome elimination (Figure 2C). (HJ X HC)(6x) X VV was made to obtain seedlings for colchicine doubling treatment. None of the six treated seedlings was doubled.

Two other crosses were also attempted in April of 1974--(AS X HJ)(8x) X HJ and (AS X HJ)(8x) X VVVV. Four heads of the Agropyron sericeum X H. jubatum amphiploid were pollinated with H. jubatum. Five days later, after no signs of floral closure, stigmatic change, or seed set, these heads were pollinated with VVVV. Four seeds rapidly developed, producing one (AS X HJ)(8x) X VVVV hybrid. The H. jubatum backcross was attempted to elucidate the genomic relationship of this cross. The H. vulgare hybrid was made for trait transfer purposes.

By the summer of 1974, healthy H. vulgare plants were repeatedly obtained under growth chamber conditions. Therefore, I attempted a cross during the summer. H. jubatum X VVVV gave a respectable seed set ranging from 0% to 90% on different spikes, but only proembryos--globular,

undifferentiated embryos--resulted from this cross. Crosses with good seed set, but with a very low number of mature hybrid plants produced is good evidence for chromosome elimination (Kasha, 1974). Again, this cross was attempted to produce a hybrid with two sets of H. vulgare chromosomes with the hope of greater hybrid fertility.

Two other crosses--HJ X VV and (HJ X HC)(6x) X VV--were started during the summer and completed in the fall with the crossing taking place in the growth chamber. These crosses were made to determine the effects of EACA, an immunosuppressant, which Bates, et al. (1974) claimed spectacular effects from. The HJ X VV cross was also made for breeding purposes and to help elucidate the genomic relationship of H. jubatum and H. vulgare.

By this time, the deleterious effects on vigor and male fertility of the H. jubatum cytoplasm on H. vulgare derivatives were quite apparent. Thus reciprocal crosses were attempted. Between August and late December, spring habit, normal H. vulgare and genetic male sterile H. vulgare plants were used. Both failed to produce any hybrid seed. Starting in early January, hybrids were produced from winter genetic male sterile diploid barley X H. jubatum, (HJ X HC)

(6x) and (AS X HJ)(8x). During August and September of 1975 seventy heads of the VV X (HJ X HC)(6x) hybrid were backcrossed with VV. Only one lateral floret developed, and this had an endosperm with normal starch deposition but no embryo.

III. COLCHICINE TREATMENT OF PLANT MATERIAL

During January to March of 1973 clones of AT X HJ and AS X HJ were divided into tiller divisions. For colchicine treatment, I chose to use eight colchicine treatments and no attempt was made to keep track of which were effective, because many factors such as vigor of material, particular species, stage of growth, temperature and lighting determine the best colchicine treatment (Elliott, 1958). One thousand vigorous tillers of each spontaneous hybrid were divided into eight nearly equal groups. Each group received one of the treatments used. Colchicine treatments used were 1 and 3-9. After treatment, tillers were carefully washed and planted in flats. Several treatments caused 50% mortality.

By June, the surviving plants were growing vigorously and beginning to head. The heads were cut off and the plants transplanted to the field. Field conditions were good for rapid tiller production and vigorous growth, but these plants failed to produce large numbers of heads over the next two years in the field. Evidently the right conditions for floral initiation never occurred. These hybrids seemed to require very long days and a moderate temperature for floral initiation.

For a few weeks after transplanting to the field a few plants continued to head. Several of these spikes produced a few seeds.

Three amphiploids of AT X HJ were obtained from the seed (Table 5). They all had good fertility under favorable growing conditions. Only one of these plants has been maintained. See Huang (1975) for cytological investigation of this amphiploid.

Thirty doubled plants of AS X HJ were obtained from the seed. Some of these were completely sterile under favorable growing conditions, while others had very good fertility. Only those with 56 chromosomes at anaphase I and a good fertility level were maintained. Only one of these

was used in crossing and chromosome studies. See Murry (1975) for cytological investigation of this amphiploid.

From the spring of 1973 to the fall of 1974, all nine doubling techniques were extensively used to double the hybrid (HJ X HC)(6x) X VV. Ten thousand plants were treated with no fertile seeds produced.

With a very similar cross (HJ X VV) three Canadian research groups (personal communications from Dr. Eric R. Kerber, Steve Symko, and Dr. Bryan Harvey) also failed to produce fertile doubles. Extensive attempts were made by all three research groups. Both Bryan Harvey and Eric Kerber used all the standard colchicine doubling techniques for several years.

There are many possible explanations for the failure to produce fertile seeds or visibly doubled sectors in these crosses. Some of these are: failure to produce doubled cells; lack of competitive ability of doubled tissue; rapid reversion of doubled cells; and sterility of doubled tissue. Lindstrom (1966), working with colchicine-treated tetraploid rye, concluded that failure to produce octaploid seeds and/or octaploid meiosis was probably due to lack of competitive ability of the octaploid tissue.

IV. CROSSING AND PROGENY PRODUCED

Crosses vary greatly in seed set, transfer survival, response to environmental stresses, etc. An understanding of how several crosses behave can be very useful in making further crosses and in interpreting the results of crosses made by other researchers. The crossing method and results of crosses including progeny produced and environmental influences are herewith discussed.

Second and Third Backcross Generations of (HJ X HC) (6x) X VV

Extensive efforts were made to produce the second backcross to *H. vulgare* of (HJ X HC)(6x) X VV. This involved careful selection and backcrossing of 2000 healthy B1.2 heads over two greenhouse seasons (1972-73 and 1973-74). Good heads of B1.2 were seldom produced before late November and by late May, greenhouse conditions were too hot for further successful crosses. Crossing was performed on a regular basis, but success was very dependent on weather conditions. Depending on environmental conditions, from 5% to 50% of the spikes would begin development of small seed(s). Usually these would live for 6 to 10 days

and cease development at the proembryo (no differentiation) stage.

In an attempt to provide a better environment for embryonic development, thirty spikes were treated with gibberellic acid by the Larter and Enns (1960) method. The treatment delayed collapse of some seeds until they were twenty-five days old, but only proembryos were produced by this treatment. This lack of success caused me to abandon gibberellic acid treatment until I used Kruse's (1967) methods on crosses with H. vulgare as female parent.

On untreated plants, chance and the combination of environmental factors, particularly temperature and lighting, allowed continued development of the embryo on rare occasions. Many of these continued growth and development until they were completely differentiated and similar to small mature embryos. Much of the chance factor probably involved elimination of certain chromosomes to produce a diploid H. vulgare (see section on chromosome elimination).

