Direct Genetic Transfers from *Aegilops squarrosa* L. to Hexaploid Wheat[1]

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ABSTRACT

Methodology of direct genetic transfer from diploid *Aegilops squarrosa* L. ($2n = 14$) to cultivated wheat (*Triticum aestivum* L.) ($2n = 42$) was explored as a possible applied plant breeding technique for rapid introgression of useful traits. One or more plants of 31 accessions of *A. squarrosa* (selected for resistance to certain pests of cultivated wheat) were crossed with 'Wichita' or 'Newton'. A total of 219 hybrid embryos were cultured on a synthetic medium to overcome interploidy hybrid embryo-endosperm incompatibility. Of the 219 embryos, 114 from crosses involving 17 accessions did not grow and were lost. However, one or more hybrids were obtained from crosses involving the 14 other accessions, and 24 F_1 hybrids were grown to maturity. The F_1s were male sterile, but backcross (BC) progenies included fully fertile and meiotically stable 42-chromosome plants. The partially fertile BC_1 plants had a chromosome number range of 40 to 50; 50% with $2n = 40$ to 42 and another 50% with $2n = 49$ to 50; the latter arose from the functioning of restitution gametes. The probability of transfer of *A. squarrosa* genes from an F_1 hybrid to a single BC_1 plant is 0.75. The BC_1 and BC_2 progenies segregated for several phenotypic traits and several lines with resistance to Hessian fly (*Mayetiola destructor* Say) biotype D, greenbug (*Schizaphis graminum* Rond.) biotype E, and leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) culture PRTUS6 were selected. Thus, the use of the embryo rescue method facilitated direct genetic transfers from *A. squarrosa* to hexaploid wheat, and thereby averted the need of using bridging species or prior synthesis of *T. turgidum*/*A. squarrosa* amphiploids to overcome interspecific cross-incompatibilities.

Additional index words: **Embryo rescue, Interspecific hybridization, *Triticum aestivum* L., Gene introgression, Disease resistance, Chromosome pairing, Germplasm, Genetic resources.**

COMMON, or hexaploid wheat [*Triticum aestivum* L. em Thell ($2n = 42$, AABBDD)], exists primarily under cultivation and is reproductively isolated from the vast reservoir of gene pools contained in the diploid and tetraploid progenitor species (A, D, and AABB genome species). Based on genomic affinities, the A, B, and D genome progenitor species constitute the homologous gene pool. Other species that carry a different genome constitute the homoeologous gene pool. The related polyploid species that carry only one of the common wheat genomes (e.g., AAGG and CCDD species) constitute a partially homologous gene pool. Genes in the homologous pool can be transferred by chromosome pairing and crossing-over, whereas special and rather complex manipulations are often necessary for the transfer of genes from the homoeologous gene pool. Gene transfer from the homologous gene pool may be complicated by the sterility caused by differences in chromosome number; cross-compatibility barriers, especially between diploid species and common wheat; complementary genes that cause seedling lethality; and the general impairment of yield potential.

In most studies of genetic transfer from diploid progenitors species to wheat, the tetraploid wheat *T. turgidum* L. var. *durum* was used as a bridging species (Sharma and Gill, 1983). Gerechter-Amitai et al. (1971), Vardi (1971), and Vardi and Zohary (1967) documented introgression from *T. monococcum* L., *Aegilops speltoides* Tausch., and *A. longissima* Schwgine. et Musch. to durum wheat via a triploid bridge. From durum wheat, genes can then be transferred to hex-

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aploid wheat (Kerber and Dyck, 1973; The and Baker, 1975). Durum wheat can also be used to transfer genes from *A. squarrosa* L. by the formation of a synthetic hexaploid wheat (Kihara et al., 1957; McFadden and Sears, 1946; Tanaka, 1959, 1961). This approach has been used to transfer several disease and insect resistance genes from *A. squarrosa* to hexaploid wheat (Dyck and Kerber, 1970; Harvey et al., 1980; Hatchett et al., 1981; Joppa, 1980; Kerber and Dyck, 1969).

