INTERGENERIC CROSSABILITY BARRIERS IN THE TRITICEAE

by

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INTRODUCTION

Recent progress in developing high yielding triticales (Zillinsky and Borlaug, 1971) and the development of chromosome engineering techniques (Sears, 1969) have increased interest in interspecific and intergeneric crosses in the Triticeae. Although some researchers are interested in developing new cereals, such as triticale, wide crosses already have made significant contributions to the gene pools of cultivated cereals. Evolutionary divergence between desirable parents, however, frequently causes unfavorable prefertilization interactions (cross-incompatibility) or abnormal seed development. The purpose of this study was to better define these barriers to crossability in wheat X rye and wheat X barley crosses and to test the effectiveness of genotype and chemical or hormonal treatments in overcoming these barriers.

LITERATURE REVIEW

Genetics of Crossability Between Wheat and Rye

Bread wheat varieties, Triticum aestivum L. var. aestivum, differ in seed set when crossed, as maternal, with rye, Secale cereale L.. The simple inheritance of this trait was first noted by Backhouse (1916) and was subsequently explored further by Taylor and Quisenberry (1935) and Vassiliev (1940).

Lein (1943), according to Riley and Chapman (1967), demonstrated there were two loci, Kr_1 and Kr_2, controlling crossability. The recessive alleles, kr_1 and kr_2, allowed seed set in wheat X rye crosses. The two loci were also reported to differ in their ability to enhance or inhibit seed set. Kr_1 had a stronger effect than Kr_2. Lein proposed a model to explain variable seed set produced by the two loci:

genotype	% seed set
$Kr_1\ Kr_1\ Kr_2\ Kr_2$	0.0
$Kr_1\ Kr_1\ kr_2\ kr_2$	10.0
$kr_1\ kr_1\ Kr_2\ Kr_2$	30.0
$kr_1\ kr_1\ kr_2\ kr_2$	60.0

Sasaki and Wada (1966), according to Lange and Wojciechowska (1976), studied the crossability of intervarietal disomic substitution lines of Cheyenne (crossability 9.1%) in a Chinese Spring background (crossability > 60.0%). Chromosome 5B was identified as having a major effect and chromosomes 4A, 1D and 7D had weaker, more variable effects.

Riley and Chapman (1967) studying wheat X rye crossability in intervarietal disomic substitution lines of Hope (crossability 0.0%)

in Chinese Spring, showed chromosomes 5A and 5B significantly lowered seed set. Kr_1 was identified on 5B and Kr_2 on 5A. The authors also demonstrated, via seed set of nulli-tetra lines, that the action of the Kr genes was due to the inhibitory nature of the dominant alleles. These lines were nullisomic for either 5A or 5B and tetrasomic for 5D. The two extra copies of 5D compensated for their absent homoeologues producing a viable plant. Neither nulli-5A-tetra-5D nor nulli-5B-tetra-5D differed from Chinese Spring in seed setting ability when crossed with rye. The authors reasoned that if the recessive alleles enhanced crossability their absence would decrease seed set. Since this did not occur, they concluded that the dominant alleles must in some way "actively inhibit" seed set.

Although inhibition of crossability is dominant, it is not complete. Lein (1943) reported that the F_1 had higher crossability than the parent with low crossability. The disomic substitution lines CS/Hope-5B and CS/Hope-5A differed in seed set and were intermediate to Hope and Chinese Spring. This led Riley and Chapman (1967) to conclude that the loci are either complementary or additive. Marais and Pienaar (1977a) recently provided additional evidence to support the above dominance and epistatic relationships.

In crosses between CS/Hope-5B and CS-ditelo-5B, the recombination frequency between the Kr_1 locus and the centromere was determined to be $11.45 \pm 3.0\%$ (Lange and Riley, 1973). No comparable experiment has been reported concerning Kr_2 and its position on 5A.

The genetics of crossability between tetraploid wheats and rye was not as simple as the bread wheat system described above. Pienaar (1973), Moss (1972) and Krolow (1970) have demonstrated the tetraploids

have generally good sed set when crossed with rye but hybrid embryos have poor germination. Substantial variation existed between cultivars and between species with regard both to the total number of seed set and to kernel development (Marais and Pienaar, 1977a; Moss, 1972; Krolow, 1970).

Diploid wheat behaved in a manner similar to the tetraploids. Seed setting ability varied and growth of hybrid embryos and endosperms were often considerably retarded (Marais and Pienaar, 1977b; Krolow, 1973; Melnyk and Unrau, 1959). No hybrid has been reported from direct crosses between diploid T. monococcum L. and Secale spp.. The desired 4x triticale amphiploids, however, have been produced by other methods (Krolow, 1973). The genus Aegilops, now classed Triticum (Morris and Sears, 1967) contains many diploid species some of which cross more readily with rye than T. monococcum (Marais and Pienaar, 1977b; Krolow, 1973; Melnyk and Unrau, 1959). Attempts have been made to classify the diploid and tetraploid wheats under the Kr system proposed for hexaploids (Marais and Pienaar, 1977a,b; Moss, 1972; Krolow, 1970), however, this effort has generated considerable debate (Lange and Wojciechowska, 1976; D'Souza, 1978) and is still unresolved.

Evolution of Crossability Barriers

An interesting model of the evolution of the cross-incompatability genes in bread wheat has been proposed by Riley and Chapman (1967). The authors noted the low crossability of European varieties and the high crossability of Chinese varieties. European cultural practices resulted in mixed populations of bread wheat and rye. Hybrid seed from crosses between these species usually had good germinability and produced vigorous,

sterile plants which acted as annual weeds. Selection favored bread wheat genotypes which would not generate their own weeds. The Chinese die not co-cultivate rye and wheat, and consequently, there was no selection for cross-incompatibility. European tetraploids, also co-cultivated with bread wheat and rye, usually had good seed set when crossed with rye. The hybrid kernels, however, were usually viable due to endosperm collapse; the result, no selection for cross-incompatibility (Riley and Chapman, 1967).

Pre-fertilization Events

In cereals, onset of pollen germination is rapid and variable (Kihara and Hori, 1966; Hoshikawa, 1960; Pope, 1937). Germination increases with larger pollen populations (Ter-Avanesian, 1978; Chandra and Bhatnagar, 1974; Brewbaker and Majumdar, 1961) and is dependent upon the age of both the pollen and the stigma (Watanebe, 1961). Boyes and Thompson (1937) reported large differences in rye pollen germination (10% vs. 60%) on low and high crossable bread wheat stigmas. Subsequent studies have not substantiated this report and instead note optimum pollen germination for wheat and rye (regardless of pistil compatability) to be about 60% (D'Souza, 1978; Lange and Wojciechowska, 1976; Zeven and van Heemert, 1970; Tozu, 1966).

In cereals pollen tubes penetrate the stigma and grow intercelluarly in the feathery stylar tissue (Chandra and Bhatnagar, 1974). The growth rate is variable and abnormalities such as corkscrewing, misorientation and tip swelling commonly occur (Lange and Wojciechowska, 1976; Luxova 1967; Kihara and Hori, 1966). Similar events have been reported in numerous wheat X rye crosses (Tozu, 1966; Zeven and van Heemert, 1970;

Lange and Wojciechowska, 1976; D'Souza, 1978). In selfed material, pollen tubes continued growth through the style and the transmitting tissue above the ovary cavity (Pope, 1937; Chandra and Bhatnagar, 1974). Although numerous pollen tubes were observed in the transmitting tissues of the style and ovary (D'Souza, 1978; Lange and Wojciechowska, 1976), few were observed in the ovary cavity (Lange and Wojciechowska, 1976). Occasionally, however, more than one tube was observed at the micropyle (Lange and Wojciechowska, 1976; Chandra and Bhatnagar, 1974).

The nature of the crossability barrier in bread wheat X rye crosses has been the subject of considerable debate and confusion. Tozu (1966) reported that differences in hexaploid wheat X rye crossability did not result from inhibition of pollen tube growth in the style but was due to some other undefined pre-fertilization barrier. Zeven and van Heemert (1970) reported pollen tubes of Secale segetalis L. near the micropyle of high and low crossable genotypes. Lange and Wojciechowska (1976) studied pollen tube growth in the disomic substitution line CS/Hope 5B (crossability ≈ 10.0%), Hope (crossability 0.0%) and Chinese Spring (crossability > 60.0%). They presented cytological evidence that the site of the Kr-gene inhibition was in the transmitting tissue of the ovary and style. D'Souza (1978) continues to disagree, however, and reported no differences in rye pollen tube growth in high and low crossable styles. D'Souza, however, did not study pollen tube growth in the ovary.

Another example of stylar cross-incompatibility in cereals has been observed by Pickering and Hayes (1976). They reported that several Hordeum vulgare L. cultivars had lower than average seed set (10% vs. 60%) after crossing with H. bulbosum L.. Inhibition of pollen tube

growth in the style was identified as the reason for reduced seed set.

Fertilization and Post-fertilization Events

The mature unfertilized embryo sac and early seed development have been extensively studied in wheat, barley, rye and triticale (Pope, 1937; Morrison, 1944; Luxova, 1967; Cass and Jensen, 1970; Bhatnagar and Chandra, 1975; Kaltsikes, 1973). Bennett et al. (1973, 1975) conducted studies of early seed development for several members of the Triticeae. Although differences occurred between studies, genotypes and environments, the overall processes and structures were similar in all selfed material.

