Triticeae III

Editor A.A. Jaradat

Cover photo credits (left to right)

Upper row:
1. *Aegilops ovata (=Triticum ovatum).* 2. *Triticum aegilopoides* (John Raupp). 3. *Triticum dicoccoides* (Moshe Feldman). 4. *Triticum monococcum* (Leonor Pena-Chocarro)

Middle row:
5. *Triticum dicoccum.* 6. *Triticum spelta* (Leonor Pena-Chocarro). 7. *Triticum durum* × *Aegilops* spp. (Natural hybrid). 8. *Triticum durum* (branched spike) (A. A. Jaradat). 9. *Triticum polonicum.* 10. *Triticum ispahanicum* (N. Watanabe).

Bottom row:
11. *Hordeum marinum.* 12. *Leymus condensatus* (Mary Barkworth). 13. *Lopophyrum elongatum.* 14. *Thinopyrum junceiforme* (P. Jauhar).

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2.1

Evolutionary relationships and gene transfer in the Triticeae

A. Mujeeb-Kazi

Plant breeders exploit conventional crop improvement efforts in order to meet the ever-increasing demands for food production. They are however, finding less and less appropriate germplasm with desired traits among cultivated crops themselves with which to make the needed improvements. Fortunately, new and useful genetic resources are being found in wild, uncultivated plant species that possess a close or distant genetic relationship to food crops. The challenge is to expediently incorporate this alien germplasm into existing food crops, and simultaneously sustain genetic diversity. In the tribe Triticeae, these goals are being addressed through intergeneric and interspecific hybridization methodologies. Facilitated by the basically critical embryo/tissue culture techniques, researchers have ingeniously produced a wide array of wide hybrids amongst the Triticeae species. The current status suggests that hybridization barriers can be readily circumvented and novel germplasm developed. Simultaneously, the conventional and molecular diagnostics have evolved to the level that alien introgression detection no longer remain too complex a process. For speeding up alien introgression and production/maintenance of genetic stock programs, use of polyhaploidy through sexual hybridization of bread wheat with maize, pearl millet, *Sorghum* and *Tripsacum* has emerged as a stable technique. The above areas form a package that impinges upon exploitation of alien genetic diversity for wheat improvement. Production outputs stand strongly associated with the evolutionary relationships present among the Triticeae species in their various gene pools. These views shall be elucidated, and will demonstrate our major applied emphasis using as a base the novel germplasm available, or being currently developed in CIMMYT.

Introduction

This paper's focus is set for achieving agricultural production targets with emphasis on bread and durum wheat. Such production targets are to be achieved by enforcing crop improvement protocols based upon utilization of genetic diversity, that is crucial for durability of stress resistances/tolerances, and for ensuring sustainability.

Though genetic diversity can be induced, for more controlled, well directed incorporation diversity naturally present in the annual and perennial Triticeae species has priority. This natural diversity resides

in the conventional wheat germplasm, and in closely or distantly related alien species sources. The species resources are distributed within gene pools, and genetic transfers can be realized for wheat improvement from these pools over short- or long-term time frames. The gene pools are structured upon the genomic constitution of the species, and are comprised of three groups: primary, secondary and tertiary.

The 'primary' gene pool species include the hexaploid landraces, cultivated tetraploids, wild *Triticum dicoccoides*, and diploid donors of the A and D genomes to durum/bread wheats. Genetic transfers from these two genomes occur as a consequence of direct hybridization and homologous recombination with breeding protocols contributing different backcrossing and selection strategies. Some cross combinations require embryo rescue, but no cytogenetic manipulation procedures are necessary. The 'secondary' gene pool is composed of the polyploid *Triticum* plus *Aegilops* species which share one genome with the cultivated wheats. The diploid species of the Sitopsis section are included in this pool, and hybrid products within this gene pool demonstrate reduced chromosome pairing. Gene transfers occur as a consequence of direct crosses, breeding protocols, homologous exchange between the related genomes or through use of special manipulation strategies amongst the non-homologous genomes. Embryo rescue is a complementary aid for obtaining hybrids. Diploid and polyploid species are members of the 'tertiary' gene pool. Their genomes are non-homologous. Hence, genetic transfers require special techniques that assist homoeologous exchanges facilitated by irradiation or callus culture mediated translocation induction.

