THE UNIVERSITY OF ADELAIDE

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WHEAT : BARLEY HYBRIDIZATION AND THE PRODUCTION AND CHARACTERIZATION OF ADDITION LINES

by

A.K.M. Rafiqul Islam

B.Sc., M.Ag. (Dacca)

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Department of Agronomy
Waite Agricultural Research Institute
Glen Osmond, South Autralia
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Dedicated to my parents,
my father the late Mozahar Uddin Ahmed
and my mother Amina Ahmed, whose sacrifice,
support and encouragement for 25 years made
it possible for me to complete the first
period of my education.

STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief it contains no material previously published and written by another person, except when due reference is made in the text.

However, this thesis comprises several papers either already published or submitted for publication. I have myself done all of the experimental work reported in these papers. The co-authors acted in their role as supervisors and have helped me in the planning of experiments and in editing the manuscripts.

Signed:

A.K.M. Rafiqul Islam

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SUMMARY

This thesis reports work on wheat:barley hybridization and the subsequent isolation and characterization of addition lines having individual pairs of barley chromosomes added to the chromosome complement of hexaploid wheat.

It was found that wheat and barley can be hybridized without difficulty when barley is used as the female parent and self-sterile F_1 hybrids with 28 somatic chromosomes were obtained using *in vitro* culture of embryos. Although no fertile sectors were produced after treatment of the F_1 hybrids with colchicine, some backcross (BC_1) seeds were obtained after pollinating them with wheat pollen. The majority of the backcross progeny were 49-chromosome heptaploids which evidently originated from fertilization of egg cells which had restituted at meiosis. Putative monosomic addition lines were isolated in the second backcross (BC_2) progeny of the self-sterile BC_1 plants. However, these plants were all self-sterile and exhibited pistillody due to an unfavourable interaction between barley cytoplasm and the wheat genome.

To overcome this problem of pistillody the more difficult reciprocal cross was attempted and although 20 hybrids were obtained in 8133 crosses, only one of them possessed the expected complement of 28 chromosomes which exhibited 28' at meiosis. The others possessed chromosome numbers varying from 21 to 36 in different plants. Presumably these abnormal plants originated from disruption of normal spindle activity during early divisions of the zygote. The 28-chromosome normal wheat x barley hybrid behaved similarly to the reciprocal hybrids in the BC_1 and BC_2 generations, except there was no evidence of pistillody and most of the BC_2 plants were self-fertile with this

cross. Five different monosomic addition lines were detected among BC_2 progeny and all of these plants were self-fertile. Disomic addition lines were isolated from among the progeny of these 43-chromosome monosomic additions and some other 44-chromosome double monosomic additions. Altogether five disomic and six ditelosomic additions were obtained from the progeny of these plants. Another disomic and a ditelosomic addition were obtained separately from three unusual hybrids exhibiting 22', 21' + 1" and 25' + 1"', at meiosis.

In this work, a new method for producing disomic addition lines from monosomic additions, was developed using Hordeum bulbosum crosses. The monosomics were crossed with H. bulbosum and 22-chromosome aneuhaploids were selected from among the progeny and disomic additions were then obtained directly from them by colchicine doubling.

The six disomic addition lines were initially designated A to F according to their sequence of isolation. Later N-banding was applied to barley chromosomes and it was found that each chromosome has a distinctive pattern, and furthermore, these patterns are all different from those exhibited by wheat chromosomes. Thus by studying the N-banding pattern of the chromosomes in the addition lines it became possible to determine which standard barley chromosome was present in each line. It was found that addition line A,B,C,D,E,F possesses standard barley chromosomes 4,7,6,1,2 and 3 respectively.

The remaining addition line (5) could not be obtained in disomic form, because chromosome 5 of barley when added to wheat results in cytological disturbances such as mosaic pollen mother cells and multi-pore pollen grains, and lines carrying it are self-sterile. The isolation of a fertile line carrying a translocation chromosome with

the short arm of chromosome 5 of barley joined to an unidentified arm of a wheat chromosome, showed that the sterility factors must be located on the long arm of chromosome 5.

It is evident that six out of the seven possible disomic additions and seven out of the 14 possible ditelosomic additions have been obtained. These addition lines will be useful in assigning genes controlling barley characters to particular barley chromosomes and also in determining the genetic similarity of individual barley chromosomes with wheat chromosomes. Furthermore, such addition lines could serve as the source material for transferring desirable characters from barley to wheat.

CHAPTER 1: GENERAL INTRODUCTION

This thesis is concerned with hybridization between wheat and barley, the two most important cereal crops of temperate agriculture. Wide crosses between different plant species and even between different genera have interested cereal breeders and botanists since before the turn of the century (for review see Riley and Kimber, 1966). The most extensive hybridizations have been between wheat (Triticum) and species from neighbouring genera, referred to in this thesis as alien species. The reason for attempting such crosses has been to try to incorporate into wheat useful characters present in related species or genera but which are lacking from wheat. Such characters include drought and disease resistance, resistance to insects and pests, winter hardiness and tolerance of poor soils. In addition certain of these crosses were made to obtain information on the evolutionary relationship between wheat and the alien species since they are believed to have evolved from the same ancestral species. The pairing behaviour of chromosomes in hybrids from such crosses was used for determining the similarity of genomes, referred to as genome analysis (see review by Lilienfeld, 1951). The other approach was to determine the genetic equivalence of individual alien chromosomes with particular wheat chromosomes by estimating the ability of such alien chromosomes to genetically compensate for the missing wheat chromosome in a substitution line.

Ever since the beginning of this century, plant breeders have been interested in crossing wheat and barley to produce a new type of crop plant. However, this has proved to be a very difficult cross to achieve, which is not surprising considering that wheat and barley must have undergone considerable evolutionary divergence since their separation from a common ancestor possibly in the Miocene-Pliocene epochs of the

Late Tertiary period (Sakamoto, 1973). Wheat was probably grown in the Middle East as early as 10,000 years ago (Riley, 1975) and barley was also domesticated at least 9,000 years ago (Harlan, 1968), or according to a recent estimate, possibly as long as 18,000 years ago (Wendorf *et al.*, 1979). In addition, they will have diverged morphologically due to their separate domestication thousands of years ago. Although both wheat and barley belong to the same taxonomic tribe called the Hordeae by Hutchinson (1934) and the Triticeae by Bowden (1959), they have been assigned to different subtribes Triticinae and Hordeinae, respectively, (Nevski, 1933)(cited by Sakamoto, 1973).

There are some agronomic characters in barley which might confer some advantage to wheat if they could be transferred to the latter. For example, barley is more tolerant of drought than wheat and some barley cultivars are claimed to be more salt-tolerant than wheat (Epstein and Norlyn, 1977). Farrer (1904) described his interest in crossing wheat and barley to produce a crop "..... with the habits, pecularities, and adaptation of ordinary barley which will produce flour from which leavened bread can be made, and in this way grain suitable for the making of bread may be produced in places where wheat cannot be grown and the world's supply of bread-making material considerably increased..." Despite the attempt of Farrer and others (Waterhouse, 1930; Gordon and Raw, 1932; Ahokas, 1970) to achieve this cross, there are no well substantiated records of success in producing a wheat:barley hybrid until 1973 when Kruse was able to produce F_1 hybrids by crossing diploid, tetraploid and hexaploid wheats with barley, using barley as the female parent. The F_1 hybrids were self-sterile as expected from such a wide cross, but Kruse did not report any attempt to produce a fertile amphiploid from them. However, he obtained one plant after backcrossing the barley x hexaploid wheat hybrid with wheat pollen.

Shortly after Kruse published his study the present author commenced a programme of hybridization of barley with wheat (mainly the hexaploid form) using barley as the female parent. The aim was to produce an amphiploid of barley and wheat. Although doubled sectors were observed following colchicine treatment of the hybrid plants, selfed seeds were never obtained. However, rare backcross seeds were obtained when the self-sterile hybrids were pollinated with the wheat parent. The majority of the backcross progenies were heptaploids which had the full complement of wheat and the haploid complement of barley chromosomes. It was anticipated that these heptaploids could be used to produce a set of addition lines having individual barley chromosomes added to wheat. Although some monosomic addition lines were obtained in this programme, the work could not be continued because of the occurrence of pistillody (transformation of anthers into ovary-like structures) due evidently to the unfavourable interaction of barley cytoplasm with the wheat nucleus. It was realized that the reciprocal hybrids should not give this problem so wheat was used as the female parent in further crosses with barley. This was a much more difficult cross to achieve and again amphiploids could not be obtained. However, in this programme it was possible eventually to produce six disomic addition lines having individual pairs of barley chromosomes added to wheat.

This thesis comprises a series of papers on the production of reciprocal wheat:barley hybrids and the subsequent isolation and characterization of disomic addition lines. A new method for producing disomic additions from monosomic additions, using haploidy induced by crossing with Hordeum bulbosum is described. Observations on the process of meiotic restitution in F_1 hybrids and the occurrence of cytological abnormalities in F_1 hybrids and their derivatives are reported. The usefulness of heterochromatic banding (N-banding) of chromosomes for the identification of individual barley chromosomes and the application

of this technique in detecting the presence of barley chromosomes in a wheat background is also demonstrated. Furthermore, a general discussion of the results obtained in this work is presented.

The following terminology is used in this thesis to facilitate description of the reciprocal crosses between wheat and barley and the types of addition lines produced:

wheat : barley crosses - used as a general term without reference to which parent was used as male or female.

barley x wheat crosses - term used when barley was the female parent.

wheat x barley crosses - term used when wheat was the female parent.

wheat - barley addition lines - refer to the addition of barley chromosomes to the wheat genome; euplasmic lines have wheat cytoplasm whereas alloplasmic lines have barley cytoplasm.

CHAPTER 2: LITERATURE REVIEW

There is a vast literature on interspecific and intergeneric hybridization in the grass family (Gramineae) and no attempt is made in this review to cover the entire field. For example, the most recently available list of such crosses in the Gramineae (Knobloch, 1968) includes more than 2,400 different hybrids, covered in 1,131 references. Instead, to set an appropriate background for the present study on wheat: barley hybrids a brief account of the taxonomic and broad genetic relationships within the Triticeae is given, followed by a general review of wide crosses involving Hordeum and Triticum with the other genera of the Triticeae. The latter review does not include a complete list of all crosses, but is chosen to illustrate the main features encountered in these wide crosses. However, the history of attempts to cross wheat and barley and the outcome of these crosses is given in detail. To complete the background information required for the present study a brief review of the production and utilization of wheat-alien chromosome addition lines is also provided.

2.1 Taxonomic relationships in the Triticeae

Taxonomically wheat and barley belong to the tribe Hordeae according to Hutchinson (1934) and the Triticeae according to Bowden (1959). Hutchinson (1934) sub-divided the Hordeae into two subtribes Triticinae and Elyminae principally on the basis of the number of spikelets at each node of the rachis. These spikelets are solitary in the former but present in clusters of two to six at each node in the latter. He included the genera Triticum, Secale, Agropyron, Aegilops and Haynaldia in the subtribe Triticinae and Hordeum, Elymus, Sitanion and Asperella in the subtribe Elyminae. Nevski (1933)(cited by Sakamoto, 1973), however

divided the tribe Triticeae into seven subtribes and placed Hordeum, Sitanion, Critesion, Crithopsis, Psathyrostachys, Taeniatherum and Cuviera in the subtribe Hordeinae. Bowden (1959) in his attempt to clarify the taxonomic and nomenclatural treatments in the Triticeae accepted Nevski's classification of placing Hordeum and the other genera in the subtribe Hordeinae. Sakamoto (1973) includes 15 genera in the tribe Triticeae which he divided into two major groups, namely the Mediterranean and Arctic-temperate groups based on their geographical distribution. The Mediterranean group are mostly annuals and include the genera Triticum, Aegilops, Secale, Haynaldia, Eremopyrum, Henrardia and Heteranthelium with a solitary spikelet, Crithopsis and Taeniatherum with two spikelets, and Psathyrostachys which has three spikelets at each rachis node and is perennial. Secale and Haynaldia also have some perennial species. The Arctic-temperate group includes Agropyron, Hordeum, Asperella, Elymus and Sitanion and they are differentiated into complex endemic species. They have two or three spikelets at each node except Agropyron which has solitary spikelets. They are mostly perennials but Hordeum includes some annuals.

Sakamoto's (loc. cit.) system of classifying the Triticeae has been adopted in the present review but only the more important members of this tribe are discussed further in this section.

The genus Triticum includes diploids (2n=14), allo-tetraploids (2n=28) and allo-hexaploids (2n=42). From genome analysis, these three groups have been assigned the genomes AA, AABB and AABBDD, respectively (see review by Morris and Sears, 1967). Similar ploidy level differences also occur within the genus Aegilops (Kihara, 1954). Although Triticum and Aegilops were considered as separate genera for a long time, more recent information indicates that some Aegilops species are not

widely different from wheat, since one and possibly a second genome of hexaploid wheat come from the genus Aegilops. Bowden (1959) proposed to expand the genus Triticum to include Aegilops on the ground that one parental diploid species of T. aestivum cannot be placed in Aegilops whilst another is included in Triticum. Morris and Sears (1967) followed Bowden's scheme and included the species previously assigned to Aegilops in Triticum.

Although up to 14 species of Secale have been reported by different authors (Bowden, 1959), only five species were subsequently recognised by Khush (1962). All of these Secale species are diploids with 2n=14. The genus Haynaldia is very small and contains only two species, H. villosa (L.) Schur and H. hordeacea (Goss. et Dur.) Hackel. (Sakamoto, 1973). The former is a diploid (2n=14) but both diploid (2n=14) and tetraploid (2n=28) forms exist in the latter.

The genus Agropyron is very complex and heterogeneous and Cauderon (1966) listed more than 150 species. Apart from diploids with 2n=14, a wide range of polyploids from 2n=28 to 2n=70 exists in the genus. The genus Elymus also displays a high level of polyploidy, the most common forms are tetraploid (2n=28), but higher polyploids with up to 84 chromosomes exist. In Sitanion, both species known are tetraploids (2n=28), and they occur only in North America (Sakamoto, 1973). Sakamoto (loc. cit.) places Agropyron, Elymus and Sitanion in a complex consisting of many taxonomically difficult and cytologically complex polyploid species. This complex was divided into five major genetic groups based on cytogenetic studies of hybrids and their geographical distribution.

There is no general agreement on the number of species in the genus Hordeum. Bowden (1959) listed 31 species and of these only one species,

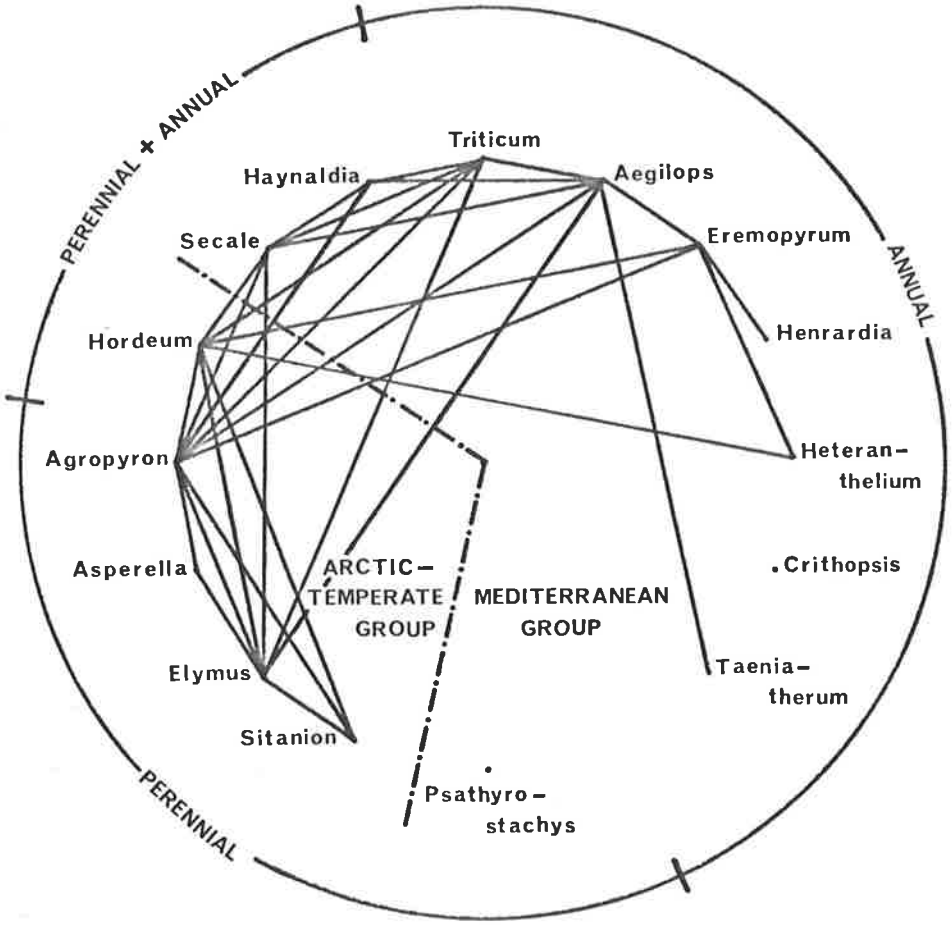
H. vulgare L. (2n=14) is cultivated (Rajhathy *et al.*, 1963). More than half of the Hordeum species are diploids (2n=14) and the rest are either tetraploids (2n=28) or hexaploids (2n=42). Only three diploids and two tetraploid species are known in Eremopyrum (Sakamoto, 1973). The tetraploids are thought to be amphiploids derived from hybrids between the diploids.

2.2 Broad genetic relationships and cross compatibilities in the Triticeae

A broad genetic relationship between the genera of the subtribe Triticinae has long been indicated from the extent of intergeneric hybrids produced within this subtribe. Although embryo culture was required to obtain some hybrids, the widespread cross-compatibility indicates that the genomes of the Triticinae, at least, are genetically and evolutionarily related. Furthermore, a common basic chromosome number of 7 in all of the genera of this subtribe adds strength to the hypothesis that they have all evolved from a common diploid ancestor. However, hybrids have also been produced in wider intergeneric combinations within the Triticeae as set out in Fig. 2.1 and Sakamoto (1973) concluded from this sort of evidence that there is a fairly good genetic and cytoplasmic compatibility within the whole tribe. Sakamoto (loc. cit.) predicted that it might be possible to produce hybrids in all possible combinations between the 15 genera he includes in the tribe Triticeae.

It is evident from Fig. 2.1 that the genera within the subtribe Triticinae are cross-compatible in all combinations. Furthermore, Triticum has been crossed with Hordeum and Elymus of the other subtribes. Similarly, Hordeum has also been successfully crossed with Triticum, Agropyron, Secale, Elymus, Sitanion, Eremopyrum and Heteranthelium. Sakamoto (1973) noted that natural intergeneric hybridization is rare

Fig. 2.1: Genetic relationships within the tribe Triticeae in terms of successful intergeneric hybrid production (after Sakamoto, 1973). Three additional intergeneric hybrids have been included namely Elymus - Aegilops (Schooler, 1966); Agropyron-Haynaldia (Cauderon, 1966) and Triticum - Hordeum (Kruse, 1973; Islam *et al.*, 1975, 1978)


PERENNIAL + ANNUAL
ANNUAL
PERENNIAL
Triticum
Haynaldia
Aegilops
Secale
Eremopyrum
Henrardia
Hordeum
Agropyron
Heteran-
thelium
Asperella
ARCTIC–
TEMPERATE
GROUP
MEDITERRANEAN
GROUP
Crithopsis
Taenia-
therum
Elymus
Sitanion
Psathyro-
stachys

among the genera belonging to the Mediterranean group within the Triticeae whereas both interspecific and intergeneric hybridization occurs very extensively in the Arctic-temperate group.

2.3 Wide crosses involving Hordeum

2.3 a) *Intergeneric crosses* - In the past, the main reason for attempting intergeneric hybridization with Hordeum was to determine the genomic and phylogenetic relationships between Hordeum and the other genera in the Triticeae. In addition there are some useful agronomic characters known to occur in other genera of the Triticeae which are lacking in barley and wide crosses have been made with the aim of incorporating these characters into barley. For example, Quincke (1940) hybridized H. vulgare with rye (S. cereale) in an attempt to incorporate into barley the winter hardiness of rye. The crosses which have been successful and some of the main features encountered in hybridizing Hordeum with other genera of the Triticeae, are described below.

Although spontaneous hybrids of Hordeum with Elymus (Bowden, 1958; Stebbins *et al.*, 1946) and Agropyron (Stebbins *et al.*, 1946; Boyle and Holmgren, 1955) are known to exist, Smith (1942) was not able to produce any hybrids artificially by pollinating several thousand florets of H. vulgare with 9 Elymus species and 5 Agropyron species. However, Korablin (1937) (cited by Price, 1968) apparently produced 107 seeds from a Hordeum x Elymus cross and Stebbins *et al.* (1946) produced one F_1 hybrid from H. nodosum L. x E. glaucus Buckl. crosses. Also Dewey (1971) succeeded in producing 7 hybrid plants from crosses between E. canadensis L. and H. bogdanii Wilensky. Bowden (1958) in his review, listed two artifical hybrids, one involving H. distichon L. x E. racemosus Lam. produced in the USSR and the other E. virginicus L. x H. jubatum L.,

produced in the USA. All of these hybrids and also those listed below were self-sterile.

Stebbins *et al.* (1946) failed to produce any hybrids from A. pauciflorum Hitche. ex Silveus x H. nodosum crosses. Boyle and Holmgren (1955), however, obtained hybrids from A. trachycaulum (Link) x H. jubatum crosses and also from the reciprocal cross. The hybrids were self-sterile but Ashman and Boyle (1955) succeeded in producing a fertile amphiploid from the A. trachycaulum x H. jubatum hybrid. Hybrids between cultivated barley and Agropyron were first reported by Kruse (1974). He crossed four diploid and three autotetraploids of H. vulgare as the maternal parent with A. repens (L.) P.B. (2n=42). The average seed set was 11.6%, and 76.6% of these seeds possessed embryos and 49% of the cultured embryos produced plants. Their hybrid status was established from somatic chromosome counts which revealed 28 and 35 chromosomes in plants derived from crosses with diploid and tetraploid barley, respectively. Among the hybrid plants, 29% died within four months and 50% of the survivors produced inflorescences. The hybrids were self-sterile and backcrosses to H. vulgare were unsuccessful.

Spontaneous hybrids of Hordeum and Sitanion were recorded by Wiebe and Smith (quoted by Smith, 1951). Wiebe and Smith also produced artificial hybrids between H. nodosum and S. jubatum J.G.Smith and Dewey (1971) obtained hybrids from S. hystrix (Nutt.) J.G.Smith and H. bogdanii crosses. All of the Hordeum - Sitanion hybrids were completely sterile.

Partial homology between the genomes of Hordeum, Elymus, Agropyron and Sitanion was suspected when some chromosome pairing was observed in these spontaneous and artificial hybrids. Dewey (1971) observed 5.40 and 5.72 bivalents at meiosis in F_1 hybrids of E. canadensis and

S. hystrix crossed with H. bogdanii, and concluded that one genome of the two in both Elymus and Sitanion is partially homologous with the genome of H. bogdanii. He also stated that some other related species such as E. glaucus, E. virginicus, S. jubatum, A. canimum (L.) Beauv., A. trachycaulum (Link) Malte, and A. dasystachyum (Hook.) Scribn. contain bogdanii-like genomes. Stebbins *et al.* (1946) considered that E. macounii Vasey represents a natural F_1 hybrid from crosses between some Agropyron species and either H. nodosum or H. jubatum. Gross (1960) was able to confirm this suggestion by producing plants morphologically similar to E. macounii from reciprocal crosses between A. trachycaulum and H. jubatum.

Hybrids between Hordeum and Secale have been reported in the Literature but it appears to be a very difficult cross to accomplish. Quincke (1940) made crosses between H. vulgare and H. jubatum with S. cereale L. cv. Crown and although he obtained apparent seed sets of 90% and 95%, respectively, none of the hybrid seeds possessed an embryo or endosperm. Thompson and Johnston (1945) reported that 80% of the ovaries were fertilized when H. vulgare was pollinated with S. cereale, but the embryos died at an early stage due to abnormal development of the endosperm. However, Brink *et al.* (1944) were successful in producing one F_1 hybrid of H. jubatum x S. cereale cv. Imperial out of 81 embryos cultured *in vitro*. Wagenaar (1959) also used embryo culture to produce two hybrids from this cross using cultivars Antelope and Sangaste of S. cereale. Similarly Morrison *et al.* (1959) used embryo culture to produce hybrids of H. californicum Covas et Stebbins, H. depressum (Scribn. and Smith) Rydb. and H. vulgare with S. cereale.

The low percentage of embryos recovered in crosses between Hordeum and Secale is commonly due to post-fertilization breakdown of the endosperm. Larter and Enns (1960) reported that even in crosses

between autotetraploids and diploids of H. vulgare the triploid embryos did not grow beyond a few days due to the gradual degeneration of the endosperm. They attempted to overcome this problem by stimulating growth of embryos and endosperm tissues with the application of gibberellic acid (GA) to the last leaf of the maternal parent before and after pollination. They found that GA applied in this way did prolong development of the embryo and endosperm. In his work with H. vulgare x S. cereale crosses, Kruse (1967) modified this technique by applying GA directly to the stigma of H. vulgare one day after pollination. Although this treatment promoted embryonic growth he obtained only two hybrids due to difficulties with the embryo culture medium.

2.3 b) *Interspecific crosses* - Hybridization within Hordeum has been carried out principally to study the genetic relationship between the different species in this group. However, wide crosses involving H. vulgare were aimed also at transferring desirable characters such as winter hardiness and disease resistance from wild species to cultivated barley. None of these attempts at gene transfer have been successful so far. Rajhathy *et al.* (1963) have pointed out the difficulty in assessing cross-compatibility among Hordeum species because the small size of the floral parts of most species makes it difficult to carry out large numbers of crosses. However, they believe that there is a general cross-compatibility between these species because despite this technical difficulty a large number of hybrids have been produced.

Morrison *et al.* (1959) obtained hybrids from only 14 out of 39 different cross combinations attempted within Hordeum. Hybrids were obtained between H. vulgare and seven other species but H. murinum L. could

not be hybridized with any of the four species used. Subsequently Rajhathy *et al.* (1963) were able to hybridize H. murinum with H. vulgare by using colchicine-doubled hexaploid and octoploid forms of H. murinum. They also succeeded in crossing H. pusillum Nutt. x H. stenostachys Godron but only when these diploids had been raised to the tetraploid level.

A common problem encountered in Hordeum interspecific crosses is a disturbance of the early developmental stage of the seed leading to degeneration of the endosperm and death of the embryo. This problem was largely overcome by artificial culture of young embryos (Konzak *et al*, 1951; Morrison *et al.*, 1959; Davies, 1960). Another problem was semi-lethality of hybrids and in crosses of H. californicum Covas et Stebbins with H. vulgare and H. bulbosum L. (Davies, 1960) and also H. murinum x H. vulgare (Rajhathy *et al.*, 1963), the hybrid plants appeared normal at first but they subsequently died within a few days or weeks.

Another common abnormality observed in Hordeum interspecific crosses is chromosome elimination in the hybrids. For example, Cauderon and Cauderon (1956) reported that in H. bulbosom (4x) x H. secalinum Schreb. (4x) hybrids, there was a tendency for secalinum chromosomes to be eliminated during growth. Rajhathy *et al.* (1963) also observed two types of progeny in H. lechleri (Steud.) Schenck x H. vulgare crosses. One type had the expected 28 chromosomes, whereas the other type possessed a haploid complement of lechleri chromosomes. Davies (1958) obtained self-fertile diploid vulgare plants from crosses of H. bulbosum (4x) with H. vulgare (4x) and concluded that such plants arose by male parthenogenesis. However, subsequently Kasha and Kao (1970) and Kao and Kasha (1970) demonstrated

that haploids and dihaploids of H. vulgare were obtained by selective elimination of the bulbosum chromosomes when H. vulgare was crossed with H. bulbosum at the diploid and tetraploid levels, respectively. In contrast, triploid hybrids were produced when diploid H. vulgare was crossed with tetraploid H. bulbosum. Subsequently similar examples of selective chromosome elimination were encountered in crosses involving other Hordeum species. In each of the following examples the chromosomes of the second named species were found to be eliminated in the developing hybrid embryos: H. arizonicum Covas (6x) x H. vulgare (2x), H. arizonicum (6x) x H. bulbosum (2x) (Islam and Sparrow, 1974); H. jubatum L. (4x) x H. bulbosum (2x), H. lechleri (6x) x H. vulgare (2x) (Rajhathy and Symko, 1974); H. parodii Covas (6x) x H. bulbosum (2x) and H. procerum Nevski (6x) x H. bulbosum (2x) (Subrahmanyam, 1977).

All of these interspecific Hordeum hybrids are self-sterile, and there are very few reports of amphiploid production from them. Schooler (1960a,b) was successful in producing amphiploids from H. compressum Griesb. x H. pusillum Nutt. and H. marinum Huds. x H. compressum hybrids by colchicine treatment. However, Morrison *et al.* (1959) were unsuccessful in producing any amphiploids even though many of the F_1 hybrids produced in their work were treated with colchicine. Although a few seeds began to develop in doubled sectors of some of the treated plants, these seeds soon degenerated.

In contrast to the above examples, hybrids between H. marinum x H. leporinum Link, H. jubatum x H. brachyantherum Nevski and H. arizonicum x H. hexaploidum Covas are partially fertile (Rajhathy *et al.*, 1963), and the authors concluded that the parental species involved in the first two crosses are conspecific whilst the parents of the last mentioned cross are very closely related. Furthermore, fertile

hybrids, result when H. vulgare is crossed with the wild barley H. spontaneum C. Koch. Rudorf and Wienhues (1951) were able to transfer mildew resistance from H. spontaneum var. nigrum to cultivated barley in a conventional backcross programme. Not only did they obtain mildew-resistant lines, but some lines were also better in yielding ability, grain quality and straw strength.

In summary, it appears that interspecific Hordeum hybrids except those involving H. spontaneum have been of little value in improving cultivated barley. However, the induction of barley haploids from H. vulgare x H. bulbosum crosses has potential in barley breeding because homozygous lines can be obtained in the F_2 generation as compared to the F_5 or F_6 in conventional breeding programmes (Reinbergs *et al.*, 1975).

2.4 Wide crosses involving Triticum

2.4 a) Triticum X Secale *crosses* - Wilson (1876) was apparently the first person to produce a hybrid between wheat and rye but at that time the hybrid was considered to be merely a botanical curiosity. Subsequently the interest in this cross became more practical with the aim of producing high-yielding amphiploids or else transferring desirable rye characters such as winter hardiness, disease and insect resistance and tolerance of poor acid soils to wheat. This led to the production of fertile amphiploids (called triticale) by doubling the chromosome number of sterile F_1 hybrids, at first relying on spontaneous doubling and later using colchicine-induced chromosome doubling (Blakeslee, 1937; Eigsti, 1938). Since the early part of this century, but especially since the 1930's, numerous workers have been producing triticales and evaluating their potential as a new crop species.

Many Triticum species have been hybridized with different rye cultivars to produce a wide range of triticales. Initially hexaploid wheats were used to produce octoploid triticales but, because of their meiotic instability and relatively poor agronomic performance more emphasis is now given to hexaploid triticales based on crossing tetraploid wheat with rye. The history of the development of triticale and its current status as a crop plant has been the subject of several recent comprehensive reviews (Larter, 1974; Tsunewaki, 1974; Muntzing, 1979) and only some of the salient features of Triticum x Secale hybridization are reviewed here.

An early finding was the difference in crossability of different hexaploid wheat parents with rye. Backhouse (1916) found that a wheat of Chinese origin gave 80% seed set in crosses with rye compared to only 0-0.21% seed set when some other wheats were used. Similarly, Leighty and Sando (1928) obtained 90.5% seed set after pollinating a Chinese wheat with rye but they obtained only 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) also observed differences in crossability among different wheat strains and were able to transfer rye crossability from the Chinese strain to other more agronomically desirable wheats by intervarietal crossing and selection. Lein (1943)(cited by Riley and Chapman, 1967) studied the genetic control of crossability of rye and showed that allelic differences at two loci are responsible. He assigned the genotype $kr_1kr_1kr_2kr_2$ for the readily crossable Chinese 466, $Kr_1Kr_1Kr_2Kr_2$ for the poorly crossable Marquis and $Kr_1Kr_1kr_2kr_2$ for the partially crossable Blausamtiger Kolben, where Kr_1 has a more marked influence on preventing crossability than Kr_2. Subsequently Riley and

Chapman (1967) studied intervarietal substitution lines involving substitution of pairs of chromosomes of poorly crossable Hope for chromosomes of readily crossable Chinese Spring and they demonstrated that Kr_1 and Kr_2 are located on chromosomes 5B and 5A of Hope, respectively, whereas Chinese Spring has recessive alleles at these loci. Lange and Wojciechowska (1976) investigated the cytological basis of differences in crossability and found that the poor crossability of Hope and the CS/Hope 5B substitution line resulted from failure of fertilization due to failure of the rye pollen tube to grow in the style base and the ovary wall of the wheat parent.

Apart from these intervarietal differences in crossability among wheats, Röbbelen and Smutkupt (1968) observed reciprocal differences in the success rate of wheat x rye crosses. With Chinese Spring wheat and Petkuser rye they obtained 61% seed set in wheat x rye crosses but only 1% seed set in the reciprocal cross. The poor result in the latter cross was attributed partly to the relatively slow growth of the wheat pollen tube in the style of rye.

