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Wheat Improvement Facilitated by Novel Genetic Diversity and *In vitro* Technology

Abdul Mujeeb-Kazi

International Maize and Wheat Improvement Center (CIMMYT), Apartado 6-641, Mexico. D.F. 06600, MEXICO

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Abstract

Extensively utilized forms of wheat improvement are various conventional plant breeding protocols. These are executed without any embryo formation complexities, embryo rescue, or plantlet regeneration constraints. Such standard approaches have significantly contributed to global wheat production and will continue to do so. Ideas, however, have been expressed about the narrow genetic base in wheat. Hence, in order to widen the gene base, novel strategies have emerged that employ *in vitro* techniques which are crucial for assisting these novel genetic diversification programmes. *In vitro* techniques also contribute to research output efficiency for all bread wheats (*Triticum aestivum:* 2n = 6x = 42, AABBDD**)** and to a lesser degree for some durum wheat (*T. turgidum:* 2n = 4x = 28, AABB) cultivars. Elucidated here are outputs of an integrated research strategy that incorporates new genetic diversity in wheat linked with *in vitro* procedural involvement.

Introduction

Novel genetic diversity for wheat improvement has been studied for over 125 years, when Wilson (1876) reported the first wide hybrid between wheat and rye. Then followed the first *Triticale* (Rimpau 1891) and the debatable wheat x barley cross (Farrer 1904). During the 1920's and 1930's were the *Triticum* x *Aegilops* combinations (Kihara 1937); then came at least two significant events that changed the course of wide hybridization research. The first was the advent of colchicine in late 1930's (Eigsti and Dustin 1955). The treatment with colchicine yielded fertile amphiploids giving new crops (Gupta and Priyadarshan 1982) or generating information about the genomes of wheat (McFadden and Sears 1946). The second event was the integration of embryo

culture technology in the 1960's (Murashige and Skoog 1962). The latter set the stage for active wide cross research with wheat (Kruse 1967, 1969, 1973, 1974a, b) and then the strategy was rigorously pursued by several others with varied basic and applied outputs (Islam et al. 1981; Sharma and Gill 1983 a, b, c; Mujeeb-Kazi and Kimber 1985; Mujeeb-Kazi et al. 1987, 1989; Mujeeb-Kazi and Asiedu 1989, 1990; Gill 1989; Wang 1989; Sharma 1995; Mujeeb-Kazi et al. 1996a, Mujeeb-Kazi 2001 a, b, 2003; Friebe et al. 1993; Jauhar 1993).

The combination of the above two developments formed an integral component for wide cross research that is basically pursued to exploit novel genetic resources of the *Triticeae,* thereby enhancing the genetic variation in the wheat crop. The novel resources reside in three gene pools and their incorporation strategies are categorized under interspecific and intergeneric hybridization (Mujeeb-Kazi and Rajaram 2002). Dewey (1984) indicated that the wealth of this Triticeae resource comprised at least 325 species, of which 250 were perennials and 75 annuals.

The species have been classified on the basis of their genomic constitution into primary, secondary and tertiary gene pools (Jiang et al. 1994). Presented here are some outputs associated with wheat x distant species hybridizations. In addition, a more recent development (Mujeeb-Kazi 2000) that enhances plant breeding efficiency with multiple ramifications across biological disciplines is elucidated, where *in vitro* technology is pivotal for research outputs.

Production of wide hybrids

Germplasm and hybrid production : Elite wheat cultivars generally are female parents in crosses with wild species. These cultivars are susceptible for a trait that limits wheat productivity and is present in the wild species. The parental germplasms, treated according to their habit (Spring, winter or facultative), are grown under controlled environments or in the field. Several dates of planting are essential in order to ensure a niche for the crosses. The simplest protocol for hybrid production involves emasculation, pollination, embryo rescue, regeneration, cytological validation, inducing amphiploidy or getting a backcross 1 (BC1) derivative (Mujeeb-Kazi et al. 1987).

Barriers are prevalent but as of the last decade these obstacles have been significantly reduced (Sharma 1995). The key manipulative procedures to ensure success of hybrid outputs have been pre- and post-pollination treatments such as (a) bud-pollination, (b) frequency of pollination, (c) variation of culture media, (d) regeneration treatment, and (e) effective colchicine induction or (f) backcrossing protocol.

The *in vitro* stage has been the single most crucial phase in wide hybridization research and variation made here alone can further enhance success. The wide array of hybrids produced so far, have generally exploited the Murashige and Skoog (l.c.) basic formulation. Some have used Taira and Larter's (1978) medium for small embryos. Additional variations have been reviewed/reported by Raghavan (1980), Young et al. (1981), Williams et al. (1987), Baum et al. (1992), that led Sharma (1995) express that further breakthroughs in wide crossing may be possible through the exploitation of growth regulators followed by embryo rescue, posing the question "which species are left to be crossed with wheat".

Wide hybrid combinations have shown a dramatic increase over the past two decades (Sharma 1995), essentially due to the methodology variations and strategies that have emerged. These protocols are now an integral part of wheat improvement across interspecific and intergeneric domains, that exploit wild species across different gene pools.

It may be safe to conclude that if there were no *in vitro* involvement, there would have been no exciting wide crossing, resulting only in limited use of genetic resources and very little gene diversification in wheat.

The gene pools of the Triticeae : Natural genetic diversity within the *Triticeae* resides within three gene pools that are structured upon the genomic constitution of the species. The three pools are primary, secondary and tertiary. For wheat improvement the primary pool species are preferred, since genetic transfers with wheat occur as a consequence of homologous recombination, yielding practical outputs very readily. Some combinations require *in vitro* assistance, of which use of the A and D genome diploids have formed a major component of our programme. These species are also influencing other global wheat improvement programmes for biotic/abiotic stresses. The secondary gene pool comprises polyploid *Triticum* and *Aegilops* species, that share one genome with wheats. Genetic transfers are slightly complex and chromosome pairing is reduced. Embryo rescue is required for obtaining F1 hybrids. The tertiary gene pool members consist of diploid/and polyploid species with non-homologous genomes. Genetic transfers are highly complex, chromosome pairing is extremely reduced, and embryo rescue is essential.

The focus of the wheat improvement in this article will be on the D genome diploid species accessions of the primary gene pool, followed by a brief mention of an *in vitro* based novel protocol for speeding up alien genetic introgression from the tertiary gene pool. Finally an in-depth elucidation of an *in vitro* dependent breeding/basic research undertaking will be discussed.

The primary gene pool (Interspecific hybridization)

Ae. tauschii (2n : 2 x = 14, DD); syn. *Ae. squarrosa, T. tauschii*, goat grass: *In vitro* technology has contributed significantly to the interspecific area of wide crosses of which *Ae. tauschii* has been the most significant. This grass has perfect homology with the D genome of bread wheat; and from the wide distribution of its numerous accessions, a large arsenal of genes can be readily introgressed (Mujeeb-Kazi 2001 b). The wide distribution and diversity of this species across Eurasia (Kimber and Feldman 1987) provides a unique oppor-tunity for exploiting new genetic variability. *Ae. tauschii* has a wide range of resistance or tolerance to biotic or abiotic stresses (Valkoun et al. 1990). This wild progenitor also appears to be a potent source of new variability for important yield components such as 1000-grain weight, increased photo-synthetic rate and bread making quality (Mujeeb-Kazi et al. 1998).

Utilization of *Aegilops tauschii* for wheat improvement

The *Ae. tauschii* accessions could be used in the following three ways for wheat improvement:

- Producing synthetic hexaploids by crossing *T. turgidum* cultivars with *Ae. tauschii* accessions.
- Crossing elite, but susceptible *T. aestivum* cultivars with resistant *Ae. tauschii* accessions and backcrossing (BC) the ABDD Fl hybrids with the elite *T. aestivum* cultivar used in the initial cross.
- Extracting the AABB genomes from commercial *T. aestivum* cultivars and then developing hexaploids by crossing them with the desired *Ae. tauschii* accessions.

