Chapter 23

Wide Crosses for Durum Wheat Improvement

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INTRODUCTION

Durum wheat (*Triticum turgidum* L.; $2n = 4x = 28$, AABB) improvement has predominantly been accomplished through conventional plant breeding methodologies and is an approach that shall continue to be the predominant procedure in the future. Novel approaches that complement plant breeding have emerged and are attracting research interest. The impacts of these novel approaches are in general considered to be quite futuristic, and one such approach encompasses wide hybridization. This distant stance is particularly valid for intergeneric hybridization and has so far received more attention than the short-term swifter product output area of interspecific hybridization. The two hybridization categorizations are dictated by the genera/species genomic designations and their location within the various Triticeae gene pools (primary, secondary, tertiary) of these annual and perennial wild species. Though wide hybrids have been attempted and studied for over 100 years, many involving *Triticum* and *Aegilops* species were produced during the 1920s and 1930s (Kihara, 1937) from which the genomic relationships of the genus were derived (Lilienfeld, 1951). It was, however, the pioneering work of the late Anton Kruse (1967, 1969, 1973) that led to an increase in research momentum associated with basic/strategic/applied activities involving bread or durum wheat combinations with species of *Agropyron, Aegilops, Elymus, Haynaldia, Heteranthelium, Hordeum,* and other Triticeae members. This intensity has magnified significantly over the past two and a half decades. Most hybridization projects have been motivated by the major desire to capture and exploit the wild Triticeae species' abundant genetic diversity in two salient areas. One method is focused on harnessing biotic/abiotic stress diversity for ensuring that crop outputs are durable and sustainable. The second and more recent route is to exploit the species diversity for generating a wealth of DNA polymorphisms to enrich the sophisticated molecular biology disciplines.

This chapter describes the structure of the Triticeae gene pools; the methodology and rationale of parent selection plus hybrid production; cytological analysis; introgression of alien diversity; the breeding strategy yielding genetic stocks or advanced derivatives; and their stability and maintenance. The focus is on durum wheats in our CIMMYT-based scenario of wide cross research activities.

THE TRITICEAE GENE POOLS: GENETIC DIVERSITY AND DISTRIBUTION

Genetic variation is crucial for plant improvement. When diversity is limited, allelic variation can be induced via mutagenesis and introduced into new germplasms. Hence, researchers may combat the biotic and abiotic stress scenarios with a chain of new cultivars to address the global food production demands. It is felt that outputs will be favorable for diploids, e.g., *Hordeum*, and complex for tetra- or hexaploid wheats. More recent research has attempted to capture alien genetic diversity channeled into cereals by the wide hybridization technology. Use of wild Triticeae relatives has the advantage over mutagenesis in that the introduction of the genetic material from the target alien source into wheat is a consequence of recombination between homologous chromosomes, and would thus place the alien segments in the best location in the recipient wheat chromosome. The ease of this process is dependent on the alien species and its location within the Triticeae gene pools.

The approximately 325 species in the Triticeae (Dewey, 1984), comprised of 250 perennials and 75 annuals, are distributed among three gene pools: primary, secondary, and tertiary. Based upon the genetic distance of these species from wheat have emerged the wide hybridization interspecific and intergeneric areas where hybrid production is common to both categories.

Gene Pools and Hybrid Production

The Primary Gene Pool

The primary gene pool species include the hexaploid land races, cultivated tetraploids, wild *Triticum dicoccoides*, and diploid donors of the A and D genomes to durum/bread wheats. In our CIMMYT wide crosses program, we have concentrated on utilizing the A and D genome accessions since their hybrids with durum wheats require embryo rescue, are generally self-sterile, and thus are not readily available for breeding programs.

The A genome accessional diversity present in several accessions of *T. boeoticum, T. monococcum,* and *T. urartu* ($2n = 2x = 14$, AA) is a potent source for durum wheat improvement by protocols associated with direct crossing [Durum wheat (AABB) × AA accession→ABA hybrid] followed by a backcross or top-cross breeding strategy on the F_1 ABA hybrid. An extension that promotes the production of genetic stocks for global utility from the ABA hybrid involves colchicine-induced doubling of the $2n = 3x = 21$ (ABA) F_1 hybrid to generate a $2n = 6x = 42$ (AABBAA) amphiploid. These hexaploids (amphiploids) can be subjected to multilocational stress screening from which descriptors can be established. We have currently generated 194 such amphiploids and screened the germplasms for several biotic stresses, including *Cochliobolus sativus, Septoria tritici, S. nodorum, Fusarium graminearum,* BYDV, and leaf and stripe rust. The potential to evaluate these hexaploids for quality alleles and other stresses is promising and can be explored further. In general, durum × A genome crosses are simplistic and of high frequency, with A to A genome transfers occurring readily as a consequence of homologous recombination in the $2n = 3x = 21$, ABA hybrid. Extensive data accumulated over decades in our program for crosses involving several durum wheat cultivars and numerous A genome accessions have given mean figures of 30 percent for seed set, leading to 80 percent embryo excision and 80 percent plantlet differentiation, with at least 70 percent colchicine-induced doubling frequency resulting in fertile AABBAA hexaploid amphiploids. Genetic exchange is inferred from the F_1 meiotic association values of the A genomes that range from a mean of 5.5 to 6.0 bivalents/meiocyte. The amphiploid stocks with four A genomes are highly fertile with an elevated bivalent pairing frequency (16.5 to 21.0), trivalency from 0 to 0.4, and quadrivalency from 0 to 1.7/meiocyte. The utilization strategy of the A genome is elucidated in Figure 23.1. Cytology of the ABA F_1 hybrid and its AABBAA amphiploid is presented in Figures 23.2 and 23.3.

The D genome diploid *Aegilops tauschii* (syn. *Ae. squarrosa, T. tauschii,* goat grass; $2n = 2x = 14$, DD) demonstrates an unparalled wealth of genetic diversity for several biotic and abiotic stresses as observed upon screening the AABBDD synthetic hexaploids (Mujeeb-Kazi, 2001b). Because of its homology with the D genome of bread wheat *(T. aestivum),* the transfers from *Ae. tauschii* are swift and are ideally integrated, analogous to events that occur in conventional crop improvement. Use of *Ae. tauschii* requires the initial cross to incorporate embryo culture; for achieving fertility, colchicine treatment facilitates the generation of the hexaploid product ($2n = 6x = 42$, AABBDD) called a "synthetic hexaploid" (SH). The hexaploid formation could also be spontaneous.

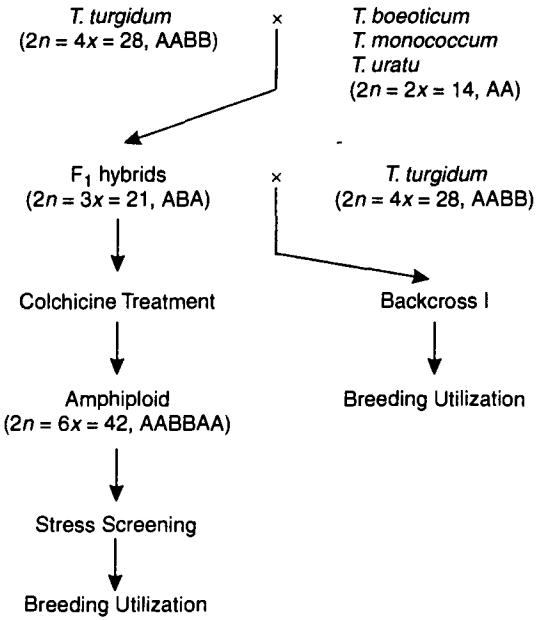

FIGURE 23.1. The utilization strategy of the various A genome diploid accessions ($2n = 2x = 14$) for durum wheat improvement.

Durum wheats lack resistance for several biotic/abiotic stresses that are well characterized. Thus, if a derived resistant SH stock involving a susceptible durum cultivar and *Ae. tauschii* accession is identified, the logical inference is that the *Ae. tauschii* parent accession is the resistance contributor. Some such SH wheats with an unequivocal contribution to resistance from *Ae. tauschii* accessions have been identified for several biotic and abiotic stresses. These are for drought, salinity, *Fusarium graminearum, C. sativus, S. tritici, S. nodorum,* barley yellow dwarf virus (BYDV), and leaf and stripe rust. Whether the D genome stress resistance diversity can be transferred to durum wheats has been the question durum breeders have often asked and are keen to see positively resolved.

We have attempted to introgress the D genome *Ae. tauschii* resistance for durum wheat improvement and have placed at a high priority the transfer of scab *(F. graminearum)* resistance genes. The strategy of transfer from D to the A genome of durum and further prebreeding advance is elucidated in the section in this chapter regarding future trends.

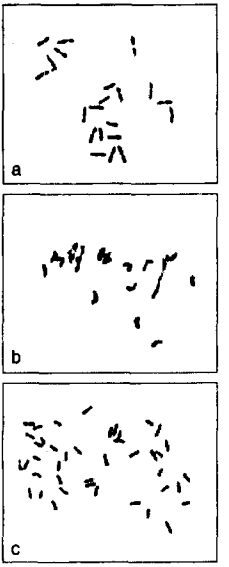

FIGURE 23.2. Cytology of durum/A genome hybrid, showing (a) an F_1 somatic cell with $2n = 3x = 21$ ABA chromosomes, (b) an F_1 meiocyte with 6 bivalents + 9 univalents, and (c) a somatic amphiploid cell ($2n = 6x = 42$, AABBAA).

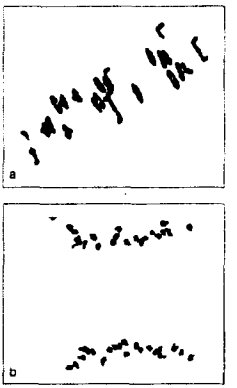

FIGURE 23.3. (a) A meiocyte of the 42 AABBAA amphiploid with 21 bivalent chromosome associations (16 rings + 5 rods), and (b) normal anaphase I 21 + 21 separation.

