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Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. × *T. tauschii*; 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement

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Abstract

Triticum tauschii (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L., 2n = 2x = 14, DD genome) with its diverse range of accessions and distribution provides a unique opportunity for exploiting novel genetic variability for wheat (*T. aestivum* L.) improvement associated with biotic/abiotic stress factors. From our working collection of 490 *T. tauschii* accessions we have so far produced 430 different synthetic hexaploids (2n = 6x = 42, AABBDD) resulting from the chromosome doubling of *Triticum turgidum* L. s. lat. × *T. tauschii* F_1 hybrids (each synthetic involving a different *T. tauschii* accession). We present here our results on hybrid production, plantlet regeneration, cytology, colchicine induced doubling of the 2n = 3x = 21 chromosome F1 hybrids, seed increase of the doubled progeny and screening for a biotic stress; *Cochliobolus sativus* Ito and Kuribay (syn. *Helminthosporium sativum* Pamm. King and Bakke); of 250 of these synthetic hexaploid (2n = 6x = 42) amphiploids. Application of the direct crossing methodology involving susceptible *T. aestivum* cultivars with resistant *T. tauschii* accessions is also alluded to.

Introduction

Triticum aestivum L. (2n = 6x = 42, AABBDD) improvement has predominantly been accomplished through conventional plant breeding methodologies and this approach shall continue to be the predominant procedure in the future. Novel approaches that complement plant breeding have emerged (Mujeeb-Kazi & Asiedu, 1989) and are attracting research interest. The impacts of novel approaches however, are futuristic. Wide hybridization, specifically intergeneric hybridization, is viewed as such. Presumably the realistic approach for exploiting alien genetic variability would be to separate the practical gains objectives into short- and long-term time-frames. The short-term benefits hold a high potential with lesser constraints. For this to materialize, interspecific hybridization stands at a priority with emphasis assigned to *T. tauschii* (Coss.) Schmalh.(*Aegilops squarrosa* auct. non L., 2n = 2x = 14, DD) because of its genetic proximity to the D genome of wheat. *T. tauschii*; unequivocally accepted as the D genome donor to *T. aestivum* (see Kimber & Feldman, 1987); is further attributed with a wide range of resistances/tolerances to biotic/abiotic factors (see Valkoun et al., 1990; Cox et al., 1992) that can contribute to *T. aestivum* improvement. One mechanism – of a few that exist – for exploiting the *T. tauschii* variation is via bridge crosses (review in Gill & Raupp, 1987) where *T. turgidum* L. s. lat. /*T. tauschii* hybrids (2n = 3x = 21, ABD) lead to generation of synthetic hexaploids (2n = 6x = 42, AABBDD) upon colchicine treatment or by spontaneous induction. Two other mechanisms deal with (i) extraction of AABB genome tetraploid plants from elite *T. aestivum* cultivars with their eventual hybridization to different *T. tauschii* accessions, and (ii) direct hybridization of *T.*

aestivum with *T. tauschii* (Alonso & Kimber, 1984; Gill & Raupp, 1987) with some methodology variations (see Valkoun et al., 1990).

We have emphasized indiscriminate hybridization of different *T. turgidum* cultivars with several *T. tauschii* accessions accompanied by ultimately screening the resulting synthetic hexaploids (SH) for *Cochliobolus sativus* Ito and Kuribay.

This paper elucidates the mechanisms of F_1 hybrid production, embryonic development, cytology, amphiploid induction and seed increase. Breeding potential of these synthetic hexaploids is further addressed through bridge crosses onto *T. aestivum*, and by direct crosses of susceptible *T. aestivum* cultivars × selected resistant *T. tauschii* accessions where resistance is inferred from the screening of the earlier produced synthetic hexaploids where the durum parents are susceptible.

Materials and methods

Germplasm

Triticum tauschii (2n = 2x = 14, DD) accessions were obtained from the CIMMYT germplasm bank in Mexico (El Batán) and from researchers in Pakistan (N. Hashmi: Nat. Agr. Res. Council, Islamabad), U.K. (C. Law: AFRC-IPSR) and USA (B. Gill: Kansas State; G. Kimber: Univ. of Missouri; R. Metzger: then at Oregon State; G. Waines: Univ. of California, Riverside). A total of 490 accessions were thus acquired and increased for seed quantity prior to their utilization by vernalization procedure (8 °C, 8h of light for 8 weeks). After the accession seed increase, additional *T. tauschii* seedlings similarly vernalized were also transplanted to the field cycles in the Mexico location of Ciudad Obregon during November to May for hybridization to *T. turgidum*.