Twenty B2.2 seeds developed to the normal H. vulgare seed size (Figure 1D) and contained completely differentiated embryos comparable in size to those of H. vulgare. These were all transferred to wick type embryo culture

media (Figure 2D). Five developed abnormally on media and died. Three remained dormant and white with an appearance of viability for at least six weeks. These embryos were left on the plant until early dough stage and dormancy seems to have been induced during starch deposition.

The remaining 12 B2.2 embryos produced mature plants (Table 3). Only one of these had a relatively high self-fertility of approximately 40% (Figure 1A). A few of the remaining plants set an occasional selfed seed. All selfed seed occurred in a hot summer greenhouse. Selfed seed and third backcross seed from all plants had no self-fertility when grown in cooler environments.

Only seven normal H. vulgare bivalents were observed in selfed and third backcross H. vulgare plants. These plants closely resembled Dr. Schooler's restorer lines (H. vulgare in H. jubatum cytoplasm) except, due to their lack of restorer genes, they failed to set selfed seed in cooler environments. Other than for cytoplasmic effects, these plants only exhibited H. vulgare characteristics.

H. jubatum cytoplasmic effects were the most distictive features of the restorer and second and third backcross H. vulgare plants. These plants were far less vigorous than

normal H. vulgare, even under ideal growing conditions. Under less than ideal growing conditions the plants often failed to head normally and died at any age from one to nine months. Under extremely unfavorable conditions, plants produced many abnormal tillers, probably due to failure of the development of main tillers. Figure 4D shows a representative example of weak and abnormal tiller production.

Extreme lack of vigor made it very difficult to use the restorer lines as parents in crosses. Thus formation of an intermating population among the restorer lines would have been nearly impossible. Therefore the H. jubatum cytoplasm seems a dead end even with restoration genes present.

(AS X HJ)(8x) X VVVV and
(HJ X HC)(6x) X HJ

These crosses are representative of the two distinctly different seed set groups of my crosses. (HJ X HC)(6x) X HJ, like several other crosses had a very good seed set, ranging from 0 to 95% on individual spikes. (AS X HJ)(8x) X VVVV had one of the highest seed sets of the low seed set group. It set a total of four seeds on four spikes.

FIGURE 4.--Parthenocarpic fruit and other cross results

A. Large parthenocarpic fruits (normal seed size) in central florets of six row *H. vulgare*

B. Smaller parthenocarpic fruit in all florets. Stigmas still feathery

C. Four hybrid seeds from VV X HJ. Other florets still with feathery stigmas 18 days after pollination

D. Selfed progeny from B2.2 with yellowing of leaves (nicofume injury) and many weak and often deformed tillers

E. Seed from [VV X (HJ X HC)(6x)] X VV

F. Seedling from HJ X VV on Norstog's agar medium II

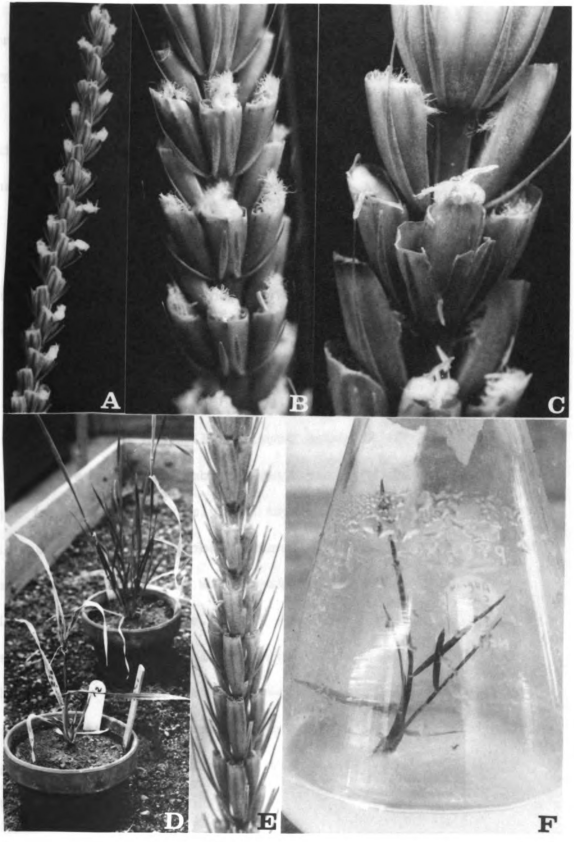


Figure 4

None of the species X species or species X amphiploid crosses fell into an intermediate category.

Seed set of the high seed set group was comparable with that expected for a cross within a species, but somewhat more susceptible to environmental influences. Crosses of the low seed set group are either extremely susceptible to environmental influences (like oat crosses in the greenhouse in Michigan) or they have fairly effective isolation mechanisms preventing fertilization.

Seed of the cross (HJ X HC)(6x) X HJ produced a reasonable amount of endosperm starch and could possibly have germinated normally without embryo culture. Thirty well-differentiated embryos were transferred to Norstog's agar medium. Only twelve of these developed into mature plants (Table 4). Probably many more mature plants would have developed on the wick medium from these completely differentiated embryos. Self fertility was 70%, which is very high for pentaploid hybrids. Morphologically, the plants were intermediate between their parents in spike characteristics and overall morphology (Figure 3A). For cytological verification and discussion of pairing, see Starks and Tai (1974).

Seed of the cross (AS X HJ)(8x) X VVVV also produced well developed embryos with some starch deposition in the endosperm. Only one mature plant developed from these embryos. Morphologically, this plant more closely resembled its female parent, although the head was somewhat different and auricles were definitely more pronounced. For cytological verification and discussion of pairing, see Murry (1975).

Other Crosses Resulting in Embryos with H. jubatum Cytoplasm

The following crosses, (HJ X HC)(6x) X VV, HJ X VV, HJ X VVVV, [(HJ X HC)(6x) X HJ] X VVVV, and (HJ X HC)(6x)X VVVV all had a relatively high seed set, which can be favorably compared with the seed set of (HJ X HC)(6x) X HJ. All crosses had a seed set ranging from 0 to 95% on different spikes. This group of crosses demonstrated the effects chromosome elimination had on the ease of obtaining mature progeny.