For any of the above techniques, it is important that the desired traits in the D genome from *Ae. tauschii* be identified since genetic factors in the A and B genomes may mask or modify its expression. However, this may not be a general rule, since earlier researchers have observed that synthetic hexaploids which were produced with a karnal bunt (KB) susceptible durum parent, expressed KB resistance of the *Ae. tauschii* accessions.

We can screen the *Ae. tauschii* accessions for their many desired attributes and then cross selected ones to *T. aestivum*. When screening the *Ae. tauschii* accessions is a major constraint, these can be hybridized first with *T. turgidum,* followed by screening the resulting synthetic hexaploids (SH) for traits of interest.

Resistance screening of the *Ae. tauschii* accessions for stresses at Poza Rica and at Toluca have been inconclusive. Growing conditions at these two Mexican locations can adversely affect the data obtained for the alien species, but logistically the sites are ideal for disease resistance screening. Because of the species screening constraints, winter habit, and the tendency for shattering (which could cause a weed problem at the above stations) of the *Ae. tauschii* accessions, it was decided to indiscriminately cross *T. turgidum* with *Ae. tauschii* accessions. This has allowed screening all resulting synthetic hexaploids more adequately for stress tolerance without having to deal with vernalization. In addition, when a positive attribute was found (the durum parent being susceptible), the breeding programme can immediately use synthetic hexaploids. Even if the synthetics express diluted resistance, the end-product resistance is far superior to that encountered in the best wheat germplasm available.

Synthetic hexaploid production

Methodology

The hybridization process is quite simple, when any of several manipulative crossing procedures (durums as the female parent) as described by Kruse (1973), Sharma and Gill (1983a), Mujeeb-Kazi and Asiedu (1990), or Riera-Lizarazu and Mujeeb-Kazi (1990) is used. For crossing cycles, the durum cultivars obtained from CIMMYT's durum breeding programmes, are planted over at least three planting intervals, so that their flowering coincides with the flowering of the *Ae. tauschii* accessions. Procedures for embryo rescue, embryo culture, and plantlet management are similar to those described by Mujeeb-Kazi et al. (1987). The plantlets are transplanted to a potted soil mix, maintained in greenhouses and cytologically validated (2n = 3x = 21, ABD).

These Fl hybrids are doubled (induced with colchicine or spontaneous) to produce 42-chromosome synthetic hexaploids (2n = 6x = 42, AABBDD; Fig. 1). The majority of plants in the population show the normal 42 chromosomes. Chromosome-count-based aneuploid individuals, are discarded (Fig. 2). Resistant or tolerant synthetic hexaploids selected on the basis of appropriate disease or stress screening, are then crossed conventionally with other bread wheat cultivars.

Direct crossing of Aegilops tauschii *with bread wheat :* The most ideal, efficient technique for exploiting *Ae. tauschii* variability for bread wheat improvement is to achieve direct transfers from resistant/tolerant *Ae. tauschii* accessions to bread wheat. This methodology rapidly produces improved BC1

derivatives with the six genomes (AABBDD), five of which (AABBD) resemble the elite wheat cultivar used in the cross (Fig. 3). With this methodology, we may have to encounter instances of aneuploidy in the BC derivatives, diminishing its value in mapping programmes for recognized quantitative traits. Cox et al. (1990) reported its numerous advantages.

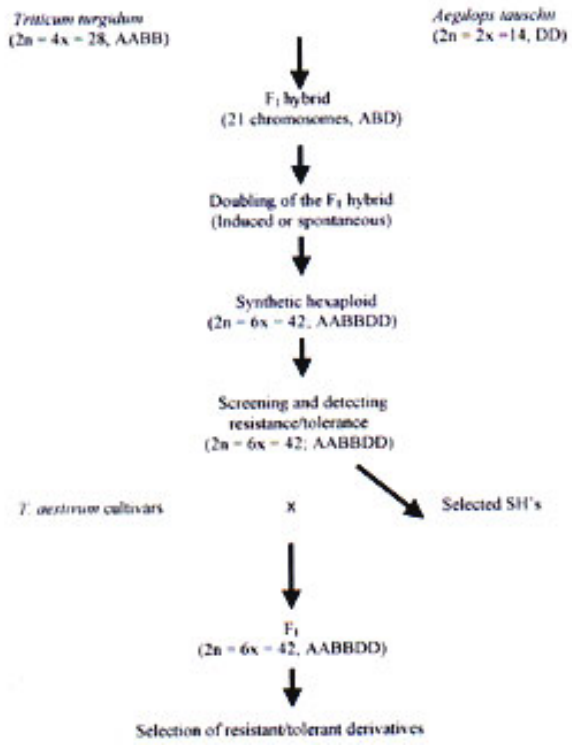

Fig. **1**. Schematic diagram showing the production of synthetic hexaploids derived from crossing, *Triticum turgidum* x *Aegilops tauschii* and their utilization.

Before direct crossing with bread wheat, reliable screening of the *Ae. tauschii* accessions for resistance and tolerance to diseases and abiotic stresses is critical. Alonso and Kimber (1984), Cox et al. (1990, 1991) and Gill and Raupp (1987) unequivocally placed priority on crossing *Ae. tauschii* directly with bread wheat cultivars. Based on the transfer of stem rust resistance from *Ae. tauschii* to the bread wheat cultivar, Chinese Spring, Alonso and Kimber (1984) determined that one backcross on to the Fl hybrids restored 92% of the genotype of the recurrent parent.

In situations where there are constraints to direct screening of *Ae. tauschii* accessions, such as lack of reliable identification of resistance or

tolerance to *H. sativum*, *F. graminearum*, *N. indica*, and salinity, screening of the synthetic hexaploids of the cross, *T. turgidum* x *Ae. tauschii* seems to be an alternative procedure, especially in case of susceptible durums. The information obtained from screening the synthetics allows targeting of specific *Ae. tauschii* accessions for direct crossing with susceptible elite bread wheat cultivars, e.g., 'Ciano T 79' and 'Kanchan' for *H. sativum* resistance, 'Seri M 82' and 'Opata M 85' for *F. graminearum* resistance, and 'Oasis F 86' or 'PBW 343" for salt tolerance. The use of these cultivars enabled duplication of the crossing successes that Alonso and Kimber (1984) reported for the cultivar Chinese Spring. Several other options are also available for achieving additional crossing successes (Gill and Raupp 1987, Riera-Lizarazu and Mujeeb-Kazi 1990).

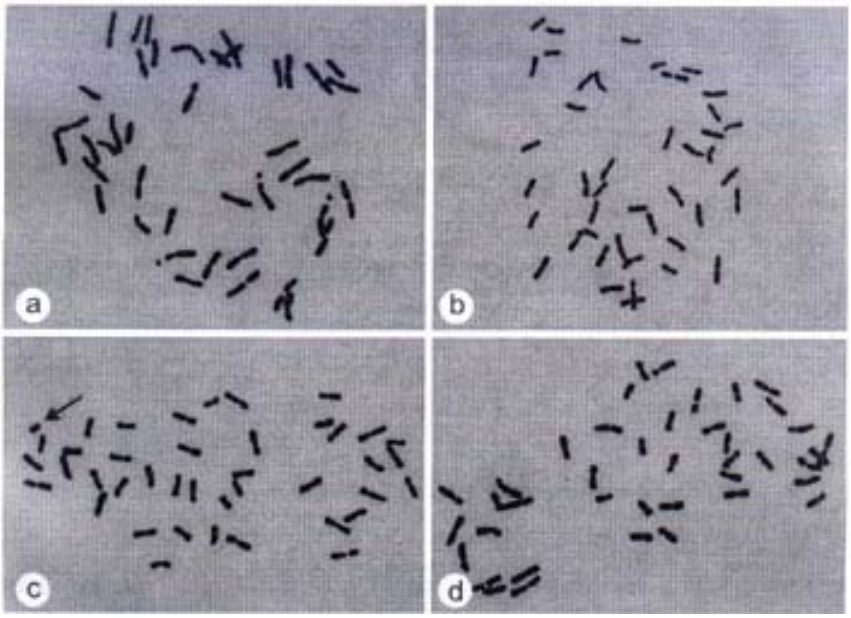

Fig. **2**. Somatic chromosome numbers of synthetic hexaploids from *Triticum turgidum* x *Aegilops tauschii* : (a) 2n = 6x = 42, (b) hyperploid with 43 chromosomes, (c) 42 with a telocentric (arrowed), and (d) hypoploid with 41 chromosomes.

