WHEAT IMPROVEMENT THROUGH WIDE HYBRIDIZATION: ENHANCING RESISTANCE/TOLERANCE TO SOME BIOTIC/ABIOTIC STRESSES

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ABSTRACT

Annual and perennial Triticeae species offer a unique genetic variability pool for wheat improvement through interspecific and intergeneric hybridization. Several annual *Triticum tauschii* (2n=2x=14, DD) accessions have been combined with *T. turgidum* (2n=4x=28, AABB) yielding synthetic hexaploids. These upon screening, exhibit varied resistances to *Helminthosporium sativum* and salinity; two aspects focussed here; where the *T. turgidum* cultivars are susceptible. Direct hybridization of the contributing *T. tauschii* resistant accessions to susceptible bread wheats now offer opportunities for rapid genetic transfers. Wheat improvement by intergeneric methodology has utilized *Thinopyrum elongatum* for *H. sativum* and *Th. bessarabicum* for salinity. Transfer strategies rely upon alien disomic chromosome additions to wheat, establishing marker diagnostics and manipulation of the *Ph* locus on chromosome 5B via a sexual wheat X maize polyhaploid crossing methodology.

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 and are attracting research interest (Mujeeb-Kazi and Asiedu 1989). Impacts of novel approaches however, are futuristic. Wide hybridization, specifically intergeneric hybridization, is viewed as such. The realistic approach for exploiting alien genetic variation is to separate the practical gains objectives into short- and long-term time-frames. The short-term benefits hold a high potential with lesser constraints and here interspecific hybridization inevitably stands at a priority with our emphasis currently assigned to *T. tauschii* (2n=2x=14 DD), which is unequivocally accepted as the D genome donor to *T. aestivum* (see Kimber and Feldman 1987). It is attributed with a wide range of resistances/tolerances to biotic/abiotic factors (see Cox *et al.* 1992; Valkoun *et al.* 1990) 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 and Raupp, 1987) with *T. turgidum* as the female parent leading to the generation of synthetic hexaploids (2n=6x=42, AABBDD). Another mechanism deals with direct hybridization of susceptible *T. aestivum* cultivars with resistant/tolerant *T. tauschii* accessions (Alonso and Kimber 1984; Gill and Raupp 1987) with some variations (see Valkoun *et al.* 1990). These two approaches have been addressed here as they impinge upon exploiting *T. tauschii* accessions for transferring salinity tolerance and *Helminthosporium sativum* resistance to *T. aestivum*. Though more complex, the intergeneric hybridization methodology provides access to a novel gene pool for wheat improvement. The group *Thinopyrum* is particularly rich as a genetic resource, has relative ease of hybridization offering adequate ploidy sources to select

appropriate alien donors for characteristics desired in wheat improvement. Our concentration has been on two diploid *Thinopyrum* species; *elongatum* for *H. sativum* and *bessarabicum* for salinity tolerance. Progress on exploitation of these gene resources far from complete shall also be reported upon.

MATERIALS AND METHODS

Interspecific Hybridization. A total of 490 *T. tauschii* accessions were acquired from several sources (Mujeeb-Kazi *et al.*, 1993) and increased for seed quantity prior to utilization in hybridization by the vernalization procedure (8°C, 8h of light for 8 weeks). Subsequently, *T. tauschii* seedlings similarly vernalized were transplanted to the field cycles in Ciudad Obregon, Mexico (November to May crop cycle) and El Batan (May to October crop cycle) for hybridization to *T. turgidum.*

Hybridization. For hybridization several *T. turgidum* cultivars obtained from CIMMYT's durum breeding program were planted over at least three planting intervals so as to flower with the *T. tauschii* accessions. The crossing (*T. turgidum* as the female parent), embryo rescue, embryo culture and plantlet managing procedures were similar to those earlier reported (Mujeeb-Kazi *et al.* 1987).