For practical end-products to be obtained, some transfer prerequisites that encompass all the three gene pool species span from hybrid production, embryo rescue, plant regeneration, cytological diagnostics, breeding methodology, and stress screening, culminating in stability of the advanced derivatives contributed by homozygosity. Based upon these prerequisites and genetic transfer ease, the primary gene pool diversity holds priority significance for wheat improvement. The species of the diploid A and D genomes contribute novel genes, allow direct recombinational exchanges with their respective genome partners to facilitate both durum and bread wheat improvement over a relatively "short-term" time frame, than what is provided by the secondary or tertiary gene pool species. The strategy and outcome of exploiting the A and D genome accessions are illustrated in the schematics of Figures la and lb.

One avenue of using the A genome accessional diversity is via bridge crossing of the amphiploids (Fig.1a). In general, the durum parents x A genome accession crosses are simplistic and of high frequency (Table 1). Meiosis of F_1 hybrids (2n=3x=21, ABA) with up to six bivalents for metaphase I chromosome associations per meiocyte is indicative of genomic exchange amongst the A genomes (Table 2). The meiotic stability of the AA BBAA, 2n=6x=42 amphiploids suggests an ease of maintenance of these genetic stocks (Table 3). The durum cultivars in these amphiploids are susceptible for the stresses being addressed in our studies. Hence, upon stress screening a resistant amphiploid implied that the particular A genome accession had contributed the expressed resistance. So far, some diversity has been identified in the AA BBAA amphiploids for *Cochilobolus sativus*, and is more extensively observed for *Septoria tritici*.

The A genome strategy has been extended to cover the D genome (Fig. lb), a genome that demonstrates an unparalleled wealth of genetic diversity for several biotic and abiotic stresses as observed upon the screening of the AABBDD (SH) synthetic hexaploids. Crossing the resistant synthetic hexaploids with elite but susceptible bread wheat (BW) cultivars has yielded resistant BW/SH

derivatives. Currently we have produced 620 SH wheats, and a wide array of these wheats are being globally utilized for wheat improvement either at the SH or at the BW/SH advanced derivative levels.

(a)

T. *turgidum* x AA Species
↓
F_1 Hybrids
(2n=3x=21, AB A)
↓
Colchicine
↓
Amphiploid (2n=6x=42, AABB AA)

(b)

T. *turgidum* x DD species
↓
F_1 hybrids
(2n=3x=21, AB D)
↓
Colchicine
↓
Amphiploid (2n=6x=42, AABB DD)
Synthetic hexaploid

Fig. 1.Schematics showing the production of A and D genome 2n=6x=42 chromosome stocks as a consequence of hybridizing durum cultivars with A genome diploids (a) *boeoticum, monococcum* or *urartu*), and D genome diploid; (b) *Aegilops squarrosa, Ae. tauschii* or *T. tauschii*) accessions.

Most advanced in our wheat breeding programs are the D genome resistances for karnal bunt *(Tilletia indica)*, *S. tritici* and *H. sativum*. Promise also exists for resistances/tolerances in this SH germplasm for leaf rust, stripe rust, mineral toxicities, drought, salinity, heat, cold, sprouting, waterlogging, high molecular weight (HMW)/low molecular weight (LMW) glutenin sub-units, powdery mildew, loose smut, cereal cyst nematode (CCN), yield and its components.. The least accessional diversity observed so far in the D genome is for scab or *Fusarium graminearum* (less than 1.0 %), but under evaluation tests conducted at one location in Mexico, the observed scab resistance is promising and superior than that of the leading bread wheat cultivars Frontana and Sumai-3 with their assemblage of four genes (Van Ginkel et al. 1996).

Table 1. Mean production frequencies of F_1 hybrids from some *Triticum turgidum* x diploid A genome species accessions. [*T. turgidum* / A genome (2n=3x=21, AB_A)]

Combination	Florets	Seed	Embryos
4X/ *T. boeoticum*	351	163	153
4X/ *T. monoccocum*	252	41	36
4X/ *T. urartu*	352	45	45

From the secondary gene pool, the potential of using *Aegilops speltoides* resistance diversity for several stresses does exist. The newly produced 2n=6x=42, AABBBB amphiploids have shown initial promise for resistance to *H sativum*, *S. tritici*, barley yellow dwarf virus (BYDV), leaf and stripe rust. However, more testing is required in order to verify this resistance.