The Secondary Gene Pool

The utilization of the secondary pool species accessions in our program are essentially similar to that of the A genome, with the focus being on the Sitopsis diploid member *Ae. speltoides*. In general, the secondary gene pool species share one genome in common to wheat, including *Aegilops* and *Triticum* species that are mostly polyhaploids. The Sitopsis diploid *Aegilops* species are related to the B genome (syn. S^b) and are sources of additional genetic diversity. Of these five diploids, we are attempting to exploit the *Ae. speltoides* accessions for improving spring durum wheats. *Aegilops speltoides* has seldom been utilized in wheat breeding because it distorts the meiotic arrangements in most F_1 hybrid combinations. On a positive note, we could envisage increased chromosomal rearrangements in the F_1 ABB hybrids produced, thereby facilitating chromosomal exchanges to support the applied goals characteristic of our breeding programs. The colchicine-induced hexaploid amphiploids ($2n = 6x = 42$; AABBBB) with four B genomes exhibit multivalent associations; bivalency is fairly prevalent, although slightly less than was observed for AABBAA amphiploids (Figure 23.4), translating into adequate fertility yielding satisfactory seed increases.

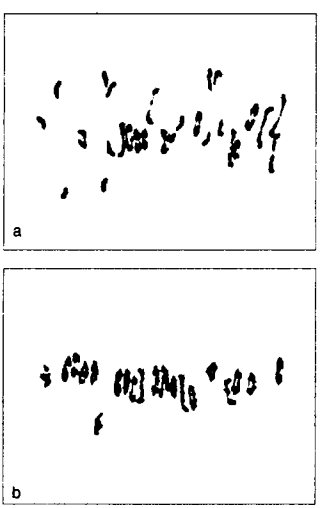

FIGURE 23.4. (a) A meiocyte of the B genome amphiploid ($2n = 6x = 42$, AABBBB) showing decreased bivalent chromosome pairing compared to that of an A genome amphiploid (b).

The diversity utilization of the *Ae. speltoides* accessions could be directly from the F_1 route or from the amphiploid base. The strategy is similar to that elucidated for the A genome resource in Figure 23.1. Limited testing so far suggests that the AABBBB amphiploids are an excellent diversity source for resistance to *S. tritici, S. nodorum,* leaf/stripe rust, *C. sativus,* powdery mildew, and BYDV, as well as an average source for *F. graminearum* (Type II). The potential benefits to address other production constraints across the varied global megaenvironments are apparently very promising for which the amphiploid stocks could be the means.

The Tertiary Gene Pool

Most of the species in the tertiary gene pool have been difficult to exploit for cereal improvement, more often being used for bread wheat. The genomes of these species are nonhomologous with those of wheat, and genetic transfers cannot be made by homologous recombination. Genomic homology does exist and is an option that enables genetic transfers through an array of tedious and complicated cytogenetic protocols that frequently delay the desired research outputs required by those seeking expedient practical agricultural gains at the farm level.

The data gathered from research efforts with bread wheat have enriched several wide cross operative technologies. Hybrid production is no longer a major obstacle (Sharma, 1995). Embryo rescue/differentiation has become more efficient, and hybrid validation techniques are of remarkable definition due to the integration of conventional (Mujeeb-Kazi and Asiedu, 1990) and molecular diagnostics (Islam-Faridi and Mujeeb-Kazi, 1995). The production of stable genetic stock has become greater in both quantity and quality (Mujeeb-Kazi, 2001a), and breeding techniques have been well integrated into the earlier exclusive cytogenetic research interests that did not focus on providing agricultural products (Mujeeb-Kazi et al., 1987, 1989). Stress germplasm screening has become more stringent/refined as well, culminating in stability of the euploid products adding to breeding efficiency (Mujeeb-Kazi, 2000). These are some crucial facets that have now become routine tools in bread wheat wide crosses. Hopefully they shall soon be amalgamated in our application goals in a durum wide crossing program.

Hybrid Production for Wide Crosses

With our success at generating over 100 bread wheat/perennial Triticeae species hybrid protocols (Mujeeb-Kazi et al., 1987, 1989; Mujeeb-Kazi,

2001), similar approaches have now led to the production of several durum × perennial/annual Triticeae species hybrids.

Interspecific Hybridization (Primary and Secondary Gene Pools): Germplasm, Hybrids, Cytology, Amphiploidy; and Phenology Descriptors

Triticum boeoticum, T. monococcum, T. urartu ($2n = 2x = 14$, AA), and *Aegilops speltoides* ($2n = 2x = 14$, BB) accessions were obtained from the CIMMYT germplasm bank in Mexico (El Batán) and from researchers in the United States (B. S. Gill, Kansas State; G. Waines, University of California, Riverside; and H. Bockelman, National Small Grains Collection [NSGC], Aberdeen, Idaho). The seed quantity of all accessions was increased by incorporating the seedling vernalization procedure (8°C, 8 h of light, for 8 weeks). After the accession seed increase, 200 of the A genome and 54 *Aegilops speltoides* accessions were similarly vernalized and then field transplanted during the crop cycles in the Mexico location of Ciudad Obregon during November to May over five consecutive years for hybridization to elite *T. turgidum* cultivars which were planted over four dates at ten-day intervals over each of the five years in order to coincide with the A and B genome pollen availability. Emasculation, pollination, embryo rescue, and regeneration procedures were similar to those reported earlier (Mujeeb-Kazi et al., 1987).

The vernalization procedure resulted in very vigorous growth of all A and B genome diploid accessions with a flowering range of 90 to 135 days. This enabled crossing with the *T. turgidum* cultivars for a majority of the accessions. Embryos rescued at 16 days postpollination from all crosses were small, translucent, generally ill defined, and floating in a watery endosperm cavity. The embryos were plated on a special medium for "small" embryos and given a 21-day, 4°C cold shock (dark) that allowed for better plantlet regeneration. Crossability data for some combinations presented in Table 23.1 indicate the general trend observed for florets pollinated, seed set, and embryos recovered, from which 95 to 100 percent of plants regenerated. The cultured embryo tubes after the cold shock (4°C) continued to be kept in the dark at 22°C. The embryos germinated within 30 to 45 days, after which the plantlets were transplanted to a soil media and maintained in the greenhouse for conducting cytology and later for inducing amphiploidy.

From each potted hybrid plantlet, root tips were collected and somatically analyzed (Mujeeb-Kazi, Jahan, and Vahidy, 1994) to validate hybridity. All genuine hybrids possessed $2n = 3x = 21$ (AAB or ABB) chromosomes with a codominant F_1 phenotype. The mean meiotic relationships

TABLE 23.1. The crossability data of *Triticum turgidum*/A genome diploid species *(T. boeoticum, T. monococcum, T. urartu)* accessions.

Cross combination	Florets pollinated	Seed set	Embryos plated
T. turgidum/T. boeoticum	80	44	41
	52	48	44
	96	24	22
	64	16	16
	39	15	15
	20	16	15
Total	351	163	153
Mean	58.5	27.2	25.5
T. turgidum/T. monococcum	56	4	2
	28	5	5
	48	6	6
	24	1	1
	48	8	7
	48	17	15
Total	252	41	36
Mean	42.0	6.8	6.0
T. turgidum/T. urartu	76	14	2
	96	20	10
	28	20	20
	48	15	9
	48	3	2
	56	3	2
Total	352	75	45
Mean	58.7	12.5	7.5

(Table 23.2) were a reflection of the diversity generated by combining several durum/A genome diploid accessions. The total mean bivalent range for the three A genome species combinations over all F_1 hybrids ranged from 5.5 to 6.0/meiocyte. These pairing trends were similar and consistent with earlier reports for AAB hybrids. Multivalency was the variable feature in the ABB F_1 hybrids and was consistent with the nature of the *Ae. speltoides* action on the *Ph* locus. After colchicine doubling of the 21 chromosome F_1 hybrids, the C-0 amphiploid seed generally possessed 42 chromosomes. Some hypo- or hyperploidy did exist that was subsequently purified by additional cytology during seed increase and maintenance.

TABLE 23.2. Mean meiotic associations of F_1 hybrids ($2n = 3x = 21$, AAB) of *Triticum turgidum*/A genome diploid accessions *(T. boeoticum, T. monococcum, T. urartu).*

	Metaphase I chromosome associations				
Cross combination	**I**	**II Rings**	**II Rods**	**Total II**	**III**
T. turgidum/T. boeoticum	9.4	4.3	1.5	5.8	—
Range	(9-11)	(2-6)	(0-3)	—	—
T. turgidum/T. monococcum	8.8	3.2	2.8	6.0	0.07
Range	(7-11)	(1-5)	(1-5)	—	(0-1)
T. turgidum/T. urartu	9.0	3.2	2.3	5.5	0.2
Range	(6-11)	(1-5)	(1-5)	—	(0-1)

All hybrid plants (maximum of seven per combination) possessing $2n = 3x = 21$ chromosomes were treated with 0.1 percent colchicine + 2.0 percent dimethyl-sulfoxide for six hours via aerated root treatment for doubling the chromosome number in order to obtain fertile amphiploids ($2n = 6x = 42$, AAAABB or AABBBB). Mitotic and meiotic analytical procedures for the F_1 and C-0 germplasms were similar to those of Mujeeb-Kazi, Jahan, and Vahidy (1994).

Production of amphiploids allows for a more reliable evaluation of the genetic value of the alien genes (Jiang, Friebe, and Gill, 1994) through the availability of a permanent germplasm base. Amphiploid instability may occur but can be cytologically discarded. The 194 AAAABB amphiploids produced had greater cytological stability (Table 23.3 shows data for some combinations) than the B genome amphiploids, in which greater univalency was observed. The predominance of bivalents with a high seed set/amphiploid enabled adequate production of seed for both the A and B genome products (Figure 23.2c and Figure 23.4) to be used for global distribution and stress testing. Maximum recombinational exchanges from the diploid accessions into durum wheat occur at F_1, with some carryover during maintenance. For speeding up utilization for breeding, each F_1 AAB or ABB hybrid could also be back- or top-crossed with *T. turgidum*.

The spikes on all hybrid plants of the colchicine-treated plants were glassine bagged upon extrusion, with seed set being the measure of colchicine-induced amphiploid induction. All amphiploid seed was germinated; it was then somatically and meiotically analyzed to validate the amphiploid status and obtain a seed increase, with glassine bagging ensuring purity.

TABLE 23.3. Mean meiotic metaphase I chromosomal associations of some A and B genome amphiploids of *Triticum turgidum* cultivars with *T. boeoticum*, *T. monococcum*, or *T. urartu* (AAAABB) and *Aegilops speltoides* (AABBBB) accessions.

