CYTOGENETIC STUDIES INVOLVING A NUCLEAR MALE-STERILITY MUTANT IN WHEAT

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STATEMENT

To the best of my knowledge and belief this thesis contains original research which has not been submitted previously for any degree at this, or any other, university. No material previously written or published by any other person, is included in this thesis except where due reference is made in the text.

M. AZHAR HOSSAIN

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SUMMARY

Since problems in the production of hybrid wheat using *timopheevi* cytoplasm were recognized, other alternatives have been sought. These alternatives include the proposed systems of Driscoll (1972), Maan and Lucken (1972), Frankowiak *et al.* (1976) and Tsunewaki *et al.* (1978).

The XYZ system (Driscoll, 1972) requires two basic components, a recessive nuclear male sterility mutant of hexaploid wheat and a compensating male fertility alien chromosome. The addition of single and double doses of a compensating male sterility alien chromosome to the male sterility mutant satisfy the requirements of the system. The Cornerstone mutant *ms1c*, (Driscoll, 1977) a male sterile hexaploid wheat mutant is the Z component of the system. Cereal rye, *Secale cereale* L. was considered to be a likely source of an alien chromosome that would compensate for the male sterility of Cornerstone.

Fertile octoploid triticale was produced by crossing Cornerstone and S.A. Commercial rye and subsequent chromosome doubling of the F_1 by colchicine, indicating that the whole rye genome can compensate for the Cornerstone male sterility. The octoploid triticale was used as the pollen parent for the backcross to *ms1c* and a fertile heptaploid (2n = 7X = 49, AABBDDR) was produced. The heptaploid was backcrossed to *ms1c* and second backcross seeds were obtained.

Among the second backcross progeny, monosomic and double monosomic additions of seven rye chromosomes were isolated and observed for their ability to compensate for *ms1c*. Rye chromosomes 2R and 4R of South Australian Commercial rye individually partially compensate for

msic. The remaining chromosomes, namely 1R, 3R, 5R, 6R and 7R failed to compensate for the male sterility of Cornerstone.

Chromosome 2R compensates approximately 56% under controlled-environment conditions. The misdivision products of chromosome 2R were analysed and it was determined that the male sterility compensating gene(s) was located on its short arm. The compensation for male sterility by the short arm of 2R is approximately 94% under the same environmental conditions. The long arm of 2R provides no compensation for male sterility. The level of sterility compensation by 2RS (94%) was compared with the fertility level of Chinese Spring (98%). The difference is at the point of statistical significance, indicating that the gene(s) for male fertility on the short arm of 2R provides full compensation for male sterility. The incomplete compensation (56%) by entire 2R appears to be due to the presence of an *antifertility* factor(s) on its long arm. Such *antifertility* factor(s) may not be distinguishable from the factors for poor vigour which are known to be located on the long arm of 2R.

The male sterility compensation by chromosome 4R is partial and is greatly influenced by the variation of environment. The genes for male fertility and rye alcohol dehydrogenase are both located on the short arm of 4R. The compensation for male sterility by the short arm of 4R is erratic. The long arm of chromosome 4R does not compensate for male sterility at all. The compensation for sterility by entire chromosome 4R or by its short arm is greatly reduced by the occasional occurrence of pistillody. Phenotypically, all the possible derivatives of 4R additions are normal except that the seeds in those derivatives, when produced, suffer from major shrivelling.

Chromosome 4A of hexaploid wheat, which bears *msIc* (Barlow and Driscoll, 1981), is considered unusual (Driscoll, 1981) in that the absence of its male fertility gene(s) cannot be compensated for by increased dosages of the other chromosomes of its homoeologous group (Sears, 1966). Further, its diagnostic N-banding pattern is more like that of a B-genome chromosome (Gerlach, 1977; Jewell, 1979). As well, it fails to pair at meiosis with chromosome 4 of diploid wheat (Chapman *et al.* 1976; Dvořák, 1976).

The homoeologous relationships of the 7 rye chromosomes with their wheat homoeologues have been established. However certain rye chromosomes have the ability to partially compensate for the wheat chromosomes of more than one homoeologous group (Koller and Zeller, 1976).

The compensation for male sterility by rye chromosome 4R is an expected event from an evolutionary view point. However, the compensation by 2R in addition to 4R was unexpected. The male fertility gene(s) on the short arm of 2R is similar to the male fertility gene(s) on chromosomes 4A, but the male fertility gene(s) on the short arm of chromosome 4R is apparently different from those two genes. This difference can be explained on the basis of two hypotheses:

Two independent genes for male fertility with different abilities might have evolved;

or

A single gene(s) may have been duplicated by way of translocation to another rye chromosome.

The addition of either 2R or 4R to *msIc* in double and single dose resulted in the production respectively of the X and Y components of the system. Except for the ditelosomic and monotelosomic additions

of 2RS, no other additions of 2R or 4R showed any promise as X and Y components of the system.

The two components with the 2RS telosome showed intolerable addition decay in the X line and an intolerably high rate of 2RS transmission through the male gametes of Y plants. Fusion of a neutral arm of another alien chromosome with the short arm of 2R may rectify the situation. The process of centric fusion (Sears, 1972) could produce such a chromosome.

The Cornerstone male sterility mutant has been transferred to tetraploid wheat by backcrossing. The mutant character is the same at both ploidy levels; however, there is a greater selection against gametes carrying the mutant at the tetraploid level than at the hexaploid level. A further attempt was made to transfer *ms1c* from the tetraploid level to the diploid level i.e., to *T. monococcum* (2n = 14, AA) but this failed. Backcrossing of the triploid hybrid by the diploid was not possible. The double triploid (AAAABB) from the cross of tetraploid *ms1c* x *T. monococcum* was fully male fertile, indicating that diploid wheat is able to compensate for the male sterility of *ms1c*.

Also, the tetraploid *ms1c* was pollinated by *Aegilops squarrosa*, the progenitor of the D genome. A colchicine-doubled hybrid of this cross was highly fertile. Thus, *Ae. squarrosa* is also capable of compensating for the male sterility of *ms1c*.

The partially fertile hexaploid triticale (2n = 42, AABBRR) has been produced by crossing the male sterile tetraploid wheat and the diploid rye cultivar Imperial, and subsequent chromosome doubling. Male sterile hexaploid triticale could be produced from this stock by the elimination of male fertility genes from the rye genome through further chromosome manipulation.

CHAPTER 1. GENERAL INTRODUCTION

Plant breeders have exploited heterosis in hybrid varieties in a number of agronomically important plants. Male sterility is usually required to facilitate commercial production of hybrid seed. Cytoplasmic male sterility has been utilized for the production of commercial hybrids in a number of crops, for example in onion and carrot (Peterson, 1970) and in maize (Jones and Everett, 1949). The outstanding agricultural example is maize (Mangelsdorf, 1955). However, hybrid maize production using male sterile Texas (T) cytoplasm has been discontinued due to the susceptibility of T cytoplasm to strain T of the Southern Corn Leaf Blight organism *Helminthosporium maydis* Nisikado and Miyake (Marcado and Lantican, 1961; Scheifele *et al.* 1970). Other alternatives, such as genic male sterility and male sterile C-cytoplasm, are being examined; the latter has been used commercially for hybrid maize production (Duvic and Noble, 1978).

Common wheat is one of the most important food crops of the world and attempts have been made to produce hybrid varieties based upon *Triticum timopheevi* Zhuk. cytoplasm (Schmidt, Johnson and Maan, 1962).

Hybrid wheat breeders are working almost exclusively with *timopheevi* cytoplasm, although many sources of cytoplasmic male sterility in wheat have been described (Maan, 1973). The other cytoplasms have associated disadvantages such as reduced plant vigour and incomplete fertility restoration.

Cytoplasmic male sterility has some disadvantages in wheat. There is a reduction in seed quality both morphologically and physiologically (Johnson, 1967). In addition, complete male fertility restoration is still not available, although many male fertility

restorer lines have been reported (Oehler and Ingold, 1966; Miri *et al.* 1970; Mihaljev, 1973). Fertility restoration of cytoplasmic male sterility (cms) is controlled by multiple genes which are influenced by the environment (Sage, 1972).

Another method of producing hybrid wheat is by the use of chemically-induced male sterility. This type of male sterility also has some disadvantages: delay in growth, reduced height, shrivelling of caryopsis and incomplete emergence of heads have been reported for some pollen inhibitors (Rowell and Miller, 1971, 1974; Jan *et al.* 1974; Borghi *et al.* 1973. Other pollen inhibiting chemicals are being examined that may be more suitable.

Due to the problem faced by plant breeders in the use of male sterility derived either from *timopheevi* cytoplasm (Wilson, 1968) or from phytochemicals, the search for alternatives led to the possible use of genic male sterility for the production of hybrid wheat. Driscoll (1972) proposed the XYZ system for the production of hybrid wheat using genic male sterility. This system involves a single Mendelian recessive gene for male sterility and a dominant male fertility restorer gene(s) on a chromosome of an alien species.

Very few reports (Pugsley and Oram, 1959; Athwall *et al.* 1967; Krupnov, 1968; Fossati and Ingold, 1970) are available on genic male sterility in hexaploid wheat because of its polyploid nature. The genic male sterile mutant of Fossati and Ingold (1970) was considered to be a suitable component for the proposed XYZ system (Driscoll, 1972). Subsequently, Driscoll (1977) isolated 'Cornerstone', a recessive genic male sterility mutant which was used for further examination of the proposed system (Driscoll, 1978). Cornerstone is an induced male-sterility mutant which is located of the α arm of

chromosome 4A of wheat (Barlow and Driscoll, 1981). There is limited information (Zeller and Baier, 1973; Koller and Zeller, 1976; Dvořák, 1980; Miller *et al.* 1982) regarding the substitution of other chromosomes for chromosome 4A of hexaploid wheat. The system requires an alien chromosome which can compensate for the sterility of Cornerstone. Diploid rye, *Secale cereale* L. (2n = 14), cultivar South Australian was thought to be a likely source of a male sterility compensating alien chromosome(s).

In order to isolate the male sterility compensating alien chromosome(s) from South Australian rye, an amphiploid was produced involving Cornerstone and South Australian rye. The colchicine-induced octoploid triticale (2n = 8 x = 56, AABBDDRR) was male fertile. Thus, the entire rye genome can compensate for the male sterility of Cornerstone. The octoploid triticale thus produced was backcrossed to Cornerstone to produce the heptaploid. This was male fertile, which demonstrated that the haploid set of rye chromosomes can compensate for the male sterility of Cornerstone. A second backcross was made to Cornerstone and amongst the second backcross progeny, plants were selected with male sterility compensating chromosome(s). The arm location of male sterility compensating factor(s) have been determined from further study of the derivatives of the male fertile backcross lines.

This study not only produced chromotypes that will be useful in producing hybrid varieties, it also produced information on the evolutionary relationships of wheat and rye chromosomes.

The attempted transfer of this mutant from the hexaploid level to the tetraploid level and the diploid level showed whether this mutant is tolerated at lower levels of ploidy. The transfer of this mutant to the tetraploid wheat *Triticum durum* Desf. (2n = 4 x = 28, AABB) has

been achieved. However, efforts to transfer this mutant from the tetraploid to the diploid level by the same method failed.

The synthetic hexaploid wheat involving male sterile durum *T. durum* and *Aegilops squarrosa* L. (2n = 14, DD) has been produced. Isolation of male fertility compensating *squarrosa* chromosome(s) in a male sterile durum background has been initiated. The relationship of *Ae. squarrosa* to the Cornerstone mutant has evolutionary implications.

CHAPTER 2. LITERATURE REVIEW

Many reports on male sterility in different species are available. Male sterility in agriculturally important crops is useful for the purpose of producing hybrid seed. Male sterility in plant species has been classified into three main groups, namely, cytoplasmic, nuclear-cytoplasmic and nuclear or chromosomal or genic. In addition to this classification, male sterility due to environmental factors (Heslop-Harrison, 1957; Bingham, 1966) or deficiency of certain essential elements (Graham, 1975) has been reported. There is a vast literature on these different types of male sterility in various plant species. A few important examples are summarized below.

2.1 Examples of male sterility

The first report of cytoplasmic male sterility in *Zea mays* L. was made by Rhodes (1931). This male sterility was entirely determined by non-nuclear elements of the maternal gametes. Subsequently, Raghaven (1951), Burton (1958) and Craigmiles (1960) reported this type of male sterility in Sugarcane, *Saccarum* spp. L., pearl millet, *Pennisetum glaucum* L. and Sudan grass, *Sorghum arundinaceum* (Desv.) Stapf variety Sudanese (stapf) Hitchc respectively. Edwardson (1956, 1970) has reviewed all the reported cases of cytoplasmic and nuclear-cytoplasmic male sterility in different plant species.

The male sterility due to nuclear and cytoplasmic variants was reported in flax for the first time by Bateson and Gaidner (1921). A similar type of male sterility is also found in onion, *Allium cepa* L. variety Cepa (John and Emsweller, 1937), orchard grass, *Dactylus glomerata* L. (Myers, 1946), Capsicum, *Capsicum annum* L. (Peterson, 1958) and Carrot, *Daucus carota* L. (Thompson, 1961). The accumulated reports on

ms in different plant species have been reviewed by Jain (1959). Driscoll and Barlow (1976) in their review noted that nuclear male sterility accounted for 25 of the first 34 reported cases of male sterility in agriculturally-important species.

Attempts have been made to induce further male sterility mutants in various crop plants. Chemicals and irradiation have been utilized for the induction of male sterility, for example, by irradiation in wheat (Fossati and Ingold, 1970; Driscoll, 1977).

The occurrence of *ms* varies from species to species depending on ploidy level. Higher number of male sterility mutants have been reported in diploid species than in polyploid species. Beedle (1932) reported fifteen genes for male sterility in maize. Rick (1948) mentioned nine cases of male sterility in tomatoes. Hockett and Eslick (1968) reported nineteen cases of genic male sterility in barley, *Hordeum vulgare* L. of which, sixteen have been proved to be non-allelic. On the contrary, reports on genic male sterility in tetraploid and hexaploid wheats are few (Bozzini, 1974; Bozzine and Scarascia-Mugnozza, 1968; Pugsley and Oram, 1959, Athwal *et al.* 1967; Krupnov, 1968; Fossati and Ingold, 1970; Driscoll, 1977; Franckowiak *et al.* 1976; Sasakuma *et al.* 1978) because of the higher ploidy level.

Generally, genic male sterility is controlled by a single recessive gene and follows a Mendalian inheritance pattern. However, evidence of a dominant gene for male sterility has been reported in cotton, *Gossypium hirsutum* L. (Murthi and Weaver, 1974) and in wheat (Sasakuma *et al.* 1978; Jingyang and Zhongli, 1982).

2.2 Description of male sterility

Male sterility in different plant species exhibits different forms. The disintegration of tapetal cells in cytoplasmic male sterile cotton (Murthi and Weaver, 1974), vascular deficiency in the stamens of cytoplasmic male sterile wheat (Joppa *et al.* 1966), delayed tapetal degeneration in flax (Dubey and Singh, 1965) and tapetal hypertrophy in maize (Duvic, 1965) are general characteristics of male sterility.

Alam and Sandal (1969) suggested that the histone in male sterile anthers might be complexed with DNA resulting in the suppression of the synthesis of specific proteins thereby causing pollen abortion in Sudan grass. De Vries and Ie (1970) found no clear cut differences between the cytoplasm of male sterile and fertile wheat anthers. Barlow (unpublished Ph.D. thesis) suggested that a pre-meiotic bio-chemical breakdown may cause male sterility in Cornerstone.

2.3 Cytoplasmic male sterility in wheat

The first example of cytoplasmic male sterility in wheat was reported by Kihara (1951). Fukasawa (1953) isolated a male sterile *T. durum* with *Ae. ovata* cytoplasm and subsequently Fukasawa (1959) transferred this male sterility to hexaploid wheat *T. aestivum*. The male sterile plants with *ovata* cytoplasm were short and late in maturity. Wilson and Ross (1962) discovered cytoplasmic male sterility factors in *T. timopheevi* with *T. aestivum* cultivar Bison. Subsequently, Wilson (1962) and Schmidt *et al.* (1962) independently discovered fertility restorer genes in *timopheevi* which restored male fertility to cytoplasmic male sterile hexaploid wheat.

Ohler and Ingold (1966) claimed that the variety Primepi can restore full fertility to male sterile hexaploid wheat with *timopheevi* cytoplasm. The fertility restoration by Primepi in *timopheevi* cytoplasm

has been claimed to be determined by a single dominant gene (Goujan and Ingold, 1967). Miller and Schmidt (1970) suggested that fertility restoration in *timopheevi* cytoplasm by variety Primepi is controlled by two incompletely dominant genes. However, Sage (1972) put forward a hypothesis regarding the restoration of male fertility in *timopheevi* cytoplasm. He postulated three major genes, dominant or partially dominant and additive, each contributing differently to fertility restoration.

In spite of the complexities involved in male fertility restoration in *timopheevi* cytoplasm, the wheat lines P1 277013 and P1 277016 (Joppa and McNeal, 1969), NP 889, NP 883 and NP 880 (Miri *et al.* 1970) and VK 64-28 (Mihaljev, 1973) have been claimed to restore full fertility to *timopheevi* cytoplasm. The *timopheevi* cytoplasm has adverse effects on the plant, among them, meiotic instability (Maan and Graken, 1968), reduction in height (Tsunewaki and Endo, 1973), shrivelled kernel (Johnson *et al.* 1967) and pre-harvest sprouting (Jönsson, 1976) are important.

2.4 Induction of male sterility using phytochemicals

The problems associated with *timopheevi* cytoplasm resulted in plant breeders showing considerable interest in the development of phytochemicals which can induce male sterility in wheat. Such phytochemicals must selectively induce sterility in the male parts of the plants without interferring with other normal functions. Treated plants can be used as female parents to produce hybrid wheat. Several experiments with different chemicals have been performed (Rowell and Miller, 1971, 1974; Borghi *et al.* 1973; Jan *et al.* 1974; Johnson and Brown, 1978) to evaluate their ability to satisfy the requirements for hybrid wheat production, however, none of the chemicals have yet been proved to be suitable for this purpose.

2.5 The proposed XYZ system for hybrid wheat production

The system involves a recessive genic male sterility gene *ms* on a wheat chromosome and a compensating male fertility factor *Ms* on an alien chromosome. The system requires three components namely X, Y and Z. All the components in the system are homozygous for wheat *ms* and contain 2, 1 and 0 doses of the male sterility compensating alien chromosome respectively.

The X component is disomic for the alien chromosome, homozygous for alien *Ms/Ms* and is pure breeding. The Y component is monosomic for the alien chromosome and produces 21-chromosome gametes and 22-chromosome gametes, however, in the 21-chromosome gametes function preferrably because of certation of the hyperploid pollen. The Z line is homozygous for wheat *ms/ms* and does not possess any alien chromosome.

Primarily, the system requires a proper amount of Z seeds which can be obtained following steps 1 and 2 in the proposed system (Driscoll, 1972; 1973). The Z seeds can be used as the female block in hybrid seed production. The male block is a normal wheat variety and hybrid seeds are heterozygous for the wheat male-fertility genes. The male sterility compensating alien chromosome is not expected to be present in the hybrid seeds.

2.5.1 Characteristics of *ms* on a wheat chromosome

The genic male sterility in wheat should behave as a single recessive Mendalian gene and should be stable in different varietal backgrounds and environmental conditions. The *ms* should not have any adverse effect on the plant phenotype and there should not be any selection against the gametes bearing *ms* in competition with the gametes bearing *Ms* allele.

2.5.2 Suitable genic male sterile mutant for the proposed system

Pugsley and Oram (1959) first reported the occurrence of spontaneous male sterile plants in an F_3 population of Kenya farmer x bearded Juvelin 48^4. The inheritance of this male sterile mutant is complex. Waninge and Zeven (1968) reported the occurrence of variation in chromosome numbers from 40 to 42 in root tips of the seedlings raised from seeds obtained from Dr. A.T. Pugsley. However, Briggle (1970) backcrossed this mutant to the variety Chancellor and isolated a stock which behaves as a single recessive gene. Driscoll (1975a) determined that the locus for male sterility in this mutant is on chromosome 4A. Athwall *et al.* (1967) reported the isolation of genic male sterile plants from a complex hybrid. The gene for male sterility in this mutant was recessive and was controlled by mutiple factors. It was assumed that the high male sterility was controlled by the additive effect of three major genes. The expression for male sterility was found to be influenced by environmental factors. Krupnov (1968) isolated a male sterile wheat mutant in the variety Saratovskaya-29. The male sterility was controlled by a single recessive gene. However, the male sterile plants were less viable than the fertile plants. Fossati and Ingold (1970) irradiated seeds of the wheat variety Probus and selected a male sterile mutant. The male sterility in the Probus mutant is controlled by a single recessive gene. The mutant locus for male sterility in this mutant has been investigated and located on chromosome 4A (Barlow and Driscoll, 1981). Cornerstone is a chromosomal male sterile mutant which has been isolated from the cross of Federation monosomic 4A by Pitic 62 following the application of 250 rads of γ-rays to the pollen of Pitic 62 (Driscoll, 1977). This male sterility mutant involves a terminal deletion of the α arm of chromosome 4A (Barlow and Driscoll, 1981). The male sterility in this mutant is controlled by a single recessive gene.

Franckowiak *et al.* (1976) isolated nine EMS-induced male sterile mutants. Most of the male sterile mutants displayed monogenic or digenic inheritance patterns for their male sterility. Sasakuma *et al.* (1978) studied the expression and inheritance pattern for male sterility in those mutants obtained from Franckowiak *et al.* (1976). They observed eleven mutants for male sterility, each of which was controlled by a single recessive gene and three of those were allelic. One dominant mutant (F S_6) for male sterility was found stable under different genetic background.

The male sterile mutants of Fossati and Ingold (1970) and Driscoll (1977) can equally qualify as suitable candidates for the proposed XYZ system (Driscoll, 1972, 1973).

2.5.3 Characteristics and probable sources of *Ms* from alien species

There are a number of restrictions on the male sterility compensating alien chromosome(s). It must not have a major adverse effect on the plant phenotype. The alien *Ms* must not be affected either by environmental factors or by genetic background. The alien chromosome possessing the *Ms* gene must refrain from pairing with wheat chromosomes. *Ms* must not be frequently transmitted through pollen from the Y line. It must not misdivide frequently in the Y line. It should have a low rate of addition decay in the X line.

Driscoll (1973) mentioned several possible sources for such an alien chromosome bearing *Ms*, based on the ability of alien chromosomes to substitute for certain chromosomes of wheat in the homoeologous groups 4 and 5. Among them, 4E of *Agropyron elongatum*, chromosomes D and 5R of *Secale cereale* L. cv. Imperial and chromosome C of *Ae. umbellulata* ZhuK. (2n = 14) were suggested. Driscoll (1981) further suggested another source for an alien male sterility compensating

chromosome outside the sub-tribe *Triticinae*. A structurally modified barley (*Hordeum vulgare* L.) chromosome was suggested as a suitable candidate for the proposed system on the basis of its ability to compensate for male sterility in the complete absence of chromosome 4A of hexaploid wheat (A.K.M.R. Islam and K.W. Shepherd, personal communication).

2.6 Crosses involving *Triticum* and *Secale* species

Wilson (1876) was apparently the first person to produce a hybrid between wheat and rye. Interest in the production of high yielding amphiploids later led to further investigation of crosses between *Triticum* and *Secale* species. The aim of hybridization between *Triticum* and *Secale* species was to transfer the desirable rye character(s) to wheat. This led to the production of hybrid amphiploids (x *Triticosecale* Wittmack).

Several *Triticum* species have been crossed with different species and cultivars of *Secale* to produce a wide range of Triticale. Primarily, octoploid triticale was produced. The octoploid triticale did not achieve popularity except in China as a useful crop (Müntzing, 1979) because of its meiotic instability (Müntzing, 1957; Tsuchiya, 1968) and poor agronomic performances. Subsequently plant breeders have concentrated their efforts on producing hexaploid triticale, involving a cross between tetraploid wheat and diploid rye. The history of development of triticale and its usefulness as a new plant species has been reviewed (Larter, 1974; Tsunewaki, 1974; Müntzing, Loc. cit.).

2.6.1 Crossability between *Triticum* and *Secale* species

Backhouse (1916) observed that a wheat of Chinese Spring origin gave 80% seed set in the cross with rye but he obtained only 0.02% seed set when Chinese Spring was replaced by other cultivars.

Similarly, Leighty and Sando (1928) obtained 90.5% seed set after pollinating a Chinese wheat with rye but they obtained 9.1% and 18.2% seed set when two plants suspected to be hybrids between Chinese wheat and another wheat variety were pollinated with rye. Taylor and Quisenberry (1935) observed differences in crossability among different wheat strains and subsequently they transferred the rye crossability from Chinese Spring to agronomically desirable wheats by intervarietal crossing. Lein (1943), cited in Riley and Chapman (1967), investigated the genetic control of the crossability of wheat with rye and showed that allelic differences at two loci are responsible. He assigned the genotype $kr_1kr_1kr_2kr_2$ for readily crossable to Chinese Spring 466, $Kr_1Kr_1Kr_2Kr_2$ for poorly crossable to Marquis and $Kr_1Kr_1kr_2kr_2$ for partially crossable to Blausamtigar Kolben. Kr_1 has a more pronounced effect on preventing crossability than Kr_2.

Riley and Chapman (1967) investigated intervarietal substitution lines involving the substitution of pairs of chromosomes of poorly-crossable Hope for the chromosomes of readily crossable Chinese Spring and showed that kr_1 and kr_2 are located on chromosomes 5B and 5A of Hope respectively. Chinese Spring has recessive alleles at these two loci. Lange and Wojciechowska (1976) observed that the poor crossability of Hope and Chinese Spring/Hope 5B substitution lines resulted from the failure of fertilization.

Beside these intervarietal differences in crossability among wheats, Röbbelen and Smutkupt (1968) found reciprocal differences in the rate of success in crosses of wheat and rye. They obtained 61% seed set in the cross between Chinese Spring and Petkus rye. But, they obtained 1% seed set in the reciprocal cross.

The differences in crossability of different species of *Triticum* and *Secale* were also noticed by Nakajima (unpublished, see the review of Tsunewaki, 1974). *T. aestivum* and *T. compactum* showed the highest crossability with *S. cereale, S. ancestrale* Zhuk. and *S. vavilovii* Grossheim. It was very difficult to cross *T. aestivum* and *S. fragile* Bieb. Larter (1974) reported that tetraploid wheat and diploid rye were first crossed by Aase in 1930. Nakajima (unpublished, see review of Tsunewaki, 1974) noticed poor crossability between nine tetraploid wheat species and seven *Secale* species. Only 0-0.4% seed set was found in the crosses involving tetraploid wheat and *S. cereale, S. ancestrale* and *S. vavilovii*. A higher percentage (0.1-69%) of seed setting was found in the reciprocal crosses, when some three *Secale* species were crossed with hexaploid *Triticum* species.

Generally, the F_1 seeds involving the cross between tetraploid wheat and diploid rye contains less endospermic tissue and as a result the F_1 seeds fail to grow on the mother plant. However, the embryo culture technique (Brink *et al.* 1944) facilitates amphiploid production.

2.7 Production of alien addition lines to hexaploid wheat

Many intergeneric amphiploids have been produced involving crosses between *Triticum aestivum* and many other members of the sub-tribe *Triticinae*. Except hexaploid triticale none of the amphiploids has proved to be a potential agricultural crop. This is due to the incorporation of undesirable genes to *T. aestivum* along with the desirable ones. This led to the production of alien addition lines to *T. aestivum* so as to exclude undesirable character(s) present in the whole genome of the alien species. Many chromosomes of alien species have been added monosomically or disomically to hexaploid wheat. A comprehensive report on addition lines of alien species has been made

by Riley and Kimber (1966) and Driscoll (1975b, 1976).