We have satisfactorily screened *Ae. tauschii* accessions for karnal bunt (KB) resistance and identified several 0% infection types. Successful crosses have been made between KB-susceptible bread wheat cultivars, Seri M 82 and Bacanora T 88 and several of these *Ae. tauschii* accessions. The above procedure is highly efficient and yields 99% normal hybrids with 2n = 4x = 28 chromosomes, setting the stage for fast applied outputs.

Extracting the AABB genomes from bread wheat cultivars : Extracting the AABB genomes from commercial *T. aestivum* cultivars and then developing hexaploids (Fig. 4) by crossing them with desired *Ae. tauschii* accessions allow a very clear analysis of the genetic contribution of the alien D genome. There is a negligible interference from recombinant segregation of the A and B genomes that is widespread in the fast hybridization procedure (Fig. 1). However, transmission of paternal chromosomes and aneuploidy in the backcross generations can complicate the process, seemingly without under-mining its research output precision.

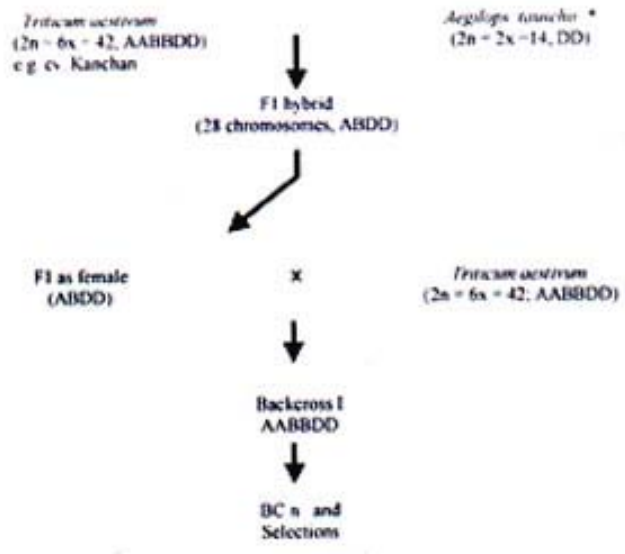

Fig. **3**. Schematic diagram demonstrating alien transfers from *Aegilops tauschii* (2n = 2x = 14, DD) to elite *Triticum aestivum* cultivars via direct crossing and backcrossing. (*This DD will give the recombined version as the end product).

Some practical contributions from the D genome via synthetic hexaploids: Reported here is the current status of stress resistance identified in these synthetics, and also the transfer of the traits of choice to elite but stress susceptible bread wheats. The stresses are *Fusarium* head scab, *H. sativum*, *N. indica, S. tritici*, and salinity.

Germplasm screening

The SH germplasm was screened for *Cochliobolus sativus* (= *H. sativum*) *F. graminearum* (Head Scab), *S. tritici,* and *N. indica* (Karnal Bunt) at Poza Rica, Toluca (Scab and Septoria), and Obregon, Mexico, respectively over 3 - 5 years. The stringent screening formed the basis of the conclusions made in this

study. All evaluations were carried out under field conditions. The synthetics were planted in hill plots. The evaluation protocols were similar to those earlier reported for *Cochliobolus sativa*, *Fusarium*, *S. tritici*, and *N. indica* (Mujeeb-Kazi 2001a).

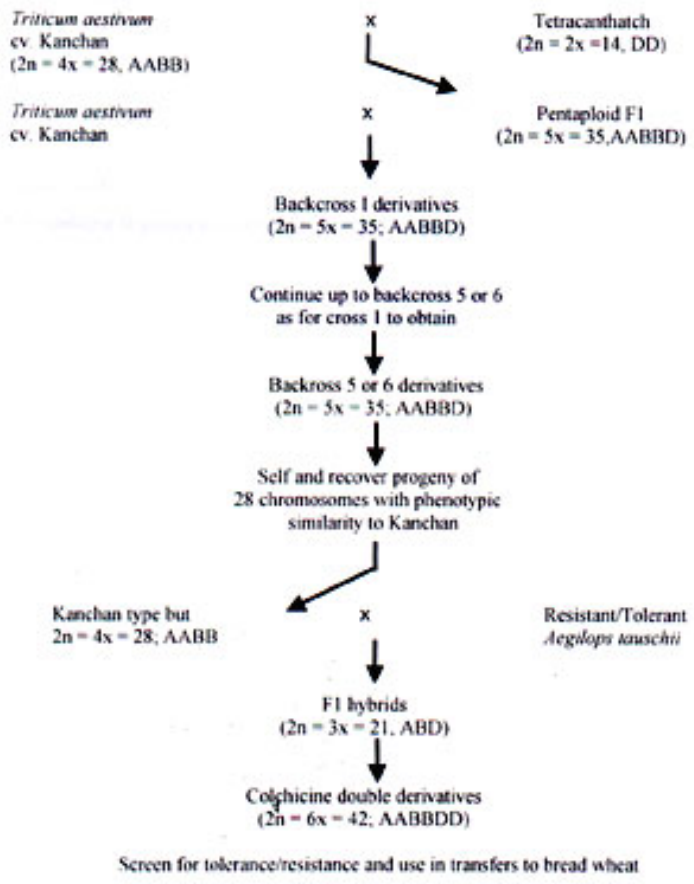

Fig. **4**. Schematic diagram showing extraction of the AABB component from an elite hexaploid wheat cultivar, derivation of a synthetic hexaploid by crossing it to *Aegilops tauschii* (2n = 2x =14; DD), and utilization of the doubled derivatives (2n = 6x = 42; AABBDD).

Abiotic stress screening for salinity was conducted under greenhouse conditions at El Batan, Mexico. The hydroponic protocol recommended by Gorham (Personal Comm.) was followed. The procedure was based upon K : Na discrimination. The seedlings tested, were grown for a period of 21 days at 50 mM NaCl.

Identification and utilization of resistant SH germplasm: From the data of the above five stress tests, resistant SH's were identified and hybridized with stress susceptible elite bread wheat cultivars. The hybrids were advanced by the pedigree method with the focus on selections for (a) plant type, (b) maturity, (c) height and (d) multiple disease resistance, keeping the global cultivation picture into consideration. Protocols for evaluation and the test sites were similar to those described for the SH germplasm above. The SH/BW or BW/SH entries from all biotic stress related germplasm were field-planted in 2 - 3 m double rows, tested and subjected to desired selections. The selected materials were stabilized, using the maize-based doubled haploid protocol (Mujeeb-Kazi 2000).

Results and Discussion

Status of synthetic hexaploid (SH) wheats

From our working collection of *Ae. tauschii* accessions that currently number about 750, we have randomly hybridized them to elite durum wheat cultivars. So far, 1000 synthetics have been produced over the past decade and from these one elite set of 95 entries has been compiled and globally distributed. Not all the 750 accessions have been combined, but attempts are in progress to accomplish this objective. There are three categories of SH's : (1) same durums with different accessions, (2) different durums with the same accession and (3) durum wheat/an accession and its reciprocal cross. In addition, beyond the 1000 synthetics are SH's from a gene-pyramiding programme. SH wheats with *Ae. tauschii* accessions of diverse nature, involved in conferring stress resistance were hybridized. Of these, a number of highly resistant F2 plants (superior resistance than their individual SH's) were used for producing stable DH derivatives. Sub-sets of SH's, involved in the various biotic and abiotic stresses, have been prepared for DNA fingerprinting, using D genome microsatellites.