	Metaphase I chromosomal associations					
Amphiploid combination	**I**	**II Rings**	**II Rods**	**Total II**	**III**	**IV**
AAAABB						
Yuk/*T. boeoticum* (1)[a]	0.4	13.8	3.8	17.6	—	1.6
Sca/*T. boeoticum* (10)	—	15.5	2.1	17.6	—	1.7
Garza/Boy//*T. boeoticum* (10)	1.4	10.3	6.2	16.5	0.4	1.6
Scoop/*T. monococcum* (98)	0.1	14.3	3.3	17.6	0.1	1.6
Scoop/*T. monococcum* (118)	0.2	14.8	2.8	17.6	0.2	1.5
CPI/—Cra[b]/4/*T. monococcum* (115)	1.0	14.1	3.7	17.8	0.2	1.2
Altar/*T. urartu* (552)	0.7	14.8	3.3	18.1	0.1	1.2
68.111—/4/Rabi[c]/5/*T. urartu* (555)	—	14.4	4.0	18.4	—	1.3
Doy/*T. urartu* (563)	0.4	15.3	2.5	17.8	—	1.5
AABBBB						
Arlin_1/*Ae. speltoides* (161)	3.4	8.6	6.5	15.1	1.2	1.2
Arlin_1/*Ae. speltoides* (158)	2.2	13.0	5.3	18.3	0.4	0.5
Arlin_1/*Ae. speltoides* (147)	9.5	5.8	8.9	14.7	0.5	0.4
Arlin_1/*Ae. speltoides* (146)	3.0	11.0	6.8	17.8	0.6	0.4
Arlin_1/*Ae. speltoides* (138)	3.5	10.8	5.5	16.3	0.9	0.8

[a]Numbers in parentheses are the accession numbers in the CIMMYT wide cross program working collection.
[b]Cultivar pedigree is CPI/Gediz/3/Goo//Jo/Cra.
[c]Cultivar pedigree is 68.111/Rgb-U//Ward/3/Fgo/4/Rabi.

From the field planting in Obregon, Mexico, some descriptors were established for the amphiploids. These were for days to flowering, plant height at maturity, awn color, pubescence, days to physiological maturity, and 1000 grain weight (Table 23.4).

Intergeneric Hybridization (Tertiary Gene Pool): Hybrid Production, Cytology, Amphiploidy, Phenology, and Backcrossing

Spikes of the durum wheat cultivars were emasculated, pollinated by the perennial species pollen one to three days after emasculation, and treated once daily for three days with 75 ppm gibberellic acid. From the seed set,

TABLE 23.4. Cross combination details of some AAAABB amphiploids and some of their morphological descriptors.

Triticum turgidum/AA genome amphiploid combination	Cross No.	Descriptors[b]					
		Flow	Pub	Ht	Awn	P.Mat	GWT
YUK/*T. boeoticum* (1)[a]	CIGM90.769	85	P	125	B	127	66.5
YUK/*T. boeoticum* (2)	CIGM90.770	85	P	125	B	127	63.3
SCA/*T. boeoticum* (3)	CIGM90.667	85	P	120	W	127	71.2
GARZA/BOY//*T. boeoticum* (10)	CIGM90.773	117	—	135	B	152	55.4
GARZA/BOY//*T. boeoticum* (12)	CIGM90.774	110	—	115	W	148	51.0
SCA/*T. boeoticum* (14)	CIGM90.671	85	P	120	B	127	52.7
AOS/*T. monococcum* (98)	CIGM90.791	110	—	120	B	150	59.5
AOS/*T. monococcum* (111)	CIGM90.793	115	—	95	B	152	51.5
BOTNO/*T. monococcum* (112)	CIGM92.465	115	—	110	B	152	46.8
SCOOP_1/*T. monococcum* (118)	CIGM90.712	100	—	130	B	127	56.6
FGO/USA2111//*T. monococcum* (119)	CIGM90.795	96	P	125	B	130	66.2
FGO/USA2111//*T. monococcum* (122)	CIGM90.796	106	P	110	B	148	62.5
DOY1/*T. urartu* (542)	CIGM90.567	110	P	125	B	148	36.5
DOY1/*T. urartu* (543)	CIGM90.568	99	P	115	B	127	44.9
DOY1/*T. urartu* (550)	CIGM90.570	100	P	105	B	148	45.9
DOY1/*T. urartu* (560)	CIGM90.573	102	P	115	B	144	50.0
DOY1/*T. urartu* (563)	CIGM90.574	96	P	125	B	127	56.1
DOY1/*T. urartu* (564)	CIGM90.575	96	P	140	W	130	57.9

[a]Numbers in parentheses are the accession numbers in the CIMMYT wide cross program working collection.
[b]Flow = days to flowering; Pub = pubescence; P = present; — = glabrous; Ht = plant height at maturity (cms); Awn = awn color, i.e., B = brown, W = whitish; P.Mat = days to physiological maturity; GWT = 1000 grain weight (g).

the embryos were excised 13 to 15 days after pollination and cultured on a special medium for small embryos (Taira and Larter, 1978). These and subsequent procedures associated with embryo differentiation, plantlet growth, transfer to Jiffy-7 peat pots, and transplantation to a potted soil mix in the greenhouse were similar to those reported by Mujeeb-Kazi and colleagues (1987), as were the environmental growth regimes of the glasshouses.

After vigorous growth, each F_1 hybrid was physically divided into four plants, and these clones grew into vigorous plants. From each clone of each F_1 hybrid, root tips were collected for somatic cytology and C-banding. The cytological procedures were essentially similar to those described by Mujeeb-Kazi, Jahan, and Vahidy (1994).

Spikes for meiotic analyses were collected in early morning hours, fixed in Carnoy's solution (6:3:1, absolute alcohol:chloroform:acetic acid) for 48 to 72 h, and stored under refrigeration (4°C) in 70 percent alcohol until use. Anthers at metaphase I were stained in alcoholic-acid-carmine for several days and crushed in 45 percent acetic acid with a drop of 2 percent aceto-carmine to enhance the coloration. Meiotic chromosome associations were analyzed at metaphase I. Cytological photography was done of quality representative cells on a black-and-white high-contrast film using a special green/yellow filter combination.

One clone of each combination was treated with a colchicine (0.05 percent) and 2.0 percent dimethyl sulfoxide (DMSO) solution for six hours using the aerated root treatment protocol of Mujeeb-Kazi and colleagues (1987) in order to produce amphiploids.

Five fully emerged spikes from each F_1 hybrid and its corresponding durum parent were characterized for spike morphology. Several self-sterile spikes from each untreated colchicine combination were back- or top-crossed by durum cultivars to obtain backcross I seed for future use.

Intergeneric Combinations Produced

Crossing between durum cultivars and perennial Triticeae species leading to seed set and putative hybrid embryo excision ranged from a low of 0.9 percent to nearly 50.0 percent. Low frequencies of success were noted for durum cultivar combinations with *Elymus fibrosus* (5.6 percent), *E. virginicus* (2.1 percent), *Elytrigia pungens* (1.5 percent), *Pascopyrum spicatum* (2.3 percent), *Psathyrostachys juncea* (1.0 percent), *Thinopyrum elongatum* (0.9 percent), *Th. scirpeum* (3.6 percent), and *Th. scythicum* (2.3 percent). In the intermediate category were those combinations where hybridization success was approximately 8.0 to 15.0 percent, i.e., durum wheat combinations with *Th. junceiforme* (12.8 percent; mean over two

cultivars), *Th. junceum* (11.8 percent; mean over three cultivars), and *Th. littorale-campestre* (12.2 percent). In almost all of these hybrid combinations the excised embryos were generally minute, translucent, and globular in shape. Embryos from the *Th. junceiforme* and *Th. junceum* combinations were somewhat larger, with a definitive scutellum. Endosperm ranged from complete absence to being watery in nature. Hybrid recovery combinations with a high frequency of success exhibited well-defined embryos, possessed copious endosperm, and produced rapidly growing, vigorous regenerants. Percentages of these hybrids were as follows: 37.2 (ssp. *acutum*), 44.0 (ssp. *glaucum*), 45.5 (ssp. *intermedium*), 43.8 (ssp. *pulcherrimum*), 30.0 (ssp. *trichophorum*), and 41.9 (ssp. *varnense*). A high rate of hybrid recoveries with similar embryo/endosperm characteristics was also obtained for the cross between cultivar Mexicali 75 and *Th. podperae* (31.4 percent).

Except for the cultivar Cappelli, the other seven cultivars combined with the various Triticeae species were all high-yielding durums. Hence desirable alien introgressions may be anticipated to yield practical outputs in a relatively short time. Several cultivars produced hybrids with *Th. intermedium,* its five subspecies, *Th. junceiforme, Th. junceum,* and *Th. scirpeum.* The other combinations in which a single cultivar was hybridized indicated cultivar crossability variations. We do not consider this an impediment since even one valid F_1 hybrid involving any cultivar can be exploited for durum improvement by utilizing other elite cultivars in the breeding protocol scheme.

Phenotype of F_1 hybrids. All hybrids were perennial, possessed a vigorous growth habit, and tillered profusely. Each hybrid was self-sterile, but female fertile and set various frequencies of backcross I seed when pollinated by durum cultivars (results not presented here). Where the number of hybrids in a combination were few, they were physically cloned, and for each F_1 at least four plants were produced.

An intermediate phenotype was a common observation for several intergeneric hybrids within the Triticeae and considered to be a valid morphological indicator of alien genetic expressivity in a wheat background (Mujeeb-Kazi et al., 1987). This codominant wheat/alien species phenotype was a common characteristic. Figures 23.5 and 23.6 show some F_1 spike phenotypes of hybrids and substantiate alien genetic expressivity in the durum background. The phenotypic parameters generally affected included spike length, spike size, spike width, reduced awn length to awn absence, lax heads with greater internodal distance, and an occasional presence of pubescence (Table 23.5).

Somatic and meiotic cytology. A simple chromosome count and satellite detail is often enough to initially validate hybridity (Figures 23.7a to f). Two satellited chromosomes (1B and 6B) present as pairs in euploid

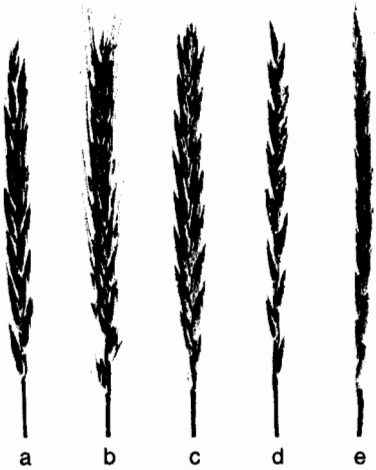

FIGURE 23.5. Codominant spike phenotype of some F_1 intergeneric durum/perennial Triticeae species showing combinations of (a) *Elymus fibrosus*, (b) *E. virginicus*, (c) *Elytrigia pungens*, (d) *Pascopyrum spicatum*, and (e) *Psathyrostachys juncea*.