The unfertilized anatropous cereal ovule contains a single monosporic, polygonum type of embryo sac (Maheshwari, 1950). Located at the micropylar end are three cells, the egg and two synergids. In squash preparations the egg nucleus was observed to be in an "early prophase-like condition" (Bennett et al., 1975). The polar nuclei lie in a large vacuolated central cell adjacent to the egg apparatus in a band of cytoplasm running from the egg apparatus to the antipodal cells (Cass and Jensen, 1970; Bhatnagar and Chandra, 1975). The polar nuclei were observed to be closely appressed to one another and also in an "early prophase-like condition" (Bennett et al., 1975). The antipodal cells lie opposite to the micropyle at the chalazal end of the embryo sac. Moss (1972) reports that antipodal size varies and that the mean number of cells per embryo sac ranged from 12 to 19.5 in Triticum spp.. The antipodals are endoploid with a mean nuclear DNA content of 52C at anthesis in T. aestivum L. cv. Chinese Spring, 4C being the amount in a somatic prophase cell (Bennett et al., 1973).

In selfed material, fertilization or deposition of the sperms inside the embryo sac has been reported to occur as early as 30 minutes and as late as 14 hours following pollination (Wojciechowska and Lange, 1977; Bennett et al., 1973, 1975; Morrison, 1954; Pope, 1937). Upon fertilization both the egg and the polar nuclei formed numerous nucleoli (Bennett et al., 1973, 1975; Bhatnagar and Chandra, 1975; Luxova, 1967) and the sperms became diffuse and formed their own nucleoli (Morrison, 1954; Luxova, 1967). Zygotes divided between 15-26 hours after pollination depending upon the genotype and the environment (Bennett et al., 1973, 1975; Morrison, 1954; Pope, 1937). The first zygotic division in Chinese Spring was transverse, with the basal suspensor cell undergoing no further divisions and the upper cell developing into the embryo (Bennett et al., 1973). Cell cycle time in the embryo varied from 11 to 19 hours and was species dependent (Bennett et al., 1975).

The polar nuclei in selfed material were fertilized at approximately the same time as the egg, but underwent division five to seven hours after pollination (Bennett et al., 1973, 1975; Wojciechowska and Lange, 1977). Early divisions were synchronous and coenocytic. Cellularization began near the micropyle at approximately 72 hours following pollination. Asynchrony was gradual and neighboring cells retained some synchrony for several days. Nuclear doubling time in the endosperm was short at first (4.5 hours) but gradually lengthened until it was equal to that of the embryo by about the fifth day (Bennett et al., 1975).

Some early researchers concluded that hybrid embryo development following interspecific or intergeneric hybridization was comparatively normal (Thompson and Cameron, 1927; Boyes and Thompson, 1937; Beaudry, 1951). Other abnormalities, most notably in the endosperm, were

identified as leading to the death of the embryo. This frequently may be the case, as evidenced by the successful use of embryo culture in rearing immature hybrid embryos (Kaltsikes, 1974; Brink et al., 1944). Embryonic abnormalities, however, may be the cause of seed failure independent of the endosperm (Wakakua, 1934; Sears, 1944; Gill and Waines, 1978). Nakamura (1966) reported a delayed first mitosis and retarded early divisions in hexaploid wheat X rye embryos. Wojciechowska and Lange (1976) reported the opposite, that cell cycle time was shortened in hybrid embryos when compared to selfed parents. Bennett et al. (1975), working with hexaploid triticale, octoploid triticale and disomic wheat-rye chromosome addition lines, reported that generally rye chromosomes lengthened cell cycle time in the embryo and endosperm. There were exceptions, however; the triticale cultivar Rosner was reported to be similar to wheat species (12.5 hours).

In hybrid endosperm giant nuclei, bridges between nuclei and lack of cellularization have been reported in interspecific and intergeneric crosses (Wojciechowska and Lange, 1977; Moss, 1972; Boyes and Thompson, 1937; Wakakua, 1934). Crosses between species or genera of different ploidy levels generally set more viable seed when the maternal parent had the higher chromosome number (Kihara and Nishiyama, 1932). In wheat X rye crosses, the higher the ploidy level of the wheat species the more viable the hybrid kernel (Krolow, 1970). This corresponds to the cytological observations of Moss (1972), who studied endosperm development in 2x, 4x and 6x wheat x rye crosses. He observed abnormal endosperm development in crosses at all three ploidy levels, but reported that the severity of the abnormalities increased at lower ploidy levels.

The degree of seed shrivelling has been proposed to be due to

chromosomal unbalance or unfavorable genome ratios between embryo, endosperm and maternal tissue (Thompson and Cameron, 1929; Boyes and Thompson, 1937; Watkins, 1932). Dhaliwal (1977) suggested that genome ratios in endosperm tissue resulting from crosses between species at the same ploidy level may account for seed failure. The cross Triticum uraratu Tum. X T. boeticum Boiss. yields shrivelled seed; the reciprocal, however, yields plump viable seed. Cytoplasmic effects were discounted by reproducing the crosses in nuclear-substitution lines. Recently, Gill and Waines (1978) have challenged Dhaliwal's conclusions and have provided evidence that in diploid Triticum and Aegilops crosses (including T. uraratu X T. boeticum and its reciprocal) factor(s) which lead to endosperm collapse were simply inherited. One gene studied, which led to endosperm collapse, was expressed only when crossed as paternal. The degree of shrivelling was dependent upon the number of copies of that gene in the endosperm, thus leading Dhaliwal (1977) to the incorrect conclusion concerning genome ratios. Kihara and Nishiyama (1932) working with interspecific Avena crosses and Schwartz (1963) with intraspecific Zea mays crosses have also suggested there is differential gene expression of male and female loci in endosperm tissue. Davies (1973) provided electrophoretic evidence for differential gene expression in embryonic tissue in reciprocal intraspecific crosses in Pisum sativum L..

Cooper and Brink (1944) and Beaudry (1951) have suggested that lack of stimulation of the antipodal cells following intergeneric hybridization could cause the observed endosperm disturbances. Antipodal cells have been hypothesized as having a nutritive role, furnishing the rapidly developing coenocytic endosperm with metabolites and rRNA for protein synthesis (Bennett et al., 1975). Antipodal enlargement just prior to

and after fertilization and their degeneration several days later correlates with the rapid growth stage of the endosperm. Their basiophyllic cytoplasm and large nucleoli also indicate a nutritive role (Bennett et al., 1975); however, biochemical or histochemical studies have yet to provide a definitive answer. Regardless of the role antipodal cells play in developing seed, some researchers did not believe their behavior was sufficiently abnormal to be the cause of endosperm breakdown (Thompson and Johnston, 1945; Morrison, 1954; Moss 1972).

Genetic Manipulation of Crossability Barriers

Cross-incompatability or post-fertilization barriers (ranging from endosperm collapse to F_1 hybrid sterility) are isolating mechanisms. They have arisen either as a consequence or a cause of speciation (Sears, 1944). Geneticists and breeders have been attempting to circumvent these barriers to expand crop gene pools. Radiations, chemicals, and tissue culture techniques have been employed with varying degrees of success (Pienaar, 1973; Bates et al., 1974; Kruse, 1973; Carlson et al., 1972).

The most successful methods to date have been those exploiting the variability within populations of interest and identifying compatible or partially compatible genotypes. In the latter case researchers frequently have employed hormonal or embryo culture techniques to obtain the desired hybrid (Kruse, 1973; Kaltsikes, 1973).

Finding compatible genotypes is easiest in closely related species or genera, where crossability barriers are often simply inherited (Backhouse, 1916; Sears, 1944; Pickering and Hayes, 1976). Compatible genotypes may be lacking, however, in more distantly related populations. In such cases, a population compatible to the two incompatible populations

may be used to transfer the traits of interest. Kimber and Sallee (1976) provided an example of the usefulness of this approach when they crossed Triticum timopheevi Zhuk. X Hordeum bogdanii. The amphiploid resulting from that cross is self-fertile and cross-fertile with T. timopheevi and T. aestivum (Wheat Newsletter, 14, 1978, p. 102). The use of this and similar amphiploids as "bridging species" may offer a practical method of transferring genes between wheat and barley.

Bridging species have received attention from breeders interested in expanding the gene pool of triticale (Krolow, 1973; Marais and Pienaar, 1977a,b). Krolow (1973) reported that 6x auto-alloploid (AAAABB) and 4x autoploid (AAAA) wheat had more viable seed when crossed with rye than their corresponding 4x and 2x progenitors. Alloploid 4x triticales (ABRR) have been produced by crossing 6x triticales (AABBRR) with rye and selfing the progeny for several generations (Krolow, 1973; Gustafson and Krolow, 1978). These 4x triticales displayed good seed set when selfed and when crossed with rye, 6x and 8x triticales. This has led Krolow (1973) to propose the use of 4x triticale as a crop, as well as, a bridging species.

The identification of D-genome chromosomes in the more successful triticales (Gustafson and Qualset, 1975) and the high crossability of Aegilops spp. with rye (Melnyk and Unrau, 1959; Krolow, 1973; Marais and Pienaar, 1977b) has led to interest in bridging species which contain D-genome chromosomes. Marais and Pienaar (1977b) reported an enhancement of seed set and kernel development in synthetic 6x alloploids derived from 4x wheats and Ae. squarrosa L. crosses. The authors provided evidence that this enhancement was not due solely to the increased ploidy level of the artificial alloploid.

The advantages of higher maternal ploidy were discussed in the chapter dealing with post-fertilization events. Exceptions to this generalization, however, do occur. The most notable being the higher crossability crossability of H. vulgare X T. aestivum as compared to its reciprocal, 5.8% vs. < 1.0% (Islam et al., 1975). The reason for this reciprocal difference was not made clear, but Kruse (1973) has suggested that it is partially due to the underdeveloped nature of T. aestivum X H. vulgare embryos. The amphiploids from Hordeum X Triticum crosses all have been male-sterile and partially female-sterile (Islam et al., 1975; Kruse, 1974; Fedak, 1978), and unfavorable nucleo-cytoplasmic interactions may be responsible (Islam et al., 1975). Siminar nucleo-cytoplasmic interactions may account for the differences in crossability as well (Maan, 1976).