Selection criteria for resistant/tolerant SH's and their derivatives

The criteria set for identifying resistance or tolerance to biotic/abiotic stress in the SH wheats were stringent for combinations designed for advancement of pre-breeding objectives. These did not exceed a 3-3 double digit score for *H. sativum* and 2-2 for *S. tritici*, 15.0% or less Type 11 infection score for *F. graminearum*, and less than 3.0% infected kernels for *N. indica*. For salinity, the tolerant germplasms selected, possessed K : Na discrimination values of greater than 2.0, where values close to 1.0 were associated with non-tolerance to the stress.

Synthetics related germplasm for biotic stress resistance

(a) *Fusarium graminearum* (Head scab)

Resistance in SH wheats and derivatives: The SH wheat lines (*T. turgidum* x *Ae. tauschii*) highly resistant (less than 15% infection) to *F. graminearum* (Type II) are presented in Table 1. Resistant BW check Sumai 3 scored between 12 and 15% less, while the moderately susceptible BW check 'Flycatcher' always showed over 20% and the durum wheat, 'Altar 84' over 40% infection. After several cycles of testing, some advanced BW/SH scab resistant derivatives were selected for Type II resistance (Table 1). These derivatives generally also possessed resistance to leaf rust, stripe rust and *S. tritici*. Each scab resistant entry selected had a disease score of less than 15%

Table 1. Promising D genome synthetic hexaploids and some SH and BW derivatives screened for head scab (Type II) at Toluca, Mexico; mean values across years.

Germplasm pedigree	Infection type II(%)
Synthetics	
YUK/(217)	11.6
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (629)	10.9
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (878)	12.7
68.111/RGB-U//WARD/3/FGO/4/RABI/5/ (882)	12.3
SORA/ (884)	13.2
68.111/RGB-U//WARD/3/FGO/4/RABI/5/(890)	12.7
CEW (895)	12.0
GAN/(180)	10.8
LCK59.61/(313)	11.8
SCOOP I/ (358)	12.9
YUK/(217)	11.6
TRN/ (700)	13.5
DOY1/(333)	12.5
DVERD_2/(1027)	13.1
MAYOOR//TK SN1081/ (222)	8.7
FLYCATCHER (Susceptible)	33.8
SUMAI-3 (Resistant)	12.0
ALTAR 84	40.8
Pre-breeding derivatives	
BCN//DOY1/ (447)	12.5
BCN/DOY1/ (447)	12.0
ALTAR 84/(224)//2*YACO	10.1
OPATA/6/RGB-U//WARD/3/FGO.. .(878)	11.6
SABUF/5/BCN/4/RABI//GS/CRA/3/(190)	11.2
SABUF/3/BCN//CETA/(895)	11.0
MAYOOR//TK SN1081/(222)	8.9
FLYCATCHER (Susceptible)	33.8
SUMAI-3 (Resistant)	12.0

*Percentage score means from 10 spikes tested.
Numbers in parenthesis = *Aegilops tauschii* accessions in wide crosses working collection.

across each test year. Sumai-3 averaged 12% over the past three test years. The most promising entries from the BW/SH combinations were further tested for three other scab categories (I, III, IV). Four were found to possess combined resistance to all the four types of scab.

(b) *Helminthosporium sativum* (syn. *Cochliobolus sativus* spot blotch)

The disease affects wheat crops across several environments stretching from Latin America, Africa, Asia to South East Asia including Bangladesh, representing a major disease location. Our Mexican screening site is the severest of all. Several SH/BW germplasms were evaluated at this location, based upon damage recorded progressively (79 to 96 d) on leaves and grain (Table 2). All lines possessed superior *C. sativus* resistance as compared to 'Mayoor' a resistant check, and 'Ciano 79', a susceptible check. The synthetic hexaploids represent diverse accessional gene pyramiding and were developed by intercrossing several *T. turgidum* /*Ae. tauschii* combinations, involving different *Ae. tauschii* accessions. From segregating F2 populations, spot blotch resistant plants were selected and hybridized with *Zea mays*. The resulting haploids (n = 3x = 21, ABD) resulting from total elimination of maize chromosomes were colchicine treated to yield homozygous doubled derivatives (2n = 6x = 42, AABBDD). The seven bread wheat (BW) genotypes were derived from various SH lines, crossed with the spot blotch susceptible bread wheat cultivars 'Bacanora', 'Opata', and 'Yaco'. Some of the best lines (SH and advanced lines) are shown in Table 2. The disease score does not exceed 3-2 and grain finish is less than 2 versus a susceptible score of 9-9 and 4, respectively.

Table 2. Characteristic of five double haploid synthetic hexaploid wheat, and seven bread wheat germplasm lines resistant to *Helminthosporium sativum*; mean values across years.

Pedigree	Infection	Grain Finish
Synthetic hexaploids		
Gan/(236)f//Doy I/ (447)	2-2	1
Gan/(236)//Ceta/(895)	2-2	2
Scoop l/(434)//Ceta/(895)	3-3	1
Doy l/(447)//Ceta/ (895)	3-3	2
68.11 l/Rgn-u/TWard/3/ Fgo/4/(629) /5/Ceta/(895)	3-3	1
Ciano 79 (Susceptible)	9-9	4
Pre-breeding advanced derivatives		
Altar/(224)//2*Yaco	3-2	1
Sabuf//Altar/(224)/3/Yaco/Croc-l/(205)	2.2	2
Bcn//Sora/(323)	2-2	2
Opata/3/Sora// (323)	3-3	2
Bcn/4/68.11 l/Rgb-u//Ward/3/ (325)	2-1	1
Bcn//Doy/(447	3-2	1
Mayoor (Resistant	9-3	2
Ciano 79 (Susceptible)	9-9	4

Numbers in parenthesis = *Aegilops tauschii* accessions in wide crosses working collection.
Infection scores rated on a double digit modified scale: the first digit indicates the height of infection where l = lowest leaf; 5 = up to mid plant; and 9 = up to flag leaf, the second digit indicates disease severity on infected leaves, where 1 = 10% coverage; 5 = 50% and 9 = 90% coverage. Grain finish scale of 1 to 5, where l = low grain infection and 5 = severe infection.

(c) *Septoria tritici* (Leaf blotch)

Septoria leaf blotch limits wheat production in high rainfall areas across 10.4 million hectares globally. *S. tritici* (syn. *Mycosphaerella graminis*) resistant synthetic hexaploids (SH) crossed with the susceptible wheat cultivars Seri M82, Yaco, Borlaug M95, Opata M85, Kauz, Papago M86, and the moderately resistant cultivar Bagula, gave advanced lines with good leaf blotch resistance (Mujeeb-Kazi et al. 2000). Ratings of *S. tritici* resistance were based upon leaf damage recorded at water, milk, and dough growth stages, using a double digit modified scoring scale (Mujeeb-Kazi et al. 2000). The disease ratings of each of the genotypes indicated their superiority over the bread wheat check cultivars (scores of 2-1 or 1.1 vs. 4.1 to 8.9). All germplasms have a good agronomic plant type and were high yielding under optimum disease-free environments.

For more information on pedigrees, disease scoring, and agronomic phenotype descriptor details of some of these registered lines, see Mujeeb-Kazi et al. (2000).

(d) *Neovossia indica* (Karnal bunt)

Several of the *Ae. tauschii* accessions in our working collection were identified as sources of karnal bunt resistance. These accessions were randomly hybridized with *T. turgidum* cultivars to yield SH wheats and resistant entries identified. The bread wheat germplasm lines were derived from the karnal bunt resistant synthetic hexaploids crossed with karnal bunt susceptible bread wheat cultivars: Flycatcher, Kauz, Yaco, Borlaug and Papago M86. Segregating generations of the crosses were advanced by the pedigree method. The mean agronomic performance of the germplasm lines over five years of field tests demonstrates an acceptable phenotype; this is considered to be an asset for breeding use.

The disease score was based on the number of infected and healthy kernels at maturity in each plot. Synthetic hexaploid and bread wheat germplasm line infections ranged from 0 up to 1.97% compared with a 30% mean infection of 'WL711', the susceptible bread wheat check cultivar. The durum wheats in the pedigrees had infection levels from 0.3 to 1.6%, while the SH wheats were immune. These germplasms offer genetic diversity of the *Ae. tauschii* accessions as well as the A and B genome diversity of the durum cultivars in SH pedigrees.