Leighty and Taylor (1924) achieved the addition of *Secale cereale* chromosomes to *T. aestivum* for the first time. They utilized the backcross method to isolate addition lines which had a specific rye character (hairy peduncle, Hp) to *T. aestivum*. O'Mara (1940) added three pairs of rye chromosomes individually to hexaploid wheat. Initially, he produced wheat-rye amphiploids, crossed the amphiploid with wheat and the heptaploid was obtained. He allowed the heptaploids to self and the plants with a single alien chromosome added to wheat ($21^{II} + 1^{I}$) were selected by observing rye character(s) in the wheat background. He isolated disomic addition lines from the selfed progeny of monosomic addition lines. Riley and Chapman (1958), Riley (1960) and Riley and Macer (1966) produced all seven possible addition lines of the variety King II of *Secale cereale* to hexaploid wheat cv. Holdfast. The individual rye chromosomes added to cv. Holdfast produced phenotypic differences from the recipient variety. Among the phenotypic differences, growth habit, hairy peduncle, leaf, spike and culm morphology were used to identify the individual rye chromosomes added to wheat. Riley (1960) observed that the added rye chromosomes did not pair frequently. The fertility of addition lines varied among themselves and were comparatively less fertile than that of Holdfast. Evans and Jenkins (1960) isolated two monosomic and five disomic additions of Dakold fall rye to the wheat variety Kharkov. Only one rye chromosome brought phenotypic changes to the recipient variety. The chromosome which produced pubescent peduncles was classified as chromosome VI. Other added rye chromosomes brought minor changes to the morphology of the recipient variety. Driscoll and Sears (1971) completed the isolation of all seven disomic addition lines of Imperial rye chromosomes to *T. aestivum* cv. Chinese Spring. They classified rye chromosomes A, B, F and G into wheat

homoeologous groups 5, 2, 6 and 3 respectively. Also, they suggested that chromosome C, D and E might belong to groups 7, 4 and 1 respectively. Bernard (1976) isolated six disomic addition lines of Petkus rye to hexaploid wheat. The individual addition lines were identified by differences in their plant morphology. All the addition lines showed chromosomal asynapsis, however, the author found no correlation between asynapsis and fertility in these lines. Chang *et al.* (1973) isolated disomic addition lines of 5R of Gator, Prolific, Imperial, Weedy 1, White and Wrens x Brazil to Chinese Spring. They demonstrated that the gene(s) for *Hp* (Hairy peduncle) is located at least 50 cross-over units from the centromere of chromosome 5R. Kimber (1967) produced six different disomic addition lines of *Ae. umbellulata* ZhuK. to Chinese Spring. Later, Chapman and Riley (1970) isolated the remaining addition line. The addition lines were phenotypically distinguishable from each other by their heights, spike characters and their maturity time. These lines are cytologically more stable than the rye addition lines. Riley *et al.* (1966) produced a monosomic addition of the *Ae. comosa* Sibth. et Sm chromosome 2M which conditioned resistance to stripe rust. Jewell and Driscoll (in press) isolated nine of fourteen possible single addition lines of tetraploid *Ae. variabilis* Eig. A complete set of seven addition lines of the diploid *Agropyron elongatum* chromosomes to Chinese Spring were isolated by Dvořák and Knott (1974). Six additions showed morphological differences. Knott (1964) produced a disomic addition line of polyploid *Agropyron elongatum* (2n = 70) to the variety Thatcher. The added chromosome carried gene(s) for stem rust resistance. Cauderon *et al.* (1973) produced six disomic additions of *A. intermedium* chromosomes to wheat. Three of the six lines individually possessed the genes for resistance to leaf rust, stem rust and stripe rust. Hyde (1953) isolated five of the seven possible disomic addition lines and six of the seven possible monosomic addition lines of *Hynaldia villosa*

to Chinese Spring. Islam *et al.* (1981) produced six of the possible seven disomic addition lines and seven of the possible fourteen ditelosomic addition lines of barley (*Hordeum vulgare* L.) cv. Betzes chromosomes to Chinese Spring. Chromosome 5 of barley carries a self-sterility gene, hence the production of the disomic addition of this particular chromosome was not possible. Individual barley chromosomes added to wheat produced different morphologies. All the addition lines showed more meiotic asynapsis and less fertility compared with the recipient variety.

The addition of alien chromosomes to wheat has not made any direct contribution to the improvement of wheat. The addition lines are useful in identifying the gene content of individual alien chromosomes (Riley and Chapman, 1958). Their study was related to the transfer of alien gene(s) to wheat by various means such as manipulation of Sears' *Ph* mutant (1977), or suppression of chromosome 5BL activity by *Ae. speltoides* (Riley *et al.* 1968), or induction of translocations involving wheat chromosomes and alien chromosomes by irradiation (Sears, 1956a; Sharma and Knot, 1966).

2.8 Substitution of wheat chromosomes by the chromosomes of alien species

There are numerous reports on the substitution of wheat chromosomes by alien chromosomes, some relevant important cases are described below:

HOMOEOLOGOUS GROUP 1

Shepherd (1973) substituted 1D of Chinese Spring disomically by chromosome E of *Secale cereale* cv. Imperial. The genetic compensation for sporophyte and pollen by the replaced chromosome was satisfactory. Rye chromosome E bears genes for rye prolamine and is genetically related

to homoeologous group 1 of hexaploid wheat. The author substituted 1B of wheat disomically by chromosome B of *Ae. umbellulata*. Riley *et al.* (1973) isolated the substitution lines $1C^{u}$(1A), $1C^{u}$(1B) and $1C^{u}$(1D) in all of which they observed some reduction in chromosome pairing at low temperature. The reduction in pairing was most obvious in the $1C^{u}$(1D) line. The authors suggested that $1C^{u}$ carries genes epistatic to those of 5D. Further, Riley, Chapman and Miller (PBI annual report, 1971) showed the relationship of chromosomes D and C of *Ae. umbellulata* to the chromosome of homoeologous groups 2 and 5, respectively. Mettin *et al.* (1973) and Zeller (1973) reported the spontaneous substitution of 1B by 1R in the variety Zorba. Due to its excellent resistance to rust, Zorba has been used as a source of rust resistance in several breeding programmes in Europe.

HOMOEOLOGOUS GROUP 2

Riley, Chapman and Macer (1966) substituted all the chromosomes of homoeologous group 2 disomically by chromosome 2M of *Ae. comosa* (2n = 14). All the substitution lines were vigorous and fertile. Chromosome 2M carries a gene(s) for stripe rust resistance. Sears (1968) successfully substituted 2B and 2D of Chinese Spring by chromosome III of Imperial rye. The Imperial rye chromosome III is now referred to as 2R. Later, Sears substituted chromosome 2A of Chinese Spring by chromosome 2R of Imperial rye (cited in Bielig and Driscoll, 1971).

HOMOEOLOGOUS GROUP 3

Bakshi and Schlehuber (1959) reported that chromosome 3D of the variety Pawnee was replaced by a chromosome of *Agropyron elongatum*. This chromosome carries a factor for rust resistance. Acosta (1961), cited in Bielig and Driscoll (1971), showed that chromosome 3R of

Secale cereale cv. Imperial (which bears a gene for wheat stem rust resistance) is related to the chromosomes of homoeologous group 3, Barber *et al.* (1968) demonstrated the relationship between chromosomes of homoeologous group 3 and Imperial chromosome G. This chromosome bears a gene for wheat stem rust resistance and also carries a gene for a fast moving esterase isozyme. Gupta (1969) found that a single rye chromosome of *Secale cereale* cv. Dakold can substitute for both 1D and 3D, demonstrating that this chromosome is related to homoeologous groups 1 and 3. A translocation involving 1R and 3R may explain this observation.

The and Baker (1970) reported the spontaneous replacement of chromosome 3A disomically by a pair of *Agropyron intermedium* chromosomes in TAF_1.

HOMOEOLOGOUS GROUP 4

Bielig and Driscoll (1973) substituted 4B of Chinese Spring by Imperial chromosome D. This line showed good vigour but was sterile. Driscoll (1975b) substituted 4B of Chinese Spring monosomically by Imperial chromosome C and this line also was sterile. Zeller and Baier (1973) analysed the spontaneous substitution wheat line W70a86 (Blaukorn) and found that 5R of *Secale cereale* had been substituted for chromosome 4A. However, the wheat line W70a86 carries five different translocations relative to the chromosomes of Chinese Spring, involving chromosomes 1A, 2A, 5A, 6A, 3B, 7B, 1D, 3D, 6D and 7D, and these translocations complicate the results obtained from this study.

Dvořák and Sosulski (1974) reported the substitution of 4D by chromosome III of *Agropyron elongatum* in which the fertility and vigour was quite normal. Rao (1975) allegedly substituted chromosome 4A disomically by Imperial rye chromosome C. The substitution line was

vigorous but was not fully fertile as compared with normal Chinese Spring. After further study the author showed that the alien chromosome was a 4A/C translocated chromosome. Koller and Zeller (1976) disomically substituted 4A by Imperial rye chromosomes CR and CRS (these are referred to as 4R and 4RS, respectively in this thesis). The 4R (4A) substitution line was only 4% fertile whereas 4RS (4A) line displayed 19% fertility. The authors further demonstrated the relationship between 4R and 7R and the wheat chromosomes of their respective homoeologous groups. They provided evidence for the existence of translocations involving 4R, 7R and 6R. Dvořák (1980) disomically substituted chromosome 4A by chromosome 4E of *Agropyron elongatum* (2n = 14). This line was vigorous but sterile. It has been suggested that chromosome 4E of *Agropyron elongatum* might have lost the critical gene(s) for male fertility in the process of evolution. The substitution lines 4E (4B) and 4E (4D) were quite normal in fertility. Miller *et al.* (1982) reported the disomic substitution of wheat chromosome 4A by chromosome A of *Ae. sharonensis*.

HOMOEOLOGOUS GROUP 5

In the history of information regarding the relationship between wheat and alien chromosomes, Kattermann (1938) was the first to report the relationship between chromosomes of homoeologous group 5 of wheat and an alien chromosome. He showed that a particular rye chromosome, which possesses the gene(s) for hairy penduncle (*Hp*), is genetically related to chromosome 5A of wheat. Later, O'Mara (1946) substitututed a pair chromosomes 1X (now 5A) of *T. vulgare* by a pair of chromosomes of *Secale cereale*. This substitution line was both male and female fertile and improved the normal *vulgare* wheat. Chapman and Riley (1970) substituted 5B of Chinese Spring by $5C^u$ of *Ae. umbellulata* and demonstrated that $5C^u$ cannot

substitute for the pairing regulator gene(s) of 5BL. Bielig and Driscoll (1970a) substituted 5B of Chinese Spring with the long arm of 5R. The results showed that the pairing regulator gene(s) of 5BL cannot be substituted by the long arm of 5R. They observed pairing between wheat and rye chromosomes in the absence of 5B. Bielig and Driscoll (1970b) isolated the substitutions of 5A, 5B and 5D by Imperial rye chromosome 5R. Rye chromosome 5R successfully restored fertility in 5R (5A) and 5R (5D) but not 5R (5B).

HOMOEOLOGOUS GROUP 6

Knott (1964) studied the stem rust and leaf rust reactions of the derivatives produced by L.H. Shebeski from the cross *T. vulgare* VIII variety Chinese Spring x (Chinese Spring x *Agropyron elongatum* (Host) Beauv. F_{13} plants derived from this cross were crossed to *T. vulgare* variety Thatcher and homozygous resistant plants were selected. Monosomic analysis revealed that both substitution lines lacked wheat chromosomes VI (now 6A). These lines showed normal vigour and fertility and yielded equal to Thatcher. Gametes carrying 20 wheat chromosomes plus 1 *Agropyron* chromosome competed well with gametes carrying 21 wheat chromosomes. Riley (1963) substituted the chromosomes of homoeologous group 6 of *T. aestivum* variety Holdfast with chromosome II of *Secale cereale* cv. King II. These lines were vigorous but showed marked loss of fertility. Johnson (1966) demonstrated the close relationship of a particular *Agropyron elongatum* chromosome with 6A and 6D of hexaploid wheat. Bielig and Driscoll (1971) isolated a number of putative substitutions for chromosome 6A and 6B. The isolation of a substitution for 6D had been made previously by Anderson and Driscoll (1967). All the substitution lines in the homoeologous group 6 were vigorous and fertile.

HOMOEOLOGOUS GROUP 7

Quinn and Driscoll (1967) confirmed that 7D in Agrus had spontaneously been disomically substituted by a pair of chromosomes of *Agropyron elongatum* (Host) Beauv. The *Agropyron* chromosome bears gene(s) for resistance to wheat leaf rust (*Puccinia recondita* ROB ex. Desin). The plants were fully fertile and vigorous. Bielig and Driscoll (1971) showed a very close relationship between the chromosomes of homoeologous group 7 and 7E of *Agropyron elongatum*. All the substitution lines were extremely vigorous and fertile. The and Baker (1970) reported that in TAF_2, a pair of 7D chromosomes have been replaced by a pair of *Agropyron intermedium* chromosomes which condition resistance to stem rust. Recently, Dvořák (1980) successfully substituted all the chromosomes of this homoeologous group by the chromosome 7E of *A. elongatum*.

2.9 The homoeologous relationship between wheat and rye chromosomes

Generally the homoeology of a particular alien chromosome with respect to the wheat chromosomes is determined by its ability to compensate for the chromosomes of a wheat homoeologous group. The homoeology of the chromosomes of diploid rye, *S. cereale* with wheat chromosomes has been determined by various workers as discussed in Section 2.8. Workers have used different rye cultivars in different wheat backgrounds and as a result the determination of homology of the 7 chromosomes of a particular rye cultivar with the 7 chromosomes of another rye cultivar has not been verified completely. Koller and Zeller (1976) showed the homology of the 7 rye chromosomes of three different rye cultivars and their homoeologous relationships with wheat chromosomes.

The homoeology between wheat and rye chromosomes becomes more complex when rye chromosomes are involved in translocations.

Riley (1955) observed that *S. cereale* differs from *S. montanum* Guss. by two reciprocal translocations involving three chromosomes. Khush and Stebbins (1961) demonstrated that the genome *S. cereale* differs from the wild species by two translocations.

Koller and Zeller (1967) demonstrated translocations involving chromosomes 4R, 7R and 6R of *S. cereale* cv. Imperial rye and showed their relationship with wheat chromosomes of homoeologous groups 4 and 7. The chromosome 4R has gained a major portion of the short arm of chromosome 7R and chromosome 7R has gained the reciprocally translocated segment of the long arm of 4R. In addition, 7R has exchanged a segment of chromosome 6R and vice versa, and as a result, 7R of Imperial rye has become a double translocated chromosome. Neither 4R nor 7R of Imperial rye can fully compensate for the chromosomes of their respective homoeologous groups due to these interchanges of genetical material.

Darvey (1973) tentatively allocated Imperial rye chromosome D to homoeologous group 4 and chromosome C to group 7 which contrasts with the Koller and Zeller (Loc. cit.) classification. Tang and Hart (1975), on the basis of the location of the structural gene for alcohol dehydrogenase, classified Imperial rye chromosome C into homoeologous group 4. Hart (1978) further demonstrated that the structural gene for rye ADH is located on the short arm of chromosome C and this arm is homoeologous to 4Aα, 4BL and 4DS of Chinese Spring. A portion of the short arm of Imperial rye chromosome D is homoeologous to 4Aβ, 4BS and 4DL. Now, it has been decided to refer to Imperial rye chromosomes CR and DR as 4R and 7R respectively (see Appels *et al.* 1982).

2.10 Genome analysis with reference to the progenitor of the A genome

Sax (1922) demonstrated that the A genome of the polyploid wheat was derived from a diploid Eikorn, *T. monococcum* L. McFadden and Sears (1944, 1946) demonstrated that the D genome of hexaploid wheat is derived from *Ae. squarrosa* L. The authors crossed *T. dicoccoides* Korn exshwent and *Ae. squarrosa* and doubled the F_1. The resulting amphiploid showed the morphological characteristics of hexaploid *T. spelta* L. Kihara (1944) as well, Riley and Chapman (1960) came to the conclusion that the D genome of hexaploid wheat had evolved from *Ae. squarrosa*.

The controversy about the progenitor of the B genome of hexaploid wheat has not yet been resolved. It is suspected that the B genome may have evolved from one or more *Aegilops* species. Several species of *Aegilops* such as *Ae. speltoides* (Sarker and Stebbins, 1956; Riley *et al.* 1958; Rees and Walters, 1965), *Ae. bicornis* (Sears, 1956b), *Ae. longissima* (Nishikawa and Furuta, 1978) and *Ae. searsii* (Feldman, 1978) have been proposed as the donor of the B genome for both tetraploid and hexaploid wheat. Peacock *et al.* (1981), using a repeated DNA sequence technique, demonstrated that chromosomes 2B, 5B, 7B and 1B of hexaploid wheat have the same repeated DNA sequences as *Ae. longissima*. They concluded that the donor(s) of the B genome must be closely related to the species in the sitopsis section of *Aegilops*.

At one time *T. monococcum* was considered to be the sole progenitor of the A genome of tetraploid and hexaploid wheat. Chapman and Riley (1966) reported that the α arm of chromosome 4A of hexaploid wheat was involved in 48% pairing with a chromosome of *T. thaoudar* (Reuter) Schiem. Feldman (1966), by using known telocentric stocks of Chinese Spring, observed that the right arm (now α arm) of chromosome 4A did not pair at all in hybrids between Chinese Spring and the amphiploid

T. timopheevi-T. tauschii. The left arm (now β arm) was involved in pairing only 29.7% of the time. However, Chapman *et al.* (1976) and Dvořák (1976) demonstrated conclusively that all chromosomes of the A genome of hexaploid wheat, except chromosome 4A, paired with the chromosomes of *T. urartu* in F_1 plants derived from crosses between telocentric stocks of Chinese Spring and *T. urartu*. K. Shepherd (personal communication) observed no pairing between telocentrics 4Aα or 4Aβ in the pollen mother cells of the hybrids involving crosses between Chinese Spring and *T. thaoudar*, *T. boeoticum* Boiss, *T. aegilopoides* Bal and *T. monococcum*.

2.10.1 Characteristic N-banding pattern of chromosome 4A and the progenitor of the A genome

The N-banding pattern of chromosome 4A is different to all other chromosomes of the A genome. Gerlach (1977) and Jewell (1979) demonstrated that all seven chromosomes of the B genome have a distinctive N-banding pattern. Chromosome 7A, which has faint terminal bands at each end, and chromosome 4A, which has heavy banding on each side of the centromere as well as a faint sub-terminal band in the β arm, are the only chromosomes of the A genome to have a distinctive N-banding pattern. Peacock *et al.* (1981) used a Ag^+ satellite probe to obtain information regarding the origin of the A genome. They found no diploid species which contains a chromosome with the distinctive pattern of sites characteristics of chromosome 4A. These observations led to the supposition that *T. monococcum* can no longer be considered the sole progenitor of the A genome. The origin of chromosome 4A of tetraploid and hexaploid wheats is still untraced. The origin of the A genome of tetraploid and hexaploid wheats may be complex.

2.10.2 Characteristic male-fertility gene(s) on chromosome 4A

Sears (1966) observed that all three possible nullisomics of homoeologous group 4 are non-vigorous. Also, nullisomic 4A tetrasomic 4B or 4D plants are normal but male sterile. Conversely, nullisomic 4B or 4D tetrasomic 4A plants are normal and fertile. This indicates that chromosome 4A has a gene(s) for male fertility which cannot be compensated for by either 4B or 4D. The male fertility gene(s) on chromosome 4A is located on the α arm since ditelocentric 4Aα is fully fertile and ditelocentric 4Aβ is male sterile.

2.11 Production of synthetic hexaploid wheat

Since the discovery of the progenitor of the D genome of hexaploid wheat (McFadden and Sears, 1944, 1946; Kihara and Lilienfeld, 1949; Riley and Chapman, 1960), the newly synthesized hexaploid wheat has been of little value to agriculture. However, evidence is available that some *Aegilops* species have been used for transferring certain desirable characters to wheat (Kimber, 1967; Karber and Dyke, 1969; Sears, 1958; Riley *et al.* 1968).

2.11.1 Meiotic restitution

Kihara and Lilienfeld (1949) obtained synthetic hexaploid wheat through the union of two unreduced gametes. The F_1 hybrids of crosses between *T. dicoccoides Spontaneo-nigrum* and *Ae. squarrosa* showed 21 univalents in the majority of their pollen mother cells. The occasional formation of 1-3 bivalents in their pollen mother cells was observed. They suggested that unreduced gametes were produced through the restitution of nuclei at the first telophase stage, followed by longitudinal division of the chromosomes during the second division. They further reported that the degree of meiotic restitution is

determined by environmental factors. Tabushi (1959) noticed asynapsis in newly synthesized hexaploid wheat. He reported various degrees of univalent formation in the pollen mother cells of true synthetic hexaploid wheat.

Maan and Sasakuma (1977) reported a high frequency of meiotic non-reduction resulting in the production of functional male and female gametes in the amphiploid of *Ae. heldreichii* Homz (2n = 14, MM) and *T. durum* Desf. Most of the pollen mother cells showed 21 univalents at metaphase I. They obtained two types of cell: one type had randomly scattered univalents like those remaining unpaired and lying off the metaphase plate at first meiotic division. This type of pollen mother cells produced asymmetrical dyads and tetrads possessing nuclei of different sizes. In the other type of pollen mother cells, all 21 univalents, each possessing two chromatids, were oriented at the metaphase plate. They subsequently divided mitotically and produced symmetrical dyads which produced functional pollen grains without undergoing further division.

Further, Maan *et al.* (1980) produced F_1 hybrids involving a species of *Aegilops* as female and *T. durum* as male. In the pollen mother cells of the F_1 hybrids asynapsis resulted in the formation of viable male and female gametes. The union of such unreduced male and female gametes resulted in seed set.

2.11.2 Addition of the chromosomes of the D genome of hexaploid wheat to tetraploid wheat

Joppa and McNeal (1972) crossed seven lines of Chinese Spring, each tetrasomic for one of the D genome chromosomes to *T. durum* Desf. cv. Wells and Lacota. Most of the F_1 plants possessed 15 pairs of

chromosomes plus six univalents. They isolated disomic addition lines of chromosomes 1D, 3D, 4D, 5D and 6D from the F_3 population. The disomic addition lines of 1D, 3D and 6D were not maintainable because they were male sterile. The disomic addition lines of 4D and 5D showed chromosomal stability, were partially male fertile, and as a result, these two addition lines were maintainable.

CHAPTER 3. METHODS AND MATERIALS

3.1 Genotypes

3.1.1 Hexaploid and tetraploid wheats

Triticum aestivum L. cv. Chinese Spring

T. aestivum cv. Cornerstone *mslc*

T. durum Desf. (Accession No. K721)

Triticale

Seeds of octoploid triticale involving the cross between *T. aestivum* L. cv. Chinese Spring and *Secale cereale* L. cv. Imperial were kindly provided by Professor C.J. Driscoll.

3.1.2 Diploid wheats and *Aegilops*

Seeds of *Triticum monococcum* L. (2n = 14, AA; WARI Accession No. 4777) and *Aegilops squarrosa* L. were kindly provided by Dr. K.W. Shepherd.

3.1.3 The following hybrid seeds were kindly provided by Professor C.J. Driscoll.

F_1 seeds: Cornerstone *mslc* x *T. durum* Desf. (2n = 28, AABB, (Accession No. K721)

First backcross seeds: (Cornerstone *mslc* x *T. durum*) x *T. durum*

3.1.4 The following diploid rye seeds (*Secale cereale* L.) were obtained from Professor C.J. Driscoll.

Secale cereale L. cv. South Australian Commercial (South Australian)

S. cereale L. cv. Imperial.

3.1.5 Seeds of each of the seven addition lines of Imperial rye chromosomes to Chinese Spring developed by Dr. E.R. Sears were obtained from Professor C.J. Driscoll.

3.2 Production of chromotypes

3.2.1 Production of amphiploids

The male sterile hexaploid wheat plants were selected from segregating populations on the basis of the morphology of their anthers and the non-dehiscent character of pollen grains of these anthers. The apex of the anther lobes of male sterile hexaploid wheat is characteristically curved inwards. Empty non-dehiscent and non-stainable pollen grains is another identifying character of male sterile plants.

F_2 seeds of Cornerstone *ms1c* (Federation/ 2^* Pitic 62/ Timgalen/3/2^* Chinese Spring, were placed on moistened filter papers in petridishes and allowed to germinate at 26°C. Following germination, the seeds were kept in a refrigerator at 4°C. for seven days. Two young seedlings were planted in 20 cm diameter plastic pots in the glasshouse. At least 50 seedlings were planted at each planting. Seedlings of South Australian rye were planted in the glasshouse on different dates to facilitate synchronized flowering times of Cornerstone and the rye. Some selected heads of male sterile hexaploid wheat were enclosed within glassine bags to prevent any possible out-crossing. Pollen grains from South Australian rye were dusted on the receptive stigmas of the primary and secondary florets of male sterile hexaploid plants.

The day after pollination, a small drop of 40 ppm gibberellic acid was applied to each pollinated floret. This procedure was repeated

on three consecutive days. 18-20 days from the date of pollination, the growing hybrid embryos were removed by dissection and cultured using the method of Cheng and Smith (1975). Young F_1 embryos were kept in the dark for 6-7 days at 20°C. and then transferred to an oven at 20°C. The young established embryos were provided with 30 lux of light for 12 hours followed by 12 hours of darkness. Seedlings at 2-3 leaf stage were taken off culture media and planted individually in 8 cm diameter plastic pots. The seedlings were grown in a growth chamber and were supplied with 20,000 to 50,000 lux of light followed by 12 hours of darkness. Seedlings at 2-4 tiller stage were removed from their individual pots, their root system washed with tap water and then treated with an aerated aqueous solution of 0.05% colchicine plus 3% dimethyl-sulphoxide for 4 hours and 30 minutes. Following colchicine treatment, the root systems of the seedlings were thoroughly washed with tap water, then each seedling was repotted in 20 cm diameter plastic pots in the glasshouse.

A majority of the F_2 seeds from the doubled sectors were allowed to grow on the mother plants. A number of embryos from F_2 seeds aged from 18-21 days were dissected out from the doubled sectors of the F_1 plants and the embryos were cultured as described above to save intergeneration time. The mature F_2 seeds of 35-40 days old were harvested and stored.

The octoploid triticales so produced with *msIc* were fertile and were used as pollen parents in the cross to Cornerstone in order to obtain first backcross seeds. The Bc_1 plants were raised following the same embryo culture technique and the chromosomal complements of the Bc_1 plants were determined from meiotic observations by the standard Feulgen method. Further, fertile Bc_1 plants were used as pollen parents in the cross to Cornerstone and second backcross seeds were obtained. The Bc_2 seeds were allowed to grow on the mother plants to full maturity.

Monosomic and double monosomic additions of the seven rye chromosomes were grown and fertile plants were isolated and were allowed to self pollinate. The male sterile plants were pollinated with normal hexaploid wheat to maintain the addition lines. The arm locations of the male sterility compensating gene(s) were determined from the misdivision products of individual male sterility compensating rye chromosomes.

3.2.2 Production of male sterile tetraploid wheat

Cornerstone was pollinated with normal durum (*T. durum* Desf. 2n = 28, AABB). Subsequently first backcross seeds were obtained by pollinating the F_1's with normal durum. The chromosome numbers of the first backcross plants were determined both from mitotic and meiotic cells. Several first backcross plants, each possessing 0-2 chromosome(s) of the D genome were grown and selfed in the glasshouse. A male sterile durum plant was isolated from the Bc_1F_2 population.

3.2.3 Production of hexaploid triticale (x *Tritico secale* Wittmack)

Male sterile tetraploid wheat *Triticum durum* Desf. (*mslc* Hossain and Driscoll, 1981) was pollinated by diploid rye (*Secale cereale* L. cv. Imperial) and the F_1 embryos were dissected out from the mother plant. Following embryo culture (Section 3.2.1) the seedlings were treated with an aqueous solution of colchicine and dimethylsulphoxide and F_2 seeds were harvested from the doubled sectors of the F_1 plants.

3.2.4 Transfer of the male sterility mutant from the tetraploid wheat to diploid wheat

The male sterile durum (Hossain and Driscoll, 1981) was pollinated with *T. monococcum*. The F_1 plants were again pollinated with *T. monococcum* to produce the first backcross generation. Following

embryo culturing (Cheng and Smith, 1975) the first backcross plants were raised in a growth cabinet under controlled-environment conditions (Section 3.2.1). Mitotic and meiotic observations were made on the first backcross plants. Both N-banding (Section 3.3.7) and Feulgen staining (Section 3.3.2) techniques were employed to observe the pairing of the chromosomes of the F_1 hybrid plants.

3.2.5 Production of synthetic hexaploid wheat with male sterile tetraploid wheat and *Ae. squarrosa*

Male sterile durum was pollinated by *Ae. squarrosa* L. var. *strangulata* (2n = 14, DD). Embryo culturing and doubling of the chromosomes of F_1 hybrid plants were carried out following the same method (Section 3.2.1). The hybrid plants were grown in a growth chamber. Meiotic observations of some of the F_1 plants were made using the Feulgen method (Section 3.3.2). Seeds set due to meiotic non-reduction divisions (See Chapter 8) and colchicine-induced doubled sectors were harvested. Euploid synthetic hexaploid (2n = 42, AABBDD) plants were selected from the F_2 population and backcrossed to male sterile durum. The first backcross plants were obtained following embryo culture. The chromosome number of Bc_1 plants were determined from observations on pollen mother cells.

The first Bc_1 plants, being male fertile were used as pollen parents to cross with male sterile durum and Bc_2 seeds were obtained. The mitotic chromosome number of Bc_2 plants were determined by Feulgen technique and the plants were allowed to grow in the glasshouse during Spring to classify for fertility.

3.2.6 Glasshouse conditions during Winter and Spring

Plants grown in the glasshouse during Winter were not provided with supplementary light. As well the day temperature inside the glasshouse fluctuated widely and was frequently below 20°C. During Spring the day temperature inside the glasshouse also varied but generally was above 20°C.

3.3 Chromosome preparation

3.3.1 The Feulgen method for chromosome study

An investigation of the chromosomal complement of Cornerstone octoploid triticale and synthetic hexaploid wheat was made by following this method. Also, some Bc_2 seeds involving crosses between male sterile durum and synthetic hexaploid were studied by this Feulgen staining method. The seeds of these stocks as referred above were allowed to imbibe overnight at 26°C. in an oven. Next morning, the seeds were transferred to a refrigerator at 4°C. and kept for approximately 52 hours. The seeds were then transferred to an oven at 26°C. and grown overnight. The following morning, the two seminal roots of each seedling were excised and placed in an aqueous solution of 1-bromonapthalene for 4 hours and 30 minutes. The root tips were then fixed in glacial acetic acid and stored at -15°C. overnight. Next morning, the root tips were hydrolysed with 1 normal hydrochloric acid at 60°C. for approximately 13-15 minutes. The hydrochloric acid was replaced by Feulgen stain. The root tips developed a pink colour after few minutes in Feulgen stain and were ready for the preparation of slides by the squash method. The cover slip was removed from the slide by freezing in liquid nitrogen (See Conger and Fairchild, 1953). The slide was immediately put into 98-100% ethalnol

at room temperature for one hour. A drop of Euparal was put over the specimen and a clean cover glass was placed on top.

