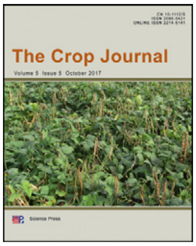
Crop Science Society of China

Available online at www.sciencedirect.com

ScienceDirect



Comparing two approaches for introgression of germplasm from *Aegilops tauschii* into common wheat☆

Thomas S. Cox[1], Jizhong Wu*, Shuwen Wang[1], Jin Cai, Qiaofeng Zhong, Bisheng Fu

Institute of Crop Germplasm and Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China

ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form
14 April 2017
Accepted 18 May 2017
Available online 29 June 2017

Keywords:
Aegilops tauschii
Direct hybridization
Origin of wheat
Synthetic hexaploid
Triticum aestivum
Wheat breeding

ABSTRACT

Allelic diversity in the wild grass *Aegilops tauschii* is vastly greater than that in the D genome of common wheat (*Triticum aestivum*), of which *Ae. tauschii* is the source. Since the 1980s, there have been numerous efforts to harness a much larger share of *Ae. tauschii*'s extensive and highly variable gene pool for wheat improvement. Those efforts have followed two distinct approaches: production of amphiploids, known as "synthetic hexaploids," between *T. turgidum* and *Ae. tauschii*, and direct hybridization between *T. aestivum* and *Ae. tauschii*; both approaches then involve backcrossing to *T. aestivum*. Both synthetic hexaploid production and direct hybridization have led to the transfer of numerous new genes into common wheat that confer improvements in many traits. This work has led to release of improved cultivars in China, the United States, and many other countries. Each approach to D-genome improvement has advantages and disadvantages. For example, production of synthetic hexaploids can incorporate useful germplasm from both *T. turgidum* and *Ae. tauschii*, thereby enhancing the A, B, and D genomes; on the other hand, direct hybridization rapidly restores the recurrent parent's A and B genomes and avoids incorporation of genes with adverse effects on threshability, hybrid necrosis, vernalization response, milling and baking quality, and other traits, which are often transferred when *T. turgidum* is used as a parent. Choice of method will depend in part on the type of wheat being developed and the target environment. However, more extensive use of the so-far underexploited direct hybridization approach is especially warranted.

Contents

☆ Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.

* *Corresponding author.*
E-mail address: wujz@jaas.ac.cn (J. Wu).

[1] Permanent address: The Land Institute, 2440 E. Water Well Rd., Salina, KS 67401, USA.

http://dx.doi.org/10.1016/j.cj.2017.05.006

1. Introduction

In the seven decades since Kihara [1] and McFadden and Sears [2] demonstrated that hexaploid wheat (*Triticum aestivum* L., genomic constitution AABBDD) arose as a natural amphiploid between the tetraploid wheat species *T. turgidum* L. (AABB) and the diploid grass *Aegilops tauschii* Coss. (DD), evidence has accumulated that common wheat is not monophyletic. That is, at the time of wheat's origin 8000 years ago, the formation of more than one interspecific amphiploid contributed to formation of wheat [3]. Although we can never know the precise number of hybridizations that were at the root of the hexaploid wheat gene pool, we know that they occurred within a small geographic range and that a very limited proportion of *Ae. tauschii*'s total diversity was incorporated into common wheat at that time.

For example, by analyzing more than 7000 single-nucleotide polymorphisms (SNP) in the *Ae. tauschii* genome, Wang et al. [4] showed that a very narrow portion of the *Ae. tauschii* gene pool accounts for the parentage of *T. aestivum*'s D genome. Whereas the geographical range of the 402 *Ae. tauschii* accessions analyzed stretched almost 4000 km from Georgia, Turkey, and Syria in the west to China in the east, accessions with the shortest genetic distance to hexaploid wheat all had geographical origins in a small sector of northwestern Iran, along 300 km of its Caspian seacoast.

In accord with the narrow sampling of D-genome diversity that occurred at the time common wheat originated, there is ample evidence that genetic diversity within wheat's D genome, which was derived from *Ae. tauschii*, is much lower than the diversity found in collections of *Ae. tauschii* germplasm. Numerous studies covering more than a quarter century have found that allelic diversity in the D genome of *Ae. tauschii* is vastly greater than that in the D genome of wheat. Studies using various DNA markers, endosperm storage proteins, and enzyme variation have all reached that conclusion [4–8]. Diversity in wheat's D genome is also lower than in its A and B genomes [9].

Ae. tauschii germplasm collections have been screened extensively for phenotypic traits as well, with the strongest focus on resistance to pathogens and pests. Variation was found for many such traits, and much work has been done to transfer some of the genes responsible into wheat. Ogbonnaya et al. [10] and Gill et al. [11] provided lists of the numerous genetic transfers that have been accomplished, with gene designations, names of germplasm releases, pedigrees, and other details. Nevertheless, the bulk of the genetic variation within the species *Ae. tauschii* has remained isolated from wheat and therefore has not been evaluated in the hexaploid background.