(e) Abiotic stress (Salinity tolerance)

K^+/Na^+ discrimination is a trait that enhances salinity tolerance in bread wheat compared to durum wheat, and is present in the wheat ancestor *Ae. tauschii*. K : Na ratios were lower in the durum parents (close to 1.0) than in the elite synthetics (over 1.0 and greater than 2.0 being selected for breeding use). This confirmed that the K : Na trait was present in the synthetics, and demonstrated its expression in durum wheats. The best-performing synthetics with high K : Na ratios are shown in Table 3.

Table 3. Best synthetic hexaploids from the elite set of 95 entries with high K:Na ratios as determined from leaf tissue samples from seedlings grown in hydroponics in 50 mMol NaCl.

Pedigree	K : Na
68.111/Rgb-U//Ward Resel/3/Stil/4/(781) †	4.5
68.11 l/Rgb-U//Ward/3/Fgo/4/Rabi/5/ (883)	3.0
68112/Ward//(369)	3.2
Altar 84/ (224)	4.9
Altar 84/ (502)	5.2
Altar 84/ (220)	3.0
Altar 84/(211)	3.5
Altar 84/ (Bangor)	3.7
Ceta/(1027)	3.5
Ceta/ (895)	3.9
Croc I/ (224)	3.9
D67.2/P66.270// (220)	3.5
D67.2/P66.270//(213)	3.4

† = *Ae. tauschii* with accession number/source in parenthesis

Conclusions

The above stress screening results are indicative of the enormous potential that *Ae. tauschii* accessions offer via utilization of their respective synthetic hexaploid products. The extension of these observations is to incorporate the resistant/tolerant synthetics in a wheat breeding programme, where desirable bread wheat cultivars will be crossed with selected SH's with a view to producing resistant/tolerant free-threshing derivatives. Some derivatives of such wheats may prove to be drought tolerant as shown by Trethowan et al. (2003). The other hexaploid wheat varieties developed by Mujeeb-Kazi et al. are those that are resistant to *S. tritici* (2000), *H. sativum* (2001 b), *N. indica* (2001 a), *F. graminearum* (2003). The extended research phase from this stage is to select those accessions that are most potent in their output performance and incorporate them in a 'direct crossing' programme to yield rapid practical

outputs. The additional thrust encompasses the use of genome and chromosome specific microsatellites, which are particularly amenable for the D genome chromosomes of *Ae. tauschii*. Since 1998 such molecular programmes are pursued vigorously in leading laboratories and already spectacular progress has been made in this direction.. It would be appropriate to emphasize that *in vitro* technology has formed a key phase for enabling the exploitation of novel gene sources for wheat improvement. This new strategy has led to varietal releases in China (2002), Spain (2003) and is offering immense molecular diversity for application of DNA based diagnostics to make breeding programmes in the near future more targeted and efficient.

The tertiary gene pool (Intergeneric hybridization)

The wild grasses in this gene pool are genomically quite diverse from wheat, difficult to hybridize, and when successfully combined exhibit little to no integeneric chromosome pairing. Hence accomplishing beneficial alien transfers is time consuming and complex. Despite these limitations significant success and advancements have been made over the past two decades in diverse wide hybridization areas that led Sharma (1995) to express confidence on the range of wide hybrids that is possible to produce globally. Crucial for this success are new hybridization technologies and *in vitro* methodologies. The basic step implicated shifts to achieve practical gains. The classical methodologies have generated genetic outputs but the maximum effect realized has only been from the spontaneously produced TIBL.IRS translocation (Rajaram et al. 1983) and to some extent the TIAL-IRS wheat germplasm.

Recent candidates, where tertiary pool species are involved, relate to wheat germplasms with the *Lr19*, *Bdv2* (Singh et al. 2001) and *H. sativum* derivative with *Thinopyum curvifolium* in its pedigree. (Mujeeb-Kazi et al. 1996 b). In attempts to override the complex phases of gene transfers via classical cytogenetics, a novel integrated strategy was structured (Mujeeb-Kazi 2001 a). The novel strategy has shown a good functionality (Mujeeb-Kazi 2003). The protocol exploits the *Ph* locus (Sears 1977) on chromosome 5B, operating on the wheat ∇ maize haploid (Mujeeb-Kazi 2000) mechanism, i.e., (a) PCR to detect *ph* plants (Qu et al. 1998), (b) fluorescent *in situ* hybridization (FISH) to detect translocations, (c) C-banding to identify the chromosomes with meiotic cytology being a support diagnostic. This protocol has the potential of producing translocations swiftly and also allows researchers to exploit previously generated germplasm stocks that are maintained in gene banks. This precludes the demanding effort of having to re-make the complex intergeneric hybrids (Mujeeb-Kazi and Rajaram 2002). CIMMYT alone maintains its 170

intergeneric hybrids, 34 amphiploids and 17 self-fertile backcross 1 combinations that can be the base for initiating the *ph* based manipulation strategy. The successful exploitation of tertiary pool species revolves around the *in vitro* step. At first it is necessary to make the complex hybrid embryos grow, if the haploid process is adopted. The haploid step, using wheat x maize crossing has developed into an efficient tool for a wheat improvement programme. The successful *in vitro* growth of the haploid wheat embryos, plays a key role in obtaining the desired wheat variety. The protocol and its role in diverse areas of wheat improvement follow.

Wheat maize based haploidy and biological applications

Polyhaploid production in wheat has relied heavily on anther culture and sexual crosses with *Hordeum bulbosum*. The occurrence of somaclonal variation, aneuploidy and genotypic specificity are major limitations of anther culture. The homoeologous group 5 crossability loci (*Kr*) influences the sexual crossings of wheat with *H. bulbosum*. Producing wheat haploids by sexual crosses of bread wheat x maize, pearl millet or *Tripsacum* has become a significant procedure on account of the production constraints of anther culture and *H. bulbosum* crosses. Currently, this procedure is being routinely used in wheat cytogenetics, wheat breeding using wide crosses, with extension of its application in genetic engineering and molecular mapping.

Recent technique advances in enhancing efficiency of haploid production utilize detached tillers from selected plants, their culture in a nutrient solution including sulfurous acid to avoid contamination, and hot water (43°C) immersion of the spikes for three minutes to effect emasculation. Hormonal treatment (100 ppm 2,4-D) is essential, for embryo rescue 14 days after pollination. The protocol is almost 100% effective for all bread wheat cultivars. It has genotypic specificity for durums and *TriticoSecale*. The contribution of D genome chromosomes is a definitive factor in the success of the cross. Mean frequencies of embryo excision are 25%, plantlet differentiation 95% with a colchicine-induced doubling range from 95 to 100%. Results of the application of techniques in wide crosses, genetics, cytogenetics, breeding, and genetic transformation are presented. Use of stored millet pollen to facilitate haploid production during the period, when wheat pollen is unavailable or scarce, will also be elucidated.

Some history

Polyhaploid plants are important in that they reduce the number of generations to fix the homozygosity of wheat and other cereal plants. A homozygous plant is obtained when the chromosomes of a polyhaploid are

doubled. Until recently, polyhaploid production in the Triticeae had relied mostly on anther culture and sexual crossings with the perennial barley relative, *H. bulbosum* . The occurence of somaclonal variation, aneuploidy, and genotypic specificity (Picard 1989) are major limitations of anther culture in polyhaploid production. The homoeologous group 5 crossability loci (*Kr1, Kr2*, *Kr3*) are limiting factors in *H. bulbosum* sexual crossings (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch and Snape 1986, 1987; Mujeeb-Kazi and Asiedu 1990). In order to avoid tissue culture-associated somaclonal variation, the sexual route to polyhaploid production appears to be more desirable; however, a substitute for the *H. bulbosum* technique was needed in order to overcome genotypic specificity. *Zea mays* (Laurie and Bennett 1986, 1988 a, c; O'Donoughue and Bennett 1988; Laurie et al. 1990; Zenkteler and Nitzsche 1984) and *T. dactyloides* (Riera-Lizarazu and Mujeeb-Kazi 1993) have emerged as alternative sexual routes for polyhaploid production in the Triticeae, and led to documented production of polyhaploid plants. There have also been successful crosses between *Z. mays* and *T. turgidum* as well as other *Triticum* and *Aegilops* spp. (O'Donoughue and Bennett 1988). Successful fertilization has also been accomplished in crosses between wheat and *Sorghum bicolor* (Laurie and Bennett 1988 a, b); *Pennisetum glaucum,* pearl millet (Laurie 1989); *Z. mays* ssp. *mexicana*, teosinte (Ushiyama et al. 1991); *H vulgare* , barley (Laurie and Bennett 1988c); and *Secale cereale* (Laurie et al. 1990).