Direct introgression from diploid species into hexaploid wheat is more difficult and may require specialized techniques. The crossability of wheat cultivars, F_1 seed abortion, F_1 hybrid lethality, and high male and female sterility of F_1 hybrids are major hurdles. The (1973) and The and Baker (1975) found that viable F_1 plants were obtained only from crosses of wild *T. monococcum* with hexaploid wheat. Hybrids with cultivated *T. monococcum* either died as seedlings or failed to flower. Dvořák (1977) successfully transferred rust resistance from *A. speltoides* to two commercial hexaploid wheat cultivars. The cross between hexaploid wheat and *A. squarrosa* is possible only by the embryo rescue technique (Alonso and Kimber, 1984; Raupp et al., 1983; Riley and Chapman, 1960).

In spite of the difficulties encountered, direct hybridization allows rapid genetic transfer of useful traits to an adapted cultivar and can be a valuable applied technique. There is, however, considerable evidence that both the A and B genomes have undergone coadaptive evolution in tetraploid wheats for millions of years, and any introduction of extant A and B genomes by hybridization is genetically a traumatic event. For example, transfer lines derived from commercial wheats with *A. speltoides* were inferior to the recurrent parent even after five backcrosses (Knott and Dvořák, 1981). Introgression from A genome progenitor species is also problematic. Recent cytogenetic evidence suggests that only six A-genome chromosomes may be present in polyploid wheats (Dvořák, 1983; Chen and Gill, 1983). Both A and B genomes in polyploid wheat are, to some extent, differentiated as compared to the extant genomes of the progenitor species. Thus, part of the useful genetic variation present in A and B genome progenitor species may be inaccessible for transfer by normal recombination methods into an elite genetic background.

Aegilops squarrosa may be the most suitable among the progenitor species for direct introgression. There is complete homology between hexaploid wheat D-genome chromosomes and those of *A. squarrosa* (Riley and Chapman, 1960). Not only is the total genetic variation in *A. squarrosa* more readily accessible, but also there is very little adverse genetic interaction between the D genome of wheat and *A. squarrosa*. Finally, there is evidence that *A. squarrosa* has greater useful genetic variability than is found in the other progenitor species (Gill et al., 1986b).

In our research on the management and utilization of wheat genetic resources, we have emphasized wild *Triticum* and *Aegilops* species as providing a readily available source of potentially useful variation for wheat improvement. We have reported on their evaluation for disease and insect resistance (Gill et al., 1983, 1985, 1986b; Hatchett and Gill, 1981, 1984). In this paper, we report experiments on rapid genetic introgression from *A. squarrosa* to wheat and its plant breeding significance.

Table 1. Number of embryos cultured, hybrids obtained, chromosome numbers, and selfed and crossed seed of F_1 and backcross progenies of 14 *Aegilops squarrosa/Triticum aestivum* hybrids.

Hybrid†	Embryos cultured‡	2n plants obtained	Chromosome no.	Seed set/plant	
				Selfed	Crossed
1642/WI	4	2(1§)	28¶	0	1
1642/2*WI		1	47	6	7
WI/1642	1	1	28	0	3
WI/1642//WI	3	3			
		1	49	18	--
		1	40	41	--
		1	40	31	--
1644/NWT	9	3			
		1	28	2	1
		1	28	3	2
		1	28	0	1
1644/2*NWT	1	1	49¶	13	--
1644/NWT//WI		1	49	10	37
1649/WI	8	1	28	0	3
1649/2*WI		1	40	35	48
1656/1645//WI	9	1	28	0	2
1656/1645//2*WI		2			
		1	40¶	109	9
		1	--	18	--
1656/1651//WI	13	2			
		1	28	0	2
		1	28	0	3
1656/1651//2*WI		3			
		1	43¶	76	3
		1	41¶	6	4
		1	49	20	2
1665/WI	9	1	28	0	0
1670/WI	8	2			
		1	28	0	0
		1	28	0	0
1675/WI	10	1	28	0	2
1675/2*WI		2(1§)			
		1	50	31	14
1680/WI	9	1§			
1687/WI	5	1§			
1691/WI	4	1	28	0	2
1691/2*WI		2			
		1	50	11	13
		1	44	3	2
1695/WI	9	4			
		1	28	0	3
		1	28	0	0
		1	28	0	1
		1	28	0	0
1695/2*WI		4(1§)			
		1	40	14	86
		1	49	9	11
		1§	39		
WI/1704	1	1	28	0	0
1704/WI	2	2			
		1	28	2	0
		1	28	0	0

† Kansas State Univ. Accession no.; NWT = cultivar Newton, WI = cultivar Wichita; the crosses were attempted with 31 accessions, only 14 accessions for which F_1 hybrids were obtained are listed here.
‡ Only embryos from 10 to 20-day-old seed from crosses between *A. squarrosa* and *T. aestivum* were cultured on synthetic medium.
§ Plants died prematurely.
¶ Meiotic chromosome pairing data of these plants is summarized in Table 3.