2. Approaches to introducing *Ae. tauschii* germplasm into the hexaploid gene pool

Two approaches to use *Ae. tauschii* for improvement of common wheat have been used. In the "synthetic hexaploid" approach, a triploid ABD hybrid between *T. turgidum* and *Ae. tauschii* is formed, and either its chromosome number is doubled with a mitotic agent, or it spontaneously produces hexaploid seeds through the formation and union of unreduced gametes to produce an AABBDD hexaploid (Fig. 1) [12,13]. This was the procedure followed by McFadden and Sears [2] when they demonstrated the process by which *T. aestivum* originated. In the second approach, usually termed "direct hybridization," *T. aestivum* plants are pollinated by *Ae. tauschii*, the resulting embryos are rescued, and those ABDD F_1 plants are backcrossed to *T. aestivum* (Fig. 2). The resulting BC_1 population segregates for chromosome number. Stable 42-chromosome AABBDD progeny are obtained through selfing or a second backcross [14].

These initial steps in the two approaches produce plant populations that can be screened for traits or markers of interest, and selected plants can be used as parental germplasm in further crosses with common wheat. Typically, synthetic hexaploids have been crossed and then backcrossed

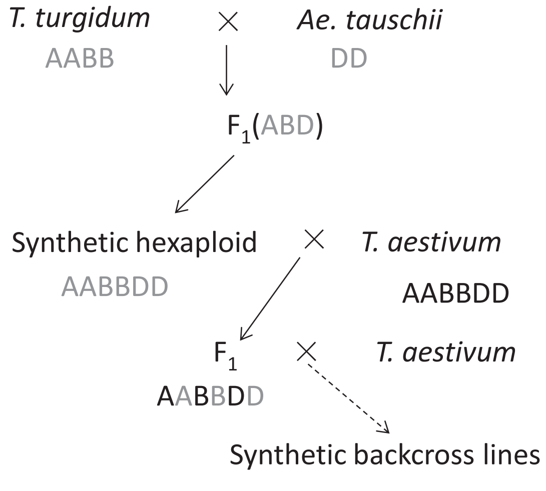


Fig. 1 – Procedure for producing synthetic hexaploids and synthetic backcross lines. The synthetic/*Triticum aestivum* F_1 plant carries one set each of A- and B-genome chromosomes from *T. turgidum* (gray), one set of D-genome chromosomes from *Aegilops tauschii* (gray), and one set each of A-, B-, and D-genome chromosomes from *T. aestivum* (black). Therefore, alleles from both donor species, whether they are advantageous or deleterious, will be segregating throughout all three genomes in selfed progeny of the F_1 plant.

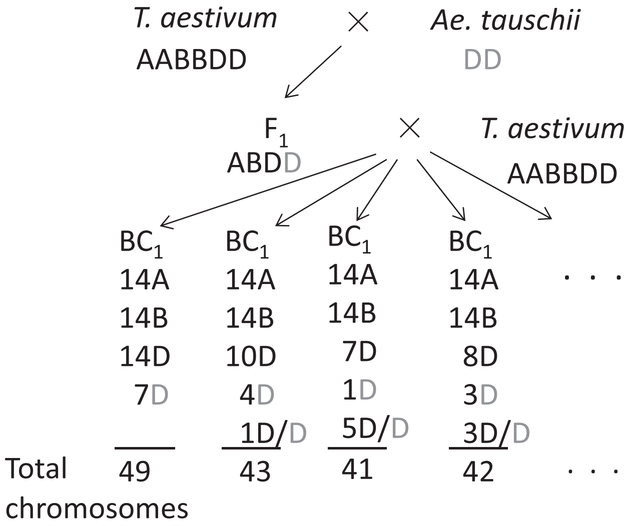


Fig. 2 – Procedure for direct hybridization and introgression between *Triticum aestivum* and *Aegilops tauschii*. All BC_1 plants carry the full complement of the *T. aestivum* parent's A- and B-genome chromosomes (black), and they carry varying numbers of D-genome chromsomes from *Ae. tauschii* (gray). BC_1 plants with 49 chromosomes result from formation of unreduced female gametes in the F_1 plant; they carry three sets of D-genome chromosomes, two of them from *T. aestivum*. Numbers and origins of D-genome chromosomes carried by other BC_1 plants depend upon recombination and transmission during gamete formation in the F_1 plant. An expected 70%–75% of the D genome and 100% of the A and B genomes in BC_1 plants are derived from *T. aestivum*. When BC_1 plants are crossed to *T. aestivum*, the bulk of the progeny have 42 chromosomes. Therefore, direct hybridization allows introgression of target genes into only one of *T. aestivum*'s three genomes with only two backcrosses.

or topcrossed to elite wheat cultivars or breeding lines to produce populations of "synthetic backcross lines" (SBLs) from which breeding lines, or in some cases cultivars, have been selected [10,13,15]. With direct hybridization, BC_1F_x, or more often BC_2F_x, selections are generally moved directly into breeding programs for use as parents [11].

Although both approaches have been used by many wheat research groups over the decades, different programs have employed them to different extents. Of the more than 1500 synthetic hexaploids that have been produced, more than 1300 were developed and released by the International Maize and Wheat Improvement Center (CIMMYT), which has also produced numerous large populations of SBLs [10,12]. Most synthetic hexaploids have had spring-type growth habit, thanks to genes contributed by their *T. turgidum* parents. The largest output of germplasm from direct hybridization has come from Kansas State University's Wheat Genetics Resource Center [11], and it has been of winter-type growth habit. Synthetic hexaploids, and to a lesser extent direct hybridization, have been used by breeding programs in China for more than 25 years [15,16].