Cytology. Root tips were collected from each putative hybrid plant and processed according to the method of Mujeeb-Kazi and Miranda (1985). F_1 hybrid plants with 2n=3x=21 chromosomes were treated with 0.1% colchicine + 2.0% dimethyl-sulfoxide for 6 hours via aerated root-treatment. The seeds selfed on these plants after treatment were germinated and somatically analyzed. For each chromosome-doubled fertile plant a seed increase followed from which a reserve of 50g was maintained while the excess seed was utilized for testing resistance/tolerance *Helminthosporium sativum*/salinity. *H. sativum* screening was conducted under field conditions in Poza Rica, Mexico (Villareal *et al.*, 1993) and salinity in hydroponics under sodium chloride stress (Mujeeb-Kazi *et al.*, 1992).

Direct Hybridization. Once synthetic hexaploids upon screening were identified as resistant/tolerant where the *T. turgidum* cultivars were susceptible in their pedigree, the contributing *T. tauschii* accessions were candidates for hybridization onto susceptible *T. aestivum* cultivars for *Helminthosporium sativum* and salinity. The hybridization and F_1 advance procedures were esentially similar to those described by Alonso and Kimber (1984), Gill and Raupp (1987).

Intergeneric hybridization. The intergeneric *T. aestivum/Th. bessarabicum* F_1 hybrid to yield backcross derivatives led to the production of *Th. bessarabicum* chromosome additions (Mujeeb-Kazi, 1992) that were characterized by various diagnostic markers for establishing homoeologous relationships. These addition lines were screened for salinity tolerance under hydroponics according to previously published procedures (Mujeeb-Kazi *et. al.*, 1992) where the salt stress was provided by sodium chloride concentrations ranging from 0 - 200 mM.

Screening of the *Th. elongatum/T. aestivum* was restricted to its amphiploid (2n=8x=56) for *H. sativum* under natural field infection in Poza Rica, Mexico.

RESULTS AND DISCUSSION

Interspecific hybridization

We indiscriminately hybridized *T. tauschii* accessions with *T. turgidum*. The synthetic hexaploids emerging as a consequence could then be screened more adequately for our objectives, will not require vernalization and where positive for an attribute (durum wheat check being susceptible) could first be used in wheat breeding programs and their indirect crosses of the contributing resistant/tolerant *T. tauschii* accesions to susceptible brea wheat cultivars.

Currently 250 synthetic hexaploids; each involving a different *T. tauschii* accession; have been produced over several cycles of hybridization. These have then undergone screening for *H. sativum* and salinity. Resistant synthetics have been identified for *H. sativum* (Table 1) and several have shown a positive response to salt stress in hydroculture (Table 2). Initial genetic studies involving crosses of susceptible bread wheats x resistant synthetic hexaploids (F_1), the reciprocal F_1, F_2 and BCI analyses suggest a simple dominant genetic control of the resistance.

Towards the development of synthetic hexaploids all genuine F_1 hybrids were stable for 2n=3x=21 chromosomes. After colchicine doubling, the C-O synthetic seed generally possessed 42 chromosomes, though some aneuploid did exist that has been subsequently purified by additional cytology and seed increase. The resistant synthetics for *H. sativum* have already entered our wheat breeding program. Those positive for salinity await utilization.

The ideal efficient technique for exploiting *T. tauschii* variability in wheat improvement requires at least two pre-requisites: (i) Reliable screening for biotic and abiotic factors, and (ii) hybridization with *Triticum* species. Direct *T. tauschii* hybridization with *T. aestivum* cultivars stands at a priority (Alonso and Kimber 1984, Cox *et al.*, 1990, 1991, Gill and Raupp 1987), since backcrosses onto F_1 hybrids readily give 11/12 (92%) of the genotype of the recurrent parent in a single growing season. This inference was drawn by Alonso and Kimber (1984) based upon stem rust transfers from *T. tauschii* into the cultivar `Chinese Spring'.