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

Table 2. Mean meiotic metaphase I association in some F_1 hybrids of *Triticum turgidum* x diploid A genome species accessions. (2n=3x=21, AB A)

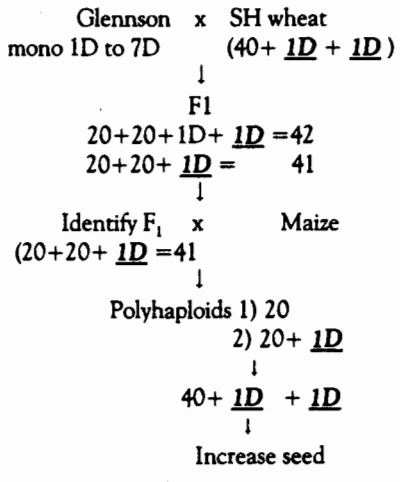
Combinations	Metaphase I associations				
	I	Rings	Rods	Total	III
4X/ *T. boeoticum*	9.4	4.26	1.53	5.79	
4X/ *T.monococcum*	8.8	3.20	2.80	6.00	0.07
4X/ *T. urartu*	9.0	3.20	2.30	5.50	0.20

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. Additionally, we must mention the potential of D genome resistance transfers to durum wheats of genes associated with salinity tolerance, drought tolerance, *S. tritici*, *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 in the presence of the *ph* locus. The bivalents are generally of the A and D genome chromosomes, and univalents of the B genome as inferred separately from meiotic C-banding data (Unpublished). 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 biotic stresses, and since we have ideal screening protocols with reliable screening locations in Mexico, priority has been assigned to these diseases.

Alien genetic transfer impacts over a "short-term" are being increasingly emphasized, and their measure would be through production outputs of research end products. Such impacts shall be a consequence of gene transfers from the closely related diploid species, and their accessional diversity. In some cases, even faster results can be obtained as exemplified by the D genome direct crossing procedures (Alonso and Kimber, 1984; Gill and Raupp, 1987). Greater efficiency emerges by mediating direct transfers with sexually-induced double haploidy (DH) for achieving rapid homozygosity (Mujeeb-Kazi and Riera-Lizarazu, 1996). The DH procedure is making significant contributions in our wide crosses program. One application of this methodology is the multilocational stress testing of homozygous advanced lines resistant to *H. sativum*. The DH approach has provided further usage, and is now being advantageously utilized for modified complete or partial monosomic analyses. The partial analysis is conducted when resistance is associated with the D genome of SH wheats (Fig. 2). The F_1 monosomics of 1D to 7D chromosomes (2n=6x=40 +1D to 40+7D) when crossed with maize, yield 21

chromosome polyhaploids with the 1 D to 7D contributions coming from resistant SH wheats. Doubling these n=3x=21 polyhaploid plants with colchicine, results in stable 42 chromosome double haploids. Each DH having the homozygous 1D to 7D chromosomes of the resistant SH parent is being analyzed for the chromosomal location(s) of the resistant gene(s). Upon screening, the non-segregating resistant DH's are attributed with having the gene(s) in them. The stable monosomic derived DH germplasm, apart from simplifying the conventional monosomic analyses, also facilitates global distribution of the developed germplasm. The germplasm enables experimental repetition without having to rebuild the analytical germplasm, as is necessary when the conventional monosomic analytical procedure is followed.

The DH role in salvaging *Ph*-based F_1 hybrids has become an option to enable *ph*- mediated alien introgression(s) without having to remake complex F_1 hybrids using the *ph* genetic stock (Sears, 1977) as the maternal parent. Since we maintain a living herbarium of F_1 hybrids of wheat and alien species (*Ph* locus present), BC_1 derivatives can be produced by pollinating these *Ph* F_1 wheat/alien hybrids with the Chinese Spring *ph ph* wheat genetic stock. The BC_1 progenies *(Ph ph)* are crossed with maize to yield polyhaploids that possess the *Ph* or *ph* locus. The entire wheat and alien chromosomal complement is represented. The *ph*-based BC_1 derivatives favored as the alien introgression germplasm, is identified at the seedling stage by a PCR-based diagnostic analysis (Gill and Gill, 1996) and enhances program efficiency. We have further extended the use of DH's for development of molecular mapping populations, stabilizing wheat transgenics coupled with chromosomal location of the transgene, and for shortening the conventional bread wheat breeding time by integrating homozygosity protocols as desired by breeder objectives.



Seven such DH's result; non-segregating resistant 1D to 7D possesses the resistant genetic control

Figure 2.Schematic showing steps associated in conducting a partial monosomic analysis where resistance is located within the synthetic hexaploid (2n=6x=42) D genome chromosomes. The monosomic parent used is susceptible to the stress. A resistant BW/SH advanced line can also be used in the test.