Thanks to CIMMYT's energetic efforts, dating back to the 1980s, to produce and utilize synthetic hexaploids, along with its strong international wheat-breeding networks, synthetic hexaploids have been more widely studied by wheat scientists and used as parents by wheat breeders than have the products of direct hybridization. But even after extensive germplasm development using both approaches, much more D-genome diversity remains to be exploited. Cluster analysis based on microsatellite data has shown that the development of the synthetic hexaploid collection left large portions of the *Ae. tauschii* gene pool, including a large lineage that was not involved in the origin of hexaploid wheat, unsampled [17].

We will now examine the contributions that synthetic hexaploid generation and direct hybridization have so far made to wheat improvement, the advantages and disadvantages of using each method in future wheat improvement, and prospects for renewed utilization of both approaches in the future to further improve the D genome of wheat.

3. Useful genes transferred from *Ae. tauschii* via synthetic hexaploids and direct hybridization

Many genes conferring resistance to pathogens and pests have been transferred into hexaploid wheat from *Ae. tauschii*. Both production of synthetic hexaploids and direct hybridization brought wide arrays of resistances into wheat, with transfers of multiple distinct genes in the case of several traits. Table 1 lists resistances to three insect species, two nematodes, and five fungal pathogens that were transferred from *Ae. tauschii* through synthetic hexaploids. Resistances to two insect species, two viruses, and five fungal pathogens have been transferred through direct hybridization. The resistances listed in Table 1 for synthetic hexaploids do not include those conferred by genes derived from *T. turgidum* or those for which the chromosomal locations of genes responsible are not yet known and that therefore may have come from *T. turgidum*. The number of items in this list is therefore conservative.

Introgression into the D genome has also had positive effects on traits related to grain productivity and end-use quality (Table 2). Here, improvements have been accomplished more often through the synthetic hexaploid route than through direct hybridization, in part reflecting the much larger global efforts in germplasm development, evaluation, and research that have gone into the former approach.

Both approaches have had significant impact on wheat genetics and breeding. Synthetic hexaploids have been used in evaluation, genetic studies, and/or as breeding parents at CIMMYT and the International Center for Agricultural Research in the Dry Areas (ICARDA) stations around the world and by other organizations in Australia, France, Japan, Mexico, Netherlands, the United Kingdom, the United States, and other countries [7,29,46,47,57,62,63], but perhaps the strongest impact on cultivar development has been in China, especially in Sichuan Province, where a series of high-yielding cultivars derived from synthetic backcross lines have been released, starting with the highly successful Chuanmai 42 in 2003 [13,15].

Meanwhile, Wheat Genetics Resource Center germplasm lines derived from direct *T. aestivum*/*Ae. tauschii* crosses have been widely used as parents by winter wheat breeders in the

Table 1 – Pathogen- and pest-resistance traits that have been improved in hexaploid wheat through introduction of genes from *Aegilops tauschii*, through either *Triticum turgidum/Ae. tauschii* synthetic hexaploid production or through direct hybridization between *T. aestivum* and *Ae. tauschii*.

Approach	Trait	Selected reference
Synthetic hexaploid	Hessian fly	Xu et al. [18]
	Greenbug	Joppa and Williams [19], Porter et al. [20], Lazar et al. [21]
	Cereal leaf beetle	Joukhadar et al. [22]
	Cereal cyst nematode	Eastwood et al. [23], Mulki et al. [24]
	Root lesion nematode	Mulki et al. [24]
	Stem rust	Kerber and Dyck [25], Marais et al. [26]
	Yellow rust	Singh et al. [27]
	Septoria leaf blotch	Mujeeb-Kazi et al. [28], Ghaffary et al. [29]
	Powdery mildew	Lutz et al. [30]
	Karnal bunt	Mujeeb-Kazi et al. [31]
Direct hybridization	Hessian fly	Raupp et al. [32], Cox and Hatchett [33]
	Greenbug	Gill et al. [34]
	Soilborne mosaic virus	Gill et al. [35], Cox et al. [36]
	Wheat spindle streak mosaic virus	Cox et al. [36]
	Leaf rust, seedling and adult	Dyck et al. [37], Kerber [38], Raupp et al. [39], Cox et al. [40,41]
	Powdery mildew	Cox et al. [36], Murphy et al. [42,43]; Yang et al. [16]
	Septoria leaf blotch	Gill et al. [11]
	Stagonospora blotch	Cox et al. [44]
	Tan spot	Brown-Guedira et al. [45]

United States and elsewhere. By 2005, such parents were in the pedigrees of 25% of elite hard winter wheat lines being tested in the U.S. Department of Agriculture-coordinated Southern Regional Performance Nursery [11]. From 1999 to 2011, 19 cultivars released by 11 breeding programs in the region had *Ae. tauschii*-derived germplasm in their pedigrees.

4. Advantages and disadvantages of the synthetic-hexaploid approach

T. turgidum and other tetraploid wheats can easily be hybridized with *Ae. tauschii*, and chromosome doubling either happens spontaneously or can be reliably induced [12,13]. The resulting synthetic hexaploid carries the entire genome of the *Ae. tauschii* parent, is meiotically stable, and can be crossed and backcrossed with any hexaploid wheat to produce large, fully fertile recombinant populations [12,15]. Originally, the primary goal was to introduce diversity into and improve the D genome; however, research on SBLs has shown that improvement in phenotype can often be attributed to genes coming from both the *Ae. tauschii* and *T. turgidum* parents, as well as interaction between genomes derived from the two parental species [10].