After fertilization occurs in any of the above crosses, chromosomes of the male parent are eliminated very early (Laurie and Bennett 1988a), thus producing a polyhaploid embryo that retains only the chromosomes of the female wheat parent. Normally, the embryo soon aborts; however, exogenous treatment with the synthetic auxin, 2,4-D promotes seed and embryo development until the embryo can be excised and plated on to a synthetic medium for continued growth and plantlet regeneration (Laurie et al. 1990).

Using this methodology, polyhaploid cereal plants have been recovered from crosses of bread wheat (*T. aestivum*) x maize (Comeau et al. 1988; Laurie and Bennett 1988 c, Suenaga and Nakajima 1989; Inagaki and Tahir 1990; Rines et al. 1990; Riera-Lizarazu and Mujeeb-Kazi 1990; Laurie and Reymondie 1991); durum wheat x maize (Inagaki et al. 1998a); wheat x pearl millet (Ahmad and Comeau 1990); bread wheat x sorghum (Ohkawa et al. 1992); bread wheat x teosinte (Ushiyama et al. 1991); barley x maize (Furusho et al. 1991); and *T. aestivum* x*Tripsacum* (Riera-Lizarazu and Mujeeb-Kazi 1993).

Some haploid production approaches

(a) Via anther culture

Anther culture, a method for haploid induction, is widely used in wheat and many other crops. Significant genotypic differences are present for anther culture response in wheat (Andersen et al. 1987). Few responding genotypes, low haploid recovery and aneuploidy have been some of the major limitations in the anther culture method. Advances in its application are still being pursued (Kisana et al. 1993).

(b) Via crosses with *Hordeum bulbosum*

Wide crosses of wheat with a bulbous wild barley (*H. bulbosum*) results in the production of immature haploid embryos of wheat after preferential elimination of *H. bulbosum* chromosomes from the hybrid zygotes (Barclay 1975). However, the crossability of wheat with *H. bulbosum* is genetically controlled by the genes *Kr1* and *Kr2* located on chromosomes 5B and 5A, respectively (Falk and Kasha 1983; Snape et al. 1979). According to the pedigrees of wheat varieties, crossable genotypes can be traced to the variety Chinese Spring or to materials of Asian origin (Falk and Kasha 1981). Both Japanese and Chinese wheat varieties, and in particular, local varieties, are highly crossable with *H. bulbosum* (Inagaki and Snape 1982; Inagaki 1986; Li and Hu 1986). Wheat genotypes carrying the dominant *Kr* gene(s) are not crossable with *H. bulbosum* and cannot produce haploid embryos. The lack of hybridization of wheat genotypes with *H. bulbosum* is due to the failure of the pollen tube to penetrate the embryo sac (Snape et al. 1980). Application of plant growth regulator, 2,4-D promotes seed setting and embryo formation, when compatible genotypes, used for crosses are unable to break the barrier of cross-incompatibility (Inagaki 1986). Since the efficiency of wheat haploid production is greatly influenced by the crossability of *H. bulbosum* on to wheat, the *H. bulbosum* method is restricted only to crossable wheat genotypes.

(c) Via maize and other sources

Ultra-wide crosses of wheat with members of the Panicoides subfamily have been attempted in alien genetic transfer. Maize (*Z. mays*) pollen can successfully hybridize wheat egg cells and produce hybrid zygotes (Laurie and Bennett 1986), irrespective of the presence of *Kr* gene(s). Maize chromosomes are rapidly eliminated from the hybrid zygotes, requiring artificial rescue of proembryos at an early developmental stage (Comeau et al. 1992; Laurie and Bennett 1987 1988c). A post-pollination 2,4-D treatment is critical to enhance embryo development in wheat x maize crosses (Suenaga and Nakajima 1989). Maize pollination and subsequent 2,4-D treatment results in the production of

immature wheat embryos capable of regenerating haploid plants, even for wheat varieties that are cross-incompatible with *H. bulbosum*. Wheat haploid production through maize crosses has been achieved using diverse wheat varieties (Inagaki and Tahir 1990; Laurie and Reymondie 1991). Some species related to maize, such as teosinte (Ushiyama et al. 1991) and eastern gamagrass (*T. dactyloides*) (Riera-Lizarazu and Mujeeb-Kazi 1993), are efficient alternative pollen donors for wheat haploid production.

Cytological evidence indicates successful fertilization and elimination of paternal chromosomes from hybrid zygotes in sorghum (*S. bicolor*) and pearl millet (*P. glaucum*) crosses, which suggests that sorghum and pearl millet are potential pollen sources for wheat haploid production (Ahmad and Comeau 1990; Comeau et al. 1992; Laurie and Bennett 1988 b; Laurie 1989). Wheat haploids were obtained at high frequencies from sorghum (Ohkawa et al. 1992) and pearl millet crosses, followed by 2,4-D treatment after pollination. However, in sorghum crosses with wheat, a strong genotypic barrier was expressed for embryo formation (Inagaki and Mujeeb-Kazi 1995). Therefore, haploid production through crosses with maize and pearl millet appears more stable than other methods because of its less pronounced genotypic effect on haploid embryo formation.

The haploid production protocol

Germplasm: Sowing times of seed materials need to be determined in order to synchronize flowering times of wheat and pollen donors. A continuous supply of pollen from pollen parents sown at one or two-week intervals should be ensured.

For intact plants: At the time of ear emergence, wheat spikes still attached to the plants are conventionally emasculated and pollinated with maize or pearl millet one day before the estimated conventional wheat anthesis. On two consecutive days after pollination, the uppermost internodes of the wheat culms with pollinated spikes get needle-injected with a 100 mg/l 2,4-D solution according to the method of Inagaki and Tahir (1990).

For detached tillers: Wheat tillers with spikes are cut at the base of each tiller from growing plants and cultured in a flask with tap water. These spikes are then emasculated and pollinated as described above. After pollination, the detached wheat tillers are cultured in a solution containing 40 g/l sucrose, 8 ml/l sulfurous acid (6% SO_2) and 100 mg/l 2,4-D and cultured until embryo rescue. Culture conditions maintained are 22.5°C, 12 h day-length and 60 - 70% relative humidity in a growth chamber.

Regeneration and rescue : At 14 days after pollination, immature embryos get aseptically excised from wheat seeds, and transferred on to a half strength Murashige and Skoog (l.c.) culture medium supplemented with 20 g/l sucrose and 6 g/l agarose. The embryos are incubated at 25°C, 12 h daylength and approximately 5000 lux light intensity. Plants regenerated from embryos are cytologically examined in root-tip mitotic preparations, stained with aceto-orcein, according to the method of Mujeeb-Kazi and Miranda (1985).

Modifications in pollen storage : Fresh pollen collected between 9:30 and 10:00 a.m. is screened through a 0.5 mm aperture sieve to remove anthers. Ten g of pollen get spread on a paper tray and dried with gentle ventilation at 35°C and 35 - 40% relative humidity. Pollen water content is reduced to 11.8% in maize and 5.3% in pearl millet in approximately two hours of drying. Pollen water content is determined from a 0.5 g pollen sample dried at 95°C for five hours. The dried pollen is then distributed among cryopreservation tubes (1.5 ml volume). These sealed tubes are immediately immersed in liquid nitrogen (–196°C). Maize and pearl millet pollen were stored for 8 and 10 months, respectively. After thawing pollen in the tubes kept in a waterbath at 38°C for five min can be used for crossing. Pearl millet pollen is better suited for storage and delayed use, since the induction frequency of haploids remains unaltered as recorded for maize (Inagaki and Mujeeb-Kazi 1994).