Though closely related genomes hold a priority for wheat improvement, additional genes from 'diverse' gene pools also offer unique resistance durability, and are anticipated to contribute to

sustainable cropping systems. The various gene sources contributing to *H. sativum* resistance elucidates this concept. These genes when pyramided, have the potential to ensure resistance durability across several locations where *H. sativum* is a wheat production constraint. From the earlier BH1146 cultivars resistance, the improvement present in the *Thinopyrum curvifolium* Chinese wheat-derived cultivars has been significantly dramatic (cvs. Chirya and Mayoor) and has remained durable across several countries for approximately ten years. However, complacency in not introgressing more diverse genes must not prevail for *H. sativum* or for any other biotic stress. In essence, the progress from BH1146 to *Thinopyrum curvifolium* and/or usage of Chinese wheat cultivar derivatives (Chirya, Mayoor), coupled with the extensive variation identified in the A, B and D genome accessions is seen as a guarantee for stability over years to come. It is a path being followed for *H. sativum* and can be extended to address other stress objectives.

The above approach, as we are approaching the 21st century, is totally removed from some wide hybridization views. Fedak et al.(1994) expressed that wide crossing for purposes of gene transfer be done as a last resort when the variability for a particular trait was exhausted or was non-existent in the primary gene pool.

We however, view wide crossing as a complementary approach, to be simultaneously conducted and integrated within conventional breeding programs. This approach contributes multiple diverse novel genes from all gene pools by pyramiding them with the conventional genetic resource present in the primary pool commonly used by breeders. Even if these are "major" alien genes, their multiplicity and diversity shall provide the advantage when they are pyramided with the conventionally available "minor" genes that recognizably contribute to durable resistance.

Table 3. Mean meiotic metaphase I association in some F_1 hybrids of A genome diploids (2n=6x=42, AABBAA). [AABB AA Meiotic association]

Diploid species in combination	Metaphase I associations					
	I	Rings	Rods	Total	III	IV
T. boeoticum	14.0	10.3	6.2	16.5	0.4	1.6
T. boeoticum		13.0	4.6	17.6		1.7
T. monococcum	0.1	14.3	3.3	17.6	0.1	1.6
T. monococcum	0.2	14.6	2.8	17.6	0.2	1.5
T. urartu		14.5	3.5	18.0		1.5
T. urartu		14.4	4.0	18.4		1.3

Distantly related species (e.g.,'tertiary' gene pool) are complex to exploit, but their potential use in crop improvement is very high. To exemplify, the contributions of *Secale cereale* in wheat improvement cannot be overlooked (Islam-Faridi and Mujeeb-Kazi, *1995;* Mujeeb-Kazi et al., 1996), and a major role of *Thinopyrum* species for BYDV resistance further attests to the use of this distant diversity (Henry et al., 1996).

Involvement of other 'tertiary' gene pool species in wheat germplasm has been the subject of a few recent reviews (Jiang et al., 1994; Sharma, *1995;* Friebe et al., 1996). We suggest that a modified tertiary gene pool transfer strategy should receive greater emphasis in the future, thus providing maximum recombination between wheat and alien species chromosomes in the early hybrid generation stages. The enhanced recombination will be a consequence of the cytogenetic *Ph* locus manipulation, irradiation,

callus induction, etc. *Ph* manipulations will involve the use of chromosome 5B genetic stocks, and of the relatively newer Ph^1 germplasm option (Chen et al., 1994). The latter warrants more exploitation.

The earlier the wheat/alien chromosomal exchanges occur in an intergeneric hybridization program involving tertiary gene pool species, the sooner appropriate breeding protocols can be incorporated, and stress screening coupled with homozygosity can be applied. Moreover, with the current molecular diagnostic strength, identification of alien introgression(s) can be easily achieved and get exploited.

Our vision is to shorten the 'tertiary' gene pool conventional genetic transfer protocols to a short-term product oriented program akin to the interspecific approach that capitalizes upon the 'primary' and 'secondary' gene pool species. The vision projected above will better address the incorporation of polygenically-controlled traits hopefully "*en-bloc*" into wheat, of which the alien *Th. elongatum* chromosomal control (3E, 4E, 7E) for salinity tolerance is one example.

Conclusions

There are 25 genera, some 400 species and a wealth of accessional variation in the Tribe Triticeae. Crop improvement reliance on this genetic diversity is high, and its effective utilization is governed by the species relationships.

To meet crop development goals, research strategies are crucial for attaining production targets over set time frames. These targets can be realized rapidly through the use of novel protocols, and by an integrated research approach that encompasses multilocational and multidisciplinary teams. Such a framework shall provide durable and sustainable products, which is the theme of this paper.

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