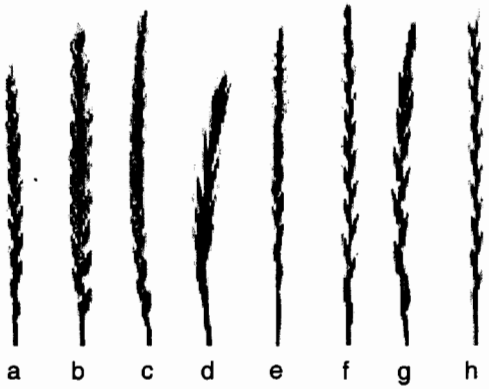

FIGURE 23.6. Codominant spike phenotype of some F_1 intergeneric durum/perennial Triticeae species showing combinations of (a) *Thinopyrum elongatum*, (b) *Th. junceiforme*, (c) *Th. junceiforme*, (d) *Th. junceum*, (e) *Th. campestre*, (f) *Th. podperae*, (g) *Th. scirpeum*, and (h) *Th. scythicum*.

TABLE 23.5. Mean spike characteristics of some F_1 hybrids between *Triticum turgidum* cultivars and perennial Triticeae species of different polyploidy levels.

Cross combinations of durum cultivars and alien species	Spike character analyzed[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Elymus fibrosus/Cocorit 71	11.7	0.5	14.0	0.8	3.1	0.5	14.0	4.5	1.1	0.1	1.3	1.1	0.3
E. virginicus/Cocorit 71	19.0	0.7	19.5	0.8	5.2	0.5	19.5	4.5	1.2	0.2	1.3	4.4	0.2
Altar 84/*Elytrigia pungens*	10.6	0.6	17.0	0.5	1.7	0.6	17.0	5.3	1.1	0.1	1.5	0.4	0.2
Laru/*Pascopyrum spicatum*	12.5	0.5	15.5	1.0	1.6	0.5	15.5	5.0	1.0	0.1	1.2	0.1	0.3
Dvergand/*Psathyrostachys juncea*	10.0	1.3	12.7	0.6	1.9	0.9	12.7	5.0	1.4	0.1	1.4	0.3	0.3
Yavaros/*Thinopyrum elongatum*	12.2	0.7	15.5	0.6	3.3	0.8	15.5	4.8	0.9	0.1	1.1	1.6	0.2
Cappelli/*Th. junceiforme*	12.0	0.7	14.6	0.7	1.8	0.5	14.6	4.6	1.4	0.0	1.5	0.7	0.3
Cocorit 71/*Th. junceiforme*	12.2	0.6	16.5	0.7	2.4	0.6	16.5	3.5	1.3	0.0	1.4	0.4	0.3
Altar/*Th. junceum*	12.8	0.5	14.0	0.6	2.8	0.3	14.0	4.0	1.2	0.1	1.4	1.1	0.2
Cocorit 71/*Th. junceum*	20.6	0.8	25.0	1.1	1.8	0.9	25.0	7.5	1.2	0.1	1.4	0.1	0.3
Croc/*Th. junceum*	12.6	0.7	10.0	0.8	3.3	0.4	10.0	4.5	1.3	0.1	1.5	1.5	0.1
Cocorit 71/*Th. littorale-campestre*	14.8	0.6	18.0	1.0	1.7	0.6	18.0	4.0	1.1	0.1	1.3	1.0	0.2
Mexicali 75/*Th. podperae*	10.5	0.4	14.0	0.9	1.3	0.4	14.0	3.5	0.9	0.1	1.0	0.3	0.3
Altar 84/*Th. scirpeum*	12.7	0.5	12.5	1.2	0.6	0.6	12.5	4.5	0.9	0.1	1.1	0.2	0.3
Croc/*Th. scirpeum*	15.6	0.7	12.0	1.2	2.4	0.7	12.0	8.5	1.1	0.1	0.8	0.1	0.2
Yavaros 79/*Th. scythicum*	10.2	0.5	14.0	0.8	1.3	0.3	14.0	4.0	0.7	0.0	1.0	0.0	0.3

Th. intermedium ssp.													
Cappelli/*Th. acutum*	24.9	0.7	22.5	1.7	1.8	0.5	22.5	5.0	1.1	0.1	1.1	0.1	0.3
Cocorit 71/*Th. acutum*	16.5	0.6	21.0	1.0	1.5	0.5	21.0	4.5	0.8	0.1	1.0	0.5	0.3
Yavaros 79/*Th. acutum*	17.9	0.5	16.0	1.2	1.8	0.5	16.0	3.5	1.3	0.0	1.3	0.3	0.2
Chen/*Th. glaucum*	18.4	0.6	22.0	0.9	1.9	0.6	22.0	5.5	1.1	0.1	1.3	0.2	0.3
Cappelli/*Th. intermedium*	11.9	0.5	13.5	1.1	1.7	0.6	13.5	4.8	1.1	0.1	1.3	0.2	0.3
Cocorit 71/*Th. intermedium*	12.0	0.5	15.0	0.8	1.9	0.4	15.0	4.5	0.8	0.1	1.0	0.7	0.2
Yavaros 79/*Th. intermedium*	15.6	0.7	16.5	0.9	2.2	l0.6	16.5	4.0	0.8	0.0	1.3	1.0	0.3
Cocorit 71/*Th. pulcherrimum*	13.6	0.6	13.0	1.4	1.7	0.5	13.0	5.8	1.0	0.1	1.1	0.1	0.3
Mexicali 75/*Th. pulcherrimum*	12.5	0.5	12.5	1.1	2.0	0.7	12.5	7.3	1.0	0.3	1.2	0.1	0.3
Dvergand/*Th. trichophorum*	17.8	0.7	13.0	1.5	2.6	0.7	13.0	8.5	1.3	0.0	1.3	0.1	0.4
Laru/*Th. trichophorum*	17.1	0.7	14.5	1.4	2.1	0.7	14.5	7.5	1.2	0.0	1.3	0.5	0.4
Mexicali/*Th. trichophorum*	16.0	0.5	13.5	1.5	1.6	0.4	13.5	5.3	1.0	0.0	1.2	0.0	0.3
Altar 84/*Th. varnense*	14.6	0.7	22.5	0.8	1.9	0.4	22.5	4.8	0.9	0.1	1.1	0.1	0.3
Cappelli/*Th. varnense*	15.3	0.6	20.5	0.9	1.8	0.5	20.5	4.5	0.9	0.0	1.1	0.1	3.2
Mexicali 75/*Th. varnense*	11.9	0.5	13.5	1.1	1.7	0.6	13.5	4.8	1.1	0.1	1.3	0.2	0.3
Laru/*Th. varnense*	20.5	0.7	18.0	1.3	2.5	0.7	18.0	7.5	1.0	0.1	1.3	0.7	3.7

[a]1 = spike length (cm); 2 = spike width (cm); 3 = nodes per spike; 4 = internode length (cm); 5 = spikelet length (cm); 6 = spikelet width (cm); 7 = spikelets per spike; 8 = florets per spike; 9 = glume body length (cm); 10 = glume awn length (cm); 11 = lemma body length (cm); 12 = lemma awn length (cm); 13 = anther length (cm).

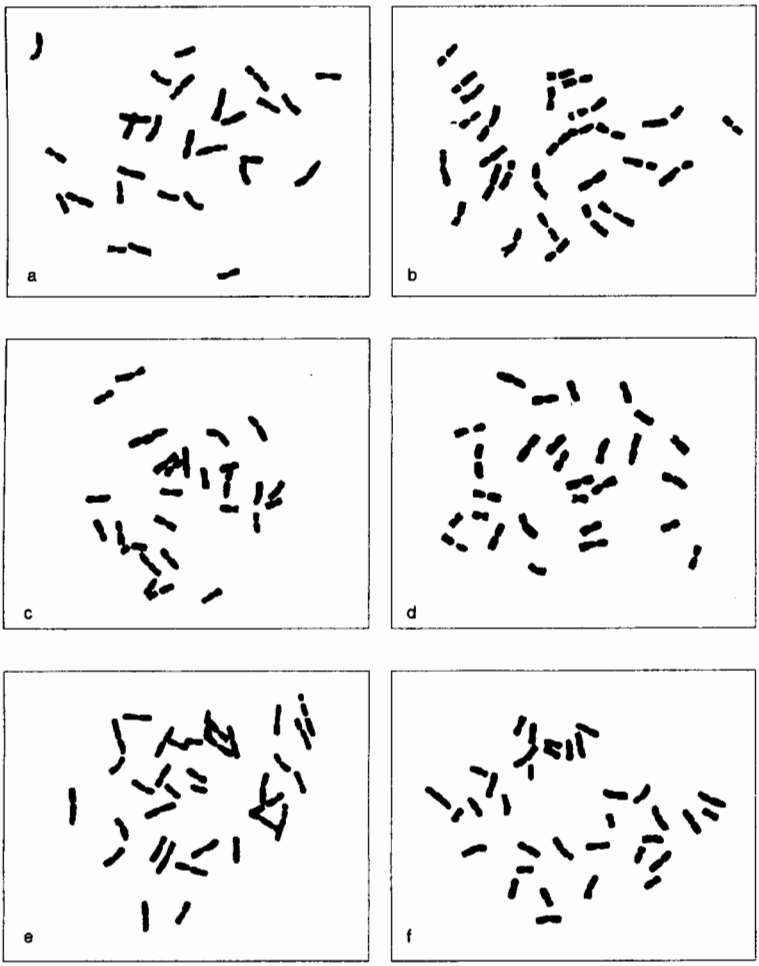

FIGURE 23.7. Somatic F_1 cells showing expression of the 1B and 6B chromosome satellites in hybrids of durum wheat with (a) *Elymus fibrosus*, and (b) *E. virginicus*. The satellited chromosomes often do not express explicitly due to amphiplasty. These are elucidated in the four combinations of durums with (c) *Th. scirpeum*, (d) *Th. scythicum*, (e) *Th. podperae*, and (f) *Th. pulcherrimum*.