Modifications in emasculation: When numbers of spikes to be emasculated are excessive, they are immersed in hot water maintained at 43°C for three min one day before pollen shed. The treatment renders anthers sterile. Spikes are then pollinated with maize or millet without removing wheat anthers.

Applications of haploidy in some biological areas

Haploidy for production/stabilization of disomic alien chromosome addition lines: In cytogenetic studies involving alien chromosomes addition to wheat to yield addition lines, a constraint was identified in the paternal transmission of the alien chromosome. Hence, 44 chromosome derivatives do not result. Often multiple disomics may be a requirement and their stability a factor in the maintenance of stocks. Haploid induction on 43 chromosome plants (42 wheat plus 1 alien) or for example 45 chromosomes (42 wheat plus 3 different aliens) yield haploids ranging from 21, 21 + 1 or 21 and different, 21 + 1, 21 + 2 or 21 + 3 combinations. These, upon doubling, result in disomic or multiple disomic additions (Fig. 5 for the disomic product). Whether such homozygous additions are more stable than those conventionally produced is a concept that needs testing.

Haploidy in cytogenetic manipulation integrated with the Ph and ph locus : The doubled haploid wheat/maize based manipulation protocol is applicable to *Ph* F1 wheat/alien F1 hybrids. This is also applicable to amphiploids and fertile BC1 combinations, where the *Ph* locus is homozygous dominant.

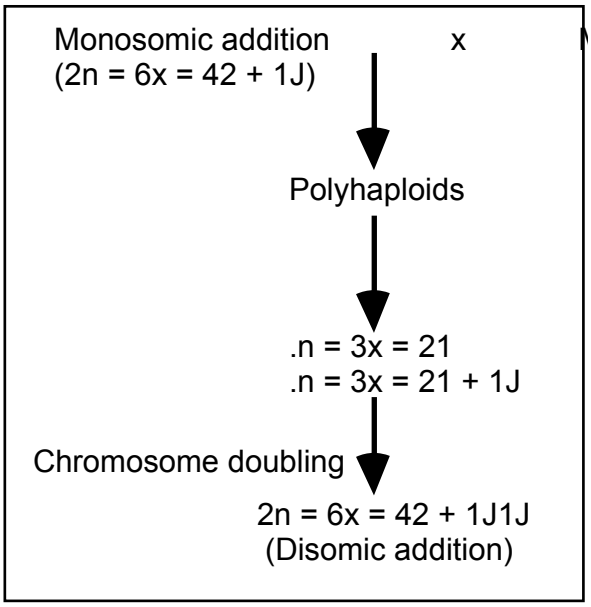

Fig. **5**. Development of a disomic alien addition line from the monosomic addition parent via the maize haploid induction protocol.

The DH role in salvaging *Ph* based F1 hybrids has become an option to enable *ph* mediated alien introgression/s without having to remake complex F1 hybrids using the *ph* genetic stock (Sears 1977) as the maternal parent. Because of our living F1 herbarium involving wheat and several alien species (*Ph* locus present), BC1 derivatives can be produced by pollinating these *Ph* F1 wheat/alien hybrids with the Chinese Spring *ph ph* wheat genetic stock. The BC1 progenies (*Ph ph*) are crossed with maize to yield polyhaploids that possess the *Ph* or *ph* locus. The entire wheat and alien chromosomal complement is represented. The *ph* based haploids derived from the *Ph ph* BC1 derivatives identified at the seedling stage by a PCR based diagnostic analysis (Gill and Gill 1996, Qu et al. 1998), can enhance the programme efficiency (Fig. 6), and allow a crop improvement programme, integrated into the breeding methodologies.

The BC1 derivatives can also be selfed and the *Ph Ph, Ph ph* or *ph ph* progeny similarly identified.

Haploidy in enhancing breeding efficiency : In self-pollinating crops, such as bread wheat (*Triticum aestivum*), breeding programmes include a three-step process for developing germplasm: (1) genetic recombination for enlarging variation, (2) identification and selection of recombinant genotypes according to their agronomic types and (3) fixation of genes in homozygous genotypes. Pedigree selection is the conventional method of accumulating genetic recombination in each generation. The heterozygosity in early generations makes the efficient identification and selection of recombinant genotypes more difficult. Repeated pedigree selection increases homozygosity, but requires many generation cycles to attain homozygosity for loci associated with agronomic traits. The single-seed descent method is used to obtain homozygous inbreds by accelerating generation cycles. Its application, however, is dependent on the growth habit of the plant materials used.

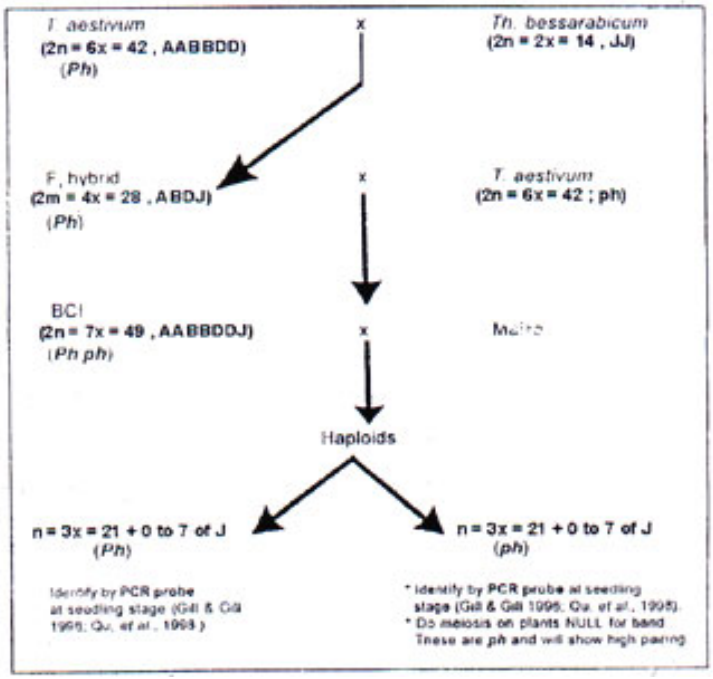

Fig. **6**. Use of a PCR probe to detect *Ph* and *ph* plants in intergeneric derivatives obtained from backcross 1 (*Ph ph*) x maize crosses.

Artificial production of haploid plants followed by chromosome doubling is a quick method for obtaining homozygous recombinant genotypes from heterozygous parental genotypes in a single generation (Nei 1963). This DH method has the advantage in that yield evaluation commences earlier and

speed up varietal development. This method also has the great advantage of increasing the efficiency of selection because doubled haploid lines do not express dominance variation and segregation within lines (Snape 1989).

Recently the yield performance of each group of ten bread wheat lines selected by doubled haploid (DH), single-seed descent (SSD) and pedigree selection (PS) methods from three F1 crosses was compared with the aim of evaluating the DH method in breeding programmes (Inagaki et al. 1998b). Their results confirmed that the DH method saves time in obtaining recombinant inbred lines ready for yield evaluation. However, a larger DH population is required to achieve the same level of genetic advance with the PS method in crosses containing a greater genetic variation.

Since the DH procedure is accurate and genotype independent in bread wheat, its application can fit almost any filial generation. The perfection of techniques allows one to work on the breeder's selected F2 or F3 plants in diverse populations for which the detached spike modification is an asset, if larger numbers are involved. Fixing lines for achieving homozygosity is also seen as a boon to assist global testing of elite lines, and economize country wheat improvement programmes.

Haploidy linked with molecular biology via production of mapping populations

Based on F1, or backcross I (BC1): The development of molecular mapping populations based upon conventional or distant species involvement has been approached by the DH route. Desired parents are crossed to produce the F1 seed. Plants raised from hybrid seeds are then crossed with maize or an alternate source to generate haploids. When a synthetic is used to develop mapping populations, one can expect high DNA polymorphism frequencies. Such mapping populations with *Ae. tauschii* as the alien source are currently being developed for resistance to drought, salinity, spot blotch and *Fusarium* head blight. The DH route offers stability, homozygosity, and these stocks can be utilized very broadly by researchers. These mapping populations can also be produced on BC1 germplasm.