Evaluation of *Ae. tauschii* accessions themselves for traits such as seed yield and size, stress tolerances, and in some cases even resistances to pests and pathogens, is often not predictive of their usefulness as parents in breeding common wheat [10]. The rapid incorporation of an accession's entire genome into a synthetic hexaploid background for trait evaluation is therefore very useful. In this and other ways, synthetic hexaploids have been used to greatly expand our knowledge of *Ae. tauschii* and wheat. The large collection produced by CIMMYT and others is a stable, reproducible genetic resource that permits trait evaluation and genetic studies to be done by researchers around the world using

Table 2 – Traits other than pathogen and pest resistance for which hexaploid wheat has been improved through introduction of genes from *Aegilops tauschii*, through either *Triticum turgidum/Ae. tauschii* synthetic hexaploid production or through direct hybridization between *T. aestivum* and *Ae. tauschii*.

Approach	Trait	Selected reference
Synthetic hexaploid	Grain yield	Mujeeb-Kazi et al. [12], Yang et al. [13], Li et al. [15]
	Kernel size	Okamoto et al. [46], Williams and Sorrells [47], Rasheed et al. [48]
	Preharvest sprouting	Lan and Yen [49], Imtiaz et al. [50]
	New HMW glutenin subunits	Lagudah et al. [51], Mackie et al. [52], Hsam et al. [53], Tang et al. [54]
	Low polyphenol oxidase and lipoxygenase	Yang et al. [55], Mares and Mrva [56]
	Milling and baking quality	Kunert et al. [57], Tang et al. [54]
	Salinity tolerance	Ogbonnaya et al. [10]
	Aluminum tolerance	Ryan et al. [58]
Direct hybridization	Grain yield	Cox et al. [41,59,60]
	Kernel weight and weight per volume	Cox et al. [41,59,60]
	Dough mixing properties	Cox et al. [41,59,60]
	New gliadin and HMW glutenin subunits; improved loaf volume	Brown-Guedira et al. [61]

common sets of genotypes. As a result, much has been learned about useful variation in all three genomes. Identification and utilization of molecular markers in particular has received a boost, thanks to the much greater allelic and phenotypic variation in synthetic hexaploid/*T. aestivum* crosses, compared with standard *T. aestivum*/*T. aestivum* crosses [64].

Nevertheless, when useful genes are introgressed from *T. turgidum* through use of synthetic hexaploids, deleterious genes can also be introduced. Genes in many *T. turgidum* lines interact with *T. aestivum* genes to induce lethal hybrid necrosis; those lines therefore cannot be used as parents of synthetic hexaploids [65]. The *vrn* alleles for winter-type vernalization response in *Ae. tauschii* are not expressed in synthetic hexaploids because epistatic *Vrn* alleles derived from *T. turgidum*. Therefore, winter wheat breeding programs have made more limited use of synthetic hexaploids than have programs developing spring or intermediate wheat cultivars. Synthetic hexaploids are homozygous for both the *q* allele from their *T. turgidum* parent and the *Tg* allele from *Ae. tauschii*; therefore, they are not free threshing. Furthermore, for 8000 years, the A and B genomes of common wheat have undergone divergent natural and artificial selection for adaptation to stresses in different ranges of environments, and the two species have also been selected in very different directions for grain-quality traits. When common wheat is crossed with a synthetic hexaploid, these gene complexes and more are disrupted across all three genomes (Fig. 1). They must be reassembled at the same time that desired segments of the *T. turgidum* and *Ae. tauschii* genomes are being transferred.

Genes in *T. turgidum* may also mask or suppress desired genes in *Ae. tauschii*, or vice versa. In discussing this, Ogbonnaya et al. [10] pointed out that amphiploidization delivers the twin genomic “shocks” of hybridity and polyploidy. Gene expression may be altered by DNA sequence elimination, methylation, transposon movement, altered regulation, or other mechanisms. In synthetic hexaploids, it is more often *Ae. tauschii*-derived genes whose expression is reduced [66].

5. Advantages and disadvantages of the direct-hybridization approach

Most common wheat cultivars or breeding lines when pollinated by *Ae. tauschii* will produce a small number of viable F_1 embryos. And almost all F_1 plants, when pollinated by common wheat, can produce at least a few backcross seed. The experience of the Wheat Genetics Resource Center is that F_1 plants produce approximately one seed per spike pollinated. Kong et al. [67] obtained similar results with Chinese wheat parents. Between 25 and 50% of the backcross plants thus produced come from fertilization of a 28-chromosome ABDD egg formed by restitution; those 49-chromosome plants carry a complete copy of the *Ae. tauschii* parent’s genome. Chromosome numbers of backcross plants formed without restitution vary but tend to cluster near 42 [68].