MATERIALS AND METHODS

Aegilops squarrosa accessions used in the crosses are listed in Table 1. These accessions contained one or more useful traits for disease and insect resistance (Gill et al., 1986b). The wheat cultivars used were 'Wichita' and 'Newton'. Both are hard red winter wheats adapted to the Great Plains re-

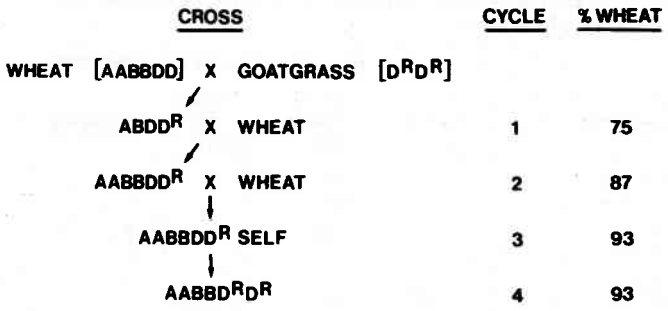

Fig. 1. A crossing scheme for direct genetic transfer from *Aegilops squarrosa* (goatgrass) into hexaploid wheat. D^R designates the goatgrass genome carrying a resistance gene that is to be transferred to wheat.

gion. Wichita is an older cultivar released in 1944. Newton was released in 1977 and has been a leading cultivar in Kansas for the last several years. For crossing purposes, emasculations and pollinations were made in the greenhouse at 24°C. Thirty-one accessions of *A. squarrosa*, three of which were present as (1656/1645) and (1656/1651) F_1 hybrids, were crossed as females with Wichita, except 1644 and 1704 where Newton wheat was the male parent. A few hybrids were produced with wheat as the female parent. The crossing scheme is shown in Fig. 1.

The F_1 seed from *A. squarrosa*/wheat hybrids aborted 12 to 18 days after pollination. To obtain F_1 plants, embryos were dissected from developing seed at 12 to 18 days under sterile conditions and cultured on a Murashige minimal organic medium (Gibco no. 510-1118, Gibco Laboratories, Grand Island, NY) supplemented with 3% sucrose, 0.4 mg/L thiamine-HCl, 100 mg/L *i*-inositol, and 10 mg/L each of the amino acids L-tyrosine, L-arginine, and glycine. The small seedlings were transferred to pots in the greenhouse and grown to maturity.

Root tip chromosomes were counted using a standard squash technique (Endo and Gill, 1984). For meiotic analysis, young spike samples were fixed in a 6:3:1 mixture of ethanol, chloroform, and acetic acid for several days at room temperature. Anther squashes were made in 1% acetocarmine.

The segregating progenies from *A. squarrosa*/wheat hybrids were evaluated for resistance to Hessian fly (*Mayetiola destructor* Say) biotype D, greenbug (*Schizaphis graminum* Rond.) biotype E, and leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) culture PRTUS6 by methods as described previously (Gill et al., 1986b).

RESULTS

A total of 219 embryos were planted on Murashige medium. One-hundred and fourteen F_1 embryos from 17 parental *A. squarrosa* accessions produced no plants (data not shown). Another 105 embryos from 14 parental *A. squarrosa* accessions (3 were represented as F_1 hybrids 1656/1645 and 1656/1651) produced 24 F_1 plants (Table 1). Three of the F_1 hybrids died, another eight F_1 hybrids set no self or cross seed, and one hybrid-1704/Wichita-set two self seeds and no cross seed. Among the remaining 12 F_1 hybrid plants, repeated pollinations produced 26 BC_1 seeds, 3 of which were highly shriveled and inviable. The F_1 hybrids, 1644/Newton and Wichita/1704, produced seven selfed seeds. The selfed seeds turned out to be octoploids with $2n = 56$ (AABBDDDD). All the remaining F_1 hybrids had $2n = 28$ (ABDD).

Table 2. Chromosome numbers, genetic segregation, and seed set (per plant) in BC_2 and BC_1S_1 plants derived from selected 49-chromosome, 43-chomosome, and 44-chromosome BC_1 plants of some *Aegilops squarrosa/Triticum aestivum* hybrids.