3.3.2 Meiotic preparation from anthers for Feulgen staining

One anther of each spikelet was checked for the proper stage of meiosis and the remaining two anthers were fixed in 3 parts ethanol and 1 part glacial acetic acid by volume. The anthers were fixed overnight at room temperature. Next morning, the anthers were hydrolysed for 12-13 minutes with 1 normal hydrochloric acid at 60°C. The hydrochloric acid was replaced by Feulgen stain and the specimen tube was kept in the dark for half an hour. The stained anthers were crushed with a needle on a clean slide and the debris from the anther wall was removed from the slide. The slide, with cover glass in place, was gently heated over a flame and then the preparation was squashed. The slide was made permanent as in Section 3.3.1.

3.3.3 Mitotic metaphase preparation for chromosome banding

The second backcross seeds obtained from the cross between Cornerstone octoploid triticale and Cornerstone were germinated at 26°C. on moistened filter paper in petridishes. After approximately 40 hours the brush end of each seed was cut off and used in a test to determine the presence of the isozyme, rye alcohol dehydrogenase. From the embryo half of individual seeds, two seminal roots were excised and placed in an aqueous solution of .07% colchicine for approximately 4 hours and 45 minutes. The root tips were then fixed in 3 parts ethanol and 1 part glacial acetic acid by volume and stored at -15°C.

3.3.4 Meiotic metaphase preparation for C-banding

One anther of each spikelet was checked for meiosis and the remaining two anthers were fixed in 3:1 and stored at room temperature for a period of not more than 2 hours. Each anther was macerated in 45% acetic acid and the debris of the anther was removed from the slide. The method described in Section 3.3.1 was followed for slide preparation and the removal of the cover glass. The slides were dried on a hot plate at 55°C. for 5-7 minutes.

3.3.5 Meiotic preparation for N-banding

The same procedure was followed as in Section 3.3.4, except the hydration was by immersion in ethanol rather than on a hot plate. The prepared slides were stored overnight in ethanol at -15°C.

3.3.6 C-banding method

The C-banding method of Vosa and Marchi (1972) was considerably modified.

I. The slides were prepared immediately after fixation.

II. The slides were dried on a hot plate at 55°C. for approximately 5-7 minutes.

III. The prepared slides were treated with a 6% barium hydroxide ($Ba(OH)_2$ $8H_2o$) solution for 5 minutes 30 seconds for mitotic preparations and 3 minutes 30 seconds for meiotic preparations. The slides were washed with distilled water.

IV. After $Ba(OH)_2$ treatment the slides were incubated in 2 x S.S.C. at 75°C. for 20 minutes. The slides were rinsed with distilled water.

V. Finally the slides were placed in a Giemsa solution for 5-7 minutes. The slides were rinsed with distilled water and then rapidly dried under a hot air stream.

3.3.7 N-banding method for chromosome banding

Jewell's (1979) method for N-banding wheat chromosomes was followed. Prepared slides were removed from 98-100% ethanol, air dried for 20-30 minutes and then immersed in 1 molar NaH_2PO_4 at 93 ± 1°C. for either 5 minutes 30 seconds (mitotic preparations) or 1 minute 30 seconds (meiotic preparations). The slides were immediately rinsed with distilled water, then stain in 7.3% V/V Gurr Improved R66 Giemsa stain for 45 minutes. The slides were rinsed with distilled water and dried quickly.

3.4 Electrophoresis

3.4.1 Analysis of alcohol dehydrogenase

Seeds of hexaploid wheat, *Triticum aestivum* L. cv. Chinese Spring, diploid rye, *Secale cereale* L. cv. South Australian Commercial, Imperial addition line chromosome C to Chinese Spring and the second backcross seeds of Cornerstone octoploid triticale to Cornerstone were allowed to germinate on moistened filter paper. After 40 hours, the brush ends of individual seeds were removed and used in an assay for rye alcohol dehydrogenase. Seeds of Chinese Spring, S.A. rye and Imperial addition C to Chinese Spring were used as controls. The remaining embryo halves of the Bc_2 seeds were retained for mitotic observations by C-banding (Section 3.3.6).

3.4.2 Analysis of esterase from leaf tissue

Initially, a double monosomic addition of S.A. rye chromosome 3R and 5R to Cornerstone was isolated by C-banding. This plant was male sterile (See Chapter 4) and it was pollinated with Chinese Spring to maintain the line. The monosomic 3R addition seedling was isolated and grown in petridishes at 20°C. for 3-5 days. Seedlings of Chinese Spring,

S.A. rye and Imperial addition chromosome G to Chinese Spring were grown simultaneously. All the seedlings as referred above were subjected to 12 hours darkness, then 12 hours in light of 30 lux. Two young leaves of the growing seedlings were used in an assay for rye estrases. The Barber *et al.* (1968) method for esterase analysis was followed.

3.4.3 Electrophoresis method

12 gel tubes, each of 12 cm length and 5 mm diameter were stored in a cleaning agent (Section 3.7.4) prior to use. The gel tubes were rinsed in distilled water, then dipped into a photoflo 200 solution and dried in the oven. One end of the gel tube was sealed with nescofilm. 0.3 ml of a 40% sucrose solution was placed in each tube, then 0.2 ml of the stacking solution (Section 3.7.5) was added without mixing with the sucrose layer. A few drops of distilled water were placed on the surface of the stacking gel to avoid dehydration. The gel tubes were put under a light to polymerize the stacking solution. When the polymerization was completed, the distilled water layer was drained off, then 2 ml of the running gel (Section 3.7.6) was added to each tube and allowed to polymerize. The tubes were then washed with distilled water, and then rinsed with a 1 x Davis electrode buffer (Section 3.6.19). The gel tubes were fixed onto a disc electrophoresis apparatus. Both cathode and anode chambers of the electrophoresis apparatus were filled with the 1 x Davis' electrode buffer. The supernatant resulting from centrifuging of the prepared samples (Section 3.7.7) was carefully dispensed into the sample space at the cathode end of the gel tube. 1 mA, 2 mA and 3 mA of current was passed through each tube for the first, second and third hours respectively. The electrophoresis was performed in a voltage refrigerator at 5^{o}C. for approximately 4 hours and 30 minutes. The

gel tubes were then removed from the apparatus. The gels were removed from the tubes and stained separately in test tubes for either alcohol dehydrogenase or esterase (Section 3.7.8, 3.7.9).

3.5 Estimation of male fertility

3.5.1 Viability test for pollen grains

Several mature anthers were collected from different spikelets of individual plants immediately before dehiscence. The anthers were positioned on moistened filter papers in petridishes and brought to the laboratory. The anthers were crushed on a clean slide in a drop of 2% potassium iodide solution. The pollen grains lying between the slide and cover glass were protected from drying by surrounding the edge of the cover glass with clear nail varnish.

3.5.2 Percentage of fertility count

Important plants were grown in a growth chamber which maintained 20°C. and intervals of 12 hours of darkness and 12 hours of light of 20000 to 50000 lux. The fertile plants were allowed to self pollinate and heads between 3 and 10 in numbers of each plant were selected at random for seed counting. Two basal and two upper florets of each head were excluded from counting due to their malformation. Seeds set on primary and secondary florets were counted. The fertility of a number of plants grown in the glasshouse during Winter and Spring were counted following the same procedure. The percentage of fertility for each spike or plant was calculated as follows:

$$\% = \frac{\text{Total number of seed set per spike or plant}}{\text{Total number of florets per spike or plant}} \times 100$$

3.6 Preparation of Chemicals

3.6.1 Colchicine for chromosome doubling of the F_1 hybrid seedlings

125 mg of colchicine and 7.5 ml of dimethylsulphoxide were dissolved in 250 ml of distilled water to give a .05% colchicine + 3% DMSO solution. The solution was occasionally used on several occasions. The solution was stored at $4^{o}C$.

3.6.2 Colchicine to increase mitotic index

0.07% of colchicine (7 mg in 10 ml of distilled water) was used to increase the mitotic index in roots of 40 hours old seedlings. Sufficient colchicine solution was used to submerge the excised roots. The same concentration of colchicine was applied to increase the mitotic index of intact roots.

3.6.3 Preparation of a stock solution of 1-bromonapthalene

1 ml of 1-bromonapthalene was dissolved in 100 ml of ethanol. The stock solution was stored in a sealed container at room temperature. The working solution consisted of 1 ml of 1-bromonapthalene dissolved in tap water and the volume made up to 100 ml. Fresh working solution was made for each run.

3.6.4 Preparation of orthophosphate

A 1 molar (15.8 gm in 100 ml of distilled water) solution of sodium orthophosphate ($NaH_2PO_4 2H_2O$) was made freshly for each N-banding run.

3.6.5 Preparation of barium hydroxide solution

6 gm of $Ba(OH)_2$ was dissolved in 100 ml of distilled water and the solution was stirred for 3-5 minutes. The solution was filtered

once through a clean tissue paper and then immediately used for slide treatment. Fresh $Ba(OH)_2$ solution was made for each C-banding run.

3.6.6 2 x SSC (Saline/sodium citrate)

A stock solution of 20 x SSC (87.17 gm of sodium chloride and 44.10 gm of sodium citrate made up to 500 ml with distilled water) was stored at -15°C. The working solution of 2 x SSC (0.3 molar sodium chloride + 0.03 molar sodium citrate) was prepared by taking 10 ml of stock solution and making it up to 100 ml. Fresh working solution was prepared for each C-banding run.

3.6.7 Fixative

The 3:1 alcohol-acetic acid fixer was prepared immediately prior to the fixation of anthers and root tips. 45% glacial acetic acid V/V was prepared and stored at room temperature.

3.6.8 Giemsa staining solution

Stock solutions of 0.5 molar $NaHPO_4 12H_2O$ (dibasic) and 0.5 molar $NaH_2PO_4 2H_2O$ (monobasic) were prepared and stored at room temperature. The stock solutions when contaminated by bacteria or mould were replaced with freshly prepared solutions. A solution of 0.03 molar Sorensen's buffer and 7.3% Giemsa was freshly prepared for each banding run adding 1.5 ml of each of the monobasic and dibasic stock solutions to a coupling jar containing approximately 20 ml of distilled water. 1.5 ml of Gurr Improved Giemsa R66 was added to the solution which was then stirred with a clean slide immediately prior to use.

3.6.9 Potassium iodide solution

2 gm of potassium iodide (KI) was dissolved in 100 ml of ethanol. This solution was stored in a sealed container at room temperature.

3.6.10 Aqueous solution of gibberellic acid

4 gm of gibberellic acid (grade III) was dissolved in 20 ml of 0.1 millimolar potassium bicarbonate ($KHCO_3$) and the volume was made up to 100 ml with distilled water, giving a solution of 40 ppm gibberellic acid.

3.6.11 THAM solution

36 gm of THAM [Tris(hydroxymethyl)aminomethane] [Tris] was dissolved in distilled water with gentle heating. The volume of the solution was made up to 100 ml with distilled water. The pH of the solution was adjusted to 8.9 at 23°C. by titration with concentrated hydrochloric acid.

3.6.12 THAM hydrochloric acid solution

5.0 gm of THAM was dissolved in 48 ml of 1 normal hydrochloric acid. The pH was adjusted to 6.9 at 23°C. and the volume was made up to 100 ml with distilled water. The solution was filtered and stored indefinitely at 4°C.

3.6.13 Acrylamide-Bis solution for running gel

28.0 gm of acrylamide and 0.735 gm of Bis-acrylamide was dissolved in 100 ml of distilled water. The solution was filtered and stored indefinitely at 4°C.

3.6.14 Acrylamide-Bis solution for stacking gel

10 gm of acrylamide and 2.5 gm of Bis-acrylamide were dissolved in distilled water. The solution was filtered and stored indefinitely at $4^{o}C$.

3.6.15 Riboflabin

5 gm of riboflabin was dissolved in 50 ml of distilled water. The solution was filtered and stored indefinitely at $4^{o}C$.

3.6.16 Ammonium persulphate

140 gm of ammonium persulphate was dissolved in 100 ml of distilled water. The solution was made fresh weekly and stored at $4^{o}C$.

3.6.17 Sucrose solution

40 gm of sucrose was dissolved in distilled water and the volume was made up to 100 ml with distilled water. The solution was made fresh weekly and stored at $4^{o}C$.

3.6.18 Extraction buffer (Carlson's modified buffer)

4.84 gm of THAM (0.1 M), 2.98 gm of potassium chloride (0.1 M) 832.4 mgm of ethylene diamene tetra acetic acid (EDTA) (0.005 M), 1.25 ml of 2-mercapto-ethanol (2-ME) (0.04 M) and 13.68 gm of sucrose (0.1 M) were mixed together. The volume was made up to 400 ml with distilled water and the solution was stored indefinitely at $4^{o}C$.

3.6.19 Reservoir buffer (electrode buffer)

Stock solution

The stock solution consisted of 28.8 gm of glycine and 5.0 gm of THAM dissolved in distilled water and the volume made up to 1000 ml,

and was stored indefinitely at 4°C. Usually the pH of this solution is 8.57 at 23°C. A working solution of this buffer was made by taking 160 ml of the stock solution and making the volume up to 1600 ml with distilled water. A fresh working solution was made for each run.

3.7 Stains

3.7.1 Tracking dye

1 mg of bromophenol blue was dissolved in 100 ml of distilled water. This stain was stored at room temperature indefinitely.

3.7.2 Stock solutions of the stain for the analysis of alcohol dehydrogenase

TRIS-HCL buffer: 0.069 molar tris-hydrochloric acid was prepared by dissolving 8.4 gm of THAM in 1000 ml of distilled water. The pH was adjusted to 7.5 at 23°C. with concentrated hydrochloric acid. The solution was stored indefinitely at 4°C.

NITRO BLUE TETRAZOLIUM: 0.006 molar nitroblue tetrazolium was prepared by dissolving 490 mgm of solid NBT in 0.5-1.0 ml of N, N-Dimethyl formamide and making the volume up to 100 ml with distilled water. The pH was adjusted to 7.5 at 23.3 with 0.01 molar hydrochloric acid. The solution was stored indefinitely at 4°C.

PHENAZINE METHOSULPHATE (PM): 0.002 molar PM was prepared by dissolving 3.065 gm of solid PM in 50 ml of distilled water. The solution was stored indefinitely at 4°C.

ETHANOL: 43.8 ml of absolute ethanol (98-100%) was made up to 100 ml with distilled water and the pH was adjusted to 7.5 at 23°C. The solution was stored indefinitely at 4°C.

3.7.3 Stock solution of the stain for the analysis of esterase

Tris-HCl buffer: 6.055 gm of THAM was dissolved in 50 ml of distilled water. The pH was adjusted to 7.0 with 1N HCl. The volume of Tris-HCl buffer was made up to 100 ml with distilled water. This solution was stored indefinitely at $4^{o}C$.

3.7.4 Preparation of the cleaning agent for gel tubes

The cleaning agent was prepared by mixing hydrochloric acid, methanol, nitric acid and distilled water in ratio 1:6:1:2 V/V.

3.7.5 Preparation of the stacking gel

0.5 ml of the THAM-hydrochloric acid (Section 3.6.12), 1 ml of the acrylamide-Bis solution (Section 3.6.14) and 2 ml of the 40% sucrose solution were mixed together. 3 µl of N,N,N',N'- tetramethyl-ethylenediamine (TEMED) was added to the mixture immediately prior to pouring the stacking gel into the gel tubes.

3.7.6 Preparation of the running gel

6.0 ml of the THAM solution (Section 3.6.11), 12 ml of the acrylamide-Bis solution (Section 3.6.13), 6 ml of distilled water and 24 ml of the ammonium persulphate solution were mixed together. 16 µl of TEMED was mixed thoroughly with above mixture immediately prior to pouring the running gel into the gel tubes.

3.7.7 Preparation of samples for electrophoresis

Either 25 mgm of the brush end of a 40 hour old seedling (for ADH) or 80 mgm of young leaf tissue (for esterase) was placed in a mortar and 0.2 ml of extraction buffer (modified Carlson's buffer) was added. A few grains of clean sand were added to the mortar to aid maceration. Following maceration with pestle, the slurry of the

sample was centrifuged in a SORVALL centrifuge (Model SS-3) for approximately 30 minutes at 10000 rpm.

3.7.8 Staining procedure for alcohol dehydrogenase

72 ml of Tris-HCl buffer (Section 3.7.2), 4 ml of the NBT, 4 ml of the ethanol solution, 0.018 gm of β-nicotinamide-adenine dinucleotide (β-NAD) and 4 ml of distilled water were thoroughly mixed. The stain was poured into the individual test tubes (Section 3.3.3) containing the gels. The gels were stained for approximately 10-15 minutes until bands appeared. The stain was replaced with 7% acetic acid and the gels stored at $4^{o}C$.

3.7.9 Staining procedure for esterase

30 mgm of α-napthyl acetate, 10 mg of β-napthyl acetate and 100 mgm of the fast blue RR were dissolved in 1.5 ml of acetone. 10 ml of the 0.5 molar Tris-HCl buffer (Section 3.7.3) and 90 ml of distilled water were added to the above solution. This stain was poured into the individual test tubes (Section 3.3.3) containing the gels and staining was allowed for 15-20 minutes. The stain was replaced with 7% acetic acid and the gels stored at $4^{o}C$.

3.7.10 Photography

All microphotographs were taken using an automatic Zeiss camera and recordak film (KODAK). Photomicrographs of banded preparations were taken using a green and yellow filter combination to increase contrast. The film was developed in 50% V/V DK 50 (KODAK) for 10 minutes at $20^{o}C$. and printing was done on gloss Kodak paper. The photomicrographs were cut and karyotypes mounted on blank photographic paper, then rephotographed by the photographic section.

CHAPTER 4. SEARCH FOR MALE-STERILITY COMPENSATION IN DIPLOID RYE

OUTLINE:

Hybrids between Cornerstone and South Australian rye (S.A. rye) were produced and treated with colchicine in order to double the chromosome number. The colchicine-induced doubled sectors of the F_1 hybrids set seeds, indicating that the whole rye genome is capable of compensating for the male-sterility of Cornerstone. The fertile octoploid triticales, thus produced, were backcrossed to Cornerstone and fertile heptaploids were produced. The heptaploids were backcrossed to Cornerstone and Bc_2 seeds were obtained. Addition lines involving the seven rye chromosomes were identified and tested for their ability to compensate for the male-sterility of Cornerstone from further backcrossing of the Bc_2 population.

METHODS AND MATERIALS (Described in Chapter 3)

RESULTS:

4.1 Production of octoploid triticale involving Cornerstone and South Australian rye

Cornerstone is maintained in a number of Australian wheat backgrounds by Professor C.J. Driscoll. Cornerstone in the Transfed background and Cornerstone in a mixed background including two doses of Chinese Spring (Section 3.2.1) were crossed with South Australian rye. The percentage of seed set was 0.5 and 22.5 in the Transfed and the mixed backgrounds, respectively (Table 4.1A). Eight of the fourteen F_1 hybrid seedlings in the Transfed background were treated with colchicine but no progeny were obtained (Table 4.1B).

In the partially Chinese Spring background, a total of 148 F_1 embryos were cultured and seedlings were raised. Another 79 F_1 hybrid

seeds were harvested when mature from the mother plants. Of the cultured F_1 seedlings, 75 died at various stages of growth (including post colchicine treatment (Table 4.1B), leaving 73 survivors.

Most of the colchicine-treated hybrid seedlings were subjected to cold treatment for at least five consecutive days to induce greater tillering. Two hundred and twenty F_2 octoploid triticale seeds (Table 4.1B) were harvested from the colchicine-treated plants.

Fifty-three F_2 octoploid triticale plants possessing from 53 to 56 chromosomes plus a telosome or an isochromosome, were planted in the glass house for crossing with Cornerstone. Thirteen F_2 (24.5%) plants were euoctoploid and the remaining plants were aneuploids.

Twenty F_3 progeny of an euoctoploid triticale were cytologically examined by the C-banding technique and they produced a maximum of 57 and minimum of 53 chromosomes. Only two plants were observed to possess the expected 56 chromosomes (Fig. 4.1A) and two plants were trisomic for rye chromosomes 2R and 4R respectively. The content of rye chromosomes varied among the individuals and structural modifications such as deletions at various points on both arms of rye chromosomes and the presence of telosomes and isochromosomes were observed in this population (Table 4.3). In rare cases, modified wheat chromosomes such as a telosome, were also observed.

4.2 Chromosome pairing behaviour and fertility in octoploid triticale

The pairing behaviour of chromosomes in 128 pmc's of three euoctoploid triticales was studied. Fourteen cells (10.9%) displayed 28 bivalents only (Fig. 4.1B) whereas the remaining 114 cells (89%) possessed from two to ten univalents (Table 4.4).

TABLE 4.1A. Percentage of seed set in crosses between Cornerstone and S.A. Commercial rye.

Cornerstone background	Total No. of florets pollinated	No. of embryos or seeds obtained	Percentage of seed set
Transfed	2688	14	0.5
Partially Chinese Spring	1008	227	22.5

TABLE 4.1B. Number of F_1 hybrids before and after the treatment with colchicine.

Cornerstone background	No. of embryos or seeds obtained	No. of seedlings which survived colchicine treatment	Total No. of F_2 seeds obtained	Average seed set per treated plant
Transfed	14	8	0	0
Partially Chinese Spring	148	73	220	3.0%

TABLE 4.2. The chromosome numbers of fifty-three F_2 Cornerstone octoploid triticales.

Chromosome number	53	53^+	54	54^+	55	55^+	56	56^+
Number of plants	5	2^a	12	3^b	11	4^c	13	3^d
Percentage of the population	9.4	3.8	22.6	5.7	20.8	7.5	24.5	5.7

NOTE:

+ = Part or modification(s) of chromosome(s) in addition to entire chromosomes

a = One plant with two telosomes and the other with two modified chromosomes

b = One plant with two telosomes and two plants with one telosome in each

c = Each plant with one telosome

d = Two plants with one telosome in each and one plant with one isochromosome

It was not determined whether the univalents represented rye or wheat chromosomes because Feulgen staining was used in this study.

Twelve plants of Cornerstone octoploid triticale possessing chromosome numbers ranging from 54 to 56 and five plants of Sears' octoploid triticale (involving Chinese Spring and Imperial rye) having similar chromosome numbers were grown in the glass house during winter and their fertility was recorded. The Cornerstone euoctoploid triticale had an average fertility level of 31.4% compared to 38.4% with the octoploid triticale of Sears' (Table 4.5).

4.3 The first backcross generation

The male fertility of the F_2 octoploid triticales involving Cornerstone demonstrates that two doses of the genome of S.A. rye can compensate for the male sterility of Cornerstone. This observation led to the hypothesis that a certain rye chromosome (or chromosomes) compensates for the male-sterility of Cornerstone. As the first step in testing the hypothesis nine of the euoctoploid triticale plants based on Cornerstone were used as the male parents to cross with Cornerstone to obtain heptaploids.

Thirty-two Bc_1 seeds were initiated following pollination of approximately 520 florets of Cornerstone. No success was achieved in obtaining Bc_1 seeds without the application of Gibberellic acid daily on the four consecutive days following pollination. All the Bc_1 seeds showed severe shrivelling when allowed to mature on the spikes. Nineteen growing Bc_1 embryos were dissected out and grown in vitro. Finally, ten Bc_1 plants developed into mature plants. These plants

TABLE 4.3. The rye chromosome complement of F_3 Cornerstone octoploid triticales determined by C-banding.

Plant No.	1R		2R		3R		4R		5R		6R		7R	
	L	S	L	S	L	S	L	S	L	S	L	S	L	S
1	*	*			*							i		
2		i							*	*	*	*		
3	*				*		D		D		*	*		
4														
5			T											
6 and 7		i			*	*		D			*	*		
8						D			D	i			*	*
9							T							
10 and 11	*	*								i	D			
12		i			D			D		*	*			
13														
14	*	*				D		D	*			*		
15	D				*									
16	D		*	*				D	D					
17 and 18		*								i	D			
19									*	i				
20	D		*	*					*	*				

Abbreviations used:

L = long arm; S = short arm; * * = entire chromosome missing; * = one arm of a chromosome missing; T = trisomic; D = major deletion; i = iso chromosome.

were grown under controlled-environment conditions and the chromosome numbers of individual Bc_1 plants were determined at the first metaphase of meiosis (Table 4.6).

Phenotypically, all the Bc_1 plants were vigorous. The plant which possessed eighteen bivalents plus five univalents was male sterile. This male sterile Bc_1 plant lacked both some wheat and some rye chromosomes. The remaining nine Bc_1 plants were partially male fertile. Only one Bc_1 plant was found to possess the whole chromosome complement of hexaploid wheat plus seven rye chromosomes i.e. it formed 21 bivalents plus 7 univalents at first metaphase of meiosis in pmc's (Fig. 4.1C). Eight Bc_1 plants contained 21 bivalents plus less than seven univalents.

Priority was given to mainly the heptaploid Bc_1 plant ($21'' + 7'$) as a pollen parent in the crosses with Cornerstone in order to obtain Bc_2 progeny. The aneuploid Bc_1 plants were also used as pollen parents but with decreasing priority as the number of rye chromosomes present was reduced.

4.4 The second backcross generation

The C-banding technique (Section 3.3.6) and an assay for rye alcohol dehydrogenase (Section 3.4.1) were employed simultaneously to determine the number of specific rye chromosomes present in the Bc_2 plants. A total of 109 Bc_2 seedlings were analysed in this study.

Ten Bc_2 seedlings were found to contain no added rye chromosomes. Fourteen seedlings possessed only one rye chromosome or segment of a rye chromosome where as twenty-six seedlings had either two entire rye chromosomes or segments of two rye chromosomes. Thirty-four seedlings possessed more than two entire rye chromosomes or parts of rye chromosomes. The number of rye chromosomes in this category

TABLE 4.4. Chromosome pairing behaviour in 128 pollen mother cells of three Cornerstone euoctoploid triticales.

1. No. of bivalents and univalents	28"	27" +2'	26" +4'	25" +6'	24" +8'	23" +10'
2. No. of cells in each type	14	26	40	26	19	3
3. Percentage of the cells of the total analysed population	10.9	20.3	31.3	20.3	14.8	2.3

TABLE 4.5. The fertility (%) of Cornerstone octoploid triticale and Sears' octoploid triticale.

Chromotype	2n = 56	2n = 55	2n = 54
Cornerstone octoploid triticale	31.4(3)	29.1(5)	31.0(4)
Sears' octoploid triticale	38.4(1)	42.9(2)	50.5(2)

NOTE:

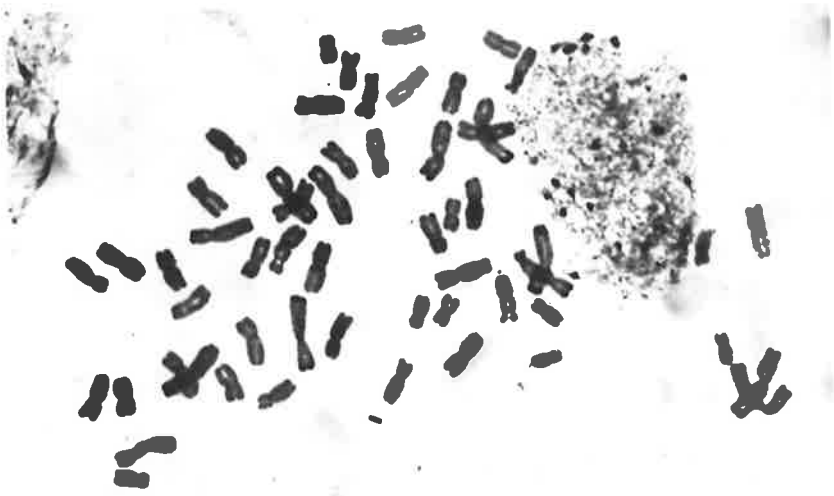
Figures in parentheses indicate the number of plants

FIGURE 4.1A-C: Chromosome complement of Cornerstone euoctoploid triticale and the first backcross plant.

A. C-banded chromosome complement of an euoctoploid triticale showing forty two wheat chromosomes and fourteen South Australian rye chromosomes. X 714

B. First metaphase of meiosis in an euoctoploid triticale showing twenty eight bivalents. X 780

C. Chromosome complement of a first backcross plant showing twenty one bivalents plus seven univalents. X 747



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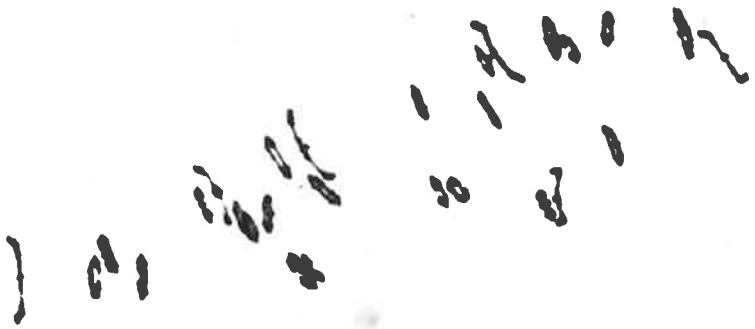

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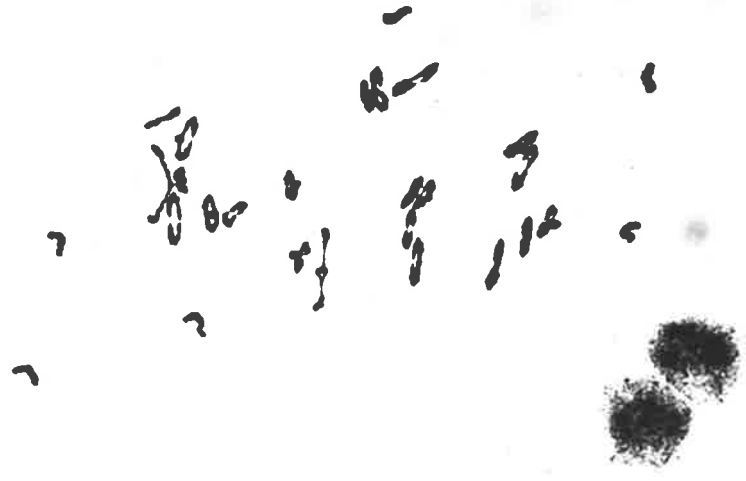


C

TABLE 4.6. Chromosome complement of first backcross plants.