T. turgidum were identified in most of the F_1 hybrids (Figure 23.7b). Satellites of the alien species were not observed in several hybrid combinations as a consequence of amphiplasty. In several other hybrids none of the satellites were observed (Figure 23.7c). Hence, when alien species with similar ploidy levels are involved in crosses, additional diagnostics such as chromosome banding or in situ hybridization become necessary to validate hybridity. Unequivocal proof of hybridity and charting the program research strategy comes from meiotic analyses that provide an accurate index of the introgression methodology to be adopted for effecting alien genetic transfers to durum wheats. The meiotic data of Table 23.6 elucidates the constraints of homologous transfers via recombination. Several species are autotetraploids *(Ps. juncea),* segmental allotetraploids, or partial autopolyploids *(Th. junceiforme, Th. scirpeum),* segmental allohexaploids *(Th. junceum),* or segmental auto-allohexaploids (*Th. podperae, Th. intermedium* with its subspecies *acutum, glaucum, pulcherrimum, trichophorum, varnense*) possessing two closely related genomes with a distinctly different third genome. Therefore, the meiotic associations require careful evaluation before any conclusion is reached to suggest wheat/alien interchanges for crop improvement. The two combinations selected for durum improvement (scab and salinity) do not exhibit any intergeneric chromosome pairing and shall require cytogenetic manipulation strategies, employing conventional or possibly some novel options (Mujeeb-Kazi, 2001a,b).

Some amphiploids and their practicality. Upon being pollinated by *Triticum* cultivars, intergeneric self-sterile F_1 *Triticum*/perennial Triticeae hybrids yield advanced derivatives for subsequent cytological and practical utilization. The latter aspect is better addressed if amphiploids of F_1 hybrids are developed first to facilitate screening for various stresses. We report here the production, cytogenetics, and maintenance of several durum wheat × perennial Triticeae species amphiploids. Normalcy of cytology existed in most amphiploid combinations, and in each, plants with high fertility measured through seed set/plant were identified. Alien species involved in these amphiploids were *T. turgidum* with *Th. elongatum, Th. intermedium* and its four subspecies *(acutum, pulcherrimum, trichophorum, varnense), Th. scythicum, Th. podperae, Th. scirpeum, Th. junceiforme,* and *Ps. juncea.* Though amphiploid production has generally capitalized on use of colchicine, alien autotetraploids *(Psathyrostachys juncea)* also yielded BCI amphiploid derivatives (Mujeeb-Kazi, Cortes, and Riera-Lizarazu, 1995) and could be an alternate route favored for complex combinations.

Production of intergeneric hybrids forms the initial step in exploiting alien genetic variability for crop improvement. The self-sterile F_1 hybrids can be advanced to yield backcross I derivatives by pollinating the F_1 plants with the same wheat parent or by using a different wheat cultivar. Addi-

tional crosses lead to advanced backcross generations allowing for the production of alien chromosome addition lines, substitution lines, and wheat/alien chromosomal translocations. Through genetic manipulation procedures these crosses have the potential of yielding subtle alien genetic exchanges (Mujeeb-Kazi et al., 1987). Amphiploids derived from F_1 intergeneric hybrids have significance for the ease of germplasm distribution as well as the facilitation of the systematic development of cytogenetic stocks (Gill, 1989; Lukaszweski, 1988).

Somatic and meiotic cytology of fertile amphiploids. Each amphiploid seed (C-0) produced after colchicine treatment of F_1 hybrid clones was germinated and somatically analyzed by root tip mitotic counts using the procedure of Mujeeb-Kazi and Miranda (1985). After the C-3 generation, at least 50 plants from each amphiploid combination were checked for somatic chromosome numbers. Meiotic analyses were conducted only on those plants that (1) best fitted the expected amphiploid count and (2) had satisfactory selfed seed production. The meiotic analyses procedures were essentially similar to those of Snow (1963) except that half anthers prechecked to be at metaphase I were stained for two to three weeks in alcoholic carmine.

Seed maintenance. Data were obtained for each amphiploid plants' seed set (C-0 to C-n) for which cytological information was also generated. From each amphiploid combination, individual plants were identified that had near-normal somatic plus meiotic cytology, a high level of seed setting, and a satisfactory growth duration so as to eliminate very late, straggly plants. Seed purity was ensured by bagging all spikes at each growth generation. In the C-0 to C-2 cycles multiple spikes were bagged to facilitate seed setting. When an adequate seed supply has been obtained (about 25 g) it is to be deposited in CIMMYT's wheat germplasm bank, and a working collection will be maintained in the wide cross program.

General observations of amphiploidy. In intergeneric hybridization programs within the Triticeae, F_1 hybrids between wheat (*Triticum aestivum* L.) and alien species have been rapidly advanced to yield backcross derivatives by pollinating the self-sterile but female-fertile F_1 plants with *T. aestivum.* Subsequent backcrosses allow for the recovery of alien disomic additions by selfing an alien monosome, double or triple monosomic lines, or by integrating polyhaploidy techniques that would circumvent alien pollen chromosome transmission constraints. More effective is the process by which a male-fertile backcross I heptaploid, upon being backcrossed to the amphiploid, instantly fixes the alien chromosomes (Lukaszewski, 1988). Apart from being an essential step in the development of alien disomic addition lines, amphiploids fix alien chromosome sets, representing a valuable resource for long-term observations and exploitation (Gill, 1989).

TABLE 23.6. Mean and range of meiotic associations at metaphase I in intergeneric hybrids of some *Triticum turgidum* L. ($2n = 4x = 28$, AABB) cultivars with different perennial Triticeae species.

Cross combination	Chromosome number	Mean meiotic chromosomal associations					
		I	II Rings	II Rods	Total bivalents	III	IV
Elymus fibrosus/Cocorit 71	$2n = 4x = 28$	27.6	0	0.2	0.2	0	0
Elymus virginicus/Cocorit 71	$2n = 4x = 28$	25.4	0.1	1.2	1.3	0	0
Altar 84/*Elytrigia pungens*	$2n = 5x = 35$	31.0	0	2.0	2.0	0	0
Laru/*Pascopyrum spicatum*	$2n = 5x = 35$	21.2	0.7	5.3	6.0	0.6	0
Dvergand/*Psathyrostachys juncea*	$2n = 4x = 28$	20.8	0.5	3.1	3.6	0	0
Yavaros/*Thinopyrum elongatum*	$2n = 3x = 21$	19.4	0	0.8	0.8	0	0
Cocorit 71/*Th. junceiforme*	$2n = 4x = 28$	17.8	0.5	4.1	4.6	0.2	0.1
Altar/*Th. junceum*	$2n = 5x = 35$	19.9	1.8	3.6	5.4	1.3	0.1
Cocorit/*Th. junceum*	$2n = 5x = 35$	20.8	1.0	4.6	5.6	1.0	0
Mexicali/*Th. podperae*	$2n = 5x = 35$	27.1	0.4	3.1	3.5	0.3	0
Altar 84/*Th. scirpeum*	$2n = 4x = 28$	19.4	2.4	1.9	4.3	0	0
Croc/*Th. scirpeum*	$2n = 4x = 28$	19.2	1.2	3.2	4.4	0	0
Yavaros/*Th. scythicum*	$2n = 4x = 28$	22.6	0.3	2.4	2.7	0	0

TABLE 23.6 *(continued)*

Cross combination	Chromosome number	Mean meiotic chromosomal associations					
		I	II Rings	II Rods	Total bivalents	III	IV
Hybrids with *Th. intermedium* ssp.							
Cocorit 71/*Th. acutum*	$2n = 5x = 35$	20.3	0.6	4.9	5.5	1.1	0.1
Yavaros/*Th. acutum*	$2n = 5x = 35$	25.0	0.4	4.0	4.4	0.4	0
Chen/*Th. glaucum*	$2n = 5x = 35$	25.0	0.1	4.0	4.1	0.6	0
Altar 84/*Th. intermedium*	$2n = 5x = 35$	29.9	0	2.4	2.4	0.1	0
Yavaros/*Th. intermedium*	$2n = 5x = 35$	29.3	0	2.5	2.5	0.1	0.1
Cocorit 71/*Th. intermedium*	$2n = 5x = 35$	32.0	0	1.2	1.2	0.2	0
Memo/Mexicali//*Th. glaucum*	$2n = 5x = 35$	23.1	0.3	5.1	5.4	0.1	0.2
Cocorit/*Th. pulcherrimum*	$2n = 5x = 35$	20.9	1.4	4.7	6.1	0.5	0.1
Mexicali/*Th. pulcherrimum*	$2n = 5x = 35$	24.7	0.5	3.9	4.4	0.5	0
Dvergand/*Th. trichophorum*	$2n = 5x = 35$	26.1	0.6	3.2	3.8	0.4	0
Laru/*Th. trichophorum*	$2n = 5x = 35$	27.9	0.3	3.1	3.4	0.1	0.1
Mexicali/*Th. varnense*	$2n = 5x = 35$	30.6	0	2.2	2.2	0	0
Altar/*Th. varnese*	$2n = 5x = 35$	31.4	0	1.8	1.8	0	0

Though the importance of amphiploids has been well emphasized, one must also recognize that constraints do exist in their production and maintenance. The genetic stocks of wheat × barley were developed (Islam, Shepherd, and Sparrow, 1981) without the assistance of its amphiploid, which was a production constraint. It was substantially later that an amphiploid from a similar combination was reported (Molnar-Lang and Sutka, 1993). An identical amphiploid production constraint is inferred for hybrids between *Elymus ciliare* and *E. trachycaulus* with *T. aestivum*. For the classic *T. aestivum* × *Aegilops variabilis* combination, amphiploid production remained evasive until recently, when callus culture mediation yielded a few regenerated F_1 plants that set seed as a consequence of meiotic restitution.

More recently, colchicine-induced doubling took on tremendous importance in our wheat × rye, interspecific *(T. turgidum* × *T. tauschii, T. urartu, T. boeoticum, T. monococcum),* and polyhaploidy (Triticeae species × maize) programs. In the maize program, where data recording was more crucial because of its novel nature, colchicine-induced doubling success (indicated by seed setting on treated plants) increased by as much as 70 percent (Riera-Lizarazu, Mujeeb-Kazi, and William, 1992). In our intergeneric hybridization program all perennial F1 hybrids have been maintained by cloning biannually since their initial production in 1980. Recently, after our improved success with colchicine application in some of our other research areas, numerous young clones of the self-sterile F_1 hybrids are regularly treated with colchicine. C-0 seed set occurs on several plants, though in very low numbers ranging between two and seven seeds per combination. Emphasis has been solely placed upon obtaining fertile seed from as many hybrids as possible (see Mujeeb-Kazi and Bernard, 1985; Mujeeb-Kazi et al., 1987, 1989 for array of germplasm available), and some durum wheat based amphiploids have been produced (Table 23.7).