When screening constraints for *T. tauschii* accessions occurred, we sacrificed efficiency for agricultural practicality in order to obtain a plausible solution. Such constraints existed for identifying with reliability resistant or tolerant *T. tauschii* accessions to *H. sativum*, and salinity. However, the *T. turgidum* cultivars x *T. tauschii* accessions leading to synthetic hexaploids did overcome this situation and gave a conclusive resistance screening data.

Screening at the synthetic hexaploid level for *Helminthosporium*, *Fusarium* and salt is viable since the *T. turgidum* cultivars (those in the pedigree) were susceptible. Screening of synthetic hexaploids; has yielded selections that have positive value for wheat improvement for both attributes. The intricacies of the A, B and D genome associations that obviously exist are circumvented and even if the resistance/tolerance effect observed is diluted in the hexaploid screened, it possesses a level recognizably higher

than what our wheat germplasm demonstrates for *H. sativum*, and salinity. We are not discounting the fact that D genome interactions with the A and B genomes of durum wheat do exist through gene suppression or enhancement mechanisms. This generalization however, may not be valid for all synthetic hexaploids, and now with the wide array of genetic diversity that we have generated, further elucidation of the D genome interactions with the A and B genomes shall inevitably emerge; presumably more explicit for simply inherited characteristics.

Table 1. Selected five synthetic hexaploids from *Triticum turgidum x T. tauschii (Aegilops squarrosa)*; 2n=6x=42; and the amphiploid of *Thinopyrum elongatum*/Goshawk "S" (2n=8x=58) resistant to *Helminthosporium sativum*.

Synthetic hexaploid pedigree and attribute	(*T. tauschii* CIMMYT) identifier number	Disease Score Leaf damage*	Seed damage**
CPT/GEDIZ/"S"/3/Goo"S"//Jo"S"/CR"S"		99	4
CPT/GEDIZ/"S"/3/Goo"S"//Jo"S" CR"S"/4/*Ae. squarrosa*	(215)	93	2
TK SN1081		98	4
TK SN1081/*Ae. squarrosa*	(222)	93	2
Gan"S"		96	3
Gan"S"/*Ae. squarrosa*	(236)	93	2
Doyl		97	3
Doyl/*Ae. squarrosa*	(446)	93	2
Doyl/*Ae. squarrosa*	(510)	93	2
Ciano 79 (Susceptible bread wheat)		99	5
BH1146 (Resistant bread wheat)		97	3
T. aestivum cv. Goshawk "S"		99	5
Th. elongatum/*T. aestivum* cv. Goshawk "S" amphiploid		93	2

*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. **Grain infection scored as: 1=low and 5=high seed blemish at embryo points.

Since resistant/tolerant synthetic hexaploids have been identified (Tables 1 to 3) the following options are available for exploiting the germplasm for wheat improvement:

(i) Exploit the hexaploids by crosses onto susceptible *T. aestivum* cultivars and select the resistant/tolerant segregants exercising initial caution associated with the necrosis genes present in the synthetics as a consequence of the *T. turgidum* cultivars; and

(ii) From the resistant/tolerant synthetic hexaploids exploit the *T. tauschii* accessions by direct crosses onto the elite but susceptible *T. aestivum* cultivars using recurrent backcrossing with *T. aestivum* parents as the procedure, coupled with cytology to extract stable 2n=6x=42 euploids.

Table 2. Selected five synthetic hexaploids from *Triticum turgidum x T. tauschii (Ae. squarrosa)*; 2n=6x=42; tested positive for the Na:K discrimination trait associated with salinity tolerance in hydroculture testing. Levels 50 days after a 50 mol m^{-3} NaCl concentration was reached.