It was relatively easy to obtain mature progeny from crosses of HJ X VV and (HJ X HC)(6x) X VV. The crosses produced fairly completely differentiated embryos. Seed of

the cross (HJ X HC)(6x) X VV had some endosperm starch deposition, while HJ X VV produced enough starch that mature plants were produced without embryo culture (Kerber, personal communication). I also produced one mature HJ X VV hybrid without embryo culture while eight hybrids were produced using embryo culture. Twelve mature (HJ X HC) (6x) X VV hybrids were produced (Table 3). Transfer success was around 30% for both crosses on agar or wick media. Lack of complete embryo development probably restricted transfer success.

Phenotypically, the hybrids of both crosses more closely resembled their female parents. Hybrids of both crosses can be easily distinguished from their respective female parents on the basis of overall plant type, slightly more H. vulgare-type heads and presence of auricles (Figure 3A-D). All the hybrid plants produced from these crosses were self-sterile.

Approximately 1/2 of the hybrids of each cross were produced using EACA, an animal-effective immunosuppressant, to test the effects of this chemical. No morphological differences were noted between the treated and nontreated hybrids. All treated progenies were just as sterile.

Colchicine treated seedlings and divisions of (HJ X HC)(6x) X VV seemed just as stubborn in producing seeds as divisions of 69-101 were. Thus in contrast to Bates, et al.'s (1974) claims, I was unable to observe any effects of EACA.

Metaphase I chromosome counts of 69-101 were all 28, thus the genomic constitution AA'A''V seemed quite stable (Starks, unpublished). This agreed with Tom Orton's preliminary embryo squash data (Table 9). Kerber (unpublished) found AA'V metaphase I counts varied from 16 to 23, so it would appear AA'V was not quite as stable.

While crosses of (HJ X HC)(6x) X VVVV, [(HJ X HC)(6x) X HJ] X VVVV and HJ X VVVV had just as high seed set, production of mature plants seemed to be strongly controlled by chromosome elimination. See Table 6 for genomic stability of this crossing series. Crosses of HJ X VVVV produced only globular undifferentiated embryos (proembryos) which were not successfully cultured on Norstog's medium. Crosses of [(HJ X HC)(6x) X HJ] X VVVV and (HJ X HC)(6x) X VVVV produced many reasonably well differentiated embryos. These embryos were essentially at the same stage of development as (HJ X HC)(6x) X VV embryos. Approximately 100 embryos of each cross were transferred to embryo culture

TABLE 6. Stability of hybrid progeny.

HYBRID	CROSS	METAPHASE I	PROGENY
AA' A" V	(HJ X HC)(6x) X VV	Stable-- all counts 28	all hybrids
VAA'	VV X HJ	variable-- 16-22*	8 hybrids--3 of which produced some haploid tillers
AA' V	HJ X VV	17-23**	all hybrids
AA' A" VV	(HJ X HC)(6x) X VVVV	stable 21	3 polyhaploids (AA'A")
		22-28	1 intermediate progeny
		variable	1 pentaploid hybrid

*Murry (1975)
**Rajhathy and Morrison (1959)

media. If the stage of embryo development were the only factor controlling production of mature plants these crosses should have produced many mature plants. The more differentiated embryos of both crosses were transferred to a wick medium. Only a small percentage of the embryos of both crosses had no development on culture media.

Approximately 1/2 of the remaining embryos of each cross produced only abnormal growth ranging from undifferentiated growth or hypocotyl development only, to abnormal roots and shoots. The other 1/2 for a brief time developed nearly normal shoots and roots. [(HJ X HC)(6x) X HJ] X VVVV embryos only maintained near-normal growth for a short time before dying. Thus no mature plants were produced from this cross. Figure 2C is a good example of how long near-normal growth usually occurred.

In contrast, embryos from the cross (HJ X HC)(6x) X VVVV produced five mature progeny: three "polyhaploids" --haploids of the amphiploid; one "pentaploid" hybrid; and one "intermediate" progeny--intermediate between the pentaploid and the polyhaploid progenies in morphology and chromosome number. Figure 3E,F shows morphological

comparisons of the plants and Table 7 of Gil Starks' unpublished data shows the cytogenetics of these plants.

The polyhaploids had the expected morphology of haploids of the amphiploid, that is, more but smaller tillers with somewhat smaller floral parts. There were no H. vulgare characteristics in these plants. For example, H. vulgare and all true H. vulgare interspecific or intergeneric hybrids that I made had auricles. Like H. jubatum, H. compressum and (HJ X HC)(6x), the polyhaploids had no visible auricles. The pentaploid hybrid was intermediate in morphology between the parents. This was more obvious when the pentaploid was compared with hybrids from (HJ X HC)(6x) X VV. The pentaploid had fewer but more robust tillers with larger, often clasping auricles and a definitely more H. vulgare-like head. Plants from the cross (HJ X HC)(6x) X VV had small auricles that were rarely clasping.

Like most hybrids from crosses with chromosome elimination, the pentaploid had a variable chromosome number at metaphase I of meiosis (Table 7 of Starks' unpublished data). The intermediate progeny also had a variable chromosome number.

TABLE 7. Starks' metaphase I counts of progeny from (HJ X HC)(6x) X VVVV.

CHROMOSOME NUMBER	POLYHAPLOIDS	INTERMEDIATE	PENTAPLOID
19			1
20			
21	80		
22		1	
23		1	
24		2	
25		7	3
26		9	4
27		6	4
28		24	6
29			1
30			4
31			4
32			1
33			2
34			4
35			1
Total number of cells	80	50	35

TABLE 8. Starks' metaphase I counts of the pentaploid and B1.2.

CHROMOSOME NUMBER	B1.2 COUNT IN 1974	PENTAPLOID 1974 COUNT	PENTAPLOID DEC. 1975 COUNT*	PENTAPLOID DEC. 1975 COUNT**
15	2			
16	2			
17	1			
18	5			
19	2	1		
20	2			
21	2			
22	5			
23	3			
24	7		1	
25	4	3	3	
26	9	4	1	
27	7	4	3	
28	9	6	10	
29	4	1	1	
30	3	4		2
31	3	4		1
32	3	1		5
33		2		1
34	1	4		1
35		1		
Total number of cells	74	35	19	10

*Count from four or five florets from two spikes.
**All counts from one floret.

Both the intermediate and pentaploid progenies changed morphologically since the 1974 data were taken. Partly due to lack of vigor, change in the intermediate progeny was difficult to describe, but this plant seemed more vigorous with some changes in overall plant type and head morphology. The pentaploid head morphology became impossible to distinguish from [(HJ X HC)(6x) X VV] X VV heads.