Chemical and Hormonal Manipulation of Crossability Barriers

Plant hormones were first suggested as a means of enhancing cross-ability by Emsweller and Stuart (1948). Larter and Enns (1961) reported that gibberellic acid (GA_3) enhanced both ovule and embryo development in crosses between autotetraploid and diploid barley. Larter and Chaubey (1965) were less successful in crossing barley X rye, although GA_3, (100 ppm) enhanced kernel set and embryo recovery. No embryos were sufficiently differentiated to germinate on the culture media. Kruse (1967, 1973) reported hybrid plants were obtained after crossing H. vulgare with S. cereale, T. aestivum and T. turgidum, when GA_3 (75 ppm) was applied to the florets twice following pollination. The H. vulgare X T. aestivum and H. vulgare X T. turgidum crosses since have been made

without the use of any chemical application (Thomas et al., 1977). Recently, Kasha et al. (1978) reported that GA_3, applied three days following pollination significantly enhanced seed set, emybryo recovery, and germinability of haploid embryos upon culturing. Effective concentrations ranged from 12.5 ppm to 150 ppm with the optimum concentrations being 37.5 ppm and 75 ppm.

Kruse (1974) claimed that treatment of H. vulgare pistils prior to pollination with 2,4-dichloro-phenoxyacetic acid (2,4-D), 10-100 ppm, eliminated pre-fertilization barriers and allowed crossing with a wide variety of distantly related grasses. Post-pollination treatments with GA_3 (75 ppm) were also necessary to obtain positive results. Crossed and treated spikes set approximately 80% seed and had 10-20% embryo recovery.

Cross-incompatibility has been hypothesized to be similar to immune responses in animals (Makinen and Lewis, 1962). Bates and Deyoe (1973) used the term stereo-specific incompatability reaction (SIR) for this hypothesized response. They suggested the use of animal effective immunosuppressants to overcome SIR. Bates et al., (1974, 1976) reported success using the chemicals in overcoming the incompatability barrier(s) between bread wheat X barley, durum wheat X barley and barley X rye. e-amino-caproic-acid (EACA), a lysine analogue, was identified as being the most effective.

EACA has been applied under varying concentrations and treatment schedules to durum wheat varieties. Bates et al. (1977) used a foliar application of 1000 ppm for two weeks prior to pollination. They reported a 45.2% increase in total seed set and a 22.3% increase in embryo formation. The effect of EACA was noted to be genotype dependent. Taira

and Larter (1977a) tested several concentrations and treatment schedules. They applied EACA by dropping the solution into the void species of spikelets with a hypodermic syringe. Treatment of pistils with EACA at 1000 ppm for three to four days following pollination was observed to have the optimum effect. EACA was not reported to enhance the number of seeds set or the number of embryos recovered. Rather, the authors reported that EACA, as well as lysine, promoted embryonic growth and development (Taira and Larter, 1977a,b).

Environmental Effects on Crossability Barriers

Maternal plant vigor and nutrient supply has been reported to affect seed set following crossing of H. vulgare X H. bulbosum (Kasha et al., 1978). Temperature significantly affected embryo development in the cross T. turgidum X S. cereale (Taira and Larter, 1977b). The authors reported that a constant 17° C temperature with an 18 hour day enhanced embryo development.

MATERIALS AND METHODS

Table Ia

Maternal Genotypes

Triticum aestivum L. var. *aestivum* (AABBDD)
cultivars: Bonza[1]
Torim[1]
Jupateco
WS 1809
Chinese Spring[1]

Triticum turgidum L. var. *durum* (AABB)
cultivars: Pinguino
Erpel
Cocorit-71

Triticum timopheevi Zhuk. var. *timopheevi* (AAGG)[1,2]
cultivar: unknown

Triticum monococcum L. (AA)
cultivar: CI 2433

Aegilops squarrosa L. (DD)[3]
cultivar: unknown

Table 1b

Paternal Genotypes

Secale cereale L. (RR)
cultivars: Prolific
Petkus
Snoopy

Secale montanum L. (RR)
cultivar: unknown

Hordeum vulgare L. (HH)
cultivar: Manker (M-16)

[1]Also used as a paternal parent as a control in cytological investigations.

[2]Mujeeb *et al.*, 1978.

[3]Also known as *Triticum tauschi* (Morris and Sears, 1967).

Growth Conditions

All material was grown in 4 x 4 inch plastic pots, two plants per pot and 12 pots per tray. Each tray consisted of one genotype. Potting mixture was composed of 3 parts soil, 1 part peat and 1 part vermiculite. All pots received commercial fertilizer upon transplantation of seedlings and again at early boot stage.

All plant material was housed in one of four environments.

1. The greenhouse during fall, winter and spring. Supplimentary lighting to maintain a 16 hour day was provided by 300 watt incandescent bulbs. Light intensity, temperature and humidity varied with the climate. Material was often started and grown in this environment but transferred to growth chambers at boot stage or upon emasculation. Most pollen parents were reared in the greenhouse, although nicking of cultivars and high summer temperatures required controlled environments.

2. Growth chamber #1 maintained a 16 hour day at about 32,000 lux. Day/night temperatures were 26.6° C/15.5° with approximately 50% relative humidity. Hexaploid and tetraploid wheat were grown in this environment from the seedling stage. The effects of maternal genotype and EACA on crossability with rye were measured. The two diploid wheat species were transferred to this environment upon reaching the boot stage and the effect of paternal and maternal genotype on crossability with rye was examined.

3. Growth chamber #2a maintained a 16 hour day at about 50,000 lux. Day/night temperatures were 15° C/12° C with approximately 60% relative humidity. Emasculated spikes of T. monococcum L. CI 2433 were transferred to this environment from the greenhouse. Individual spikes were clipped from plants and placed in fresh tap water and changed daily.

Various plant growth substance treatments were applied.

4. Growth chamber #2b maintained a 24 hour day at about 50,000 lux. Temperature remained constant at 17° C with approximately 60% relative humidity. The photoperiod was proposed by Bennett et al. (1973) as a means of synchronizing fertilization events for cytological investigations by "piling up" receptive florets on a spike. Bonza was transferred to this growth chamber in early boot stage, prior to meiosis, and crossed with either M-16 barley or Bonza.

Emasculation and Pollination Procedures

Florets were emasculated when anthers were yellow-green. Attempts were made to be as uniform as possible with pollination occurring three days later.

Rye pollen was collected in the morning at anthesis by tapping a spike over a petri dish. The pollen was liberally applied to wheat stigmas within the half hour with a camel hair brush.

Barley and wheat pollen was collected just prior to anthesis. Ripe individual anthers were crushed over several florets. This manner of pollination subsequently was revealed to be inadequate for cytological investigations. Insufficient pollen populations were often present on stigmas to induce optimal levels of germination (Brewbaker and Majumdar, 1961; Ter-Avanesion, 1978). Consequently some Bonza X M-16 crosses were repeated but in the greenhouse rather than growth chamber #2b.

Chemical and Hormonal Treatments

EACA was applied to hexaploid and tetraploid wheat genotypes to determine its effect on crossability with rye. The concentration of

1000 ppm was suggested by Bates et al. (1977) and Taira and Larter (1977a). The solution was dropped into emasculated florets with a hypodermic syringe (Taira and Larter, 1977a). The void spaces around the pistil and between the primary and secondary florets were filled to overflowing. Quantification of the amount applied was not practical. EACA was applied to the hexaploid wheat cultivars for three evenings prior to pollination. EACA was applied to the tetraploid wheat cultivars, however, for three evenings following pollination, as suggested by Taira and Larter (1977a). In all cases an approximately equal number of spikes was crossed as a control. Treatments were randomized within each genotype by the toss of a coin.

The plant growth substances 2,4-D and GA_3 were used in an attempt to enhance crossability between T. monococcum and Secale spp.. Due to the extreme variability of maturity of florets on a spike, most were pollinated two and four days after emasculation (spikes in experiments of paternal and maternal genotype effects all received one pollination). There were four treatments:

1. GA_3 (75 ppm), two post-pollination application; all spikes pollinated with Prolific.
2. 2,4-D (50 ppm), a single pre-pollination application; includes spikes pollinated with Prolific and Petkus.
3. 2,4-D (50 ppm) + GA_3 (75 ppm) applied as in 1 and 2; includes spikes pollinated with Prolific and Petkus.
4. untreated, dry control; all spikes pollinated with Prolific

Embryo Culture and Scoring of Kernel Development Resulting from Wheat X Rye Crosses

Spikes were collected between 10 and 22 days following pollination.

Table II

Casein Hydrolysate Media

KH_2PO_4	900.00[1]
KCl	750.00
$MgSO_4$	366.00
$MnSO_4 \cdot H_2O$	3.00
H_3BO_3	0.50
$ZnSO_4 \cdot 7H_2O$	0.50
Na $MoO_4 \cdot 2H_2O$	0.03
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
Thiamine-HCl	1.00
Nicotinic acid	1.00
Pyridoxine-HCl	1.00
m-Inositol	100.00
Succinic acid[2]	440.34
$CaCl_2 \cdot 2H_2O$	750.00
EDTA-Fe	25.00 g/l
Sucrose	51.30 g/l
Casein hydrolysate	2.50 g/l
Agar[3]	9.50 g/l

[1]All quantities in mg/liter unless otherwise stated.
[2]Or 500 mg/l malic acid; in both cases adjust to pH = 5.5.
[3]Autoclave 15 min at 115°C.

Seeds were scored under a dissecting scope and placed in one of four categories:

1. stimulation, embryo and endosperm absent or degenerate.
2. endosperm only, endosperm tissue of on some degree of development (or degeneration) present but embryo absent.
3. embryo only, embryonic tissue of some form present but endosperm tissue absent.
4. embryo + endosperm, both present, although in varying degrees of development.

Embryo length was measured for Chinese Spring X Prolific and Pinguino X Prolific crosses collected 22 days following pollination. In the lesser differentiated hybrid embryos the mass diameter was measured. An ocular micrometer at 60x magnification was used for all measurements.