Hybrid†	$2n$ BC_1 plant	BC_2/BC_1S_1 plants analyzed	$2n$	Genetic segregation‡	Seed set
					no./plant
1656/1645//2*WI/3/NWT	49	6			
(BC_2)		1	41§	HfR	170
		1	43		8
		1	48	HfR	116
		1	43		31
		1	43§	HfR	85
		1	44		
1656/1651//2*WI	43	10			
(BC_1S_1)		1	42§	HfR, Rc	175
		1	44	W^I, Rc, gr	150
		1	44	W^I	168
		1	42	W^I	140
		1	42¶		190
		1	43+t		205
		1	43	W^I	118
		1	44	W^I, v.gr.	11
		1	43	Rc	175
		1	43	W^I	80
1691/3*WI	44	5			
(BC_2)		1	42§	LrR, HfR	33
		1	44		20
		1	44§	HfR	41
		1	42	LrR	38
		1	42	LrR	10

† Kansas State Univ. Accession no.; NWT = cultivar Newton, WI = cultivar Wichita.

‡ Genetic segregations: HfR = Hessian fly resistance, Rc = red coleoptile, W^I = nonwaxy stem, gr = grassy, v.gr = very grassy, LrR = leaf rust resistance.

§ 42-chromosome, homozygous resistant plants were isolated in the progeny of these plants.

¶ Metaphase I pairing 0.3I + 2.4II(rod) + 18.4II(ring).

Twenty BC_1 plants were grown; 3 died before flowering and the remaining 17 plants were partially self-fertile (Table 1). Backcrosses were not attempted on all plants, but when they were attempted, using the recurrent parent either as female or male, BC_2 seed was obtained. There was a tendency for seed shriveling among the self seed of most BC_1 plants.

The BC_1 plants showed a chromosome number range of 39 to 50; the only 39-chromosome plant died as a seedling (Table 1). Among the predominant chromosome number classes, five had $2n = 40$, five had $2n = 49$, and two had $2n = 50$. There was one plant each with chromosome numbers of 41, 43, 44, and 47. Thus, about 50% of the plants had chromosome numbers of $2n = 40$ to 43 and another 50% were $2n = 49$ to 50. These plants were nearly always the most fertile compared with plants of other chromosome numbers such as $2n = 44$ and $2n = 47$.

Data on chromosome numbers, genetic segregations, and seed set per plant were scored in BC_2 plants derived from two groups of $2n = 40$ to 44 and $2n = 47$ to 50 chromosome BC_1 plants. A sample of data on BC_2 plants derived from a 49-chromosome and a 44-chromosome BC_1 plant is given (Table 2). The BC_1 plants with $2n = 40$ to 43 chromosomes were highly fertile and a range of chromosome numbers and fertility data on 10 BC_1S_1 plants derived from a 43-chromosome BC_1 plant is also presented (Table 2). Among the hybrids, the BC_2 plants derived from the $2n = 47$ to 50 BC_1 plants had an average chromosome number

Table 3. Metaphase I chromosome pairing in F_1 and BC_1 hybrids of *Aegilops squarrosa/Triticum aestivum*.

Hybrid†	Generation	Genomes	Chromosome no.	Chromosome association‡ I	II (rod)	II (ring)	III	IV
1642/WI	F_1	ABDD	28	14.0	2.6	4.1	0.1	0.1
1644/2*NWT	BC_1	AABBDDD	49	3.9	2.5	15.8	2.8	–
1656/1645//2*WI	BC_1	AABBDD	40	3.0	3.2	15.3	–	–
1656/1651//2*WI	BC_1	AABBDD	43	2.5	4.2	15.5	0.2	0.2
	BC_1	AABBDD	41	4.1	5.5	12.2	0.5	–

† Kansas State Univ. Accession no.; NWT = cultivar Newton, WI = cultivar Wichita.
‡ Average of 20 pollen mother cells.

of $2n = 45$ (range 41 to 49). The BC_2 plants derived from the $2n = 40$ to 44 BC_1 plants had an average chromosome number of $2n = 41$ (range 35 to 47). Selections were made for vigorous, highly fertile, 42-chromosome plants among the selfed progeny of BC_1 (BC_1S_1 to BC_1S_5) and BC_2 (BC_2S_1 to BC_2S_5) plants. These plants were meiotically stable and chromosome pairing data is shown for one 42-chromosome BC_1S_1 plant (see footnote, Table 2).