Low seed set on F_1 plants is a potential bottleneck for retention of a desired gene; however, given a 25% chance of a restitution gamete, recovery of as few as four viable BC_1 seed confers a 98% chance of at least one BC_1 plant carrying the gene. Transmission rates of *Ae. tauschii* alleles during backcrossing, and recombination between loci occur as predicted under the assumptions of non-preferential segregation and complete homology between D-genome chromosomes from the two parents [69]. Therefore, the mean proportion of the D genome derived from *Ae. tauschii* in the BC_1 generation is expected to be between 0.25 and 0.30. The BC_2 plants are mostly euploid, with an expected *Ae. tauschii* contribution of 0.125 to 0.150. However, because the A and B genomes are entirely derived from the wheat parent, 94%–96% of the wheat parent’s full hexaploid genome is expected to be restored by the BC_2 generation.

The above events lead to the chief advantage conferred by the direct hybridization approach: rapid introduction of new germplasm into the D genome accompanied by complete conservation of the *T. aestivum* parent’s A and B genomes (Fig. 2). Loci in the A and B genomes governing important characteristics such as vernalization and photoperiod response, threshability, and plant height, along with quantitative traits related to productivity, stress tolerance, and grain quality, remain fixed while the D genome undergoes segregation. BC_2 and most BC_1 plants are free- threshing, but plants that still carry the *Tg* allele from *Ae. tauschii* may be somewhat more difficult to thresh. Resistance genes expressed in the *Ae. tauschii* parent are usually expressed similarly in the hexaploid background. In studies at Kansas State University, grain yields, kernel weights, and milling and baking quality traits of BC_2-derived lines derived from direct hybridization were found similar to those of the recurrent parent under disease-free conditions, but significantly better (as much as 50% higher in the case of grain yield) under infection by a pathogen to which they were resistant [41,59,60].

Direct hybridization also has some disadvantages. Unlike synthetic hexaploids, direct backcross derivatives do not immediately form a fixed, reproducible collection of germplasm lines carrying intact introduced D genomes; however, their progeny, after a few generations of self-pollination and selection, can be maintained, shared, studied, and utilized more widely [59]. With about 95% of the wheat genome being restored, direct crossing is a somewhat conservative approach, and the ability to keep the *T. aestivum* A and B genomes intact comes with a potential cost: the missed opportunity to introduce useful variation from both *Ae. tauschii* and tetraploid wheat species in a single hybridization.

6. Future incorporation of *Ae. tauschii* germplasm

Genomic diversity studies have shown that *Ae. tauschii* is a vast storehouse of potentially useful genetic diversity far larger than the gene pool that has been exploited to date. Furthermore, in examining *Ae. tauschii*’s genome sequence, Jia et al. [70] found 1219 protein-coding genes potentially involved in disease resistance, 485 potentially involved in abiotic stress tolerance, 216 potentially involved in cold tolerance, and 14 transcription factors associated with drought-tolerance genes. All of these totals were greater than for the same gene categories in the diploid species rice, sorghum, or maize.

Despite this potential, new hybridization for both synthetic hexaploid formation and production of hexaploid/diploid hybrids has tapered off in recent years. In a recent review of the utilization of synthetic hexaploids, Ogboyanna et al. [10] argued that “there is a need to widen the *Ae. tauschii* accessions sources by including collections from areas of particular stresses, for example, drought and thus broaden the variability for such stress tolerance in addition to choice of *Ae. tauschii* on the basis of genotype diversity. These accessions could enter future direct crossing programs that could target breeding goals more effectively” [10]. In doing this, they suggested that increased effort be directed toward direct *T. aestivum*/*Ae. tauschii* hybridization, writing, “We opine that direct crossing for wheat improvement has not received the attention it deserves. Apart from precision transfers into the D genome only, the speed with which advanced lines of good agronomic phenotype are recovered warrants more attention to this strategy.” Ogbonnaya et al. [10] further suggested that under-represented *Ae. tauschii* parents should be hybridized more widely not only with common wheat and durum but also with other tetraploids, including the *T. turgidum* subspecies *carthlicum*, *dicoccum*, and *dicoccoides*.

Use of *Ae. tauschii* parents collected from the fringes of the species’ geographical distribution can introduce DNA sequences highly diverged from those introduced during common wheat’s origin. The novel variation could be especially useful in wheat improvement if the *Ae. tauschii* parent is locally adapted. Chinese researchers, for example, have long made use of *Ae. tauschii* accessions collected within China. Lan and Yen [49], for example, produced a synthetic hexaploid by crossing a Chinese durum cultivar with a Chinese *Ae. tauschii* accession. In reviewing the use of *Ae. tauschii* for wheat improvement in China over the years, Lang et al. [71] recommended that the species be exploited even more fully in the future. *Ae. tauschii* accessions and synthetic hexaploids resistant to *Fusarium* head blight [72] would be of special interest in China.

A renewal of efforts to capture useful diversity from *Ae. tauschii* in hexaploid germplasm can take full advantage of recent advances in sequencing the D genome [4,70]. Commenting on the results of Wang et al. [4], Gill [73] wrote, “This paper by Wang et al. will be of great interest to plant breeders who need to choose the most diverse *Ae. tauschii* accessions for genetic introgression into wheat. Is this the last word on the origin of the wheat D genome? Probably not, but now that we are homing in to the place where bread wheat originated, perhaps a more thorough sampling of these regions or previously collected samples from these areas may provide additional data.”