Results of these crosses are good examples of the effects of chromosome elimination on a crossing series. Thus these results can be useful in interpreting the results of other researchers. For example, Morrison, et al. (1959) transferred 1400 embryos of H. murinum X VV. These were well developed embryos, but produced almost identical results to those of [(HJ X HC)(6x) X HJ] X VVVV. Earlier, this research group had produced a hybrid, which later broke down into H. vulgare and H. murinum sectors (Hamilton, 1955). Taken as a whole, I consider this good evidence that chromosome elimination was operating in this H. murinum-H. vulgare hybrid.

Crosses with H. vulgare as Female

In May of 1973, the cross VVVV X (HJ X HC)(6x) was attempted on a limited scale. Although the six heads were massively pollinated, the florets remained open and receptive. Both lack of floral closure and lack of stigma collapse are good evidence of nonfertilization. Thus I assumed that this was good evidence this cross was improbable. Later, when the extreme disadvantage of H. jubatum cytoplasm became all too obvious, other crosses with H. vulgare as the female were attempted.

Preliminary attempts with crosses of VV X HJ and VV X (HJ X HC)(6x) gave similar results. All florets not obviously injured by excessive drying or poor emasculation remained open and appeared receptive for many days. Lack of stigma collapse was taken to indicate continued receptive condition (Figure 4B,C). Crossing on a large scale was then undertaken with the aid of a spring type, genetic male sterile population and a winter type, male sterile population. These populations had the added advantage of genetic diversity for many traits and thus possible variability for receptivity to foreign pollen.

Crossed heads were also treated with gibberellic acid (GA_3). Kruse (1967) was highly successful in using this chemical in achieving interspecific and intergeneric Hordeum hybrids.

Crossing onto spring type male sterile plants was soon stopped after difficulties occurred with incomplete sterility and frequent development of parthenocarpic fruit. I use the term "parthenocarpic fruit" for fruits appearing to have only maternal tissue development. Sometimes small parthenocarpic fruit were produced by spring type plants even without gibberellic acid treatment and without pollination. Gibberellic acid treatment seemed to be necessary to stimulate parthenocarpic fruit development in the winter type male sterile plants. Partial genetic control of parthenocarpic fruit development was indicated by the more frequent and more uniform development in the spring type population.

The external morphology of parthenocarpic fruits was highly variable (Figure 4A,B) with only one character remaining constant. All developing parthenocarpic fruits had noncollapsed stigmas. Lack of stigma collapse could be observed in all parthenocarpic fruit for at least ten days.

Size of parthenocarpic fruits ranged from 1/3 normal to normal seed size. On some heads only the central florets developed into parthenocarpic fruits. Most parthenocarpic fruits could easily be distinguished from normal seed, but a few were very similar to normal seeds.

Three hundred fruits were dissected. Internal morphology was less variable than external morphology. Fruits generally appeared to have a spongy proliferation of ovary wall cells, which made it very easy to distinguish parthenocarpic fruits from normal seeds. All three hundred dissected fruits had no signs of embryo or endosperm.

Fifty winter type male sterile heads were massively pollinated with H. jubatum. Florets remained open and stigmas failed to collapse for many days. Only two spikes developed hybrid seeds. One spike had ten hybrid seeds, while the other had four hybrid seeds. Figure 4C shows the spike with four hybrid seeds and other florets with non-collapsed stigmas. The picture was taken eighteen days after pollination and still some florets had noncollapsed stigmas. Embryos were transferred immediately after this picture was taken. The hybrid embryos were nearly as big as H. vulgare selfs, and well-differentiated. Usually the

scutellum was deformed in that it curved back and partially covered the shoot end of the embryonic axis. I assumed this was a geotropic response, since all hybrid embryos of this cross were in water sac-like endosperms which appeared to allow considerable freedom of embryonic movement.

The ten most normal appearing embryos were transferred to a wick medium and all developed into mature plants. Eight of these were normal hybrids. Three of these hybrids sometimes produced haploid H. vulgare tillers (Figure 3D). The other two remaining plants were neither normal hybrids nor haploid H. vulgares. These plants remained very short for many months and failed to head. They lacked H. jubatum leaf hairs and had less distinct auricles than true hybrid progeny. Eventually these plants produced heads morphologically identical to normal hybrids. Probably these plants were true hybrids but had phenotypic suppression of normal hybrid pubescence and auricle expression. I based this statement on some callus culture results of true hybrid tissue. Regeneration plantlets from callus formed from true hybrid tissue have the same failure of expression of the H. jubatum pubescence and hybrid auricle type. Metaphase I chromosome counts of one of these plants

were mostly 21. Thus it seemed reasonable to assume this plant was a true hybrid with phenotypic suppression of some characters.

The remaining four embryos were transferred to a callus promoting medium. Three embryos soon became diseased and died. The remaining embryo rapidly callused and was turned over to Dr. Tom B. Rice for experimental purposes.

Seventy winter type male sterile heads were massively pollinated with (HJ X HC)(6x). Four spikes set a total of 17 seeds. Seed sets were 9, 5, 2, and 1 seed per spike. Seeds in this cross were very similar to those of VV X HJ. In both crosses embryos were nearly normal H. vulgare size, well differentiated and had some overgrowth of the shoot tip of the embryonic axis by the scutellum. In both cases the endosperm remained a water sac with very little or no starch deposition. All seventeen embryos were transferred to a wick medium. Fourteen of these developed into normal hybrid plants (Table 3). The other embryos failed to develop into mature plants. Morphologically, these hybrids and VV X HJ hybrids were very similar to their respective reciprocal crosses. But these hybrids

were distinctly more vigorous than their reciprocal crosses with cytoplasm from H. jubatum. Vigor is dependent on growing conditions and thus is not a good character to distinguish reciprocal crosses grown under different micro-environmental conditions. The most consistent morphological difference between reciprocal crosses was that hybrids with cytoplasm from H. vulgare had a somewhat wider lemma.

One hundred and eighty winter type male sterile heads were massively pollinated with (AS X HJ)(8x). Again, essentially all florets remained open and stigmas failed to collapse for many days. Six spikes set a single seed per spike. Four of these seeds had large, well-differentiated embryos in a water sac-like endosperm. Again some overgrowth of the embryonic axis by the scutellum occurred. All four of these embryos were transferred to a wick medium. Seedling development in all four cases soon became abnormal, resulting eventually in death.

The remaining two embryos were transferred at a much earlier stage of development. Although embryos had a partially developed scutellum, they were so small that proper orientation on a wick medium was left up to chance. One of these hybrids developed into a mature plant.