The six durum-based amphiploids ranged from a hexaploid ($2n = 6x = 42$) to a decaploid ($2n = 10x = 70$) ploidy level. Five of these were products of colchicine induction except for durum/*Ps. juncea*. Good cytological and fertility stability is expressed (Figures 23.8 and 23.9; Table 23.8), and we should be able to attain the required seed amounts for our multipurpose objectives expressed earlier for the *T. aestivum* amphiploids much faster for durum combinations. Seeds of the *E. fibrosum*/durum amphiploid were more slender than those of other amphiploid combinations but possessed well-filled endosperm with an elongated seed shape. Though colchicine treatment is common for amphiploid induction, other options could also be considered. Where alien autotetraploids are used in initial crosses to yield F_1 hybrids, backcrossing these F_1 hybrids leads to BC_1 derivatives that represent an amphiploid status (Mujeeb-Kazi, Cortes, and Riera-Lizarazu, 1995).

TABLE 23.7. Details of some annual/perennial Triticeae species amphiploids with various durum wheat (*Triticum turgidum* L.) cultivars.

Durum cultivars and alien species combinations	Expected chromosome number	Current generation	Chromosome range observed	Seed amount (g)
ANNUAL TRITICEAE				
Laru/*Aegilops variabilis*	$2n = 8x = 56$	C-7	54-57	15
Arlin/*Ae. variabilis*	$2n = 8x = 56$	C-7	55-56	15
Altar/*Ae. variabilis*[a]	$2n = 8x = 56$	C-7	55-56	15
Bia/*Ae. variabilis*	$2n = 8x = 56$	C-7	55-56	15
Ceta/*Ae. ventricosa*	$2n = 8x = 56$	C-7	52-57	10
Capelli/*Ae. ovata*[a]	$2n = 8x = 56$	C-7	55-56	25
Capelli/*Ae. triuncialis*	$2n = 8x = 56$	C-7	53-57	10
Capelli/*Ae. speltoides*	$2n = 6x = 42$	C-7	36-43	5
PERENNIAL TRITICEAE				
Elymus fibrosus/Cocorit 71	$2n = 8x = 56$	C-9	55-56	10
Altar 84/*Thinopyrum scirpeum*	$2n = 8x = 56$	C-9	55-56	10
Capelli/*Th. acutum*	$2n = 10x = 70$	C-9	55-70	20
Yavaros/*Th. acutum*	$2n = 10x = 70$	C-9	55-70	20
Cocorit 71/*Th. acutum*	$2n = 10x = 70$	C-9	55-70	20

Yavaros 79/*Th. intermedium*	$2n = 10x = 70$	C-9	55-71	15
Cocorit 71/*Th. intermedium*	$2n = 10x = 70$	C-9	55-68	15
Mexicali 75/*Th. intermedium*	$2n = 10x = 70$	C-9	55-67	15
Capelli/*Th. intermedium*	$2n = 10x = 70$	C-9	55-68	15
Cocorit 71/*Th. junceiforme*[a]	$2n = 8x = 56$	C-9	55-57	10
Cocorit 71/*Th. pulcherrimum*	$2n = 10x = 70$	C-9	58-68	10
Mexicali 75/*Th. pulcherrimum*	$2n = 10x = 70$	C-9	59-71	10
Mexicali 75/*Th. podperae*	$2n = 10x = 70$	C-9	64-71	10
Mexicali 75/*Th. trichophorum*	$2n = 10x = 70$	C-9	58-70	10
Mexicali 75/*Th. varnense*	$2n = 10x = 70$	C-9	58-70	10
Yavaros 79/*Th. varnense*	$2n = 10x = 70$	C-9	58-70	10
Capelli/*Th. varnense*	$2n = 10x = 70$	C-9	67-70	10
Arlin/*Th. glaucum*	$2n = 10x = 70$	C-9	65-70	15
Croc_1/*Th. glaucum*	$2n = 10x = 70$	C-9	65-70	10
Yavaros 79/*Th. glaucum*	$2n = 10x = 70$	C-9	64-71	10
Dverd_2/*Th. glaucum*	$2n = 10x = 70$	C-9	65-69	10
Arlin/*Th. acutum*	$2n = 10x = 70$	C-9	65-69	10
Altar 84//*Th. acutum*/*Th. intermedium*	$2n = 10x = 70$	C-9	64-72	15
Croc_1//*Th. acutum*/*Th. intermedium*	$2n = 10x = 70$	C-9	63-70	15
Laru//*Th. acutum*/*Th. intermedium*	$2n = 10x = 70$	C-9	65-68	15

TABLE 23.7 *(continued)*

Durum cultivars and alien species combinations	Expected chromosome number	Current generation	Chromosome range observed	Seed amount (g)
Arlin//*Th. acutum/Th. intermedium*	$2n = 10x = 70$	C-9	66-69	15
Arlin_1/*Th. junceiforme*	$2n = 8x = 56$	C-9	55-57	15
Altar 84/*Th. junceiforme*[a]	$2n = 8x = 56$	C-9	54-56	15
Croc_1/*Th. junceiforme*[a]	$2n = 8x = 56$	C-9	54-56	15
Altar 84/*Elytrigia pungens*	$2n = 10x = 70$	C-9	66-68	15
Yavaros 79/*Th. scirpeum*[a]	$2n = 8x = 56$	C-9	55-56	15
Laru/*Pascopyrum spicatum*	$2n = 10x = 70$	C-9	65-71	5
Dverd/ *Th. trichophorum*	$2n = 10x = 70$	C-9	65-71	10
Croc_1/*Th. trichophorum*	$2n = 10x = 70$	C-9	65-71	10
Rok/Kml//*Th. trichophorum*	$2n = 10x = 70$	C-9	66-70	10
Laru/*Th. trichophorum*	$2n = 10x = 70$	C-9	65-70	10
Altar 84/*Th. varnense*	$2n = 10x = 70$	C-9	67-71	10
Altar 84//*Th. acutum/Th. intermedium*	$2n = 10x = 70$	C-9	68-72	10
Laru/*Th. varnense*	$2n = 10x = 70$	C-9	67-71	10
Dverd_2/*Psathyrostachys juncea*[a]	$2n = 6x = 42$	C-9	41-42	25
Yavaros 79/*Th. elongatum*[a]	$2n = 6x = 42$	C-3	41-42	25

[a]Indicates combinations for practical agricultural use.

FIGURE 23.8. Somatic cells of the amphiploids of durum wheats with (a) *Aegilops variabilis* and (b) *Thinopyrum trichophorum* with 56 and 68 (2 telocentrics) chromosomes. Amphiploids with 42 to 56 chromosomes are more stable than higher ploidy levels.

TABLE 23.8. Mean meiotic metaphase I chromosomal associations of some durum wheat amphiploids with perennial Triticeae species.

Combination of *T. turgidum* cultivars with alien species	Somatic count	Metaphase chromosomal associations					
		I	II Rings	II Rods	III	IV	Xta/ cell
Elymus fibrosum/Cit71[a]	2*n* = 56	7.30	14.75	9.45	1.10	—	—
Laru[a]/*Th. trichophorum*	2*n* = 66	21.20	12.00	7.95	1.50	0.10	35.45
Memo/Mexi[a]//*Th. junceiforme*	2*n* = 57	5.90	12.10	12.60	1.30	0.20	38.00
Mexi[a]/*Th. pulcherrimum*	2*n* = 70	3.60	27.20	5.90	—	0.05	60.45
Yav[a]/*Th. acutum*	2*n* = 68	6.15	21.55	9.15	0.15	—	52.55
Yav[a]/*Psathyrostachys juncea*	2*n* = 42	9.20	11.80	4.60	—	—	28.20

[a] *T. turgidum* cultivars.

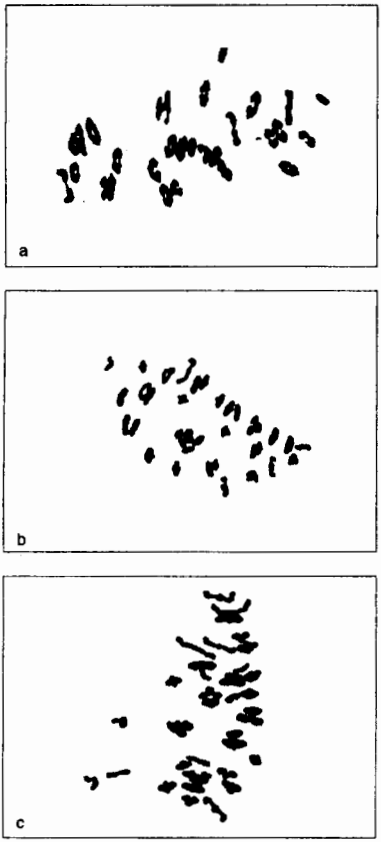

FIGURE 23.9. Meiocytes of durum/alien species amphiploids showing (a) 27 bivalents + 2 univalents, (b) 34 bivalents + 2 univalents, and (c) 34 bivalents + 2 univalents. High bivalency is associated with greater fertility and high seed production in the amphiploids.

We have subjected the amphiploids to a screening protocol for several biotic/abiotic stresses and attach priority to some combinations with *Th. elongatum, H. villosa, Th. scirpeum, E. fibrosum, Th. junceiforme, L. mollis,* and *Ps. juncea* for durum wheat improvement. These species express significant alien genomic diversity (E, N, S, and H) that has an advantage for stress stability parameters. We are also complementing this with the

diversity from interspecific sources which, in order of priority, include the D-genome, A-genome, and some of the Sitopsis group diploids.

The alien introgression methodology is receiving more attention because the stress objectives being addressed by us are complex. Consequently, earlier introgression approaches need consideration. These are being implemented with emphasis around the *Ph* locus. Except for some enhanced homologous pairing of the F_1 with *Th. scythicum*, none of the other hybrids provide evidence of such a trend.

From the acquired amphiploids, the *H. villosa* combination is suited to our needs, since it brings a novel genome to complement prevalent diversity being used for incorporation. For durum wheat, some breeding objectives relate to incorporation of resistance to *F. graminearum*, *H. sativum*, salinity, barley yellow dwarf virus (BYDV), and quality improvement. It appears that initially the diploids *Th. elongatum* ($2n = 2x = 14$), *Ps. juncea* ($2n = 2x = 14$), and *H. villosa* ($2n = 2x = 14$) would be ideally suited to provide such genetic variation. Next would be the tetraploids *Ps. juncea* and *Leymus mollis*. We are still a long way from effecting incorporation of this diversity into durum wheats, but efforts are underway. It will be advantageous to induce F_1 level exchanges and use 'Cappelli' durum possessing the *ph1c* gene to facilitate alien introgression for durum breeding program utility.