Metaphase I (MI) chromosome pairing was studied in five F_1 and BC_1 hybrids (Table 3). As expected, the average chromosome pairing in the F_1 plant was 7.0 bivalents and 14.0 univalents, indicating complete homology between D genomes present in wheat and *A. squarrosa*. Four BC_1 hybrids showing a range of chromosome numbers were analyzed for chromosome pairing. A 49-chromosome BC_1 hybrid gave an average of 3.9 I + 18.3 II + 2.8 III. A 43-chromosome BC_1 hybrid showed a few univalents (2.5 I) and quadrivalents (0.2 IV), the latter indicating tetrasomy for one of the D genome chromosomes. A 41-chromosome hybrid showed 0.5 III, indicating trisomy for one of the D genome chromosomes, which also means it was monosomic for at least two chromosomes. Multivalents may also have been due to translocation differences. One 40-chromosome BC_1 plant, which probably was double monosomic for two chromosomes, gave an average chromosome pairing of 3.0 I + 18.5 II. The fact that 50-chromosome BC_1 plants were encountered indicates chromosome nondisjunction and may also explain the existence of trisomy and tetrasomy in hexaploid derivatives as observed above.

Certain kinds of cytological abnormalities such as highly polyploid cells and chromosome fragmentation were observed in the root tips of some plants. One 40-chromosome BC_1 plant gave a 35-chromosome BC_2 plant that was highly sterile. Similarly, the frequency of univalents was greater in some plants than would be otherwise expected. This indicates genetic interaction akin to hybrid dysgenesis during interspecific hybridization (Kidwell, 1983).

The resistance of various *A. squarrosa* strains to Hessian fly, greenbug, and leaf rust has been reported previously (Gill et al., 1986b). Segregating progenies from various *A. squarrosa*/wheat hybrids were scored for their reaction to races of the above pests (Table 4). The exact source of resistance in wheat strains derived from 1656/1645 and 1656/1651 hybrid *A. squarrosa* accessions was not known and this data is not included in Table 4. Both Newton and Wichita wheat cultivars

Table 4. Reaction to disease and insect races in *Aegilops squarrosa*-derived wheat lines.

Accessions, hybrids, and controls†	Hybrid generation (2n)	Reaction to disease and insect race‡ Leaf rust culture PRTUS6	Hessian fly biotype D	Greenbug biotype E
NWT (Control 1)		99P	S	S
WI (Control 2)		99P	S	S
1642		14C	R	S
1642/WI	F_1(28)	nt	nt	nt
1642/WI	BC_1 (47)	nt	nt	nt
1642/WI	BC_1S_1 families	nt	4 (S) 1 (Seg)	nt
1644		88C	R	S
1644/NWT	F_1 (28)	nt	nt	nt
1644/NWT//WI	BC_1 (48)	nt	nt	nt
1644/NWT//WI	BC_1S_1 plants	nt	4 (R) 16 (S)	nt
1644/NWT//WI	BC_1S_1 families	nt	5 (S) 10 (Seg) 5 (R)	nt
1649		01N	Seg	S
1649/WI	F_1 (28)	14C	nt	nt
1649/WI	BC_1 (40)	nt	nt	nt
1649/WI	BC_1S_1 plants	9 (99P) 1 (78X) 5 (23X–56X)	S	nt
1649/WI	BC_2 plants	5 (88P) 2 (56X) 2 (23X)	S	nt
1675		03C	S	R
1675/WI	F_1 (28)	nt	nt	nt
1675/WI	BC_1 (40)	nt	nt	nt
1675/WI	BC_1S_1 families	7 (88P) 1 (Seg)	nt	3 (Seg) 2 (S)
1691		000	Seg	S
1691/WI	F_1 (28)	78X	nt	nt
1691/WI	BC_1 (50)	nt	nt	nt
1691/WI	BC_2 plants	3 (99P) 4 (78X) 2 (67X)	nt	nt
1691/WI	BC_2S_1 families	nt	5 (Seg)	nt
1695		03C	R	R
1695/WI	F_1 (28)	88P	nt	nt
1695/WI	BC_1 (49)	nt	nt	nt
1695/WI	BC_2 (43–47) plants	5 (99P)	nt	2 (Seg) 3 (S)
1695/WI	F_1 (28)	88P	nt	nt
1695/WI	BC_1 (40)	nt	nt	nt
1695/WI	BC_1S_1 plants	8 (99P)	8 (R) 2 (S)	nt