Chromosome complement*	No. of plants
21" + 7'	1
21" + 6' + t'	1
21" + 6'	2#
21" + 5' + t' + t'	2#
21" + 5' + t'	2#
21" + 4' + t'	1
18" + 5'	1

* Abbreviations used:

21" = 21 bivalents; 7' = seven univalents;

t' = a telosomic univalent

\# May not involve the same rye chromosomes

ranged from two and a half to five and a half. Modifications of rye chromosomes, mainly deletions, formation of telosomes and isochromosomes, were present in some of the Bc_2 progeny. Only one Bc_2 individual possessed 41 wheat chromosomes plus one rye chromosome. The remaining Bc_2 individuals could not be classified into any specific category due to poor quality of preparation. Although, only forty Bc_2 individuals possessed one or two added rye chromosomes, the chromosome complement of fifteen Bc_2 plants was fully determined (Table 4.7).

C-banded karyotype of South Australian rye

The karyotype of South Australian rye is based primarily on the first published C-banded karyotype of Imperial rye (Gill and Kimber, 1974), except that the modification of Koller and Zeller (1976) involving the transposition of 4R and 7R has been followed. Confirmation of the identity of some rye chromosomes was made from correlating changes in specific plant characters or biochemical markers, with the addition of the particular rye chromosome to the wheat genome. This is essential because of the karyotypic variations between different rye varieties (see Darvey and Gustafson, 1975; Lelley *et al.* 1978). The plant characters used were reduction in leaf width and generally poor vigour associated with chromosome 2R (Sears, 1968), a particular rye esterase which is associated with chromosome 3R (Barber *et al.* 1968), rye alcohol dehydrogenase which is associated with chromosome 4R (Tang and Hart, 1975) and pubescent penduncle which is associated with chromosome 5R (Kattermann, 1938). Chromosome 1R shows a characteristic nucleolar C-band, chromosome 6R displays a distinctive C-banded pattern which is common to many rye cultivars. Chromosome 7R has been distinguished from 3R and 4R on the basis of absence of the structural genes for a fast moving rye esterase and rye alcohol dehydrogenase. The karyotype and ideogram of South Australian rye is described as follows (Fig. 4.2).

1R has an arm ration of 1.5:1 and approximately equal amounts of telomeric banding in the two arms. It also possesses a prominent band immediately proximal to the nucleolar organizing region on the short arm and an intestitial band on the long arm.

2R has an arm ratio of 1.4:1 and a large telomeric band on the short arm and two minor terminal bands on the long arm. The long arm bears a minor interstitial band. This chromosome results in narrow leaves and generally very poor vigour when added to wheat.

3R has an arm ratio of 1.4:1 with a larger telomeric band on the long arm than on the short arm. There is no interstitial band. This chromosome has the structural gene for a fast moving rye esterase.

4R has an arm ratio of 1.6:1 and has a large telomeric band in each arm with the band on the short arm slightly smaller than that on the long arm. There is no interstitial band. This chromosome bears the structural gene for rye alcohol dehydrogenase.

5R has an arm ratio of 2.0:1 and a telomeric band on the short arm only. The long arm has two interstitial bands. The chromosome bears the gene for a pubescent penduncle.

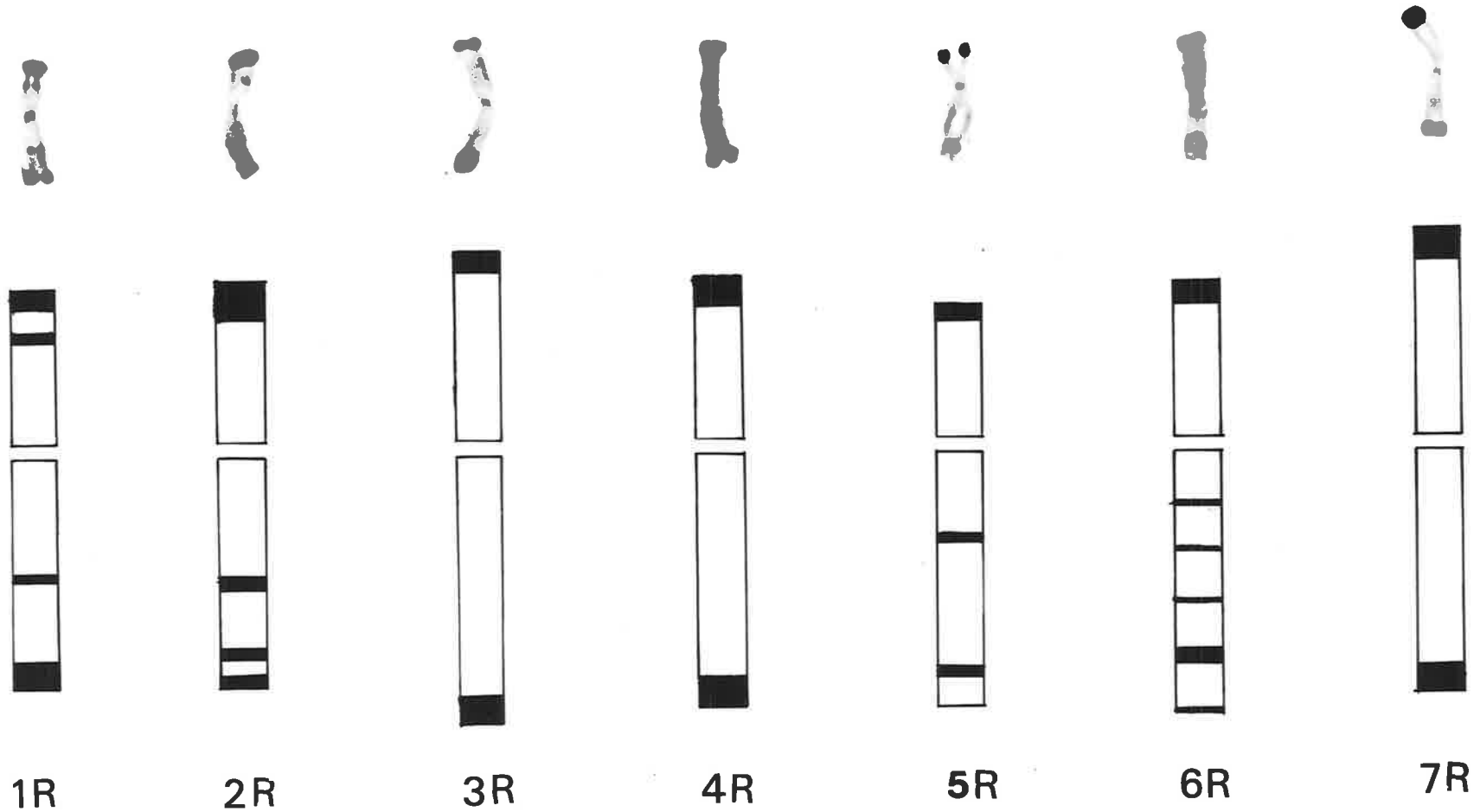
6R has an arm ratio of 1.7:1 and a large telomeric band on the short arm and a slight telomeric band on the long arm. The long arm bears four minor interstitial bands.

7R has an arm ratio of 1.2:1 and has a large telomeric band on each arm with the band on the long arm slightly smaller than that on the short arm. There are no interstitial bands.

FIGURE 4.2: C-banded karyotype of South Australian rye and its ideogram.

Top row - C-banded mitotic karyotype of South Australian rye.

Bottom row - Ideogram of the haploid karyotype of South Australian rye based on the karyotype shown in the top row.


1R
2R
3R
4R
5R
6R
7R

4.4.1 Analysis of the second backcross plants

Of the fifteen Bc_2 plants which were fully analyzed (Table 4.7) four of them were found to be partially fertile; two of the plants possessed a singly added 2R chromosome, one of these plants also possessing a modified 6R chromosome. The other two plants possessed a singly added 4R chromosome, one of these plants also possessing a singly added 6R chromosome. The fertility of the monosomic addition 2R and monosomic addition 4R plants is recorded separately in Tables 4.8 and 4.16 respectively. The progeny of monosomic 2R and monosomic 4R additions were subjected to further study, as described in (Section 4.5) and (Section 4.6.1).

The plants having added rye chromosomes 1R, 3R, 5R, 6R and 7R produced abortive pollen grains and all were male-sterile. However, these plants were found to be female fertile when backcrossed with normal hexaploid wheat pollen. Therefore, South Australian rye chromosomes 1R, 3R, 5R, 6R and 7R can not compensate for the male sterility of Cornerstone.

4.5 Analysis of progeny from self pollination of tne monosomic 2R addition plant

The chromosome complements of 139 offspring of the monosomic 2R addition plant was screened in root tips by applying the C-banding technique. This screening was done in order to isolate the disomic 2R addition plant for examining its fertility level. Altogether fourteen different chromotypes (Table 4.9) were detected. 52 of them apparently contained no portion of chromosome 2R. Three plants were disomic and 36 plants were monosomic for 2R (Figs. 4.3A, 4.3B). The remaining 46 plants formed misdivision products of chromosome 2R; 26 plants contained the short arm of 2R as telosome (Fig. 4.3C) where as only 2 plants

TABLE 4.7. Cornerstone single and double monosomic addition lines classified by rye chromosomes present and fertility.

Rye chromosomes present*	Fertility*
1R#	S
1R + 3RL(iso)	S
2R	PF
2R + 6Rm	PF
3R + 5R	S
4R	PF
4R + 6R	PF
5R	S
5R + 7R	S
6R + f	S
7R + 5RL + f	S

* Abbreviations used:

S = sterile; PF = partially fertile;
3RL(iso) = a long arm isochromosome of chromosome 3R;
6Rm = a modified chromosome 6R involving a deletion of part of the long arm; f = a small fragment;
5RL = a long arm telosome of chromosome 5R.

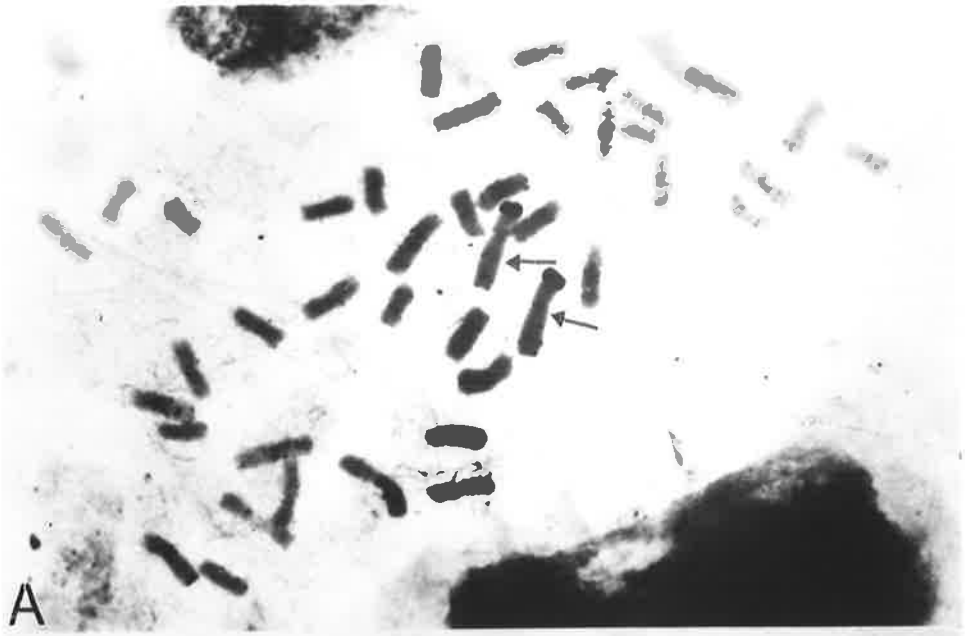
\# Five such plants observed

FIGURE 4.3A-C: C-banded mitotic chromosome complement of disomic 2R, monosomic 2R and monotelosomic 2RS addition plants.

A. C-banded mitotic chromosome complement of a disomic 2R addition plant showing forty two wheat chromosomes plus two 2R chromosomes of South Australian rye (Arrow). X 953

B. C-banded mitotic chromosome complement of a monosomic 2R addition plant showing forty two wheat chromosomes plus chromosome 2R of South Australian rye (Arrow). X 953

C. C-banded mitotic chromosome complement of a monotelosomic 2RS addition plant showing forty two wheat chromosomes plus a 2RS telosome (Arrow). X 953



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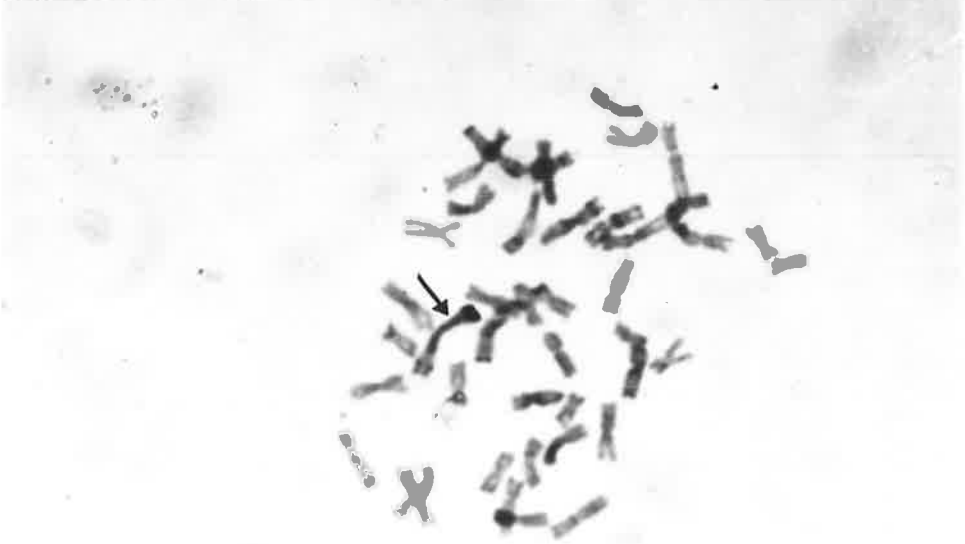

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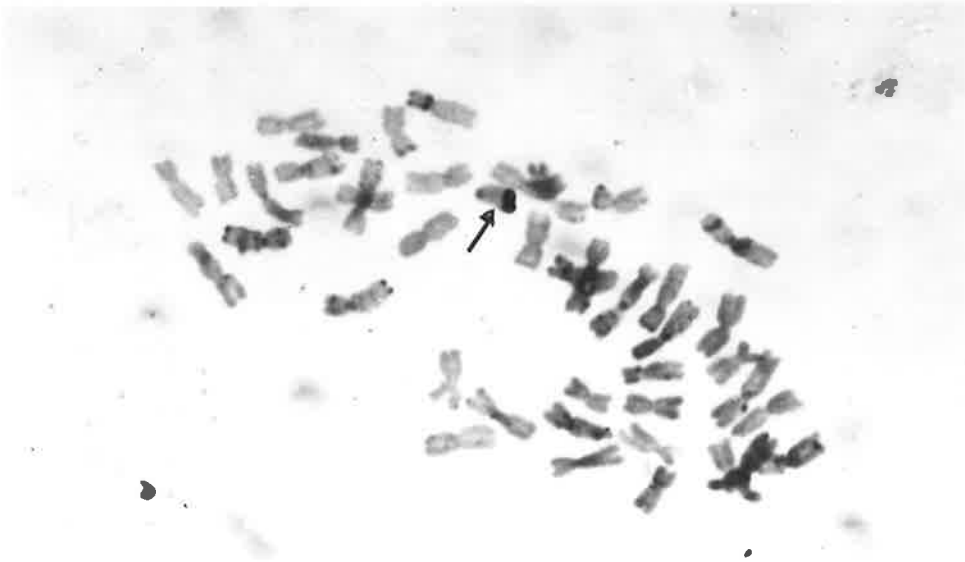


C

possessed the long arm of 2R. Two other plants of interest possessed a modified form of chromosome 2R as monosomic additions; one modification of 2R involved the loss of very small terminal segment of the long arm (Fig. 4.4B). The other modified 2R had lost a large terminal segment of the long arm. The break point for the deletion was observed to be just below the interstitial C-band present on the long arm (Fig. 4.4C).

4.5.1 Plant phenotype of disomic 2R addition lines

Initially, one disomic 2R addition plant was grown under controlled-environment conditions. It grew normally during the first 2-4 weeks and produced a limited number of narrow leaves. The subsequent growth rate was poor, the plant remained stunted and the plant failed to produce any tiller. This plant was transferred into a cool chamber at 4°C and kept there for five days in order to induce tillering. Subsequently, the plant was returned to the controlled-environment conditions. Only two tillers eventuated and one tiller produced a spike. Following self-pollination nine seeds were obtained. Two more 2R disomic addition plants were isolated from 139 offspring and grown under controlled-environment conditions. A very small number of seeds were obtained from one of the plants. Additionally twenty disomic 2R addition plants were grown under a range of environmental conditions (controlled-environment conditions, in the glass house during Spring, Summer and Winter) during the course of this study, none of them were vigorous (Fig. 4.5) and all showed the same plant phenotype as the original disomic 2R addition plant. Some of the disomic 2R addition plants died before heading.

4.5.2 Male sterility compensation by chromosome 2R and plant phenotype of the 2R misdivision products

All the monosomic 2R addition plants grown under controlled-environment conditions and in the glass house during Spring initially displayed moderate growth but later grew slowly. The plant phenotype of monosomic 2R addition plants was more normal than disomic 2R addition plants (Fig. 4.5). The original monosomic 2R addition plant grown under controlled-environment conditions displayed 51% fertility (Table 4.8). One group of six monosomic 2R addition plants displayed fertility levels ranging from 51.1% to 60.0% (Table 4.10) and another group of six plants displayed fertility levels ranging from 49.0% to 60.7% when grown in the glass house during Spring (Table 4.10). These two groups displayed similar fertility levels and the average fertility of all plants was 56% as compared with the control var. Chinese Spring (100%) (Table 4.15). Therefore, the average fertility of monosomic 2R addition plants grown under two different environments is similar.

An analysis of variance was performed on the fertility data (after arc sign transformation) for plants of Chinese Spring (control) and monosomic 2R additions grown in the controlled-environment conditions and glass house in Spring. The analysis showed that these were significantly different in both environments ($P < .001$). That is, the fertility of monosomic 2R plants grown in either controlled-environment conditions or glass house during Spring is significantly less than that of Chinese Spring.

Two monoisosomic 2RL addition plants were grown under controlled-environment conditions. Both of the plants grew extremely slowly and their spikes were smaller than those of the monosomic 2R addition plants. Also, these spikes possessed a very few florets (10-14). The anthers of both plants were very small and contained few pmc's. A majority of

TABLE 4.8. Fertility of a monosomic 2R addition plant grown under controlled-environment conditions.

Spike No.	Total No. of florets/spike	Total No. of seeds/ spike	Percentage of seed set in each spike
1	16	7	43.7
2	24	9	37.5
3	16	12	75.0
4	15	12	80.0
5	22	7	31.8
6	20	2	10.0
7	14	11	78.5
8	16	13	81.2
Total	143	73	51.0

TABLE 4.9. Chromosome complement of individuals in the progeny of self-pollinated Cornerstone monosomic 2R addition.

Chromosome complement*	Number of plants	Percentage of population
42 wheat + rye chromosome		
None	52	37.4
2R (disomic)	3	2.1
2R	35	25.1
2RS	26	18.7
2RS(iso)	9	6.4
2RL	2	1.4
2RL(iso)	3	2.1
2R + 2RS	2	1.4
2R + 2RL(iso)	1	0.7
2RS + 2RS(iso)	1	0.7
2RS + 2RL(iso)	1	0.7
2RS(iso) + 2RL(iso)	1	0.7
Modified 2R	2#	1.4
41 wheat + rye chromosome 2R	1	0.7
	139	

* Abbreviations used:

2R = entire rye chromosome 2R; 2RS = a short arm telosomic; 2RL(iso) = a long arm isochromosome.

\# These involved different lengths of deletion of the long arm.

TABLE 4.10. Fertility of monosomic 2R addition plants grown under controlled-environment conditions and in the glasshouse.

Controlled-environment conditions

Plant No.	Total No. of florets/spike	Total No. of seed set/spike	Percentage of seed set in each plant
1	153	80	52.2
2	78	48	51.2
3	78	43	55.1
4	192	121	63.0
5	50	30	60.0
6	86	47	54.6

Glasshouse (during Spring)

1	88	44	50.0
2	78	45	57.6
3	31	17	54.8
4	96	54	56.2
5	104	51	49.0
6	84	51	60.7

the pmc's appeared to be empty when stained with potassium iodide solution and there was no sign of any nuclei. Nevertheless a few pmc's had undergone meiosis. These two plants produced abortive pollen grains and as a result both plants were male sterile. However, they produced seeds when the spikes were pollinated with normal hexaploid wheat.

4.5.3 Phenotypes of plants having monosomic and disomic additions of a modified 2R

Two modified monosomic 2R addition plants (Section 4.5, Table 4.9, Figs. 4.4B, 4.4C) were grown under controlled-environment conditions. These plants did not differ phenotypically from each other nor from the monosomic 2R addition plants. One disomic modified 2R addition plant (involving a major deletion, Fig. 4.4C) was grown under controlled-environment conditions and it showed the same plant phenotype as disomic 2R addition plants.

4.5.4 Plant vigour and fertility of 2RS derivatives

Plants possessing 2RS as a monotelosome or monoisosome or combination of both were grown under controlled-environment conditions. The growth habit of these chromotypes was normal in all respects (Fig. 4.5). These plants produced several tillers with normal leaves and culms, and their spikes were larger and more normal than those of monosomic 2R, disomic 2R and disomic modified 2R addition plants grown under the same conditions. Furthermore, the spikes of the monotelosomic and monoisosomic 2RS addition plants produced a greater number of florets (Fig. 4.5) than observed with monosomic and disomic 2R addition plants.

Nine monotelosomic 2RS, two monoisosomic 2RS and one monotelosomic 2RS plus a monoisosomic 2RS addition plant were grown

under controlled-environment conditions during the course of this study. The 2RS derivatives produced normal numbers of viable pollen grains and anthers shed pollen, resulting in seed set. These fertile plants were allowed to self-pollinate and fertility in those chromotypes was recorded and compared with the fertility of Chinese Spring. Very little variation in the fertility level was observed among different spikes of the same plant (Table 4.11) and also between plants of the same chromotype (Table 4.15).

The average fertility of nine monotelosomic 2RS addition plants (94%) and seven monosomic 2R addition plants (56%) under controlled-environment conditions was compared with the fertility level of Chinese Spring (Table 4.15). The analysis of variance (Section 4.5.2) showed that there was a significant difference ($P < .001$) between the fertility of monotelosomic 2RS addition plants and monosomic 2R addition plants. There was a small difference (just significance at the 5% level) between the fertility of Chinese Spring and that of monotelosomic 2RS addition plants. This demonstrates that the monotelosomic 2RS addition plants are almost as fertile as normal Chinese Spring.

Two monotelosomic 2RS addition plants grown in the glass house during Spring displayed similar fertility levels to those of monotelosomic 2RS addition plants grown under controlled-environment conditions (Tables 4.11, 4.15). Variation in the fertility among the spikes of the same plant and between the two plants was found in the glass house grown plants (Table 4.12).

4.5.5 Breeding behaviour of monotelosomic 2RS addition plants

The breeding behaviour of monotelosomic 2RS addition plants were examined in order to test the possibility of using 2RS as the Y component for the system.

TABLE 4.11. Fertility of two monotelosomic 2RS addition plants grown under controlled environment conditions. These two plants were selected on the basis of highest and lowest fertility compensation.

Plant No.: 1

Spike No.	Total No. of florets/spike	Total No. of seed set/spike	Percentage of seed set in each spike
1	38	38	100.0
2	32	31	96.8
3	34	34	100.0
4	34	33	97.0
5	32	32	100.0
6	34	34	100.0
7	26	23	88.4
8	24	24	100.0
9	36	36	100.0
10	30	30	100.0
Total	320	315	98.4

Plant No.: 2

1	20	18	90.0
2	24	21	87.0
3	20	16	80.0
4	26	23	88.4
5	18	16	88.8
6	20	18	90.0
7	16	10	62.5
8	16	14	87.5
Total	160	136	85.0

TABLE 4.12. Fertility of two monotelosome 2RS addition plants grown in the glasshouse during Spring.

Plant No.: 1

Spike No.	Total No. of florets/spike	Total No. of seeds/spike	Percentage of seed set in each spike
1	30	29	96.6
2	28	28	100.0
3	20	20	100.0
4	20	15	75.0
5	26	22	84.6
6	26	21	80.7
7	32	30	93.7
8	32	32	100.0
9	32	30	93.0
Total	246	227	92.0

Plant No.: 2

1	26	23	88.4
2	36	34	94.4
3	32	30	93.7
4	28	26	92.8
5	26	22	84.6
6	24	20	83.3
7	28	25	89.2
8	28	27	96.4
9	26	24	92.3
Total	254	231	90.9

Four monotelosomic 2RS addition plants were included in this study. Plants No. 7, 8, 11 and 15 were identified as monotelosomic 2RS additions in the progeny of the original monosomic 2R addition and they were allowed to self-pollinate. Their progeny were cytologically examined using the C-banding technique.

Family No. 7: Twenty-five plants of this family were examined (Table 4.12). Thirteen of those reverted to the euploid i.e. Cornerstone. Ten plants had the parental chromotype. One plant was a monoisosomic 2RS addition line and the other was a disomic 2RS addition line.

Family No. 11: Seven plants were studied, two of them retained the telosome and five reverted to the euploid.

Family No. 15: Among the twenty-five analysed plants, fourteen reverted to the euploid, two retained 2RS as an isochoromsome and the remainder retained the telosome.

Family No. 8: An interesting phenomenon was noticed in the progeny of this family. The original plant No. 8 had the normal wheat complement plus a 2RS telosome. This was confirmed both mitotically and meiotically by the C-banding technique. Fifty-six progeny of this plant were examined. Twenty-nine were euploid, twelve retained the original telosome and three had an isochromosome of 2RS. Ten plants possessed a translocated monosome as indicated by C-banding and their ability to pair with a wheat chromosome(s). Seven of these translocations involved a small segment of an unknown wheat chromosome(s) joined to 2RS (Fig. 4.4E). Three others appeared to have 2RS joined to a larger wheat chromosome segment. As well, two disomic addition plants involving translocated monosomes were isolated. None of these translocations have yet been identified. All plants involving the

FIGURE 4.4A-E: The karotypes and the ideograms of chromosome 2R and modified 2R of South Australian rye. (Modifications mainly due to deletions and a translocation with a wheat chromosome.)

Top row - Karyotypes of entire 2R, modified 2R (minor and major deletions on the long arm of chromosome 2R), 2RS telesome and the translocated 2RS monosome.

Bottom row - Ideograms of the above karyotypes labelled A, B, C, D and E respectively. The translocated wheat segment is shown by the dotted line.

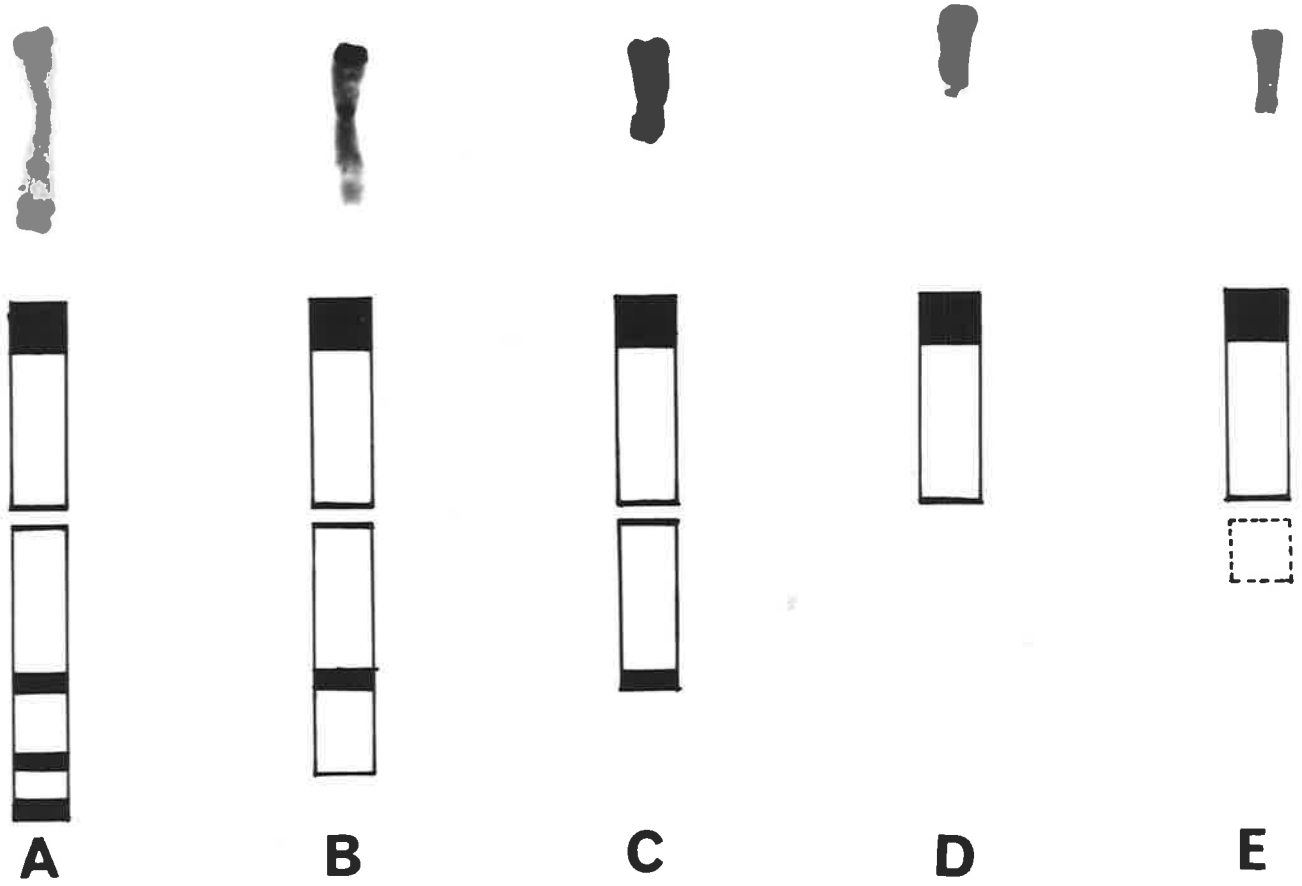

A
B
C
D
E

FIGURE 4.5: Plant morphologies of disomic 2R, monosomic 2R and monotelosomic 2RS addition plants and the spike sizes of each chromotype.