UTILIZATION AND PRACTICALITY OF WIDE CROSS GERMPLASM

Interspecific Germplasm

From the wide array of AAAABB and AABBBB wheats produced, field plantings for establishing some descriptive parameters were completed. The descriptors demonstrated extensive genetic diversity for plant height, flowering date, grain-fill duration, awn color, days to physiological maturity, and 1000-grain weight. Some "selected" A genome amphiploid combinations possessing good agronomic plant types and their descriptors are presented in Table 23.4. Similar data (not shown here) for AABBBB were also generated. Utilization of these selected germplasms for durum wheat improvement will be advantageous if the selections further express high levels of resistance to biotic/abiotic stresses.

The amphiploids embody a wide array of genetic diversity of A and B genome accessions. They are all spring type in habit, which facilitates their easier practical utilization, seed increase, conservation, and global distribution. The germplasm further provides a unique gene pool for evaluating the A and B genome response toward a wide range of biotic/abiotic stress con-

ditions. The international distribution of amphiploids has additional merit following screening by global collaborative/national agricultural programs plus other research programs for different stresses, as the variation can be readily incorporated into their locally adapted germplasm. The current promising use of this germplasm is for leaf rust, *S. tritici*, and *Fusarium* head scab resistance (Type II). All the durum cultivars in these amphiploids are highly to moderately susceptible, allowing for alien accessional diversity for resistance to be readily identified in both the A and B genome amphiploids.

Intergeneric Germplasm

The hybrids produced by us (Table 23.9) comprise alien Triticeae species that are promising for resistances/tolerance to several biotic/abiotic stresses. Some of these have been used extensively in bread wheat improvement (e.g., *Agrotricum, Th. bessarabicum, Th. curvifolium, Th. distichum, Th. elongatum, Th. junceiforme, Th. ponticum*), yielding germplasm/cultivar releases such as the cultivars Luan, Mayoor, Tia, Chirya, Rohtas 90, Pasban 90, Azubi Ciat, Oasis, Buitre, and Thelin 1 and 2. However, the impact of alien genetic diversity in durum wheat improvement has been limited and slower to realize. Durum/species hybrids produced by researchers are few so far. Their validation has been provided, potential utility in agriculture emphasized, but practical gains not fully realized. Based upon existing data, we have selected two combinations in our applied program focusing on scab resistance (a major limitation for durum production), with other stresses complementing the breeding strategy. Of these, the advancement of one cross *(T. turgidum/Th. elongatum)* is highlighted.

The F_1 hybrid possessed $2n = 3x = 21$ chromosomes (Figure 23.10a) with univalency dominating meiotic analyses (Figure 23.10b). The diploid species has a unique C-banding pattern (Figure 23.11a). A stable amphiploid ($2n = 6x = 42$) has been obtained (Figure 23.11b,c), and its backcross derivatives have yielded large monosomic or double monosomic populations ($2n = 4x = 28 + 1$ or 2 alien) from which the complete disomic addition set has been extracted (Figure 23.12a,b). The accession used is similar to that combined with a bread wheat (cv. Goshawk) and exhibits Type II scab resistance (<15 percent).

The durum cultivars used in backcrossing or top-crossing were all amenable to maize-mediated haploid production, which was integrated with generation of haploids from the 29 or 30 chromosome monosomic populations in the anticipation that DH-based addition lines would be more stable

TABLE 23.9. Hybridization details of successful combinations between *Triticum turgidum* L. cultivars and alien perennial Triticeae species under greenhouse conditions.

T. turgidum cultivar	Alien species	Florets pollinated	Seeds set	No. of embryos excised	No. of plants obtained
Cocorit 71	*Elymus fibrosus*[b]	36	6	3	2
Cocorit 71	*E. virginicus*	48	4	3	1
Altar 84	*Elytrigia pungens*	66	8	3	1
Laru	*Pascopyrum spicatum*	44	7	2	1
Dvergand[a]	*Psathyrostachys juncea*[b]	100	2	1	1
Yavaros[a]	*Thinopyrum elongatum*	110	13	7	1
Arlin[a]	*Th. junceiforme*[b]	24	17	9	2
Cocorit 71[a]	*Th. junceiforme*[b]	46	32	14	7
Altar 84	*Th. junceum*	44	28	15	6
Cocorit 71	*Th. junceum*	48	30	16	6
Croc	*Th. junceum*	44	30	14	4
Cocorit 71	*Th. littorale-campestre*	74	32	30	9
Mexicali 75	*Th. podperae*	70	61	49	22
Altar 84[a]	*Th. scirpeum*	44	18	5	2
Croc	*Th. scirpeum*	40	19	3	1
Yavaros[a]	*Th. scythicum*	132	68	23	3
Combinations with *Th. intermedium* subspecies					
Cocorit 71	ssp. *acutum*[b]	48	33	29	17
Yavaros[a]	ssp. *acutum*[b]	52	38	33	18
Arlin[a]	ssp. *acutum*	48	31	21	13
Cappelli	ssp. *acutum*	51	43	39	26
Chen	ssp. *glaucum*	100	74	55	44
Cocorit 71	ssp. *intermedium*	128	98	68	56
Yavaros 79	ssp. *intermedium*	88	70	59	43
Cappelli	ssp. *intermedium*	20	14	10	8
Cocorit 71	ssp. *pulcherrimum*	54	32	21	15
Mexicali 75[a]	ssp. *pulcherrimum*[b]	96	73	62	40
Yavaros 79	ssp. *pulcherrimum*	74	62	55	43
Mexicali 75	ssp. *trichophorum*	24	12	7	3
Croc	ssp. *trichophorum*	22	14	9	5
Dvergand	ssp. *trichophorum*	46	33	26	18
Laru[a]	ssp. *trichophorum*[b]	24	18	11	7

TABLE 23.9 *(continued)*

T. turgidum cultivar	Alien species	Florets pollinated	Seeds set	No. of embryos excised	No. of plants obtained
Cappelli	ssp. *trichophorum*	24	16	12	9
Altar 84[a]	ssp. *varnense*	46	40	32	24
Cappelli	ssp. *varnense*	54	38	28	19
Laru	ssp. *varnense*	30	22	17	13
Mexicali 75	ssp. *varnense*	30	20	15	11

[a]Combinations with BCI seed obtained.
[b]Combinations with amphiploid seed obtained.

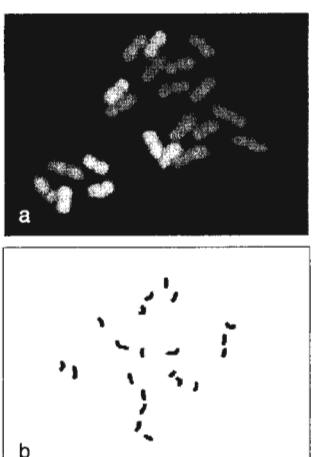

FIGURE 23.10. Cytological details of a durum/*Thinopyrum elongatum* hybrid from the F_1 to disomic addition line production. Shown is (a) F_1 with $2n = 3x = 21$ ABE in FISH and (b) F_1 meiosis with higher univalency.

than those obtained by selfing the monosomic additions. These additions will form the primary stage in identifying the alien chromosomal contributions for scab resistance.

An alternative cytogenetic manipulation route has also been addressed to counter the possibility that Type II scab resistance could be on more than a single alien chromosome. This strategy involves crossing the amphiploids

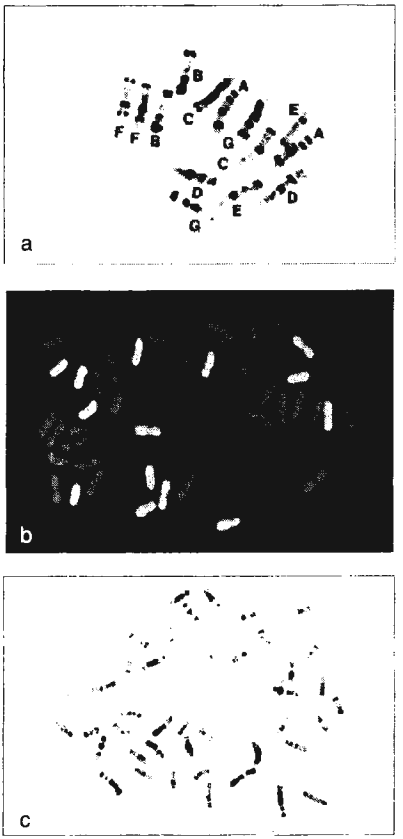

FIGURE 23.11. The durum/*Thinopyrum elongatum* combination showing (a) a C-banded mitotic cell of *Th. elongatum* ($2n = 2x = 14$, EE), (b) A mitotic amphiploid cell ($2n = 6x = 42$, AABBEE) in FISH, and (c) Giemsa C-banded amphiploid cell.

with the *ph1c* durum stock (cv. Capelli) and selecting *ph1c* haploids from the heterozygote F_1 product. Doubled haploids with *ph1c ph1c* will be the germplasm source with desired homologous (wheat/alien) exchanges as previously obtained for bread wheats (Mujeeb-Kazi, 2001a). Recovery of euploid durum wheats ($2n = 4x = 28$) with alien introgressions bestowing scab resistance is still quite distant but is feasible (see Figure 23.13).

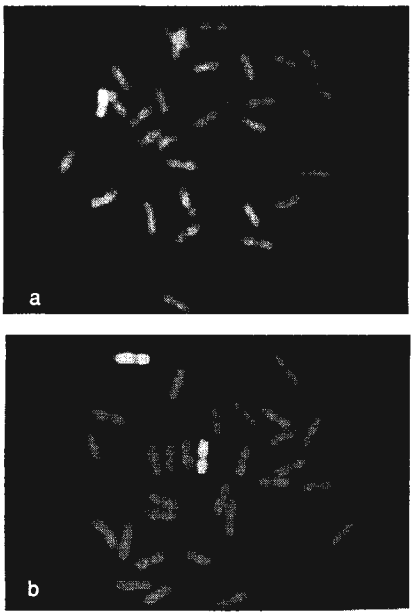

FIGURE 23.12. Backcross derivatives from durum/*Th. elongatum*//durum showing (a) monosomic alien addition line ($2n = 4x = 28 + 1$) in FISH, and (b) disomic addition line in FISH ($2n = 4x = 28 + 1$ alien pair = 30). Durum wheat DNA was used for blocking and *Th. elongatum* DNA as the labeled probe.