† Kansas State Univ. Accession no.; NWT = cultivar Newton, WI = cultivar Wichita.
‡ nt = not tested, R = resistant, S = susceptible, Seg = segregating R and S plants. Each family contained 10 plants. Leaf rust reaction was scored using the infection-type coding method of Browder and Young (1975). The first code portrays relative sporulation on a scale of 0 to 9 and the second code shows relative lesion size on a scale of 0 to 9; the third (alphabetic) describes infection type: X = indefinite, C = chlorosis, P = pale, and N = necrosis. Scores were 000 = immune; 01C–23X = highly resistant; 56X –67X = moderately resistant; 78X–99P = were susceptible.

were susceptible to Hessian fly, greenbug, and leaf rust. The BC_1S_1 plants, BC_2 plants, and plants in BC_2S_1 families resistant to leaf rust were identified in hybrids of *A. squarrosa* accessions 1649, 1675, and 1691 with Wichita. However, the expression of resistance was reduced in all cases. Segregating progenies from other leaf rust resistant *A. squarrosa* accessions were either not tested or resistant plants were not identified.

Five *A. squarrosa* accessions were resistant or segregating for resistance to Hessian fly. Resistant plants were identified in the progenies of hybrids of acces-

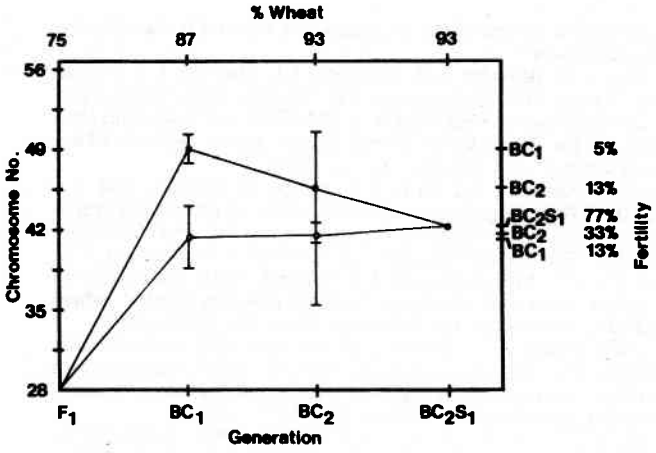

Fig. 2. Pathway of cytogenetic transfer from *Aegilops squarrosa* into *Triticum aestivum* including hybrid generations, chromosome number, percent wheat, and fertility of two kinds of BC_1 plants ($2n$ = 47–50 group and $2n$ = 40–43 group) and derived BC_2 and BC_2S_1 families. Fertility was calculated on the basis of seed set per family as a percentage of seed set of the recurrent parent (Wichita) that had 300 seeds per plant.

sions 1642, 1644, 1691, and 1695 with wheat. Two accessions, 1675 and 1695, were resistant to greenbug. Segregating resistant (R) plants were isolated in wheat strains derived from both accessions. Stable 42-chromosome breeding lines homozygous for genes for resistance to Hessian fly (four lines with different sources of resistance), greenbug (two lines), and leaf rust (three lines) were isolated and are under field evaluation. One hard red winter wheat Hessian fly–resistant line derived from *A. squarrosa* accession 1644, has been released as a germplasm (Gill et al., 1986a) and other germplasm releases will be made in the future.

Various progenies also segregated for a variety of other morphological and physiological traits. A detailed analysis of the segregation of various traits was not undertaken; some data are given in Table 2. Some obvious traits, such as red coleoptile, red culm, leaf pubescence, nonwaxiness, and tough glume (nonthreshing spike), were derived from *A. squarrosa*. A variety of growth habits were observed; some had tall erect leaves and others had a more spreading growth habit and tillered profusely. Grassy clump and dwarf plants were encountered in some progenies. A variety of responses to vernalization and photoperiod were encountered. Variations in seed size and shriveling were observed.