Embryo culture was used primarily in attempts to rear hybrid embryos from CI 2433 X rye crosses. Random samples of embryos from other wheat X rye crosses were cultured also on the media formulated by Drs. Taira and Larter of the University of Manitoba. Its ingredients are listed in Table II.

Cytology

Fluorescent microscopy was used to follow the course of pollen tubes in the feathery cereal style. The analine blue technique of Martin (1959) was used, with the exception that styles were cleared in 1 N NaOH for 3 hours, rather than 8 N NaOH for 8 hours. Spikes were collected and fixed in a solution of formaldehyde, acetic acid and 95% ethanol (FAA, 1:1:8) priot to pollination and at various intervals following pollination. Preparations were examined with a Reichert incident fluorescent system.

A blue exciter filter (3-BG-12), a barrier filter (1-GG-9 + 2-OG-515) and a mercury-vapor lamp were used to yield UV light with wavelength from 350-450 nanometers. Callose fluoresced a bright greenish-blue. Bonza X Prolific, Chinese Spring X Profific, Bonza X M-16 and Bonza X Bonza were examined using this technique.

Serial sections were examined to follow the course of the pollen tubes in the ovary and to study fertilization and subsequent events. Bonza X M-16 and Bonza X Bonza crosses were the focus of this study but Bonza X Prolific, Jupateco X Prolific, T. timopheevi X Prolific, T. timopheevi X T. timopheevi, Bonza selfed and Torim selfed were examined as well. Spikes were fixed in Bouins solution (Sass, 1951) prior to pollination and 2, 8, 24, 48, and 96 hours following pollination. A few intermediate timings were also observed. An ethanol-butanol series was used to dehydrate and clear the tissues. Due to the brittle hairs on the top of the ovary sectioning was difficult. Satisfactory, although not excellent, results were obtained by:

1. Using Paraplast Plus, an imbedding media with DMSO (dimethyl-sulfoxide) and plastic polymers, as well as, paraffin (melting pt., 56° C).

2. Cutting thicker sections, approximately 13-17 microns.

3. Cooling the mounted specimens in a freezer for 30 minutes prior to sectioning.

Slides were stained in either Delafield's fast-ripening or Harris's hematoxylin (Sass, 1951) and counter stained with either fast green or eosin-y (1.0% in ethanol).

RESULTS AND DISCUSSION

Crossability of Hexaploid Wheat X Rye

Chinese Spring has been identified as a double homozygous recessive ($kr_1kr_1kr_2kr_2$, crossability > 60.0%) (Riley and Chapman, 1967). The observations of this study (Table III) coincided with the reports for Chinese Spring. The other four genotypes were phenotypically classified as double homozygous dominant ($Kr_1Kr_1Kr_2Kr_2$, crossability < 1.0%). The lack of intermediate genotypes was not surprising in so small a sample. Lange and Wojciechowska (1976) reviewed the many wheat X rye crossability studies and estimated that 80% of T. aestivum genotypes were $Kr_1Kr_1Kr_2Kr_2$.

Due to low seed set in the poorly crossable combinations, the data were insufficient to determine if genetic variability existed among these five genotypes, for the ability to develop viable seed upon fertilization. Pienaar (1973) reported, however, that this variability was present within T. aestivum.

EACA (1000 ppm) applied prior to pollination had no effect either in enhancing or in inhibiting seed set in these genotypes when crossed with rye (Table III). The inhibitory action of the dominant Kr alleles was not affected by the immunosuppressant. It was of interest to note that in the absence of the inhibitory alleles (i.e., Chine Spring) EACA also had no effect.

Due to the low seed set of Bonza, Jupateco, WS 1809, and Torim, it was possible only to evaluate the effect of EACA on hybrid seed development for Chinese Spring X Prolific crosses. EACA had no effect on the number of seeds with an embryo and/or an endosperm (Table III). Treated hybrid embryos from the same cross showed no enhancement in development as measured by their length (Table IV).

Table III

Hexaploid Crossability A. Effect of Maternal Genotype and EACA on Crossability with Prolific Rye.

Female Parent and Treatment	# Spikes	# Florets	Total Seed Set	%[1]	Stimulation	%[2]	Endosperm Only	%[2]	Embryo Only	%[2]	Endosperm + Embryo	%[2]
Chinese Spring control	13	418	339	81.10[a3]	7	2.07[a]	8	2.36[a]	12	3.54[a]	312	92.04[a]
Chinese Spring EACA	14	467	365	78.16[a]	8	4.93[a]	4	1.10[a]	20	5.48[a]	323	88.49[a]
Bonza control	14	380	4	1.05[b]	-	-	-	-	-	-	4	100.00
Bonza EACA	15	408	3	0.74[b]	-	-	2	66.70	-	-	1	33.30
Torim control	10	210	2	0.95[b]	-	-	-	-	-	-	2	100.00
Torim EACA	11	226	8	0.88[b]	-	-	-	-	-	-	2	100.00
WS 1809 control	9	201	1	0.50[b]	-	-	-	-	-	-	1	100.00
WS 1809 EACA	8	168	1	0.60[b]	-	-	-	-	-	-	1	100.00
Jupateco control	12	278	0	0.00[b]	-	-	-	-	-	-	-	-
Jupateco EACA	9	216	1	0.47[b]	-	-	-	-	-	-	1	100.00
Total H			(9 d.f.) 62.22**		(8 d.f.) 2.26		(8 d.f.) 5.05		(8 d.f.) 5.10		(8 d.f.) 12.30	
Genotype H			(4 d.f.) 61.82**									
EACA vs. Control			(1 d.f.) 0.01									

$H \sim X^2$ $\quad {}_9X^2_{0.10} = 14.68 \quad {}_8X^2_{0.10} = 13.36 \quad {}_4X^2_{0.10} = 7.78 \quad {}_1X^2_{0.10} = 2.71$

[1]Percent of florets.
[2]Percent of seed set.
[3]Figures followed by the same letter are NS ($\alpha = 0.10$).
**Significant at $\alpha = 0.01$.

Table IV

Effect of EACA on Embryonic Length of Chinese Spring X Prolific Embryos.

Treatment	n	$\bar{X}$	S^{2*}	$S_{\bar{x}}$	t
Control	39	1.734 mm	0.0936	0.005 mm	0.366^{NS}
EACA	36	1.698 mm			

$(73)|t|_{(0.10)} > 1.995$

*Bartlett's test for heterogeneity of variance, NS ($\alpha = 0.10$)

The environment has been reported to affect interspecific (Kasha et al., 1978) and intergenic (Taira and Larter, 1977b) crossability in cereals. The greenhouse environment in January, cool with low light intensity, however, had no effect on the cultivar Bonza when compared to growth chamber #1 (Table V). Most qualitative traits are independent of minor environmental changes and the Kr genes are probably no exception.

The mechanism of the Kr alleles was demonstrated by Riley and Chapman (1967) to be of an inhibitory nature. Unfortunately, the biochemistry of this inhibtion has yet to be defined. Bates and Deyoe (1973) hypothesized that cross-incompatibility, in general, may be a stereo-specific inhibition reaction (SIR). This inhibition, according to the authors, could be caused by protein-protein or possibly protein-phenolic interactions. The inhibiting molecule(s) were visualized to interact with proteins/enzymes of incompatible pistils to control fertilization and embryogenesis. In later studies, Bates (1975) and Bates et al. (1974) reported favorably on the use of immunosuppressants in overcoming SIR. It was suggested that the immunosuppressants interfered with the production of these hypothesized inhibiting molecules. EACA was identified as being the most effective (Bates et al., 1974).

It appears from the studies reported here (Table III), that EACA has not affected the Kr alleles. This does not, however, rule out Bates and Deyoe's (1973) hypothesis for this particular cross-incompatability system and other immunosuppressants or treatment schedule may have some effect.

Hogenboom (1975) has proposed another interesting system to explain the many different manifestations of crossability barriers. He terms

Table V

Hexaploid Crossability B. Effect of EACA and Environment on Bonza X Prolific Rye Crosses.

Treatment	# Spikes	# Florets	Total Seed Set	%[1]	Stimulation	%[2]	Endosperm Only	%[2]	Embryo Only	%[2]	Endosperm + Embryo	%[2]
Growth Chamber #1 control	14	380	4	1.05	-	-	-	-	-	-	4	100.0
Growth Chamber #1 EACA	15	408	3	0.74	-	-	2	66.7	-	-	1	33.3
Greenhouse control	15	350	9	2.57	-	-	3	33.3	-	-	6	66.7
Greenhouse EACA	16	348	4	1.15	-	-	2	50.0	2	50.0	-	-
Total H (3 d.f.)				1.06				2.14		2.93		5.64

$H \sim X^2 \quad 3X^2_{0.10} = 6.25$

[1] Percent of florets.

[2] Percent of seed set.

it "incongruity," a mismatching of genetic systems. According to this hypothesis, co-evolution of sexual partners (pollen-pistil, embryo-endosperm, etc.) results in specific metabolic requirements and supplies. Closely related species or genera cross since their genetic systems have not fully diverged (Hogenboom, 1975).

One could argue that Hogenboom's model does not apply to the Kr-system. Riley and Chapman (1967) demonstrated that pollen tubes are inhibited by the dominant alleles, rather than having an essential metabolite supplied by the recessive alleles. Present knowledge in molecular biology, however, informs us that single genes (proteins) may regulate metabolic process4s. This regulation may be accomplished by interactions between a regulatory protein (Kr-gene product) and an enzyme. Regulatory proteins also bind with DNA and affect gene expression (in this case the Kr locus may be either the DNA that the protein binds to or the DNA that codes for the regulatory protein). In any case, it is clear that the dominant Kr alleles do not have to code for a protein that "actively inhibits" pollen tube growth, as surmised by Riley and Chapman (1967). Rather, the Kr-locus may produce a protein that regulates the synthesis of a metabolite(s) required for rye pollen tubes and not for wheat pollen tubes. The additive nature and unequal effects of Kr_1 and Kr_2 could be attributed to enzyme kinetics and different affinities of a dimorphic regulatory protein.