Synthetic hexaploid pedigree and attribute	(*T. tauschii* CIMMYT) identifier number	K:Na ratios
ROK"S"/KMLI"S"		1.2
ROK"S"/KMLI"S"//*Ae. squarrosa*	INTERVER-214	7.7
PBW 34		1.2
PBW 114/*Ae. squarrosa***		13.3
CPT/GEDIZ/3/GOO//JO"S"/CR"S"		1.1
CPT/GEDIZ/3/GOO//JO"S"/CR"S"//*Ae. squarrosa*	INTERVER-206	16.4
MEX//VIC/YAV		1.5
MEX//VIC/YAV/*Ae. squarrosa*	INTERVER-343	17.7
DOY 1		0.7
DOY 1/*Ae. squarrosa*	INTERVER-510	3.5

* K:Na discrimination ratios; higher values positive for salinity tolerance.

** Synthetic obtained from H. Dhaliwal. Instead of the durum PBW114 we have used PBW34 in the evaluation, since both are susceptible.

Table 3. Hydroculture screening of some addition lines of *Th. bessarabicum* in wheat: Dry weight and K:Na ratios, 50d stress at 150 mol m^{-3}

	Dry wt	K/Na
CS	4.5	4.5
CS/*Th*	3.7	9.2
3J	1.0	7.9
3J/7J	2.7	4.3
6J	2.1	7.3
7J	1.4	7.2
Yecora	1.1	3.7

Using this information we have now targeted *T. tauschii* accessions (Tables 1 to 3) for direct hybridization with susceptible and elite *T. aestivum* cultivars. These are cultivars

'Ciano 79' and 'Bacanora' for *H. sativum* and 'Oasis', 'Yecora', 'Ciano 79' for salt tolerance. Over 200 F_1 hybrids were obtained and predominantly all had the expected 2n=4x=28, ABDD constitution. Only three hybrids had 27 chromosomes. Two backcrosses and selfings should forge the way to euploid 42 chromosome plant status and their screening for resistance.

New synthetics covering more *T. tauschii* accessions than our present 250 are also being produced, with emphasis subsequently placed on achieving direct transfers from *T. tauschii* targeted accessions to *T. aestivum*. These approaches are anticipated to contribute to the availability of additional genetic variability for wheat breeding utilization, germplasm conservation and global distribution. "International distribution" of "synthetic hexaploids" has merit for screening in national agricultural programs having different objectives and varied adapted germplasm.

Intergeneric Hybridization

Salinity tolerance. In our initial screening (Mujeeb-Kazi *et al.*, 1992) the positive influence on salinity of several *Th. bessarabicum* chromosomes was apparent from hydroculture evaluations (Table 3). Major contribution was from the disomic addition lines 3J, 6J, and 7J that gave desirable dry weight and greater than 1 sodium:potassium values as did also the amphiploid of *T. aestivum/Th. bessarabicum* (2n=8x=56) compared to the wheat check. Though manipulation from such a stage is possible to effect desirable gene transfers we consider it preferable to set up a *Phph* BCI stage or produce a new F_1 hybrid with the *ph* locus, and then culminate with polyhaploid based homozygous methodology.

This inforced genetic manipulation approach is anticipated to hasten the transfer methodology where the conventional addition line, substitution line and introgression stages are apparently overly complex because of several genes distributed over several alien chromosomes. Multiple disomics is a consideration for *ph* based manipulation accompanied by polyhaploid induction but does not stand higher in priority than the initial *ph* based manipulation.

Helminthosporium sativum. *Th. elongatum* is known for its salt tolerance potential but we are exploiting it in addition for its resistance to *H. sativum*. The *Th. elongatum/T. aestivum* cv. Goshawk "S" (2n=8x=56) amphiploid expresses a high level of resistance which is encouraging and leads to the influence that alien genes express in the amphiploid where Goshawk "S" wheat is susceptible (Table 1). Addition lines of *Th. elongatum* are now being produced coupled with crosses onto the amphiploid of the *ph* mutant as a futuristic look were the inheritance to be complex for the alien genes from *Th. elongatum*. The synthetic hexaploid source however, showed segregation data fitting simple dominant genetic control but this generalization cannot extend to include *Th. elongatum* at this stage.

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