Chromosome elimination could possibly explain these results. The much lower mature plant production frequency of some crosses in which chromosome elimination operated seemed to indicate that embryos in the process of losing chromosomes had a much lower survival frequency. Early embryo transfer results in a higher percentage of hybrids (Kasha, 1974). In a similar cross, (AT X HJ)(8x) X VV, Huang (1975) found chromosome number in metaphase I of meiosis was variable. Thus possibly this cross was also unstable. Morphologically, plants of the cross VV X (AS X HJ)(8x) were similar to (AS X HJ), but they did have distinctly more H. vulgare-like auricles. This hybrid had a stable chromosome number of 35 in metaphase I of meiosis. So it was impossible to make any interpretations on whether chromosome elimination was operating in this cross.

All the hybrids with H. vulgare cytoplasm were distinctly more vigorous than their reciprocal crosses. Thus vigor of H. vulgare derivative lines should be good.

V. HYBRID SEED DEVELOPMENT

Normal seed development involves normal development of many tissues. This process is set in motion by

pollination and fertilization (Maheshwari, 1950 and Nitsch, 1971). Initially, activity is most intense in these maternal tissues: the diploid integuments, nucellus and ovary wall and the haploid antipodal cells. In the cross Hordeum jubatum X Secale cereale, Cooper and Brink (1944) attributed failure of normal endosperm development to failure of the antipodal cells to develop normally. Thompson and Johnston (1945) attributed breakdown of hybrid seed development directly to irregular development of the endosperm, including formation of giant nuclei and slowing down of the mitotic cycle.

These authors seem to imply normal embryo and endosperm development are strongly interdependent. In actual fact, the embryo and endosperm follow independent and autonomous developmental sequences and interaction between the two systems is not a prerequisite for normal formation of either tissue. Results of interspecific crosses support this statement.

Interspecific crosses in Datura sometimes produce seeds with only endosperm or embryo. From the results of several Triticeae interspecific and intergeneric crosses Ivanovskaya (1962) concluded there was no relationship

between the degree of embryonic development and production of endosperm tissue in mature seeds.

The results of my crosses also agree with the autonomous developmental idea. Completely differentiated embryos occurred in the crosses VV X HJ, VV X (HJ X HC)(6x), and VV X (AS X HJ)(8x) with no or only a watery sac-type endosperm development. These embryos were at least as completely differentiated as those of (HJ X HC)(6x) X HJ or HJ X VV. In these latter crosses enough endosperm starch deposition occurred that normal germination of the seed was possible.

To complicate the matter even more, crosses between different ploidy levels of the same species can result in severe disturbances of the endosperm and embryo. Larter and Enns (1960) found hybrid embryos of tetraploid X diploid H. vulgare were so small that even with the aid of embryo culture only a very few normal seedlings could be produced.

Whatever the cause of developmental breakdown, there is no indication of incompatibility between the embryo and the maternal tissues. In fact, results of crosses between different ploidy levels of the same species is very good evidence against incompatibility between these tissues.

Incompatibility due to maternal tissue also seems very unlikely, because embryos usually have more normal development than the endosperm. It would seem any incompatibility with maternal tissue would more severely affect tissues having a greater proportion of their genetic material from the paternal parent.

Thus transfer should be normally delayed until collapse of the endosperm tissue occurs. This allows for production of more completely differentiated embryos, which when embryo cultured usually produce many more hybrid plants.

I observed two exceptions to this rule. Those exceptions were due to these intervening factors: seed dormancy and chromosome elimination. Prevention of seed dormancy probably only requires transfer before much endosperm starch deposition occurs. Transfer time in the case of chromosome elimination must be the best compromise between greater developmental problems with young embryos and the increasing proportion of older embryos mixed up with chromosome loss problems. In some crosses, e.g. HJ X VVVV, chromosome elimination was so severe that there was no optimum time for transfer.

VI. CHROMOSOME ELIMINATION AND EVIDENCE FOR THE H. VULGARE BACKCROSS OF (HJ X HC)(6x) X VV AND THE PENTAPLOID HYBRID FROM (HJ X HC)(6x) X VVVV HAVING THE SAME BASIC GENOMIC CONSTITUTION

This section deals with the gradual change in phenotype and metaphase I chromosome number of the pentaploid hybrid from (HJ X HC)(6x) X VVVV. I did not find any similar examples in the literature on Hordeum. Obviously, rapid change from hybrid to a haploid of one of the parents is easily observed and easily documented for publication. H. bulbosum-H. vulgare hybrids have become a classic example of this. These hybrids have also become a classic example of the cause of this rapid change, chromosome elimination. Chromosome elimination also causes many other conditions, which Kasha (1974) has listed as indirect evidence for chromosome elimination. These expressions of chromosome elimination in interspecific or intergeneric crosses are: 1) evidence of genome balance, e.g. triploid hybrid VBB is essentially stable, but VB, VVBB, and VVB hybrids are unstable; 2)good seed set, but seeds non-viable without embryo transfer; 3)

chromosome instability of hybrids leading to mosaics, chimeral sectors or variable chromosome number in metaphase I; and 4) production of hybrids and haploids. Obviously, some of these factors, taken individually, could be caused by other variables.

Direct evidence for chromosome elimination can be obtained from embryo squash counts. For example, Subrahmanyam and Kasha (1973) made a very convincing argument for chromosome elimination in VB hybrids from this type of count. Their results were: 1) near hybrid counts for most very young embryos and 2) a gradual reduction in chromosome number of most counts over the next few days until most counts were near haploid level. Most counts began near a hybrid level, so production of a reasonable number of haploids was not due to male or female parthenogenesis. The gradual reduction in chromosome number of most counts shows chromosomes were lost gradually and not by whole genomes at a time. Embryo squash counts were also made on the essentially stable VBB hybrids. Much less variation in chromosome number occurred in these embryos.

My crosses exhibited the following expressions of chromosome elimination:

(1) VAA'A" from (HJ X HC)(6x)X VV or reciprocal was completely stable; VAA' from HJ X VV or reciprocal was slightly unstable; AA'VV from HJ X VVVV produced only proembryos; AA'A"VV from (HJ X HC) (6x) X VVVV was somewhat unstable, exhibiting variable metaphase I counts and production of polyhaploids, intermediate progeny and a hybrid.

(2) Very good seed set, but very low production of mature plants through embryo transfer, while closely related crosses produced mature plants relatively easily with the aid of embryo culture.

(3) Variable metaphase I counts.

(4) Production of hybrids and haploids.