Hybridization, embryo rescue and plantlet regeneration

Elite 48 durum wheat (*T. turgidum*) cultivars were planted over 4 dates at 10 day intervals in order to niche with *T. tauschii* pollen availability. Emasculation, pollination, embryo rescue and regeneration procedures were similar to those reported earlier (Mujeeb-Kazi et al., 1987, 1989).

Cytology of hybrids, colchicine doubling and cytology of amphiploids

From each potted hybrid plantlet, root-tips were collected and somatically analyzed (Mujeeb-Kazi & Miranda, 1985) to validate hybridity. All hybrid plants (7 per combination) possessing 2n = 3x = 21 chromosomes were treated with 0.1% colchicine + 2.0% dimethyl-sulfoxide for 6 hours via aerated root-treatment for doubling the chromosome number in order to obtain fertile amphiploids (2n = 6x = 42).

Additional hybrid plants of each combination where more than 7 were present were not treated with colchicine. The spikes on all hybrid plants were glassine bagged upon extrusion. Seed set on these spikes was the measure of amphiploid induction (colchicine induced or spontaneous). All amphiploid seed was germinated, somatically analyzed to validate the amphiploid status and for obtaining a seed increase.

C. Sativus disease screening

The 250 synthetic hexaploid combinations, their durum parents, the two susceptible 'Ciano 79' and resistant (BH 1146) bread wheat cultivars were each planted in a 2m double row in Poza Rica, Mexico in November 1992 for *C. sativus* screening. The location is a natural severe epidemic site. Disease evaluations were based upon foliar infestation and grain blemish at maturity. A double digit scale measured foliar infestation, where the first digit equated to the height of infection and the second digit with the infection severity. Scale gradations were 1 to 9. For the height of infection a score of 5 was for plants with infection upto the plant center and for a score of 9 infection had spread to the flag leaf. A disease severity score of 1 was for infected leaves exhibiting low disease symptoms, whereas a 9 score reflected total leaf destruction. Grain infection at maturity was scored on a 1 to 5 scale with 1 being low and 5 being a high seed blemish at embryo points.

Results and discussion

The vernalization procedure resulted in very vigorous growth of *T. tauschii* 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 were rescued at 18–20 days post-pollination

Table 1. Crossability, plant regeneration and colchicine induced doubled seed data for some synthetic hexaploids between *Triticum turgidum* and *T. tauschii* accessions

T. turgidum/T. tauschii combination and CIGM cross number	Florets pollinated	Seeds set	Embryos excised	Plants	Doubled progeny
CROC_1/*T.tauschii* (168)CIGM87.2755	24	4	4	1	46
Altar 84/*T.tauschii* (178)CIGM88.1168	48	5	5	1	22
Doy 1/*T.tauschii* (188)CIGM88.1175	72	10	9	3	45
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T.tauschii* (191)CIGM88.1179	48	4	4	3	55
Duergand/*T.tauschii* (214)CIGM86.951	24	13	13	10	60
D67.2/P66.270//*T.tauschii* (223)CIGM88.1219	48	10	9	3	24
Gan/*T. tauschii* (236)CIGM88.1228	48	3	3	1	13
Mexi/Vic//Yav79/3/*T.tauschii* (434)CIGM88.1335	48	16	6	5	40
LCK59.61/*T.tauschii* (344)CIGM90.816	24	9	9	2	26

from all crosses. These were small, translucent, ill-defined and floating in a watery endosperm cavity. Consequently the embryos were plated on Taira & Larter's (1978) medium for small embryos, which coupled with a 15 day 4 °C cold shock (dark) allowed better regeneration than the MS medium generally used for well-defined embryos. Crossability data for some combinations is presented in Table 1 to indicate the general trend observed for seed set, embryos recovered, plants regenerated, and the seed number of doubled plants after one generation of increase. In intergeneric hybrids it is frequently observed that all seeds set after crossing may not possess embryos, a trend that prevailed here also. The embryo size was minute in all cases, translucent and devoid of scutellar shape. Embryos were generally floating in a watery endosperm. Upon excision and plating of the embryos in the sterile culture media tubes, a cold shock (4 °C) in the dark was given for 15 days. The cultured embryo tubes were kept further in the dark at 22 °C after the cold treatment. The embryos usually germinated within 30 days after which the plantlets were transplanted into a soil media and maintained in the greenhouse for conducting cytology and inducing amphiploidy.