The numerous documented transfers from *Ae. tauschii* into the D genome of wheat in past decades (Tables 1 and 2), the vast portions of this species’ gene pool that have not yet been utilized, and the increasingly powerful genomic tools available for improvement of wheat all point toward a renewed effort to introgress diverse *Ae. tauschii* germplasm into common wheat. Choice of introgression method, synthetic hexaploid or direct hybridization, will depend in part on the type of wheat being developed and the target environment. But more extensive use of direct hybridization is especially warranted, considering that the approach has so far been underexploited and that it rapidly recaptures the bulk of the *T. aestivum* parent’s genome in early backcross progeny.

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2016YFD0100102-3), the Recruitment Program of High-end Foreign Experts of State Administration of Foreign Experts Affairs (GDT20163200028), and the Independent Innovation of Agricultural Science and Technology of Jiangsu Province [CX(15)1001].

REFERENCES

[1] H. Kihara, Discovery of the DD analyser, one of the ancestors of *Triticum vulgare*, Agric. Hortic. 19 (1944) 889–890.

[2] E.S. McFadden, E.R. Sears, The origin of *Triticum spelta* and its free-threshing hexaploid relatives, J. Hered. 37 (1946) 81–89 (107–116).

[3] K.S. Caldwell, J. Dvorak, E.S. Lagudah, E. Akhunov, M.C. Luo, P. Wolters, W. Powell, Sequence polymorphism in polyploid wheat and their D-genome diploid ancestor, Genetics 167 (2004) 941–947.

[4] J.R. Wang, M.C. Luo, Z.X. Chen, F.M. You, Y.M. Wei, Y.L. Zheng, J. Dvorak, *Aegilops tauschii* single nucleotide polymorphisms shed light on the origins of wheat D-genome genetic diversity and pinpoint the geographic origin of hexaploid wheat, New Phytol. 198 (2013) 925–937.

[5] J. Dvorak, M.C. Luo, Z.L. Yang, H.B. Zhang, The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat, Theor. Appl. Genet. 97 (1998) 657–670.

[6] T.S. Cox, Deepening the wheat gene pool, J. Crop. Prod. 1 (1997) 1–25.

[7] J.C. Reif, P. Zhang, S. Dreisigacker, M.L. Warburton, M. van Ginkel, D. Hoisington, M. Bohn, A.E. Melchinger, Wheat genetic diversity trends during domestication and breeding, Theor. Appl. Genet. 110 (2005) 859–864.

[8] E.L. Lubbers, K.S. Gill, T.S. Cox, B.S. Gill, Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*, Genome 34 (1991) 354–361.

[9] J.A. Chapman, M. Mascher, A. Buluç, K. Barry, E. Georganas, A. Session, V. Strnadova, J. Jenkins, S. Sehgal, L. Oliker, J. Schmutz, K.A. Yelick, U. Scholz, R. Waugh, J.A. Poland, G.J. Muehlbauer, N. Stein, D.S. Rokhsar, A whole-genome shotgun approach for assembling and anchoring the hexaploid bread wheat genome, Genome Biol. 16 (2015) 26.

[10] Synthetic hexaploids: harnessing species of the primary gene pool for wheat improvement, in: F.C. Ogbonnaya, O.M. Abdalla, A. Mujeeb-Kazi, A.G. Kazi, S.S. Xu, N. Gosman, E.S. Lagudah, D. Bonnett, M.E. Sorrells, H. Tsujimoto, J. Janick (Eds.), Plant Breeding Reviews, Vol. 37, John Wiley & Sons, Inc., Hoboken, New Jersey, USA 2013, pp. 35–122.

[11] B.S. Gill, B. Friebe, W.J. Raupp, D.L. Wilson, T.S. Cox, R.G. Sears, G.L. Brown-Guedira, A.K. Fritz, Wheat Genetics Resource Center: the first 25 years, Adv. Agron. 89 (2006) 73–136.

[12] A. Mujeeb-Kazi, A. Gul, M. Farooq, S. Rizwan, I. Ahmad, Rebirth of synthetic hexaploids with global implications for wheat improvement, Crop Pasture Sci. 59 (2008) 391–398.

[13] W.Y. Yang, D.C. Liu, J. Li, L.Q. Zhang, H.T. Wei, X.R. Hu, Y.L. Zheng, Z.H. He, Y.C. Zou, Synthetic hexaploid wheat and its utilization for wheat genetic improvement in China, J. Genet. Genomics 36 (2009) 539–546.

[14] B.S. Gill, W.J. Raupp, Direct genetic transfers from *Aegilops squarrosa* L. to hexaploid wheat, Crop Sci. 27 (1987) 445–450.

[15] J. Li, H.S. Wan, W.Y. Yang, Synthetic hexaploid wheat enhances variation and adaptive evolution of bread wheat in breeding processes, J. Syst. Evol. 52 (2014) 735–742.

[16] W.Y. Yang, Y. Yu, X.R. Hu, B.H. Wu, C. Yen, J.L. Yang, Y.L. Zheng, Direct transfer of powdery mildew resistant gene from *Aegilops tauschii* into bread wheat and its genetic expression, Southwest China, J. Agric. Sci. 13 (2000) 15–20 (in Chinese with English abstract).

[17] N. Gosman, H. Jones, R. Horsnell, A. Kowalski, G. Rose, L. Everest, A. Bentley, S. Tha, C. Uauy, D. Novoselovic, R. Simek, B. Kobiljski, A. Kondic-Spika, O. Mitrofanova, Y. Chesnokov, A. Greenland, Comparative analysis of D-genome diversity in *Aegilops tauschii*, common bread wheat (*Triticum aestivum*) & synthetic hexaploid wheat, in: S. Dreisigacker, S. Singh (Eds.),Proceedings of the 21st International Triticeae Mapping Initiative Workshop, Mexico City, Mexico 2011, pp. 31–32.