Preliminary embryo squash results also agreed with the chromosome elimination hypothesis (Table 9). Orton found the mean chromosome number of (HJ X HC)(6x) X VVVV embryos was depressed from the expected zygotic chromosome

TABLE 9. Orton's preliminary embryo squash counts of (HJ X HC)(6x) X VVVV.

DAYS AFTER POLLINATION	CHROMOSOMES PER CELL																	MEAN CHROMOSOME NUMBER
	Less Than 21	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	Greater Than 35	
1	0	2	1	0	3	1	1	1	2	1	2	0	1	1	0	3	1	28.35
2	0	2	0	1	0	2	2	3	1	4	2	2	1	3	0	1	2	30.7
3	0	1	0	0	0	2	2	1	1	1	2	0	1	2	3	4	1	30.6
4	2	1	1	2	0	0	0	1	1	3	6	0	2	2	1	3	1	30.0
5	0	0	0	2	0	1	1	1	3	1	1	1	2	0	0	4	5	32.4
6	4	2	0	0	3	1	3	1	2	0	1	1	2	0	3	3	1	27.9
7	3	2	0	0	0	0	1	0	5	1	1	1	3	0	1	4	2	28.8*
8	2	0	1	2	2	0	0	2	5	3	3	4	0	3	0	4	0	28.3

*Mean chromosome number for seven day old embryos of (HJ X HC)(6x) X VV is 26.7.

number of 35. This suppression was significant at the .05 level for all days except day five. Only a relatively small portion of this decrease in zygotic chromosome number could be attributed to reduction in gametic chromosome number of (HJ X HC)(6x) (Starks and Tai, 1974) and H. vulgare(4x) (Orton, unpublished). Thus by definition, chromosome elimination had occurred. Chromosome elimination had also caused considerable instability in chromosome number and the variance in chromosome number increased with the age of the embryos.

Gupta and Gupta (1973) found a gradual transition in phenotype and chromosome number in a cross of tobacco similar to that expressed by the pentaploid. Nicotiana suaveolens (2n=32) was crossed with N. glutinosa (2n=24). In hybrid tissues, N. glutinosa traits were dominant. For the first few weeks the plants maintained a N. glutinosa phenotype. Gradually, the phenotypic expression began to shift toward the N. suaveolens phenotype. Root tip and premeiotic anther squashes showed a high average frequency of aberrant mitotic anaphases (12% with bridges; 11% with fragments). Metaphase I counts showed a range of 24-29 chromosomes in a single flower. Mitotic metaphase,

examined in petals, also showed a similar variation ranging from 24-29. The ranges in mitotic and meiotic counts seemed to reflect prior mitotic abnormalities. Also, metaphase I counts seemed representative of the somatic variation of nearby tissues. By the use of mitotic karyotype, the authors were able to show that the transitional change in phenotype was positively correlated with loss of N. glutinosa chromosomes. Their results indicated: 1) metaphase I count variation corresponded to somatic variation of nearby tissues, 2) loss in characters of one parent corresponded to loss in chromosome number, particularly loss in chromosomes of that parent, and 3) mitotic and meiotic counts reflected preceding mitotic abnormalities.

It is only reasonable to assume that many of these findings also apply to Hordeum material. Comparision of the tobacco results with those available for the H. bulbosum-H. vulgare material also shows evidence of actual loss of chromosomal material. Triploid VBB hybrids have about 5% of their somatic cells with micronuclei. This comparatively low frequency closely agrees with the small variation in chromosomal number in embryo counts of this hybrid.

The frequency of micronuclei in shoots of the diploid VB hybrid was much higher and more variable, which was in close agreement with embryo squash counts. The metaphase I chromosome number range was large for both hybrids, but the variance for VB was considerably higher. This seems to reflect the preceding mitotic abnormalities of these hybrids. Thus it would appear metaphase I counts are representative of the somatic variation of surrounding tissues.

The greater variability in micronuclei of the VB hybrids possibly indicates that once the chromosome number deviated enough from the hybrid constitution more rapid loss of chromosomes occurred. This agrees with the cross results indicating H. bulbosum chromosomes exert a stabilizing effect for their own retention. This would also effectively explain the rapid change in phenotype of some VB tillers.

Researchers working with the H. bulbosum-H. vulgare hybrids found little or no reciprocal differences in chromosome elimination. I found definite maternal effects in H. vulgare-H. jubatum crosses. Metaphase I counts of material with a hybrid phenotype from HJ X VV and VV X HJ

are essentially the same. Murry (1975) found a metaphase I range of 16-22 for my VV X HJ hybrids and Rajhathy and Morrison (1959) found a range of 17-23 for their HJ X VV hybrids. Thus it appeared that the same basic somatic variability was expressed in hybrid tissue of both crosses. None of my nine HJ X VV hybrids produced haploid H. vulgare tillers. Three of my eight VV X HJ hybrids produced haploid H. vulgare tillers. This would seem to indicate that in H. jubatum cytoplasm there was a strong lack of competitive ability of cells, tissues and tillers losing too many H. jubatum chromosomes. The extreme lack of vigor of H. vulgare derivatives in a H. jubatum cytoplasm strongly supports this conclusion.

The pentaploid (HJ X HC)(6x) X VVVV hybrid was produced in the spring of 1974. The first H. vulgare backcross of (HJ X HC)(6x) X VV-Bl.2- had been produced by Chung Lee in December of 1969. Thus, before I began my study the Bl.2 had nearly three years to stabilize phenotypically and in chromosomal variability. This plant has remained basically stable in phenotypic expression since then. Its phenotype was as stable as any of these hybrids of the wild species.

By the fall of 1974, these plants could be compared cytologically and morphologically. Starks (unpublished) found metaphase I chromosome number varied from 15-34 for the Bl.2 and from 19 to 35 for the pentaploid progeny (Table 8). In 1974 the pentaploid had the expected phenotypic characters to go with a larger culm diameter. The pentaploid had fewer but larger tillers, which were usually taller and had a larger head. The pentaploid also had a more H. vulgare-type head and the auricles were usually more pronounced. For all these characters the pentaploid expression was more like H. vulgare.

The pentaploid underwent a gradual shift in morphology until by December of 1975, head morphology of the pentaploid and Bl.2 were essentially identical. That is, heads removed from both plants and mixed could not be distinguished. Expression of the other characters was very similar for the two plants. These characters were much more affected by microenvironmental factors, particularly within pot competition factors.

Clones of both the pentaploid and Bl.2 were divided and paired comparisons made in two environments. Again, other morphological traits appeared essentially identical.