Top row - Plant morphologies of disomic 2R (left), monosomic 2R (middle) and monotelosomic 2RS addition plants (right). X 0.1

Bottom row - The spike size of each chromotype shown in top row. X 0.8

translocations displayed normal plant phenotypes and equal fertility to that of the monotelosomic 2RS addition plants grown under controlled-environment conditions.

Although a limited number (101) of self-pollinated progeny from the four families were examined cytologically, the breeding behaviour of 2RS was seen to vary among the four different plants respectively (Table 4.13). The selfed seed produced on monotelosomic 2RS additions identified in the families 7, 8, 11 and 15 (Table 4.13) were grown in the field during Spring 1982 to obtain further information on the breeding behaviour of 2RS. None of these 919 progeny grown were examined cytologically, but it was assumed that fertility indicated the presence of 2RS. The breeding behaviour of the four families varied significantly ($P < .001$) between the families with fertile plants ranging from 10.9% to 46.7% in different families (Table 4.14).

4.5.6 Chromosome pairing behaviour and fertility of disomic 2RS addition plants

The pairing behaviour of two 2RS telosomes in the pmc's of a ditelosomic 2RS addition plant was studied by the C-banding technique. The two telosomes were found to remain as univalents at first metaphase of meiosis in 8% pmc's. Phenotypically, the ditelosomic 2RS addition plant was as vigorous as monotelosomic 2RS addition plants. The fertility of this plant was 81% grown under glass house conditions during Spring.

4.5.7 Breeding behaviour of ditelosomic 2RS addition plants

Progeny of the normal ditelosomic 2RS addition plant from family No. 7 was examined cytologically by the C-banding technique. Among the fifty progeny analysed twenty-six (52%) retained the parental

TABLE 4.13. Chromotypes and number of progeny obtained following self pollination of four monotelosome 2RS addition plants. The percentage of plants in each family is shown in parentheses.

Origin of 2RS addition plants	Euploid	Monotelosomic addition	Monoisosomic addition	Ditelosomic addition	Total No. of plants
Family No. 7	13(52.0)	10(40.0)	1(4.0)	1(4.0)	25
" *8	29(65.9)	12(27.2)	3(6.8)	-	44
" 11	5(74.4)	2(28.5)	-	-	7
" 15	14(56.0)	9(36.0)	2(8.0)	-	25
Total	61(60.3)	33(32.6)	6(5.9)	1(0.9)	101

NOTE:

* Ten translocated 2RS monosomes, two translocated 2RS disomes involving unidentified wheat chromosome(s) were also isolated from this family. These translocations have not been shown in this table but have been discussed (Chapter 4, Section 4.5.5).

TABLE 4.14. The breeding behaviour and χ^2-test for homogeneity of monotelosome 2RS addition plants of four families grown in the field during Spring. These plants were not cytologically examined (see Section 4.5.5).

Origin of 2RS addition plants	Fertile	Sterile	Total	$\chi^2 = 1348$ ($P < .001$)
Family No. 7	28	228	256	
" 8	60	119	179	
" 11	69	190	159	
" 15	105	120	225	
Total	162	657	919	

chromosome complement, twenty (40%) lost one of the 2RS telosomes and four (8%) lost both telosomes.

The disomic translocated 2RS was not included in this study because it pairs with a wheat chromosome(s) and as a result it has no significance for hybrid wheat production using the XYZ system (Driscoll, 1972).

4.5.8 Somatic instability of the derivatives of 2R and the formation of chimeras

In the C-banded mitotic preparations, it was observed in a number of plants, that one root tip possessed a single 2RS telosome, while another root tip had none. In another plant, one root tip possessed 2RS as a telosome and another root tip had an isochromosome of 2RS. Two other plants had been classified as monotelosomic and monoisosomic additions of 2RS respectively, but at maturity, both plants were male-sterile. Subsequent observations at meiosis confirmed the loss of the telosome and the isochromosome from the plants. One monotelosomic 2RS addiiton plant produced eighteen tillers, ten of which were male-sterile and the remaining eight were almost fully fertile. One addition line possessing both arms of 2R as isochromosomes were grwon under controlled-environment conditions. This plant grew quite vigorously and it was almost fully fertile. It was suspected that the isochromosome of the long arm of 2R may have been lost during growth. This was confirmed later when the progeny of this plant were examined by the C-banding technique. Some of the progeny contained 2RS as an isochromosome but none contained 2RL as an isochromosome or telosome.

TABLE 4.15. Summary of fertility of Cornerstone addition lines involving 2RS and its misdivision products grown under controlled-environment conditions and glass house during Spring. (Includes data from Tables 4.8, 4.10, 4.11 and 4.12.)

Percentage of seed set on each plant

Chromotype	Controlled-environment conditions		glass house	
	Individual plants	Mean(% of Control)	Individual plants	Mean(% of Control)
Euploid Chinese Spring (control)	98, 98, 97	100	96, 94, 94	100
Cornerstone plus				
2R	63, 60, 55, 55, 52, 51	56	61, 57, 56, 55, 50, 49	56
2RL (iso)	0, 0			
2RS	98, 98, 95, 93, 93, 91, 91, 87, 85	94	91, 92	94
2RS (iso)	95, 95	98		
2RS + 2RS (iso)	92			

Abbreviations used: 2RS = short arm of chromosome 2R;
(iso) = isochromosome of long or short arm;
2RL = long arm of chromosome 2R.

These observations demonstrate that some telosomes and isochromosomes resulting from the misdivision of chromosome 2R are lost mitotically and some of them form chimeral tissue.

4.5.9 The transmission rate of 2RS telosomes in male gametes

The rate of transmission of the 2RS telosome was determined by pollinating Cornerstone with pollen of three (7, 11, 15) of the four families of monotelosomic 2RS addition plants. The resulting 107 F_1 seeds were not examined cytologically but were sown in the glass house during Spring. Eleven plants were fertile and it was assumed that the fertility was due to the presence of 2RS. Thus, the average transmission rate of 2RS was 10%.

4.6 Compensation of Cornerstone male sterility by chromosome 4R of South Australian rye

Under controlled-environment conditions, it was found that one dose of chromosome 4R can compensate for the male sterility of Cornerstone at an approximate level of 49% (Table 4.16). The progeny of the monosomic 4R addition plant were studied to obtain further information on the ability of the chromosome to compensate for male sterility. C-banding and electrophoretic assay for rye ADH were employed to identify the two arms of chromosome 4R.

4.6.1 Progeny of the self-pollinated monosomic 4R addition line

A total of 80 seedlings from a self-pollinated monosomic 4R addition plant were examined cytologically and electrophoretically. Three disomic 4R addition plants were isolated but one of them lacked one arm of an unknown wheat chromosome. Twenty-four progeny (30%) were monosomic 4R additions and twenty-eight plants (35%) reverted to the euploid Cornerstone. Eleven monotelosomic and one monoisosomic additions of the short arm of 4R were isolated. These plants possessed the rye ADH marker bands (Fig. 4.6). Seven monotelosomic and three monoisosomic additions of the long arm of 4R were isolated and these plants lacked rye ADH bands. Three other plants were isolated, one of which was monosomic for 4R and monotelosomic for 4RS and the other two plants were monosomic for 4R and monotelosomic for 4RL. Twenty-five plants (31.2%) of the analysed population involved misdivision products of chromosome 4R (Table 4.17).

4.6.2 Fertility of disomic 4R addition plants

Two disomic 4R addition plants (Section 4.6.1, Table 4.17, Fig. 4.7A) were grown under controlled-environment conditions. These two plants showed normal plant phenotype and the fertility of both plants

TABLE 4.16. Fertility of a Cornerstone monosomic 4R addition plant grown under controlled environment conditions.

Spike No.	Total No. of florets/spike	Total No. of seed set/spike	Percentage of seed set/spike
1	28	12	42.8
2	24	14	58.3
3	20	11	55.0
4	20	9	45.0
5	20	8	40.0
6	6	5	27.8
7	24	14	58.3
8	18	9	50.0
9	20	13	65.0
Total	192	95	49.4

FIGURE 4.6A-D: Fig. A-D showing the alcohol dehydrogenase zymogram phenotypes produced by different chromotypes. Migration was towards the anode from the origin as indicated by the arrow.

A. Euploid Chinese Spring.

B. South Australian rye.

C. Chinese Spring-Imperial rye disomic addition line of chromosome C.

D. Cornerstone-South Australian rye addition lines possessing chromosomes 4R and 4RS.

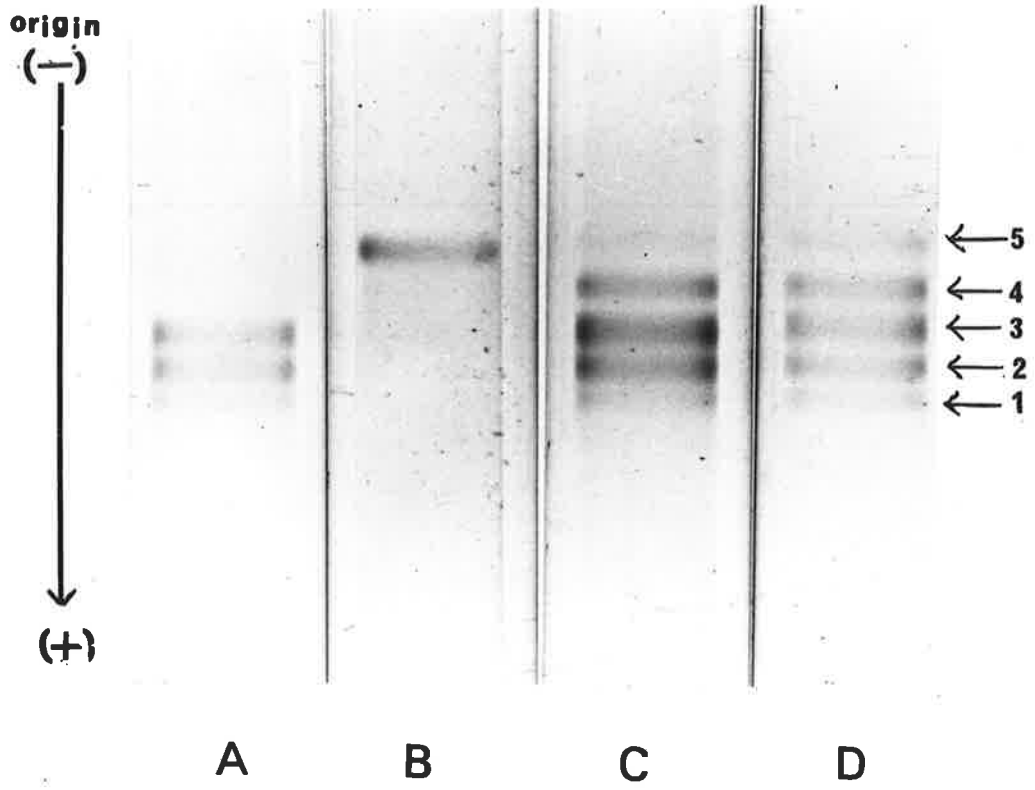

origin
(−)
(+)
5
4
3
2
1
A
B
C
D

TABLE 4.17. Chromosome complement of individuals in the progeny of a self-pollinated Cornerstone monosomic 4R addition.

Rye chromosomes in addition to 42 wheat chromosomes	No. of plants	Percentage of the population
None	28	35.0
4R (disomic)	3	3.7
4R	24	30.0
4RS	11	13.7
4RS(iso)	1	1.2
4RL	7	8.7
4RL(iso)	3	3.7
4R + 4RS	1	1.2
4R + 4RL	2	2.5

Abbreviations used:

S = short arm of chromosome 4R; L = long arm of chromosome 4R; (iso) = isochromosome of either short arm or long arm.

was recorded (Table 4.20). There was pronounced shrivelling of seeds on these two plants and sometimes it was difficult to distinguish between sterility and seeds which had shrivelled at an early stage. The fertility of one of the plants was counted early before the onset of seed shrivelling and it was found to be 67%. On the other plant, the fertility was counted after the completion of seed shrivelling and it gave only 45%. The remaining disomic 4R addition plant (lacking one arm of an unidentified wheat chromosome) showed extremely low fertility and only 12 seeds were produced on 320 florets when grown under glass house conditions during spring.

4.6.3 Fertility of monosomic 4R and monotelosomic 4RS addition plants grown under different environmental conditions

Four monosomic 4R addition plants (Fig. 4.7B) were planted in the glass house in the first week of August 1981 and the fertility of those plants was recorded (Table 4.20). It was noticed that the earlier tillers produced fewer seeds compared to the late tillers. A wide range of variation in fertility among the tillers of a plant and between two plants was observed (Table 4.18). The range of fertility in those four plants varied from 35% to 61% (Table 4.20) giving an average fertility of 46% as compared with control (100%).

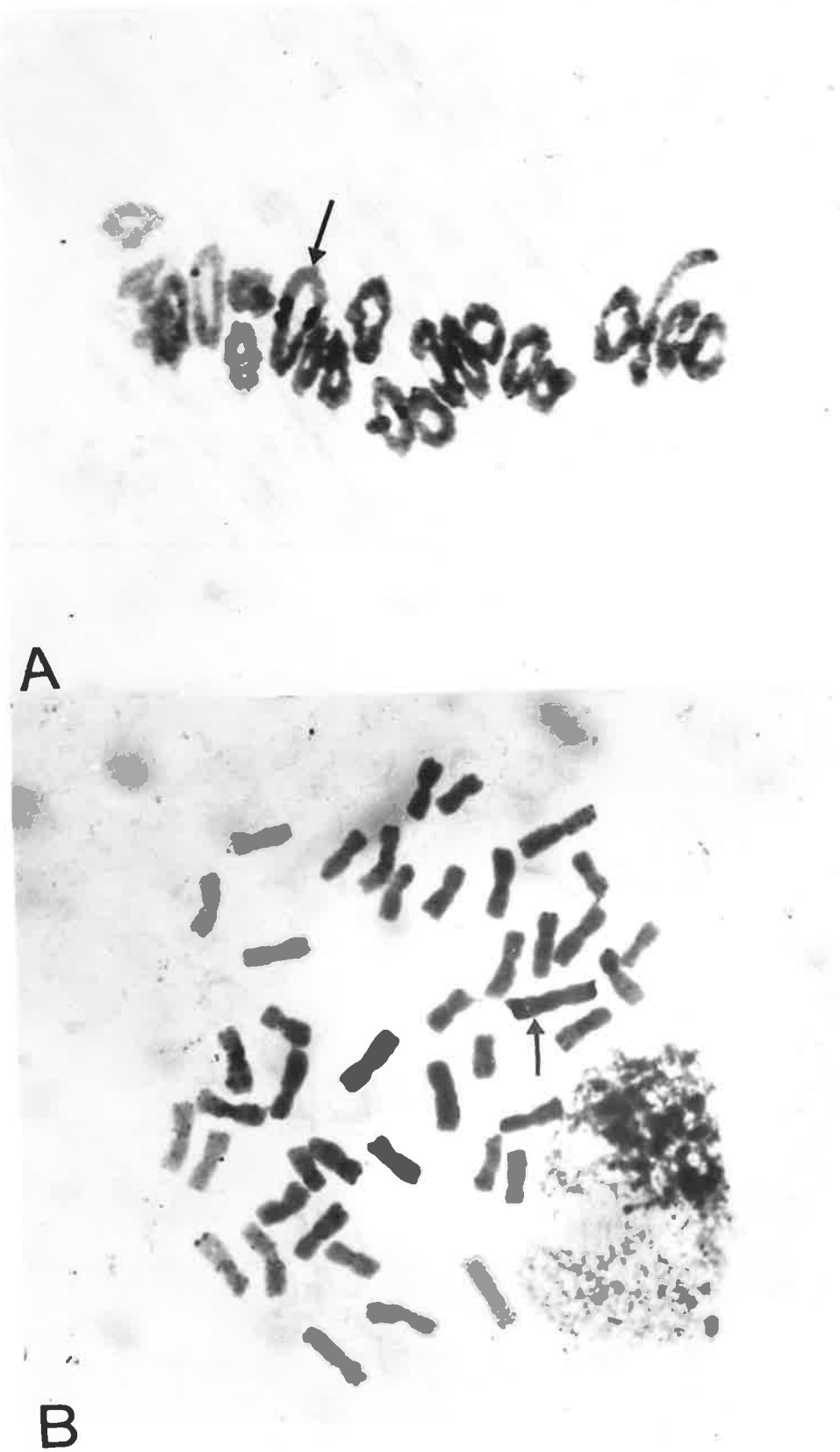
Seven euploid (Cornerstone) and two monotelosomic 4RL addition plants were grown under controlled-environment conditions. All nine plants were vigorous and each of those plants produced several tillers but none of them was male fertile (Table 4.20). Observations on the pmc's of those two 4RL addition plants confirmed the presence of the 4RL telosome.

Hence, the 4RL telosome does not possess any gene(s) for male fertility which can compensate for the male-sterility of Cornerstone.

FIGURE 4.7A-B: C-banded meiotic chromosome complement of a disomic 4R addition plant and C-banded mitotic chromosome complement of a monosomic 4R addition plant.

A. C-banded meiotic chromosome complement of a disomic 4R addition plant showing twenty one wheat bivalents (unbanded) and a ring bivalent (banded) of chromosome 4R (Arrow). X 1260

B. C-banded mitotic chromosome complement of a monosomic 4R addition plant. Chromosome 4R of South Australian rye is shown (Arrow). X 1227


A
B

Also, 4RL does not possess any deleterious gene(s) which has an adverse effect on the plant vigour.

Two monotelosomic and one monoisosomic 4RS addition plants were planted in the glass house in the last week of August 1981 and began heading in late spring (October). The majority of the anthers of the first few tillers in those plants failed to dehisce properly. A minimum of 2% and a maximum of 8% seed set was observed in the few early tillers of each plant. However, the fertility was much higher in the latter tillers and this was presumed to be correlated with an increase in temperature inside the glass house. The average fertility of four late tillers from one monotelosomic 4RS addition plant and eight late tillers from the other one was 57% and 80%, respectively. The monoisosomic 4RS addition plant displayed a similar pattern of fertility compensation. These observations led to the supposition that the fertility of monotelosomic and monoisosomic 4RS addition plants is greatly influenced by the environment.

To check this idea, a further two monosomic 4R, two monotelosomic 4RS and one monoisosomic 4RL addition plants were grown under controlled-environment conditions. However, all five plants displayed some degree of pistillody and all plants were male-sterile (see Section 4.5.6, Table 4.20). These pistilloid plants started heading in early summer.

Subsequently, two monosomic 4R addition plants, one from a parent exhibiting good fertility, and the other from a parent exhibiting poor fertility, and three monotelosomic 4RS addition plants were grown under controlled-environment conditions. The two monosomic 4R addition plants displayed fertilities of 60% and 26%, respectively (Table 4.20). The three monotelosomic 4RS addition plants showed fertilities ranging

TABLE 4.18. Fertility of two monosomic 4R addition plants grown in the glasshouse during Spring. These two plants have been selected to show maximum and minimum fertility.

Plant No. 1

Spike No.	Total No. of florets/spike	Total No. of seeds/spike	Percentage of seed set/spike
1	20	8	40.0
2	20	15	75.0
3	20	8	40.0
4	22	15	68.1
5	20	14	70.0
6	22	16	72.7
7	18	11	61.1
Total	142	87	61.2

Plant No. 2

1	18	6	33.3
2	20	8	40.0
3	22	9	40.9
4	26	9	34.6
5	24	8	33.3
6	20	6	30.0
7	18	6	33.3
Total	148	52	35.1

from 84% to 32% (Table 4.20). A wide range of variation in the fertility of tillers of each of those plants was also observed (Table 4.19). The anthers in the plant which displayed only 32% fertility occasionally failed to dehisce properly.

A group of three monosomic 4R and ten monotelosomic 4RS addition plants were planted in the glass house, in summer (mid February) 1982 and flowered in winter. One of the monosomic 4R addition plants displayed pistillody and as a result the plant was sterile. The fertility in the remaining two monosomic 4R addition plants was 17% and 10% respectively (Table 4.20). One of the monotelosomic 4RS addition plants lost the telosome and as a result the plant was male-sterile. A wide range of variation in the fertility of the remaining nine monotelosomic 4RS addition plants was observed in the glass house during winter 1982. Again, the first few tillers of each plant displayed extremely low fertility. The fertility of each plant increased as the temperature inside the glass house increased (Fig. 4.8). The fertility in those plants ranged from 80% to 7% (Table 4.20).

4.6.4 Fertility of monoisosomic and diisosomic 4RS addition plants

Three monoisosomic 4RS addition plants were grown in the glass-house during winter. These plants were isolated from the progeny of the first monoisosomic 4RS addition plant (which displayed variable fertility during spring 1981). These plants also showed very low fertility in their first few tillers and high fertility in the late tillers. The average fertility of those plants ranged from 34% to 17% (Table 4.20).

A single diisosomic 4RS addition plant displayed 55% fertility when grown under controlled-environment conditions. Following the

TABLE 4.19. Fertility of two monotelosomic 4RS addition plants grown under controlled environment conditions. These two plants have been selected to show maximum and minimum fertility.

Plant No. 1

Spike No.	Total No. of florets/spike	Total No. of seeds/spike	Percentage of seed set/spike
1	18	13	72.2
2	18	16	88.9
3	22	18	81.8
4	18	16	88.8
5	18	16	88.8
6	20	16	80.0
7	20	19	95.0
8	22	17	73.3
9	18	16	88.8
Total	174	147	84.4

Plant No. 2

1	22	8	36.3
2	20	9	45.0
3	26	3	11.0
4	16	8	50.0
5	18	10	55.5
6	20	1	5.0
7	20	12	60.0
8	18	4	22.2
9	20	2	10.0
Total	180	57	31.6

transferring of the plant to the glass house (during winter) the plant produced a few new tillers. The prevailing weather conditions were not favourable for good pollen development but the fertility of those new tillers was 89% (Table 4.20).

Two plants possessing both 4R complete and 4RL were grown separately under two different environmental conditions. The plant which was grown under controlled-environment conditions displayed a fertility of 29% and the other plant grown in the glass house during winter displayed only 4% fertility (Table 4.20).

4.6.5 Pistillody in 4R derivatives and somatic instability of the telosomes

Plants of different chromotypes such as monosomic 4R, monotelosomic 4RS and monoisosomic 4RL addition plants showed pistillody to varying degrees when grown under controlled-environment conditions and in the glass house during winter. In extreme cases the three stamens turned into feathery stigma like structures. In other cases, only the tips of the anthers turned into stigma like structures. None of these pistilloid plants were fertile (Table 4.20). Observations on both mitotic and meiotic cells in those pistilloid plants indicate that there was no loss either in the wheat or the rye chromosomes.

A number of monotelosomic 4RS addition plants were grown under different environmental conditions. Except one, all the plants were found to possess the telosome in pmc's.

4.6.6 Seed shrivelling

The morphology of the seeds of monosomic 4R addition lines and their fertile derivatives were examined. The seeds on the spikes of all

fertile derivatives were examined. The seeds on the spikes of all fertile derivatives of 4R except 4RL grew well up to approximately 18 to 21 days after anthesis at which time seed shrivelling began and continued until maturity. The developing seeds lost their normal plumpness and uniformity. The greatest seed shrivelling was observed in the self-pollinated seeds of the disomic 4R addition plants, even when grown under controlled-environment conditions. In some instances at maturity it was difficult to decide whether the florets were male sterile or whether fertilization had occurred followed by early seed shrivelling.

4.6.7 Polyembryony (Multiple seedlings)

Among the germinated self-pollinated seeds of monotelosomic 4RS addition plants, one seed produced a twin of equal size (Fig. 4.8). This led to further investigation of this abnormality. In a total of sixty-three self-pollinated seeds from two monotelosomic 4RS addition plants which were germinated, eight seeds each produced a single coleoptile enclosing two seedlings (Fig. 4.8). The twin seedlings produced plants with similar phenotypes. Another seed produced three independent coleoptiles. The third coleoptile of the triplet was comparatively small and very slow growing. The third seedling of this triplet died after planting in a pot in the glass house. A few duplets were examined cytologically at meiosis. All were euploids and male sterile.

TABLE 4.20. Summary of fertility of addition lines of 4R and its misdivision products grown under controlled-environment conditions and in the glass house during Spring and Winter. (Includes data from Tables 4.15, 4.16, 4.18 and 4.19.)

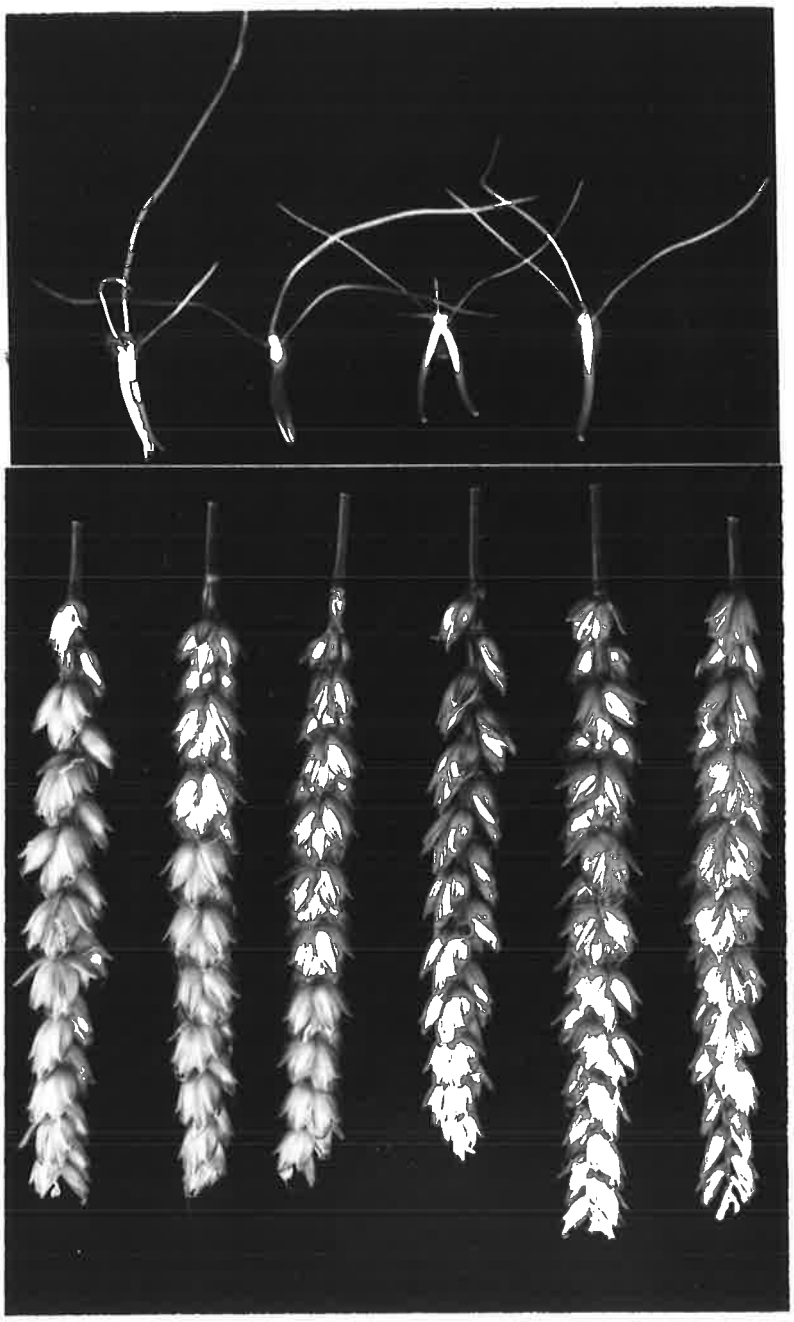
Chromotype	Percentage of seed set on each plant					
	Controlled-environment conditions		glass house			
			Spring		Winter	
	Individual plants	Mean(% of Control)	Individual plants	Mean(% of Control)	Individual plants	Mean
Euploid Chinese Spring	98, 98, 98	100	96, 94, 94	100		
Cornerstone plus						
4R (disomic)	67(A),45(B)	57				
4R	60, 49, 26, P, P	28	61, 37, 41, 35	46	17, 10, P	9
4RL	0, 0					
4RL (iso)	P					
4R + 4RL	29				4	
4RS	84, 80, 32, P, P	40			80, 55, 26, 22, 12, 11, 10, 8, 7	27
4RS (iso)					34, 21, 17	24
4RS (diiso)	55#				89#	

Abbreviations used: P = nil seed set due to pistillody; A = before seed shrivelling; B = after the completion of seed shrivelling; # = initially the plant was grown under controlled-environment conditions but later was shifted to the glasshouse (during Winter); iso = isochromosome of short or long arm; diiso = two isochromosomes of the same arm.