[18] S.S. Xu, X. Cai, T. Wang, M.O. Harris, T.L. Friesen, Registration of two synthetic hexaploid wheat germplasms resistant to Hessian fly, Crop Sci. 46 (2006) 1401–1402.

[19] L.R. Joppa, N.D. Williams, Registration of Largo, a greenbug resistant hexaploid wheat, Crop Sci. 22 (1982) 901–902.

[20] K.B. Porter, G.L. Peterson, W.D. Worrall, M.E. McDaniel, N.A. Tuleen, D.S. Marshall, L.R. Nelson, Registration of TXGH10563B, TXGH10989 and TXGH13622 greenbug-resistant wheat germplasm lines, Crop Sci. 29 (1989) 1585–1586.

[21] M.D. Lazar, W.D. Worrall, K.B. Porter, N.A. Tuleen, Registration of eight closely related wheat germplasm lines differing in biotype E greenbug resistance, Crop Sci. 36 (1996) 1419.

[22] R. Joukhadar, M. El-Bouhssini, A. Jighly, F.C. Ogbonnaya, Genome-wide association mapping for five major pest resistances in wheat, Mol. Breed. 32 (2013) 943–960.

[23] R.F. Eastwood, E.S. Lagudah, R. Appels, M. Hannah, J.F. Kollmorgen, *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*), Aust. J. Agric. Res. 42 (1991) 69–77.

[24] M.A. Mulki, A. Jighly, G.Y. Ye, L.C. Emebiri, D. Moody, O. Ansari, F.C. Ogbonnaya, Association mapping for soilborne pathogen resistance in synthetic hexaploid wheat, Mol. Breed. 31 (2012) 299–311.

[25] E.R. Kerber, P.L. Dyck, Resistance to stem and leaf rust of wheat in *Aegilops squarrosa* and transfer of a gene for stem rust resistance to hexaploid wheat, in: S. Ramanujam (Ed.), Proceedings of the 5th International Wheat Genetics Symposium, February 23–28, Indian Society of Genetics & Plant Breeding, New Delhi, India 1978, pp. 358–364.

[26] G.F. Marais, G.F. Potgieter, H.S. Roux, An assessment of the variation for stem rust resistance in the progeny of a cross involving the *Triticum* species *aestivum*, *turgidum*, and *tauschii*, S. Afr. J. Plant Soil 11 (1994) 15–19.

[27] R.P. Singh, J.C. Nelson, M.E. Sorrells, Mapping *Yr*28 and other genes for resistance to stripe rust in wheat, Crop Sci. 40 (2000) 1148–1155.

[28] A. Mujeeb-Kazi, L.I. Gilchrist, R.L. Villareal, R. Delgado, Registration of 10 wheat germplasms resistant to *Septoria tritici* leaf blotch, Crop Sci. 40 (2000) 590–591.

[29] S.M.T. Ghaffary, J.D. Faris, T.L. Friesen, R.G.F. Visser, T.A.J. van der Lee, O. Robert, G.H.J. Kema, New broad-spectrum resistance to *Septoria tritici* blotch derived from synthetic hexaploid wheat, Theor. Appl. Genet. 124 (2012) 125–142.

[30] J. Lutz, S.L.K. Hsam, E. Limpert, F.J. Zeller, Chromosomal location of powdery mildew resistance genes in *Triticum aestivum* L. (common wheat). 2. Genes *Pm*2 and *Pm*19 from *Aegilops squarrosa* L, Heredity 74 (1995) 152–156.

[31] A. Mujeeb-Kazi, G. Fuentes-Davila, R.L. Villareal, A. Cortes, V. Rosas, R. Delgado, Registration of 10 synthetic hexaploid wheat and six bread wheat germplasms resistant to karnal bunt, Crop Sci. 41 (2001) 1652–1653.

[32] W.J. Raupp, A. Amri, J.H. Hatchett, B.S. Gill, D.L. Wilson, T.S. Cox, Chromosomal location of Hessian fly-resistance genes *H22*, *H23*, and *H24* derived from *Triticum tauschii* in the D genome of wheat, J. Hered. 84 (1993) 142–145.

[33] T.S. Cox, J.H. Hatchett, Hessian fly-resistance gene *H26* transferred from *Triticum tauschii* to common wheat, Crop Sci. 34 (1994) 958–960.

[34] B.S. Gill, D.L. Wilson, W.J. Raupp, J.H. Hatchett, T.L. Harvey, T.S. Cox, R.G. Sears, Registration of KS89WGRC4 hard red winter wheat germplasm lines with resistance to Hessian fly, greenbug, and soilborne mosaic virus, Crop Sci. 31 (1991) 246.

[35] B.S. Gill, J.H. Hatchett, T.S. Cox, W.J. Raupp, R.G. Sears, T.J. Martin, Registration of KS85WGRC01 Hessian fly-resistant hard red winter wheat germplasm, Crop Sci. 26 (1986) 1266–1267.