Haploidy in wheat transformation for stability of gene expression: The stability of the DH products has also been applied in conferring this attribute to wheat transformants. It is recognized that transformants may tend to be unstable in that the gene expression in the selfed progenies of transformants may not remain consistent. Could it be due to segregation as a consequence of incipient heterozygosity, apart from other reasons like methylation? Having a

homozygous DH transformant, initially positive for the transgene expression, will hopefully address this question of expressive gene stability over each selfed DH analysis, and allow the comparison of germplasms with normal selfed derivatives (Fig. 7).

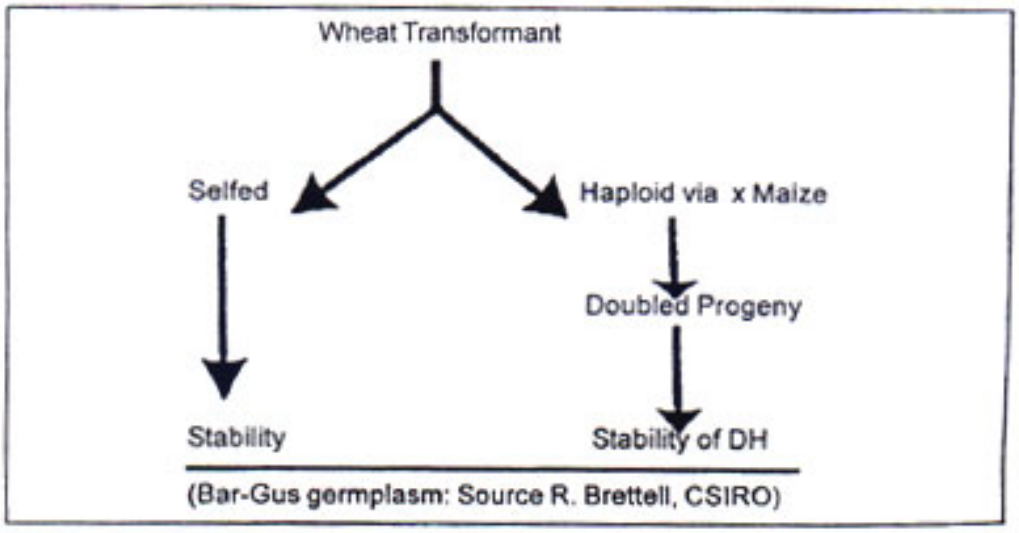

Fig. 7. Production of DH derivatives from wheat transformants to evaluate the gene expression stability.

Haploidy for conducting genetic monosomic analysis: The DH approach is being effectively used to conduct genetic analyses for identifying physical gene locations through complete or partial monosomic analysis. The partial analysis is conducted when resistance is associated with the D genome of synthetic hexaploid (SH) wheats or their resistant SH/susceptible bread wheat derivatives that are resistant. This resistance in the derivatives is attributed to the D genome chromosomes of *Ae. tauschii* (Fig. 8). The F1 monosomics of 1D to 7D chromosomes (2n = 6x = 40 + 1D to 40 + 7D), when crossed with maize yield 21 chromosome polyhaploids with the 1D to 7D contributions derived from resistant SH x BW progeny. Doubling these polyhaploid plants (n = 3x = 21) with colchicine, results in the origin of stable double haploids with 42 chromosome. Each DH now possesses the homozygous 1D to 7D chromosomes of the resistant SH parent. Thereafter, they are analyzed for the location/s of the resistant gene/s on appropriate chromosomes. Upon screening, the non-segregating resistant DH's are attributed to have these gene/s in them. Apart from simplifying the conventional monosomic analysis, the stable monosomic derived DH germplasm also facilitates global distribution of the

developed germplasm. The germplasm enables experimental repetition without having to re-build the analytical germplasm, as is necessary when the conventional monosomic analytical procedure is followed.

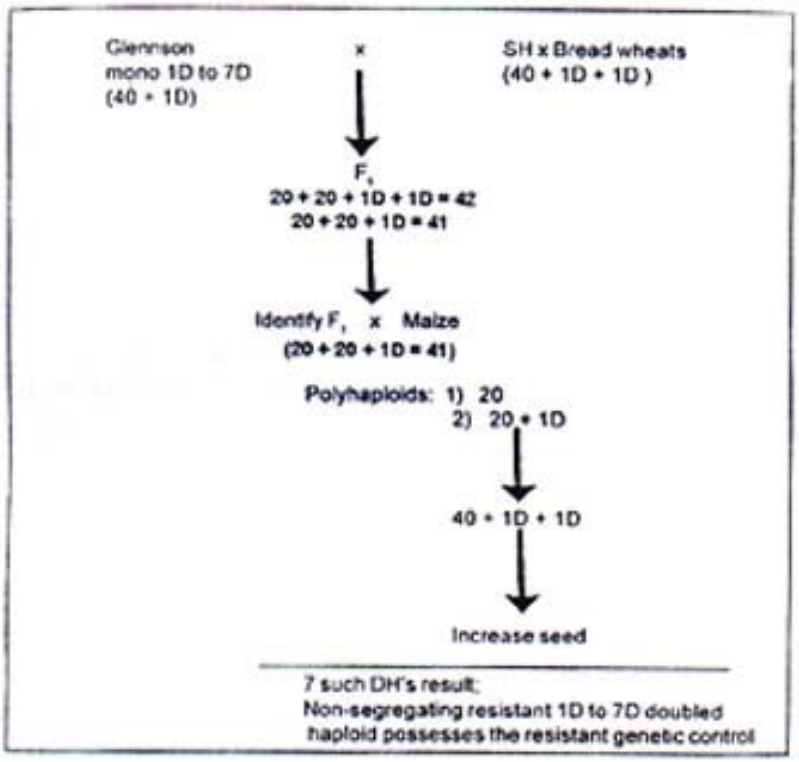

Fig. **8**. Steps involved in conducting a partial D genome based monosomic analysis utilizing doubled haploidy.

Constraints recognized for the wheat x maize haploid procedure

Bread wheat germplasm of diverse growth habit (spring, winter, facultative) all respond to the haploid induction procedure using maize or other species as pollen sources. This makes its application attractive and routine in bread wheat improvement. Genotypic specificity is non-existent, and production frequencies render the utilization of these techniques quite simple for crop improvement. A similar trend, however, is not seen so far for durums or *TriticoSecale* (Almouslem et al. 1998, Inagaki et al. 1998a).

Manipulations to enhance frequencies of haploid production have been positive (Inagaki et al. 1998) but breaking barriers to render the incompatible types compatible has not been achieved. The D genome and some of its

chromosomes decidedly have an influence as observed by Almouslem et al. (1998), Inagaki et al. (1998a) for durums and Inagaki et al. (1997) for *TriticoSecale*. The positive contributions of D genome to haploid production are also apparent from durum and their derived SH (durum x *Ae. tauschii*) crosses with maize. The haploid production is significantly higher in the SH's as compared with their durum parents that lack the D genome.

Conclusion

Crosses between wheat (*T. aestivum*) and maize are an effective means of producing wheat polyhaploids. Haploid generation is independent of the source of maize pollen and of the recipient wheat cultivar, though some pollen source preference does exist. Application of the haploid procedure is useful in breeding programmes, genetic analysis, developing mapping populations, producing cytogenetic stocks, and as an off-shoot in assisting wide crossing programmes. Techniques that complement satisfactory hybridization product frequencies strongly favor the use of detached spikes, sucrose as a nutrient source and sulfurous acid for overcoming contamination. Post pollination 2,4-D treatment remains crucial. Use of stored maize pollen gives positive results with a low frequency. Improved frequencies are obtained when stored pearl millet pollen is used. The technique gives a variable response with durum wheats and x *TriticoSecale*.

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