All genuine F_1 hybrids were stable for $2n = 3x = 21$ (ABD) chromosomes. After colchicine doubling the C-0 synthetic seed generally possessed 42 chromosomes, though some hypo- or hyper-ploidy did exist that has been subsequently purified by additional cytology and seed increase. The resistant synthetics for *C. sativus* have already entered our wheat breeding program.

From the wide array of SH wheats produced, field plantings have been utilized for the evaluation of agronomic parameters including the yield potential and its components. Based upon these characteristics Villareal et al. (1994) demonstrated extensive genetic diversity for plant height, flowering date, grain-fill duration, days to physiological maturity, above ground biomass at maturity, 1000-grain weight, spikes/m^2 and higher grain yield; the latter of these ranging from 0.89 upto 8.01 t/ha. Utilization of this germplasm for wheat improvement shall presumably be at an advantage if the more agronomically desirable SH wheats are exploited that further express high levels of resistance to biotic/abiotic stresses as opposed to using resistant but poor agronomic types. From our screening of 250 SH wheats in Poza Rica for *C. sativus*, diversity of resistance in the various SH wheats was observed. Some of these results highlighting a few SH wheats are provided in Table 2. The durum cultivars involved in these SH combinations were generally susceptible both for the leaf infection and seed blemish parameters. A resistant SH wheat after screening is consequently interpreted as being so because of the respective *T. tauschii* accessions involvement.

Based upon the agronomic evaluations (Villareal et al., 1994) and the *C. sativus* disease screening data (Table 2) elite SH types have been identified for wheat improvement (Table 2). Those SH wheats with a leaf score of 95 or less, and a seed damage of 3 or less are considered as preferred resistant donors for wheat improvement. The score damage for the respective durum parents involved in the SH wheats of Table 2 are listed in Table 3 and are generally more susceptible than the derived SH progeny. This SH bridge is advantageous for crop improvement since it allows not only the *T. tauschii* resistance to be exploited but

Table 2. Some synthetic hexaploids (SH) for *Triticum turgidum* × *T. tauschii (Ae. squarrosa)*; 2n = 6x = 42; evaluated for *Cochliobolus sativus*

T. turgidum cultivar/*T. tauschii* accession(*SH*) combination and its CIGM cross number	Disease Score		
	Leaves*		Seed**
	a	b	
Croc_1/*T. tauschii* (168)CIGM87.2755	97	99	4
Altar 84/*T. tauschii* (178)CIGM88.1168	96	97	3
Altar 84/*T. tauschii* (188)CIGM87.2765	97	99	4
Doy 1/*T. tauschii* (188)CIGM88.1175	93	94	2
Rabi//GS/CRA/3/*T. tauschii*(190)CIGM88.1178	93	94	2
Altar 84/*T. tauschii* (191)CIGM87.2766	96	97	3
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (191)CIGM88.1179	96	97	3
SORA/*T. tauschii* (191)CIGM88.1180	97	99	4
SORA/*T. tauschii* (192)CIGM90.540	96	97	4
CPI/Gediz/3/Goo//Jo69/CRA/4/*T. tauschii* (193)CIGM88.1183	93	95	3
CPI/Gediz/3/Goo//Jo69/CRA/4/*T. tauschii* (196)CIGM88.1186	94	96	4
Altar 84/*T. tauschii* (198)CIGM87.2768	97	99	4
Altar 84/*T. tauschii* (205)CIGM87.2770	97	99	4
CPI/Gediz/3/Goo//Jo69/CRA/4/*T. tauschii* (208)CIGM88.1194	93	95	3
Croc_1/*T. tauschii* (210)CIGM87.2754	97	99	4
Altar 84/*T. tauschii* (211)CIGM87.2771	97	99	4
Croc_1/*T. tauschii* (213)CIGM86.947	97	99	4
Duergand/*T. tauschii* (214)CIGM86.951	96	97	4
Rok/Kmli//*T. tauschii* (214)CIGM86.959	93	94	2
CPI/Gediz/3/Goo//Jo69/CRA/4/*T. tauschii* (215)CIGM88.1204	93	95	3
Yuk/*T. tauschii* (217)CIGM90.561	93	94	2
Altar 84/*T. tauschii* (220)CIGM87.2760	97	99	4
Duergand/*T. tauschii* (221)CIGM86.953	96	97	4
D67.2/P66.270//*T. tauschii* (223)CIGM88.1219	96	97	4
Altar 84/*T. tauschii* (224)CIGM86.941	96	96	3
Arlin_1//*T. tauschii* (225)CIGM86.956	98	99	4
Gan/*T. tauschii* (236)CIGM88.1228	93	94	2
Duergand/*T. tauschii* (247)CIGM88.1237	96	97	4
Mexi/Vic//Yav79/3/*T. tauschii* (434)CIGM88.1335	92	92	2
Doy 1/*T. tauschii* (447)CIGM88.1344	92	93	2
Yav/Sco//Jo69/CRA/3/Yav79/4/*T. tauschii* (498)CIGM88.1356	94	96	3
Doy 1/*T. tauschii* (510)CIGM88.1360	93	95	3
Doy 1/*T. tauschii* (511)CIGM88.1363	93	94	3
Doy 1/*T. tauschii* (515)CIGM90.566	93	94	3
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (521)CIGM90.529	93	95	3
Gan/*T. tauschii* (522)CIGM88.1370	93	95	3
Yar/*T. tauschii* (524)CIGM89.474	94	95	3
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (629)CIGM90.590	92	93	2
Ceta/*T. tauschii* (850)CIGM89.552	93	94	2
Yuk/*T. tauschii* (864)CIGM90.760	95	97	3
Ceta/*T. tauschii* (872)CIGM89.555	93	94	3
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (878)CIGM89.559	92	93	2
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (882)CIGM89.561	93	95	3
68.111//RGB-U/Ward/3/FGO/4/Rabi/5/*T. tauschii* (890)CIGM89.564	94	95	3
Rabi//GS/CRA/3/*T. tauschii*(891)CIGM90.602	94	95	3
Rabi//GS/CRA/3/*T. tauschii*(895)CIGM90.603	93	94	3
Rabi//GS/CRA/3/*T. tauschii*(904)CIGM90.605	92	93	2
Croc_1/*T. tauschii* (518)CIGM86.944	93	94	3