FIGURE 4.8: Seed setting in different tillers and emergence of multiple seedlings following self-pollination of monotelosomic 4RS addition plants.

Top row - Spikes of a monotelosomic 4RS addition plant arranged according to the age of the tiller ranging from the earliest tiller (extreme left) to the last (extreme right). X 0.8

Bottom row - Emergence of mulitple seedlings from a single seed. Normal seedlings (extreme left) and various duplets (towards right). X 1



DISCUSSION

OUTLINE:

The production of F_1 hybrids between Cornerstone and South Australian rye and the subsequent isolation of euoctoploid triticale assembled the chromosome complement of hexaploid wheat and diploid rye. The isolation of heptaploid (Bc_1) plants resulted in the addition of the haploid chromosome complement of the rye genome to Cornerstone. The Bc_2 population included plants having from zero to five and a half rye chromosome(s) added to Cornerstone.

4.7 Crossability

The initial cross between Cornerstone (in Transfed background) and South Australian rye resulted in very poor seed set, possibly due to dominant genes for non-crossability Kr_1Kr_1 Kr_2Kr_2 which were detected in the variety Holdfast (Riley and Chapman, 1967). Chinese Spring has the recessive alleles for crossability, hence it is readily crossable to alien species. The transfer of the male-sterility of Cornerstone to a partially Chinese Spring background and subsequent hybridization with South Australian rye resulted in higher seed set (Table 4.1A). This result can be compared with the result of Backhouse (1916), Leighty and Sando (1928), Riley and Chapman (1967) and Snape *et al.* (1979). These workers obtained 80%, 90.5%, 74.3% and 88.2% seed set, respectively, in crosses between Chinese Spring and *Secale cereale*.

4.7.1 Chromosome complements of Cornerstone octoploid triticale

The Cornerstone euoctoploid triticale had the expected chromosome content of 2n = 8x = 56. The chromosome constitution of 53 F_2 plants and 20 F_3 plants derived from these euoctoploids was determined and the frequency of euploid progeny was 13 (24.5%) and 2 (10%), respectively. The remaining plants of the populations were aneuploid.

In the F_2 population the frequency of hypoploidy (69.8%) was much higher than the frequency of hyperploidy (5.66%). Further, occurrence of secondary aneuploidy was also observed within the same population.

Aneuploidy in octoploid triticale is not uncommon. For example, Krolow (1962) cited in Tsuchiya (1974) found that only 16.7% of the analysed population was euoctoploid and the remaining population was aneuploid. He found the chromosome numbers of 306 plants ranged from 41 to 58. Weimarck (1973) observed 31% and 50% euploid plants in five primary and eight selected strains, respectively.

The frequency of euploids and anouploids in the F_2 population (Table 4.2) of Cornerstone octoploid triticale was intermediate to the frequencies observed in these two studies referred to above.

Reports in the literature on the chromosome constitution of octoploid triticales using the C-banding technique are extremely limited and poor in quality. Weimarck (1974) studied the chromosome complement of a strain of octoploid triticale by C-banding. She found elimination of rye and wheat chromosomes in the ratio of 1:3 approximately in a population of twenty-one plants. However, she did not report any euploid, hyperploid or secondary aneuploid types. However, in the present work, C-banding analysis of an F_3 population of Cornerstone octoploid triticale has demonstrated the presence of all of these types of plants plus some showing secondary aneuploidy and modification to the structure of rye chromosomes (Table 4.3).

The instability of the normal chromosome complement in octoploid triticale and its tendency to revert to hexaploid wheat is due to the production of abnormal gametes resulting from irregular meiosis. Meiotic abnormality in octoploid triticale includes the

formation of univalents and occasionally multivalents which result in further abnormalities such as laggards at anaphase I. The variation in the numbers of univalents in pmc's has been studied by Müntzing (1939), Riley and Chapman (1957), Riley and Miller (1970) and Weimarck (1973). The number of univalents varied from 0-14 per pmc in most of the strains studied. However, Weimarck (loc. cit) recorded up to twenty univalents in some pmc's indicating that wheat chromosomes were involved in the formation of univalents.

The frequency of complete chromosome pairing in the pmc's in the octoploid triticale is variable. The reported minimum and maximum frequency of 28 bivalents was 8.6% and 61.1% respectively (see Tsuchiya, 1974 for review). The chromosome pairing behaviour in three F_2 Cornerstone euoctoploid triticale has been estimated (Table 4.4) and these are within the range of values reported by other workers.

4.7.2 Fertility of Cornerstone octoploid triticale

The compensation for the male-sterility of Cornerstone by the whole genome of rye was an important early finding in the present study. The fertility of octoploid triticale is determined by several factors including the parental genotype, the chromosome number, meiotic stability and generation of selfing. The fertility levels in euoctoploid triticale involving Cornerstone and South Australian rye and Sears' euoctoploid triticale involving Chinese Spring and Imperial rye is similar (Table 4.5). Hence, the genome of South Australian rye at least partially compensates for the male-sterility of Cornerstone wheat.

4.8 Compensation for the male-sterility of Cornerstone by chromosomes 2R and 4R of South Australian rye

The monosomic additions of chromosomes 2R and 4R partially compensated for the male-sterility of Cornerstone when grown under controlled-environment conditions (Tables 4.8, 4.16) indicating that two different rye chromosomes can compensate individually for the missing male fertility gene(s) of the α arm of chromosome 4A of hexaploid wheat.

It is widely accepted that the members of the sub-tribe *Triticinae* have evolved from a common progenitor and these species have, to a large extent, conserved the integrity of their chromosomal structure and gene content throughout the process of evolution (see Hart, 1979).

It was, therefore, expected that chromosome 4R might carry a male fertility gene(s) which could compensate for the male-sterility of Cornerstone. Both chromosome 4A of hexaploid wheat and 4R of South Australian rye have conserved the structural genes for wheat and rye alcohol dehydrogenase, respectively (Hart, 1973; Tang and Hart, 1975) and a male fertility gene(s) (from this study) throughout the process of evolution.

The compensation for the male-sterility of Cornerstone by chromosome 2R of South Australian rye is a new finding.

4.8.1 Breeding behaviour of the monosomic 2R addition line

The monosome 2R misdivided (Darlington, 1939) frequently and as a result 33% of the derivatives involved misdivision products (Table 4.9). The monosome 5A of Chinese Spring misdivides 39.7% at first telophase of meiosis (Sears, 1952a). Morris *et al.* (1969)

reported the misdivision of 5A, 5B and 5D in different varieties and found that the rate of misdivision ranged from 2.5% to 23.6%. Chromosome 5D misdivided always with a lower frequency than chromosomes 5A and 5B. Anderson and Driscoll (1967) observed 13% misdivision of an *Agropyron elongatum* (Host) Beauv chromosome, when present as a monosomic substitution for wheat chromosome 6D.

Thus, the misdivision rate of monosome 2R although high, is within the range of published figures.

4.8.2 Analysis of the male sterility compensation by chromosome 2R

The male sterility compensation in Cornerstone by entire chromosome 2R of South Australian rye tested in two different environments is incomplete compared to the fertility level of normal Chinese Spring (Section 4.5.2), whereas the short arm of 2R is significantly different and gives almost complete compensation for this male sterility (Section 4.5.4). These two chromotypes differ by the presence of the long arm in the former chromotype and its absence in the latter types. Hence, it is apparent that the long arm of 2R is responsible for the reduction of male sterility compensation in the monosomic 2R addition plants. It is concluded that the long arm of 2R possesses one or more factors which causes reduction in male sterility compensation in Cornerstone by chromosome 2R and it has been designated as an *antifertility* factor(s) (see Hossain and Driscoll, 1983).

Phenotypically, the monosomic 2R addition plants are not vigorous and the disomic 2R addition plants are extremely vigorous. The monosomic and disomic modified 2R addition plants had phenotypes similar to those involving the entire 2R addition. Hence, the *antifertility* factor(s) may be associated with the factor(s) for general poor vigour. These factors must be located between the centromere and the interstitial C-band of the long arm.

The alien addition lines of the homoeologous group 2 display a similar plant phenotype (Riley and Chapman, 1958; Sears, 1968) to that of the disomic addition lines of 2R of South Australian rye. Dvořák and Knott (1974) observed that the general poor vigour of *Agropyron elongatum* chromosome 2E addition lines is controlled by the long arm of this chromosome. Therefore, the results from this study confirm that the gene contents of the long arm of 2R and 2E are similar in function.

4.8.3 Breeding behaviour of 2RS telosome additions

The chromosome 2R and the modified chromosome 2R disomic and monosomic addition plants were considered not to be very promising components for the XYZ system. It was thought that the 2RS telosome might be more suitable for this system. This led to an investigation of the breeding behaviour of monotelosomic and ditelosomic 2RS addition plants and the pollen transmission rate of the 2RS telosome from Y plants to Z plants.

The retention of 2RS as a telosome or an isochromosome in the progeny resulting from self-pollination was 38.5% (32.5 + 5.9) (Table 4.13) and the rate of 2RS transmission through the pollen was approximately 10% (Section 4.5.9). According to Sears (1954), the average retention of a wheat monosome in the egg is 25%, therefore, the retention of 2RS in the eggs appears to be similar to that of a wheat monosome.

The 101 progeny from self-pollinated monotelosomic 2RS addition plants were cytologically examined, using the C-banding technique, and selfing resulted in the production of various chromotypes (Table 4.13). Further, 919 progeny from four different families were

tested in the field for their breeding behaviour and significantly different ($P < .001$) results were obtained with the different families.

The ditelosomic 2RS addition plants were unstable and exhibited rapid addition decay (Section 4.5.7). This was presumably due to the telocentric nature of the chromosome.

4.9 Analysis of male sterility compensation by chromosome 4R in different environments

The main feature of the male sterility compensation by chromosome 4R was the variability of this compensation between environments and between plants within environment (Table 4.20). The average fertility of five monosomic 4R addition plants grown under controlled-environment conditions was 28% and the average fertility of four 4R addition plants grown in the glass house during Spring was approximately 46% (Table 4.20). Two plants grown under controlled-environment conditions were sterile due to pistillody and as a result the average fertility of monosomic 4R addition plants grown under controlled-environment conditions was less than the fertility of glass house grown plants. The average fertility of three monosomic 4R addition plants grown in the glass house during Winter was the lowest (Table 4.20). The average fertility of two disomic 4R addition plants grown under controlled-environment conditions was 57% (Table 4.20). The fertility of individual plants grown under three different environmental conditions varied to a great extent among themselves. Therefore, the fertility of either monosomic or disomic 4R addition plants varies with changes in environment and is much reduced compared to the fertility of Chinese Spring.

The compensation for the male-sterility of Cornerstone by chromosome 4R of South Australian rye confirms the report of Koller and Zeller (1976). They obtained 4% fertility in 4R (4A) and 19% in 4RS (4A) substitution lines. The higher fertility compensation by chromosome 4R to Cornerstone is probably due to the greater loss of chromosome 4A chromatin in the substitution lines. Chromosome 4E of *Agropyron elongatum* was expected to substitute for chromosome 4A of hexaploid wheat but it can only compensate for the plant vigour but not for the male fertility gene(s) of 4Aα (Dvořák, 1980). Dvořák (loc. cit) suggested that chromosome 4E of *Agropyron elongatum* might have lost its male fertility gene(s) during the process of evolution. Chromosome 5R of *Secale cereale*, belonging to a different homoeologous group, is reported to substitute for chromosome 4A in terms of vigour and fertility in a wheat line W70a86 (Blaukorn) (Zeller and Baier, 1973). This could mean that chromosome 5R of wheat line W70a86 is different from 5R of South Australian rye or that the male fertility gene(s) of chromosome 4Aα has been translocated to another chromosome in Blaukorn.

Analysis of male sterility compensation by 4RS derivatives under different environmental conditions

The average fertility of five 4RS addition plants (including two sterile plants due to pistillody) was approximately 40% and the average fertility of nine 4RS addition plants grown in the glass house during Winter was approximately 27% (Table 4.20). Thus, just as with the entire 4R addition, the male sterility compensation by 4RS is incomplete and varies in different environments. Also, with 4RS, male sterility compensation was influenced by the age of tillers; the older tillers which developed at higher temperature displayed higher fertility. A dramatic increase in fertility was observed in the 4RS

addition plants grown under glass house conditions during Spring as compared to Winter. Increased doses of 4RS as in the iso and diiso additions did not give greater compensation for the male-sterility of Cornerstone (Table 4.20).

Therefore, the male sterility compensation by 4RS or its derivatives is variable, inconsistent and partial. The long arm of chromosome 4R does not possess any gene(s) which has a marked effect on plant vigour since the 4R addition plants and all its derivatives including 4RL are vigorous. However, the long arm appears to contribute negatively to the compensation of male-sterility in Cornerstone. No monosomic or disomic 4R addition plants were observed to give more than 67% compensation for the male-sterility of Cornerstone, whereas, some of the 4RS addition plants grown under controlled-environment conditions or in the glass house in Winter, gave more than 80% compensation. The late tillers of 4RS addition plants grown in Spring have also demonstrated high fertility. Koller and Zeller (1976) observed the negative effect of the long arm of 4R in their substitution lines since the substitution CRS (= 4RS) for 4A gave higher (19%) compensation than the CR (= 4R) substitution (4%).

4.9.1. Pistillody in monosomic 4R addition plants and their derivatives

The occurrence of pistillody was not restricted to any specific chromotype or environment. Pistillody caused sterility and as a result the average fertility in the 4R derivatives involved in this study was reduced. Sears (1954) reported that Chinese Spring nullisomic 6B or 7A usually showed considerable transformation of stamens to pistils in some of the florets and these were sterile. Kihara and Tsunewaki (1961) observed pistillody when the nucleus of

T. durum was added to the cytoplasm of *Ae. caudata*. Fisher (1972) reported that transformation of stamens to pistils was induced in Opal Spring wheat (*T. aestivum*) by shifting plants from 16 hours to 10 hours photoperiod when stamen primordia were visible on the first floret of the most advanced spikelet of the main shoot. Islam *et al.* (1975) observed pistillody due to incompatibility between the nucleus of hexaploid wheat and the cytoplasm of barley. They suggested that the environment had an effect on pistillody. Pistillody due to the addition of *Ae. sharonensis* chromosome 4SL to Chinese Spring has been reported (Miller *et al.* 1982).

Pistillody in this study has been found in monosomic 4R, monotelosomic 4RS and monoisosomic 4RL addition plants grown under controlled-environment conditions and in the glass house during Winter. No loss of any wheat chromosome has been observed in this material. The pistillody in these chromotypes appears to be due to an interaction between genotype and environment, the genotype being provided by 4RS or 4RL.

4.9.2 Seed shrivelling and polyembryony in the 4RS addition plants and derivatives

All of the 4R additions and derivatives (4RL was not observed because of male sterility) showed seed shrivelling. Maximum seed shrivelling occurred with the disomic 4R addition plants. It is assumed that the genes for seed shrivelling are located on both arms of chromosome 4R as seeds of the disomic 4R were more shrivelled than those of the 4RS isochromosome addition. This result confirms the reports of Darvey (1973) and Köller and Zeller (1976) on seed shrivelling.

Multiple seedlings emerged from 14.2% of the self-pollinated seeds of monotelosomic 4RS addition plants. No other tested chromotype was found to produce more than one seedling when both short and long arms were present. Apparently, there is a gene(s) on the short arm of 4R which causes a physiological upset resulting in polyembryony and 4RL bears a gene(s) that contradicts this.

4.10 General discussion

The members of the sub-tribe *Triticinae* have evolved from a common progenitor and have retained the basic chromosome number of n = 7. The evolutionary pathways of divergence and convergence resulted in the production of amphiploids (polyploids) in which the gene content of the ancestral parent is duplicated and preserved. The best example is hexaploid wheat (*T. aestivum*). Similar gene content among the three genomes of hexaploid wheat has been experimentally proved by nullisomic-tetrasomic compensation analysis (Sears, 1966). Diploid rye is expected to possess and to preserve a similar gene content to that of hexaploid wheat. The similarity of gene content in rye has been proved experimentally by various workers using different wheat-rye substitution lines and the homoeology between wheat and rye chromosomes has been established in the first approximation.

The diploid rye genome can at least partially compensate for the male-sterility of Cornerstone. The fertility of Cornerstone euoctoploid triticale is similar to the fertility of Sears' euoctoploid triticale. The effect of the deleterious gene(s) present on the long arm of 2R is not expressed in either octoploid triticales or heptaploid (Bc_1) because of the interaction with a gene(s) present on another rye chromosome(s).

The Cornerstone male-sterility mutant, *msIc*, involves 4A of hexaploid wheat, therefore, it was expected that 4R might compensate for the male-sterility of this mutant. However, chromosome 2R of South Australian rye can also compensate for this male sterility. This observation is not surprising when one considers the unusual characteristics of chromosome 4A (Driscoll, 1981) and the translocations in *Secale cereale* cv. Imperial relative to *S. montanum* Guss (Koller and Zeller, 1976).

The male sterility compensating genes are present on the short arms of both chromosomes 2R and 4R. The gene(s) on the short arm of 2R is well buffered against the variation of environmental conditions. It compensates very well and the fertility of 2RS addition plants is almost equal to that of normal wheat. This demonstrates that the male fertility gene(s) on 2RS is similar to the deleted male fertility gene(s) on the α arm of Cornerstone 4A. The male fertility gene(s) on the short arm of chromosome 4R is very sensitive to the variation of environment, mainly temperature. The male fertility gene(s) on 4RS is neither similar to the gene(s) on 2RS nor to the gene(s) referred to on the α arm of chromosome 4A.

4.11 Suitability of 2R and 4R addition lines as X and Y components for the XYZ system of producing hybrid wheat

The three disomic addition lines involving entire 2R, modified 2R and entire 4R can be considered as X lines for the proposed XYZ system. The X lines involving entire 2R and modified 2R are not acceptable for several reasons. The disomic addition lines of both chromotypes are slow growing, extremely poor in vigour, late in maturity, produce limited tillers and moreover are not fully fertile. A third possible candidate is the disomic addition of chromosome 4R.

Phenotypically, the X line involving this chromosome is vigorous but it has disadvantages including incomplete male sterility compensation and severe seed shrivelling. These two factors eliminate the disomic 4R addition line as an acceptable X line.

Isolation of ditelosomic stocks of 4RS have not yet been made but the X line involving 4RS can be judged as unacceptable on the basis of observations on additions of isochromosomes and diisochromosomes of 4RS. Seed shrivelling, incomplete fertility compensation and sensitivity to variation in environment are the major problems with these lines.

The remaining alternative is the utilization of ditelosomic and monotelosomic additions of 2RS as X and Y lines, respectively. Phenotypically, these lines are almost normal in all respects and their compensation for male sterility in the tested environments is excellent. The morphology of the seeds in both lines is normal. However, these lines are not free from some disadvantages, these being, higher rate of addition decay and higher rate of 2RS transmission through male gametes from the Y line to the Z line. However, these two disadvantages can probably be nullified by fusing a neutral alien arm to 2RS by the process of centric fusion (Sears, 1972).

CHAPTER 5. TRANSFER OF THE CORNERSTONE MALE-STERILITY MUTANT FROM HEXAPLOID WHEAT TO TETRAPLOID WHEAT

INTRODUCTION:

An attempt has been made to transfer the Cornerstone male-sterility mutant from hexaploid wheat to tetraploid wheat to examine its segregation pattern at the tetraploid level. Cornerstone *ms1c* is a recessive nuclear male-sterility mutant (Driscoll, 1977), supposedly due to a terminal deletion of the segment of the α arm of chromosome 4A possessing a male fertility gene(s) (Barlow and Driscoll, 1981). It is possible to transfer this male-sterility mutant from hexaploid wheat to tetraploid wheat because both wheats have in common the A and B genomes. The possible transfer of this mutant from hexaploid wheat to tetraploid wheat has some significance in the production of male-sterile hexaploid triticale by chromosome manipulation.

An attempt could be made to further transfer this male-sterility mutant from tetraploid wheat to diploid wheat to examine its expression at the lowest ploidy level.

MATERIALS AND METHODS: (described in Chapter 3, Section 3.2.2)

5.1 Results

The F_1 seeds from the cross between male-sterile hexaploid wheat and tetraploid wheat, *Triticum durum* and the first back-cross seeds (male-sterile hexaploid wheat x *Triticum durum*) x *T. durum* were provided by Professor C.J. Driscoll. The chromosome complement of the fourteen Bc_1 plants were examined at mitosis and meiosis by the Feulgen method (Chapter 3, Section 3.3.1). The plants numbered 8-14 (Table 5.1) were not retained for further investigation because some

TABLE 5.1. The chromosome complement of the fourteen first backcross plants.

Plant no.	Mitosis	Meiosis
1	30e	$14^{II} + 2^{I}$
2	30e	$14^{II} + 2^{I}$
3	30e	$14^{II} + 1^{I} + 1$(iso)
4	29e	$14^{II} + 1^{I}$
5	29e	$14^{II} + 1^{I}$
6	29e	$14^{II} + 1^{I}$
7	28e	14^{II}
8	36e	-
9	34e	-
10	32e	$IV + 12^{II} + 4^{I}$
11	31e	$13^{II} + 5^{I}$
12	28 + 1 telo	-
13	28e	$13^{II} + 2^{I}$
14	28e	-

NOTE:

e = entire chromosome

Plant No. 8 to 14 were not retained beyond first backcross generation

possessed more than thirty chromosomes and formed multivalents, some were deficient in chromosomes of the A and/or the B genome and some of them died at the seedling stage. Plants numbered 1 to 7 were allowed to self-pollinate and the somatic chromosome numbers of the resultant seeds were determined from root-tip mitosis. A number of seeds from each of the seven families were planted and all of the resultant thirteen seedlings possessed 28 chromosomes. These plants were tested for their fertility (Table 5.2) and all were male fertile except one which was male sterile (from family 4). Meiosis in pmc's from this plant was normal but it produced abortive pollen grains in small anthers that failed to dehisce (Fig. 5.1). Although three bagged heads failed to set any seed, the plant was found to be female fertile when pollinated with normal durum. Further an additional three male sterile plants were isolated from a total of sixty-four plants (family 4). One of the male sterile plants was pollinated with normal durum and subsequently fifteen Bc_2 plants were grown. Meiotic observations were made on six of those Bc_2 plants and they showed fourteen normal bivalents, proving that all the A and B genome chromosomes had been retained and that chromosomes of the A and B genome had not been replaced by chromosomes of the D genome.

Further, Bc_2F_2 offspring from two Bc_2 plants were scored for fertility and in one family three out of seventy-nine progeny and in another family one out of nineteen were male sterile. Except for their male sterility these four plants appeared to be phenotypically normal. A total of one hundred and seventy-eight plants involving three families (one Bc_1F_2 and two Bc_2F_2) were examined and eight male sterile plants were isolated (Table 5.3).

The segregation pattern of Cornerstone male sterility at the hexaploid level varies from genotype to genotype (Driscoll, unpublished).

The segregation pattern of this mutant in a mixed genotype was fifty seven fertiles to eleven steriles at the hexaploid level (Table 5.3).

A Fisher's Exact test has been performed on the segregation patterns of this mutant at the hexaploid and tetraploid levels (Table 5.3). The segregation pattern of this mutant at the tetraploid level and the hexaploid level are significantly different ($.001 < P < .01$).

5.2 Discussion

The Cornerstone mutant is thought to have a deletion on the terminal end of the α arm of chromosome 4A of hexaploid wheat (Barlow and Driscoll, 1981). The male sterility of this mutant has been transferred successfully to tetraploid wheat by back crossing. The ratio of fertile plants to sterile plants at the tetraploid level is much higher than at the hexaploid level. The hexaploid wheat differs from tetraploid wheat by its additional possession of the D genome, which perhaps, provides a buffer, which in effect reduces selection against the mutant gametes at the hexaploid level.

It is not known whether the supposed deleted segment of 4Aα has lost any gene(s) other than the male fertility gene(s). Phenotypically, the male sterile tetraploid wheat does not show any evidence of the loss of a gene(s) other than the gene(s) for male fertility. Any postulated loss of a gene(s) in addition to the male fertility gene(s) may be possible to detect at the diploid level since diploid wheat possesses only one genome which may not provide a buffer for the tolerance of this mutant.

An attempt has been made to transfer the Cornerstone male sterility mutant from tetraploid wheat to diploid wheat (See Chapter 6).

TABLE 5.2. The number of male fertile and male sterile plants in seven selected BC_1F_2 and two BC_2F_2 families.

Progeny	Family No.	Fertile	Sterile
BC_1F_2	1	13	0
	2	12	0
	3	29	0
	4	76	4
	5	15	0
	6	13	0
	7	15	0
BC_2F_2	1	76	3
	2	18	1

TABLE 5.3. Segregation pattern of the Cornerstone male sterility mutant at the hexaploid and tetraploid levels and homogeneity test.

Level of ploidy	Fertile	Sterile	Total	.00 < P < .01**
6X	57	11	68	—
4X	170	8	178	

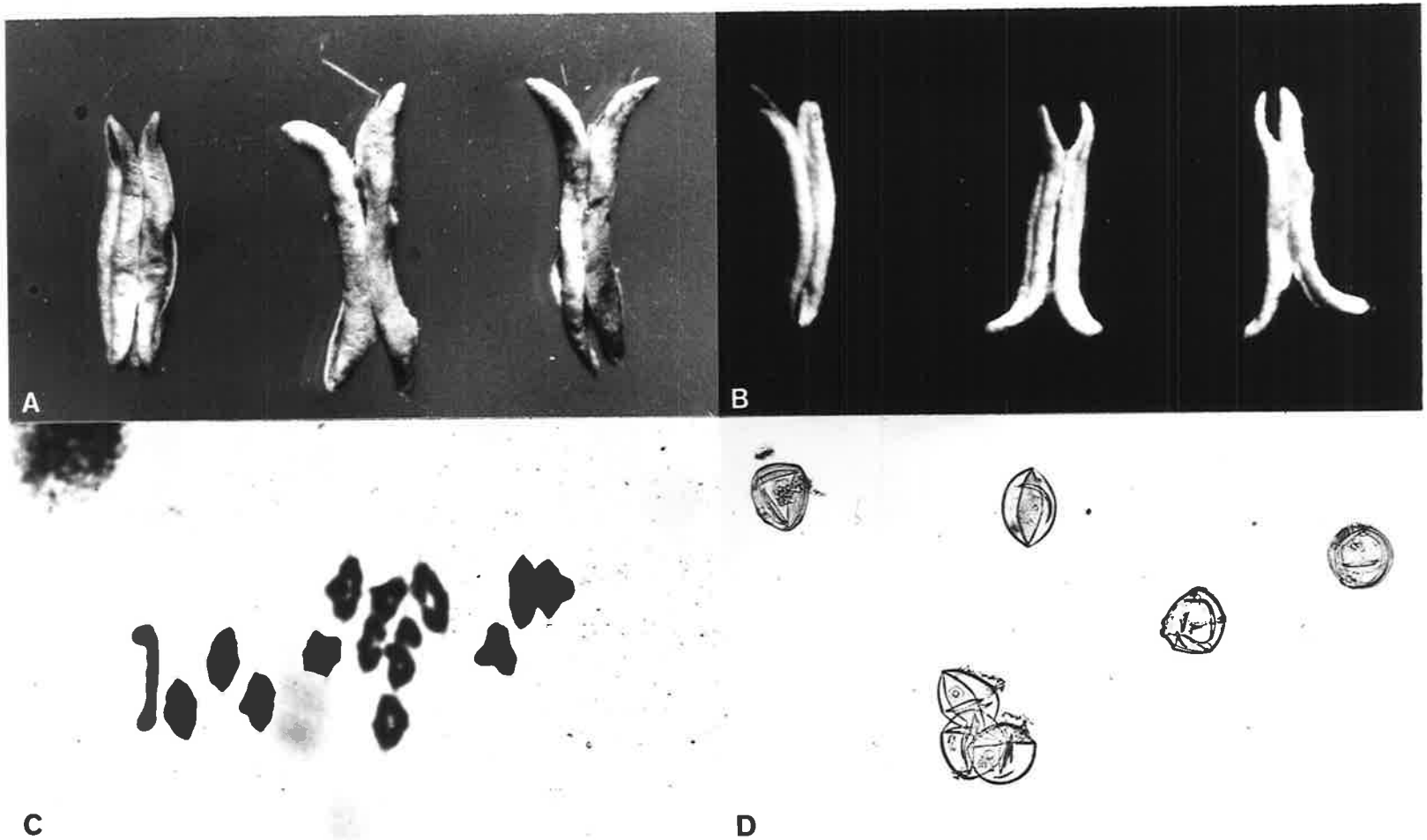
FIGURE 5.1A-D: Anthers of normal and mutant tetraploid wheat plants, first metaphase of meiosis and the abortive pollen grains of mutant tetraploid wheat.

A. Three anthers of a normal tetraploid wheat. X 11.2

B. Three anthers of a mutant tetraploid wheat. X 8.5

C. First metaphase of meiosis showing fourteen normal bivalents in pmc's of a mutant tetraploid wheat. X 950

D. Abortive pollen grains of a mutant tetraploid wheat. X 216


A
B
C
D

CHAPTER 6. THE ATTEMPTED TRANSFER OF THE CORNERSTONE MALE STERILITY MUTANT FROM TETRAPLOID WHEAT TO DIPLOID WHEAT

INTRODUCTION:

The aim of transferring the Cornerstone male sterility mutant from tetraploid wheat to diploid wheat was to examine the tolerance and breeding behaviour of the mutant at the diploid level. It is assumed that the Cornerstone male sterility mutant could be transferred from tetraploid wheat to diploid wheat because both wheats have in common the A genome.