This is, of course, pure speculation as are the hypotheses of others. No convincing evidence to explain the biochemical nature of cross-incompatability in grasses has been produced. This is curious, for the Kr system in T. aestivum offers an ideal "model system." The genetics have been well documented and the traits of interest are simply inherited.

The availability of chromosome substitution lines of Hope into a Chinese Spring background simplifies the production of isogenic lines and would allow research in a well defined genotype. The use of this model system would not only facilitate studies concerning cross-incompatability, but could also offer an opportunity to study the evolution of a gene. Kr_1 and Kr_2 are probably homoeologous, although proof of this requires a linkage study on Kr_2 similar to the one conducted on Kr_1 (Lange and Riley, 1972). Isolation of a protein (Kr gene product) either inhibitory, enzymatic or regulatory (a large assumption) would allow comparison of Kr_1 and Kr_2 on a molecular level. This would be of academic interest in its own right, but could also shed light on the evolution of the Triticeae.

Crossability of the Tetraploid Wheats X Rye

Krolow (1970) and Pienaar (19739 reported differences between genotypes of tetraploid wheat species in the number of seed set when pollinated with rye. Genotypes also differed in their ability to develop viable seed. The studies reported here confirm their observations. All four genotypes were significantly different in seed set when pollinated with rye (Table VI). Seed development was observed to be genotype-dependent as well. In all four genotypes, embryo and endosperm development appeared to be largely independent of each other. This was evidenced primarily by the high incidence of seeds in the "embryo only" and "endosperm only" categories. In addition, some seed with moderately well developed endosperm tissue lacked embryos. Occasionally, seed which had fairly large differentiated hybrid embryos (approximately 2.0 mm in length as compared to $2.5 \pm .03$ mm in 4x wheat selfs 22 days after

Table VI

Tetraploid Crossability. Effect of Maternal Genotype and EACA on Crossability with Prolific Rye.

Female Parent and Treatment	# Spikes	# Florets	Total Seed Set	% [1]	Stimulation	% [2]	Endosperm Only	% [2]	Embryo Only	% [2]	Endosperm + Embryo	% [2]
Pinguino control	23	527	107	20.3^{b}[3]	8	7.5^{b}	22	20.6^{a}	21	19.6^{bc}	56	52.3^{a}
Pinguino EACA	17	425	79	18.6^{b}	4	5.1^{b}	17	21.5^{a}	11	13.9^{c}	47	59.5^{a}
Cocorit control	16	367	34	9.3^{c}	13	38.2^{a}	5	14.7^{b}	9	26.5^{b}	7	20.6^{b}
Cocorit EACA	15	346	37	10.7^{c}	12	32.4^{a}	5	13.5^{b}	8	21.6^{c}	12	32.4^{a}
Erpel control	22	441	18	4.1^{c}	2	11.1^{b}	2	11.1^{b}	10	55.6^{a}	4	22.2^{ab}
Erpel EACA	17	351	8	2.28^{c}	1	12.5^{b}	0	00.0^{b}	3	37.5^{a}	4	50.0^{ab}
T. timopheevii[4] control	17	378	257	68.0^{a}	74	28.8^{a}	63	24.5^{a}	84	32.7^{a}	37	14.4^{b}
T. timopheevii[4] EACA	19	396	246	62.12^{a}	56	22.8^{a}	80	32.5^{a}	59	24.0^{a}	51	20.7^{b}
Total H (7 d.f.)				92.98**		26.42**		19.18**		14.97*		32.22**
Genotype H (3 d.f.)				92.33**		25.04**		18.59**		9.03*		26.91**
EACA vs. Control H (1 d.f.)				0.01^{NS}		0.14^{NS}		0.10^{NS}		3.64†		1.16^{NS}

$H \sim X^2$ $_7X^2_{0.10} = 12.02$ $_3X^2_{0.10} = 6.25$ $_1X^2_{0.10} = 2.71$

[1] Percent of florets.
[2] Percent of seed set.
[3] Figures followed by the same letter are NS ($\alpha = 0.10$)
[4] Mujeeb et al 1978.

** Significant at $\alpha = 0.01$
* Significant at $\alpha = 0.05$
† Significant at $\alpha = 0.10$

pollination) were totally devoid of any observable endosperm tissue. It is possible, however, that in these latter cases some endosperm tissue was present at an earlier stage, but was exhausted by the developing embryo.

Krolow (1970) has suggested that a Kr system exists in wheat other than T. aestivum. The large differences, between and within species, in total seed set observed in this and other studies (Krolow, 1970; Pienaar, 1973; Marais and Pienaar, 1977a,b) would seem to support this view. The nature of hybrid seed seed development at the 4x and 2x level, however, clouds the issue. It is not clear whether a stimulated ovary results from fertilization or from pollen tube growth in the ovary (Heslop-Harrison, 1978). It is equally unclear if all fertilization events yield at least a stimulated ovary. The variable nature of seed set at this ploidy level and the lack of distinct crossability groups underline this point. None the less, it was Moss's (1972) opinion, based on cross-ability data and cytological investigations, that two systems are operating in the 4x and 2x wheats that govern crossability with rye: a pre-fertilization system (similar if not identical to the Kr system of T. aestivum) and a post-fertilization system governing seed development. The behavior of the T. timopheevi genotype used in this study provides evidence supporting Moss' views. T. timopheevi, of the four genotypes studied, had the highest total seed set but the poorest ability to develop seed with an "embryo + endosperm". It is unlikely that a genotype would be on both extremes of seed set and seed development if at least two systems were not operating. Although this does not provide proof that there are pre-fertilization and post-fertilization mechanisms operating, it is certainly an interesting observation of a confusing

situation. A definitive answer could come from a thorough cytological investigation of high and low crossable genotypes, coupled with an inheritance study of those genotypes. Biochemical procedures that may be developed in studies on the Kr system of T. aestivum could also aid in the resolution of this question.

Application of EACA (1000 ppm) had no significant effect on total seed set when applied to these four genotypes following pollination with Prolific rye (Table VI). The effect of EACA on seed development, however, revealed an enhancement of the "embryo + endosperm" category at the expense of the "embryo only" category for Cocorit-71 (Table VI). As expected, the total number of seeds with an endosperm ("endosperm only" plus "embryo + endosperm") increased significantly in treated spikes over controls (46.0% vs 35.6%, H = 19.28**) as determined on an equi-seed set basis. Taira and Larter (1977) reported that EACA had no effect on endosperm development in the genotypes used in their studies.

It is necessary to mention a failure that occurred in the heating system in February, 1978 which caused many Prolific plants to suffer a cold shock. The shock evidently caused some pollen sterility. This occurred when the T. turgidum cultivars were being crossed with Prolific and probably accounts for the lower than expected seedset in Cocorit-71 (L. S. Bates, personal communication). Since all treatments were randomized within each genotype, this should have no effect on the conclusions of that study.

Taira and Larter (1977a) report an enhancement of embryonic development for hybrid embryos treated with EACA. No enhancement of growth or development was observed in Pinguino X Prolific embryos. The mean embryonic length in control and in treated hybrid embryos was identical

to two significant digits (Table VII). The measure of embryonic development is not straightforward. Taira and Larter's (1977a,b) method of ranking embryos into three classes based on size and differentiation is a subjective judgement and questionable. The method employed in the study reported here is dependent upon size only. Even though embryonic size is probably correlated with differentiation, there are exceptions. Small embryos resulting from T. timopheevi crosses occasionally germinated when cultured and conversely, large ones often did not. It would seem more profitable to determine the effects of different genotypes and treatments on embryonic development by culturing hybrid embryos and comparing germination rates. This method was recently used by Kasha et al. (1978) in their studies on GA_3 and crossability between H. vulgare and H. bulbosum. This method, however, has limitations as well, for small changes in media contents may affect growth of different embryonic genotypes.

Crossability of the Diploid Wheat X Rye

In the past, efforts have been made to hybridize T. monococcum with various diploid Secale spp. (Krolow, 1970, 1973). Those attempts failed as did the ones reported here. Although 90 embryos were cultured only one germinated, produced roots and died. None of the others even developed into callus tissue. Embryos were extremely small, approximately 0.2 mm in diameter, and completely devoid of any observable differentiation, such as the development of a coleoptile, coleorhiza or scutellum. Endosperm development was severely retarded; out of 485 seeds dissected only one contained some very watery endosperm tissue (the result of a CI 2433 X Petkus cross examined 19 days post-pollination).

Table VII

Effect of EACA on Embryonic Length of Pinguino X Prolific Embryos.

Treatment	n	$\bar{X}$	S^{2*}	$S_{\bar{X}}$	t
Control	34	1.362 mm	0.3250	0.158 mm	0.025^{NS}
EACA	21	1.358 mm			

$(53)\,|t|_{(0.10)} > 2.006$

*Bartlett's test for heterogeneity of variance, NS ($\alpha = 0.10$)

Plant growth substances, particularly GA_3 and 2,4-D have been reported to aid in overcoming crossability barriers (Larter and Enns, 1961; Kruse, 1973, 1974; Kasha et al., 1978). In the cross of CI 2433 X Prolific, GA_3 was not observed to have any beneficial effect (Table VII). The action of GA_3 on hybrid embryos is not yet understood. It is not known whether GA_3 acts on embryonic tissue directly or enhances development by increasing the supply of nutrients from the ovary. Evidence that the former case may be involved has been provided by Norstog (1974). His studies show increased germination of immature barley embryos when GA_3 was included in the medium. The need for further studies on GA_3, as well as other gibberellins, seems apparent. The failure of GA_3 in facilitating the growth of CI 2433 X Prolific embryos, when applied to pistils following pollination, may be due to the severe underdeveloped nature of those embryos.