Table 2. Continued.

T. turgidum cultivar/*T. tauschii* accession(*SH*) combination and its CIGM cross number	Disease Score		
	Leaves*		Seed**
	a	b	
PBW114/*T. tauschii* (Introduction from India)	93	94	3
Ceta/*T. tauschii* (895)CIGM89.567	92	92	2
Arlin_1//*T. tauschii* (308)CIGM90.811	97	98	4
LCK59.61/*T. tauschii* (344)CIGM90.816	97	99	4
CPI/Gediz/3/Goo//Jo69/CRA/4/*T. tauschii* (358)CIGM90.817	93	95	3
Rabi//GS/CRA/3/*T. tauschii*(457)CIGM90.832	92	94	2
Ciano 79 (Susceptible Bread Wheat)	97	99	5
BH 1146 (Resistant Bread Wheat)	95	97	3

*Two-digit scoring system: first digit = height of infection; i.e. five = upto center of plant, 9 = upto the flag leaf; second digit = disease severity on infected leaves, 1 = low and 9 = total leaf destroyed. a = score at early milk stage, b = score at dough stage.
**Grain infection scored as: 1 = low and 5 = high seed blemish at embryo points.

also incorporates the genetic diversity of the A and B genomes of the respective durum wheat cultivars.

The *T. tauschii* accessions contributing to *C. sativus* resistance in the SH wheats have become the parental sources of another methodology of their exploitation for wheat improvement through direct susceptible *T. aestivum*/resistant *T. tauschii* accession crosses. This approach has been advocated as the ideal efficient technique for exploiting *T. tauschii* variability for wheat improvement.

Direct *T. tauschii* hybridization with *T. aestivum* cultivars is accepted as a priority (Alonso & Kimber, 1984; Gill & Raupp, 1987; Cox et al., 1990, 1991), since backcrosses onto the ABDD F_1 hybrids (2n = 4x = 28) by the same *T. aestivum* cultivar readily gives 11/12 (92%) of the genotype of the recurrent parent in a single growing season. This inference was drawn by Alonso & Kimber (1984) based upon stem rust transfers from *T. tauschii* into the *T. aestivum* cultivar 'Chinese Spring'.