Species differences in crossability were also observed by Nakajima (unpublished) in both Triticum and Secale (see review by Tsunewaki, 1974). When five species of Triticum were crossed with six different Secale species, marked differences in crossability were observed among the wheats. T. aestivum and T. compactum showed the highest crossability, particularly when crossed with S. cereale, S. ancestrale Zhuk. and S. vavilovii Grossheim. T. aestivum was found to be the most difficult species of wheat to cross with S. fragile Bieb. According to Larter (1974), tetraploid wheat (T. durum) and rye were first hybridized by Aase in 1930. These crosses usually result in badly shrivelled grain which often fails to germinate. The introduction of embryo culture techniques (Knudson,

1922; Brink *et al.*, 1944) has facilitated the production of many new combinations of tetraploid wheat x rye hybrids. However, Nakajima (loc. cit.) observed poor crossability between nine tetraploid wheat species and seven Secale species, and particularly with S. cereale, S. ancestrale and S. vavilovii, where only 0-0.4% seeds were obtained. However, the same three Secale species showed more crossability (0.1 to 69%) with hexaploid Triticum species.

2.4 b) Triticum *X* Aegilops *crosses* - Some modern taxonomic treatments of the Triticinae (Bowden, 1959; Morris and Sears, 1967) do not recognize Aegilops as a separate genus but include it in Triticum. Although many workers have adopted this new classification, it is not yet universally accepted. Since Aegilops is not a major interest in the present work and as this classification was used in most of the literature reviewed, the designation Aegilops has been retained here for convenience.

The earliest recorded cross between Triticum and Aegilops was Ae. ovata x T. aestivum made by Godron in 1854. Subsequently species belonging to these two genera have been hybridized extensively for many different reasons. The main interest in these crosses initially was to determine the genomic relationship between Triticum and Aegilops. Sax and Sax (1924) observed that hybrids between Ae. cylindrica Host and T. aestivum (AABBDD) form seven bivalents at meiosis whereas there is little or no chromosome pairing in Ae. cylindrica x T. turgidum (AABB) hybrids (Gaines and Aase, 1926). Although these observations indicated that the D genome was present in Ae. cylindrica, the identity of the diploid contributing this D genome was not known because of the tetraploid nature of Ae. cylindrica. Subsequently Sears (1941) synthesized an amphiploid of

Ae. caudata L. and Ae. squarrosa L. and McFadden and Sears (1944) noted that this amphiploid resembled Ae. cylindrica, suggesting that Ae. cylindrica must be a natural amphiploid from these two species. From meiotic pairing behaviour in F_1 hybrids of Ae. caudata x T. aestivum, Kihara and Lilienfeld (1935) concluded that the caudata genome cannot be homologous to the D genome of T. aestivum. Attempts were then made to cross Ae. squarrosa with tetraploid wheat. Although McFadden and Sears (1944, 1946) failed to produce hybrids from Ae. squarrosa x T. dicoccum Schrank crosses, they were successful with the reciprocal cross. In addition they produced an amphiploid from a T. dicoccoides Körn. x Ae. squarrosa hybrid and this resembled T. spelta L. Thus they concluded that the D genome of hexaploid wheat must have come from Ae. squarrosa. Kihara (1944) (cited in Lilienfeld, 1951), also came to a similar conclusion on genomic and morphological grounds. Subsequently Riley and Chapman (1960) confirmed the equivalence of the genome of Ae. squarrosa to the D genome of wheat by demonstrating that a T. aestivum x Ae. squarrosa F_1 hybrid generally forms seven bivalents and 14 univalents at meiosis.

The B genome of wheat is also suspected to have been derived from one or more Aegilops species. Sarkar and Stebbins (1956) suggested on morphological grounds that Ae. speltoides or diploids similar to this species could be the possible donor of the B genome of wheat. Riley *et al.* (1958) agreed with this suggestion on the basis of karyotype, chromosome pairing in hybrids with tetraploid wheat and the geographical distribution of Ae. speltoides and the wild forms of diploid wheat. Kimber and Athwal (1972) demonstrated that variation occurs in different accessions of Ae. speltoides with respect to their ability to suppress the control exerted by chromosome 5B on preventing homoeologous pairing

in hexaploid wheat. Low and high pairing accessions were identified and when the low pairing line was crossed to wheat there was no evidence of homologous pairing at meiosis. Furthermore, when the amphiploid was produced, bivalent pairing only was observed. Thus they concluded that Ae. speltoides cannot be the donor of the B genome of wheat. They suggested, however, that the B genome could be a mixture of genomes derived from intercrosses of two or more amphiploids which originated from hybridization of diploid wheat with other species.

Many amphiploids involving Triticum and Aegilops species have been produced (Sears, 1959; Bell *et al.*, 1955), but none of them have shown agronomic potential. Bell *et al.* (loc. cit.) and Riley and Kimber (1966) reported that the Aegilops characters were strongly expressed in Triticum x Aegilops amphiploids indicating the epistatic nature of such characters. Moreover the amphiploids inherited the primitive spike characters such as tough glumes, and brittle rachis from the Aegilops parent and seed fertility was usually low compared to that of the parents. However, although these amphiploids did not have any direct agronomic value, they have proved very useful as a means of transferring disease resistance from some Aegilops species to wheat as described below.

Kimber (1967a) used a conventional backcross programme to transfer eye spot (Cercosporella herpotrichoides Fron.) resistance from the D genome of Ae. ve ntricosa Tausch to wheat relying on meiotic recombination between homologous chromosomes in these two species. This was achieved by crossing the susceptible T. aestivum cultivar Falcon as the female parent with a synthetic hexaploid from a T. turgidum L. x Ae. ventricosa hybrid. Resistant plants were selected in the subsequent backcross progeny. Kerber and Dyck (1969) used a similar method to transfer seedling leaf rust resistance from Ae. squarrosa to wheat. In

this case the synthetic hexaploid was produced from hybrids involving the tetraploid (AABB) component of a hexaploid wheat Canthatch and Ae. squarrosa R.L. 5289. In a similar way, they subsequently transferred to wheat, adult-plant leaf rust resistance from Ae. squarrosa R.L. 5271 (Dyck and Kerber, 1970) and stem rust resistance from Ae. squarrosa R.L. 5288 (Kerber and Dyck, 1978).

Sears (1956) used X-ray irradiation to induce a chromosome translocation for transferring leaf rust resistance from Ae. umbellulata to wheat. First he produced a monoisosomic addition line having the complete chromosome complement of wheat plus one added isochromosome of Ae. umbellulata Zhuk. carrying rust resistance. He then treated these plants with X-rays prior to meiosis. Pollen from the irradiated plants was used to pollinate untreated normal wheat plants and resistant lines were selected from among the backcross progeny. Some of these resistant plants were found to possess translocation chromosomes having a segment of umbellulata chromosomes joined to a wheat chromosome. A line with a terminal segment of umbellulata chromosome translocated onto chromosome 6B of wheat and having resistance to leaf rust was subsequently released as a breeders' line, named Transfer (Sears, 1961, 1963).

Riley *et al.* (1968) used the 5B suppressing effect of the Ae. speltoides genome for inducing pairing and recombination between an Ae. comosa Sibth. et Sm. chromosome (2M) carrying yellow rust resistance and T. aestivum chromosomes. This was achieved by crossing a monosomic wheat - Ae. comosa addition line carrying chromosome 2M with Ae. speltoides Tausch. The 29-chromosome F_1 hybrid was pollinated with wheat and rust resistant plants were selected from among the progeny. These were backcrossed twice more to wheat and a homozygous translocation

line with yellow rust resistance was eventually isolated. Recently Dvorák (1977) transferred leaf-rust resistance from Ae. speltoides to the susceptible wheat cultivars Neepawa and Manitou by first crossing each of these cultivars with Ae. speltoides and then backcrossing the F_1 hybrids and subsequent backcross derivatives with the respective wheat parents five times.

The process of introgression from diploid Aegilops species to tetraploid wheat, thought to occur widely in nature was studied by Vardi and Zohary (1967) using T. durum Desf. x Ae. longissima S. & M. F_1 hybrids. These sterile triploid hybrids were produced artificially and then planted in the field among T. durum plants. Backcross seeds were produced on these hybrids by natural pollination and the majority of the resultant plants were pentaploid or near pentaploid and most of them were almost self-sterile. In the subsequent backcross generation, there was a decrease in chromosome number towards the tetraploid level in some plants and an increase in chromosome number of others towards the hexaploid level. They concluded that introgression of Ae. longissima characters into wheat could occur in two ways, firstly by gene transfer from Ae. longissima to the tetraploid wheat and secondly by synthesis of a new hexaploid by addition of the longissima chromosomes to the tetraploid.

2.4 c) Triticum *X* Agropyron *crosses* - One interest in Triticum and Agropyron crosses was the possibility of producing perennial wheat. Most of the crosses were made in the USSR and unfortunately detailed information on this work is not available to the author because it has been published in Russian in non-accessible journals (Knobloch, 1968). Tsitsin (Cicin) and Lubimova (1959) reported that they have produced perennial wheat and forage wheat from hybridization of T. aestivum and

T. durum with A. elongatum (Host) P.B. and A. glaucum Roem. & Schult. The perennial wheat M2 (2n=56) derived from T. aestivum (2n=42) x A. glaucum (2n=42) crosses was a non-lodging type and it was resistant to fungal diseases, and also possessed a high percentage of protein compared to the winter wheat parent. Furthermore, the protein content of hay from the forage wheat was also equal to that in the grain of soft wheat. Unrau (1958), however, questioned the claim of Tsitsin (1946), that he had been able to produce perennial wheats on two grounds. Firstly, because he doubted whether the so-called perennial wheats should be considered to be wheats and secondly because the perennial habit was generally associated with grass-like characters.

Other workers have been interested in Triticum - Agropyron crosses because of the possibility of transferring disease resistance, drought resistance, winter hardiness and alkali resistance from Agropyron to wheat. The majority of the successful crosses have been achieved with A. elongatum (2n=70) and A. intermedium (Host) P.B. (2n=42). A. trichophorum (Link) Richt. and A. glaucum have also been crossed with wheat but they are considered to be a subspecies and a synonym, respectively, of A. intermedium (Gaul, 1953). Wakar (1934) obtained hybrids from crossing T. aestivum with A. elongatum but had no success with A. glaucum. However, Armstrong (1936) found that A. glaucum could be crossed more readily with tetraploid wheat than with the hexaploids.

It appears to be very difficult to cross A. repens (L.) P.B. (2n=42) with Triticum, because Armstrong (loc. cit.) did not obtain any hybrid seeds after pollinating 1442 florets of A. repens with Triticum. Furthermore, Cauderon (1958) was unsuccessful in her attempts to cross tetraploid wheat with A. repens and diploid A. elongatum. However, Jenkins and Mochizuki (1957) were able to produce hybrids between T. durum and diploid A. elongatum.

Differences in seed development have been observed in crosses between Agropyron species and tetraploid and hexaploid wheats. Verushkine and Shechurdine (1933) obtained normal seeds from crosses of A. elongatum and A. intermedium with T. durum, but the endosperm aborted in seeds obtained from the equivalent crosses involving T. aestivum. Armstrong (loc. cit.) obtained more viable seeds when tetraploid wheat was crossed with A. glaucum than when it was crossed with A. elongatum, whereas with hexaploid wheat better results were obtained with A. elongatum than with A. glaucum.

Although Triticum - Agropyron amphiploids have not been of direct agronomic value, they have been useful as vehicles for transferring disease resistance from Agropyron to wheat. For example, Knott (1961) irradiated spikes of rust-resistant monosomic addition lines with either gamma rays or X-rays to transfer stem-rust resistance from A. elongatum to wheat. Sharma and Knott (1966) using similar methods transferred leaf-rust resistance from A. elongatum to wheat. Sears (1972a,b) was able to transfer leaf-rust resistance from two different A. elongatum chromosomes to wheat by first adding the appropriate Agropyron chromosome to wheat and then inducing homoeologous pairing between this chromosome and wheat chromosomes by removal of chromosome 5B of wheat.

2.4 d) Triticum *X* Haynaldia *crosses* - The main interest in hybridizing Haynaldia with Triticum was to transfer the disease resistance present in Haynaldia to wheat. Sando (1935) found that Haynaldia hybridizes readily with diploid and tetraploid wheats such as T. aegilopoides Forsk., T. timopheevi Zhuk., T. dicoccoides, T. dicoccum, T. durum, T. polonicum L. and T. turgidum., but not with the hexaploid wheats, T. aestivum, T. spelta and T. compactum Host. Sears (1953)

noted that Kostoff succeeded in making direct hybrids between hexaploid wheat and Haynaldia in 1937, but Sears was not able to produce such a hybrid without first making the bridging cross T. dicoccoides x H. villosa (L.) Schur. The amphiploid from this hybrid was crossed with T. aestivum to produce a plant with 42 wheat chromosomes and seven univalents from H. villosa. Tsunewaki (1974) reported that T. aestivum could be hybridized directly with H. villosa but no experimental details were given. Halloran (1966) also obtained rare seeds (1.2%) by crossing T. aestivum mono 5B with H. villosa. Although the seeds were very shrivelled he was able to produce hybrid plants from them. The 28-chromosome hybrid showed very little pairing of chromosomes (0.5"/cell) but the pairing was much higher in the 27-chromosome hybrids where an average of 4.8"/cell and occasional trivalents (0.8"/cell) and quadrivalents (0.7^{iv}/cell) were observed. As Halloran (loc. cit.) had not observed any quadrivalents in nulli-5B haploids of wheat, he interpreted this increased pairing and particularly the formation of quadrivalents, as evidence of pairing between the chromosomes of H. villosa and those of T. aestivum. Although he was optimistic that useful characters of Haynaldia could be transferred to wheat using homoeologous recombination, there is no report as yet of such a transfer having been achieved.

2.4 e) Triticum *X* Elymus *crosses* - As with Triticum x Agropyron hybridizations, most of the work on Triticum - Elymus hybridization was carried out in the USSR (Knobloch, 1968) and detailed information about their work is not available. Cicin (Tsitsin) and Petrova (1958) reported their results from crossing several species of Triticum with E. giganteus Vahl. The most success was achieved with T. durum x E. giganteus crosses and 88 hybrid plants were obtained. Only a single

hybrid was obtained from T. persicum Vav. x E. giganteus crosses but attempts to hybridize T. aestivum, T. compactum and T. turgidum with E. giganteus were unsuccessful. Although the hybrid seeds obtained from these crosses failed to germinate, plants were obtained from artificial culture of hybrid embryos. The hybrids obtained from T. durum x E. giganteus crosses resembled the Elymus parent in morphology and were resistant to rust. However, the plants varied in their resistance to mildew and ergot. The single hybrid obtained from T. persicum x E. giganteus crosses was intermediate in morphology between the parents and was resistant to both rust and mildew. All of these hybrid plants were self-sterile.

2.5 Hybridization of Hordeum with Triticum

Australia's pioneer wheat breeder William Farrer (1904) appears to be the first person to publish a report on wheat:barley hybridization. However, in this report he states that Mr. Maddox of Tasmania had succeeded in producing wheat:barley hybrids before him, but no other reference to this work is known. Farrer obtained one small shrivelled seed from pollination of 12 florets of the wheat Blount's Lambrigg with a strain of Nepaul barley which he named Bald Skinless barley. The presumptive hybrid plant was self-fertile and resembled the wheat parent, except that it had lighter green leaves and weaker stems at the juvenile stage of growth. No conspicuous differences between the plants were observed in the next generation excepting some plants had weak stems. Plants of the next generation (F_3) resulting from selected plants of the previous generation were very uniform in growth and morphology and Farrer gave the varietal name Bobs to this group of plants. Since Blount's Lambrigg was susceptible to stem rust Farrer also crossed 10-12 florets from each of several stem-rust resistant Fife varieties of wheat with the same Nepaul barley. These crosses were unsuccessful but from

the reciprocal crosses seven small, shrivelled seeds were obtained on the barley parent. The leaves of most of these presumptive hybrid plants were narrower, less stiff and darker than those of the barley parent, but otherwise they resembled the barley parent.

Farrer reasoned that if Bobs had barley parentage it might show more affinity for barley pollen and his assistants crossed it with Nepaul barley. Although 23 seeds were obtained, no information is available on the morphology and breeding behaviour of any plants grown from them.

In Farrer's work, the hybrids always resembled the ovule parents in being either barley-like or wheat-like and in both cases the plants were self-fertile. Thus it is highly likely that the presumptive hybrids resulted from chance self-pollinations or uncontrolled out-crosses of some florets of the female parent, rather than from true wheat:barley hybridization.

Another wheat variety Canberra was claimed by Pridham (1914) to have come from crossing Federation wheat as the female parent with Volga barley. Later Waterhouse (1930) attempted to repeat these supposed wheat:barley crosses, using both of the barley parents Nepaul and Volga used by Farrer and Pridham, and several wheat cultivars including Federation. Only five seeds were obtained after 961 wheat florets were pollinated twice with barley. However, all of these seeds were considered to have been derived from uncontrolled self-fertilization. Thus Waterhouse was sceptical about barley being involved in the production of Bobs and Canberra.

Gordon and Raw (1932) reported that although many workers had attempted to hybridize wheat and barley, in all cases there was some

doubt on whether the cross had been achieved. They also noted that numerous attempts to repeat the supposed wheat:barley crosses of Farrer were also unsuccessful, without specifying who these workers were. In their paper, Gordon and Raw provided a detailed description of the wheat:barley hybridizations attempted at the State Research Farm, Werribee, Victoria from 1913 onwards. They stated that although their presumptive hybrids showed segregation of some characters in the F_2 and later generations, and there was some indication of chromosome unbalance, the evidence obtained was not sufficient to prove their hybrid origin.

They reported that in 1913, 1915 and 1922, they made three separate series of crosses between Bobs wheat and Golden Grain barley. A few putative hybrids were obtained in each series but the F_1 plants were always wheat-like and many of them resembled Bobs closely and, furthermore, they were all self-fertile. In the F_2, and later generations, many of these putative hybrid plants exhibited segregation for characters such as chaff colour, degree of awning, maturity date and plant height (dwarf forms). However, the segregations were irregular and did not correspond to any simple Mendelian ratios. They stated that there was no sign of any barley characters in this material.

Additional crosses were attempted in 1922 using Comeback wheat as the female parent and Golden Grain barley as the male parent. Out of four plants obtained, one was identical to Comeback and three were dwarf forms which did not reach maturity.

They also attempted the reciprocal cross using a 6-row barley cultivar Cape as the female parent in crosses to the wheats Hard Federation and Indian 7. Altogether three seeds were obtained and all of them gave barley-like plants which differed from the barley parent in being 2-rowed and in some other characters like darker plant colour.

Again the F_1 hybrids were fully fertile and showed segregation for 2-row vs. 6-row in F_2 and later generations, but no wheat characters were evident.

Additional crosses were made between wheat and barley cultivars after 1922, but these failed except one seed was obtained from the cross Brevet wheat x White Hull-less barley. The F_1 plant did not resemble either parent closely being later than the wheat parent and having different leaf colour, auricle size and spike characters compared to the wheat parent. Segregation for these characters was observed in the F_2, again with no definite ratios. The plants were all distinct wheat-types and again no barley characters were evident. In this case, all F_2 plants showed a marked degree of sterility but this was considered to be due to thrip infestation rather than hybrid sterility, because the wheat parent was also partially sterile. The F_2 plants were examined cytologically and most had the normal wheat complement but some irregularities such as extra chromosomes and lagging univalents at anaphase I and II were also reported.

Gordon and Raw's (loc. cit.) interpretation of all of these observations was that some unknown factor was causing an upset in the chromosome balance of the ovule parent. They were convinced that the supposed hybrids had not come from the uncontrolled outcrosses because of the similar behaviour of progeny in the three series of crosses using Bobs as the maternal parent and the frequent occurrence of irregular segregation ratios. However, since their putative hybrids always resembled the maternal parent, either wheat-like or barley-like, and were always self-fertile, this reviewer has little doubt that all of these plants originated from uncontrolled outcrossings. The irregular segregation ratios could be due to many factors such as the poor expressivity of characters like colouration of plant organs,

and maturity time, or otherwise due to the complex inheritance of other characters such as the degree of awning (Tsunewaki, 1966). The occurrence of 2-row barley types in the progeny from 6-row barley could be explained simply by outcrossing with a 2-row barley, since the 2-row character is dominant over the 6-row form (Rohde and Pulham, 1960). The dwarf forms of wheat observed can also be explained simply by outcrossing. Specific genes are known to control the production of these dwarf forms (grass clumps) in wheat and some cross combinations give dwarf forms in F_1, as observed with Comeback, and others give dwarfs in the F_2 as observed with Bobs (Hermsen, 1967). The most extreme hypothesis of Gordon and Raw (loc. cit.) was the one invoked to explain the origin of self-fertile hybrid plants and the apparent presence of extra barley chromosomes at meiosis in their progeny. They hypothesized that chromosome doubling had occurred in the ovule of the wheat parents due to an upset caused by the introduction of barley chromosomes and the barley chromosomes were then gradually eliminated from the plants. They have not, however, indicated as to when chromosome doubling is supposed to have occurred - before or after fertilization?. From the cytological figures presented in their paper, it is most likely that the 'extra' chromosomes they believed to be barley chromosomes are in fact division products of unpaired wheat univalents which are known to occur frequently in intervarietal crosses in wheat due to asynapsis (Person, 1956). Thus, in summary, there is no good evidence that any of the putative hybrids obtained by Gordon and Raw represent crosses between wheat and barley, but instead they most likely came from uncontrolled outcrosses with other wheat and barley types.

Another unsuccessful attempt at making wheat:barley hybridizations was reported by Ahokas (1970). Generally wheat was used as the female parent but the reciprocal cross was also tried. Several novel treatments

including pollinating wheat with fresh barley pollen along with wheat pollen inactivated by freezing and thawing, application of gibberellic acid and R Nase to the stigma before and after pollinations were tried but no hybrids were obtained.

The first well-substantiated record of successful wheat:barley hybridizations comes from the work of Kruse (1973). He obtained 10 embryos from T. aestivum x H. vulgare crosses but none of them grew into plants. However, he was successful in producing hybrid plants with the reciprocal cross. He used the 2-row H. vulgare cultivars Bomi, Bonus, Emir and Lofa as the female parents and plants of T. aestivum (cultivars Koga, Cato and Starke), T. dicoccum and T. monococcum L. as the male parents. His technique involved excising spikes from the barley parents before anthesis and growing the detached spikes in water. Emasculation was done soon after spike excision and pollination with wheat was carried out 1-2 days later. Gibberellic acid (75 ppm) was applied to each floret one day after pollination and this was repeated on the following day. The seeds failed to mature on the plants and embryos were dissected out 12 days after fertilization and cultured on whole barley endosperm tissue placed on agar culture medium. The most difficult combination to hybridize was H. vulgare with T. monococcum where the success rate was only 0.25%. With H. vulgare x T. dicoccum and H. vulgare x T. aestivum crosses, the success rate was 1% and 3%, respectively, except with one particular combination of vulgare x aestivum which gave only 1% success. Approximately 90% of the hybrid seeds contained embryos. The somatic chromosome number of the hybrids using T. monococcum, T. dicoccum and T. aestivum as the wheat parent was 14, 21 and 28, respectively, as expected from the chromosome numbers of the parents. The satellited chromosomes of H. vulgare could be identified in somatic metaphase preparations in H. vulgare x T. dicoccum and H. vulgare x T. aestivum hybrids. The F_1 plants were vigorous in growth and resembled the wheat parents in morphology. However, the plants were self-sterile and Kruse obtained

only one backcross plant after pollinating the H. vulgare x T. aestivum F_1 hybrid with T. aestivum.

Shortly after Kruse published the above report on Hordeum x Triticum hybrids, the present author also succeeded in producing F_1 hybrids from H. vulgare x T. dicoccum, H. vulgare x T. durum and H. vulgare x T. aestivum and a report on H. vulgare x T. aestivum hybrids and their derivatives was published (Islam *et al.*, 1975). At this time Bates *et al.* (1974) claimed to have produced hybrids between durum wheat and barley and bread wheat and barley with the aid of immunosuppressant drugs injected into the female parent before pollination. The somatic chromosome number in root tips of the presumptive hybrids varied from 18-21 in durum wheat x barley crosses and 21-36 in bread wheat x barley crosses. They reported that these abnormal plants were self-fertile. However, it is not possible to evaluate these claims because full experimental details were not provided in their publication. Since their supposed hybrid plants were fertile, the possibility that these plants may instead have been aneuploid forms of wheat arising from mitotic abnormalities induced in embryonic cells by the immunosuppressant drugs, should be investigated. In a subsequent report Bates *et al.* (1976) stated that wheat:barley hybrids obtained after treatment with immunosuppressants lose the barley chromosomes early in embryogenesis. Later on these workers (Thomas *et al.*, 1977) produced F_1 hybrids from H. vulgare x T. turgidum and H. vulgare x T. aestivum crosses, without applying any pre- or post-pollination chemical treatments. However, the rate of cross success was generally very low excepting in crosses with Manker barley where 1.7% and 2.5% seed set was obtained with turgidum and aestivum wheat, respectively. The rate of embryo culture success was also low as only seven plants were obtained out of 32 embryos cultured. The hybrid plants had the expected somatic chromosome constitution of 21 and 28 chromosomes, respectively, although some mitotic and meiotic instabilities were noted. These abnormalities

included occasional root tip cells, with only 14 chromosomes in H. vulgare x T. turgidum hybrids, and PMC's with one or more micronuclei. These plants were all self-sterile and attempts to induce seed set by colchicine doubling were unsuccessful.

Fedak (1977) also obtained hybrids from H. vulgare x T. aestivum crosses using the same Betzes barley and Chinese Spring wheat parents used by the present author previously (Islam *et al.*, 1975). He obtained 11.8% cross success but only 9% of the embryos grew into mature plants. The F_1 hybrids possessed 28 somatic chromosomes but some PMC's were found to possess as few as 21 univalents at metaphase I of meiosis whereas others had many bivalants and occasional complex configurations such as a hexavalent. As the total pairing observed in these hybrids exceeded the combined pairing reported by other authors in wheat and barly haploids, Fedak concluded that this increased pairing was due to the presence in barley of minor suppressors which partially overcome the normal activity of chromosome 5 in preventing homoeologous pairing in wheat.

Halloran (pers. comm.) in 1968 succeeded in producing a 28-chromosome F_1 hybrid from T. aestivum (cv. Chinese Spring) x H. spontaneum crosses without the aid of embryo culture. The F_1 hybrid possessed 28-chromosomes and was intermediate in phenotype to the parents but all attempts to obtain backcross seed with wheat pollinations were unsuccessful.

Besides hybridization between cultivated species of Triticum and Hordeum, other attempts have been made to cross Triticum with wild species of Hordeum. Smith (1943) did not obtain any seed after pollinating several hundred florets of three varieties of T. durum with H. brevisubulatum Link. However, seeds were obtained when T. aestivum cv.

Chinese Spring was crossed with diploid and tetraploid strains of H. bulbosum (Barclay, 1975). As the seeds did not mature on the plants, the embryos were dissected out and cultured on nutrient agar medium. The derived plants were found to be haploids of wheat with 21 somatic chromosomes instead of 28- or 35-chromosome hybrids. He obtained some evidence that the bulbosum chromosomes were selectively eliminated in the developing hybrid embryos as was found to occur in H. vulgare x H. bulbosum crosses (Kasha and Kao, 1970). Chapman *et al.* (1976) extended these studies by pollinating 10 cultivars of wheat with diploid and tetraploid H. bulbosum but no seed was obtained. This problem of non-crossability was investigated further by crossing inter-varietal substitution lines with H. bulbosum (see earlier review with rye crosses). Very few seeds were obtained after pollinating the Chinese Spring (Hope 5B) substitution line. Although more seeds were obtained from pollination of the Chinese Spring (Hope 5A) substitution line, the percentage success was much less than that obtained with normal Chinese Spring wheat. Although the results were not conclusive, Chapman *et al.* considered it highly likely that genes Kr_1, and Kr_2 which control the crossability of wheat with rye (Riley and Chapman, 1967) also control the crossability of wheat varieties with H. bulbosum. Subsequently Snape *et al.* (1979) provided further evidence that these same loci on chromosomes 5B and 5A of Hope wheat control its crossability with H. bulbosum and furthermore chromosome 5D of Hope was found to reduce crossability with H. bulbosum to a minor extent. Also they demonstrated that some Australian wheat cultivars can be crossed with H. bulbosum with low frequency of success in contrast to the complete lack of crossability with the European wheat cultivars used.

In other Hordeum and Triticum crosses, Kimber and Sallee (1976) obtained one seed after pollinating 40 florets of T. timopheevi with

H. bogdanii. The seed matured on the plant and germinated normally to produce a hybrid with 21 somatic chromosomes. Although some PMC's possessed up to four rod bivalents at metaphase I, the average frequency of bivalents was less than one. The occurrence of chromosome pairing and the vigorous growth and abundant tiller production of the self-sterile hybrid led the authors to suggest that possibly there may be some homoeology between the genome of H. bogdanii and one of the genomes of T. timopheevi. Martin and Chapman (1977) produced another hybrid from H. chilense Roem. & Schult. x T. aestivum (cv. Chinese Spring) crosses using embryo culture. The F_1 hybrid possessed 28 somatic chromosomes and meiosis was normal with mean chromosome pairing of 1.97" per cell. The hybrid was self-sterile, but some selfed seeds were obtained in colchicine doubled sectors. Subsequently plants were grown from these seeds and they were found to be amphiploids exhibiting 28 bivalants at meiosis (Chapman and Miller, 1978). The amphiploids were self-sterile when grown under artificial light but were partially fertile when raised under natural light. Heptaploids with 49 chromosomes were produced by crossing the amphiploid reciprocally with Chinese Spring and those with barley cytoplasm were poorly fertile whereas those with wheat cytoplasm had good fertility. The heptaploids were further backcrossed with Chinese Spring wheat and 43-chromosome putative monosomic addition lines were selected from among the backcross progeny. Eight monosomic addition lines in wheat cytoplasm and six in barley cytoplasm were obtained. The monosomic addition lines in barley cytoplasm were reported to be male-sterile. In another programme Cauderon *et al.* (1978) produced an F_1 hybrid from H. vulgare x T. timopheevii Zhuk. crosses using embryo culture. The hybrid possessed 21 somatic chromosomes and exhibited mostly 21' in PMC's at metaphase I of meiosis and was self-sterile.

Thus it can be concluded from the above observations that repeated attempts to hybridize wheat and barley were unsuccessful for a period of

more than 70 years. But after Kruse succeeded in producing hybrids between these two species in 1973, wheat has been crossed successfully by other workers not only with H. vulgare but also with other wild Hordeum species including H. bogdanii, H. bulbosum and H. chilense.

2.6 Addition of alien chromosomes to hexaploid wheat

Many intergeneric amphiploids have been produced between Triticum aestivum and other members of the Triticinae but so far none of them except triticale have had any direct value in agriculture. When the whole genome of an alien species is added to wheat many undesirable characters are usually transferred along with the desirable characters required from the species. Consequently attention has been given to adding individual alien chromosome to the genotype of wheat, so as to reduce the number of such undesirable characters incorporated into wheat. Many addition lines having individual alien chromosomes added either monosomically or disomically to wheat have been produced to date. Riley and Kimber (1966) have provided a comprehensive review of the alien addition lines produced to that time.

The first alien addition line, possessing a single chromosome of S. cereale added to T. aestivum was produced by Leighty and Taylor (1924) by selecting plants with a hairy peduncle from among selfed progeny of first backcross plants derived from crossing the wheat x rye F_1 hybrid with T. aestivum. Subsequently, O'Mara (1940) used a systematic procedure to add three pairs of rye chromosomes disomically to common wheat. This procedure involves producing the wheat-alien amphiploid, then backcrossing it to wheat to produce a 49-chromosome heptaploid which forms 21" + 7' at meiosis. The heptaploids are allowed to self and plants with a single alien chromosome added to wheat (21" + 1') are selected initially by the presence of certain rye characters. These monosomics are then selfed and rare 44-chromosome disomic additions which form 22" at meiosis

are selected from among the progeny.

Riley and Chapman (1958), Riley (1960) and Riley and Macer (1966) used a slightly different procedure to produce a set of seven disomic additions having individual pairs of King II rye chromosomes added to Holdfast wheat. They obtained only a small percentage of disomics among progeny of selfed monosomics. The different rye chromosomes in individual addition lines caused distinctive modifications to the normal phenotype of Holdfast, for such characters as growth habit, hairy peduncle, leaf, culm and spike morphology. Hence the different lines could be distinguished from each other and also from the wheat parent. Besides these changes in morphology, one addition line was found to be resistant to powdery mildew and another line was resistant to both powdery mildew and wheat yellow rust. Subsequently,Riley (1960) observed that chromosome pairing in the disomic addition lines was less regular than in Holdfast wheat and the rye chromosomes tended to be more asynaptic. The fertility of the addition lines varied but was always lower than that of the wheat parent.

Evans and Jenkins (1960) obtained five disomic and two monosomic lines representing addition of the seven different chromosomes of Dakold fall rye to Kharkov wheat. They obtained disomics from progeny of selfed monosomics with an average frequency of 1.5%. They found that five of the rye chromosomes were easily identified in the wheat background but the other two could be identified only with difficulty. But unlike the King II rye addition lines, only one of the addition lines could be identified by its changed morphology i.e. the chromosome VI addition possessed pubescent peduncles. The other addition lines only showed minor quantitative changes in plant morphology.

Driscoll and Sears (1971) reported the production of a set of seven disomic additions of Imperial rye chromosomes to Chinese Spring wheat.

One of these addition lines was found to possess the slow moving esterase variants of rye. Six lines having disomic additions of Petkus rye chromosomes to wheat (FEC 28) were produced by Bernard (1976), and these lines could be identified by their individual differences in morphology. The addition lines showed more chromosome asynapsis at meiosis than present in the wheat parent but there was no correlation between failure of pairing and reduction in fertility of these lines. Chang *et al.* (1973) added chromosome 5R from six different rye cultivars, Gator, Imperial, Prolific, Weedy I, White and Wrens x Brazil disomically to wheat, to map genes controlling quantitative characters on the rye chromosomes.