2,4-D has no effect on embryo recovery either when used alone or in addition to treatment with GA_3. Table VIII reveals the drastic increase in seed set, primarily of stimulated ovaries, accompanying 2,4-D treatment. The treatment of an emasculated, nonpollinated control revealed that this stimulation was not the result of pollination, but rather a parthenocarpic response to the auxin. Kruse (1974) reported that 2,4-D applied prior to pollination "broke down" the prefertilization barrier between H. vulgare and a wide variety of distantly related grasses (Avena sativa and T. monococcum to name two of the more closely related species). He reported "approximately 80.0% seed set and 10-20% embryo formation" (Kruse, 1974). It is not possible, however, to properly evaluate his conclusions because he has not published the crossability data.

Table VIII

Diploid Crossability A. Effect of 2-4-D and GA_3 on Seed Set and Kernal Development in CI 2433 X Prolific Crosses.

Treatment	# Spikes	# Florets	Total Seed Set	% [1]	Stimulation	% [1]	Embryo	% [1]
2-4-D + GA_3	10	166	131	78.9[a2]	129	77.7[a]	2	1.2
2-4-D	5	78	68	87.2[a]	66	84.6[a]	2	2.6
GA_3	10	164	29	17.7[b]	23	14.0[b]	6	3.7
Control	10	162	31	19.1[b]	24	14.8[b]	7	4.3
Total H (3 d.f.)			25.21**		25.11**		0.19NS	
2-4-D H (1 d.f.)			25.00**		25.00**			
GA_3 H (1 d.f.)			1.24NS		0.28NS			

$H \sim X^2$ $\quad {}_3X^2_{0.10} = 6.25$ $\quad {}_1X^2_{0.10} = 2.71$

[1] Percent of florets.

[2] Figures followed by the same letter are NS ($\alpha = 0.10$)

** Significant at $\alpha = 0.01$.

The three cultivars of S. cereale and the single cultivar of S. montanum were crossed with CI 2433 in attempts to obtain a hybrid and to determine paternal genotype effects on crossability. No significant differences existed between these four genotypes in seed set or in kernel development when crossed, as paternal, with CI 2433 (Table IX). Despite the results of this study, there can be little doubt as to the effect paternal genotype has in some wheat X rye crosses (Taira and Larter, 1977a; Marais and Pienaar, 1978), as well as, other interspecific and intergeneric crosses (Gill and Waines, 1978).

Aegilops squarrosa L. has been successfully crossed with rye on several occasions (Melnyk and Unrau, 1959; Krolow, 1973; Marais and Pienaar, 1977b). It has been suggested that the D-genome is more compatible with the R-genome than is the A-genome with the R-genome (Krolow, 1973; Marais and Pienaar, 1977b). The two genotypes studied here, one of each species, would support that allegation. Although Ae. squarrosa X Prolific set significantly more seed than CI 2433 X Prolific, no significant differences were observed in kernel development (Table X). Ae. squarrosa X Prolific crosses, however, did develop more embryos. The lack of statistical significance may be due to the unusually high variability of maturity of florets on a spike that was a characteristic of CI 2433. Ae. squarrosa X Prolific embryos were more highly developed and usually contained scutellum, coleoptile and coleorhiza tissues. No endosperm tissue was observed in any of the seeds, which were dissected 10-20 days following pollination. One embryo (of 10 cultured) germinated; however, that vial became contaminated and the plantlet died upon subculturing. It is not known whether or not the plantlet was a hybrid, a haploid or a parthenogenic event.

Table IX

Diploid Crossability B. Effect of Paternal Genotype on CI 2433 X Rye Crosses.

Male Parent	# Spikes	# Florets	Total Seed Set	%[1]	Stimulation	%[2]	Embryo	%[2]
Petkus	9	136	46	33.8	28	60.9	18	39.1[3]
Prolific	20	309	86	27.8	53	61.6	13	38.4
Snoopy	7	84	23	27.4	11	47.8	12	52.2
S. montanum	18	280	71	27.3	36	50.7	35	49.3
Total H (3 d.f.)				4.86		0.56		0.56

$H \sim \chi^2 \qquad {}_3\chi^2_{0.10} = 6.25$

[1]Percent of florets.

[2]Percent of seed set.

[3]Contained one seed with "embryo + endosperm".

Table X

Diploid Crossability C. Effect of Maternal Genotype on Crossability with Prolific Rye.

Female Parent	# Spikes	# Florets	Total Seed Set	%[1]	Stimulation	%[2]	Embryo	%[2]
Aegilops squarrosa	8	142	102	71.8	42	41.2	60	58.8
CI 2433	20	309	86	27.8	53	61.6	13	38.4
Total H (1 d.f.)			14.94^{**}		2.02^{NS}		2.02^{NS}	

$H \sim X^2$ $\quad {}_1X^2_{0.10} = 2.71$

[1]Percent of florets.

[2]Percent of seed set.

**Significant at $\alpha = 0.01$

Krolow (1973) has demonstrated an alternative procedure for obtaining tetraploid triticales other than direct crossing of diploid Triticum and Secale spp.. He backcrossed hexaploid triticale with rye and selfed the partially fertile progeny for several generationa. Although the alloploids (ABRR) which were obtained using this procedure were relatively stable and set seed when crossed with rye or 6x and 8x triticale, they did not cross with other diploid wheat species (Krolow, 1973; Gustafson and Krolow, 1978). This was probably due to the fact that they were mixed alloploids. Consequently, the production of hybrids between diploid Triticum spp. (including Aegilops) and rye is, therefore, still desirable.

It is unlikely that procedures similar to those repored here will be of much practical value if the cross T. monococcum X Secale cereale is to contribute to the triticale gene pool. Utilization of different tissue culture techniques, particularly liquid media, may initiate the formation of callus from the small undifferentiated hybrid embryos (Raghaven, 1976; Norstog, 1970). Polyhaploid plants could then be generated from this callus. Sampling of more genotypes within T. monococcum and S. cereale may produce the compatible parents. Closely related species, such as T. boeticum or T. uraratu, also offer another alternative.

Cytological Studies of Cross-Incompatibility and Seed Development

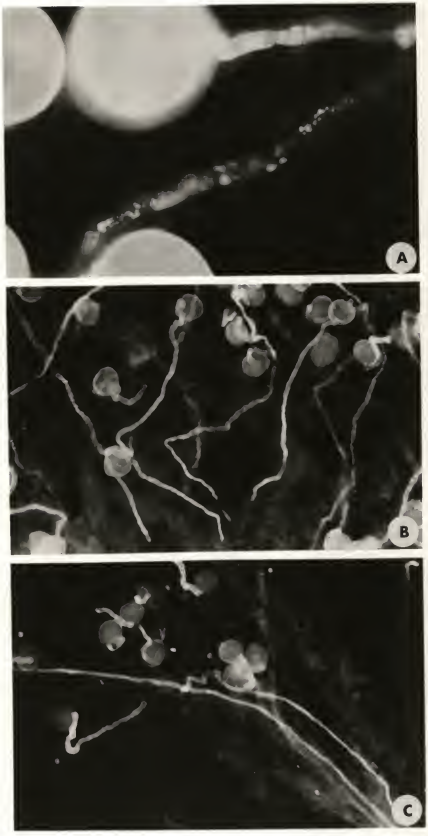
There have been numerous studies dealing with pollen tube growth and early seed development in selfed material and in a variety of wide crosses in the Triticeae. This study was undertaken to define cytologically the barrier(s) to crossability between T. aestivum, when

crossed as maternal, with H. vulgare. In addition studies of selfed material and a limited number of wheat X rye crosses were examined to gain knowledge about normal events and other documented abnormal events.

The technique of Martin (1959) was used to study pollen tube growth in the feathery cereal stigma and style. This procedure capitalizes on the fact that callose, β-1,3-polyglucan (Aspinall and Kestler, 1957) is produced in pollen tube cytoplasm (Cresti and van Went, 1976) and in the presence of analine blue fluoresces when illuminated with ultraviolet light (λ = 350 nanometers). Granules of callose may be observed in the cytoplasm of young pollen tubes (Figure 1A). Cresti and van Went (1976), working with Petunia hybrida, observed similar granules to form the typical callose plugs at the rear of the pollen tube cytoplasm and to merge with the plasma membrane forming a distinct layer of callos between the plasma membrane and the cell wall. In cereals this pollen tube wall callose appears with time and eventually fluoresces so brightly as to obscure the plugs beneath it (Figure 1B).

No apparent differences were observed in pollen germination or in pollen tube growth through the stigma and style, when comparing selfed material and Bonza X M-16 crosses. Pollen tube growth was variable and abnormalities were frequent (Figure 1C). M-16 barley pollen tubes almost always reached the base of the style, although frequently they commenced their growth from basal stigmatic hairs. Similar studies conducted on Bonza X Prolific and Chinese Spring X Prolific crosses yielded similar results. These latter observations on hexaploid wheat X rye crosses coincide with those on early pollen tube growth conducted by other researchers (Zeven and van Heemert, 1970; Lange and Wojciechowska, 1976; D'Souza, 1978).