MATERIALS AND METHODS: (discussed in Chapter 3, Section 3.2.4)

6.1 Results

Twenty-eight F_1 hybrid (2n = 21, AAB) seeds involving the cross between male sterile tetraploid wheat and diploid wheat *T. monococcum* L. (WARI Accession No. 4777) were obtained after pollinating one hundred and fifty spikelets of male sterile tetraploid wheat. The chromosome numbers of eight F_1 triploid hybrid seedlings were determined from their root-tip cells. Each F_1 triploid hybrid possessed 21 chromosomes. The hybrid plants were grown in the glass house during Spring and examined for their fertility.

6.2 The chromosome pairing behaviour in F_1 triploid hybrids

Both Feulgen staining and N-banding techniques (Chapter 3, Sections 3.3.2, 3.3.5) were employed to study the pairing behaviour of chromosomes at first metaphase of meiosis in pmc's. The chromosomes of the F_1 triploid hybrids formed six bivalents and nine univalents at the first metaphase of meiosis in most of the pmc's (Fig. 6.1A). The N-banding technique facilitated the identification of the chromosomes

which remained as univalents at first metaphase. Seven chromosomes of the B genome plus chromosome 4A of tetraploid wheat (chromosome 4A of tetraploid wheat has been derived from hexaploid wheat) remained as univalents. Apart from the eight N-banded univalents, one univalent was observed which did not stain with Giemsa N-banding. This univalent is assumed to be the counterpart of chromosome 4A of tetraploid wheat (Fig. 6.1B). However, occasionally, the long arm of 2B has been seen to pair with a chromosome of *T. monococcum* forming an open bivalent. The same arm of 2B has been observed to form an open trivalent. The chromosome(s) involved in pairing with the long arm of 2B was found consistently to be unbanded by N-banding. The F_1 triploids produced abortive pollen grains and as a result they were male sterile.

6.3 First backcross

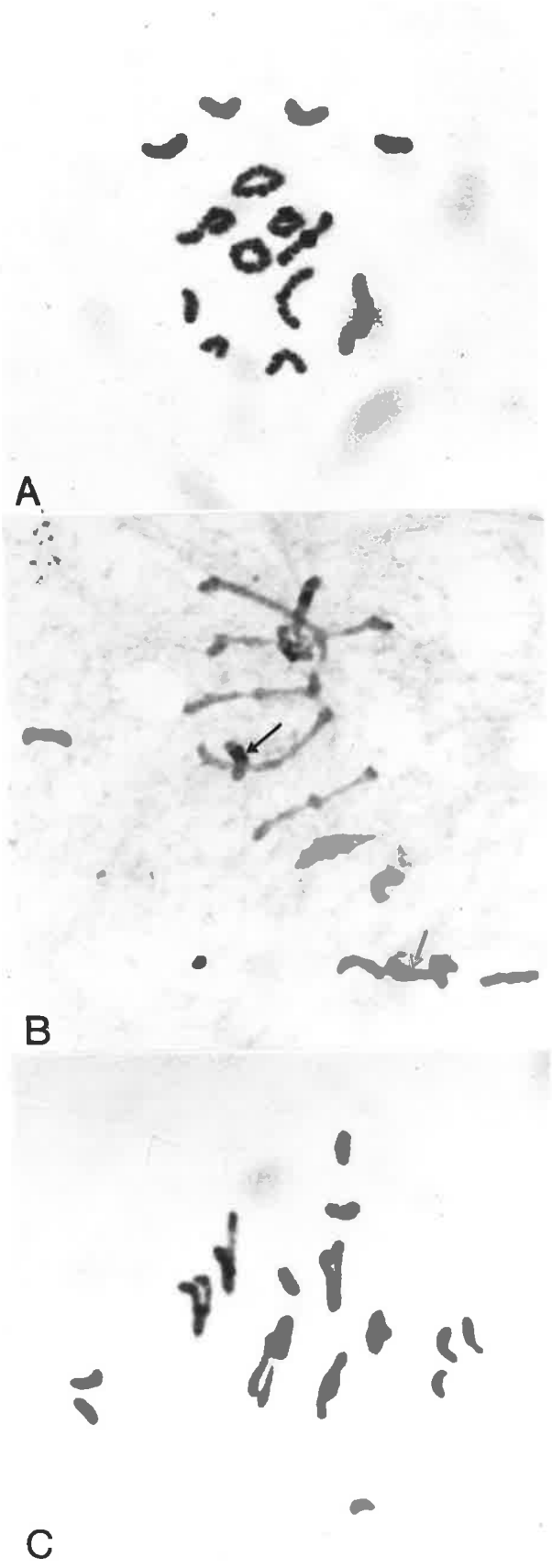
The triploid F_1 plants were pollinated with the pollen of *T. monococcum* in an attempt to obtain Bc_1 plants. Seven hundred and twenty florets of triploid F_1 hybrids were pollinated with *T. monococcum* repeatedly and three only viable Bc_1 seedlings were raised following embryo culture. The three Bc_1 plants were grown under controlled-environment conditions. The chromosome numbers of the three Bc_1 plants were determined at first metaphase of meiosis. Each Bc_1 plant possessed 28 chromosomes and these chromosomes formed trivalents, bivalents and univalents in their pmc's (Fig. 6.1C). All three Bc_1 plants were considered to have come from unreduced eggs of triploid F_1 hybrids. Phenotypically, the Bc_1 plants were vigorous but produced abortive pollen grains. As a result, all the Bc_1 plants were male-sterile. No further backcrossing was carried out with these first backcross plants.

FIGURE 6.1A-C: Meiotic chromosome complement of the F_1 hybrids involving the cross between male sterile durum and *T. monococcum* and the first backcross to *T. monococcum*.

A. Diakinetic stage in the pmc's of the F_1 hybrids involving the cross between male sterile durum and *T. monococcum* showing six bivalents and nine univalents. (Feulgen preparation). X 866

B. N-banded meiotic metaphase chromosome complement of the genotype above showing six bivalents and nine univalents. Seven univalents of the chromosomes of the B genome plus the univalent of chromosome 4A of mutant tetraploid (Big arrow) are N-banded. Chromosome 4 of *T. monococcum* remained as an unbanded univalent (Small arrow). X 866

C. The meiotic metaphase chromosome complement of the first backcrossed plant, (male sterile durum x *T. monococcum*) x *T. monococcum*, showing five trivalents, two bivalents and nine univalents. X 866


A
B
C

6.4 Compensation for the male sterility of Cornerstone by *T. monococcum*

HISTORY:

In the results (Chapter 6, Section 6.1) it has been mentioned that the triploid F_1 hybrid plants possessed AAB = 21 chromosomes. Chromosome 4A of tetraploid wheat did not pair with its counterpart chromosome 4 of *T. monococcum*. The five triploid F_1 hybrid seedlings were treated with colchicine to obtain a higher mitotic index. The N-banding technique was used to band the chromosomes of the haploid B genome in the triploid F_1 hybrids. It was expected that it would be easier to identify all N-banded chromosomes of the B genome as well as chromosomes 4A and 7A since each chromosome of the B genome was present singly. Dr. D.C. Jewell treated the seedlings with colchicine and the treated seedlings were planted in the glasshouse following washing with water. Three plants died at the seedling stage and two plants produced at least thirty effective tillers each. With the exception of two tillers in one of the treated plants, all were sterile. The two spikes of this one plant shed pollen and set seed in all spikelets. This incident suggests that after colchicine treatment, the two tillers emerged from a colchicine-induced doubled sector of the triploid F_1 hybrid plant and as a result they set seeds. Subsequently, two more seedlings raised from similar genotype triploid F_1 hybrids were treated with colchicine and another two seedlings of the same genotype were not treated with colchicine and grown as controls. The colchicine treated F_1 plants produced a number of tillers and some of the tillers produced viable pollen. These tillers produced several F_2 seeds. The untreated plants did not produce any seeds. Further, several F_2 plants were raised from the colchicine-induced F_2 seeds and each plant was found to be completely male fertile.

6.5 Discussion

Since the discovery of *T. monococcum* as a progenitor of the A genome of common wheat (Sax, 1922), the validity of this species as a progenitor has not been questioned. Chapman and Riley (1966) observed that the telocentrics of chromosome 4A cv. Chinese Spring had paired with one of the chromosome of *T. thaoudar*. Feldman (1966) observed that the β arm of chromosome 4A paired in 29.7% of pmc's in hybrids with *T. timopheevi*.

Chapman *et al.* (1976) and Dvořák (1976) observed no pairing homology between chromosome 4 of *T. urartu* and the telocentrics of chromosome 4A of Chinese Spring. The double ditelocentrics of chromosome 4A of Chinese Spring have never been found to be involved in pairing with any chromosome of *T. monococcum, T. aegilopoids* or *T. thaoudar* (K.W. Shepherd p.c.). The observations of Feldman (1966) that the β arm of chromosome 4A of Chinese Spring paired with a chromosome of *T. timopheevi* has been confirmed in this laboratory (K.W. Shepherd p.c.). These observations contrast with those of Chapman and Riley (1966) and subsequently Miller *et al.* (1981) who have clarified the pairing behaviour of telocentrics of hexaploid chromosome 4A with its diploid counterparts. Miller and Reader (1980) recorded that in hybrids between Chinese Spring euploid and diploid wheats, some plants displayed the formation of seven bivalents in their pmc's indicating that chromosome 4A of Chinese Spring might have paired in some plants. However, the seventh pair may involve homoeologous pairing and possibly not involve chromosomes 4 of diploid wheat and 4A of hexaploid wheat. The application of the N-banding technique in this study demonstrates the pairing behaviour of chromosome 4A of hexaploid wheat and diploid wheat. In this study, chromosome 4 of *T. monococcum* and chromosome 4A of tetraploid wheat have never been observed to be

paired. The chromosome 4A of tetraploid wheat was identified by N-banding in pmc's. This chromosome possesses N-bands on each side of the centromere and a faint terminal N-band in the β arm. Peacock *et al.* (1981) used a silver satellite probe to investigate the origin of the A genome. They found no diploid species which possesses a chromosome with the distinctive pattern of sites characteristic of chromosome 4A of common wheat.

The results obtained by Chapman *et al.* (1976) and Dvořák (1976) and this study further confirm that chromosome 4A of tetraploid (also hexaploid) wheat is different from chromosome 4 of diploid wheat. The history of the origin of chromosome 4A of tetraploid and hexaploid wheat is still not known. It has been suggested that the origin of chromosome 4A of tetraploid and hexaploid wheat might have a complex history (Peacock *et al.* 1981).

The attempted transfer of the Cornerstone male-sterility mutant from tetraploid wheat to diploid wheat failed because chromosome 4A of tetraploid wheat does not pair with chromosome 4 of *T. monococcum*, as a result of which, recombination is not possible. This restricted the probable isolation of a male sterile diploid wheat. Another reason for failure in transferring this male sterility mutant from tetraploid wheat to diploid wheat may be due to the differential selection for unreduced female gametes that has been noticed in all the Bc_1 plants in this study. Apart from this, it is doubtful whether this mutant would be tolerable at the diploid level.

CHAPTER 7. PRODUCTION OF MALE-STERILE HEXAPLOID TRITICALE

INTRODUCTION

Male sterility in crop plants is important in respect of developing composite crosses and for the production of hybrid seeds. Male sterility has been used in several agriculturally important crop plants such as maize (Jones and Everett, 1949) onion and carrot (Peterson, 1970) to produce hybrid seeds on a commercial scale and thereby to exploit heterosis for yield.

The aim in using male-sterile tetraploid wheat in crosses with diploid rye is to eventually produce male-sterile hexaploid triticales. However, the initial hexaploid triticale derived from such crosses is expected to be fertile because it is likely that the rye genome will compensate for the male-sterility of tetraploid wheat. Hence, to obtain male sterile triticale it will be necessary to remove the male fertility restoring gene(s) from the rye genome. These genes have been found to be located on chromosome 2R and 4R of S.A. rye (see Chapter 4).

METHODS AND MATERIALS (Described in Chapter 3, Section 3.2.3)

7.1 Results

Fifteen F_1 hybrids involving the cross between male sterile tetraploid wheat and diploid rye cv. Imperial were raised following embryo culture (Chapter 3, Section 3.2.1). The F_1 hybrid seedlings were treated with colchicine as described in Chapter 3 Section 3.2.1. Twelve F_1 hybrid plants survived and a total of ninety-five F_2 hexaploid triticale seeds were obtained from the colchicine-induced doubled sectors of the hybrid plants. This result confirmed that the whole rye

genome can compensate for the male sterility of tetraploid wheat. Several F_2 triticales thus produced were examined cytologically by Feulgen staining and found to possess 42 chromosomes in their root tip cells. This stock is the starting point for the production of male sterile triticale.

7.2 Procedure for producing male sterile hexaploid triticale

Since rye chromosome 2R and 4R of S.A. rye each individually partially compensate for the male sterility of Cornerstone (Chapter 4), it is assumed that the compensation by the whole genome of Imperial rye is due to similar genes. Providing that there are no chromosome translocation differences between S.A. rye and Imperial rye, then it is expected that chromosomes 2R and 4R of Imperial rye will also be involved in the compensation for the missing male fertility gene(s) on the α arm of chromosome 4A of tetraploid wheat.

On this basis, the production of male sterile hexaploid triticale from this stock will require a two step procedure.

Step 1 will require the substitution of wheat chromosome 2D for the male sterility compensating rye chromosome 2R. This could be achieved readily by crossing Armadillo * type of hexaploid triticale in which chromosome 2R has been substituted by chromosome 2D (Gustafson and Zillinsky, 1973). Selection should be made for a plant type which lacks chromosome 2R and is homozygous for wheat *ms*.

The identification of such a plant could be made on the basis of its fertility level. Plants with wheat *ms* will display relatively low level of fertility (See Chapter 4, Table 4.20). Any plant with one or more doses of wheat *Ms* is expected to have near full fertility and hence it should be relatively easy to find the desired plant.

Step 2 will require a cultivar in which chromosomes 2R and 4R have been replaced by wheat chromosome 2D and 4D respectively. Recently, Gill *et al.* (1981) have isolated a triticale line (TL 24) which is lacking rye chromosomes 2R and 4R. This stock could be crossed with the plant type obtained in step 1. Following crossing, the F_1 plants will be heterzygous for wheat *Ms* and will exhibit 20 bivalents plus two univalents at meiosis, one univalent for chromosome 4D and other for rye chromosome 4R. These F_1 plants should be back crossed with triticale line TL 24. Several Bc_1 plants with 42 chromosomes, but lacking chromosome 4R should be grown and selfed. The Bc_2F_2 progeny should be grown and tested for male fertility. A male sterile hexaploid triticale with 21 bivalents could be isolated from this population.

The actual size of population required cannot be predicted since Cornerstone male sterility mutant segregates differently both at tetraploid and hexaploid wheat level (See Chapter 5, Table 5.3).

CHAPTER 8. PRODUCTION OF SYNTHETIC HEXAPLOID WHEAT WITH MALE STERILE TETRAPLOID WHEAT AND *AE. SQUARROSA*

INTRODUCTION

The Cornerstone male sterility is not compensated by any of the chromosomes of hexaploid wheat. On the other hand, it is compensated at least partially by the genome of diploid rye (See Chapter 4). Thus the question arises of whether the genome from *Ae. squarrosa*, the accepted donor of the D genome of hexaploid wheat (*T. aestivum*), can also compensate for the male sterility of Cornerstone.

The aim of the present study was to cross male sterile hexaploid wheat with *Ae. squarrosa* and to produce an amphiploid to find if a newly introduced D genome can compensate for the male sterility originally derived from Cornerstone. If compensation occurred, it was desired to isolate and identify which chromosome(s) of *Ae. squarrosa* is involved in the fertility compensation.

Further, if the D genome of *Ae. squarrosa* can compensate for the male sterility of Cornerstone, this will have important implications for understanding of change of gene(s) for male fertility of the D genome following the introgression into hexaploid wheat.

8.1 Results

In the initial cross, 240 florets of homozygous male sterile tetraploid wheat, *T. durum* was pollinated with *Ae. squarrosa* var: *Strangulata* . Twenty one F_1 hybrid embryos were dissected out and cultured *in vitro*. Sixteen of these resultant plants were treated with colchicine to double the chromosome number and were grown under controlled-environment conditions (Chapter 3, Section 3.2.1). Three of the colchicine-treated F_1 hybrids were examined cytologically.

The meiotic pairing behaviour was recorded in 110 pmc's from six anthers and there was no evidence of chromosome doubling. Most of pmc's (90.9%) displayed 21 univalents, but on the other hand one (3.6%) or two (5.4%) open bivalents in addition to 19 and 17 univalents, respectively. Two types of chromosome behaviour was observed at first metaphase of meiosis in pmc's from these plants. In one type, the univalents were randomly scattered around the cell and generally away from the metaphase plate (Fig. 8.1A). The other type, possessed 21 univalents aggregated at metaphase plate (Fig. 8.1B) and univalents had two clearly visible chromatids and resembled mitotic metaphase chromosomes (Fig. 8.1C). It was deduced that these univalents divided longitudinally (Fig. 8.1D) to produce symmetrical dyads that matured to give functional microspores without undergoing a second division. Nearly all the anthers of the F_1 plants shed pollen grains and as shown in Table 8.1 the majority of the spikelets set seeds in these plants following self pollination. The chromosome constitution of forty-seven randomly selected F_2 seeds was determined by root tip analysis. A maximum of 42 and minimum of 38 chromosomes were found in the individuals of this population (Table 8.2). A number of F_2 plants possessing 42 chromosomes were grown and they were fully fertile.

8.2 First backcross to male sterile durum

The newly synthesized hexaploid wheat was fully fertile and ten euploid F_2 plants were used to pollinate the male sterile tetraploid wheat in order to obtain Bc_1 progeny. Fifty two Bc_1 seeds were produced from such cross. Although the Bc_1 seeds possessed well-developed endosperm tissue, in most cases the embryos were poorly developed and often, consisted of merely a clump of undifferentiated cells. Only three Bc_1 plants were obtained from these embryo cultures and these were grown under controlled-environment conditions. Each of the plants

exhibited fourteen bivalents plus seven univalents at first metaphase of meiosis in pmc's. All of the Bc_1 plants were fertile and they were used to pollinate male sterile durum to produce Bc_2 seeds. Only thirty-two Bc_2 seeds were obtained because of the flowering of the parents were not well synchronized.

8.3 Second backcross population

Fourteen of the Bc_2 plants were grown in the glass house during spring to examine their fertility. The chromosome numbers in those plants ranged from 28 to 31 (Table 8.3). Two of the Bc_2 plants with 31 chromosomes had a grass clump phenotype and did not produce any spike. The remaining Bc_2 plants were phenotypically normal during vegetative growth but all of them were male sterile. Meiosis was examined in pmc's from some of these plants and they exhibited fourteen bivalents plus 0 to 2 univalents in different plants. Each of these twelve Bc_2 plants produced abortive pollen grains and they were male sterile. It was not possible to identify which chromosomes of *Ae. squarrosa* were present as univalents in those Bc_2 plants because the chromosomes of *Ae. squarrosa* can not be identified by N-banding (Gerlack, 1977).

8.4 Discussion

The number of seed set in colchicine-treated F_1 hybrid plants (Table 8.1) was unexpectedly high. It was not clear how much of the seed was due to the colchicine treatment or due to the meiotic restitution which has been observed in the pmc's. Chromosome doubling was not detected in any of the anthers analysed from the three different plants. Also, sectors of fertility were not observed in any of the colchicine-treated plants, unlike with the colchicine treated wheat x rye hybrids. Rather, seed setting was observed in almost every floret

TABLE 8.1. Seed set in colchicine-treated F_1 hybrids derived from crosses between male sterile durum and *Ae. squarrosa*.

No. of F_1 plants	Total No. of heads in F_1 plants	Total No. of florets	Total No. of seeds	Percentage of seed set/ spikelet
9	73	920	719	0.78

TABLE 8.2. Chromosome complement of randomly-selected F_2 progeny of synthetic hexaploid wheat plants produced with male sterile tetraploid wheat and *Ae. squarrosa*.

Somatic chromosome numbers	No. of F_2 plants of each chromotype	Percentage of the population
42 e	25	53.19
41 e	13	27.65
40 e	2	4.25
39 e	2	4.25
38 e	6	12.90

TABLE 8.3. Somatic chromosome complement of male-sterile BC_2 progeny derived from the crosses between synthetic hexaploid and male-sterile tetraploid wheat.

Somatic chromosome number	No. of plants in each group
28 e	3
29 3	5
30 e	4
31 e	2

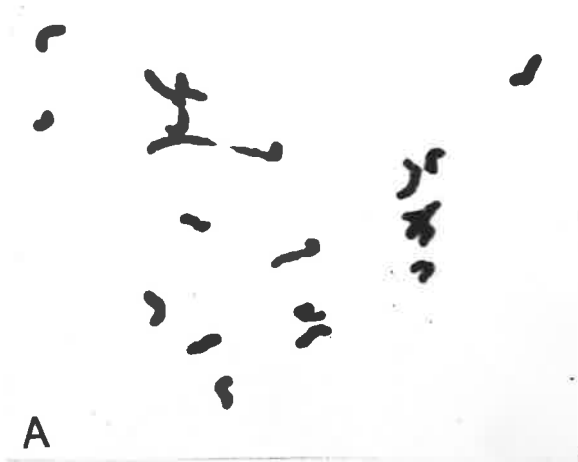
FIGURE 8.1A-D: Meiotic restitution in pmc's of F_1 hybrids involving the cross between male sterile tetraploid wheat and *Ae. squarrosa*.

A. Metaphase I exhibiting one open bivalent and nineteen univalents. This kind of pmc's resulted in the production of dyads possessing different sizes of micronuclei. X 865

B. Restituted nucleus showing all the univalents aggregated at the metaphase plant. X 865

C. End of metaphase I and the individual chromatids are evident. X 865

D. Chromatids are separated which resulted in formation of pollen grains with an unreduced chromosome number. X 865



A

B

C

D

of the spike. Hence, it seems likely that meiotic restitution might have played a role in the higher percentage of seed setting in the absence of sectors of fertility. Since, this high level of seed setting was not anticipated, all of the F_1 hybrid plants were treated with colchicine to obtain seeds of the synthetic hexaploid, and no untreated plants were left for comparison. However, there is some other indirect evidence that meiotic restitution might have been important in obtaining seed set on the F_1 hybrids. One F_1 hybrid plant derived from a cross between normal male fertile durum and *Ae. squarrosa* var. *Typica* was grown in isolation without colchicine treatment and it set on an average 2-4 seeds/spike. Similar seed setting has also been reported in untreated F_1 hybrid plants involving cross between durum and several *Aegilops* Spps (Kihara and Lilienfeld, 1944; Maan and Sasakuma, 1977).

Irrespective of whether the seed set was due to colchicine treatment or meiotic restitution, the full fertility in synthetic hexaploid wheat and pentaploid (Bc_1) clearly indicated that the D genome of *Ae. squarrosa* when added to male sterile durum can compensate for the male sterility of Cornerstone thought to be due terminal deletion of chromosome arm 4Aα

The compensation of male sterility in tetraploid wheat by the whole D genome or its haploid complement from *Ae. squarrosa* is contrast with the non-compensation of male sterility by the whole D genome in Cornerstone itself. This difference can only be explained that the male fertility gene(s) of the D genome from *Ae. squarrosa* in hexaploid wheat, *T. aestivum* might have lost in the process of evolution. The process by which this change of gene(s) has occurred is not known.

CHAPTER 9. LITERATURE CITED

AASE, H.C. (1930). The cytology of *Triticum*, *Secale* and *Agropyron* hybrids with reference to phylogeny. Res. Stud. Sta. Col. Wash. 2 : 1-60.

ACOSTA, A.C. (1961). The transfer of stem rust resistance from rye to wheat. Ph.D. thesis, Univ. of Missouri, pp. 56. Cited in Bielig, L.M. and Driscoll, C.J. (1971) Production of substitution lines in *Triticum aestivum*. Can. J. Genet. Cytol. 11 : 429-436.

ALAM, S. and SANDAL, P.C. (1969). Electrophoretic analysis of anther proteins from male-fertile and male-sterile Sudan grass, *Sorgham vulgare* var. *Sudanese Piper*. Crop Sci. 9 : 157-189.

ANDERSON, L.M. and DRISCOLL, C.J. (1967). The production and breeding behaviour of a monosomic alien substitution line. Can. J. Genet. Cytol. 9 : 399-403.

APPELS, R., GUSTAFSON, J.P. and MAY, C.E. (1982). Structural variation in the heterochromatin of rye chromosomes in Triticales. Theo. and Appl. Genet. 63 : 235-244.

ATHWALL, D.S., PAUL, P.S. and MINOCHA, J.L. (1967). Genetic male sterility in wheat. Euphytica 16 : 354-360.

BACKHOUSE, W.O. (1916). Note on inheritance of "Crossability". J. Genet. 6 : 91-94.

BAKSHI, J.S. and SCHLEHUBER, A.M. (1959). Identification of a substituted chromosome pair in a *Triticum agropyron* line. Proc. Okla. Aca. Sci. 39 : 16-21.

BARBER, H.N., DRISCOLL, C.J., LONG, P.M. and VICKERY, R.S. (1968). Protein genetics of wheat and homoeologous relationships of chromosomes. Nature 218 : 450-452.

BARLOW, K.K. and DRISCOLL, C.J. (1981). Linkage studies involving two chromosomal male-sterility mutants in hexaploid wheat. Genetics 98 : 791-799.

BATESON, W. and GAIRDNER, A.E. (1921). Male sterility in flax subject to two types of segregation. J. Genet. 11 : 269-276.

BEADLE, G.W. (1932). Genes in maize for pollen sterility. Genetics 17 : 413-431.

BERNARD, M. (1976). Etúde des caractéristiques cytologiques, morphologiques et agronomiques de six lignées d´addition Blé-seigle. Ann. Amel. plantes 26 : 67-91.

BIELIG, L.M. and DRISCOLL, C.J. (1970a). Substitution of rye chromosome 5RL for chromosome 5B of wheat and its effect on chromosome pairing. Genetics 65 : 241-247.

BIELIG, L.M. and DRISCOLL, C.J. (1970b). Substitution of rye chromosome 5R for its three wheat homoeologues. Genet. Res. 16 : 317-323.

BIELIG, L.M. and DRISCOLL, C.J. (1971). Production of alien substitution lines in *Triticum aestivum*. Can. J. Genet. Cytol. 13 : 429-436.

BIELIG, L.M. and DRISCOLL, C.J. (1973). Release of a series of MAS lines. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 147-150.

BINGHAM, J. (1966). Varietal response in wheat to water supply in the field, and male sterility caused by a period of drought in a glasshouse experiment. Ann. Appl. Biol. 57 : 365-377.

BORGHI, B., BOHOLI, F. and BOGGINI, G. (1973). Induction of male sterility in wheat with ethephon for hybrid wheat seed production. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo), pp. 337-343.

BOZZINI, A. (1974). Radiation-induced male sterility in *durum* wheat. *In* polyploidy and induced mutations in plant breeding. IAEA, Bari, Italy, pp. 23-25.

BOZZINI, A. and SCARASCIA-MUGNOZZA, G.T. (1968). A factor for male sterility inherited as a Mendalian recessive. Euphytica, 17 Suppl. No. 1 : 83-86.

BRIGGLE, L.W. (1970). A recessive gene for male sterility in hexaploid wheat. Crop Sci. 7 : 693-696.

BRINK, R.A., COOPER, D.C. and AUSHERMAN, L.E. (1944). A hybrid between *Hordeum jubatum* and *Secale cereale* reared from an artifically cultivated embryo. J. Hered. 35 : 67-75.

BURTON, G.W. (1958). Cytoplasmic male sterility in pearl millet (*Pennisetum glaucium* L.) R. Br. Agronom. J. 1967. Two subsequent sources of cytoplasmic male sterility in pearl millet and their relationship to Teft 23A. Crop Sci. 7 : 208-211.

CAUDERON, Y., SAIGUE, B. and DAUGE, M. (1973). The resistance to wheat rusts of *Agropyron intermedium* and its use in wheat improvement. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 401-407.

CHANG, T.D., KIMBER, G. and SEARS, E.R. (1973). Genetic analysis of rye chromosomes added to wheat. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 151-153.

CHANG, T.Y. and SMITH, H.H. (1975). Organogenesis from callus culture of *Hordeum vulgare*. Planta 123 : 307-310.

CHAPMAN, V. and RILEY, R. (1966). The allocation of the chromosomes of *Triticum aestivum* to the A and B genomes and evidence on genome structure. Can. J. Genet. Cytol. 8 : 57-63.

CHAPMAN, V. and RILEY, R. (1970). Homoeologous meiotic chromosome pairing in *Triticum aestivum* in which chromosome 5B is replaced by an alien homoeologue. Nature (London) 226 : 376-377.

CHAPMAN, V., MILLER, T.E. and RILEY, R. (1976). Equivalence of the A genome of bread wheat and that of *Triticum urartu*. Genet. Res. (Camb.) 27 : 69-76.

CONGER, A.D. and FAIRCHILD, L.M. (1953). A quick freeze method for making smear slides permanent. Stain Technol. 28 : 281-283.

CRAIGMILES, J.P. (1960). Development, maintenance and utilization of a male sterile Suden grass. Abst. Ann. Soc. Agron. (Chicago), pp. 46.

DARLIGTON, C.D. (1939). Misdivision and genetics of the centromere. J. Genet. 37 : 341-364.