A complete set of seven lines having disomic additions of Ae. umbellulata chromosomes to Chinese Spring wheat is available and six of these were produced by Kimber (1967b) and the remaining one by Chapman and Riley (1970). The authors noted that the umbellulata chromosome did not cause any noticeable increase in pairing failure of the wheat chromosomes in any of these monosomic addition lines. The disomic addition lines were phenotypically distinguishable from Chinese Spring wheat in height, maturity and spike characters, and one line was resistant to leaf rust of wheat. The fertility of the disomic additions, except for one line, was similar to that of the wheat parent. Furthermore, the addition lines were quite stable and gave mostly 44-chromosome progeny. Incomplete sets of disomic additions have also been produced from other Aegilops species. Riley *et al.* (1966) added Ae. comosa chromosome 2M carrying resistance to stripe rust monosomically to wheat and obtained disomic additions in its progeny with a fequency of 7%. Dover (1973b) produced four disomic and one monosomic addition of Ae. mutica Boiss. chromosomes to Chinese Spring wheat. The mutica chromosome in the monosomic addition line induced a high degree of pairing of chromosomes at metaphase I of meiosis and also resulted in meiotic abnormalities including mosaic PMC's

and multipore pollen grains (Dover and Riley, 1973). Chromosomes from the tetraploid species Ae. variabilis Eig. have been added to wheat and three different disomic and eight different monosomic addition lines are now available (Jewell and Driscoll, pers. comm). One of these addition lines (O) shows good resistance to cereal cyst nematode (Jewell, 1974). Dosba *et al.* (1978) used a bridging cross to produce five different alloplasmic disomic addition lines involving the addition of chromosome pairs from the M^v genome of Ae. ventricosa Tausch. to the wheat cultivar Moisson. Two of these lines (B and E) possessed resistance to eye spot and two lines (A and B) were resistant to cereal cyst nematode. However, all of these lines were poor in fertility and all except one were reported to be relatively unstable.

A complete set of seven disomic additions having the chromosomes of diploid A. elongatum added to Chinese Spring wheat was isolated by Dvorák and Knott (1974). Six of these seven lines are different in morphology to the wheat parent. Five of the lines have lower fertility than wheat and all seven of them show instability. In another programme, a disomic addition line was produced having a chromosome carrying stem rust resistance from polyploid A. elongatum (2n=70) added to Thatcher wheat (Knott, 1964). Also Wienhues (1963) added a chromosome carrying yellow rust resistance from another polyploid species of Agropyron (A. intermedium, 2n=42) disomically to wheat. In this case disomics were recovered from among the progeny of selfed monosomic additions with a frequency of 3-5%. Cauderon *et al.* (1973) produced six disomic addition lines having A. intermedium chromosomes added to wheat. These lines were all phenotypically different from each other in such characters as tillering propensity, spike morphology and leaf hairiness. Three of these lines possessed resistance to leaf rust, stem rust and stripe rust.

Hyde (1953) used a heptaploid made by Sears (1953) to produce five disomic additions and one monosomic addition of the chromosomes of H. villosa to hexaploid wheat. The recovery of disomics was 3.4% from the progeny of selfed monosomics. The different addition lines were identified from the morphology of the added chromosomes and the characteristic phenotype of the plants. All of the addition lines except one were meiotically stable.

Although none of these addition lines have been of direct use in agriculture, they have been useful for determining the gene content of individual alien chromosomes isolated from the rest of the chromosome complement (Riley and Chapman, 1958). Furthermore, they have been very useful in determining the genetic similarity (homoeology) between specific chromosomes of the alien species and wheat. This was determined by assessing the ability of individual alien chromosomes to genetically compensate for particular wheat chromosome in alien substitution lines (Riley 1963b; Riley *et al.*, 1968; Sears, 1968; Thé and Baker, 1970; Bielig and Driscoll, 1971; Shepherd, 1973; Dvorák and Sosulski, 1974; Koller and Zeller, 1976). Furthermore, some of the addition lines have also been used successfully for transferring desirable characters such as disease resistance from the alien species to wheat. This has been achieved by a variety of methods including the induction of translocations between the alien chromosome and the wheat chromosomes using ionizing radiations (Sears, 1956; Knott, 1961; Sharma and Knott, 1966), and the induction of homoeologous pairing either by removing chromosome 5B of wheat (Riley and Kimber, 1966; Sears, 1972a,b) or suppressing its effect by crossing the addition line plants with certain strains of Ae. speltoides (Riley *et al.*, 1968). The recent isolation of an X-ray induced mutant line of wheat (Ph mutant) which allows pairing between homoeologous chromosomes (Sears, 1977) has provided another means of incorporating alien genetic variation into wheat without the need to remove chromosome 5B of whea

In summary, chromosomes from several species of the subtribe Triticinae have been added either monosomically or disomically to the chromosome complement of wheat, and these addition lines have proved very useful in cytogenetical studies and wheat improvement programmes However, when the present studies were begun there was no example known where chromosomes from species outside the Triticinae have been added to wheat.

CHAPTER 3: ADDITION OF INDIVIDUAL BARLEY CHROMOSOMES TO WHEAT

A.K.M.R. Islam, K.W. Shepherd and D.H.B. Sparrow. Proc. 3rd Int. Barley Genet. Symp. (Garching, BRD), pp. 260-270 (1975)

INTRODUCTION

The possibility of crossing wheat with barley has interested cereal breeders since 1900 but despite many attempts (Farrer, 1904; Pridham, 1914; Waterhouse, 1930; Gordon and Raw, 1932; Ahokas, 1970) there are no well substantiated records of any success until the recent work of Kruse (1973). Initially Kruse pollinated hexaploid wheat with diploid barley and although a few hybrid seeds were produced, their embryos died in culture. However, when the reciprocal cross was made by transferring pollen from diploid, tetraploid and hexaploid wheat on to emasculated barley spikes he succeeded not only in obtaining seeds but also in culturing the embryos to give viable F_1 hybrids plants. The hybrid status of the plants was confirmed by their morphology, somatic chromosome constitution and self-sterility. The main limitation of this pioneering work of Kruse relates to the sterility of the hybrids, for he does not report any attempt to produce an amphiploid from them and he could obtain only one seed when they were pollinated with the wheat parent.

The initial aim of our work was to cross wheat and barley and to produce the amphiploid from the hybrid. Given the amphiploid, it was anticipated that the method described by O'Mara (1940) for adding rye chromosomes to wheat, could be used to produce a set of addition lines having individual pairs of barley chromosomes added to the chromosome complement of wheat. Such lines are expected to be of value to wheat and barley geneticists and possibly wheat breeders. Thus the lines would provide a new means of associating genes controlling barley

characters with particular barley chromosomes, provided of course that the barley character is expressed in a wheat background genotype (*cf.* O'Mara, 1940). Furthermore, the lines might enable studies to be undertaken on the evolutionary relationship of wheat and barley chromosomes by determining the effect of substituting particular barley chromosomes for particular wheat chromosomes (*cf.* Sears, 1968). Finally, and perhaps most importantly, such addition lines would be the starting point for transferring desirable characters from barley to wheat (*cf.* Sears, 1956 and Riley *et al.*, 1968).

MATERIALS AND METHODS

The barley (Hordeum vulgare) parents used in the crossing programme were all 2-row and included three local cultivars (Ketch, Clipper, Prior) and one from Europe (Betzes). The main hexaploid wheat (Triticum aestivum) parent was Chinese Spring but four Australian cultivars Gabo, Falcon, Heron and Halberd were used to a limited extent.

The crossing procedure employed was similar to that of Kruse (1973). Barley tillers were excised above the second node approximately one day before anthesis, and placed immediately in a liquid nutrient culture solution (modified from Hoagland and Arnon, 1938) in conical flasks. The nutrient solution was changed every 48 hours and the tiller ends were freshly cut at each change.

The barley spikes were emasculated soon after transfer to nutrient culture, and were covered with glassine bags to prevent uncontrolled pollination. Two days later the florets were pollinated with wheat pollen. Beginning at 24 hours after pollination a small drop of gibberellic acid solution (25 ppm) was applied daily to each barley floret to promote embryo growth.

The crossing procedure was later simplified in that emasculation and pollination of the barley spike was performed on the intact plant, and the hybrid seeds were allowed to continue growth on the plant. Alternatively the spikes were excised and transferred to nutrient solution 4-6 days after pollination.

Irrespective of the crossing procedure used, the developing seeds turned yellow 12-18 days after pollination and it was necessary to culture the embryos to obtain plants. The seeds were sterilised by immersion in 70% ethanol for a few seconds, followed by 4-5 minutes in a Na- hypochlorite solution (1.25%). After washing the seeds in sterile water, the embryos were removed under sterile conditions and placed on wheat or barley endosperm (14-18 days old) which previously had been placed on an agar medium (modified from Morrison *et al.*, 1959) in culture bottles. These bottles were kept in the dark at 20°C until the embryos germinated. The hybrid seedlings were then grown at 20°C in a 12 hour photoperiod until they reached the one or two leaf stage when they were transplanted into pots containing soil.

Attempts were made to produce barley-wheat amphiploids by treating the F_1 plants at the 3-4 tiller stage with 0.1% colchicine for up to 72 hours using Bell's (1950) tiller-capping method.

The chromosome constitution of hybrids, and the derived plants, was determined at both mitosis and meiosis. For mitotic studies root tips were pre-treated for 3-4 hours in water saturated with α - bromonaphthalene, fixed in glacial acetic acid overnight and then subjected to the standard Feulgen squash procedure. For meiotic studies individual anthers containing pollen mother cells (PMC's) at metaphase I were fixed in a 3:1 mixture of ethanol and glacial acetic acid and squash preparations were made as for root tips.

RESULTS

F_1 hybrid

No seeds were obtained when six detached spikes of Chinese Spring wheat were pollinated with Ketch barley. However when barley was used as the ovule parent we had no difficulty in producing barley x wheat hybrid seed (Table 3.1). Furthermore, approximately 50% of the embryos in these seeds survived dissection and culture to give a total of 67 viable hybrid plants. It is not valid to attribute differences in these crossing results to the particular parents involved, since effects due to changes in seasonal conditions and crossing procedure are confounded with parental differences. However, upon inspecting our results within seasons and within crossing procedures we obtained greater success with Chinese Spring as a pollen parent than with Gabo, Halberd or Heron. There is evidence that Betzes may be a superior ovule parent to the Australian barley cultivars used. We noted that the seed set following pollination of intact barley spikes was not significantly different from that obtained on detached tillers, but the latter procedure seemed to promote growth of the hybrid embryos.

The F_1 plants in their morphology resembled wheat more than barley, particularly in spike characters (Fig. 3.1), as observed by Kruse (1973). Their hybrid status was checked by chromosome counts on approximately one-third of the plants. The plants had 28 somatic chromosomes as expected while at meiosis in PMC's there were usually 28 univalents (Fig. 3.2a), but some PMC's possessed up to three bivalents, including an occasional ring bivalent. Rare PMC's contained many more than 28 chromosomes, including up to 23 ring bivalents, indicating that some chromosome doubling had occurred possibly resulting from abnormal premeiotic mitosis.

TABLE 3.1: Census data on barley x wheat hybridizations - October 1973 to December 1974

Cross combination	No. of florets pollinated	Seeds obtained No. (%)	No. of embryos cultured	No. of plants obtained
Ketch x CS*	512	56 (10.9)	34	18
Clipper x CS	576	56 (9.7)	43	21
Prior x CS	390	42 (10.8)	29	14
Betzes x CS	162	25 (15.4)	12	6
Ketch x Gabo	421	13 (3.1)	8	4
Clipper x Gabo	191	5 (2.6)	4	2
Prior x Gabo	341	1 (0.3)	0	0
Betzes x Gabo	22	2 (9.1)	1	0
Ketch x Heron	140	2 (1.4)	1	0
Clipper x Heron	53	0 (0.0)	0	0
Prior x Heron	219	1 (0.5)	0	0
Ketch x Falcon	59	4 (6.8)	3	2
Clipper x Falcon	65	2 (3.1)	2	0
Prior x Falcon	54	0 (0.0)	0	0
Ketch x Halberd	176	2 (1.1)	0	0
Clipper x Halberd	82	0 (0.0)	0	0
Prior x Halberd	145	0 (0.0)	0	0
Betzes x Halberd	43	0 (0.0)	0	0
Total	3651	211 (5.8)	137	67

*CS = Chinese Spring

Fig. 3.1: Spikes of barley and wheat parents, and hybrids. x 0.5

K = Ketch barley
CS = Chinese Spring wheat

3.1

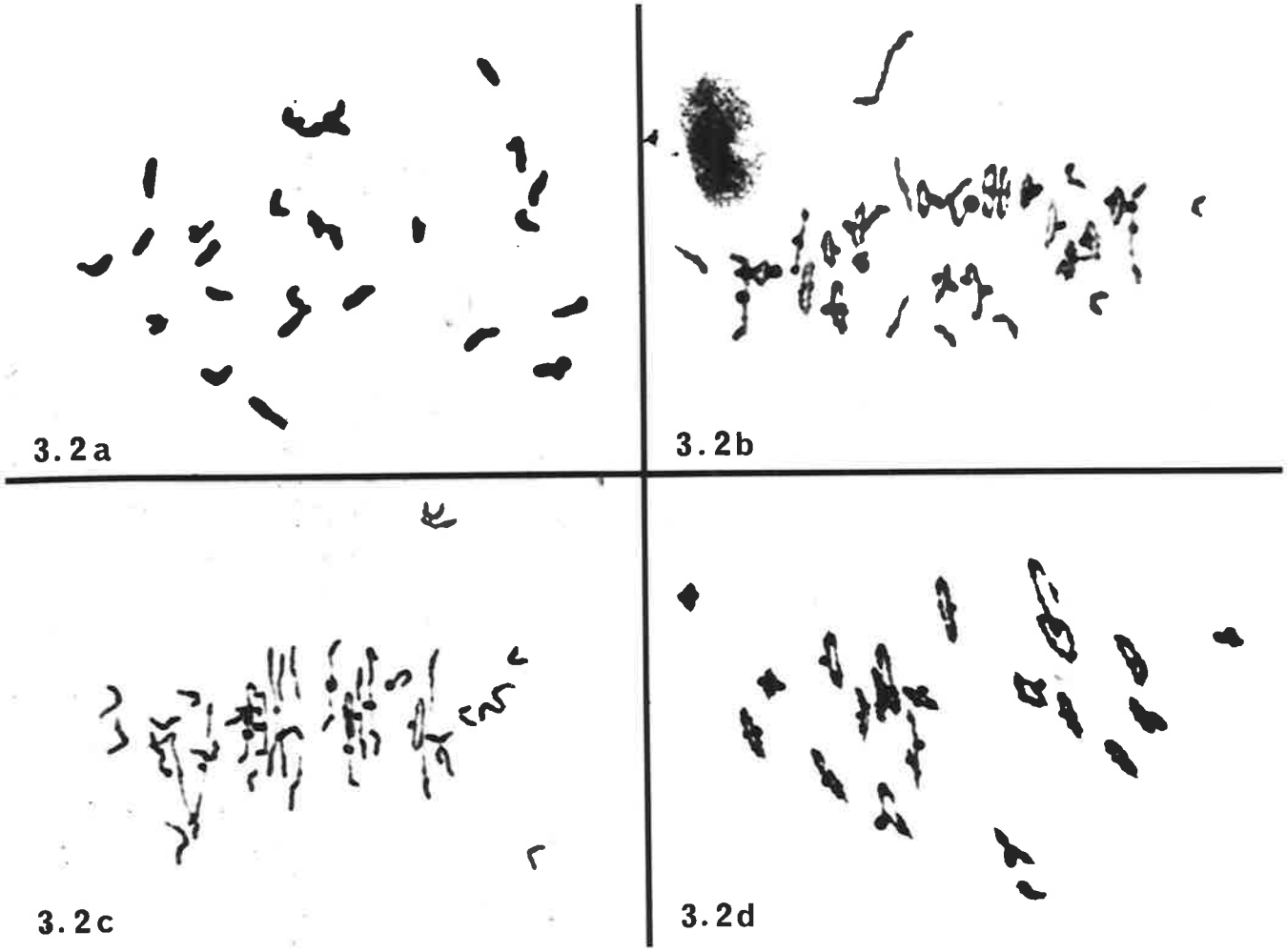
Fig. 3.2a-d: Metaphase I in PMC's from barley x wheat material.

a. F_1 hybrid Ketch x CS showing 28 univalents. x 900

b. 1st backcross plant from (Ketch x CS) x CS showing 21" + 7' in a PMC. x 1040

c. Another PMC from the same plant showing 15" + 19'. x 1060

d. Monosomic addition line from (Ketch x CS) x CS^2 showing $21"_W + 1'_B$. x 950



3.2a

3.2b

3.2c

3.2d

The attempt to produce a barley x wheat amphiploid failed, as all 67 F_1 plants treated with colchicine remained self-sterile and no viable pollen was observed in any of their anthers. However, these plants were partly female fertile when they were pollinated with the wheat parent, giving backcross seed (BC_1) which, unlike the F_1 seed, developed to maturity on the hybrid without requiring special treatment (Table 3.2).

Whereas most of the F_1 plants had normal floral morphology, a few plants exhibited partial pistillody. The spikes on these plants possessed a variable number of florets having one or two stamens replaced by pistil-like structures.

First backcross generation

Twenty-seven BC_1 plants were grown from 3, 10 and 14 seeds produced on Clipper x CS, Ketch x CS and Prior x CS hybrids, respectively. The plants resembled Chinse Spring in morphology (Fig. 3.1), except that pistillody was widespread, many spikes exhibited increased awn development and three plants produced speltoid spikes.The expression of pistillody was much more pronounced than in the F_1 generation but, nevertheless, it was variable in its incidence. Thus four plant produced normal bisexual florets only, while at the other extreme four plants produced pistilloid florets only (Fig. 3.3). The remaining 19 plants exhibited partial pistillody, in that some florets were completely bisexual whereas others were completely or partially pistilloid. Commonly these plants were completely pistilloid initially, but produced a few anthers in spikes from the late tillers.

The chromosome number of 12 of the BC_1 plants was determined from root tip mitoses and nine of them possessed 49 chromosomes, whereas

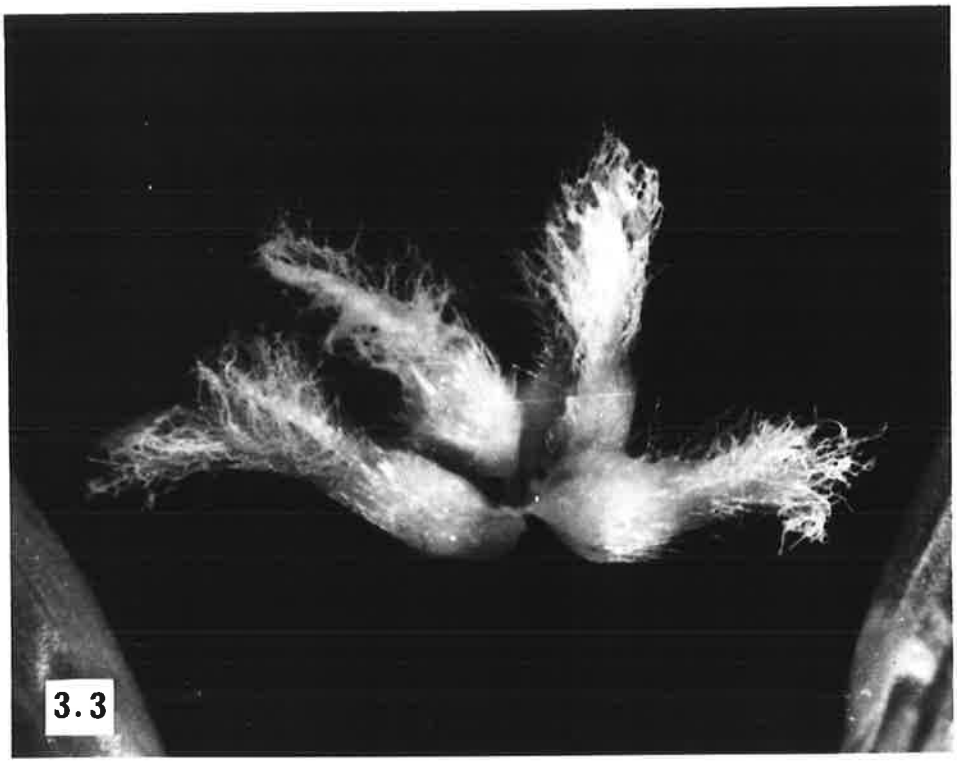
TABLE 3.2: Seed set in first backcross generation

Cross	Spikes pollinated	Seeds obtained	Seeds/spike
(Ketch x CS) x CS*	62	40	0.7
(Clipper x CS) x CS	54	14	0.3
(Prior x CS) x CS	130	91	0.7
(Betzes x CS) x CS	10	5	0.5
(Ketch x Gabo) x Gabo	20	24	1.2
(Clipper x Gabo) x Gabo	6	3	0.5

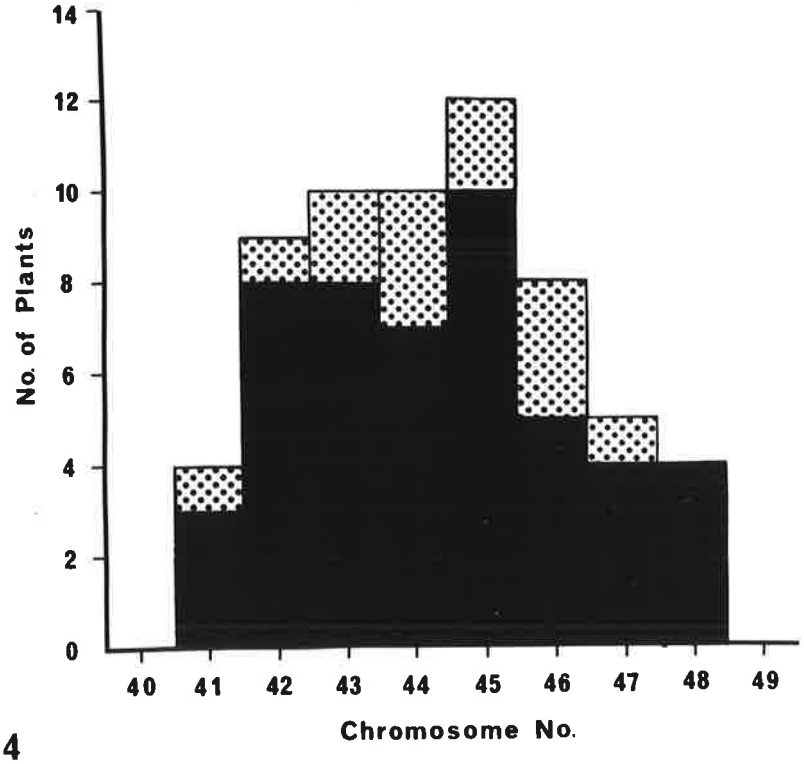
*CS = Chinese Spring

Fig. 3.3: Pistilloid floret of 1st backcross plant (Ketch x CS) x CS possessing 4 pistil-like structures instead of the normal 3 stamens and 1 pistil. x 17.5

Fig. 3.4: Somatic chromosome numbers in 2nd backcross progeny : (Ketch x CS) x CS^2 (46 plants) and (Clipper x CS) x CS^2 (16 plants). Plants represented by stippled area possessed one telo in the chromosome complement



3.3


14
12
10
8
6
4
2
0
No. of Plants
40
41
42
43
44
45
46
47
48
49
Chromosome No.

3.4

the others had 47 or 48 chromosomes. These observations suggested that the majority of the functional egg cells in the F_1 hybrids possessed a 28- chromosome restitution nucleus which produced 49-chromosome heptaploids when fertilized with wheat pollen. Meiotic studies were required to confirm their heptaploid make-up, but the widespread occurrence of pistillody limited the material available for such analysis. Furthermore, even when PMC's at metaphase I were located, frequently it was difficult to deduce their chromosome constitution because of asynapsis. Despite these difficulties, it was possible to confirm that several BC_1 plants were heptaploid since they exhibited a maximum chromosome association of 21" + 7' in some PMC's (Fig. 3.2b), even though other meiocytes of the same plants possessed up to 19 univalents (Fig. 3.2c). Since heptaploids are of key importance to the production of addition lines by O'Mara's (1940) method, we could now proceed with our programme even though we had failed to obtain the amphiploid. The BC_1 plants were again self-sterile, but seeds (BC_2) were obtained by backcrossing with wheat pollen (Table 3.3).

Second backcross generation

The chromosome numbers of BC_2 plants were determined from root tips in a search for 43- chromosome plants, representing putative monosomic addition lines. As expected, a wide array of chromosome numbers was observed among the 62 plants examined (Fig. 3.4) including eight plants with 43 complete chromosomes. It was planned to check the chromosome constitution of the BC_2 plants at meiosis but the widespread occurrence of pistillody severely restricted this programme. In general, pistillody was more pronounced than among BC_1 plants, and at least 50% of the BC_2 plants remained pistilloid throughout flowering.

TABLE 3.3: Seed set in second backcross generation

Cross	Spikes pollinated	Seeds obtained	Seeds/spike
(Ketch x CS)* x CS^2	120	161	1.3
(Clipper x CS) x CS^2	51	38	0.7
(Prior x CS) x CS^2	35	14	0.4

*CS = Chinese Spring

However, there was no consistent pattern in the expression of pistillody in the other plants. There was a tendency for plants with higher chromosome numbers (47 or 48) to produce more anthers, but there were some exceptions. Furthermore, some plants with 43 to 46 chromosomes exhibited complete pistillody initially and partial pistillody in late spikes, whereas a few plants even showed the reverse trend.

Our progress in determining the chromosome constitution at meiosis of the eight 43- chromosome plants is summarized below and in Table 3.4.

Plant No.1 - This plant was completely pistilloid during the first 6 to 7 weeks of flowering, but it produced some anthers in spikes from the late tillers. Metaphase I in PMC's from these anthers showed 21" + 1' regularly in many cells (Fig. 3.2d). Clearly, this plant has one barley chromosome added to the wheat complement and it represents a monosomic addition line. Further backcrossing of the plant with wheat pollen produced 30 seeds, which should include some 43-chromosome individuals to maintain the line.

Plant No.2 - This plant was unusual in that it produced three anthers in all florets. However, meiosis was very abnormal and the majority of the PMC's exhibited a high degree of polyploidy, with much chromosome stickiness. The maximum chromosome association observed in non-polyploid PMC's was 19" + 5'. However, one PMC had 42" + 2' which could represent either a binucleate cell having 21" + 1' in each nucleus, or a uninucleate cell having one polyploid nucleus. If the former possibility is correct, this plant would also be a monosomic addition line. Only nine seeds formed on 50 spikes pollinated with wheat

TABLE 3.4: Characteristics of plants with 43 chromosomes

Plant No.	Morphology of floret	Meiotic pairing	Backcross progeny seeds
1	Pistilloid (late anthers)	21" + 1'	30
2	Normal	19" + 5' 42" + 2'	0
3	Normal	20" + 3'	50
4	Pistilloid (late anthers)	20" + 3'	0
5-8	Pistilloid	not observed	0

pollen. Unfortunately all of the seeds aborted early, presumably due to abnormal meiosis in the ovules giving eggs with unusual chromosome numbers.

Plants No. 3 and 4 - Although these plants differed in their expression of pistillody (Table 3.4) they both showed a maximum chromosome association of 20" + 3' in PMC's. Hence we cannot decide whether they are monosomic addition lines which have some asynapsis or whether they have 41 wheat chromosomes and two barly chromosomes. We hope to determine the chromosome constitution of plant No.3 from analysis of its progeny seed, obtained following pollination with wheat.

Plants No. 5 - 8 - These plants have remained completely pistilloid, but we are still checking them for the appearance of late anthers.

DISCUSSION

The present study, and that of Kruse, have demonstrated that wheat and barley can be hybridized without difficulty, provided that barley is used as the female parent. Furthermore, hybrid plants can be obtained if the developing embryos are first treated with gibberellic acid and later transferred to artificial culture. Our rate of success in making hybridizations, which averaged 5.8% over all crosses and reached a maximum of 15.4% when Chinese Spring was crossed with Betzes, was much greater than the maximum value of 3% obtained by Kruse. The high success rate (10.9%) of Chinese Spring when crossed with barley parallels the earlier discovery that Chinese Spring has superior cross-ability with rye (Riley and Chapman, 1967).

Recently Bates *et al.* (1974) reported briefly on their success in making crosses between durum wheat x barley and bread wheat x barley, but only when the female parent was injected with an immunosuppressant

before pollination. Unexpectedly the hybrids were fertile and had mitotic chromosome numbers ranging from 18 to 21 and from 21 to 36 in the above two hybrids, respectively. We cannot evaluate the significance of these results without knowing the full experimental details, but it is clear from our work that immunosuppressants are not essential for success at least when barley is used as the ovule parent.

Despite attempts to double the chromosomes of barley x wheat hybrids with colchicine, we failed to produce an amphiploid. This was unexpected since the same colchicine treatment applied to barley haploids had resulted in diploid seeds in 90% of the treated plants (Islam, unpublished). Initially we thought that chromosome doubling may be occurring in spike primordia of the hybrids, but the doubled sectors remained undetected because of male sterility induced by an unfavourable nucleo-cytoplasmic interaction. However, the seeds which developed on spikes of colchicine-treated hybrids following pollination with wheat were distributed at random rather than in sectors, indicating that colchicine-induced chromosome doubling had not occurred. Instead, this seed distribution suggests that the fertile eggs are unreduced gametes resulting from meiotic restitution, as found in other interspecific hybrids (Wagenaar, 1968a).

The failure to produce an amphiploid forced us to change our approach for producing addition lines. Instead of utilizing the amphiploid to pollinate wheat and produce a heptaploid with wheat cytoplasm, we had to make use of the backcross heptaploid which possessed barley cytoplasm. Thus our programme was modified towards adding individual barley chromosomes to the wheat chromosome complement in barley cytoplasm (alloplasmic), instead of the more usual procedure of adding the alien chromosome to the wheat complement in

wheat cytoplasm (euplasmic). However, the use of a heptaploid with barley cytoplasm introduced the unforeseen difficulty of pistillody.

Pistillody was not a serious problem on the F_1 hybrids, but as the proportion of barley chromosomes was reduced in subsequent backcrosses to wheat, pistillody became more pronounced, indicating that it is induced by an incompatibility between the nucleus of hexaploid wheat and barley cytoplasm. However, in a few of the second backcross plants, the degree of pistillody was not closely related to their chromosome content, suggesting that the environment is also having an effect. Additional evidence for this comes from the large number of backcross plants which exhibited a change in floret morphology from pistilloid to normal, and rarely from normal to partially pistilloid, during their flowering period. However, we have not been able to correlate the degree of expression of pistillody in our material with any one environmental factor, as have other authors (Kihara, 1951; Fisher, 1972).

Pistillody has prevented meiotic analysis of more than half of our putative addition lines. Furthermore, we found that the pistilloid florets were sterile, as observed by Kihara and Tsunewaki (1961) when the nucleus of T. durum was added to the cytoplasm of Ae. caudata.

Despite these difficulties, we have produced at least one monosomic addition line, identified by the regular occurrence of 21" + 1' at metaphase I in PMC's. This addition line resembles wheat in most respects, except for pistillody, but since it was grown out of season in 1975, a detailed description of its morphology is not warranted. We plan to make such observations on its progeny. It appears from limited observations that few morphological features of barley will be

expressed in the addition lines and we are investigating the potential value of seed proteins and isozymes for identifying barley chromosomes in a wheat background. Already we have found that barley prolamins may be used to identify at least one barley chromosome in wheat, since the prolamins are detectable in the endosperm of the heptaploids and some of the second backcross progeny.

It is evident that the problems encountered in producing wheat-barley addition lines have arisen because the original heptaploid had barley rather than wheat cytoplasm. Hence we have initiated a large-scale crossing programme to obtain hybrids using wheat as the female parent. So far we have obtained only 8 hybrid plants after pollinating over 150 wheat spikes with barley, and several of these hybrids possess peculiar chromosome constitutions. However at least one plant with 28 chromosomes was obtained and first backcross seeds are now being produced.

It is anticipated that the progeny will include a heptaploid with wheat cytoplasm and, if so, we should be able to obtain addition lines without the difficulties previously encountered.

CHAPTER 4: ISOLATION AND CHARACTERIZATION OF EUPLASMIC WHEAT-BARLEY CHROMOSOME ADDITION LINES

A.K.M.R. Islam, K.W. Shepherd and D.H.B. Sparrow
(Submitted for publication)

SUMMARY

Hybridization of Chinese Spring wheat and Betzes barley using wheat as the female parent gave only 1.3% seed set compared to 15.4% obtained with the reciprocal cross made earlier. Furthermore, only one of the 20 F_1 hybrids obtained possessed the normal complement of 28 chromosomes. The others had unusual chromosome numbers ranging from 21 to 36 in different plants. The 28-chromosome normal hybrid was backcrossed to wheat to produce a heptaploid in the first backcross generation (BC_1) and subsequently monosomic (21" + 1') and double monosomic (21" + 1' + 1') additions of barley chromosomes to wheat were isolated in the BC_2 generation. The monosomic additions could be divided into five different phenotypic groups and disomic additions were isolated from among their progeny with a very low frequency (0.63%). However, some near-disomic additions (21" + 1t") obtained from the progeny of selfed monosomics yielded both disomic and ditelosomic additions in their progeny with a much higher frequency. A sixth addition line was obtained independently from three unusual F_1 hybrids exhibiting 22', 21' + 1" and 25' + 1"' at meiosis.

Altogether six of the seven possible disomic additions and seven of the 14 ditelosomic additions of barley chromosomes to wheat have been produced. The chromosome 5 addition could not be obtained in a disomic form because the plants carrying this chromosome are self-sterile. The addition lines were initially characterized by their morphological differences from the wheat parent, and subsequently from isozyme studies and heterochromatic banding (N-banding) of chromosomes. Most of these

lines showed more asynapsis at meiosis than the wheat parent and all, except addition line 4, were less fertile than the wheat parent.