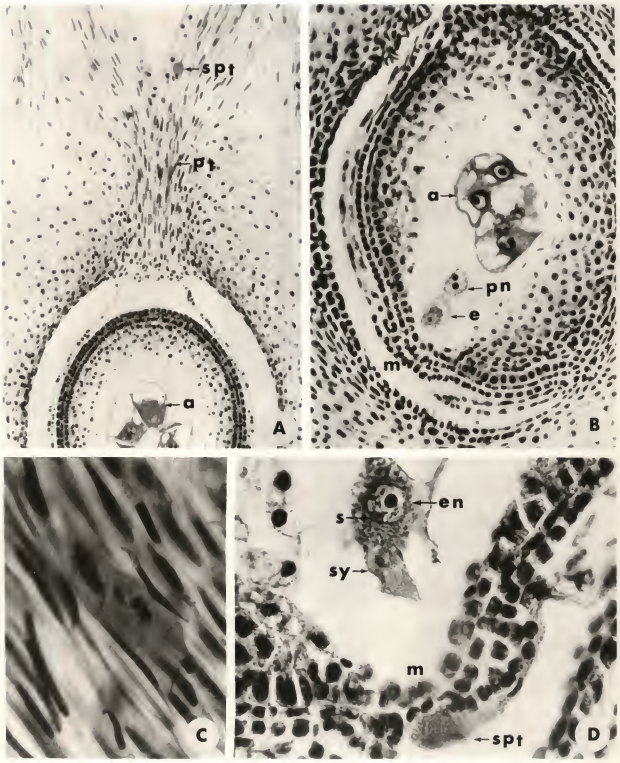
Figure 1. A. Chinese Spring X Chinese Spring (706x, 15 min post-pollination). Callose granules in pollen tubes. B. Bonza X M-16 (179x, 1 hr post-pollination). Pollen tube growth in stigmatoid hairs and pollen tube wall callose formation. C. Bonza X M-16 (179x, 1.5 hr post-pollination). Pollen tube growth in the transmitting tissue of the style.


A
B
C

Serial sections stained with analine blue were found to be ineffectual in following pollen tube growth in the transmitting tissues of the ovary and in the ovary cavity. This was due to the background fluorescence of the ovule and transmitting tissues. This technique was used by Zeven and van Heemert (1970) in their studies of rye pollen tube growth in incompatible wheat ovaries. It was concluded that they mistook vascular bundles for pollen tubes. This was due to the fact that phloem cell walls also contain callose and fluoresce in a similar manner to pollen tubes (Currier, 1957). Vascular bundles lie close to the micropyle situated in the ovary wall. Pollen tubes, however do not grow through the portions of the ovary wall indicated in their micrographs. Zeven and van Heemert's error would have been avoided had they properly screened emasculated nonpollinated controls.

Serial sections stained with hematoxylin and eosin-y (H-E) were also employed to follow pollen tube growth in the ovary. This procedure, however, proved to be less than satisfactory as well. This was due primarily to the fact that pollen tubes were usually stained only slightly more intensely with eosin-y (or fast green) than the transmitting tissue through which they grew (Figure 2A). Overstaining or cutting thick sections, a necessity in order to obtain undamaged ribbons, made the pollen tubes virtually indistinguishable from the maternal tissue. This was especially a problem in the transmitting tissue of the ovary, where cells contained darkly stained elongate nuclei. The difficulty in staining pollen tubes using these and similar procedures has also been noted by Lange and Wojciechowska (1976) and is probably at least partially the source of the confusion concerning the mechanism of the Kr genes (Boyes and Thompson, 1937; Tozu, 1966; Moss, 1968, 1972; Lange and

Figure 2. A. Bonza selfed (110x, approximately 6 hr post-anthesis). Pollen tube growth in the transmitting tissue of the ovary; pt (pollen tube), spt (swollen pollen tube tip), a (antipodal cell). B. Bonza X M-16 (175x, 24 hr post-pollination). Unfertilized embryo sac; m (micropylar region), e (egg cell), pn (polar nuclei), a (antipodal cells). C. Bonza X Bonza (700x, 6.5 hr post-pollination). Swollen pollen tube tip in transmitting tissue of the ovary with male nuclei. D. Torim selfed (441x, approximately 8 hr post-anthesis). Swollen pollen tube tip in the ovary cavity of a fertilized pistil; spt (swollen pollen tube tip), m (micropyle), sy (synergid), en (egg nucleus), s (sperm).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Wojciechowska, 1976). Different cytological methods need to be developed for studying pollen tube growth. Transmitting electron microscopy (TEM) has been used successfully in cotton (Jensen and Fischer, 1970) and would probably be of value in this work as well.

Pollen tubes were observed in the ovary cavity following compatible pollinations on only three occasions (out of 50 examined). No pollen tubes were observed in this portion of the ovary in any of the incompatible pollinations (Figure 2B). The lack of numerous pollen tubes in the ovary cavity of selfed pistils was also observed by Lange and Wojciechowska (1976). They suggested that it was due to the pollen tube fragments "floating off" the slides as they were processed. In the study reported here this possibility was minimized by the use of Mayer's affixative (Sass, 1951) and a Tissue Tech slide stainer which minimizes the handling of the slides. The low frequency of pollen tubes in the ovary cavity following self pollination is curious considering the large number of pollen tubes which grow into the transmitting tissue of the ovary directly above the cavity.

The most striking feature of pollen tube growth in selfed material was the appearance of swollen pollen tube tips. Although never frequent almost all fertilized pistils contained one or two of these structures. Unlike other pollen tubes, these stained well (with eosin) and occasionally all three male nuclei could be observed (Figures 2A and 2C). These swollen tube tips were observed most commonly in the transmitting tissues of the style and ovary; they were seen less frequently in the ovary cavity (only twice, Figure 2D) and in the feathery stigmatoid tissue. These apparently inhibited pollen tubes were observed to occur in the compatible T. timopheevi X Prolific crosses, as well as in selfed

material. Curiously they were not observed in any of the 38 Bonza X M-16 crosses or the nine incompatible T. aestivum X Prolific crosses (Jupateco X Prolific, crossability = 0.50%; Bonza X Prolific, crossability = 1.00%; Tobari X Prolific, crossability = 15%). Lange and Wojciechowska (1976) also reported observing these structures but in the styles of incompatible T. aestivum X S. cereale crosses, as well as, in compatible crosses and selfed material.

In Bonza X Bonza crosses fertilization events and early seed development were observed to occur at approximately the same rate as reported for other T. aestivum genotypes (Morrison, 1954; Moss, 1968, 1972; Bennett et al., 1973; Lange and Wojciechowska, 1976). Deposition of the sperms inside the embryo sac had occurred by two hours post-pollination. This was characterized by a change in the stainability of one of the synergids, indicating that pollen tube cytoplasm had been deposited inside that cell. Frequently the vegetative nucleus could be observed in this cell as well (Figures 3A and 3B). Concomitant with these events rod-like sperm could be observed to have migrated to the egg nucleus and to the polar nuclei (double fertilization). The sperm associated with the egg slowly fused with the egg nucleus and a distinct mass of chromatin could be observed in the nucleus as late as eight hours post-pollination. The other sperm and the polar nuclei fused much more rapidly and the primary endosperm nucleus was observed to undergo division by about six hours post-pollination. Pistils fixed 24 hours after pollination revealed a 16-32 nucleated coenocytic endosperm and a 1-2 celled zygote (Figure 3C). The endosperm had over 130 nuclei by 48 hours post-pollination (Figure 3D) and cellularization had begun in the tissue surrounding the four to eight celled embryo (Figure 4A). Later stages

Figure 3. A. Bonza X Bonza (700x, ? hr post-pollination). Fertilized embryo sac; m (micropyle), sy (synergid), s (sperm), e (egg), p (primary endosperm nucleus). B. Bonza X Bonza (110x, 6.5 hr post-pollination). Vegetative nucleus in synergid. C. Bonza X Bonza (441x, 24 hr post-pollination). Zygote and coenocytic endosperm; z (zygote), end (endosperm nucleus), a (antipodal cell). D. Bonza X Bonza (110x, 48 hr post-pollination). Developing endosperm and degenerating antipodal cells; a (antipodal cell), end (endosperm nucleus).

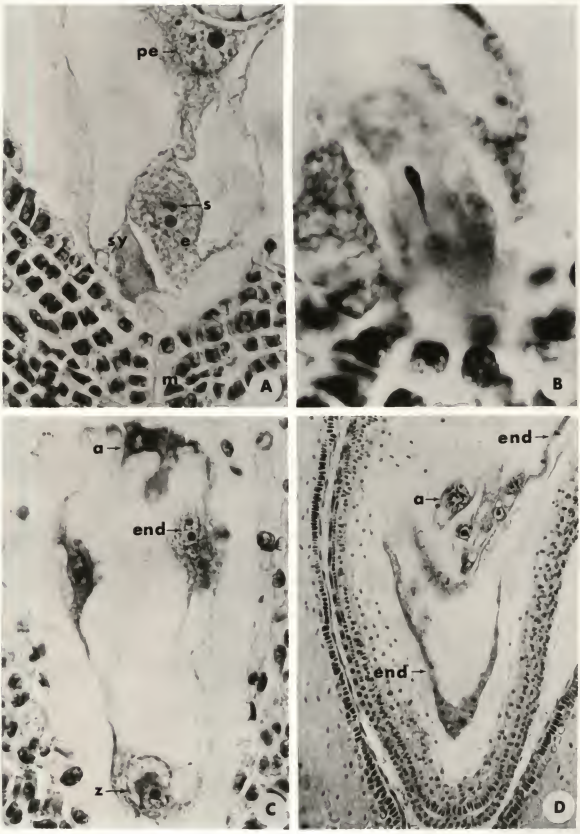
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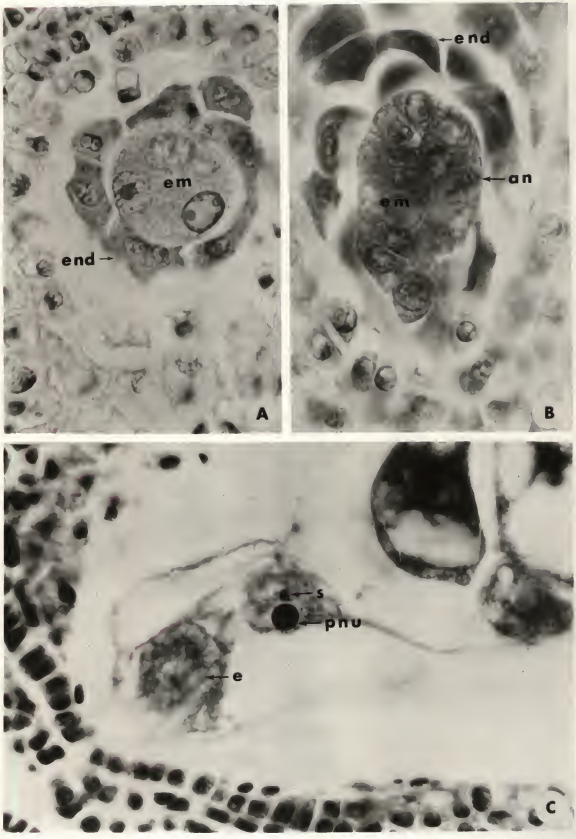
Figure 4. A. Bonza X Bonza (700x, 48 hr post-pollination). Micropylar region of developing seed; em (embryo), end (endosperm cell). B. Bonza X Bonza (441x, 96 hr post-pollination). Developing pear shaped embryo; em (embryo), an (anaphase nucleus), end (endosperm cell). C. Bonza X M-16 (529x, 24 hr post-pollination). Polar nuclei and possible barley sperm; e (egg, out of focus), pnu (polar nucleolus), s (possible sperm).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of seed development were characterized by the continued growth of the embryo and a slower, increasingly asynchronous, growth of the endosperm (Figure 4B). Antipodal cells, which had large densely staining cytoplasms at the time of fertilization were clearly degenerating 48 hours later.