The pentaploid expressed a somewhat greater variation in head type indicating it was not as yet completely stable phenotypically. From a mixture of heads of both pentaploid clones, some pentaploid partial reversion heads could be separated out. The rest of the heads of the pentaploid were still impossible to distinguish from the heads of the Bl.2.

In the winter of 1975, another set of metaphase I counts were made on the pentaploid by Gil Starks (Table 8). One count involving 19 cells was from four or five florets from two spikes. This count probably represented a chance event of finding two spikes with essentially the same chromosome variation. The remarkably small range also indicated that variation within a spike was small. The other count of ten cells came from one floret on another spike. Again there was a remarkably narrow range in chromosome number. It would seem that selection for the pentaploid's 1974 phenotype could still be partially successful.

Morphologically, the pentaploid hybrid had lost its more intense expression of H. vulgare characteristics until it could not be distinguished from the Bl.2. From

morphological observations it appeared that many of the cells had lost several H. vulgare chromosomes. The 1975 preliminary cytological observations were too biased (the sample was unrepresentative of the range and variation present in the whole clonal population of cells) to draw any conclusions. Development of considerable variability in chromosome number would seem reasonable for unstable hybrids having stable intermediate forms. For example, the tetraploid progenies of (HJ X HC)(6x) X VV with one set of H. vulgare chromosomes were stable. Loss of H. vulgare chromosomes in pentaploid progenies would tend toward this type of intermediate form. The variation present in embryo squash counts of the (HJ X HC)(6x) X VVVV cross strongly supported the idea that pentaploid progenies could develop the chromosome variation found in the Bl.2 progeny.

I can postulate only one viable hypothesis on how the pentaploid progeny could develop the same phenotype as the Bl.2. Fortunately this hypothesis also has a good explanation for the other observed results. I postulate the Bl.2 and the pentaploid are basically both of pentaploid constitution. This requires that the Bl.2 was

produced from the fusion of a tetraploid egg from the tetraploid hybrid with a normal pollen grain from diploid H. vulgare. The tetraploid egg could be produced from a doubled sector or could be an unreduced egg.

The extremely high sterility of the (HJ X HC)(6x) X VV hybrid supports the conclusion that the Bl.2 originated from a tetraploid egg. In the cross Triticum durum X Aegilops longissima, Vardi and Zohary (1967) found all viable T. durum backcross progenies were pentaploids and thus originated from unreduced eggs. The frequency of viable progeny closely corresponded with the frequency of restitution nuclei (all chromosomes end up in one nucleus at the end of meiosis I). Restitution nuclei have not been observed in the tetraploid (HJ X HC)(6x) X VV hybrid, but this does not preclude the idea that this mechanism or some other mechanism could not on rare occasions produce unreduced eggs. Chung Lee treated 69-101 with colchicine, so a small doubled sector could also have been produced. Thus production of a Bl.2 pentaploid seems quite reasonable; in fact, it is probably the most likely type of progeny.

The pentaploid constitution hypothesis easily explains why the Bl.2 and the pentaploid have the same phenotype now. It also fits nicely with the observed metaphase I chromosome variation of the Bl.2. Furthermore, it offers a reasonable explanation for the origin of 69-101-1. 69-101-1 and the intermediate progeny have considerable gross morphological similarities. 69-101-1, originating as a chromosome elimination product from a pentaploid zygote seems much more reasonable than from selfing of the extremely sterile (HJ X HC)(6x) X VV hybrid.

In summary, observation of how the pentaploid hybrid has slowly changed over time gives an example of how the Bl.2 probably originated.

Since there is excellent evidence for the Bl.2 having a pentaploid constitution, the next section deals with the breeding implications of this hypothesis.

VII. BREEDING IMPLICATIONS OF THE HYPOTHESIS THAT THE Bl.2 HAS A PENTAPLOID CONSTITUTION

The diploid H. vulgare genomic constitution of the 12 second H. vulgare backcross progenies presents a

difficult obstacle to the production of the desired parental material for an intermating population. The parental material should ideally be a series of plants with reasonable fertility and all expressing part of the desired improvements in the gentic background of the recipient species. Thus the plants could be essentially a diploid H. vulgare with one or two H. jubatum chromosomes. These plants could be cyclically intermated with selection pressure to maintain the desired H. jubatum chromosomes and to fix desired translocations when they occur.

There are two possible explanations for the failure to produce the desired parental material. These possible mechanisms must explain why the only viable mature progeny have a diploid H. vulgare constitution. The possible mechanisms are: 1) All viable eggs have a haploid H. vulgare constitution and 2) Viable eggs have some wild chromosomes plus a haploid H. vulgare set, but the only progeny to survie to maturity have all the wild chromosomes eliminated.

Either one of these mechanisms would have profound implications on the chances of getting the desired parental material. Thus knowing which is operating could be extremely important. Without knowing the genomic

constitution of the Bl.2, it is impossible to evaluate which is the more likely mechanism operating in the material. Since the only viable hypothesis on the genomic constitution of the Bl.2 is the pentaploid hypothesis, I have evaluated the results in terms of the Bl.2 being a pentaploid.

Chromosome elimination is a logical explanation because the most likely viable egg gametes from an AA'A"VV progeny are gametes with essentially two or more full genomic sets, for example, AV, A'V, A"V, and A'V. I base this statement on the genomic constitution overserved in backcross progeny of other Triticeae crosses (Linienfeld, 1951; Kihara, 1963; Furuta and Tanaka, 1970; Vardi, 1973; and Vardi, 1974). Fusion of these types of viable eggs with a pollen grain from diploid H. vulgare would produce zygotes which could be compared with those produced in species crosses. Kasha (1974) showed the genomic constitution of the embryo was the most important factor in determining embryo chromosomal stability and the genomic constitution of the female parent had surprisingly little effect.

AA'A"V from (HJ X HC)(6x) X VV was at the genomic balance point (Table 6). VAA' from VV X HJ was slightly underbalanced (not enough wild chromosomes) and had

considerable chromosomal variation occurring at metaphase I (a range of 16-22; Murry, 1975). These hybrids also occasionally produced haploid H. vulgare tillers. Thus progeny of this constitution tended to lose wild chromosomes. AA'A"VV progeny from (HJ X HC)(6x) X VVVV were slightly overbalanced and tended to lose H. vulgare chromosomes. AA'VV progeny from HJ X VVVV only developed to the proembryo stage, suggesting excessive problems with chromosome elimination. The most viable eggs (excluding haploid H. vulgare eggs) would be poorly balanced genomically and chromosome elimination would be expected to be a strong force.