These lines will be useful for associating barley genes with particular barley chromosomes, in determining the genetic similarity of individual barley chromosomes with wheat chromosomes and possibly for transferring desirable agronomic characters from barley to wheat.

INTRODUCTION

Beginning with William Farrer (1904), Australia's pioneer wheat breeder, many attempts have been made to hybridize wheat and barley (Waterhouse, 1930; Gordon and Raw, 1932; Ahokas, 1970) with the aim of producing a new type of crop plant combining desirable features from these two cereals. However, there are no well-substantiated records of any success until the recent work of Kruse (1973). He transferred pollen from diploid, tetraploid and hexaploid wheat on to emasculated spikes of barley and obtained a few hybrid seeds in each case. Furthermore, he was able to produce viable hybrids with the aid of embryo culture. The F_1 hybrids were self-sterile as expected and Kruse obtained only one backcross plant after pollinating the barley x hexaploid wheat hybrid with wheat.

Following this pioneering work of Kruse, attempts were made in our laboratory to hybridize wheat and barley using wheat as the female parent instead of barley. After an initial failure to obtain any seed from a limited number of crosses, barley was then used as the female parent and F_1 hybrids were obtained without difficulty, especially with the combination Betzes barley x Chinese Spring wheat (Islam *et al.*, 1975).

The primary aim of our work was to produce an amphiploid from the hybrid which could be used in further crosses to produce wheat-barley addition lines, following O'Mara's (1940) method for adding individual

rye chromosomes to wheat. It was anticipated that these addition lines would be useful for determining the gene content of barley chromosomes, determining the evolutionary relationship between wheat and barley chromosomes, and possibly for transferring desirable characters from barley to wheat. However, all attempts at producing an amphiploid from barley x wheat F_1 hybrids failed, but some 49-chromosome progeny (heptaploids) were obtained after backcrossing them with wheat pollen. These heptaploids formed 21" + 7' at meiosis indicating that the fertile egg cells in the hybrids must have possessed a 28-chromosome restitution nucleus (Islam and Shepherd, 1980a). The heptaploids were again backcrossed with wheat pollen and a few 43-chromosome monosomic addition lines were isolated from among their progeny. However, these plants exhibited partial or complete pistillody and were all self-sterile.

Pistillody was observed to occur sporadically in the F_1 hybrids, but it became progressively more pronounced as the proportion of barley to wheat chromosomes was reduced in the BC_1 and BC_2 generations. Clearly this pistillody was due to an incompatibility between the nucleus of hexaploid wheat and barley cytoplasm and thus our attempts to produce alloplasmic wheat-barley addition lines proved to be unprofitable.

To overcome this widespread occurrence of pistillody in barley x wheat hybrids, it was decided to renew efforts to make the reciprocal cross so that the F_1 hybrids and derivatives would have the cytoplasm of wheat instead of barley. The present paper describes the production of these wheat x barley hybrids and our progress towards the isolation and characterization of euplasmic disomic addition lines from their progeny.

MATERIALS AND METHODS

The main hexaploid wheat parent used was Chinese Spring but two other cultivars, Gabo and Tobari-66 were used to a limited extent. Tobari-66 was obtained from the CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) organization in Mexico and the other two parents were from stocks held at the Waite Agricultural Research Institute. The barley parents included two local cultivars, Ketch and Clipper, and one of European origin, Betzes.

In making crosses, the wheat spikes were emasculated 1-2 days before anthesis and immediately covered with glassine bags to prevent uncontrolled pollination. Two days after emasculation the spikes were pollinated with barley. One day after pollination a droplet of 25 ppm gibberellic acid was applied to each floret and this was repeated each day for 6-8 days, and after a further 6-8 days the embryos were dissected out and transferred to an artificial culture medium. The culture medium was prepared using the basic ingredients of the medium used by Morrison *et al.* (1959) with added trace elements according to the formulation of Norstog (1973) and 0.2 gm/l of glutamic acid. The pH of the medium was adjusted to 5.0. The embryos were placed on 14-18 day old wheat or barley endosperm tissues which earlier had been dissected out aseptically and planted on the agar medium in culture bottles.

The chromosome constitution of the F_1 hybrids and derivative plants was determined both from root tip cells and pollen mother cells (PMC's). For mitotic studies the root tips were pre-treated for 4 to 5 hours in water saturated with α-bromonaphthalene and then fixed in glacial acetic acid overnight. For meiotic studies individual anthers with PMC's at metaphase I were fixed in a 3:1 mixture of ethanol and

glacial acetic acid. A standard Feulgen staining and squash procedure was used with both root tips and PMC's to prepare material for cytological examination.

Most of the F_1 hybrids were treated with 0.1% colchicine for approximately 72 hours using Bell's (1950) tiller-capping method, in an attempt to induce chromosome doubling.

The growth habit, plant morphology and spike characters of the addition lines were recorded on several plants of each line planted singly in potting compost in 30 cm diameter pots, and grown under natural photo-period in a glasshouse in spring. The seed set on 10 spikes from two or three plants of each addition line was measured also, as an index of their fertility.

RESULTS

The detailed results obtained from hybridizing wheat and barley using wheat as the female parent and the progress towards the isolation of euplasmic addition lines from the hybrid derivatives, are described below.

F_1 hybrids

It was much more difficult to cross wheat and barley using wheat as the female parent than it was with the reciprocal cross where hybrids were obtained readily (Islam *et al.*, 1975). The best results were with Chinese Spring wheat and Betzes barley but only 1.3% of the crosses were successful (Table 4.1) compared to 15.4% in the reciprocal cross (Islam *et al.*, loc. cit.). In the present study, Betzes was a better pollen parent than the Australian cultivars Ketch and Clipper and Chinese Spring was a better pistillate parent than the cultivars Gabo and Tobari-66. Although it was very difficult to obtain seeds from these crosses, using wheat as females, the success rate in culturing

TABLE 4.1: Census data on wheat x barley hybridizations

Cross combination	No. of florets pollinated	Seeds obtained No.	Seeds obtained (%)	No. of embryos cultured	No. of plants obtained
CS* x Ketch	2731	7	(0.2)	3	1
CS x Betzes	3381	44	(1.3)	19	19
CS x Clipper	902	3	(0.3)	0	0
Gabo x Betzes	759	0	(0.0)	0	0
Gabo x Clipper	182	1	(0.5)	0	0
Tobari-66 x Betzes	178	0	(0.0)	0	0

*CS = Chinese Spring wheat

embryos (91%) was better than with the reciprocal cross (49%).

Although 20 hybrid plants were obtained in the present programme, their somatic chromosome number and chromosome pairing pattern at meiosis revealed that only one of them was a proper F_1 hybrid having the expected 28 somatic chromosomes which form 28' at meiosis (Fig. 4.1a). The other 19 hybrids possessed somatic chromosome numbers ranging from 21 to 36. Three of these plants were wheat haploids with 21 chromosomes, five plants possessed a haploid complement of wheat chromosomes plus one (Fig. 4.1b) two (Fig. 4.1c) and four to six different barley chromosomes (Islam and Shepherd, 1980b). The remaining 11 plants had very abnormal chromosome constitutions including duplication and deficiency of some wheat and barley chromosomes. For example, one hybrid plant had 35 somatic chromosomes and formed 15" + 5' at meiosis (Fig. 4.1d).

The normal F_1 hybrid grew vigorously and resembled the wheat parent in morphological characters as was also observed with the reciprocal cross (Islam *et al.*, 1975). The spikes on this plant possessed short awns especially in the upper half of the spike (Fig. 4.2a). The 21-chromosome plants resembled Chinese Spring closely excepting for having narrow stems and spikes (Fig. 4.2e) as expected with wheat haploids. The florets on the spikes of the 23-chromosome plant were somewhat larger in size than those produced by the haploids (Fig. 4.2d). However, the other F_1 plants with very abnormal chromosome constitution produced spikes with numerous abnormalities including excessive awn development, supernumerary spikelets and malformed florets (Figs. 4.2b,c). All of the F_1 hybrids were self-sterile. The normal F_1 hybrid and three of the unusual hybrids have been used in the production of addition lines and only the results obtained with these four hybrids are included in this paper.

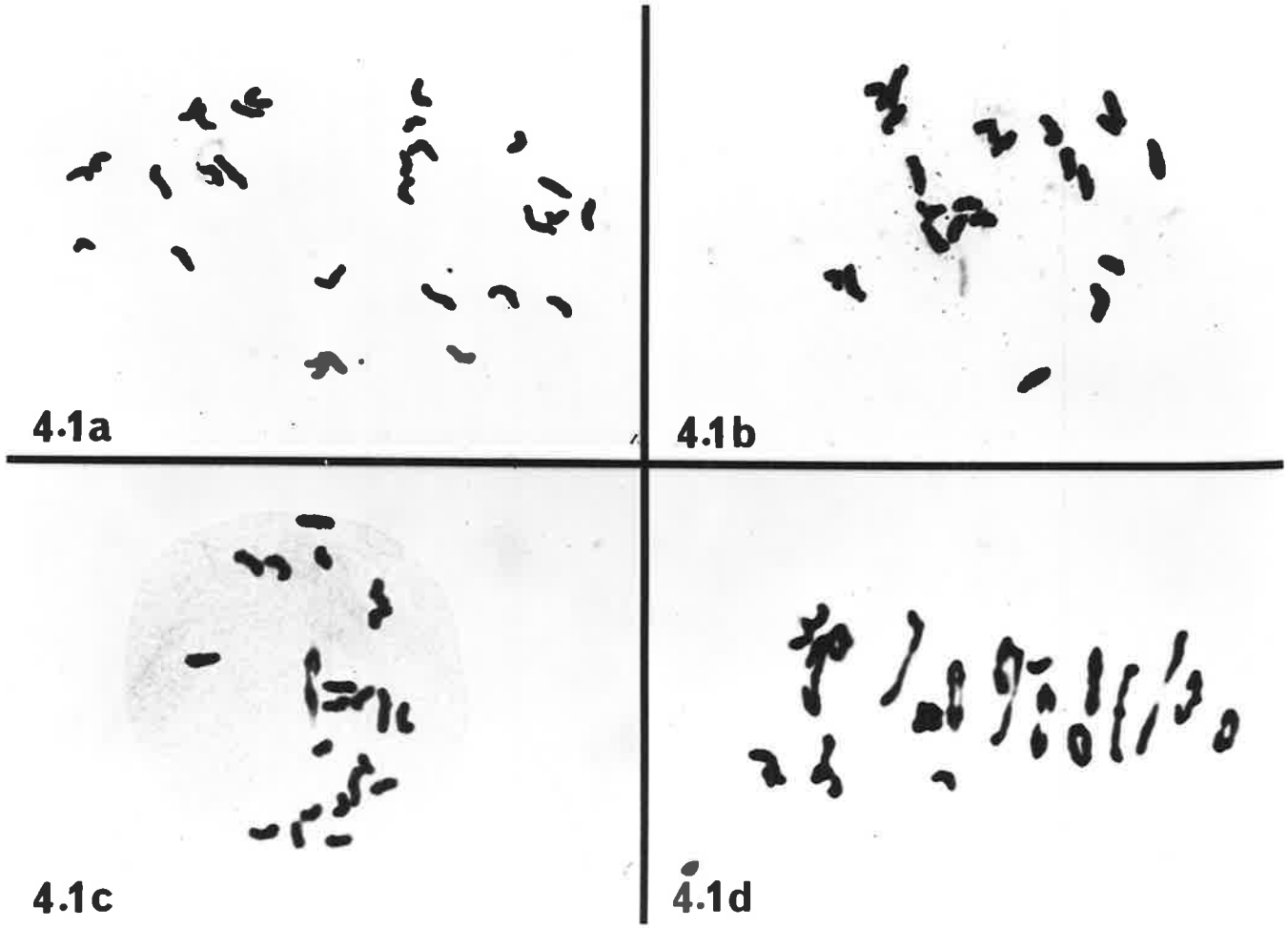
Fig. 4.1a-d: Chromosome configurations at metaphase I in PMC's of some wheat x barley F_1 hybrids

a. 28-chromosome normal hybrid (28'). x 900

b. 22-chromosome hybrid (22'). x 1040

c. 23-chromosome hybrid (21' + 1"). x 920

d. 35-chromosome hybrid (15" + 5'). x 1230



4.1a

4.1b

4.1c

4.1d

Fig. 4.2: Spike morphology of the wheat parent and some wheat x barley F_1 hybrids. x 0.75

C.S. = Chinese Spring parent
a = 28-chromosome normal hybrid
b = 31-chromosome hybrid
c = 27-chromosome hybrid
d = 23-chromosome hybrid
e = 21-chromosome 'hybrid' (haploid wheat)

4.2 F_1 Hybrids

The cytological behaviour and possible origin of the other unusual hybrids are described in detail elsewhere (Islam and Shepherd, 1980b).

The somatic chromosome number of the normal F_1 hybrid was not determined from root tip cells, but 28 chromosomes were observed at mitosis in young ovarian tissue. The majority of the PMC's possessed 28 chromosomes at metaphase I of meiosis but some mosaic cells with aneuploid and polyploid numbers were also observed (Islam and Shepherd, 1980b). Among the 28-chromosome PMC's, the majority exhibited 28' (Fig. 4.1a) but others possessed one or more bivalents with a maximum of five occurring rarely. The average pairing of chromosomes in the 28-chromosome cells was 26.51' + 0.72" + 0.015"' in 128 cells.

An attempt was made to produce an amphiploid from this F_1 hybrid with colchicine treatment. Although doubled sectors with 56 chromosomes were observed at meiosis in some anthers, no seed set was obtained. However, the plant was found to be partly female fertile and seeds were obtained after pollinating it with the wheat parent. Altogether 99 first backcross (BC_1) seeds were obtained with an average of 3.5 seeds/spike, and the seeds matured on the spikes.

Progeny obtained from the normal F_1 hybrid

First backcross generation - Altogether 41 BC_1 plants were grown and their somatic chromosome numbers ranged from 35-76, with 17 (41%) plants being heptaploids with 49 somatic chromosomes. Presumably these plants arose from fertilization of unreduced egg cells resulting from meiotic restitution, as already described (Islam and Shepherd, 1980a). Although there was considerable asynapsis at metaphase I, each of these plants had some PMC's which exhibited 21" + 7', thus confirming their heptaploid status. In addition, these plants exhibited some chromosome mosaicism in PMC's, as described elsewhere (Islam and Shepherd, 1980b).

The 49-chromosome BC_1 plants usually produced fewer tillers than the F_1 hybrid, but they had broader leaves, thicker culms and more compact spikes with larger florets. However, there was some variation between the heptaploid plants for these characters indicating some influence of environment.

In contrast to the fertile heptaploid obtained from wheat x rye crosses (Riley and Chapman, 1958) and wheat x Agropyron crosses (Dvorák and Knott, 1974) the wheat x barley heptaploids were self-sterile like the heptaploids from the reciprocal cross. However, BC_2 seeds were easily obtained after pollinating them with wheat.

Second backcross generation - The somatic chromosome constitution of 240 BC_2 plants was determined from root tip analyses in a search for 43-chromosome putative monosomic addition lines. A wide range of chromosome numbers (35-70) was observed as was found with the reciprocal cross. Altogether 35 plants with 43 somatic chromosomes were detected and 25 of these plants were monosomic additions which exhibited either 21" + 1' (20 plants) or 1^{iv} + 19" + 1' (5 plants) at meiosis. The other 10 plants showed more complicated pairing patterns, including two which exhibited very abnormal meiosis with extreme chromosome mosaicism in PMC's, (Islam and Shepherd, 1980b). The monosomic addition lines were all self-fertile but some of the other BC_2 plants were self-sterile.

The 25 monosomic addition plants could be classified into five different groups based on their morphological differences from normal Chinese Spring wheat and from each other. It was assumed that these groups represented the additions of five different barley chromosomes to wheat and consequently the search for disomic additions was restricted initially to progeny from one or two plants from each of these groups.

A few double monosomic additions (21" + 1' + 1') selected among the BC_2 progeny were also included in the search for disomic additions.

Isolation of disomic and ditelosomic addition lines - The above source material has been used in several different ways to produce the required disomic addition lines. First, root tips from the progeny of selfed monosomic additions were screened cytologically to detect rare 44-chromosome plants, and the results are given in Table 4.2. Although 317 (40%) of the progeny apparently possessed a complete barley chromosome, or part of one, only five disomic addition lines forming 22" at meiosis, were detected.

However, the 25 plants with 43 + t chromosomes in root tips were more useful than the plants with 43 chromosomes as a source of disomic additions. Eleven of these plants were examined at meiosis, and eight of them exhibited 21" + 1t" whereas the other three showed 20" + 1"' + t'. The former plants are near-disomic additions and they proved to be very valuable since, besides being an alternative source of disomic addition lines, they have also yielded ditelosomic addition lines. Six of the 14 possible different additions of barley ditelocentrics to wheat were isolated from among the progeny of such plants.

Although the difference was not significant ($0.2>P>0.1$), the double monosomic (21" + 1' + 1') additions gave a higher yield of disomic addition lines (2.0%) than the simple monosomic additions (0.63%). Besides providing the opportunity of obtaining two different addition lines the expected advantage of using double monosomics for obtaining disomic additions comes from the likelihood that 22-chromosome pollen carrying the extra barley chromosome will suffer less of a competitive disadvantage in fertilization in double monosomic additions than in simple monosomics, because the proportion of normal 21-chromosome wheat pollen having a

TABLE 4.2: No. of plants with indicated chromosome constitution among progeny of monosomic and double monosomic additions

Parent	Chromosome constitution of progeny								Total progeny
	Root tips:	41	42	42+t or 43	43+t	44			44+t	
	PMC's:					21"+2'	22"	20"+1"'+1'		
Monosomic (21"+1')		13	459	278	25	-	5†	9	-	789
Double monosomic (21"+1'+1')		-	34	52	6	4	2†	1	2	101

†Disomic addition lines

selective advantage is expected to be lower in double monosomics as compared to simple monosomics.

Progeny obtained from other F_1 hybrids

Three unusual hybrids with 22, 23 and 28 chromosomes were also used to produce disomic addition lines and their cytological behaviour has already been described (Islam and Shepherd, 1980b).

One line was produced directly from colchicine treatment of the 22-chromosome F_1 hybrid which exhibited 22' at meiosis. This hybrid possessed a haploid complement of wheat chromosomes plus a single barley chromosome and thus the seeds obtained from doubled sectors produced 44-chromosome progeny which showed 22" at meiosis.

Similarly another disomic addition was also obtained directly following pollination of the 23-chromosome F_1 hybrid, exhibiting 21' + 1" at meiosis, with the wheat parent. The majority of the backcross plants possessed 44 chromosomes and showed 22" at meiosis. Thus the F_1 hybrid must have had a haploid complement of wheat chromosomes plus a homologous pair of barley chromosomes and must have formed restituted egg cells with 23 chromosome which gave 44-chromosome disomic addition lines directly, when pollinated with wheat. Subsequently the barley chromosome in one of these lines was found to be markedly heterobrachial, unlike any of the chromosomes in Betzes. It was suspected that this chromosome may possess a pericentric inversion with break points near the end of one arm and near the centromere of the other arm to account for its pronounced asymmetry. This suspicion was confirmed later when it was found that the N-banding pattern of this chromosome matched that of chromosome 4 of barley except for a long inverted segment involving the centromere (Islam, unpublished).

Disomic addition lines were also obtained from the 28-chromosome hybrid which exhibited 25' + 1''' at meiosis. Each of two seeds produced on this plant after pollination with wheat, gave 47-chromosome BC_1 plant which exhibited 21'' + 5' at meiosis and were self-fertile. A supposed disomic addition line with 44 somatic chromosomes and 22'' at meiosis was recovered directly from cytological screening of the progeny of one of these plants. Two other progeny from the same BC_1 plant with 22'' + and 22'' + 2' at meiosis also produced supposed disomic additions (22'') i the next generation of selfing. Two of these three plants with 22'' wer alike in morphology, but the third plant showed increased awn developmer Subsequent tests with these plants, described below, showed that the first two plants were in fact the same addition line whereas the other plant w not an addition line but had 20'' of wheat and 2'' of barley chromosomes.

Isozyme studies showed that the first two plants had barley alcoho dehydrogenase (ADH) whereas the other plant with increased awn developme possessed ADH and glutamic oxaloacetic transaminase (GOT) isozymes of barley, known to be controlled by different chromosomes (Hart *et al.*, 1). Also N-banding showed that chromosome 6B of wheat was absent in the awne plant (Islam, unpublished). Hence this plant was deficient for chromos ne 6B of wheat and possessed two homologous pairs of barley chromosomes. Thus the original BC_1 plant exhibiting 21'' + 5', instead of having 21 pai of wheat chromosomes must have had only 20 wheat pairs, one pair of barl chromosomes and one wheat chromosome (6B) as a univalent. In two of th 44-chromosome progeny obtained from this plant, the univalent wheat chromosome was present as a pair and thus these plants were true disomic additions. However, in the third 44-chromosome plant isolated, the whe t univalent apparently had been lost and another pair of barley chromosome was present, thus giving the plant a constitution of 20'' of wheat and 2' of barley chromosomes.

Besides the disomic addition, a ditelosomic addition for one arm of this chromosome was recovered from the same BC_1 plant. This ditelosomic was obtained from a near-disomic addition (21" + 1t") which had been detected in the progeny of a selfed monosomic addition, which in turn had been isolated from among the progeny derived from backcrossing the BC_1 plant to wheat.

Characterization of the addition lines

The first disomic addition line was isolated from the 23-chromosome F_1 hybrid described above and this line was assigned the letter A. The other three disomic additions recovered subsequently from the F_1 hybrids exhibiting 22' and 25' + 1"' at meiosis, closely resembled addition line A and it was thought that they probably involved the same barley chromosome.

A total of 15 disomic additions were recovered from the five other groups of monosomic additions and these lines were given the arbitrary designations B to F in order of their isolation between groups. Altogether there were five separate isolations of disomic additions in group B, three in group C, four in group D, two in group E and one in group F.

Before characterizing the addition lines it was necessary to prove their authenticity and their individuality. This was accomplished by crossing an addition line from each of the six groups with wheat and also completing a half diallel of intercrosses between the different addition lines. At meiosis, the progenies from these crosses gave 21" + 1' and 21" + 2', respectively, thus confirming that each of the six lines were in fact different.

The morphological features which distinguished the monosomic additions from Chinese Spring were accentuated in the disomic additions, and the plant and spike characters of these lines along with

the parents, are shown in Figs. 4.3 and 4.4, respectively. The principal distinguishing features of the disomic addition lines when grown under glasshouse conditions are described below. The characteristics of the available ditelosomics are also described.

Addition line A - Plants of the disomic addition are shorter (approx. 15 cm) in height than Chinese Spring, have thicker culms and a more erect habit. The leaves are broader than those of Chinese Spring and tend to be darker green in colour. They tend to be later flowering and produce apically awnleted spikes, which often have supernumerary spikelets. The spikes are erect, mid-dense and produce short and oval grains. In the basal half of the spikes, there are commonly 5-7 grains/ spikelet.

The ditelosomic addition available (long arm) is morphologically similar to the disomic addition in having apically awnleted spikes.

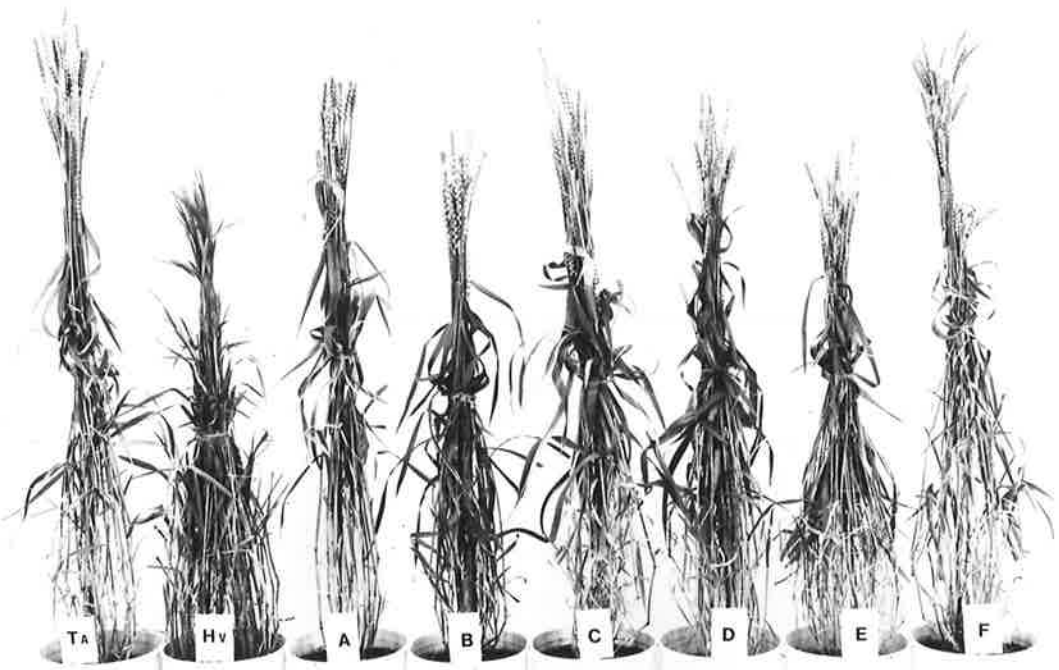
Addition line B - Plants of the disomic addition are also shorter (approx. 20 cm) than Chinese Spring and have even thicker culms and darker green leaves than addition line A. The plants are erect and have spikes which are longer and more lax than those of Chinese Spring, with larger florets and longer anthers. The grains are mid-long to long, oval and larger in size than the wheat parent. These spikes are more prone to shattering.

The ditelosomic addition available (long arm) resembles the disomic addition in having lax spikes with larger florets.

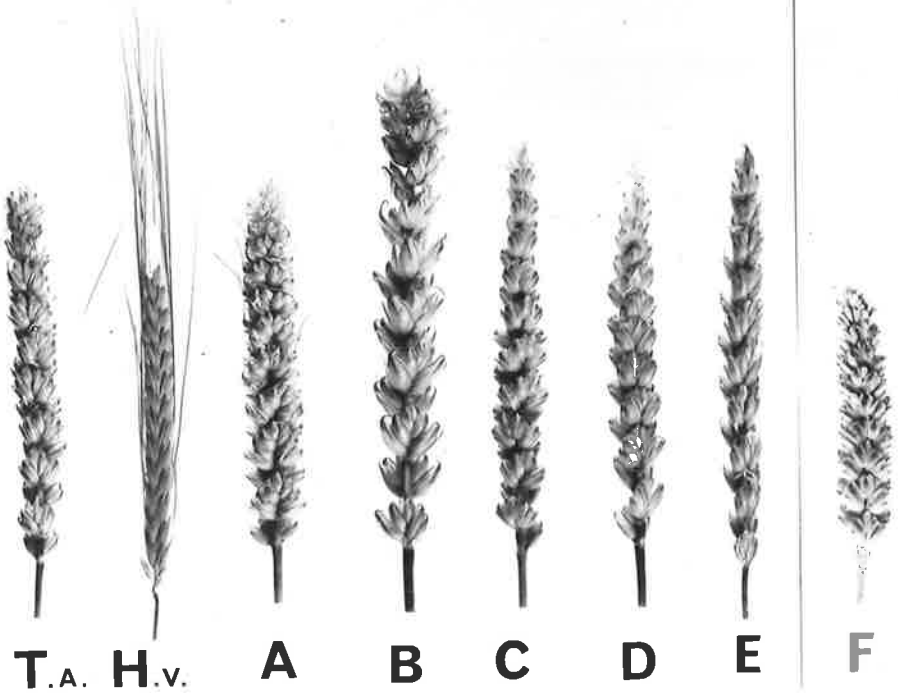
Addition line C - Plants of the disomic addition are later in heading than Chinese Spring. The spikes are erect but taper towards the tip and the beaks on the lemma of some florets protrude outwards in contrast to the normal inwardly-curved beaks of Chinese Spring. The grains are short and oval and are produced mainly in the lower half of the spike.

Fig. 4.3: Plant morphology of Chinese Spring wheat (T.A.); Betzes barley (H.V.); Disomic additions A,B,C, D,E and F (line F is near-disomic with 21" + 1t"). x 0.06

Fig. 4.4: Spike morphology of Chinese Spring wheat (T.A.); Betzes barley (H.V.); Disomic additions A,B,C, D,E and F.x0.05



4.3



4.4 T.A. H.V. A B C D E F

The ditelosomic addition available (satellited arm) produces tapered spikes like the disomic addition but the spike is smaller and there is no outward protrusion of the beaks of the lemma.

Addition line D - Plants of the disomic addition are bushy at the juvenile stage, but have normal growth habit at maturity. The plants tiller more profusely than Chinese Spring and flowering continues over a longer period. The spikes are erect, mid-dense and have larger florets with longer anthers, particularly in the basal half. Seed set is low but again the majority of the seeds are located in the basal half of the spike. The grains are mid-long and larger in size than those of Chinese Spring.

Both ditelosomics are available and one (long arm) produces larger spikes like the disomic addition but the fertility is higher. The other line (short arm) produces smaller spikes than the disomic but the fertility is again higher.

Addition line E - Plants of the disomic addition are also bushy at the juvenile stage and tiller profusely, but they are readily distinguished from addition D by their narrow leaves and prostrate growth habit. Subsequently the plants develop slender culms and are a little later in heading than Chinese Spring. The spikes are inclined, narrow and have numerous awnlets. The spikes do not thresh as freely as those of Chinese Spring and the grains are short to mid-long and elliptical in shape.

The ditelosomic addition available (short arm) has much larger spikes than the disomic addition and its fertility is also higher.

Addition line F - The disomic addition plants resemble Chinese Spring in culm and leaf characters more than any of the other addition lines but they usually produce more tillers. They head a little later than Chinese Spring and the spikes are erect to inclined, but have variable density and length. In some plants the spikes resemble those of Chinese Spring, whereas in others the spikes are much smaller in size and more dense than Chinese Spring. The grains are short and oval.

The ditelosomic addition available (short arm) produces spikes which are indistinguishable from those of Chinese Spring.

Although the addition lines are all morphologically different from the wheat parent, they do not exhibit any obvious morphological characters of barley and therefore gross morphology has little or no analytical value for identifying which specific barley chromosomes are present in the wheat background. Biochemical characters were then studied in an attempt to overcome this problem and to characterize further the addition lines. Barley prolamins (hordeins) have been used to identify one barley chromosome (G) (Islam *et al.*, 1975), but when present in wheat this chromosome causes sterility (Islam and Shepherd, 1980b) and thus it has not been possible to produce a disomic addition involving chromosome G. Several barley isozymes have also proved useful in identifying the barley chromosomes present in addition lines (Hart *et al.*, 1980) and a summary of these results is given in Table 4.3.

Although these isozyme studies helped to characterize four different addition lines, they did not reveal which barley chromosomes, in terms of their standard nomenclature, were present in the addition lines. This problem was solved by applying heterochromatic banding (N-banding) to the somatic chromosomes of disomic barley, barley trisomics and the addition lines (Islam, 1980). These results allowed the arbitrary

TABLE 4.3: Disomic wheat-barley addition lines and the morphological and biochemical characters associated with them

Addition line		Morphological differences from Chinese Spring	Biochemical* characters of barley
Initial designation	Barley chromosome present (Islam, 1980)		
A	4	Thick culms and apically awnleted spikes	ADH
B	7	Thick culms and lax spikes with larger florets and longer anthers	-
C	6	Tapered spikes, with outwardly-curved beaks on lemmas	GOT and AMP
D	1	Many tillered with prolonged flowering habit and poor fertility	EST-1,-2 and EP
E	2	Slender culms with narrow leaves and narrow, elongated florets	-
F	3	Similar to Chinese Spring but the spikes are usually smaller and more dense	EST-3,-4
G	5	Not obtained because of sterility	Hordeins

*ADH = Alcohol Dehydrogenase; GOT = Glutamic Oxaloacetic Transaminase; AMP = Aminopeptidase; EP = Endopeptidase; EST = Esterase

From Hart *et al.* (1980) and Islam and Shepherd (1980b).

designations given to these addition lines initially to be replaced, as shown in Table 4.3 with the standard numbering system used for barley chromosomes (Burnham and Hagberg, 1956; Ramage *et al.*, 1961). Thus this table provides the proper designations for the addition lines and a summary of the features which can be used to characterize them.

Meiotic pairing, fertility and stability of the addition lines

To determine whether the added barley chromosomes have any effect on the chromosome pairing behaviour of wheat, the pairing configurations in the six monosomic addition lines available were compared to those occurring in Chinese Spring wheat. The distribution of univalents in the classes 0 and 2 in wheat were compared with the distribution of univalents in the classes 1 and 3 or more, for each of the monosomic additions, in a 2 x 2 contingency table using a X^2 test of independence. As shown in Table 4.4 monosomic additions A and B showed much more asynapsis than Chinese Spring but the difference was significant only with addition A ($0.05>P>0.02$). The level of asynapsis in the other four monosomic addition lines was similar to that observed in Chinese Spring wheat.

The frequency of unpaired chromosomes in the disomic additions was much greater with additions A and D compared to the other lines. However, it is not clear whether the univalents involve mostly unpaired barley chromosomes or some wheat chromosomes. With addition line A, at least, some wheat chromosomes must have failed to pair to give the cells with four univalents (Table 4.5).

There was no evidence of any meiotic pairing between wheat and barley chromosomes in any of the PMC's examined, from the monosomic and disomic addition lines.