In the Bonza X M-16 crosses only one pistil (fixed 24 hours post-pollination) showed any evidence of fertilization. In Figure 4C what is apparently a condensed sperm is observed situated adjacent to the polar nuclei. If it is a sperm, it has not fused with the polar nuclei, nor has the apparatus migrated towards the embryo sac, as normally occurs about two hours after pollination (Luxova, 1967). No sperm or evidence of fertilization, such as an increase in the number of nucleoli, was noted in the egg cell. The egg (or possibly zygote) could be fertilized, however, and merely dormant. Significantly, the synergids have not changed their stainability, consequently, what appears to be a sperm may in fact be an artifact.

A limited study was made of seed development in T. timopheevi X Prolific crosses. Double fertilization occurred in all but one of the eight pistils examined. Seed development in the other seven pistils was variable in rate but within the range of selfed material.

An abnormality in one of the selfed pistils deserves comment. A fertilized embryo sac fixed 24 hours after pollination contained no endosperm development. This pistil was subjected to the photoperiod suggested by Bennett et al. (1973, 1975). The failure of this pistil to develop an endosperm may be just a random abnormality, however it could be a consequence of the 24 hour photoperiod. Bennett et al. (1973, 1975) suggested use of the photoperiod to synchronize receptive

florets on a spike. In their studies with several genera no such abnormalities were observed. Fortunately, the crosses between wheat and barley were conducted in two environments (Growth Chamber #2b and the Greenhouse). The one wheat pistil which may have been fertilized by barley resulted from material crossed in the greenhouse.

SUMMARY

Significant maternal genotypic differences were observed between wheat cultivars at all three ploidy levels in total seed set when crossed with rye and at the tetraploid level significant differences were observed between cultivars in their ability to develop embryos and/or endosperm tissue. The behavior of T. timopheevi X S. cereale cv. Prolific spikes, which had the best total seed set and the poorest ability to develop seed with "embryo + endosperm" provided circumstantial evidence that at least two mechanisms control crossability at the tetraploid level; one of which may be similar to the Kr system in T. aestivum.

EACA (1000 ppm, applied 3 evenings prior to pollination) was not observed to have any significant effect on total seed set or kernel development in compatible or incompatible T. aestivum X S. cereale crosses.

EACA (1000 ppm, applied 3 evenings post-pollination) was not observed to have any significant effect on total seed set or embryo development in the four tetraploid genotypes tested. A genotype dependent effect, however, was observed in endosperm development. In T. turgidum cv. Cocorit-71 X S. cereale cv. Prolific crosses the number of seed with endosperm tissue significantly increased in treated over control crosses.

Plant hormone treatments and different pollen parent genotype had no effect in overcoming the crossability barriers between T. monococcum and Secale spp.. Although numerous seed was set and 107 embryos were recovered, attempts to rear these embryos on artificial media failed.

Barley pollen was observed to germinate and grow in the stigmatoid

hairs and styles of wheat pistils. Further growth of the pollen tubes through the ovary was difficult to follow; however, no evidence of fertilization was observed in 49 of the 50 pistils examined. It was concluded, therefore, that the primary barrier to crossability between T. aestivum and H. vulgare is due to the failure of pollen tube growth in the ovary.

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APPENDIX

Statistical Procedures

The experiments involving different genotype and treatment effects on wheat X rye crossability were completely randomized designs. Analysis of variance (ANOVA) was to be used to determine differences in seed set (% of florets/spike) and seed development (% of seeds/spike). Upon completion of the experiments, however, heterogeniety of variance between cultivars was noted. Graphical representation revealed that the sample means and variances were not independent (Figure 5), as is the case with normal populations (Fryer, 1966). Various algebraic transformations (arc-sin, square root and logarithmic) did not alleviate the problems. It was concluded that ANOVA was an inappropriate technique, since at least two of its assumptions were not met.

The Kruskal-Wallis test (K-W), as described by Conover (1971) was employed as it is analogous to a one way ANOVA. This nonparametric procedure makes no assumptions concerning the shape of the populations sampled and tests only whether they are all identical or whether one or more is different. The statistic is as follows:

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{C} \frac{R_i^2}{n_1} - 3(N+1)$$

where C = the number of treatment combinations[1]

n_i = the number of samples (spikes) in a treatment combination

N = total number of samples (spikes)

$^2R_{1.}$ = the rank sum of the ith treatment combination

[1]Chinese Spring X Prolific - control is one treatment combination.

[2]Each spike is ranked according to % (% seed/florets, % stimulation/seed set, etc.).

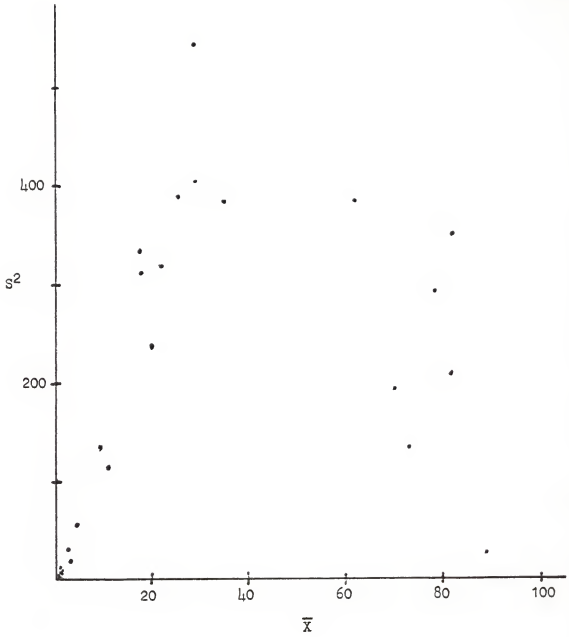

Figure 5. Relationship between sample mean and variance for crossability data.
$\overline{X}$, mean (% seed set/spike).
S^2, variance (% seed set/spike).

H is distributed approximately as a X^2 with 1 - C degrees of freedom. Significance levels read directly from the X^2 tables provide a conservative test (Conover, 1971). The asymptotic relative efficiency (ARE) of the Kruskal-Wallis procedure as compared to the F-test has been calculated to be 0.955 (Conover, 1971), when assumptions of the latter are met. Main effects may be estimated by adding rank sum totals and recalculating the K-W statistic, however, the true level of α is not known for these comparisons (Conover, 1971). Accurate multiple comparisons were made when H (total) was found to be significant, by performing two sample comparisons using the K-W procedure. The K-W test is comparable to the Mann-Whitney test in these situations (Conover, 1971). Interactions were tested by making similar comparisons between control and treated plants within each genotype.

The above analysis, although more meaningful than the parametric procedures used previously, still is inadequate. The problem arises due to small sub-sample size (number of florets/spike or number of seeds/spike) used in calculating percentages. The samples consequently varied from 100% to 0% within a treatment combination, especially in the seed development categories. While use of ranks partially solved this problem, increasing the sub-sample size from one to several spikes would probably alleviate it. Kasha et al. (1978) did just that in their crossability study between H. vulgare and H. bulbosum. Those authors, however, continued to use parametric procedures.

The t-test was used to determine differences in length of hybrid embryos in treated and control crosses (Fryer, 1966).

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INTERGENERIC CROSSABILITY BARRIERS IN THE TRITICEAE

by

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Crossability of 6x, 4x and 2x wheat with rye was studied with respect to genotype and chemical treatments. Maternal genotype influenced total seed set at all three ploidy levels and in the 4x cultivars significantly influenced kernel development as well.

ε-amino-caproic-acid (EACA) had no effect on total seed set or embryo development in Triticum aestivum L. (1000 ppm X 3 pre-pollination) or T. timopheevi Zhuk. and T. turgidum L. var. durum (1000 ppm X 3 post-pollination) cultivars when crossed with Secale cereale L.. EACA, however, significantly increased the number of seeds with endosperm tissue in T. turgidum L. cv. Cocorit-71 X S. cereale L. cv. Prolific crosses.

The plant growth substances 2,4-D (50 ppm X 1 pre-pollination) and GA_3 (75 ppm X 2 post-pollination) failed to overcome the barriers to crossability between T. monococcum L. cv. CI 2433 X S. cereale L. cv. Prolific. Different pollen genotypes within S. cereale L. and an accession of S. montanum L. failed to overcome these barriers as well.

The primary barrier to crossability in T. aestivum L. X Hordeum vulgare L. was identified to be due to the failure of pollen tube growth in the ovary.