DARVEY, N.L. (1973). Genetics of seed shrivelling in wheat and triticale. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 155-159.

DARVEY, N.L. and GUSTAFSON, J.P. (1975). Identification of rye chromosomes in wheat-rye addition lines and Triticale by heterochromatin bands. Crop Sci. 15 : 239-243.

DE VRIES, A.P.H. and IE, T.S. (1970). Electron microscopy on anther tissue and pollen of male sterile and fertile wheat (*Triticum aestivum*). Euphytica 19 : 103-120.

DRISCOLL, C.J. (1972). XYZ system of producing hybrid wheat. Crop Sci. 12 : 516-517.

DRISCOLL. C.J. (1973). A chromosomal male-sterility system of producing hybrid wheat. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 669-674.

DRISCOLL, C.J. (1975a). Cytogenetic analysis of two chromosomal male-sterility mutants in hexaploid wheat. Aust. J. Biol. Sci. 28 : 413-416.

DRISCOLL, C.J. (1975b). First compendium of wheat-alien chromosome lines. A. Wheat newsl. 21 : 16-21.

DRISCOLL, C.J. (1976). Second compendium of wheat-alien chromosome lines. A. Wheat newsl. 22 : 4-5.

DRISCOLL, C.J. (1977). Registration of Cornerstone male-sterile wheat germplam. Crop Sci. 17 : 190.

DRISCOLL, C.J. (1978). Induction of use of the "Cornerstone" male sterility mutant in wheat. Proc. 5th Int. Wheat Genet. Symp. (New Delhi, India), pp. 499-502.

DRISCOLL, C.J. (1981). Perspective in chromosome manipulation. Phil. Trans. R. Soc. Lond. B 292, 535-546.

DRISCOLL, C.J. and BARLOW, K.K. (1976). Male sterility in plants : induction, isolation and utilization. *In* induced mutation in crossbreeding, IAEA : Vienna, pp. 123-131.

DRISCOLL, C.J. and SEARS, E.R. (1971). Individual addition of the chromosomes of Imperial rye to wheat. Agron. Abst. pp.6.

DUBEY, D.K. and SINGH, S.P. (1965). Mechanism of pollen abortion in three male sterile lines of flax (*Linnum usitatissimum* L.) Crop Sci. 15 : 121-124.

DUVICK, D.N. (1965). Cytoplasmic sterility in corn. Adv. Genet. 3 : 161-174.

DUVICK, D.N. and NOBLE, S.W. (1978). Current use and future of cytoplasmic male sterility for hybrid seed production. *In* Maize breeding and Genetics. D.B. Walden (edi.), pp. 265-277.

DVOŘÁK, J. (1976). The relationship between the genome of *Triticum urartu* and the A and B genomes of *Triticum aestivum*. Can. J. Genet. Cytol. 18 : 371-377.

DVOŘÁK, J. (1980). Homoeology between *Agropyron elongatum* chromosomes and *Triticum aestivum* chromosomes. Can. J. Genet. Cytol. 22 : 237-259.

DVOŘÁK, J. and KNOTT, D.R. (1974). Disomic and ditelosomic additions of diploid *Agropyron elongatum* chromosomes to *Triticum aestivum*. Can. J. Genet. Cytol. 16 : 399-417.

DVOŘÁK, J. and SOSULSKI, F.W. (1974). Effects of additions and substitutions of *Agropyron elongatum* chromosomes on quantitative characters in wheat. Can. J. Genet. Cytol. 16 : 627-637.

EDWARDSON, J.R. (1956). Cytoplasmic male sterility. Bot. Rev. 22 : 676-738.

EDWARDSON, J.R. (1970). Cytoplasmic male sterility. Bot. Rev. 36 : 341-320.

EVANS, L.E. and JENKINS, B.C. (1960). Individual *Secale cereale* chromosome additions to *Triticum aestivum*. 1. The addition of individual "Dakold" fall rye chromosomes to "Kharkov" winter wheat and their subsequent identification. Can. J. Genet. Cytol. 2 : 205-215.

FELDMAN, M. (1966). Identification of unpaired chromosomes in F_1 hybrids involving *Triticum aestivum* and *T. timopheevi*. Can. J. Genet. Cytol. 8 : 144-151.

FELDMAN, M. (1978). New evidence on the origin of the B genome of wheat. Proc. 5th Int. Wheat Genet. Symp. (New Delhi, India), pp. 120-132.

FISHER, J.E. (1972). The transformation of stamens to ovaries and of ovaries to inflorescens in *Triticum aestivum* L. under short-day treatment. Bot. Gaz. 133 : 78-85.

FOSSATI, A. and INGOLD, M. (1970). A male sterile wheat mutant in *Triticum aestivum*. Wheat inform. Serv. (Kyoto) 30 : 8-10.

FRANCKOWIAK, J.D., MAAN, S.S. and WILLIAMS, N.D. (1976). A proposal for hybrid wheat utilizing *Aegilops squarrosa* L. cytoplasm. Crop Sci. 16 : 725-728.

FUKASAWA, H. (1953). Studies on restoration and substitution of nucleus in *Aegilotricum*. 1. Appearance of male sterile durum in substitution cross. Cytologia 18 : 167-175.

GERECHTER-AMITAI, Z.K., WAHL, I., VARDI, A. and ZOHARDY, D. (1971). Transfer of stem rust seedling resistance from wild diploid eikorn to tetraploid *durum* wheat by means of triploid hybrid bridge. Euphytica 20 : 281-285.

GERLACH, W.L. (1977). N-banded karyotypes of wheat species. Chromosoma (Berl.) 62 : 49-56.

GILL, B.S. and KIMBER, G. (1974). The Giemsa C-banded karyotype of rye. Proc. Nat. Acad. Sci. (U.S.A.) 71 : 1247-1249.

GILL, B.S., GREWAL, M.S., SING. M. and SANDHA, G.S. (1981). Chromosome substitution in hexaploid triticale (x *Triticosecale* Wittmack). Sabrao 13 : 33-38.

GOUJON, C. and INGOLD, M. (1967). Restauration de fertile de quelques ligneas male steriles de bles hexaploides. Comptes Rendus Acad. Sci. (Paris) 264 : 334-336. Cited in Edwardson, J.R. (1970) cytoplasmic male sterility. Bot. Rev. 36 : 341-420.

GRAHAM, R.D. (1975). Male sterility in wheat plants deficient in copper. Nature 254 : 514-515.

GUPTA, P.D. (1969). Studies on transmission of rye substitution gametes in common wheat. Ind. J. Plant Breeding 29 : 163-172.

GUSTAFSON, J.P. and ZILLINSKY, F.J. (1973). Identification of D-genome chromosomes from hexaploid wheat in a 42-chromosome triticale. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 225-232.

HART, G.E. (1973). Homoeologous gene evolution in hexaploid wheat. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 805-810.

HART, G.E. (1978). Chromosomal arm location of Adh-R1 and acid phosphatase structural gene in Imperial rye. Cereal Res. Comm. 6 : 123-133.

HART, G.E. (1979). Genetical and chromosomal relationships among the wheat and their relatives. Stadler Symp. Vol. 11, pp. 9-30.

HESLOP-HARRISON, J. (1957). The experimental modification of sex expression in flowering plants. Biol. Rev. 32 : 38-90.

HOCKETT, E.A. and ESLICK, R.F. (1968). Genetic male sterility in barley. 1. Nonallelic genes. Crop Sci. 8 : 218-220.

HOSSAIN, A. and DRISCOLL, C.J. (1981). Transfer of Cornerstone male sterility mutant to tetraploid wheat and hexaploid and octoploid triticales. Can. J. Genet. Cytol. 23 : 493-496.

HOSSAIN, A. and DRISCOLL, C.J. (1983). Fertility compensation of Cornerstone male sterility of wheat by rye. *Genetics* (in press).

HYDE, B.B. (1953). Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. Amer. J. Bot. 40 : 174-182.

ISLAM, A.K.M.R., SHEPHERD, K.W. and SPARROW, D.H.B. (1975). Addition of individual barley chromosomes to wheat. Proc. 3rd Int. Barley Genet. Symp. (Garching, BRD), 260-270.

ISLAM, A.K.M.R., SHEPHERD, K.W. and SPARROW, D.H.B. (1981). Isolation and characterization of Euplasmic wheat-barley chromosome addition lines. Heridity 48 : 161-174.

JAIN, S.H. (1959). Male sterility in flowering plants. Bibliogr. Genet. 18 : 101-166.

JAN, C.C., QUALSET, C.O. and VOGT, H.E. (1974). Chemical induction of sterility in wheat. Euphytica 23 : 78-85.

JEWELL, D.C. (1979). Chromosome banding in *Triticum aestivum* cv. Chinese Spring and *Aegilops variabilis*. Chromosoma (Berl.) 71 : 129-134.

JINGYANG and ZHONGLI (1982). Discovery and determination of a dominant male-sterile gene and its importance in genetics and wheat breeding. Scientia Sinia (Series B). Vol. XXV No. 5, 507-520.

JOHNSON, R. (1966). The substitution of a chromosome from *Agropyron elongatum* for chromosomes of hexaploid wheat. Can. J. Genet. Cytol. 8 : 279-292.

JOHNSON, R.R. and BROWN, C.M. (1978). Use of DPX 3778 to produce hybrid wheat seed. Crop Sci. 18 : 1026-1028.

JONES, J.A. and EMSWELLER, S.L. (1937). A male sterile onion. Proc. Amer. Sci. Host. Sci. 34 : 582-585.

JONES, D.F. and EVERETT, H.L. (1949). Hybrid field corn. Conn. Agr. Expt. Sta. Bull. 531 : 3-29.

JÖNSSON, J.Ö. (1976). Pre-harvest sprouting problems in hybrid wheat breeding. Cereal Res. Comm. 4 : 115-119.

JOPPA, L.R., McNEAL, F.H. and WELSH, J.R. (1966). Pollen and anther development in cytoplasmic male sterile wheat (*Triticum aestivum* L.). Crop Sci. 6 ; 296-297.

JOPPA, L.R. and McNEAL, F.H. (1969). Pollen fertility restoration by three spring wheat (*Triticum aestivum* L.) of the male sterility induced by *T. timopheevi* cytoplasm. Crop Sci. 9 : 839-840.

JOPPA, L.R. and McNEAL, F.H. (1972). Development of D-genome disomic addition lines of durum wheat. Can. J. Genet. Cytol. 14 : 335-340.

KATTERMANN, G. (1938). Uber konstante, halmbehaarte stämme aus Weizen-Rogen-Bastardierungen mit 2n = 42 chromosomen. Z. ind. Abst. Vererbunsgl. 74 : 354-375.

KERBER, E.R. and DYKE, P.L. (1969). Inheritance in hexaploid wheat of rust resistance and other characters derived from *Aegilops squarrosa*. Can. J. Genet. Cytol. 11 : 639-647.

KERBER, E.R. and DYKE, P.L. (1973). Inheritance of stem rust resistance from diploid wheat (*Triticum monococcum*) to tetraploid and hexaploid wheat and chromosome location of the gene involved. Can. J. Genet. Cytol. 15 : 397-409.

KIHARA, H. (1944). Die entdechung des DD-Analy sators beim weizen. Asri. and Hort. Tokyo 19 : 889-890.

KIHARA, H. (1951). Substitution of nucleus and its effect on genome manifestation. Cytologia 16 : 177-193.

KIHARA, H. and LILIENFELD, F. (1949). A new synthesized 6X-wheat. Proc. 8th Int. Cong. Genet. (Heriditas Suppl. Vol.) 307-319.

KIHARA, H. and TSUNEWAKI, K. (1961). Pistollody of *Triticum durum* induced by an alien cytoplasm. Seiken Zihô 12 : 1-10.

KIMBER, G. (1967). The addition of the chromosomes of *Aegilops umbellulata* to *Triticum aestivum* (Var. Chinese Spring) Genet. Res. (Camb.) 9 : 111-114.

KHUSH, G.S. and STEBBINS, G.L. (1961). Cytogenetic and evolutionary studies in Secale. 1. Some new data on the ancestry of *Secale cereale*. Amer. J. Bot. 48 : 723-730.

KNOTT, D.R. (1964). The effect on wheat of an *Agropyron* chromosome carrying rust resistance. Can. J. Genet. Cytol. 6 : 500-507.

KOLLER, O.L. and ZEER, F.J. (1976). The homoeologous relationships of rye chromosomes 4R and 7R with wheat chromosomes. Genet. Res. (Camb.) 28 : 177-188.

KROLOW, K.D. (1962). Aneuploidie und fertilität bei amphiploiden weizen-Roggen-Bastarden (Triticale). 1. Aneuploidie und selektion auf fertilität bei octoploiden triticale Forman. Zeits. Pflanzenzucht 48 : 177-196.

KRUPNOV, V.A. (1968). Genic male sterility in Common wheat (*Triticum aestivum*). Soviet Genetics 4 : 1300-1305.

LANGE, W. and WOZCIECHOWSKI, B. (1976). The crossing of common wheat (*Triticum aestivum* L.) with cultivated rye (*Secale cereale* L.) 1. Crossability pollen grains germinations and pollen tube growth. Euphytica 25 : 609-620.

LARTER, E.N. (1974). A review of the historical development Triticale, *In* Triticale : First man made cereal. C.C. Tsen (ed.) pp. 35-52.

LEIGHTY, C.E. and TAYLOR, W.J. (1924). Natural and artificial hybrid of a Chinese Spring wheat and rye. J. Agric. Res. 28 : 567-576.

LEIGHTY, C.E. and SANDO, W.J. (1928). Natural and artificial hybrids of a Chinese Spring wheat and rye. J. Hered. 19 : 23-27.

LEIN, A. (1943). Die genetische Grundlage der kreuzbarkeit zwischen weizen und Roggen. Zeit. f. Ind. Abst. -u. Verebgsl. 81 : 38-62. Cited in Riley and Chapman, V. (1967), The inheritance in wheat of crossability with rye. Genet. Res. (Camb.) 9 : 259-267.

LELLEY, L., JOSIFEK, J. and KALTSIKES, P.J. (1978). Polymorphism in the Giemsa C-banding pattern of rye chromosomes. Can. J. Genet and Cytol. 20 : 307-412.

MAAN, S.S. (1973). Cytoplasmic variability in *Triticinae*. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 367-373.

MAAN, S.S. and McGRACKEN, E.D. (1968). Meiotic instability of Common wheat strains derived from *Triticum timopheevi* Zhuk. crosses. Euphytica 17 : 445-450.

MAAN, S.S. and SASAKUMA, T. (1977). Fertility of amphiploids in *Triticinae*. J. Hered. 88 : 87-94.

MAAN, S.S. and LUCKEN, K.A. (1972). Increasing male sterility - male fertility restoration systems for hybrid wheat research. Crop Sci. 12 : 360-364.

MAAN, S.S., SASAKUMA, T. and TSUJU, S. (1980). Fertility of intergeneric hybrids in *Triticinae*. Seiken Zihô 29 : 78-86.

MANGELSDORF, P.C. (1955). Hybrid corn: Its genetic basis and its significance in human affairs. *In* Genetics in the 20th century. (Eds. L.C. Dunn) The McMillon Co. N.Y. pp. 555-571.

McFADDEN, E.S. and SEARS, E.R. (1944). The artificial synthesis of *Triticum spelta*. Res. Gen. Soc. Amer. 13 : 26-27.

McFADDEN, E.S. and SEARS, E.R. (1946). The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J. Hered. 37 : 81-89, 107-116.

MERCADO, A.C. Jr. and LANTICAN, R.M. (1961). The susceptability of cytoplasmic male sterile lines of corn to *Helminthosporium maydis* Nisik and Miyak. Philipine Agriculturist 45 : 235-243.

METTIN, D., BLÜTHNER, W.D. and SCHLEGEL, G. (1973). Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 709-713.

MIHALJEV, I.M. (1973). A new fertility restorer source in Common wheat. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 375-379.

MILLER, T.E. and SCHMITD, K.W. (1970). Inheritance of gene controlling male fertility in two different lines of wheat. Agron. Abst. *1970* : 23.

MILLER, T.E. and READER, S.M. (1980). Variation in the meiotic chromosome pairing of hybrids between hexaploid and diploid wheat. Cereal Res. Comm. 8 : 477-483.

MILLER, T.E., SHEPHERD, K.W. and RILEY, R. (1981). The relationship of chromosome 4A of diploid to that of hexaploid wheat. Cereal Res. Comm. 9 : 324-329.

MILLER, T.E., HUTCHISON, J. and CHAPMAN, V. (1982). Investigation of a preferentially transmitted *Aegilops sharonensis* chromosome in wheat. Theor. Appl. Genet. 61 : 27-33.

MIRI, K.K., AMAWATE, J.B. and JAIN, H.K. (1970). NP839, NP883 and NP880 : New sources of fertility restoration in male sterile wheat. Wheat Inform. Serv. (Kyoto), 31 : 9-11.

MORRIS, R., SCHMIDT, J.W., JOHNSON, V.A. and TAIRA, T. (1969). Aneuploid studies at the University of Nebraska. EWAC Newsletter, 2 : 55-56.

MURTHI, A.N. and WEAVER, J.B. Jr. (1974). Histological studies on five male sterile strains of upland cotton. Crop Sci. 14 : 658-663.

MÜNTZING, A. (1939). Studies on the properties and production of rye-wheat amphiploids. Heriditas 25 : 387-430.

MÜNTZING, A. (1957). Cytogenetic studies in rye wheat (Triticale). Proc. Int. Genet. Symp. Tokyo, 1956. (Cytologia suppl.) 51-56.

MÜNTZING, A. (1979). Triticale : Results and Problems. Suppl. to A. Pflanzenzuchtg. 10 : 1-103.

MYERS, V.M. (1946). Effect of Cytoplasm and gene dosage on expression of male sterility in *Dactylus glomerata*. Genetics 31 : 225-226.

NISHIKAWA, K. and FURUTA, Y. (1978). DNA content of nucleus and individual chromosomes and its evolutionary significance. Proc. 5th Int. Wheat Genet. Symp. (New Delhi, India), pp. 133-140.

OEHLER, E. and INGOLD, M. (1966). New cases of male sterility and new restorer sources in *Triticum aestivum*. Wheat Inform. Serv. (Kyoto), 22 : 1-3.

O'MARA, J.G. (1940). Cytogenetic studies on triticale. 1. A method for determining the effects of individual *Secale* chromosomes on *Triticum*. Genetics 25 : 401-408.

O'MARA, J.G. (1946). The substitution of a specific *Secale cereale* chromosome for a specific *Triticum vulgare* chromosome. Rec. Genet. Soc. Amer. 15 : 62-63.

O'MARA, J.G. (1953). The cytogenetics of Triticale, Bot. Rev. 19 : 587-605.

PEACOCK, W.J., GERLACH, W.L. and DENNIS, E.S. (1981). Molecular aspects of wheat evolution : Repeated DNA sequences. *In* Wheat science today and tomorrow. (Edi. Evans, L.T. and Peacock, W.J.) Camb. Univ. Press, pp.41-60.

PETERSON, P.A. (1958). Cytoplasmically inherited male sterility in capsicum. Amer. Nat. 92 : 111-119.

PETERSON, C.E. (1970). Utilizing cytoplasmic male sterility in the development of hybrid onions and carrots. Eucarpia-Sect. Hort., Versailles, pp. 7-17.

PUGSLEY, A.T. and ORAM, R.N. (1959). Genic male sterility in wheat. Aust. Pl. Breed. and Genet. Newsl. 14 : 10-11.

QUINN, C.J. and DRISCOLL, C.J. (1967). Relationships of the chromosomes of common wheat and related genera. Crop Sci. 7 : 74-75.

RAGHAVEN, T.S. (1951). Cytoplasmic inheritance in *Saccarum*. Curr. Sci. 20 : 138-140.

RAO, P.M.V. (1975). Homoeologous relationship of Imperial chromosome C with wheat chromosome 4A. Cereal Res. Comm. 3 : 103-109.

REES, H. and WALTERS, M.R. (1965). Nuclear DNA and evolution of wheat. Heridity 20 : 73-82.

RHODES, M.M. (1931). Cytoplasmic inheritance of male sterility in *Zea mays*. Science 73 : 340-341.

RICK, C.M. (1948). Genetics and development of nine male sterile tomato mutants. Hilgardia 18 : 599-633.

RILEY, R. (1955). The cytogenetics of the differences between some *Secale cereale* species. J. Agric. Sci. 46 : 377-383.

RILEY, R. (1960). The meiotic behaviour, fertility and stability of wheat-rye chromosome addition lines. Heridity 14 : 89-100.

RILEY, R. (1963). Cytogenetics and plant breeding. *In* Genetics today. (Ed. S.J. Greets) Proc. 11th Int. Genet. The Hague 3 : 681-688.

RILEY, R. and CHAPMAN, V. (1957). The comparison of wheat-rye and wheat-Aegilops amphiploids. J. Agric. Sci. 49 : 246-250.

RILEY, R. and CHAPMAN, V. (1958). The production of phenotypes of wheat-rye chromosome addition lines. Heridity 12 : 301-315.

RILEY, R. and CHAPMAN, V. (1960b). The D genome of hexaploid wheat. Wheat. Inform. Serv. 11 : 18-19.

RILEY, R. and CHAPMAN, V. (1967). The inheritance in wheat of crossability with rye. Genet. Res. (Camb.) 9 : 259-267.

RILEY, R. and KIMBER, G. (1966). The transfer alien genetic variation to wheat. Ann. Rep. Pl. Breed. Inst. (Camb.) 1964-64, pp. 6-36.

RILEY, R. and MILLER, T.E. (1970). Meiotic chromosome pairing in Triticale. Nature 227 : 82-83.

RILEY, R. and MACER, R.C.F. (1966). The chromosomal distribution of the genetic resistance of rye to wheat pathogens. Can. J. Genet. Cytol. 8 : 640-653.

RILEY, R., CHAPMAN, V. and JOHNSON, R. (1968). The incorporation of alien disease resistance in wheat by genetic interference with the regulation meiotic chromosome synapsis. Genet. Res. (Camb.) 12 : 199-219.

RILEY, R., CHAPMAN, V. and MACER, R.C.F. (1966). Homoeology of an Aegilops chromosome causing stripe rust resistance. Can. J. Genet. Cytol. 8 : 616-630.

RILEY, R., CHAPMAN, V. and MILLER, T.E. (1973). The determination of meiotic chromosome pairing. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 731-738.

RILEY, R., UNRAO, J. and CHAPMAN, V. (1958). Evidence on the origin of the B genome of wheat. J. Hered. 49 : 91-98.

ROWELL, L.P. and MILLER, D.G. (1971). Induction of sterility in wheat with 2-chloroethyl phosphoric acid (Etherel). Crop Sci. 11 : 629-631.

ROWELL, L.P. and MILLER, D.G. (1974). Effect of 2-chloroethyl phosphonic acid (Ethephon) on female fertility two wheat varieties. Crop Sci. 14 : 31-34.

SAGE, G.C.M. (1972). The inheritance of fertility restoration in male sterile wheat carrying cytoplasm derived from *Triticum timopheevi*. Theo. Appl. Genet. 42 : 233-243.

SARKAR, P. and STEBBINS, G.L. (1956). Morphological evidence on the origin of the B genome in wheat. Amer. J. Bot. 42 : 292-304.

SASAKUMA, T., MAAN, S.S. and WILLIAMS, N.D. (1978). EMS-induced male sterile mutants in Euplasmic and alloplasmic Common wheat. Crop Sci. 18 : 850-853.

SAX, K. (1972). Sterility in wheat hybrids. 11. Chromosome behaviour in partially sterile hybrids. Genetics 7 : 513-552.

SCHIEFELE, G.L., WHITEHEAD, W. and ROW, C. (1970). Increased susceptability to Southern Leaf Spot (Helminthosporium Maydis) in bred lines and hybrids of maize with Texas male sterile cytoplasm. Plant Dis. Rep. 54 : 501-503.

SEARS, E.R. (1952a). Misdivision of univalents in Common wheat. Chromosoma 4 : 535-550.

SEARS, E.R. (1952b). The behaviour of isochromosomes and telocentrics. Chromosoma 4 : 551-562.

SEARS, E.R. (1954). The aneuploids of Common wheat. Mo. Agri. Expt. Sta. Res. Bull. 575.

SEARS, E.R. (1956a). Transfer of leaf rust resistance from *Ae. umbellutata* to wheat. Brookhaven Symp. in Biol. 9 : 1-22.

SEARS, E.R. (1956b). The B genome of *Triticum*. Wheat Inform. Serv. 4 : 8-10.

SEARS, E.R. (1966). Nullisomic-tetrasomic combinations in hexaploid wheat. *In* Chromosome manipulations and plant genetics (Edi. by R. Riley and K.R. Lewis). Oliver and Boyd, Edinburgh : 29-45.

SEARS, E.R. (1968). Relationships of chromosome 2A, 2B and 2D with their rye homoeologue. Proc. 3rd Int. Wheat Genet. Symp. (Canberra, Aust.), pp. 53-61.

SEARS, E.R. (1972). Chromosome engineering in wheat. Stadler Genet. Symp. 4 : 23-38.

SEARS, E.R. (1977). An induced mutant with homoeologous pairing in Common wheat. Can. J. Genet. Cytol. 19 : 585-593.

SCHMIDT, J.W., JOHNSON, V.A. and MAAN, S.S. (1962). Hybrid wheat. Nebraska Expt. Sta. Quart. 9 : 9.

SHARMA, D. and KNOT, D.R. (1966). The transfer of leaf-rust resistance from *Agropyron* to wheat by irradiation. Can. J. Genet. Cytol. 8 : 137-143.

SHEPHERD, K.W. (1973). Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 745-760.

SNAPE, J.W., CHAPMAN, V., MOSS, J., BLANCHARD, C.E. and MILLER, T.E. (1979). Crossabilities of wheat varieties with *Hordeum bulbosum*. Heridity 42 : 291-298.

TABUSHI, J. (1957). Chromosome pairing in the progenies of synthesized 6X-wheat, ABD No. 4. Wheat Inform. Serv. 6 : 10.

TABUSHI, J. (1959). Aneuploids found in synthesized wheats, ABD Nos. 4 and 5. Wheat Inform. Serv. 9-10 : 33.

TANG, K.S. and HART, G.E. (1975). Use of isozymes as chromosome markers in wheat-rye addition lines and in Triticale. Genet. Res. (Camb.) 26 : 187-201.

TAYLOR, J.W. and QUISENBERRY, K.S. (1935). Inheritance of rye crossability in wheat hybrids. J. Amer. Soc. Agron. 27 : 149-153.

THE, T.T. (1973). Chromosome location of genes controlling stem rust resistance transferred from diploid to hexaploid wheat. Nature. New Biol. 241 : 256- .

THE, T.T. and BAKER, E.P. (1970). Homoeologous relationships between two *Agropyron indermedium* chromosomes and wheat. Wheat Inform. Serv. 31 : 29-31.

THE, T.T. and BAKER, E.P. (1973). Misdivision of an *Agropyron elongatum* chromosome. Wheat Inform. Serv. 36 : 19-20.

THOMPSON, D.J. (1961). Studies on the inheritance of male sterility in the carrot. Proc. Amer. Soc. Hort. Sci. 78 : 332-338.

TSUCHIYA, T. (1968). Chromosome variations in some strains of hexaploid triticale. Wheat Inform. Serv. 27 : 7-8.

TSUCHIYA, T. (1974). Cytological stability of triticale. *In* Triticale: First man made cereal. C.C. Tsen (ed.), pp. 62-89.

TSUNEWAKI, K. (1974). Triticale research in Japan. *In* Triticale: First man made cereal. C.C. Tsen (ed.), pp. 21-33.

TSUNEWAKI, K. and ENDO, T. (1973). Genetic relatedness among five cytoplasms in *Triticum* and *Aegilops*. Proc. 5th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 391-397.

TSUNEWAKI, K., MUKAI, Y. and ENDO, R. (1978). On the decent of the cytoplasms of polyploid species in *Triticum* and *Aegilops*. Proc. 5th Int. Wheat Genetic. Symp. (New Delhi, India), pp. 261-281.

VOSA, C.G. and MARCHI, P. (1972). Quinacrine fluorescence and Giemsa staining in plants. Nature 237 : 191-192.

WANINGE, J. and ZAVEN, A.C. (1968). Chromosome numbers in Pugsley's male sterile wheat. Euphytica 17 : 378-380.

WEIMARCH, A. (1973). Cytogenetic behaviour in octoploid triticale. 1. Meiosis, aneuploidy and fertility. Heriditas 74 : 103-118.

WEIMARCH, A. (1974). Elimination of wheat and rye chromosomes in a strain of octoploid *Triticale* as revealed by Giemsa banding technique. Heriditas 77 : 281-286.

WILSON, S.A. (1976). Wheat and rye hybrids. Trans. Bot. Soc. Edinb. 12 : 286-288.

WILSON, J.A. (1968). Problems in hybrid wheat breeding. Euphytica 17 (Suppl. No. 1) : 13-33.

WILSON, J.A. and ROSS, W.M. (1962). Male sterility interaction of *Triticum aestivum* nucleus and *Triticum timopheevi* cytoplasm. Wheat Inform. Serv. 14 : 29.

WILSON, J.A. (1962). Milestone. De Kalb Ag. Assoc. Letter Oct. 26. 1962,

ZELLER, F.J. (1973). 1B/1R wheat-rye chromosome substitutions and translocations. Proc. 4th Int. Wheat Genet. Symp. (Columbia, Mo.), pp. 209-221.

ZELLER, F.J. and BAIER, A.C. (1973). Substitution des Weizenchromosomenpaares 4A durch des Roggenchromosomenpaar 5R in dem weihenstephaner weizenstamm W70 a 86 (Blaukorn). Z. Pflanzenzüchtg. 70 : 1-10.