TABLE 4.4: Frequency of univalents at metaphase I in the monosomic addition lines and the wheat parent

Genotype	No. of plants used	No. of PMC's	No. (%) of univalents					
			0'	1'	2'	3'	4'	5'
Parent								
CS†	3	117	112 (95.7)	-	5 (4.3)	-	-	-
Monosomic addition								
A	2	139	-	117 (84.2)	-	22* (15.8)	-	-
B	5	298	-	267 (89.6)	-	31 (10.4)	-	-
C	5	173	-	168 (97.1)	-	5 (2.9)	-	-
D	4	151	-	144 (95.3)	-	6 (4.0)	-	1 (0.7)
E	5	213	-	202 (94.8)	-	11 (5.2)	-	-
F	2	137	-	129 (94.2)	-	7 (5.1)	-	1 (0.7)

† CS = Chinese Spring wheat

* Significantly more asynapsis than present in Chinese Spring ($0.05>P>0.02$)

TABLE 4.5: Chromosome pairing configurations at metaphase I in the disomic addition lines and the wheat parent

Genotype	No. of plants used	No. of PMC's	No. (%) of PMC's exhibiting				
			21"	20"+2'	22"	21"+2'	20"+4'
Parent							
CS†	3	97	93 (95.9)	4 (4.1)	-	-	-
Disomic addition							
A	3	186	-	-	143 (76.9)	35 (18.8)	8 (4.3)
B	2	69	-	-	63 (91.3)	6 (8.7)	-
C	2	51	-	-	46 (90.2)	5 (9.8)	-
D	2	79	-	-	67 (84.8)	12 (15.2)	-
E	3	140	-	-	138 (98.6)	2 (1.4)	-
F	1	80	-	-	72 (90.0)	8 (10.0)	-

† CS = Chinese Spring wheat

Although the monosomics and disomics were grown in different years, similar fertility differences from the wheat parent are apparent within each group (Table 4.6). The disomic additions except addition line A, were all less fertile than Chinese Spring. Although disomic addition line A was the least stable meiotically it was clearly the most fertile of the lines. Addition line D had the lowest fertility of any line, both in monosomic and disomic condition, even though meiosis in the monosomic addition was as regular as in Chinese Spring. Thus the decreased fertility in this addition line could not be wholly ascribed to meiotic irregularity but presumably has a genetic component also. The anthers in this line tend to be non-dehiscent and this could have contributed to the decreased fertility.

The stability of these addition lines in terms of their breeding behaviour is set out in Table 4.7. There was no close correspondence between stability and meiotic behaviour since addition line B was the least stable but showed similar meiotic behaviour to addition lines C and F. Furthermore, addition line A exhibited the most irregular meiosis but stability was intermediate between B and the other additions.

DISCUSSION

The present work when compared with the earlier study (Islam *et al.*, 1975) demonstrated a marked reciprocal difference in the success rate of hybridizing wheat and barley. Thus the Betzes x Chinese Spring cross made earlier gave 15.4% hybrid seed set whereas the same parental combination in the reciprocal cross yielded only 1.3% seed in the present study. McFadden and Sears (1944, 1946) also reported a reciprocal difference in crosses between Ae. squarrosa L. and T. dicoccum Schrank. They failed to obtain any hybrid seed when Ae. squarrosa was used as the female parent but they had no difficulty in making the

TABLE 4.6: Fertility* of the monosomic and disomic addition lines

Addition line	Grains/spikelet†		Grains/spike††	
	Monosomic	Disomic	Monosomic	Disomic
A	3.31 ± 0.09	3.62 ± 0.16	66.3 ± 2.3	74.7 ± 4.5
B	2.58 ± 0.17	2.63 ± 0.13	39.9 ± 3.2	42.7 ± 2.7
C	3.18 ± 0.22	2.54 ± 0.11	59.6 ± 4.6	50.7 ± 3.7
D	2.03 ± 0.18	1.68 ± 0.16	27.8 ± 2.4	29.2 ± 2.8
E	2.95 ± 0.09	2.62 ± 0.17	52.1 ± 1.8	44.5 ± 1.9
F	2.66 ± 0.05	2.88 ± 0.11	41.2 ± 1.0	46.0 ± 2.5

* = Average of 10 spikes

Chinese Spring gave figures of 3.70 ± 0.18† and 65.2 ± 2.8††, when grown under the same conditions as the disomic addition lines

TABLE 4.7: Chromosome constitution of progeny of the selfed disomic addition lines

Addition line	No. of progeny analysed	% progeny with indicated chromosome number				
		42	43	44	45	others
A	50	4.0	6.0	86.0	-	4.0
B	30	-	23.0	74.0	3.0	-
C	31	-	7.0	93.0	-	-
D	36	-	-	97.0	3.0	-
E	35	-	4.0	92.0	4.0	-
F	40	-	3.0	97.0	-	-

reciprocal cross. Röbbelen and Smutkupt (1968) observed a similar reciprocal difference in crosses involving Chinese Spring wheat and Petkuser rye. They obtained 61% seed set in wheat x rye crosses but only 1% seed set in the reciprocal cross. The low seed set obtained in the latter cross was attributed partly to the relatively slow growth of wheat pollen tubes in the style of rye. However, the reason for lower seed set in the wheat x barley cross is not known. Furthermore, it should be noted that the present work differs from those cited above in that the best cross success was obtained when the lower ploid parent was used as the female. It is well known that Chinese Spring wheat crosses more readily with rye than do most other wheat cultivars, because it possesses the recessive alleles of the crossability genes Kr_1 and Kr_2 located on chromosomes 5B and 5A, respectively, (Riley and Chapman, 1967). Chapman *et al.* (1976) and Snape *et al.* (1979) provided evidence that the same gene loci may control crossability of wheat with H. bulbosum. Although we have no direct evidence on this, it is possible that these genes may also be involved in crossability with barley.

An unexpected problem encountered with wheat x barley crosses was the abnormal chromosome constitution of most of the F_1 hybrids in contrast to the regular 28-chromosome F_1 hybrids obtained in the reciprocal cross. The cause of these abnormal hybrids is unknown but it has been postulated that they may arise from spindle abnormalities at the early zygotic divisions of the hybrid embryos possessing wheat cytoplasm (Islam and Shepherd, 1980b).

The failure to obtain fertile sectors on the normal 28-chromosome wheat x barley hybrid cannot be ascribed to the failure of colchicine to achieve chromosome doubling, since doubled sectors were observed at meiosis in at least one of the spikes of the treated plant. The BC_1 plants (heptaploids) were self-sterile in contrast to the similar

heptaploids from wheat x rye crosses (Riley and Chapman, 1958) and wheat x Agropyron elongatum crosses (Dvorák and Knott, 1974) which are both self-fertile. The BC_2 plants on the other hand segregated for self-fertility depending on whether they possessed chromosome 5 of barley (Islam and Shepherd, 1980b). Plants lacking chromosome 5 were always self-fertile including one plant which had 47 + t chromosomes. Thus it is likely that the self-sterility of the heptaploids and the failure to obtain fertile sectors in colchicine doubled F_1's is due to the presence of chromosome 5. Furthermore, it was determined that the sterility factor(s) is present on the long arm of barley chromosome 5 since a translocation line was obtained having the short arm of barley chromosome 5 joined to an unidentified arm of a wheat chromosome and this line is self-fertile (Islam and Shepherd, 1980b). However, sterility due to cytoplasmically-induced pistillody which occurred in the barley x wheat hybrids and their derivatives (Islam *et al.*, 1975) was not observed in any of the plants produced in the present programme since they all possessed wheat cytoplasm.

The low frequency of disomic additions among the progeny of the monosomic additions was not unexpected, since it is well known that such monosomics will produce a high proportion of euploid pollen and this pollen will have a distinct competitive advantage over any 22-chromosome pollen bearing the alien chromosome in achieving fertilization. The transmission rate of alien chromosomes in 22-chromosome pollen in a wheat background, was found to vary with different alien species. Riley and Chapman (1958) and Evans and Jenkins (1960) reported a low transmission rate for the rye chromosomes in selfed monosomic additions of rye to wheat. Sears (1956) estimated the transmission frequency of an Ae. umbellulata chromosome ($6C^u$) in selfed monosomics to be only 1.3%. However, a high transmission rate of 25% for an A. intermedium telocentric was reported by Knott (1964).

Double monosomic additions seem to be a better source than simple monosomic additions for the isolation of disomic additions. Thus besides a tendency for a higher frequency of disomics from the double monosomics they also provide the opportunity to obtain two different addition lines. The higher frequency of disomic progeny obtained from double monosomics in the present study, although not significant, is nevertheless expected on theoretical grounds because the frequency of euploid pollen with a competitive advantage will be less in these compared to simple monosomics. Hyde (1953) also used double monosomics to produce five disomic additions of Haynaldia chromosomes to wheat. Cauderon (1966) obtained 13% disomics from among the progeny of a selfed double monosomic addition of Agropyron chromosomes to wheat. However, one particular Agropyron chromosome (TAF 2) was found to be transmitted through the pollen with high frequency. On the other hand, Evans and Jenkins (1960) failed to isolate any disomic additions of rye chromosomes from the double monosomic additions used in their study.

In the present study, plants forming 21" + 1t" at meiosis proved particularly useful because they not only yielded disomic additions and ditelosomic additions, but these occurred with high frequency in their progeny.

In characterizing the disomic addition lines at least five of them can be recognized by their distinctive morphological differences from Chinese Spring with respect to growth habit, culm and leaf width and spike morphology. Similar morphological changes have been noted by Riley and Chapman (1958), Kimber (1967b) and Dvorák and Knott (1974) with wheat-rye, wheat-Aegilops and wheat-Agropyron addition lines, respectively. However, most of the phenotypic modifications in wheat-barley addition lines are quantitative in nature as was noted also by Riley and Chapman

(1958) with wheat-rye addition lines. There are some similarities between the phenotypic alterations caused by rye chromosomes when added to wheat and the barley chromosome additions described in this study. For example, barley addition 2 resembles rye addition 2R (= III, Riley and Chapman, 1958; = B, Sears, 1968) and tetrasomics 2A and 2D of wheat (Sears, 1954) in having narrowing of all organs. This observation raises the possibility that just as this rye chromosome is known to be homoeologous with group 2 chromosomes of wheat (Koller and Zeller, 1976), chromosome 2 of barley may also be related to these chromosomes.

Although their altered phenotype helped in the recognition of individual addition lines, these characteristics had no analytical value for determining which particular barley chromosome had been added to wheat. Biochemical characters such as barley prolamins and barley isozymes have proved much more useful in this respect (Hart *et al.*, 1980). However, the final characterization of the addition lines in terms of the standard numbering system used for barley chromosomes depended on comparing the heterochromatic banding pattern of the extra chromosome in these addition lines with the banding pattern of the extra chromosome in trisomic barley lines (Islam, 1980). Thus it became possible to replace the arbitrary letter designations A to F with the standard numbers assigned to barley chromosomes (Burnham and Hagberg, 1956; Ramage *et al.*, 1961) and it is recommended that future reference to these lines should use these standard numerical designations, at least until their homoeologous relationship with wheat chromosomes is determined.

The meiotic behaviour, fertility and breeding behaviour of the addition lines are of interest because not only do they indicate the degree to which the barley chromosomes integrate with the genetic system of wheat, but they also influence the ease with which these lines can be maintained.

The extent of asynapsis of chromosomes at metaphase I was similar to that occurring in the wheat parent, except with addition line 4 and to a lesser extent in addition line 7. Riley (1960) observed similar variation in meiotic pairing behaviour in different wheat-rye additions. However, Kimber (1967b) reported only occasional pairing failures in his wheat-Aegilops disomic addition lines.

None of the wheat-barley addition lines were completely stable, but there was no close relationship between stability and meiotic regularity as was also observed by Riley (1960) in wheat-rye addition lines. The stability was, however, within the range reported in wheat-rye (Riley, 1960) and wheat-Agropyron (Cauderon *et al.*, 1973; Dvorák and Knott, 1974) addition lines. The lack of stability in addition line 7 makes it essential to check its chromosome constitution in each generation to maintain the integrity of this line.

The estimates of fertility come only from a few plants grown under glasshouse conditions but there was no close correspondence between fertility and degree of meiotic stability. It is likely that genetic factors are more important in determining fertility than the degree of chromosome pairing. Thus if pairing failure occurs with the alien chromosomes, this will tend to increase the frequency of euploid pollen which will result in increased rather than reduced fertility. In general, all of the disomic addition lines have good fertility except for addition line 1, but even in this case it is better than the fertility of rye addition III (Riley, 1960). However, addition line 5 could not be obtained because this barley chromosome causes sterility when added to wheat. Furthermore, this line cannot even be maintained as a monosomic addition because of its extremely low female fertility. It can, however, be maintained as a double monosomic addition whenever chromosome 6 of barley is also present (Islam and Shepherd, 1980b).

To summarize it is clear from the different tests applied that six of the seven possible disomic additions and seven of the 14 possible ditelosomic additions of barley chromosomes to wheat, have been produced in this laboratory. These addition lines will be useful in determining the gene content of individual barley chromosomes. Already their value for locating genes controlling barley isozymes to particular barley chromosomes has been shown (Hart *et al.*, 1980). Furthermore, we are interested in substituting barley chromosomes for individual wheat chromosomes to determine whether wheat and barley chromosomes might have retained the same gene synteny relationship during their separate evolutionary development. Again the chromosomal location of the same isozymes in wheat and barley is expected to help this programme by indicating which wheat and barley chromosome to include in the substitution tests. Finally and more importantly it may be possible to use the wheat-barley addition lines to transfer some desirable agronomic characters of barley to wheat.

CHAPTER 5: MEIOTIC RESTITUTION IN WHEAT:BARLEY HYBRIDS

A.K.M.R. Islam and K.W. Shepherd. Chromosoma (Berl.) In press (1980)

ABSTRACT

Meiotic restitution occurs in pollen mother cells (PMC's) of reciprocal F_1 hybrids between wheat and barley. In occasional PMC's, all or most of the 28 chromosomes assemble at the equatorial plate at metaphase I, but instead of undergoing anaphase I separation they reform into a mass of chromatin to form a restitution nucleus. Some of these restituted nuclei undergo a regular second division and dyads are produced among other non-restituted cells which have reached the tetrad stage of division. Other restituted nuclei fail to undergo a second division and then the PMC's appear as monads among neighbouring tetrads. Both the monads and dyads are expected to produce microsporocytes with the diploid complement of chromosomes. Chromosomes which fail to become included in the restituted nucleus form separate micronuclei and, depending on whether they undergo a regular second division or not, the PMC's containing them eventually appear as tetrads, triads or dyads. These partially restituted nuclei are expected to produce unreduced gametes, deficient for one or more chromosomes. It is postulated from these observations that restitution in wheat:barley F_1 hybrids depends on a high frequency of univalent accumulation at the equatorial plate at metaphase I and the subsequent failure of the chromosomes to undergo anaphase I separation.

INTRODUCTION

There are numerous reports in the literature referring to the partial fertility of a range of interspecific and intergeneric hybrids, even though these hybrids exhibit little or no chromosome pairing at meiosis. The fertility of these hybrids has been ascribed to the production of unreduced gametes arising from some form of meiotic restitution. Since this process confers partial fertility on otherwise sterile F_1 hybrids it has obvious evolutionary significance as emphasized by Harlan and de Wet (1975) in a recent review.

It is evident from the literature that restitution can arise in a variety of ways. For example in F_1 hybrids between Triticum polonicum Vav. x Haynaldia villosa (L.) Schur, Kihara and Nishiyama (1937) observed that while PMC's formed equatorial plates, the chromosomes failed to move to the poles in some of them so allowing restitution to occur. In Oryza sativa L. x O. officinalis Wall. hybrids, Li *et al.* (1964) found occasional PMC's which possessed nuclei located to one side of the equator and the chromosomes from these nuclei did not undergo normal first division separation, possibly due to a delay in spindle formation. They concluded that the chromosomes migrate by some process to one pole only and that during interkinesis the cell is divided by a cell plate into nucleated and anucleated halves. Second division takes place in the nucleated half and unreduced gametes are eventually produced from these second division products. In Triticum crassum (Boiss.) Aitch. & Hemsl. x T. turgidum L. hybrids Wagenaar (1968a,b) showed that the chromosomes assemble at the equatorial plate in some PMC's, but do not separate at anaphase I thus allowing restitution to occur. The restituted nuclei undergo a normal second division and dyads are produced at a stage when the other PMC's have

reached the tetrad stage. He concluded that the unreduced gametes are derived from these dyads. Ramanna (1974) reported that meiotic restitution occurred in diploid Solanum phureja Juz. et Buk. when spindle fusion occurred at metaphase II in some PMC's, giving rise to dyads with a diploid chromosome number instead of haploid tetrads. Moreover, in a large proportion of the other PMC's, the nucleus of one cell of the dyad, resulting from a normal first division, restituted at metaphase II so producing a triad instead of a tetrad. The restituted half of the dyad is again expected to produce an unreduced gamete. Maan and Sasakuma (1977) describe yet another method of meiotic nonreduction in Aegilops heldreichii Holzm. x Triticum durum Desf. hybrids. They observed that in some PMC's the univalents were organized at the equatorial plate at metaphase I and they divided equationally and separated in some cells to give symmetrical dyads, which matured into functional unreduced microspores without undergoing a second meiotic division.

Meiotic restitution has also been seen in Triticum x Secale hybrids (Liljefors, 1935; Muntzing, 1939; Riley and Chapman, 1958) and Triticum x Agropyron hybrids (Cauderon, 1958) but the mechanism of restitution in these hybrids was not determined.

Thus, in the relatively few hybrids studied, a diverse array of mechanisms for meiotic restitution has been revealed. An opportunity to obtain further information on the process of meiotic restitution came from our work on hybrids between hexaploid wheat and diploid barley. Although the 28-chromosome F_1 hybrids from wheat x barley and barley x wheat crosses were completely self sterile, these plants were partly female fertile and when pollinated with the hexaploid wheat parent, gave rare backcross seed (BC_1). The chromosome numbers

in the BC_1 progeny varied from 45 to 51, with a mode at 49, suggesting that the functional egg cells in the F_1 hybrids possessed 28- or near 28-chromosome restitution nuclei. This conclusion was confirmed when it was found that the 49- chromosome progeny formed 21" + 7' at meiosis. Although the frequency of apparent restitution was low, as deduced from BC_1 seed set on F_1 spikes (2.2 - 3.5 seeds/spike), it was decided to make a study of microsporogenesis in wheat:barley hybrids in an attempt to determine the cytological basis of meiotic restitution in this very wide cross involving members of different subtribes.

MATERIALS AND METHODS

Reciprocal F_1 hybrids of Triticum aestivum cv. Chinese Spring and Hordeum vulgare cv. Betzes were produced as described earlier (Islam *et al.*, 1975; 1978). Most of the observations were made with barley x wheat crosses as only one reciprocal hybrid was available. In addition some observations were made on barley x wheat hybrids possessing cytoplasm derived from the wild grass Hordeum bulbosum L. This hybrid was produced by first crossing diploid H. bulbosum as female with Betzes barley which resulted in haploid Betzes barley in bulbosum cytoplasm following selective elimination of the bulbosum chromosomes in the hybrid embryos (Kasha and Kao, 1970). The haploid plants were then treated with colchicine and the spikes originating from doubled sectors were pollinated with Chinese Spring wheat to produce the barley x wheat hybrid with bulbosum cytoplasm.

The hybrids were cultured in pots under optimal growing conditions in a glasshouse in spring and early summer. Spikes covering a range of maturities were excised and one anther from each floret was

smeared in aceto-orcein to determine the stage of development. Whenever the required stage was detected the other two anthers from the same floret were removed and fixed in a freshly prepared mixture of ethanol (3 parts) and glacial acetic acid (1 part). Anthers with PMC's undergoing meiosis were either smeared directly in 1% aceto-orcein or hydrolysed in 1N HCl at 60°C for 8 minutes and then stained in Feulgen before smearing in aceto-orcein. Anthers with post-meiotic stages were directly smeared in aceto-orcein after fixation.

In interspecific hybrids of Triticum, Wagenaar (1961a,b) concluded that univalents accumulate progressively at the equatorial plate since he observed that the average number of univalents at the equatorial plate increases with advance in stage of development of the anthers. He classified anthers containing PMC's at metaphase I into several developmental stages and a modified form of his classification was used in the present study as follows:

Medium : Anthers with PMC's at metaphase I only.

Late : Anthers with PMC's at metaphase I, anaphase I and telophase I.

Very late : Anthers with PMC's showing stages from metaphase I to anaphase II.

There was some chromosome mosaicism in the PMC's in our hybrids and only those PMC's with the full complement of 28 chromosomes were included in the analyses.

RESULTS

Florets with PMC's at metaphase I were found more easily in the barley:wheat F_1 hybrids than in normal wheat. With hybrid spikes at

the appropriate stage of development it was possible to find PMC's at metaphase I throughout the day and also in the primary or secondary florets of two to three adjacent spikelets. By contrast, in normal wheat, PMC's at metaphase I can usually be found only over a short period of the day, and furthermore, the same stage of meiosis does not usually extend to the corresponding florets of adjacent spikelets. Although no measurements were made on the timing of meiosis in hybrids it was evident that metaphase I is more prolonged in the hybrids than in normal wheat. This apparent lengthening of the meiotic process in the hybrids could be due to the high frequency of univalents in these cells, as already postulated by Wagenaar (1961a,b) to account for the prolonged period of metaphase I in some Triticum interspecific hybrids.

At metaphase I of meiosis in the hybrids the majority of the PMC's possessed 28 univalents, though a few exhibited from 1 (Fig. 5.1a) to 4 bivalents, giving an average of 0.7 bivalents per cell.

At an early stage of metaphase I, the majority of PMC's showed a tendency for univalents to aggregate within the cell, but in a few PMC's the univalents appeared to be randomly scattered. When aggregated the univalents were located in a loose line between the two poles (Fig. 5.1b).

With the advancement of metaphase I the univalents were found to assemble at the equatorial plate in some PMC's. All 28 chromosomes accumulated at the plate in some cells (Fig. 5.1c), whereas in others one or more univalents lagged behind (Fig. 5.1d). The accumulation of chromosomes at the plate was recorded in PMC's from medium, late and very late anthers and the results are given in Table 5.1. Clearly there was a progressive accumulation of chromosomes at the first

Fig. 5.1a-h: Meiotic restitution in PMC's of wheat x barley (WxB) and barley x wheat (BxW) F_1 hybrids.

a. Metaphase I exhibiting one bivalent (arrow) and 26 univalents (BxW). x 790

b. Chromosome aggregation at early metaphase I. Note that the univalents tend to be located in a line between the poles (BxW). x 145

c. Late metaphase I with all chromosomes aggregated on the plate (BxW). x 900

d. Incomplete aggregation of chromosomes at the plate in the two PMC's (arrows) at metaphase I. The other non-restituted PMC's have reached the dyad stage and each component cell possesses one or more nuclei, often including some micronuclei (BxW). x 145

e. End of metaphase I with one univalent off the plate. Individual chromatids are now evident, but bivalents have not separated (WxB). x 790

f. Early stage of nuclear restitution occurring in the central PMC (BxW). x 790

g. Late stage of restitution with re-formation of mass of chromatin (BxW). x 1130

h. Two monads (M) with restitution nuclei accompanied by a dyad (D) and a near-tetrad (T) cell (BxW). x 220

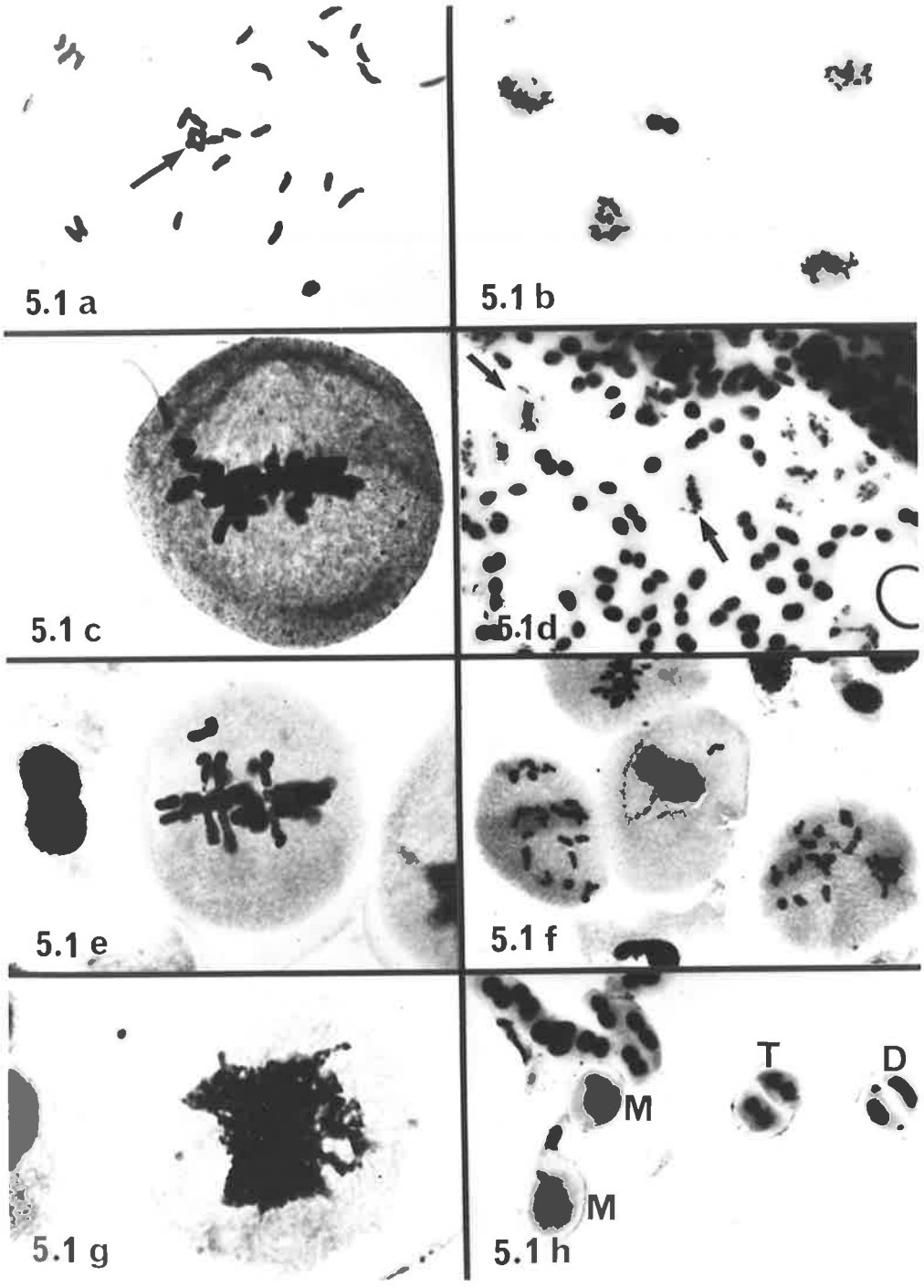

5.1 a
5.1 b
5.1 c
5.1d
5.1 e
5.1 f
5.1 g
T
D
M
M
5.1 h

TABLE 5.1: Accumulation of chromosomes at the equatorial plate at metaphase I in F_1 hybrids

Anther stage	No. of PMC's observed	Average No. of chromosomes at the plate	Percentage of maximum possible
Metaphase I			
Medium	113	13.6	48.6
Late	98	20.3	72.5
Very late	38	22.5	80.4
End of Metaphase I	15	25.1	89.6

metaphase plate as the anthers advanced developmentally. It was difficult to determine the end of metaphase I because even where occasional bivalents were present at the plate they failed to separate. However, metaphase I was considered to have ended when individual chromatids could be distinguished within the univalents. At this stage (Fig. 5.1e) 89.6% of the chromosomes were recorded at the equatorial plate (Table 5.1) but only a few such cells were available for observation.

Although the chromatids became evident at the end of metaphase I, they failed to separate and no anaphase I division took place in these cells. Instead the chromosomes became more diffuse and reformed into a mass of chromatin (Fig. 5.1f,g), around which a single nuclear membrane developed. Sometimes, one or more univalents which failed to be included in the main nucleus formed micronuclei. These either remained in the same cell or else were cut off by the formation of a cell plate.

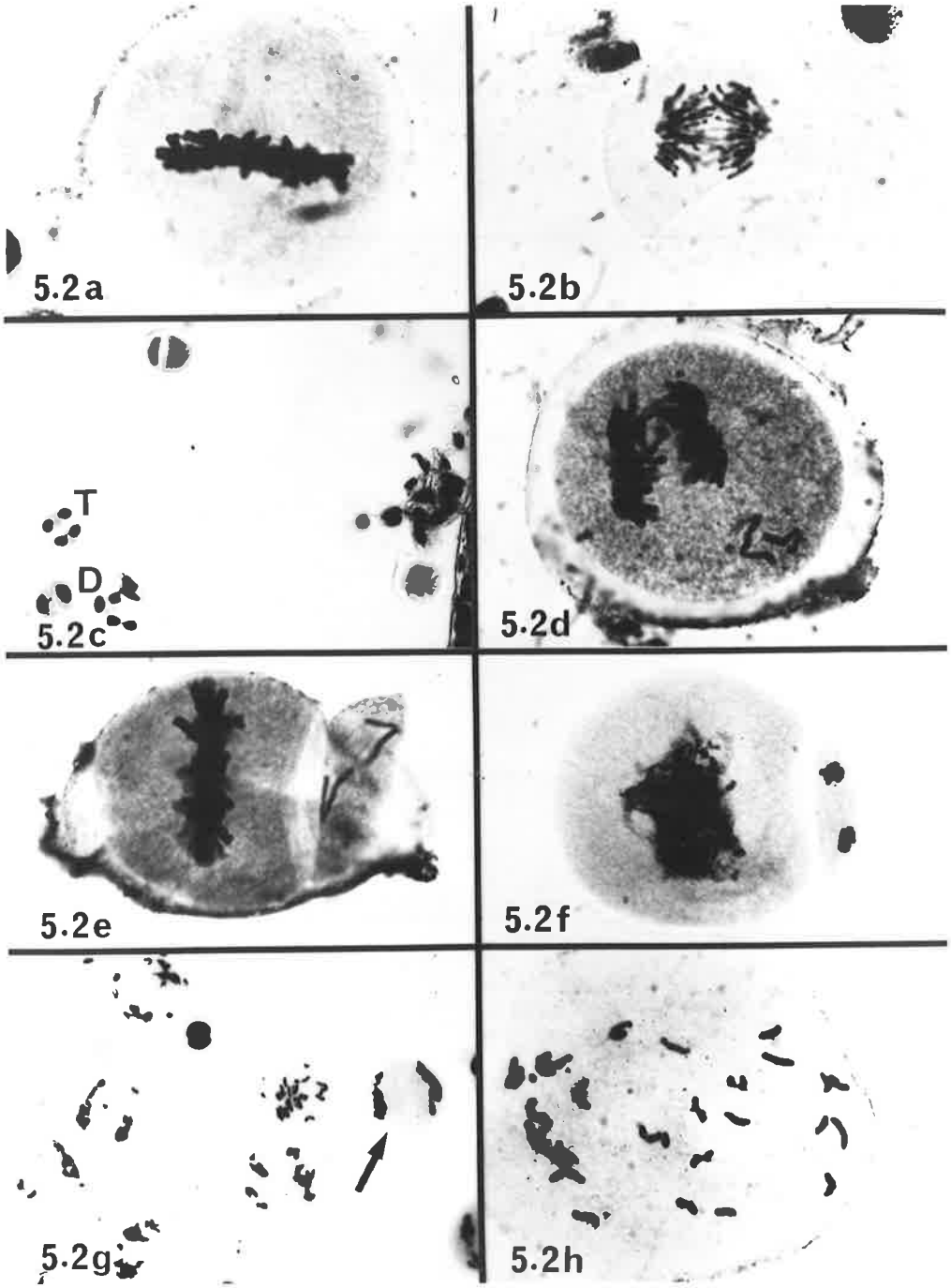
The subsequent development of PMC's containing restitution nuclei followed one of three different patterns:

Type 1. In many PMC's the restituted nucleus failed to undergo a second division and formed a monad, whereas neighbouring cells had proceeded to the dyad or near-tetrad stage of division (Fig. 5.1h). Such a monad is expected to have the potential to produce an unreduced gamete with a diploid chromosome number.

Type 2. In other PMC's the restituted nucleus divided and the chromosomes underwent regular metaphase II (Fig. 5.2a) and anaphase II (Fig. 5.2b) stages. Thus regular dyads were

Fig. 5.2a-h: Meiotic restitution in PMC's of barley x wheat F_1 hybrids.

a. Restitution nucleus at metaphase II stage. x 790
b. Restitution nucleus at anaphase II stage. x 790
c. The symmetrical dyad (D) adjacent to the near-tetrad (T) is presumed to have arisen from a restitution nucleus following regular anaphase II separation. x 170
d. Both the restituted nucleus and the micronucleus show anaphase II separation. x 790
e. Restituted nucleus at metaphase II whereas the micronucleus to the right has proceeded to anaphase II. x 790
f. Near-triad formed from completed second division of the micronucleus and failure of division of the restitution nucleus. x 790
g. End of first division in non-restituted cells. Note one cell (arrow) has nearly equal distribution of chromosomes at the poles, and it is expected to form a symmetrical dyad. x 225
h. Strong spindle activity in univalents remaining at the equatorial region at metaphase I. Note that the univalents respond by misdivision rather than equational separation. x 790


5.2a
5.2b
T
D
5.2c
5.2d
5.2e
5.2f
5.2g
5.2h

formed among neighbouring cells nearing the tetrad stage of division (Fig. 5.2c). Each cell of these dyads is again expected to produce microsporocytes with an unreduced diploid chromosome number.

Type 3. In PMC's having a restitution nucleus and a micronucleus, the restitution nucleus and the micronucleus either both underwent a regular second division (Fig. 5.2d) or the second division failed in one of them (Fig. 5.2e). Usually second division failed to occur in the restitution nucleus and division of the micronucleus gave rise to an asymmetrical triad (Fig. 5.2f).