Using this approach we have now targeted some resistant *T. tauschii* accessions for direct hybridization with susceptible but elite *T. aestivum* cultivars like 'Ciano 79' and 'Bacanora'. The schematic of Fig. 1 illustrates the crossing and advance procedure. The BCI selfed derivatives may be readily exploited for screening, though a second backcross to *T. aestivum* does become necessary when the BCI plants have a much higher than 42 chromosome composition. The procedure nevertheless is a extremely swift way of incorporating alien genetic diversity for agricultural end-product usage.

Table 3. Triticum turgidum L. (2n = 4x = 28, AABB) cultivars involved in synthetic hexaploid combinations screened for *Cochlibolus sativus*

T. turgidum cultivars	Disease Score		
	Leaves*		Seed**
	a	b	
Croc_1	99	99	4
Altar 84	99	99	4
Doy 1	96	97	3
Rabi//GS/CRA	99	99	4
68.111//RGB-U/Ward/3/FGO/4/Rabi	97	98	4
SORA	96	98	4
CPI/Gediz/3/Goo//Jo69/CRA	96	97	4
Duergand	99	99	4
Rok/Kmli	99	99	4
Yuk	97	98	3
D67.2/P66.270	99	99	4
Arlin_1	97	99	4
Gan	96	96	3
Mexi/Vic/Yav79	97	97	3
Yav/Sco//Jo69/CRA	99	99	4
Ceta	97	97	3
LCK59.61	97	98	5

*Two-digit scoring system: first digit = height of infection; i.e. 5 = up to center of plant, 9 = up to the flag leaf; second digit = disease severity on infected leaves, 1 = low and 9 = total leaf destroyed. a = score at early milk stage, b = score at dough stage.
**Grain infection scored as: 1 = low and 5 = high seed blemish at embryo points.

Conclusions

New synthetics covering more *T. tauschii* accessions than the presently screened 250 are being produced, and we anticipate incorporating our entire 490 *T. tauschii* entry working collection in this SH wheat form. So far 430 synthetics have been produced. The SH wheats embody a wide array of genetic diversity of *T. tauschii* accessions. They are all spring type in habit, and offer an easier source for practical utilization, conservation and global distribution for the SH germplasm. The SH germplasm further provides a unique gene pool for evaluating the *T. tauschii* response towards a wide range of biotic/abiotic stress conditions. The international distribution of synthetic hexaploids has additional merit that following screening by national agricultural programs for different objectives the variation can be readily incorporated into their local adapted germplasm.

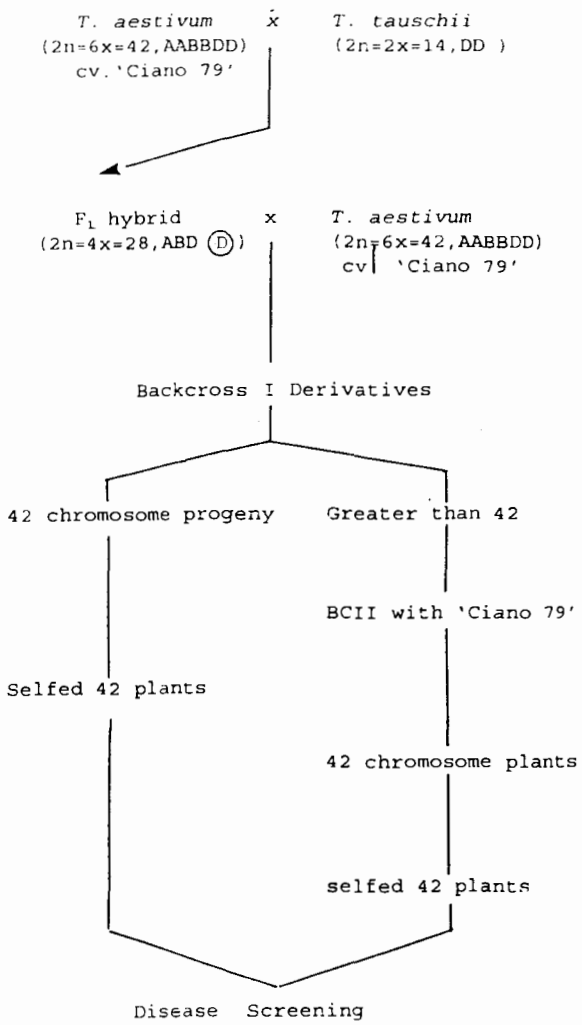

Fig. 1. A schematic showing the crossing scheme of *Triticum aestivum/T. tauschii* direct hybridization and backcross advance.

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