It has been demonstrated that direct introgression from *A. squarrosa* into hexaploid wheat is a valuable applied plant breeding technique. The salient cytogenetic and breeding aspects of the transfer scheme are shown in Fig. 2. The embryo rescue method was successfully used to obtain F_1 hybrids and backcross progenies from crosses between 14 accessions of *A. squarrosa* and hexaploid wheat. About 50% each of the BC_1 plants had a range of chromosome number of $2n$ = 40 to 43 and $2n$ = 47 to 50, and were partially fertile. The probability of gene transfer from an F_1 hybrid to a single BC_1 plant is 0.75. The partial fertility of BC_1 plants, including 49-chromosome plants, virtually assures the gene transfer to BC_2 plants. Forty-two chromosome, meiotically stable, and fully fertile plants, with 90% of the genes from the recurrent hexaploid wheat, were recovered in selfed progenies of BC_2 plants. Thus, fertile and hexaploid segregants were obtained more rapidly (in fewer hybrid generations) than may be possible by using other methods that require prior synthesis of AABBDD-genome amphiploids involving each of the *A. squarrosa* (DD) accessions and *T. turgidum* (AABB) as a bridging species.

DISCUSSION

In spite of the fact that *A. squarrosa* contains the D genome found in common wheat, interspecific hybridization between the two species is difficult and only a few tetraploid hybrids have been reported (Alonso and Kimber, 1984; Riley and Chapman, 1960; Raupp et al., 1983). Alonso and Kimber (1984) used common wheat as the female parent, whereas in this study *A. squarrosa* was the female parent in most crosses. Alloplasmic wheat populations were isolated from eight different accessions with no discernible cytoplasmic effects on plant growth or fertility.

There are both advantages and disadvantages in choosing the direction of the cross. When *A. squarrosa* is the female parent, seed set is extremely high (ca. 90%); however, hybrid seed abortion is severe and it is necessary to rescue the embryos 10 to 12 days following pollination. The recovery of culturable embryos (ca. 30%) and the frequency of embryos developing into plants (ca. 10%) are low. In the reciprocal cross, seed set is low (0 to 5%); however, hybrid seed abortion is less severe and embryo rescue can be delayed until Day 20. The 20-day embryo is well developed and can be cultured easily.

In addition to hybrid seed abortion, high sterility of tetraploid F_1 hybrids (ABDD) is another major obstacle. Considering that the probability of transferring a single gene to a BC_1 plant through a normal gamete is 0.5, low BC_1 seed set could be a serious problem if it was not for the fact that there is a 50% probability that the gamete may be of the restitution type (ABDD). Thus, the overall probability of the transfer of any gene to a BC_1 plant is 0.75. The BC_1 plants were partially fertile (average seed set was 15 to 40 seeds per plant) and gene transfer to BC_2 plants was virtually assured.

Grassy clump dwarf, caused by complementary genes, was encountered in some progenies but was not a serious problem. Cytological abnormalities such as chromosome breakage and asynapsis were encountered in only a few populations. Different degrees of seed shriveling were noticeable in some progenies. In spite of these minor problems, direct introgression was successful in generating breeding populations that were vigorous, highly fertile, and of good agronomic type. Transfer of useful disease and insect resistance genes was accomplished within the span of 1 yr.

The reasons for the quick recovery of the desirable agronomic types are twofold. First, the A and B genomes of the adapted parent were not affected because they are not present in *A. squarrosa*. Second, the D genome of the wheat parent shows almost complete homology with the D genome of *A. squarrosa* and as a result there was little adverse genetic interaction in the hybrid populations. The D genomes could recom-

bine and possibly infuse heterotic vigor (intergenomic and intragenomic heterosis) in the buffered background of the A and B genomes of the adapted parent. Favorable genotypes can be selected under appropriate conditions. Theoretically, direct introgression may be used for rapid introduction of desirable traits such as disease or insect resistance into an elite, commercial cultivar. The resulting breeding populations may also allow selection of other desirable traits. More significantly, controlled introgression permits experimental studies in quantitative and population genetics and plant breeding methodology as it relates to breeding of a polyploid crop.

Based on experimental data and more recent experience with wheat/*A. squarrosa* hybrids, the following points should be emphasized with respect to the crossing scheme shown in Fig. 1. Preferably, F_1 hybrids should be produced with wheat as the female parent to improve the viability of F_1 embryos. At least two BC_1 seeds should be obtained for each hybrid combination for the transfer of the desired gene with a probability of success greater than 90%. The partially fertile BC_1 plants should be used as males in a subsequent backcross with hexaploid wheat as the recurrent female parent, to recover meiotically stable 42-chromosome BC_2 plants in the cytoplasmic background of hexaploid wheat. In the pollen, there is a strong selection pressure against aneuploid gametes and 21-chromosome gametes will be preferentially transmitted. The BC_2 plants should be allowed to self and the desired gene may be recovered in homozygous condition in subsequent selfed generations.

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