Since some of the restituted nuclei are deficient for one or more chromosomes they will give rise to microsporocytes without the full complement of 28 chromosomes. The frequency of occurrence of restitution cells of Types 1 and 3, as determined simply by counting at the tetrad stage of division, is given in Table 5.2. The frequency of Type 2 restitution nuclei could not be so determined because at the tetrad stage they cannot be distinguished from other symmetrical dyads which could have come from non-restituted cells in which chance distribution has given nearly equal numbers of chromosomes at each pole at the end of first division (Fig. 5.2g).

In the other non-restituted PMC's at the first division, a majority of univalents were located near the polar region but the remainder were scattered. Subsequently the chromosomes became diffuse and these PMC's formed dyads with one or more nuclei, often including some micronuclei, in each component cell (Fig. 5.1d). Some of these dyads did not divide further, whereas others divided to give either triads or tetrads.

TABLE 5.2: Frequency of PMC's exhibiting restitution nuclei at tetrad stage of meiosis

Hybrid	No. of PMC's observed	Observed Frequency	
		Full restitution No.(%)	Incomplete restitution No.(%)
Barley x Wheat	486	50 (10)	60 (12)
Wheat x Barley*	515	16 (3)	35 (7)

*The single wheat x barley hybrid studied was grown in a different year, and in a different season, from the reciprocal hybrids.

Although the above observations were restricted to microsporogenesis, we can assume that similar processes occur during megasporogenesis so that functional female gametes arise from meiotic restitution. The number of seeds obtained from backcrossing the reciprocal hybrids to wheat is presented in Table 5.3. It is apparent that 52% and 41% of the BC_1 plants having barley and wheat cytoplasms, respectively, originated from the fertilization of fully restituted egg cells. On average more BC_1 seeds/spike was obtained with the hybrid having wheat cytoplasm than with those hybrids having barley cytoplasm, but, since only one wheat x barley F_1 hybrid was studied, this difference may simply be an environmental effect. On the other hand the very poor seed set (0.07 seeds/spike) obtained with several barley x wheat F_1 hybrids possessing bulbosum cytoplasm, could well be due to the cytoplasm having an influence on the restitution process.

DISCUSSION

From our observations of microsporogenesis in reciprocal wheat: barley hybrids, it is clear that restitution takes place when cells have a high accumulation of univalent chromosomes at the equatorial plate late in metaphase I and when the chromosomes do not separate at anaphase I. Thus restitution in wheat:barley hybrids is similar to that observed by Wagenaar (1968a,b) in T. crassum x T. turgidum hybrids. Although there was evidence of strong centromeric activity in those chromosomes at the equatorial region in wheat:barley PMC's having a mainly polar accumulation of chromosomes (Fig. 5.2h), the equatorially aligned univalents usually mis-divided instead of dividing equationally. Thus there was no evidence to suggest that meiotic nonreduction might occur following the simultaneous equational division at anaphase I of all univalents, as observed by Maan and Sasakuma (1977) in Ae. heldreichii x T. durum hybrids.

TABLE 5.3: Seed set on F_1 hybrids after backcrossing with wheat pollen and the chromosome constitution of the progeny

F_1 hybrid	No. of spikes pollinated	No. of seeds obtained	Average seed/ spike	Percentage of progeny* with chromosome no: 45-48	49	50-52
Barley x Wheat	30	68	2.27	38	52	10
Barley (*bulbosum* cyt.) x Wheat	45	3	0.07	100	0	0
Wheat x Barley	28	99	3.54	44	41	15

*Based on 29 of the 68, all 3, and 41 of the 99 seeds, respectively.

In the T. crassum x T. turgidum hybrids (Wagenaar, 1968a), the bivalents present in cells showing equatorial plate formation normally separated at anaphase I and moved to the poles thus resulting in chromosome deficient gametes. Thus it was postulated that fully restituted nuclei could only be obtained from cells possessing 35 univalents at meiosis. In contrast, only a few cells showing equatorial accumulation of chromosomes possessed bivalents in the wheat:barley hybrids, and these bivalents generally failed to separate at the end of metaphase I. Thus restituted cells in the wheat:barley hybrids which were deficient for one or more chromosomes probably resulted from the failure of the univalents to aggregate at the equatorial plate rather than from separation at anaphase when present as bivalents. Further indirect evidence in support of this suggestion, comes from genetic data obtained from an F_1 wheat x barley hybrid with only 23 somatic chromosomes instead of the normal 28 (Islam *et al.*, 1978). This hybrid possessed a haploid complement of wheat chromosomes plus one pair of barley chromosomes and at meiosis formed 21' + 1'' regularly. When it was backcrossed to wheat, 75% of the BC_1 seeds possessed a full complement of wheat chromosomes plus the pair of barley chromosomes even though the latter always formed a ring bivalent which regularly assembled at the equatorial plate at metaphase I in the F_1 hybrid. It is apparent from these genetic data that the bivalent failed to separate in a great majority of the restituted egg cells.

Another observation in wheat:barley hybrids which differed from those of Wagenaar (1968a,b) in Triticum intercrosses was the failure of some restituted nuclei to undergo a second division, resulting in monads occurring among other PMC's at the tetrad stage. These monads are expected to produce microsporocytes with a diploid chromosome number.

Evidence in support of the conclusion that these monads are derived from restitution nuclei which have failed to undergo second division, comes from the presence of asymmetrical triads, with one large cell and two small cells, in these same anthers. It is inferred that the triads arose through failure of second division in a restitution nucleus whilst a companion micronucleus divided to give two small daughter cells. Monads of the type observed in wheat:barley hybrids were also described by Kihara and Nishiyama (1937) in T. polonicum x H. villosa hybrids.

In conclusion we may speculate on the possible cellular mechanism controlling restitution in wheat:barley hybrids. Presumably the key factor in restitution is the failure of chromosomes to separate at anaphase I in these hybrids and this in turn may be due to the prolonged period of metaphase I in cells undergoing irregular meiosis. Wagenaar (1961a,b) has demonstrated that anaphase I is not initiated until a certain number of univalents have accumulated at the equatorial plate in Triticum interspecific hybrids and he postulated that the level of accumulation required may be specific for each hybrid. Furthermore, it is well known that the kinetochores of chromosomes can only act for a limited period as attachment points for microtubules (Bajer and Molè-Bajer, 1972). Thus, if the time required to reach the critical level of accumulation of univalents at the equatorial plate in wheat:barley hybrids exceeds the period of activity of kinetochores, this could result in failure of anaphase movement of both univalents and the rare bivalents formed in these hybrids.

CHAPTER 6: PRODUCTION OF DISOMIC WHEAT-BARLEY CHROMOSOME ADDITION LINES USING HORDEUM BULBOSUM CROSSES

A.K.M.R. Islam and K.W. Shepherd
(Short paper, submitted for publication)

INTRODUCTION

After O'Mara (1940) described a systematic procedure for adding individual pairs of rye chromosomes to wheat, many other workers have used a similar approach to add chromosomes of several other alien species to wheat. These addition lines have had many uses including assigning genes in the alien species to particular chromosomes, determining the genetic similarity (homoeology) between alien chromosomes and particular wheat chromosomes and also for transferring desirable characters such as disease resistance from the alien species to wheat.

In producing addition lines it has usually been relatively easy to produce 43-chromosome monosomic addition lines from a 49-chromosome heptaploid, but it has often been difficult to isolate the disomic addition lines from these monosomics. This difficulty stems from the fact that in monosomic additions, although the transmission rate of the extra chromosome in female gametes is expected to be approximately 25%, the 22-chromosome pollen bearing the alien chromosome usually competes very poorly with 21-chromosome euploid wheat pollen in effecting fertilization of the egg. For example, Sears (1956) obtained only 1.3% disomics from among the progeny of a selfed monosomic addition of an Aegilops umbellulata chromosome (6 C^u) to hexaploid wheat. Furthermore, Mochizuki (1963) could not recover even a single disomic addition plant from screening 1055 progeny of a selfed monosomic addition line having an Agropyron elongatum chromosome added to tetraploid wheat.

There are some exceptions to this pattern and Knott (1964) and Cauderon (1966) reported a transmission rate of 25% and 20% for a telocentric and a univalent chromosome of A. intermedium when present as monotelosomic or monosomic additions to wheat, respectively. Also, there are other examples where a specific Aegilops chromosome has almost 100% transmission through the pollen in monosomic addition lines involving particular chromosomes of Ae. triuncialis (Endo and Tsunewaki, 1975), Ae. longissima and Ae. sharonensis (Maan, 1975) and Ae. caudata (Endo, 1978; Endo and Katayama, 1978) added to common wheat. The preferential transmission of the Aegilops chromosome in both the male and female gametes results from inviability of all gametes not bearing the alien chromosomes.

In those numerous examples where it is difficult to obtain disomics from selfed monosomics, Chang *et al.* (1973) suggested that if pollen of the monosomic could be cultivated to give haploid plants, then many of these would be 22-chromosome aneuhaploids which could provide disomic addition lines upon chromosome doubling. Subsequently, Barclay (1975) demonstrated that when Chinese Spring wheat is pollinated with diploid and tetraploid Hordeum bulbosum, the bulbosum chromosomes are selectively eliminated during embryo development giving wheat haploids and this suggested to the present authors another possible way of producing disomic addition lines without having to rely on transmission of the alien chromosome through the pollen. Thus if the 22-chromosome eggs in monosomic additions could be fertilized with H. bulbosum and there was preferential elimination of the bulbosum chromosomes, 22-chromosome aneuhaploids would result and these could give disomic additions after chromosome doubling.

In the present study disomic, near-disomic and monosomic wheat-barley addition lines have been hybridized with H. bulbosum to test the

feasibility of this procedure for obtaining disomic lines. We were particularly interested to test the efficiency of this method for producing disomic wheat-barley addition line 3, since in earlier cytological screening only one disomic was found in 100 progeny from the near-disomic (21" + 1t") addition line.

MATERIALS AND METHODS

The plant material used as the pistillate parent in crosses with H. bulbosum included reciprocal wheat:barley F_1 hybrids and monosomic, near-disomic and disomic addition lines having chromosomes of Betzes barley added to Chinese Spring wheat and which had been produced in this laboratory (Islam *et al.*, 1975, 1978, 1980). Both diploid (2n=14) and tetraploid (2n=28) forms of H. bulbosum were used as the pollen parent and they were obtained as accessions CPI 18968 and CPI 14804, respectively, from the Plant Introduction Centre, Division of Plant Industry, C.S.I.R.O., Canberra City, A.C.T., Australia.

The plant material used in crosses was cultured in potting compost in 30cm pots under optimal growing conditions in a glasshouse in spring and early summer. The procedures used for emasculation, pollination and embryo culture were similar to those used earlier in making wheat x barley crosses (Islam *et al.*, 1978, 1980). However, because anthesis in H. bulbosum commences early in the morning, the bulbosum pollen used in crosses did not always come from freshly dehisced anthers. Sometimes pollen from partly dehisced older anthers was used.

The somatic chromosome constitution of the parent and progeny plants was determined from root tips which were taken from potted plants and pretreated in α-bromonaphthalene, fixed in glacial acetic acid and then stained by the Feulgen method. Meiotic preparations were made from anthers

fixed in freshly made 1:3 acetic acid-alcohol and stained by the same Feulgen method.

To induce chromosome doubling, seedlings with four to five tillers were treated for up to 72 hours with 0.1% colchicine in 2% Dimethyl sulphoxide using Bell's (1950) tiller-capping method.

For isozyme studies, the methods of Hart *et al.* (1980) were used.

RESULTS AND DISCUSSION

The seed set obtained after crossing the addition lines with tetraploid H. bulbosum pollen is shown in Table 6.1. The crosses involving disomic addition lines 2 and 4 and monosomic addition line 6 were all carried out within a three week period in late spring and the percentage seed set was similar with each line. However, the crosses with near-disomic addition 3 were made in early spring, so it is not clear whether the lower rate of success is due to genotypic or environmental differences. With all addition lines, there was much variation in the degree of seed set on individual spikes just as observed by Miller and Chapman (1976), with crosses between wheat monosomics and H. bulbosum.

When pollen from freshly dehisced anthers was used in crosses, the frequency of seeds with embryos was greater than when older pollen was used, since the latter pollen often induced ovary development only. The percentage of embryos recovered varied with the different addition lines but since separate batches of pollen were used to fertilize these lines it cannot be decided whether the differences are genotypic or due to batch differences in the pollen. Monosomic addition 6 appeared to be a better parent than the disomics used, since a higher proportion of its seeds possessed embryos and, furthermore, the embryos tended to be larger than those obtained from the disomics. However, in contrast to the usual

TABLE 6.1: Progeny obtained after pollinating disomic and monosomic wheat-barley addition lines with H. bulbosum (2n = 28)

Pistillate parent	MI configurations in PMC's	No. florets pollinated	Seeds obtained No. (%)	Embryos cultured No. (%)	No. plants obtained
Disomic Addition					
2	22''	141	41 (29)	12 (29)	7
4	22''	140	31 (22)	12 (38)	2
3*	21''+1t''	196	25 (13)	14 (56)	10
Monosomic Addition					
6	21''+1'	256	70 (27)	50 (71)	30

* = Near-disomic addition

response with disomics, a single spike of disomic addition 2 gave eight seeds with larger-sized embryos suggesting that the chromosome constitution of the embryos may not be the most important factor in determining their development.

The chromosome constitution of the progeny obtained from these crosses is given in Table 6.2. As 22-chromosome aneuhaploids were recovered from each of the disomic and near-disomic addition lines tested, it is evident that the barley chromosomes are recognized separately from H. bulbosum chromosomes when elimination of the latter chromosomes takes place in the developing embryos. The occurrence of a 21-chromosome plant among the progeny of near-disomic addition 3 seems to be inconsistent with this conclusion, but this plant could have originated from an egg cell which lacked the barley chromosome because of asynapsis or non-disjunction in the megaspore mother cell.

None of the 41 progeny examined had more than 22 chromosomes in their root tips and hence the elimination of bulbosum chromosomes must have been complete. Similarly, Miller and Chapman (1976) observed complete elimination of bulbosum chromosomes in 134 out of 136 progeny from monosomics of wheat crossed with H. bulbosum. They suspected that at least one bulbosum chromosome might have been retained in the other two plants.

The recovery of 22-chromosome plants from monosomic addition line 6 with a frequency of 19% was of special interest since it showed that there was no strong selection against embryos with 22 chromosomes in this monosomic at least. However, Miller and Chapman (1976) in their study of crosses between monosomic wheat and H. bulbosum found that there was some selection against the 20-chromosome aneuhaploid embryos, compared to the normal 21-chromosome euhaploid embryos.

TABLE 6.2: Chromosome constitution of progeny from crosses between wheat-barley addition lines and H. bulbosum (2n=28)

Addition line	MI configurations in PMC's	No. of progeny with indicated chromosome constitution		
		21	21+t	22
Disomic 2	22"	0	0	6
Disomic 4	22"	0	0	2
Near-disomic 3	21"+1t"	1	3	3
Monosomic 6	21"+1'	21	0	5

Approximately 20% of the embryos obtained from crosses of monosomic and disomic addition lines with H. bulbosum pollen had two shoot apices and one other seed contained a twin embryo, but the cause of these developmental abnormalities is not known.

At meiosis the progeny plants showed mostly univalents (21', 21' + t', 22'), but some pairing was observed in occasional PMC's similar to that occurring in normal Chinese Spring haploids. Doubled sectors were obtained in most of the plants treated with colchicine and disomic and ditelosomic addition lines with 22" and 21" + t" have been obtained. Proof that the required barley chromosomes were present in the disomic additions 3, 4 and 6 produced from these H. bulbosum crosses was obtained by detecting barley Esterases, ADH and GOT isozymes, respectively, in these lines.

An attempt was also made in the present study to use H. bulbosum crosses to propagate the difficult to produce wheat x barley normal F_1 hybrid. It was argued that if rare restitution egg cells in wheat x barley F_1 hybrids could be fertilized with H. bulbosum pollen, and if the bulbosum chromosomes were selectively eliminated, an embryo with the F_1 chromosome constitution should result. However, no seeds were obtained after crossing 300 florets of the wheat x barley F_1 hybrid with pollen of diploid H. bulbosum instead of the tetraploid which was not available at that time. However, pollen from tetraploid H. bulbosum was used on 910 florets of the reciprocal F_1 hybrid, and 11 seeds were obtained but only one possessed a small embryo which did not germinate. Although no positive results were obtained from these crosses there was some encouragement for the future when it was found that trigeneric hybrids could be produced easily from crossing these wheat:barley hybrids with rye. (Islam, unpublished).

To summarize, the present work demonstrated a new method of producing alien disomic addition lines which does not depend on rare transmission of the alien univalent through the pollen of monosomic addition lines. Consequently where the recovery of disomics from progeny of selfed monosomics is very low (Sears, 1956; Evans and Jenkins, 1960; Islam *et al.*, 1980), this bulbosum method may be much more efficient than the conventional method of screening cytologically a large number of their progeny. This method will also be useful where an alien telocentric is transmitted preferentially over the complete chromosome. For example, Sears (1968) obtained only two substitution plants with 20" + 1" 2R among 41 progeny of a plant with 20" + 1t" 2R. A similar problem was encountered in our attempts to select a disomic addition in the conventional manner from among the progeny of near-disomic addition of chromosome 3 of barley. Only one 22" plant was recovered from among 100 of its progeny whereas with the bulbosum crosses three out of the seven embryos obtained had 22'.

An important question remaining is whether this method can be applied more widely to the production of disomic addition lines with other alien species. Obviously this method can only be used when the wheat parent is crossable with H. bulbosum and so far such wheats are restricted to Chinese Spring and a few Australian cultivars (Snape *et al.*, 1979). Furthermore, use of this procedure with other alien species will depend on finding whether the individual alien chromosomes to be added to wheat are retained during the elimination of the bulbosum chromosomes in developing embryos.

CHAPTER 7: IDENTIFICATION OF WHEAT-BARLEY ADDITION LINES WITH N-BANDING OF CHROMOSOMES

A.K.M.R. Islam, Chromosoma (Berl.) 76 : 365-373 (1980)

ABSTRACT

The seven chromosomes of barley (Hordeum vulgare) have been identified individually by their distinctive N-banding pattern. Furthermore all of the barley chromosome N-banding patterns were found to be recognizably different from those exhibited by wheat chromosomes, making it possible to identify individual barley chromosomes when present in a wheat background. N-banding has therefore been used to identify the individual barley chromosomes present in (a) reciprocal wheat:barley F_1 hybrids, including some with abnormal chromosome constitution, and (b) a set of wheat-barley addition lines produced in this laboratory. The value of N-banding for detecting translocations between wheat and barley chromosomes and for isolating lines possessing a pair of barley chromosomes substituted for a particular pair of wheat chromosomes is also demonstrated.

INTRODUCTION

Recently we have produced six of the seven possible addition lines having individual pairs of barley chromosomes added to the chromosome complement of hexaploid wheat (Islam *et al.*, 1978). The uniqueness of each of the six lines was established initially from differences in their spike morphology and their isozyme pattern Hart *et al.*, 1980), and subsequently by chromosome pairing behaviour at meiosis in progeny derived from intercrosses between the

lines. These six different addition lines were arbitrarily designated A, B, C, D, E and F in relation to their serial isolation. Thus the problem remained of relating the barley chromosome present in the addition lines with the standard numbering system assigned to barley chromosomes based on the accepted linkage groups as determined by cytogenetic studies with trisomics and the translocation stocks (Burnham and Hagberg, 1956; Tsuchiya, 1960, 1961; Ramage *et al.*, 1961). The present paper is concerned with resolution of this problem.

Only three of the seven barley chromosomes can be recognized easily by their morphology in conventional somatic metaphase preparations. Chromosome 5 is the smallest in the complement and the two nucleolar organizer (N.O.) chromosomes, No. 6 and 7, are readily detected. Linde-Laursen (1975) demonstrated that a C-banding method can be used to identify each of the barley chromosomes and this was confirmed by Noda and Kasha (1976) and Vosa (1976). Although this technique produced good results with barley itself, it is known that all wheat chromosomes also band extensively with C-banding (Gill and Kimber, 1974). Furthermore the centromeric and near centromeric banding that occurs on both the barley chromosomes and on many of the wheat chromosomes makes it difficult to distinguish barley chromosomes in a wheat background. The situation is very different in wheat-rye addition lines where the characteristic telomeric C-banding in most rye chromosomes (Darvey & Gustafson, 1975) facilitate their identification. In addition the C-banding procedure is time consuming. Recently Gerlach (1977) applied to wheat the N-banding method of Funaki *et al.* (1975), which is simpler and less time consuming to apply than C-banding, and he was able to identify 9 of the 21 chromosomes. The present study was undertaken to find whether N-banding could be used to identify barley chromosomes particularly when they are present in a wheat background.

This paper describes the N-banding patterns of barley chromosomes and the various ways in which N-banding has helped analyse the chromosome constitution of wheat:barley hybrids and addition lines.

MATERIALS AND METHODS

1. Plant material studied

The barley examined with N-banding was material derived from the cultivars Betzes and Shin Ebisu 16. Besides normal disomics, observations were made on some primary trisomics and telo-trisomics of these cultivars (Betzes - chromosomes 1, 2, 3, 4, 6 and 7; Shin Ebisu 16 - chromosomes 2L, 3, 4 and 7). The seeds of these stocks were kindly supplied by Dr. R.T. Ramage and Dr. T. Tsuchiya, respectively.

Also examined were wheat:barley hybrids and derivative material all of which had been produced in this laboratory. It included (a) reciprocal F_1 hybrids between Betzes and Chinese Spring wheat, (b) six disomic addition lines possessing different pairs of Betzes chromosomes added to the chromosome complement of Chinese Spring wheat (Islam *et al.*, 1978), (c) four lines having different telocentric pairs of Betzes chromosomes added to Chinese Spring wheat, (d) four different near disomic addition lines having one complete barley chromosome plus a telocentric of the same chromosome from which the four above-mentioned ditelosomic lines were isolated.

2. Cytological procedure

Roots from 2-3 days old seedlings grown at 27°C were treated with 0.05% colchicine for 3-4 hours at 27°C. The root tips were then fixed in a freshly prepared mixture of acetic acid/ethanol (1:3) and stored at 4°C. Immature spikes chosen for ovary tissue examination were

treated in iced water at 2°C for 20-24 hours before being placed in the fixative.

In making root tip and ovary squashes the fixed material was first macerated in 45% acetic acid on microscope slides. After applying the cover glass the preparations were gently heated and then squashed to spread the chromosomes. The cover glass was then removed after freezing with liquid nitrogen. The preparations were dehydrated in 95-100% ethanol, either at room temperature for 2-3 hours or left overnight in ethanol at -15°C. Finally, the slides were air dried before applying the N-banding procedure.

3. N-banding

Gerlach's (1977) procedure for N-banding was followed, with some slight modification. The air-dried slides containing barley material were incubated in 1M NaH_2PO_4 pH 4.2 for 3-3½ minutes at 92 ± 1°C. For wheat:barley hybrids and addition lines the treatment time was increased to 4-5 minutes at 94 ± 1°C. After rinsing in distilled water, the slides were stained for 30-40 minutes in 7% V/V Gurr's Improved Giemsa R66 in 1/15 M Sørensen phosphate buffer (pH 6.8). After rinsing again in distilled water, the slides were air dried and mounted in immersion oil for observations.

4. Ideogram construction

To construct an ideogram of barley chromosomes, photomicrographs were taken of 10 well-spread N-banded cells and the lengths of the chromosome arms were measured from the photographs. Following Tuleen (1973), each chromosome arm in the cell was expressed as a percentage of the total sum of the lengths of all the chromosomes in that cell. Using Burnham and Hagburg's (1956) measurement of the long arm of

chromosome 6 (62.1 units) as a standard, the length of the long arm of chromosome 6 in the present observations was multiplied by a factor to bring it to 62.1. Measurements of the other chromosome arms were then multiplied by this same factor. The arm lengths and ratios thus determined were used to construct the ideogram.

RESULTS

Barley

The N-banded karyotype of Betzes barley is shown in Fig. 7.1. The distinctive banding patterns allow the chromosomes to be arranged in seven pairs. All seven barley chromosomes possess centromeric or near centromeric banding and most of the chromosomes also have several interstitial bands.

The identity of the barley chromosomes exhibiting these different banding patterns was determined by examining N-banded preparations of trisomic lines. For example, the N-banded karyotype of Betzes trisomic 3 is shown in Fig. 7.2, and the banding pattern of barley chromosome 3 is identified by its presence in triple dose.

The banding pattern exhibited by barley chromosomes is shown in Fig. 7.3 and described below. Chromosome 1 has medium centromeric bands in both arms and two interstitial bands in the long arm. Chromosome 2 has small centromeric bands in both arms with three interstitia bands in the short arm and two interstitial bands in the long arm. The banding intensity in this chromosome is light. Chromosome 3 has a large centromeric band, accompanied by one small proximal band in the short arm and a small centromeric band and a diffuse interstitial band in the long arm. Chromosome 4 is the most heavily banded of all the

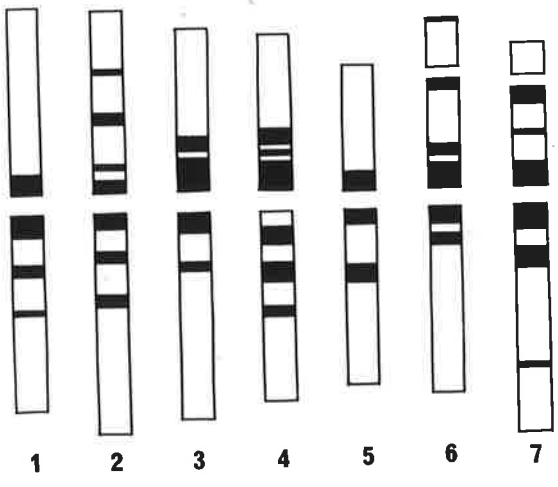
Fig. 7.1: N-banded mitotic chromosomes of Betzes barley. x 1540

Fig. 7.2: N-banded mitotic chromosomes of Betzes trisomic 3. Chromosome 3 (numbered) can be easily identified. x 1540

Fig. 7.3: Ideogram of the N-banded mitotic chromosomes of Betzes barley.

7.1

7.2



7.3

barley chromosomes. One large centromeric band and two small proximal bands are present in the short arm. No centromeric band is evident in the long arm, but three interstitial bands are present. Chromosome 5 possesses small centromeric bands in both arms and a very conspicuous interstitial band in the long arm. Both chromosomes 6 and 7 possess centromeric bands in both arms and a band in the N.O. region of the short arm. The band in the N.O. region of chromosome 7 is, however, more pronounced than the corresponding band in chromosome 6. In addition one proximal band is present in both arms of chromosome 6. Chromosome 7 also possesses a distinctive interstitial band in the long arm and a faint band in a distal position in each arm.

The bands show some variation in intensity between slides and between cells on the same slide. Sometimes faint interstitial bands observed in less condensed chromosomes do not show up in a more condensed stage. Not clearly evident in some of the preparations were the most distal band in each arm of chromosome 2, the telomeric band in the short arm of chromosome 6 and the distal band in the long arm of chromosome 7.

The banding pattern of Shin Ebisu 16 was similar to that of Betzes. Minor differences were present in the interstitial band in the long arm of chromosome 3, which is more pronounced in Shin Ebisu 16 than the diffuse band in that region in Betzes. Additionally the distal band in the long arm of chromosome 4 of Betzes was absent from Shin Ebisu 16. Similar small intervarietal differences in banding were observed by Linde-Laursen (1975, 1978a) and Noda and Kasha (1976, 1978) in C-banded preparations of barley. However, Vosa (1976) reported even larger differences between barley cultivars.

From N-banded preparations of telotrisomic 2L of Shin Ebisu 16, the chromosome arm present was identified and the validity of the arm designation was checked. Telotrisomic 2L agreed with the long arm of chromosome 2, as was also reported by Linde-Laursen (1978b) from C-banded preparations.

Wheat:barley hybrids

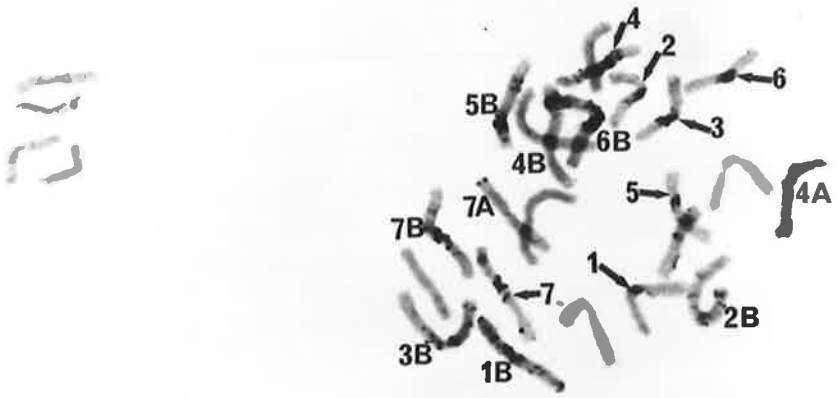
The F_1 wheat:barley hybrids could only be obtained from cultured embryos (Islam *et al.*, 1975) and only a limited number of root tips were therefore available. Hence, dividing ovarian tissue was used as a source of somatic metaphase chromosomes.

From N-banded preparations (Fig. 7.4) of the F_1 barley x wheat hybrids all the seven barley chromosomes (arrowed) were easily identified and distinguished from the nine N-banded wheat chromosomes. These hybrids had 28 chromosomes, whereas the reciprocal F_1 wheat x barley hybrids possessed an array of chromosome numbers varying from 21 to 36 in different plants (Islam *et al.*, 1978). The identity of these chromosomes could not be determined from conventional cytological studies, nor could their constitution be resolved by test crosses to wheat since no viable seeds were obtained. However, with N-banding of one such F_1 hybrid with 33 somatic chromosomes it was possible to establish that it possessed duplicate members of wheat chromosome 1B and barley chromosomes 1, 2, 4, 5, 6 and 7. This hybrid was also deficient for wheat chromosome 4B. Although N-banding can be used to trace all barley chromosomes, only the seven chromosomes of the B genome and two chromosomes of the A genome of wheat, can be distinguished with this procedure (Gerlach, 1977).

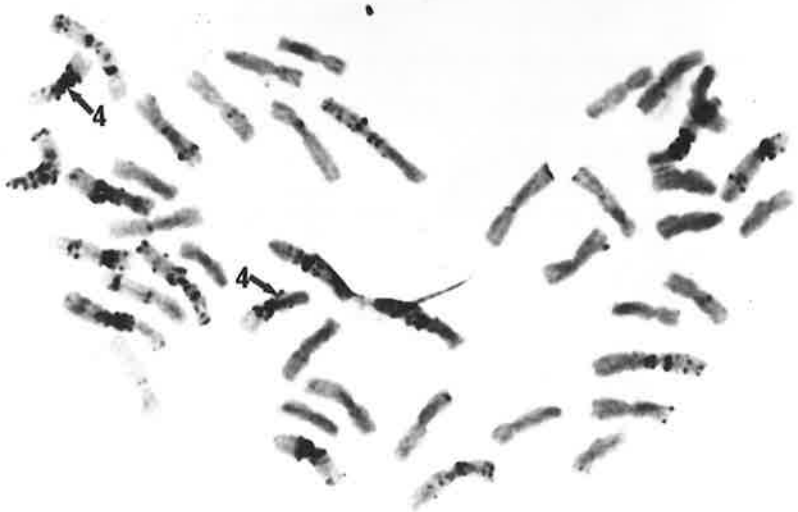
The value of N-banding was also revealed when establishing the identity of the precise chromosome pair present in each of the six disomic

Fig. 7.4: N-banded somatic chromosomes of a 28-chromosome barley x wheat F_1 hybrid (one chromosome is outside the frame). The barley chromosomes are numbered 1 to 7 and the banded wheat chromosomes are designated 1B, 2B etc. x 1220

Fig. 7.5: N-banded somatic chromosomes of wheat–barley addition line A. Barley chromosome 4 is arrowed. x 1220



7.4



7.5

wheat-barley addition lines. It was possible to identify the barley chromosomes by relating their banding patterns to that of the trisomics of barley. The addition lines previously designated with letters as A, B, C, D, E and F were found to possess standard barley chromosomes 4, 7, 6, 1, 2 and 3, respectively. An N-banded cell of an addition line having chromosome 4 of barley is shown in Fig. 7.5, where it can be easily identified from the 18 N-banded wheat chromosomes. It was also possible with N-banding to establish the arms involved in four ditelosomic addition lines. This was achieved by banding the 42 + 2t chromosome ditelosomic along with the 43 + t chromosome near disomic additions. The latter plants were helpful in confirming which chromosome arm was present in the ditelosomic additions because the telo was present together with the complete barley chromosome.

Finally it was found that N-banding could be used to detect translocations involving wheat and barley chromosomes. One such translocation line was recovered in the course of the production of the addition lines. From gel electrophoresis of prolamin proteins it was known only that the translocation line possessed most of the barley prolamins and thus involved part of chromosome 5 of barley. With N-banding it was established that the short arm of chromosome 5 of barley was involved in a translocation with an unknown wheat chromosome which does not band with this technique.

DISCUSSION

Gerlach (1977) noted some correspondence between his N-banded preparations and the C-banding preparations of Chinese Spring wheat published by Gill and Kimber (1974). In the present study although there was some agreement between N-banding of barley and C-banding of

earlier studies, the correspondence was far from exact. Linde-Laursen (1975) reported the absence of centromeric bands from both arms of chromosome 2 and from the long arm of chromosome 3. Noda and Kasha (1976, 1978) also failed to observe any centromeric band in the long arm of chromosome 5. In the present observations centromeric bands were observed in both arms of all the barley chromosomes except for the long arm of chromosome 4. Moreover, the band position, number and thickness of bands in chromosome 2, 4 and 6 differed to some extent from the C-banded preparations of Linde-Laursen (1975) and Noda and Kasha (1978). The present N-banding pattern did not agree with the C-banding pattern observed by Vosa (1976). From my results and also from those of Linde-Laursen (1975, 1978a) and Noda and Kasha (1976, 1978) it appears that Vosa's designation of chromosome 6 and 7 should be interchanged.