SOME PRACTICAL TRENDS FOR THE FUTURE

Use of New Tetraploids

S. tritici Roberge ex Desmaz (leaf blotch) is a fungal disease that limits wheat production in high-rainfall areas and affects 10.4 million hectares globally. Apart from desirable diversity in the conventional wheat germplasm primary gene pool, diploid species accessions also possess high levels of resistance. The A genome sources are *T. monococcum, T. boeoticum,* and *T. urartu*, and the D genome diploid donor is *Ae. tauschii*. Both diploids have been combined with elite durum wheat cultivars to generate AAAABB and AABBDD hexaploids that upon screening for leaf blotch in Toluca, Mexico, have unequivocally demonstrated a 1-1 to 2-2 level of re-

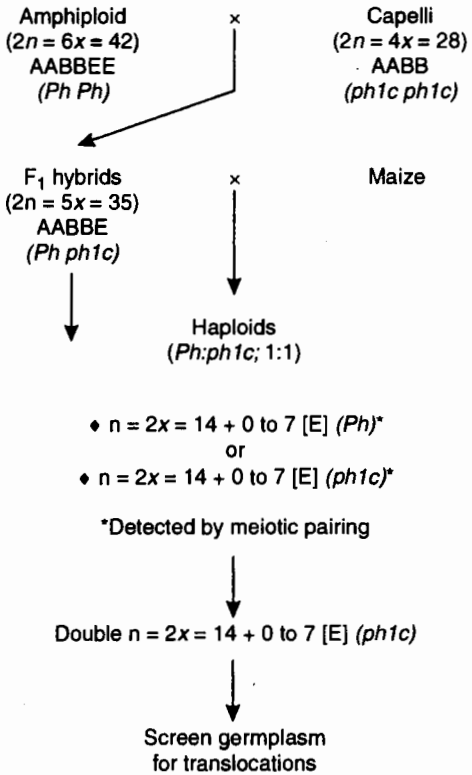

FIGURE 23.13. Schematic elucidating wheat/alien exchange products via a *ph1c* manipulation and haploidy strategy.

sistance (susceptible score being 9-9 on a double-digit (Zadoks, Chang, and Konzak, 1974) scoring system. This has enabled us to conclude that the diploid A and D genome accessions contribute to the blotch resistance in the hexaploid genetic stocks. Either hexaploid source can be independently utilized for wheat improvement, with transfers going to the A or D genome of bread wheat, respectively. To facilitate efficient crop improvement efforts, gene pyramiding is advantageous; one option is to provide stocks that already possess combined resistance sources, leading us to produce such germplasm. Selected were some A genome and D genome accessions that had contributed superior leaf blotch resistance to their respective hexaploids.

All had disease scores of 1-1 or 2-1. Hybrids were made between these A and D species where the F_1 ($2n = 2x = 14$, AD) combinations had 14 univalents at meiosis.

Accessional crossability was less than 1.0 percent, but some genotypic variation existed, giving frequencies up to 2.8 percent. Schedules for F_1 growth and colchicine treatment were standard, as was the validation of the doubled product ($2n = 4x = 28$, AADD) cytologically. Figure 23.14 documents the spike morphology and the meiotic relationship of one AADD amphiploid combination. The various combinations made are listed in Table 23.10, where the blotch score of each diploid is also indicated. Fertility of the tetraploids is satisfactory. These stocks will enable simultaneous transfers of blotch resistance genes into the A and D genome chromosomes of the recipient bread wheat cultivars thus adding to efficiency of dual source transfers.

D Genome Transfers to Durum

Progress in durum wheat/alien species hybridization has not been as exhaustive as bread wheat. However, the need to diversify the durum genetic base is crucial and can be achieved by incorporating the diversity of the primary, secondary, and tertiary gene pools. The intergeneric recombination constraints presumably can be overcome by using the *ph1c* 'Capelli' genetic stock, but this will require additional investigation to fit agricultural goals.

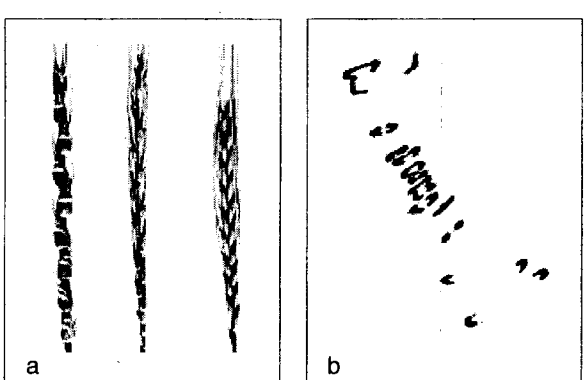

FIGURE 23.14. (a) Left to right: Spike morphology of *Aegilops tauschii* ($2n = 2x = 14$, DD), a new tetraploid ($2n = 4x = 28$, AADD), and the A genome ($2n = 2x = 14$, AA) donor; and (b) a meiocyte of the AADD tetraploid with 14 bivalents at metaphase I.

TABLE 23.10. A and D genome parental accessions used in the production of AADD tetraploids.

A genome diploid	Disease score[a]	D genome diploid	Disease score[a]
T. monococcum (111)[b]	1-1	*Ae. tauschii* (458)[b]	2-1
T. monococcum (111)	1-1	*Ae. tauschii* (319)	1-1
T. monococcum (111)	1-1	*Ae. tauschii* (323)	1-1
T. monococcum (111)	1-1	*Ae. tauschii* (273)	1-1
T. monococcum (111)	1-1	*Ae. tauschii* (321)	1-1
T. monococcum (111)	1-1	*Ae. tauschii* (434)	2-1
T. monococcum (111)	1-1	*Ae. tauschii* (222)	1-1
T. monococcum (111)	1-1	*Ae. tauschii* (319)	1-1
T. boeoticum (56)	1-1	*Ae. tauschii* (219)	1-1
T. boeoticum (56)	1-1	*Ae. tauschii* (1029)	2-1

[a]Leaf blotch scores of each diploid indicated based upon the contribution in AAAABB and AABBDD hexaploids; double digit score: the first digit indicates height of infection, where 5 = up to mid-plant and 9 = up to flag leaf; the second digit indicates disease severity on infected leaves, where 1 = low and 9 = total leaf destroyed.
[b]*Ae. tauschii* accession number in CIMMYT's wide crosses working collection.

For interspecific durum wheat improvement, A and B genome diversity through their AAAABB or AABBBB amphiploid routes allows for cross combinations to be made between the resistant amphiploids and elite durum cultivars, and will facilitate introgression/exploitation of resistant traits in breeding programs by utilizing appropriate breeding protocols. This alien-diversity-based durum wheat improvement program is currently in its infancy, but we do anticipate contributions for resistant transfers to be achieved for durums.

More challenging is the exploitation of D genome resistances for durum wheat improvement, and at a high priority would be the transfer of scab (*F. graminearum*) resistance genes. In addition, we must mention the potential of D genome resistance transfers to durum wheats of genes associated with salinity tolerance, drought tolerance, *S. tritici, S. nodorum, H. sativum,* and BYDV resistance, with quality being an integral part in all A, B, and D genome accessional transfers. These genomic transfers would be a consequence of recombinational events due to the preferential A and D genomic chromosome pairing represented as seven bivalents in the presence of the *ph1c* locus (Figures 23.15 and 23.16). The bivalents are generally of the A and D genome chromosomes, and univalents of the B genome as inferred

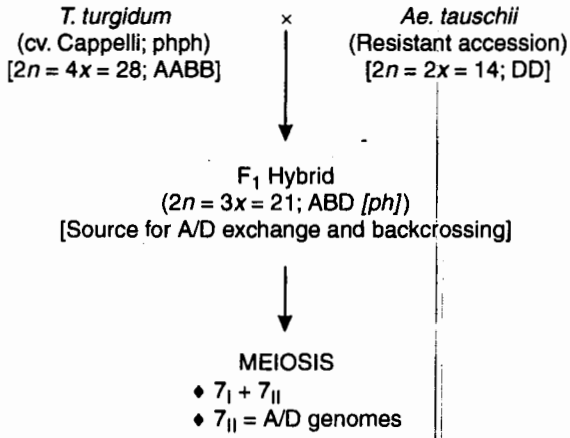

FIGURE 23.15. The strategy schematic showing the use of the D genome diversity of *Aegilops tauschii* accessions for durum wheat improvement with Capelli *ph1c ph1c* as the mediating cultivar.

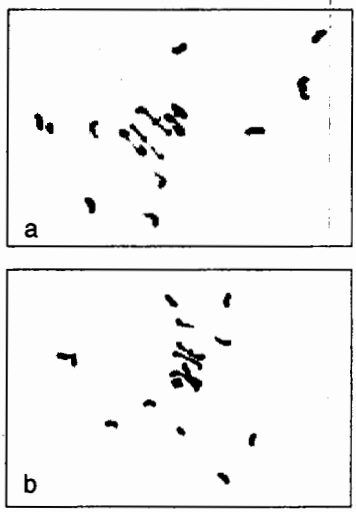

FIGURE 23.16. Meiosis of $2n = 3x = 21$, ABD *ph1c* F_1 hybrid showing preferential genomic pairing indicated by high bivalency (up to seven pairs).

separately from meiotic C-banding data. Our current tester system to demonstrate the D to A genome genetic exchange efficacy is for *H. sativum* and *S. tritici* from some D genome resistant accessions. Durum wheat cultivars are highly susceptible to both of these biotic stresses, and since we have ideal screening protocols with reliable screening locations in Mexico, priority has been assigned to these diseases. Similar work to improve durum wheat for *Fusarium* head scab is also underway.

CONCLUSION

The utilization of the alien Triticeae species for durum wheat improvement encompasses both interspecific and intergeneric hybridization procedures. The location of these species in the gene pools will chart the course of incorporating the unique genetic diversity somewhat more rapidly from interspecific combinations, since greater genomic similarity is prevalent. For durability of resistance in a crop, the distant species also are a potent source; their exploitation, though more complex, is justified. In this chapter the research efforts presented have addressed both of these aspects and incorporated essential details that are crucial to produce, validate, and advance wide cross combinations with a link toward agricultural practicality. The new genetic stock will serve as a source to utilize for future research and development. Integration of new techniques with cytogenetic manipulation, doubled haploidy, and translocations, complemented by molecular markers, will produce abundant genetic diversity from wide crosses. These can be used in conjunction with the conventional breeding procedures that harness cultivar variations and with sources such as *Triticum dicoccum* or *T. dicoccoides,* which have already been shown to combine readily.

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