At this point, it was important to determine the chance of obtaining haploid H. vulgare eggs from AA'A"VV hybrids. This required making some assumptions and using the binomial expansion. I chose to first estimate the upper limit for production of haploid H. vulgare eggs. The most favorable circumstances for production of haploid eggs would be to have perfect pairing of the H. vulgare chromosomes (a 7-7 split at anaphase I) and no pairing of the H. jubatum-H. compressum (AA'A") chromosomes. Thus the AA'A" chromosomes would distribute randomly to the

poles. The population of egg gametes would all have a haploid H. vulgare constitution plus H. jubatum-H. compressum chromosomes determined by the binomial expansion of $(1/2 + 1/2)^{21}$. The chance of any one egg having only a haploid H. vulgare constitution is $(1/2)^{21}$, or approximately $5X10^{-7}$. Since approximately 80,000 florets of the Bl.2 were pollinated, the chance of producing one diploid H. vulgare was 80,000 $(5X10)^{-7}$ or approximately .04.

The chance of producing twelve diploid H. vulgare progenies from pollination of 80,000 florets is 5.5 X 10^{-26}. This comes from the Poisson distribution, where $u^{12}/12!e^{u}$ is the chance for 12 such events in any one sample (Sokal and Rohlf, 1969). U can be found by solving $u/e^{u}=.04$, where e=2.71828 and .04 is the chance for one event. U is approximately equal to .0417. Thus, even making these ridiculously ideal assumptions, the chance of producing 12 haploid H. vulgare eggs is so close to zero it can be ignored. Therefore, it would appear that chromosome elimination played a very important role in preventing production of the desired parental material by classical backcrossing methods in this cross.

FUTURE DIRECTION

This study has shown that material having a cytoplasm from H. jubatum is a dead end and chromosome elimination is a powerful force in determining numbers and type of mature progenies produced from many of the crosses. Both of these factors have a profound influence on the chances of producing the desired parental material for an intermating population. Some progress has been made with material having H. vulgare cytoplasm. Techniques including tissue culture are being utilized to take advantage of chromosome elimination in the production of the desired parental material for an intermating population to transfer traits to H. vulgare.

Dr. Tom B. Rice has found it relatively easy to callus, subculture and regenerate large numbers of plantlets of these hybrids. He has used colchicine treatment of callus cells of VV X HJ hybrids to produce amphiploid material of this cross. Root tips of roughly one half of the plantlets from treated callus material have the

amphiploid chromosome number. A single H. vulgare backcross seed was produced from a mature plant from one of these plantlets. This seed appeared to have a completely normal endosperm with plenty of starch, but no embryo was present.

These results indicate there are no major barriers to the production of callus cultures, treatment of callus cells, and regeneration of large numbers of plantlets. These plantlets can be very successfully grown to maturity. These are all necessary steps in the use of tissue culture to produce an amphiploid of VV X HJ and the subsequent use of tissue culture to help produce the desired parental material for an intermating population from this amphiploid.

At present, I consider two different approaches to be the most promising prospects for the production of this parental material from this hypothetical, at present, (VV X HJ)(6x) amphiploid. The first approach is production of embryos of the backcross to H. vulgare and the subsequent production of large numbers of plants with different chromosome number from these embryos by utilizing tissue culture techniques to callus embryos and regenerate large numbers of plantlets. Chromosome elimination will provide the

directional driving force for the production of material with different chromosome numbers. Hopefully, many of the plants will be essentially back to a diploid H. vulgare constitution, but the direction chromosome elimination would operate in embryos of VVAA' genomic constitution can not be determined from the available cross data.

The second approach involves use of callus culture of the amphiploid to produce large numbers of plantlets with different chromosome numbers. Again, chromosome elimination will provide the directional driving force for the production of material with different chromosome numbers. Hopefully, many of the plants will be essentially back to a diploid H. vulgare constitution. Since chromosome elimination is operating in the VV X HJ hybrids in the direction of loss of H. jubatum chromosomes, it would seem quite reasonable to assume H. jubatum chromosomes would also be lost in its amphiploid.

I am very hopeful that one of these approaches will eventually produce the desired parental material for an intermating population, which will lead to the transfer of traits to H. vulgare.

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APPENDIX

TABLE A. Norstog's agar medium II.

COMPONENT	AMOUNT
	g/liter medium
Major minerals	
KH_2PO_4	0.91
KCl	0.75
$MgSO_4 \cdot 7H_2O$	0.74
$CaCl_2 \cdot 2H_2O$	0.74
Trace elements and vitamins*	mg/liter medium
$MnSO_4 \cdot H_2O$	3.0
H_3BO_3	0.5
$ZnSO_4 \cdot 7H_2O$	0.5
$CoCl_2 \cdot 6H_2O$	0.025
$CuSO_4 \cdot 5H_2O$	0.025
$NaMoO_4$	0.025
Inositol (meso-)	50.0
Thiamine·HCl	0.25
Ca-pantothenate	0.25
Pyridoxine·HCl	0.25
Iron source*	mg/liter medium
Sequestrene (sodium ferric diethylenetriamine pentaacetate)	16.0
Amino acids	g/liter medium
L-Glutamine	0.4
L-Alanine	0.05
L-Cysteine	0.02
L-Arginine	0.01
L-Leucine	0.01
L-Phenylalanine	0.01
L-Tyrosine	0.01
Other	
Malic acid**	1.0
Difco Purified Agar	6.0
Sucrose	34.2

*100X stock solution is useful

**Dissolve malic acid in 30 ml H_2O and adjust pH to 5.0 with NH_4OH

TABLE B. Wick embryo culture medium.

MAJOR MINERALS	g/liter medium
Na_2SO_4	0.8
$Ca(NO_3)\cdot 4H_2O$	0.58
$MgSO_4\cdot 7H_2O$*	0.33
KNO_3	0.08
KCl	0.005
$NaH_2PO_4\cdot H_2O$	0.038
MICRONUTRIENTS**	**mg/liter medium**
$MnSO_4$	0.45
$ZNSO_4$	0.6
H_3BO_3	0.00375
KI	0.03
Glycine	3.0
Thiamine·HCl	0.1
Ca-pantothenate	2.5
$CuSO_4\cdot 5H_2O$	0.025
$NaMoO_4$	0.025
$CoCl_2\cdot 6H_2O$	0.25
IRON SOURCE**	
Sequestrene (sodium ferric diethylenetriamine pentaacetate)	16.0
SUGAR SOURCE	**g/liter medium**
Sucrose	20.0

*Dissolve separately.
**100X stock solution is useful.
Adjust complete medium to pH 5.4.