From the analysis of multiple translocation stocks of barley, Tuleen (1973) and Künzel (1975) raised doubts about the correspondence between the numbers assigned to the linkage groups and the standard karyotype. Similarly Noda and Kasha (1978) and Linde-Laursen (1978b), using C-banded preparations, also found inconsistency between the numbering of the standard karyotype and the trisomics. The present results for the trisomic lines of Betzes and Shin Ebisu 16 are in agreement with those of Noda and Kasha (1978) who found that the extra chromosome in trisomic 1 is not the longest chromosome of the barley complement but corresponds in length and arm ratio to chromosome 3 of the standard karyotype, whereas chromosome 2 is the longest barley chromosome. Also I agree with Noda and Kasha (1978) that chromosome 3 (trisomic 3) probably corresponds to chromosome 1 of the standard karyotype. That is, when the arm ratios of chromosome 1 and 2 observed by Tjio and Hagberg (1951) were compared with chromosome 3 of Tuleen

(1973), Noda and Kasha (1978) and those in the present study, chromosome 3 with a more dissimilar arm ratio was found to match better with chromosome 1 of the standard karyotype. In my work trisomes 4, 6 and 7, however, matched well with the corresponding chromosome of the standard karyotype, whereas trisomic 5 was not available for analysis. As the trisomic numbering is more generally accepted in the literature and because the genetic linkage groups are based on it, the barley chromosomes involved in the addition lines were identified and numbered according to the chromosome involved in the trisomics irrespective of the karyotype numbering.

The merits of N-banding were very evident when attempting to resolve the chromosome constitution of the various hybrid stocks. Because of the distinctive banding patterns of individual barley chromosomes, and the lack of correspondence with any wheat chromosomes, they were easily identified in the F_1 barley x wheat hybrids. N-banding was even more useful when determining the chromosome constitution and hence the probable origin of the unusual F_1 hybrids obtained when wheat was used as the female parent. These abnormal hybrids probably arose following mitotic disturbances during early zygotic divisions of the F_1 hybrid embryos (Islam and Shepherd, 1980b). An N-banding study of one of these F_1 hybrids with 33 somatic chromosomes clearly indicated a duplication of some wheat and barley chromosomes and deficiencies of some wheat chromosomes. This technique was also of great value for identifying the barley chromosome involved in the disomic wheat-barley addition lines. From isozyme studies, six different barley isozymes were located in four different addition lines but the identity of the respective barley chromosome was not known. Similarly it was difficult to recognise the barley chromosome arm involved in the

ditelosomic addition lines from somatic metaphase preparations, because of the lack of any distinctive features apart from the nucleolar organizers on chromosomes 6 and 7. With N-banding, the individual barley chromosome and the arm involved could be identified. The technique will also be useful for identifying translocations involving the nine wheat chromosomes (the ones that band with this technique) and any barley chromosome. With translocations involving the other 12 wheat chromosomes only the barley chromosome will be known. One such line involving the short arm of chromosome 5 of barley has already been identified.

The N-banding method is currently being applied to isolate lines where individual pairs of barley chromosomes are substituted for specific wheat chromosome pairs. Already a putative substitution of chromosome 4 of barley for 4A of wheat has been detected in progeny from a double monosomic stock, using isozyme studies, and it was possible to confirm the presence of chromosome 4 of barley and the absence of 4A of wheat in this plant by N-banding.

CHAPTER 8: CYTOLOGICAL ABNORMALITIES IN WHEAT:BARLEY HYBRIDS AND THEIR DERIVATIVES

A.K.M.R. Islam and K.W.Shepherd (Submitted for publication)

ABSTRACT

Cytological abnormalities were observed at two stages of development in wheat:barley F_1 hybrids and some of their backcross (BC) derivatives. Although the barley x wheat F_1 hybrids regularly possessed 28 somatic chromosomes in root tips, the reciprocal hybrids possessed an array of chromosome numbers ranging from 21 to 36 in different plants. Some chromosome mosaicism, including polyploidy was observed in pollen mother cells (PMC's) of all of the barley x wheat and some of the reciprocal hybrids. These same hybrids also exhibited abnormalities at the tetrad stage and possessed multipore pollen grains. The BC_1 plants also exhibited similar cytological abnormalities during microsporogenesis, whereas the BC_2 plants segregated for their occurrence. The BC_2 plants which possessed chromosome 5 of barley showed abnormalities and were self-sterile, whereas plants lacking this chromosome exhibited normal cytological behaviour and were self-fertile. Isolation of a translocation chromosome revealed that the gene(s) responsible for these abnormalities must be located on the long arm of chromosome 5. All of the cytological abnormalities observed can be explained if it is assumed that normal spindle function is disrupted, (1) during early divisions of the zygote in F_1 hybrids but for some unknown reason, only when they possess wheat cytoplasm, and (2) at premeiotic mitoses and meiosis in PMC's of reciprocal F_1 hybrids and their derivatives, whenever chromosome 5 of barley is present.

INTRODUCTION

It is well known that distant hybridization between plants frequently leads to developmental abnormalities in the progeny. For example, hybrid inviability and weakness, hybrid sterility and hybrid breakdown are common in such plants (see review by Stebbins, 1958). Also, cytological disturbances including abnormal mitosis and meiosis have often been described in distant hybrids. In the present paper these disturbances are briefly reviewed and those occurring in our work on wheat:barley hybrids are described.

Mitotic instability in one form or other has been widely reported in distant hybrids. In some interspecific crosses involving Hordeum species (Kasha and Kao, 1970; Islam and Sparrow, 1974; Rajhathy and Symko, 1974; Subrahmanyam, 1977) and Triticum aestivum L. em. Thell. x Hordeum bulbosum L. crosses (Barclay, 1975) the chromosomes of one of the parental species are eliminated during early development of the embryo and this results in the production of haploids of the other species. Selective elimination of chromosomes during plant development has also been observed in some Nicotiana interspecific crosses (Ar-rushdi, 1957; Moav, 1961; Gupta, 1968; Gupta and Gupta, 1973) resulting in some morphological changes in the hybrid plants.

Another manifestation of mitotic instability in distant hybrids is the occurrence of variable somatic chromosome numbers within and between plants. For example, Yang (1965) observed variable chromosome numbers within individual plants of amphiploids from N. otophora Griseb., N. sylvestris Speg. *et* Com. and N. tomentosiformis Goodsp. crossed with N. tabacum L., and Forsberg and Wang (1971) observed similar variation in progeny from crosses between amphiploids of Avena abyssinica Hochst. and A. strigosa Schreb. with thirteen varieties of A. sativa L.

Furthermore, in N. megalosiphon Heurek and Muell.-Arg. (2n=40) x N. glutinosa L. (2n=24) crosses, the F_1 hybrids had from 30 to 34 chromosomes in different plants (Satyanarayana and Subhashini, 1964).

Chromosome number mosaicism in PMC's is another abnormality which is commonly observed at meiosis in distant hybrids and particularly in the derived amphiploids. Love (1938) observed mosaic PMC's in derivatives from T. aestivum x T. durum Desf. crosses. Also Sachs (1952) detected mosaicism in PMC's of amphiploids involving intercrosses between species of Triticum, Aegilops and Agropyron and he ascribed these to pre-meiotic spindle abnormalities. Thomas and Peregrine (1964) similarly observed mosaics in amphiploids of Avena species.

Other irregularities have been observed at meiosis and they include formation of restitution nuclei, for example in T. crassum (Boiss.) Aitch. and Hemsl. x T. turgidum L. hybrids (Wagenaar, 1968a), clumping of chromosomes in Elymus x Secale hybrids (Heneen, 1963) and Lolium x Festuca hybrids (Gymer and Whittington, 1975), and breakage of meiotic chromosomes in Bromus interspecific hybrids (Walters, 1950; 1952). Also multipolar spindles were observed by Percival (1930) in Aegilops x tetraploid wheat hybrids and by Lee *et al.* (1971) in hybrid derivatives from crosses involving three different Hordeum species.

Another effect of wide crossing on meiosis is the high pairing observed in hybrids of T. aestivum with some strains of Ae. speltoides (Tausch) Gren. ex Richter (Riley *et al.*, 1961), Ae. mutica Boiss. (Riley, 1963a) and Ae. longissima Schweinf. and Muschl. (Mello-Sampayo, 1971). In each of these cases the alien genome possesses genes which suppress the inhibitory effect of chromosome 5B of wheat on the pairing of homoeologous chromosomes.

In our work on wheat:barley hybrids some pronounced cytological abnormalities were observed in somatic cells and in sporogenous tissue. Although the barley (♀) x wheat (♂) F_1 hybrids regularly possessed the expected 28 somatic chromosomes in root tip cells, variable numbers ranging from 21 to 36, were observed in the somatic tissues of plants from the reciprocal cross. Subsequently other cytological disturbances were observed during microsporogenesis in many of these F_1 hybrids and their progeny. These cytological abnormalities are described in this paper and evidence is presented to show that the occurrence of meiotic and post-meiotic abnormalities, at least, is correlated with the presence of barley chromosome 5.

MATERIALS AND METHODS

The plant material examined is listed below and included reciprocal wheat:barley F_1 hybrids and progeny derived from them by backcrossing once (BC_1), twice (BC_2) or thrice (BC_3) to wheat and selfing of some of these backcross derivatives. In addition, other intergeneric hybrids were produced to compare the number and distribution of germ pores in their pollen grains with that of wheat:barley hybrids.

1. Reciprocal wheat:barley F_1 hybrids

Crosses using barley as the female parent - Altogether 12 F_1 plants from crosses between barley cultivars Ketch, Clipper, Prior and Betzes and the wheat cultivar Chinese Spring and two plants from crosses between Ketch barley and Gabo wheat, were examined cytologically.

Crosses using wheat as the female parent - Nineteen F_1 hybrids from Chinese Spring x Betzes crosses and one (No.12, Table 8.1) from Chinese Spring x Ketch crosses, were examined.

2. Progeny derived from F_1 hybrids

With barley x wheat hybrids, BC_1 seeds were obtained by backcrossing the self-sterile F_1 plants with wheat pollen. The BC_1 plants were further backcrossed to wheat to give the BC_2 generation.

With wheat x barley F_1 hybrids, since many of the hybrids had highly abnormal chromosome constitutions, they were completely sterile. However, a few of the hybrids produced seed when backcrossed with wheat pollen and BC_1, BC_2 and BC_3 plants were raised from these crosses.

3. Other intergeneric hybrids

F_1 hybrids of H. arizonicum Covas x Chinese Spring; Chinese Spring x Ae. umbellulata Zhuk., Chinese Spring x Ae. speltoides (high pairing) were also examined to a limited extent. The seeds of H. arizonicum (AC 34), Ae. umbellulata (AC 7817) and Ae. speltoides (AC 7038) were kindly supplied by Dr. R. Takahashi, Professor C.J. Driscoll and Dr. E.P. Baker, respectively, and our acquisition numbers are given in the parentheses.

Since all of the wheat:barley F_1 hybrids were derived from excised embryos grown in artificial culture (Islam *et al.*, 1978), root tips for cytological analyses were not available until after the plants had been transferred to soil in pots. Actively growing root tips were pretreated in a saturated solution of α-bromonaphthalene in tap water for 4 to 5 hours in the dark at 20°C. The root tips were then fixed in glacial acetic acid either for a few hours at room temperature or overnight at 4°C. Immature spikes chosen for ovary tissue examination were treated in iced water at 2°C for 20-24 hours before being fixed. Somatic chromosome counts were made from squash preparations following hydrolysis in 1N HCl and Feulgen staining. Anthers with PMC's in meiotic

and post-meiotic stages were individually fixed in glacial acetic acid/ethanol (1:3), and stored at 4°C until used. However, whole spikes were fixed for studies of archesporial divisions in very young anthers. Feulgen staining was used for prophase and metaphase stages of meiosis in PMC's. Anthers with premeiotic archesporial divisions and post-meiotic stages were squashed in 1% aceto-orcein. Observations on pollen grain morphology were made mostly on unfixed material.

The procedures for extraction and electrophoretic separation of the seed proteins were similar to those employed by Shepherd (1968). The endosperm and testa tissue from portion of a single grain was crushed and treated with 0.2 ml of 2M Urea for approximately 16 hours at 4°C. The samples were then centrifuged and the clear supernatant from each was loaded into individual slots in the gel. The gel was made of 12% hydrolysed starch in aluminium lactate buffer (pH 3.1) and 2M Urea. Electrophoresis was usually carried out for a period of 8 hours at 12.0 v/cm. The gels were sliced into two and then stained with nigrosine in methanol, water and acetic acid (60:140:2) to reveal the proteins.

RESULTS

Chromosome number in roots of F_1 hybrids

Eighty F_1 hybrids from crosses between four barley cultivars Ketch, Clipper, Prior and Betzes as female parent and Chinese Spring wheat as the pollen parent, and 20 reciprocal hybrids between Chinse Spring wheat and Betzes and Ketch barley have been produced so far. The somatic chromosome number of 12 of the barley x wheat hybrids and 19 of the reciprocal hybrids (Table 8.1) were determined from root tip mitoses. In most cases, the counts were restricted to a few cells in one to three

TABLE 8.1: Somatic chromosome constitution, meiotic behaviour and fertility of wheat (♀) x barley (♂) F_1 hybrids

Hybrid No.	Somatic chromosome No. Root tip	Ovary tissue	Meiosis in PMC's Chromosome No.	Pairing behaviour	Female fertility *
1	--	28	$28+M^{\emptyset}$	28' & others†	+
2	21	--	21	21'	+
3	21	--	21	21'	+
4	21	--	21	21'	+
5	22	--	22	22'	+
6	23	23	23	21'+1"	+
7	25	25	25	25'	+
8	27	--	27	25'+1"	+
9	27,28	--	28	25'+1"'	+
10	34,35	34,35	34+M	11"+12' & others	-
11	33	33	33+M	10"+13' & others	-
12	32,35	35	35+M	14"+ 7' & others	-
13	31,32	31	31+M	5"+21' & others	-
14	31,32	--	31	Max. 6"+19'	-
15	35,36	--	36	Max.11"+14'	-
16	33,34	--	M	complex	-
17	26,27	27	M	complex	
18	32	--	--	--	-
19	33	--	--	--	-
20	34,36	--	--	--	-

$M^{\emptyset}$ = mosaic PMC's present
† = other configurations occur in mosaic PMC's
*+ = fertile - = sterile
-- = no observations

root tips due to the paucity of actively growing roots in the potted plants.

All of the barley x wheat F_1 hybrids examined had the expected chromosome number of 28, representing the sum of the haploid chromosome complements of wheat and barley, and there was no variation in number in the different root tips from a given plant. However, the reciprocal hybrids behaved very differently and they possessed an array of chromosome numbers, varying from 21 to 36 in different plants. Furthermore, within some of these plants the chromosome number varied by one, two or three in different root tips from the same plant. Cells within a given root generally possessed the same number of chromosomes, but an occasional inconsistency was observed. Although variation within a root could have been due to cell rupture and loss of one or more chromosomes during squashing, the occurrence of variation between root tips confirmed that chromosome mosaicism does occur in the root tip tissues of at least some of these F_1 hybrids.

To determine the cause of the unusual chromosome constitution of these plants it would have been necessary to study the earliest divisions of the embryo. However, this was impracticable because of the very low percentage (1.3%) seed set when wheat is pollinated with barley. Instead we determined the chromosome number in cells of two 10-12 days old wheat x barley embryos and a uniform chromosome count was obtained in all of the scorable cells (8-10) of each embryo with one embryo having 33 chromosomes and the other 34 chromosomes. Thus it is likely that the abnormal chromosome constitutions detected in these wheat x barley hybrids arose very early in embryonic development.

Since some small variations in the number of chromosomes in root tips occurred in the hybrid seedlings with abnormal chromosome

constitution, the somatic chromosome number present in later-formed tissues was examined to find whether the chromosome constitution became fixed during development of the plant. Young ovaries were collected from one to three different spikes from eight wheat x barley F_1 hybrids and squash preparations were made. In all cases a close correspondence existed between the chromosome number in the root and in the ovarian cells of a given plant (Table 8.1). With one exception, where 34 and 35 chromosomes were observed in different ovaries of the same spike, all the ovaries examined from a single spike and from different spikes on the same plant, had the same chromosome number.

Meiotic behaviour of F_1 hybrids

The behaviour of the F_1 hybrids at meiosis varied greatly depending on their chromosome constitution, as described below:

Normal 28-chromosome F_1 hybrids - All of the barley x wheat F_1 hybrids, and one wheat x barley F_1 hybrid (No.1, Table 8.1) possessed the expected 28 somatic chromosomes representing the full haploid complements of wheat and barley, and all of these hybrids exhibited similar meiotic behaviour. The majority of the PMC's possessed 28 chromosomes which occurred mainly as univalents at metaphase I but occasional bivalents were formed (average 0.7"/cell). A small proportion of these PMC's formed restitution nuclei during meiosis as described earlier (Islam and Shepherd, 1980a,b). However, there was some chromosome mosaicism in these hybrids and from 10 to 20% of the PMC's were hypoploid (Fig. 8.1a,b), hyperploid (Fig. 8.1c) or polyploid. The frequency of polyploid PMC's in these hybrids (Table 8.2) was much lower than that of the aneuploid PMC's. There was a higher level of chromosome pairing in these abnormal PMC's than in those with 28 chromosomes, and it will be argued in the discussion that this increased pairing is not due to the

Fig. 8.1a-h: Meiosis in PMC's of wheat x barley (WxB) and barley x wheat (BxW) F_1 hybrids and BC_1 [(WxB) x W] derivatives.

a. Hypoploid cell from 28-chromosome F_1 hybrid (BxW) exhibiting 6'. x 880
b. Hypoploid cell from 28-chromosome F_1 hybrid (WxB No.1) exhibiting 4" + 17'. x 780
c. Hyperploid cell from 28-chromosome F_1 hybrid (BxW) exhibiting 6" + 21'. x 780
d. Non-mosaic cell from 34-chromosome abnormal F_1 hybrid (WxB No.10) exhibiting 11" + 12'. x 550
e. Extreme chromosome mosaicism in an abnormal F_1 hybrid (WxB No.17). x 120
f. Mosaic cell (polyploid) from the same hybrid with approximately 8 times the basic number, exhibiting 96". x 550
g. Early prophase stage from the same hybrid showing PMC's with multiple nuclei and nuclei of irregular size. x 120
h. Non-mosaic cell in a BC_1 plant exhibiting maximum chromosome association of 21" + 7'. x 780

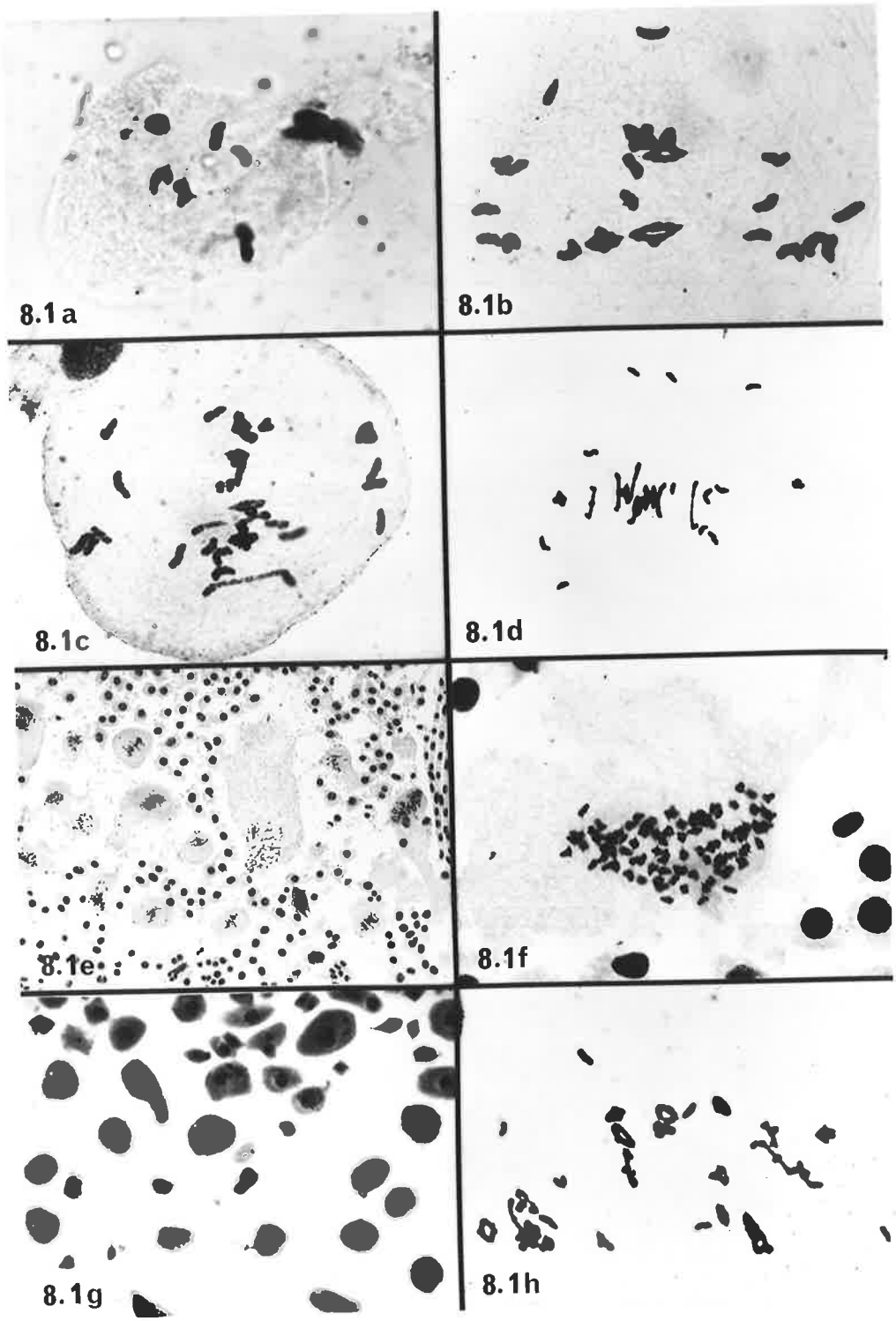

8.1a
8.1b
8.1c
8.1d
8.1e
8.1f
8.1g
8.1h

TABLE 8.2: Frequency of polyploid PMC's at metaphase I in some wheat:barley hybrids and their derivatives

Genotype	Somatic chromosome No.	Meiotic pairing behaviour	Barley* prolamins in seeds	No. of PMC's observed	Polyploid No.	PMC's (%)
F_1 hybrids						
Betzes x C.S.	28	28' & others†	+	510	4	(0.8)
C.S. x Betzes (No.1)	28	28' & others	+	662	16	(2.4)
C.S. x Betzes (No.17)	26-27	complex	+	211	49	(23.2)
C.S. x Betzes (No.6)	23	21'+1"	-	312	0	(0.0)
BC_1						
(C.S. x Betzes) x C.S.	49	21"+7' & others	+	2089	77	(3.7)
BC_2						
(C.S. x Betzes) x $C.S._2$	43	complex	+	249	48	(19.3)
(C.S. x Betzes) x $C.S._2$	44	21"+2' & others	+	479	20	(4.2)
Selfed BC_2						
Disomic addition line (Barley chromosome 4)	44	22"	-	232	0	(0.0)

*+ = Present; - = Absent; † = Other configurations occur in mosaic PMC's.

occurrence of homoeologous pairing but is due to the presence of homologous chromosomes arising from abnormal premeiotic mitoses in archesporial cells. Although these F_1 hybrids were self-sterile, rare backcross seeds were obtained after they were pollinated with wheat.

Abnormal F_1 hybrids with duplication and/or deficiencies of chromosomes - The remaining 19 wheat x barley F_1 hybrids fell into this category and they could be further subdivided into those which gave rare backcross seed when pollinated with wheat (hybrid Nos. 2-9, Table 8.1) and those which appeared to be completely sterile (hybrid Nos. 10-20, Table 8.1).

From an analysis of the pairing behaviour of chromosomes at meiosis in the eight female-fertile hybrids and in their backcross progeny, it was deduced that all hybrids except No.9, possessed the full haploid complement of wheat chromosomes and a variable number of different barley chromosomes ranging from 0 (hybrid Nos. 2,3,4) to 6 (hybrid No.9). Because F_1 hybrid No.9 gave a BC_1 plant which formed 21" + 5' at meiosis and because later isozyme analysis of BC_1 selfed progeny showed that one of these pairs is barley chromosome 4 substituted for a pair of wheat chromosomes, it could be deduced that hybrid No.9 must have possessed 20 wheat chromosomes and 8 barley chromosomes, including three doses of chromosome 4 which formed a regular trivalent. Furthermore, since hybrid No.6 gave a disomic addition line involving barley chromosome 4 directly when backcrossed to wheat (Islam *et al.*, 1978) the regular bivalent in this hybrid must have been a pair of barley chromosome 4. Although these eight hybrids possessed unusual and unexpected chromosome numbers, meiosis in their PMC's was perfectly regular with no sign of chromosome mosaicism or polyploidy (Table 8.2). The pairing configurations observed at metaphase I simply reflected their chromosome

constitution, with, for example, hybrids No.6 and No.9 producing a regular bivalent and trivalent, because of the presence of barley chromosome 4 in two and three doses, respectively. Also it is relevant to record here, for the purpose of future discussion, that none of these hybrids possessed chromosome 5 of barley as deduced from subsequent tests for the presence of prolamins in their progeny seed.

Meiotic behaviour in the remaining 11 hybrids which were sterile was more complex and three different patterns were recognized. In the first group (hybrids Nos. 10,11,12 and 13, Table 8.1) the chromosome number of the majority of the PMC's, although unusual, was the same as that of the somatic tissues. There was often a high degree of bivalent pairing at metaphase I, thought to be due to the presence of homologous chromosomes, but the degree of pairing varied widely in different PMC's. For example, with hybrid No.10 the metaphase I chromosome association of the majority of PMC's varied from a maximum of 11" + 12' (Fig. 8.1d) to 8" + 18', presumably due to asynpasis or desynapsis in the latter cells. However, some of the other PMC's in this plant, and in the other hybrids in this group, exhibited chromosome mosaicism similar to that observed in hybrid No.1.

Meiosis in the second group of hybrids (Nos. 14 and 15, Table 8.1), was similar to that described above, except that there was no evidence of chromosome mosaicism among the PMC's.

Meiosis in the third group of plants (Nos. 16 and 17, Table 8.1) was very abnormal with a high percentage of polyploid PMC's (Table 8.2) and a very complex chromosome mosaicism (Fig. 8.1e). PMC's with the somatic chromosome number were rare. For example, with hybrid No.17 PMC's showed metaphase I configurations ranging from hypoploid cells with 16' to polyploid cells with 56" and even 96" (Fig. 8.1f)

representing approximately 4- and 8-fold increases in chromosome number, respectively. Other complex configurations such as 1''' + 8'' + 8', 3'' + 18' and 7'' + 4' were observed in different PMC's from this plant.

The chromosome constitution of hybrids Nos. 10-20 could not be resolved in backcrosses to wheat because they were completely sterile. However, N-banding was applied to hybrid No.11 and it was found that it was deficient for at least chromosome 4B of wheat and possessed at least one pair of wheat chromosomes (1B) and 6 pairs (1,2,4,5,6,7) of barley chromosomes (Islam, 1980). Similarly N-banding of hybrid No.16 revealed the presence of duplicate copies of some chromosomes including one pair of chromosome 5 of barley.

Since the origin of chromosome mosaicism in PMC's was thought to be due to abnormal mitoses in archesporial cells, an attempt was made to study these premeiotic mitoses in young anthers. It was very difficult to obtain good preparations at this stage and only a few observations were made on young anthers from plants showing chromosome mosaicism. Anaphase bridges and occasional tripolar divisions were observed in some archesporial cells and there was a clear indication of variation in ploidy level because of the marked differences in the size of some interphase nuclei. Other evidence that severe mitotic irregularities had occurred during archesporial divisions comes from the highly irregular size and nuclear content of PMC's at early prophase (Fig. 8.1g).

Cytological behaviour of first backcross (BC_1) plants

In plants having 49 chromosomes the majority of PMC's also possessed 49 chromosomes, but only a few of these formed the expected maximum chromosome association of 21'' + 7' (Fig. 8.1h) at metaphase I. However, some chromosome mosaicism including aneuploid and polyploid

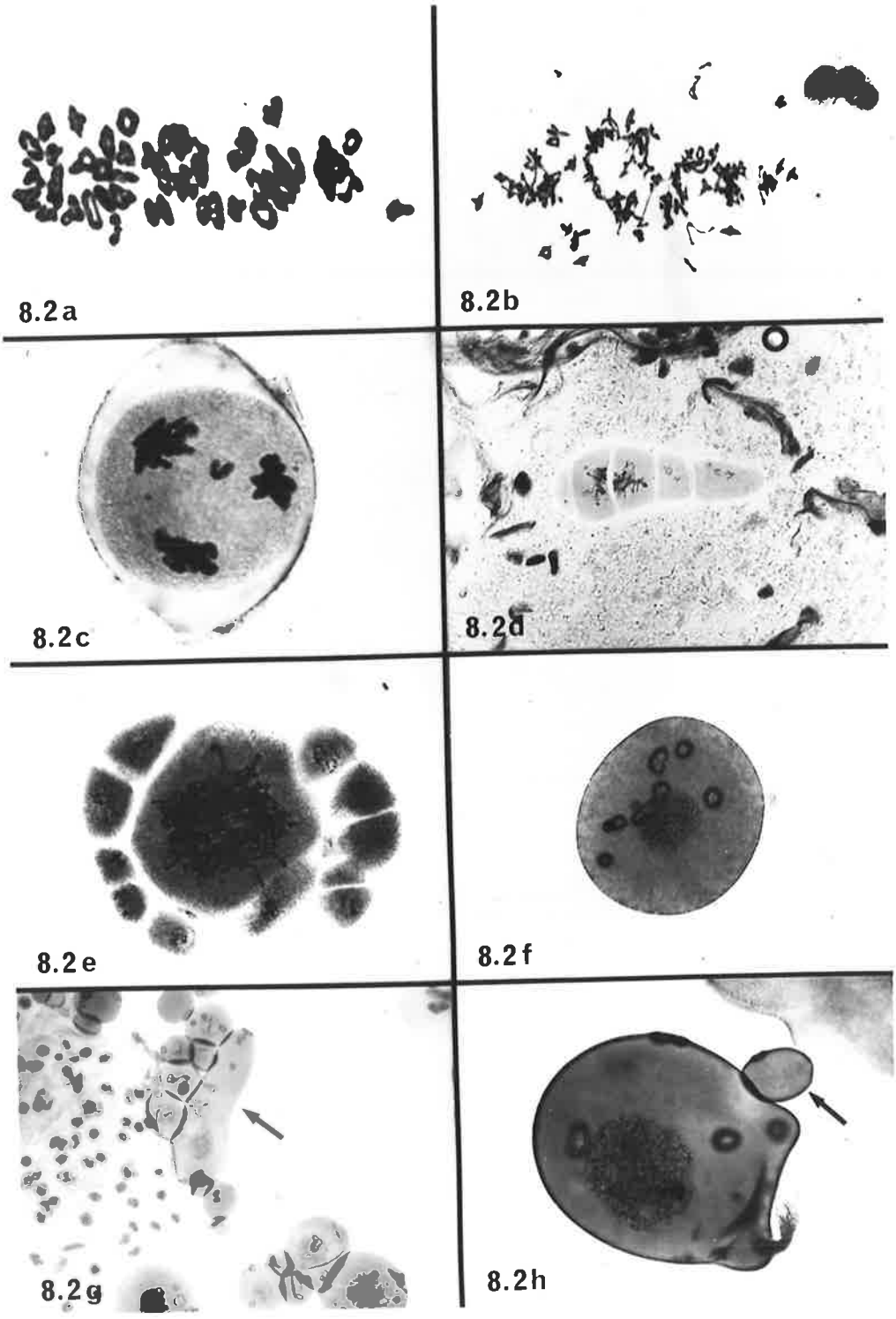
PMC's was also observed in all of the BC_1 plants studied. For example, PMC's with 5" + 8', 1"' + 7" + 5', 1^{iv} + 3"' + 10" + 10' and 49" (Fig. 8.2a) were observed in one plant. The polyploid PMC's (Table 8.2) showed mostly bivalent pairing but rare multivalents were also seen. Clumping of chromosomes occurred in many PMC's and it was difficult to determine their chromosome constitution. Although the BC_1 plants were self-sterile, backcross seeds were obtained after pollination with wheat.

Cytological behaviour of second backcross (BC_2) plants and later generations

In order to facilitate the isolation of addition lines, many of the BC_2 seeds were screened for the presence of barley prolamins as a marker character for the presence of barley chromosome 5 (Oram *et al.*, 1975) in the segregating BC_2 progeny. These tests provided the first indication that a particular barley chromosome was associated with at least some of the cytological abnormalities in wheat:barley hybrids, since all of the 65 BC_2 plants derived from seeds possessing barley prolamins were self-sterile whereas 127 plants from seeds lacking the barley prolamins were self-fertile. Furthermore, meiosis was found to be regular in the self-fertile plants but similar abnormalities to those observed in BC_1 plants were present in the self-sterile plants. The extent of meiotic abnormalities occurring in the BC_2 plants carrying barley prolamins varied depending on their chromosome constitution. Those with higher chromosome numbers (46-49) resembled the BC_1 plants in their cytological behaviour, whilst among those with 43-45 chromosomes, there was marked variation in their cytology. Plants with 43 somatic chromosomes, and a few with 44 or 45 chromosomes exhibited extreme cytological abnormalities, similar to those present in F_1 hybrids Nos. 16 and 17. Thus there was complex chromosome mosaicism in PMC's including many polyploid cells (Table 8.2) with approximately 2,4 (Fig. 8.2b) or even 8 times the somatic chromosome number, and it was not possible to deduce the pairing

Fig. 8.2a-h: Meiotic and post-meiotic stages in the normal wheat x barley (WxB) F_1 hybrid and BC_1 [(WxB) x W] and BC_2 [(WxB) x W^2] derivatives.

a. Polyploid PMC with two times the basic chromosome number (49") from a BC_1 plant. x 920
b. Polyploid PMC with approximately four times the basic chromosome number from a BC_2 plant with 43 somatic chromosomes and having extreme chromosome mosaicism in PMC's. x 460
c. Tripolar division in PMC of F_1 hybrid (WxB). x 740
d. Linear pentad from a BC_2 plant having some chromosome mosaicism in PMC's. x 180
e. Abnormal polyad from a BC_2 plant having extreme chromosome mosaicism in PMC's. x 460
f. Multipore pollen grain with 8 germ pores from the same BC_2 plant. x 460
g. Multicelled pollen grain (arrowed) from the same BC_2 plant. x 180
h. Pollen grains from the same BC_2 plant; the macro grain possesses a nucleus and 4 germ pores, the micro grain (arrowed) possesses one germ pore but no nucleus is evident. x 740


8.2a
8.2b
8.2c
8.2d
8.2e
8.2f
8.2g
8.2h

behaviour of the chromosomes in plants with 43 somatic chromosomes because of this extreme mosaicism. Such plants were completely male sterile and only marginally female fertile.

However, some of the BC_2 plants having barley prolamins in their seed and 44 to 45 somatic chromosomes showed much less meiotic irregularity than those described above (Table 8.2). In these plants a satellited univalent was regularly observed at meiosis and isozyme analysis of backcross progenies obtained from two of these plants revealed the presence of barley glutamic oxaloacetic transaminase isozymes which are known to be controlled by genes on the satellited chromosome 6 of barley (Hart *et al.*, 1980). Thus chromosome 6 of barley appears to reduce the drastic effects of chromosome 5 of barley on cytological behaviour when both chromosomes are present in wheat:barley hybrids. The self-fertile BC_2 plants continued to produce self-fertile plants in subsequent generations, whilst the self-sterile plants continued to show segregation for cytological disturbances and self-sterility in BC_3, and subsequent backcrosses, depending on whether the progeny possessed barley prolamins in their seed or not. However, an exceptional plant was detected among the progeny from a self-sterile BC_2 plant, which possessed barley prolamins in its seed but had normal meiosis and was self-fertile. Further analysis of the progeny from this plant by N-banding (Islam, 1980), and chromosome pairing behaviour when backcrossed to normal wheat, showed that it contained a translocation chromosome having apparently the entire short arm of chromosome 5 of barley joined to an unidentified arm of a wheat chromosome. This plant provides confirmatory evidence that prolamins are controlled by gene(s) on the short arm of chromosome 5 (Shewry *et al.*, 1980; Wiberg, 1974) and indicates that the gene(s) controlling the cytological abnormalities must be on the long arm of this chromosome.

Post-meiotic abnormalities

Tetrad stage - Besides monads, dyads and triads which were found to result from meiotic restitution (Islam and Shepherd, 1980a), polyads were observed at the tetrad stage of meiosis in all of the 28-chromosome normal F_1 hybrids and the abnormal F_1 hybrids exhibiting chromosome mosaicism. Multipolar spindles were observed infrequently during meiosis in F_1 hybrids (Fig. 8.2c) and these could be responsible for the origin of some of these polyads. The BC_1 plants besides producing regular tetrads also showed some abnormalities similar to those in the F_1 hybrids. The BC_2 plants segregated for this character such that the self-fertile plants produced mostly regular tetrads whereas the self-sterile plants exhibited some abnormalities and the degree of abnormality expressed depended on their chromosome constitution. The most pronounced abnormalities occurred in the self-sterile plants which possessed 43 to 45 somatic chromosomes and which exhibited extreme chromosome mosaicism including many polyploid PMC's. Besides the occurrence of linear triads and polyads (Fig. 8.2d) large uninucleate monads, and polyads with a large cell surrounded by many smaller cells (Fig. 8.2e) were often observed.

Pollen grains - The wheat and barley parents regularly produced normal pollen grains with one or rarely two germ pores. However, a proportion of the pollen grains from many of the wheat:barley hybrids and their derivatives exhibited various abnormalities including multiple germ pores (Table 8.3). The extensive pollen grain abnormalities observed could not be ascribed to the lack of chromosome pairing in the wheat:barley hybrids since wheat and barley haploids, which showed much asynapsis produced only single pore pollen in the case of barley and more than 90% single pore pollen in the case of wheat. However, as shown

TABLE 8.3: Number of germ pores in pollen of representative wheat:barley hybrids and derivatives, their parents and some other intergeneric F_1 hybrids

Genotype	Somatic chromosome No.	Chromosome mosaicism in PMC's*	No. of pollen grains observed	% of pollen grains with pore numbers indicated					
				1	2	3	4	5-10	11-17
Parents									
C.S. wheat †	42	-	1017	99.3	0.7				
C.S. haploid	21	-	357	91.9	7.3	0.8			
Betzes barley	14	-	500	100.0					
Betzes haploid	7	-	500	100.0					
F_1 hybrids									
Betzes x C.S.	28	+	241	58.9	21.6	14.9	2.5	2.1	
C.S. x Betzes (No.1)	28	+	439	54.5	25.9	15.5	3.2	0.9	
C.S. x Betzes (No.11)	33	+	270	41.8	30.7	11.9	9.6	6.0	
C.S. x Betzes (No.9)	27,28	-	246	84.1	11.8	3.7	0.4		
BC_1									
(C.S. x Betzes) x C.S.	49	+	379	56.1	18.7	12.7	7.2	5.0	0.3
BC_2									
(C.S. x Betzes) x $C.S._2^2$	43	+	203	45.3	18.7	8.9	9.8	13.8	3.5
(C.S. x Betzes) x $C.S._2^2$	44	+	483	72.0	16.2	6.8	3.3	1.7	
(C.S. x Betzes) x $C.S._2^2$	47	-	506	97.2	2.8				
Selfed BC_2 and BC_3									
Disomic addition 4	44	-	691	94.1	5.9				
Translocation line	42	-	877	96.1	3.8	0.1			
Other F_1 hybrids									
C.S. x Ae. speltoides	28	-	566	100.0					
C.S. x Ae. umbellulata	28	-	335	93.4	6.0	0.6			
H. arizonicum x C.S.	42	-	368	97.8	2.2				

†C.S. = Chinese Spring; *+ = Present; - = Absent.

by the representative examples given in Table 8.3, there was an exact correspondence between the occurrence of chromosome mosaicism in PMC's which in turn is correlated with the presence of chromosome 5 of barley, and the production of a high frequency of multi-pored pollen grains, in plants belonging to the F_1, BC_1, BC_2 and BC_3 generations. Although the majority of multipore pollen grains had 2 to 4 randomly scattered pores some had up to 8 (Fig. 8.2f) or in extreme cases even 17 pores. The plants with the most abnormal meiosis also possessed the highest percentage of multipore pollen grains. Furthermore, a large number of multicellular pollen grains (Fig. 8.2g) were observed in these plants which made accurate determination of pore numbers difficult. In contrast to the above plants, those plants which lacked chromosome mosaicism in PMC's, including some of the F_1 and BC_2 and BC_3 plants, all of the addition lines and the translocation line possessing the short arm of barley chromosome 5 translocated onto a wheat chromosome, did not produce pollen grains with significantly more germ pores than present in wheat haploids (Table 8.3). Furthermore, mostly single-pored pollen grains were observed in the other intergeneric hybrids examined, including Chinese Spring wheat x Ae. speltoides hybrid which exhibited a high degree of homoeologous pairing at metaphase I (average 14 chiasmata/cell). The number of pores in pollen grains was not dependent on the number of micronuclei present as was observed also by Dover (1972) in hybrids between Ae. mutica and T. aestivum. Some of the small cells protruding from large cells were found to have a nucleus without any pore and in occasional cells a pore was seen without any visible sign of a nucleus (Fig. 8.2h).

DISCUSSION

A feature of the cytology of wheat:barley hybrids was the difference in somatic chromosome constitution of hybrids from the reciprocal crosses. In contrast to the normal 28-chromosome hybrids regularly obtained when barley was used as the female parent in crosses with wheat, only one of the twenty hybrids examined from the reciprocal cross possessed such a chromosome constitution. The remaining hybrids possessed a diverse array of chromosome numbers ranging from wheat haploids with 21 chromosomes to plants possessing up to 36 chromosomes with deficiencies of some wheat and barley chromosomes and duplications of others.

These observations raise two questions of immediate interest: one relates to the reason for the difference in somatic chromosome constitution of reciprocal hybrids, and the other relates to the nature of the process responsible for producing the abnormal chromosome make-up of all but one of the wheat x barley hybrids. Considering the second question first, it should be noted that three of the F_1 plants were 21-chromosome wheat haploids. Since it is known that wheat haploids are regularly produced when Chinese Spring wheat is hybridized with diploid or tetraploid H. bulbosum (Barclay, 1975), we must consider whether a similar process is responsible for haploid production in both studies. Although the precise mechanism responsible for producing wheat haploids in wheat x H. bulbosum crosses is not known, there is some evidence that the bulbosum chromosomes are selectively eliminated in the early embryonic stage (Barclay, loc. cit.). However, the sequence of events leading to preferential elimination of bulbosum chromosomes is best documented in H. vulgare x H. bulbosum crosses (Kasha and Kao, 1970; Bennett *et al.*, 1976) which result in barley haploids. It was observed that the elimination of bulbosum chromosomes from the embryo could begin in the

first zygotic division and it usually continued over several subsequent divisions. Bennett *et al.*, (loc. cit.) quantified this process and found that from 0 to 3 chromosomes were lost at each division with the maximum rate of elimination occurring in 3-day old embryos and elimination was usually complete by day 5. However, the cause of this selective elimination was not determined in either study. Bennett *et al.*, (loc. cit.) simply recorded that bulbosum chromosomes sometimes failed to congress at metaphase and/or failed to reach the poles at anaphase. Although it is not known whether a similar process of selective elimination of barley chromosomes was responsible for the wheat haploids obtained from wheat x barley crosses, we can be confident that the majority of the chromosome abnormalities observed in these hybrids did not arise from such a process. Thus in contrast to the uniform end product of complete elimination of one set of parental chromosomes in the wheat x H. bulbosum and barley x H. bulbosum crosses, no consistent end product was observed with wheat x barley crosses. Besides the complete elimination of a set of barley chromosomes in a few hybrid plants, other plants were deficient for some wheat and barley chromosomes and possessed extra doses of others.

Although there was some small variation in the number of chromosomes in the somatic tissues of a few of the hybrids, there was a much greater variation in chromosome number between plants. These observations suggest that the abnormal chromosome constitution of wheat x barley hybrids occurs very early in development, perhaps in the first zygotic division, and remains relatively stable subsequently. If the abnormalities occurred over several divisions then we might expect as much variation in the chromosome number of different tissues within a hybrid plant as observed between different hybrids.

Although there is no direct information on the mechanism responsible for the unusual chromosome constitution of wheat x barley hybrids, we may speculate that it is due to spindle malfunction during early zygotic divisions, or perhaps just the first division of the zygote. Chromosome loss could arise from failure of certain chromosomes to move to the pole at anaphase or from the occurrence of a multipolar spindle. The most likely cause of duplicated chromosomes is their non-disjunction during mitosis so that both chromatids are included in one of the daughter cells, leading to a duplication in one daughter cell and a deficiency in the other. If one of these cells has a selective advantage over the other and provided no further abnormalities occurred, then subsequent cell division would result in a mature embryo with an unusual but constant somatic chromosome constitution. This was practically demonstrated in the scorable cells of two 10-12 days old wheat x barley F_1 hybrid embryos, where a constant chromosome count of 33 and 34, respectively, was observed in individual embryos.

Similarly we have no direct information on the cause of the difference in chromosome constitution of the reciprocal crosses. The wheat and barley chromosomes apparently divide regularly when present in the zygote and embryonic cells containing barley cytoplasm but for some unknown reason the same chromosomes divide most irregularly when present in similar cells with wheat cytoplasm. However, it is likely that this irregular partitioning of the chromosomes is restricted to the first division, or the first few divisions, of the zygote for the reasons given above. Another example which seems to be consistent with this idea is the one wheat x barley hybrid (No.1, Table 8.1) with a stable and normal complement of 28 chromosomes. If the first zygotic division alone is susceptible to disturbance, then we can imagine that in this

particular cross, involving exactly the same genotypes as used in the other crosses which gave abnormal hybrids, the cell environment in the zygote may by chance have been conducive to normal mitosis, and having survived this critical first division the full complement of 28 chromosomes was retained in the subsequent divisions to give a normal hybrid. On the other hand if several successive divisions of the zygote are susceptible to disturbance then it would be more difficult to obtain a normal hybrid, because of the reduced probability that a chance favourable cellular environment for normal mitosis could persist through each of several critical divisions.

Another major feature of the abnormal cytological behaviour of wheat:barley hybrids and their derivatives was the occurrence of chromosome number mosaicism in the PMC's of many of the plants examined. In this case, unlike chromosome number variation in somatic tissues, mosaicism in PMC's occurred in plants derived from both wheat x barley and barley x wheat crosses. However, its occurrence was restricted to those plants which possessed chromosome 5 of barley, or at least the long arm of this chromosome. Thus it was present in the normal 28-chromosome F_1 hybrids, the 49-chromosome BC_1 plants and all of the BC_2 plants possessing barley prolamins and presumably a complete chromosome 5 of barley. Conversely, chromosome mosaicism was not present in the F_1 hybrids (Nos. 2-9, Table 8.1) nor the BC_2 and later generation plants lacking barley prolamins and presumably complete chromosome 5 of barley also. Among the F_1 hybrids which were female sterile (Nos. 10-20, Table 8.1) six of them showed mosaicism in PMC's whereas two of them did not, and it would be of interest to know whether this difference was also correlated with the presence or absence of chromosome 5 of barley. It is known from N-banding that two of these hybrids (Nos. 11 and 16, Table 8.1) showing mosaicism in PMC's do possess chromosome 5, but unfortunately we have no information on the chromosome constitution of

the others.

Besides the observed association between the presence of the long arm of barley chromosome 5 and the occurrence of chromosome mosaicism in PMC's, this chromosome arm was also associated with abnormalities in tetrad development and the occurrence of pollen grains with multiple germ pores.

A key question raised by all of these observations is how does chromosome 5 of barley, or at least the gene(s) on the long arm of it, influence cellular events to give the cytological abnormalities observed?. From the nature of the disturbance seen, there can be little doubt that this barley chromosome when present in a wheat background genotype, results in modified spindle structure and function during premeiotic mitoses in archesporial cells and meiosis in PMC's. Some further clues on its possible mode of action come from relating the cytological abnormalities observed in wheat:barley hybrids to those known to be induced in wheat and wheat hybrids by disruption of the spindle with colchicine application at known stages during the development of the anther (Dover, 1972; Dover and Riley, 1973). For example, the polyploid PMC's containing approximately 2-fold (Fig. 8.2a), 4-fold (Fig. 8.2b) and even 8-fold (Fig. 8.1f) increases of the somatic chromosome number and showing mostly ring bivalent formation at metaphase I are consistent with non-functioning of the spindle during the final, penultimate and third last mitotic division in archesporial cells, respectively, similar to the findings of Dover and Riley (1973) when 0.5% colchicine was applied to immature spikes of wheat and wheat hybrids. Furthermore, the occurrence of hypoploid and hyperploid PMC's in wheat:barley hybrids is another indication of malfunctioning of the spindle in archesporial cells; in this case we can assume partial disruption of the spindle

during premeiotic mitosis leading to irregular distribution of chromosomes as observed by Dover and Riley (1973) when dilute colchicine (0.01%) was applied prior to the last premeiotic mitosis in anthers of wheat x rye and wheat x Ae. mutica hybrids. They argued that the colchicine treatment induced multipolar spindles during the last mitotic division giving the observed chromosome mosaicism in PMC's of the hybrids. Similar multipolar spindles may have produced the chromosome mosaicism observed in PMC's from some of the wheat:barley hybrids, but if such a process is to account for both hyperploid as well as hypoploid PMC's there must also have been some non-disjunction of homologues on these multipolar spindles. However, non-disjunction of some homologues on bipolar spindles could also account for most of the mosaic PMC's observed in wheat:barley hybrids. Dover and Riley (1973) observed higher levels of chromosome pairing in the mosaic PMC's but it is not clear from their studies whether this increase in the different hybrids studied is due to pairing between homoeologous chromosomes or homologues or both. Many of the mosaic PMC's observed in the present study also showed increased chromosome pairing such as ring bivalents (Figs. 8.1b and 8.1c) and occasional trivalents and even higher associations. In contrast to Fedak (1977), who claimed that homoeologous pairing occurred in 28-chromosome barley x wheat F_1 hybrids, we believe that the increased pairing observed in mosaic PMC's, in most cases at least, represents an association between homologous chromosomes, which have come from non-disjunction during premeiotic mitoses. It is difficult to prove this assertion, but the very low degree of chromosome pairing occurring in the 28-chromosome PMC's (average 0.7"/cell) in normal F_1 hybrids indicates that the barley genome does not normally induce homoeologous pairing when added to wheat. Furthermore, the increased pairing which occurred in the hyperploid and some of the hypoploid PMC's, usually

involved symmetrical associations including frequent ring bivalents, rather than the heteromorphic associations expected if homoeologous pairing was occurring.

In addition to these effects on premeiotic mitoses in archesporial cells, there is evidence that chromosome 5 of barley also influences the spindle during meiosis. Thus in plants possessing chromosome 5 and having mosaic PMC's, occasional multipolar spindles were observed at anaphase I (Fig. 8.2c) and linear polyads (Fig. 8.2d), complex polyads (Fig. 8.2e) and multipore pollen grains (Fig. 8.2f) were common. Spindle malfunctioning is again implicated since an identical suite of abnormalities was observed by Dover (1972), after 0.01% colchicine had been applied to wheat spikes during premeiotic interphase and meiotic prophase.

The association of several cytological abnormalities with one particular barley chromosome in wheat:barley hybrids has a parallel with the findings of Dover (1973a,b) with wheat-Ae. mutica addition lines. One chromosome (M) of Ae. mutica when added to wheat was found to induce homoeologous chromosome pairing at meiosis, a high degree of chromosome mosaicism in PMC's and abnormal multipore pollen. Thus these two chromosomes appear to have identical effects in inducing mosaicism in PMC's and multipore pollen grains and Dover and Riley (1973) believe there is a common basis for these two abnormalities through the establishment of multiple pole determinants. Furthermore, each of these chromosomes influences the pattern of meiotic pairing in PMC's but apparently in different ways, since Ae. mutica chromosome M induces homoeologous pairing whereas chromosome 5 of barley seems to result in homologous pairing most likely due to non-disjunction of chromosomes during premeiotic mitoses.

Dover (1973a,b) reviewed the evidence from colchicine studies, and behaviour of species crosses in the Triticinae, on the relation between abnormal chromosome pairing at meiosis, the occurrence of mosaic PMC's and multipore pollen and concluded that there was common causal basis for all of these abnormalities by way of spindle abnormalities. However, some observations in the present study show that these events are not always correlated. Thus in the Chinese Spring x Ae. speltoides F_1 hybrid there was clear evidence of homoeologous pairing at meiosis, but there was no sign of mosaicism in 50 PMC's examined, nor any multipore pollen in mature anthers (Table 8.3). Although meiosis in the Chinese Spring x Ae. umbellulata F_1 hybrid was not studied, only a small percentage of 2 and 3-pore pollen was observed as in haploid wheat. Similarly the H. arizonicum x Chinese Spring F_1 hybrid did not show any mosaicism in PMC's nor any multipore pollen grains.

The discovery that chromosome 5 of barley has a disruptive effect on premeiotic mitoses in archesporial cells and meiosis in PMC's, raises the question of whether this chromosome may also be responsible for the irregular somatic chromosome constitution of wheat x barley F_1 hybrids. It is difficult to get critical evidence on this possibility since any plant possessing chromosome 5 will have abnormal meiosis and hence produce gametes with a wide range of chromosome numbers which in turn could give progeny with unusual and unexpected chromosome constitutions, without the need for an abnormal division in the zygote. However, because the occurrence of abnormal chromosome constitution is restricted to wheat x barley F_1 hybrids and the meiotic abnormalities occur in any plants containing chromosome 5 irrespective of the source of cytoplasm, we believe that these two abnormalities are due to different causes. One possible way of testing this hypothesis would be to determine whether wheat plants carrying chromosome 6 of barley as an extra chromosome, when

pollinated with barley, give F_1 hybrids with a more regular chromosome constitution than obtained with normal wheat x barley crosses. If chromosome 5 acts by influencing early divisions of the zygote then we could expect chromosome 6 to reduce this effect just as it does in the archesporial divisions.

Finally, the cytological abnormalities observed at two stages of development in wheat:barley hybrids indicate the need for caution in interpreting the meiotic pairing configurations observed in wide crosses between plants. The occurrence of a high degree of meiotic chromosome pairing in PMC's may merely reflect the presence of homologous chromosomes in PMC's due either to abnormal mitosis in the early zygotic division, or irregular chromosome distribution during premeiotic mitosis giving mosaic PMC's.

CHAPTER 9: GENERAL DISCUSSION

It is clear from the findings reported in this thesis and other recent studies, that seeds of wheat:barley hybrids do not mature on the plant due to degeneration of endosperm tissues, and hybrid plants can be obtained only if the young embryos are cultured artificially (Kruse, 1973; Islam *et al.*, 1975, 1980; Thomas *et al.*, 1977; Fedak, 1977, 1980). This is the most likely reason why the early attempts of Farrer (1904), Waterhouse (1930) and Gordon and Raw (1932) to hybridize wheat and barley did not succeed. These workers only looked for mature seeds and thus they would have missed any true hybrid seeds that may have degenerated before maturity. Kimber and Sallee (1976), however, obtained a mature seed from T. timopheevi x H. bogdanii crosses and, furthermore, the seed germinated normally and produced a hybrid plant. H. bogdanii is closely related to some Elymus and Sitanion species (Dewey, 1971) but its relationship to barley is unknown. However, the development of endosperm tissue in hybrid seeds involving H. bogdanii crosses might be different from that occurring in wheat:barley crosses.

The rate of success using barley as the female parent in crosses to wheat was much higher than obtained with the reciprocal cross (Islam *et al.*, 1975, 1980; Fedak, 1980). This contrasts with the behaviour of Ae. squarrosa x tetraploid wheat crosses (McFadden and Sears, 1944, 1946) and rye x common wheat crosses (Röbbelen and Smutkupt, 1968), where the rate of cross success was much more using the species with higher ploidy as the female parent. Recently Snape *et al.* (1978) demonstrated that the genes Kr_1 and Kr_2 which control crossability of wheat and rye (Riley and Kimber, 1967) also control crossability of wheat with H. bulbosum. Although no direct evidence was obtained in the present work on the control of crossability of wheat and barley, it is

possible that the same system might be operative since the cross success was mugh higher with Chinese Spring wheat than with the two other commercial wheat cultivars used (Islam *et al.*, 1980). Further information on this could be obtained by crossing Chinese Spring/Hope substitution lines with barley. If the same Kr_1 and Kr_2 genes present in chromosomes 5B and 5A of Hope control crossability with barley, the cross success of Chinese Spring/Hope 5B and Chinese Spring/Hope 5A substitution lines should be lower than the rate of success obtained with the other substitution lines.

Although barley x wheat hybrids were obtained with relative ease compared with the reciprocal cross, the occurrence of pistillody and male sterility in the hybrid derivatives prevented them being used to produce disomic addition lines. However, pistillody was of variable occurrence in these derivatives, unlike with T. durum plants having Ae. caudata cytoplasm where all florets were transformed into pistil-like structures (Kihara and Tsunewaki, 1961). Furthermore, in the current study pistillody did not seem to be influenced by any particular environmental factor whereas pistillody in T. aestivum plants with Ae. caudata cytoplasm was most pronounced when the plants were grown under long days (Kihara, 1951). Male sterility was also observed in barley x wheat hybrids produced in bulbosum cytoplasm (Islam and Shepherd, 1980a) instead of barley cytoplasm. Although pistillody did not occur in this case the BC_1 and BC_2 plants having bulbosum cytoplasm were all self-sterile thus indicating that there is also an unfavourable interaction between this cytoplasm and the wheat nucleus.

There are many other examples known where male sterility is induced in wheat by an alien cytoplasm. For example, Maan and Lucken (1971) observed that common wheat plants having rye cytoplasm are male-

sterile. However, the addition of one or more rye chromosomes was found to restore fertility to these plants. Similarly, male sterility was observed when the nucleus of common wheat plants was substituted in the cytoplasm of several Aegilops species, namely Ae. triuncialis (Endo and Tsunewaki, 1975), Ae. longissima and Ae. sharonensis (Maan, 1975) and Ae. caudata (Endo, 1978; Endo and Katayama, 1978). In each case, one particular Aegilops chromosome was found to restore fertility in its own cytoplasm when present along with the wheat chromosomes. However, the Aegilops chromosomes restored fertility through having exclusive preferential transmission through the male and female gametes of the plants possessing them and the gametes lacking the critical Aegilops chromosome were inviable. There is no evidence that any barley chromosomes can restore fertility to barley x wheat hybrid derivatives in barley cytoplasm, since the BC_1 and subsequent backcross plants possessing the entire complement of wheat chromosomes and from one to seven barley chromosomes were invariably self-sterile.

Although pistillody was not observed in the Chinese Spring wheat x Betzes barley F_1 hybrids and their derivatives, the chromosome constitution of the majority of the F_1 hybrids was abnormal (Islam and Shepherd, 1980b). Fedak (1980) recently reported some results from crosses between the same wheat and barley parents and in contrast to the above he obtained just five normal 28-chromosome F_1 hybrids. In his study, the plants were raised in controlled environment cabinets at 16 hours photoperiod, whereas in the present study the plants used in crosses were all grown under normal glasshouse conditions in spring and early summer with day lengths varying from 11 to 14 hours. However, it is unlikely that the different results obtained in these two studies could be due just to these environmental differences. Instead, the comparative success rate in culturing hybrid embryos may be more

important. Fedak (1980) obtained a culture success rate of only 3% (5 hybrid plants out of 152 embryos) whereas the very high success rate of 91% (20 hybrid plants out of 22 embryos) was obtained in the present study. Thus the chromosome constitution of 97% of the embryos obtained by Fedak was not determined, and there may have been some embryos with unusual chromosome constitution among them. It should be noted that the ratio of normal plants obtained to total embryos formed is similar in both studies (5:152 vs. 1:22). Thus the two sets of results can be reconciled if it is assumed that only embryos with a normal complement of 28 chromosomes survived embryo culture in Fedak's study whereas the embryos recovered in the present study represented a random sample of all types produced. Although there is no direct evidence available, the most likely mechanism responsible for the unusual hybrids obtained in the present study is spindle abnormalities occurring during early zygotic divisions, or perhaps just the first division of the zygote.

F_1 hybrids with an abnormal chromosome make-up are not restricted to the crosses between Chinese Spring wheat and Betzes and Ketch barley reported in this thesis. Recently, similar results were obtained in crosses between Chinese Spring and the 2-row barley cultivars Golden Promise and Manker, a 6-row cultivar Morocco and also H. spontaneum (Islam and Shepherd, unpublished). Similarly, hybrids with an abnormal chromosome constitution were obtained by crossing T. durum as the female parent with Betzes and Golden Promise barley, whereas an F_1 hybrid with the expected 21 chromosomes was obtained from T. timopheevi x Manker barley crosses (Islam and Shepherd, unpublished).

The 28-chromosome normal F_1 hybrid and three other F_1 hybrids each with an unusual chromosome constitution were used to produce six disomic addition lines having different individual pairs of barley

chromosomes added to the wheat chromosome complement. The frequency of disomic additions obtained among the progeny of selfed monosomics was very low (0.63%) as was also reported in similar studies with rye (Riley, 1960; Evans and Jenkins, 1960) and Aegilops (Sears, 1956) chromosomes. The selection of plants with 43 + t somatic chromosomes, and which formed 21" + 1t" at meiosis, from among the progeny of monosomic additions proved very useful in the present study. Thus on selfing them both disomic and ditelosomic additions were obtained with relatively high frequency among their progeny. However, the use of H. bulbosum crosses to produce 22-chromosome aneuhaploids from a monosomic addition line and subsequent doubling of the chromosome number with colchicine, was found with one example (barley chromosome 6) to be an even more efficient method for producing disomic addition lines.

It was found that chromosome 5 of barley when present in a wheat background induced meiotic and post-meiotic abnormalities which resulted in self-sterility. These observations are similar to those of Dover (1973a,b) who found that a particular chromosome (M) of Ae. mutica results in cytological abnormalities when added to wheat. Despite their similarity in inducing cytological abnormalities when present in a wheat background, there were some differences in the abnormalities produced by each chromosome. Thus the Ae. mutica chromosome induced homoeologous pairing between wheat chromosomes. Although plants with barley chromosome 5 usually showed increased pairing in some PMC's, this has been ascribed to homologous pairing between chromosomes duplicated by non-disjunction in pre-meiotic mitoses rather than pairing between homoeologues.

Having produced six of the seven possible disomic wheat-barley additi lines, it is important to consider how these lines might be used in future genetic and breeding studies. As first indicated by Riley and

Chapman (1958), wheat-alien addition lines are useful for assigning genes of the alien species to particular chromosomes, provided these genes are epistatic to those of wheat. The wheat-barley addition lines have already proved useful for assigning genes controlling biochemical characters in barley to particular barley chromosomes. Thus Hart *et al.* (1980) have used these addition lines to assign genes controlling ADH to chromosome 4, GOT-2 and AMP to chromosome 6, EP and EST-1, 2 to chromosome 1 and EST-3, 4 to chromosome 3 of barley. Furthermore, it has been possible to assign the genes controlling these biochemical characters to particular arms of the barley chromosomes using the ditelosomic addition lines available. In the case of genes controlling barley seed proteins, the isolation of a translocation chromosome having the short arm of barley chromosome 5 translocated on to an unidentified wheat chromosome arm has revealed that the barley prolamins are located on the short arm whereas the long arm of this chromosome possesses gene(s) for sterility (Islam and Shepherd, 1980b) and barley glutelins (Lawrence and Shepherd, unpublished). Obviously these lines will be very useful for locating genes controlling many other barley characters provided they are epistatic to wheat characters.

These addition lines can also be used to produce substitution lines which would allow the genetic similarity of particular wheat and barley chromosomes to be determined. The isozyme studies of Hart *et al.* (1980) have provided an indication of which barley chromosomes might be genetically equivalent to the wheat chromosomes. Thus barley chromosomes 1, 4 and 6 and wheat chromosomes of homoeologous groups 7, 4 and 6, respectively, carry equivalent isozyme loci. The ability of the products of barley and wheat genes controlling ADH isozymes, and also GOT-2 isozymes, to associate into active heterodimers, led Hart *et al.* (1980) to conclude that the structure of the barley gene products is

as similar to the products of the wheat homoeoalleles as the wheat products are to each other.

Recent studies have revealed that barley chromosome 4 compensates well for wheat chromosome 4A and similarly barley chromosome 6 compensates very well for wheat chromosomes 6A, 6B and 6D, in alien substitution lines (Islam and Shepherd, unpublished) and thus these two barley chromosomes,at least, can be accommodated into the wheat homoeologous groups. Furthermore, since it is now known that the short arm of chromosome 5 of barley carries prolamins (Islam and Shepherd, 1980b) whereas the long arm carries glutelins (Lawrence and Shepherd, unpublished), this chromosome must be related to the group 1 chromosomes of wheat which carry equivalent genes on their short arm (Shepherd, 1968) and their long arm (Bietz *et al.*, 1975). The morphological similarity of barley disomic addition 2 to rye disomic addition 2R (Riley and Chapman, 1958; Sears, 1968; Koller and Zeller, 1976) and wheat tetrasomics 2A and 2D (Sears, 1954) in having narrow leaves, stems and spikes, suggests barley chromosome 2 might be homoeologous with the wheat chromosomes of homoeologous group 2.

Furthermore, the studies of Hart (1979) showed that structural genes controlling GOT-2 and AMP-1 isozymes are both located on a single chromosome in barley, A . elongatum and S. cereale and the three chromosomes of the homoeologous group 6 of wheat. Thus it is evident that although barley and wheat must have undergone considerable divergence during the hundreds of thousands or perhaps even millions of years since their separation from a common ancestor (Sakamoto, 1973), gene synteny relationships still exist between them and possibly with some of the other genera of the tribe Triticeae.

The most difficult and challenging, but potentially most rewarding, task remains. That is to find whether any agronomic characters of barley are expressed in a wheat background and whether they confer some advantage to wheat. If so, the addition lines would realise their greatest potential by acting as the starting point for transferring the desirable barley characters into wheat. One such character of interest is the reported tolerance of some barley cultivars to high level of salinity (Epstein and Norlyn, 1977). Since wheat is susceptible to salinity, it would be advantageous if this barley character could be transferred and utilized in wheat. Some work on this problem has already commenced at the Waite Agricultural Research Institute, Adelaide, South Australia. Another example, currently being investigated at the University of California, Davis, California, is the possible transfer of resistance to barley yellow dwarf virus (Qualset, 1975) from barley to wheat (Qualset, pers. comm.). However, the potential hazards of using the same genes for resistance in two major cultivated crops need to be given careful consideration, since this practice will create genetic uniformity across crops and hence increase their genetic vulnerability.

If barley characters are found which would be advantageous in wheat, they could be transferred to wheat by first producing the appropriate addition line and then applying methods similar to those used by Sears (1956), Riley and Kimber (1966) and Riley *et al.* (1968) for transferring desirable characters from other alien species to wheat.

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