[36] T.S. Cox, M.E. Sorrells, G.C. Bergstrom, R.G. Sears, B.S. Gill, E.J. Walsh, S. Leath, J.P. Murphy, Registration of KS92WGRC21 and KS92WGRC22 hard red winter wheat germplasms resistant to wheat spindle-streak mosaic virus, wheat soilborne mosaic virus, and powdery mildew, Crop Sci. 34 (1994) 546.

[37] P.L. Dyck, E.R. Kerber, Inheritance in hexaploid wheat of adult-plant leaf rust resistance derived from *Aegilops squarrosa*, Can. J. Genet. Cytol. 12 (1970) 175–180.

[38] E.R. Kerber, Resistance to leaf rust in hexaploid wheat: *Lr32*, a third gene derived from *Triticum tauschii*, Crop Sci. 27 (1987) 204–206.

[39] W.J. Raupp, S. Singh, G.L. Brown-Guedira, B.S. Gill, Cytogenetic and molecular mapping of the leaf rust resistance gene *Lr39* in wheat, Theor. Appl. Genet. 102 (2001) 347–352.

[40] T.S. Cox, W.J. Raupp, B.S. Gill, Leaf rust-resistance genes *Lr41*, *Lr42*, and *Lr43* transferred from *Triticum tauschii* to common wheat, Crop Sci. 34 (1994) 339–343.

[41] T.S. Cox, R.K. Bequette, R.L. Bowden, R.G. Sears, Grain yield and breadmaking quality of wheat lines with the leaf rust resistance gene *Lr41*, Crop Sci. 37 (1997) 154–161.

[42] J.P. Murphy, S. Leath, D. Huynh, R.A. Navarro, A.N. Shi, Registration of NC96BGTD1, NC96BGTD2 and NC96BGTD3 wheat germplasm resistant to powdery mildew, Crop Sci. 38 (1998) 570–571.

[43] J.P. Murphy, S. Leath, D. Huynh, R.A. Navarro, A.N. Shi, Registration of NC97BGTD7 and NC97BGTD8 wheat germplasms resistant to powdery mildew, Crop Sci. 39 (1999) 884–885.

[44] T.S. Cox, W.W. Bockus, B.S. Gill, R.G. Sears, T.L. Harvey, S. Leath, G.L. Brown-Guedira, Registration of KS96WGRC40 hard red winter wheat germplasm resistant to wheat curl mite, stagnospora leaf blotch, and *Septoria* leaf blotch, Crop Sci. 39 (1999) 597.

[45] G.L. Brown-Guedira, T.S. Cox, W.W. Bockus, B.S. Gill, R.G. Sears, Registration of KS96WGRC38 and KS96WGRC39 tan spot-resistant hard red winter wheat germplasms, Crop Sci. 39 (1999) 596.

[46] Y. Okamoto, A.T. Nguyen, M. Yoshioka, J.C.M. Iehisa, S. Takumi, Identification of quantitative trait loci controlling grain size and shape in the D genome of synthetic hexaploid wheat lines, Breed. Sci. 63 (2013) 423–429.

[47] K. Williams, M.E. Sorrells, Three-dimensional seed size and shape QTL in hexaploid wheat (*Triticum aestivum* L.) populations, Crop Sci. 54 (2014) 98–110.

[48] A. Rasheed, X.C. Xia, F.C. Ogbonnaya, T. Mahmood, Z. Zhang, A. Mujeeb-Kazi, Z.H. He, Genome-wide association for grain morphology in synthetic hexaploid wheats using digital imaging analysis, BMC Plant Biol. 14 (2014) 128.

[49] X.J. Lan, C. Yen, An amphidiploid derived from a Chinese landrace of tetraploid wheat, Ailanmai, crossed with *Aegilops tauschii* native to China with reference to its utilization in wheat breeding, J. Sichuan Agric. Univ. 10 (1992) 581–585 (in Chinese with English abstract).

[50] M. Imtiaz, F.C. Ogbonnaya, J. Oman, M. van Ginkel, Characterization of QTLs controlling genetic variation for pre-harvest sprouting in synthetic backcross derived wheat lines, Genetics 178 (2008) 1725–1736.

[51] E.S. Lagudah, F. Macritchie, G.M. Halloran, The influence of high-molecular-weight subunits of glutenin from *Triticum tauschii* on flour quality of synthetic hexaploid wheat, J. Cereal Sci. 5 (1987) 129–138.
[52] A.M. Mackie, P.J. Sharp, E.S. Lagudah, The nucleotide and derived amino acid sequence of a HMW-glutenin gene from *Triticum tauschii* and comparison with those from the D-genome of bread wheat, J. Cereal Sci. 24 (1996) 73–78.
[53] S.L.K. Hsam, R. Kieffer, F.J. Zeller, Significance of *Aegilops tauschii* glutenin genes on breadmaking properties of wheat, Cereal Chem. 78 (2001) 521–525.
[54] Y.I. Tang, W.Y. Yang, J.C. Tian, J. Li, F. Chen, Effect of HMW-GS 6 + 8 and 1.5 + 10 from synthetic hexaploid wheat on wheat quality traits, Agric. Sci. China 7 (2008) 1161–1171.
[55] S.J. Yang, S.G. Liu, J. Li, W.Y. Yang, Genetic detection of *PPO* gene in synthetic hexaploid wheat-derived populations, Mol. Plant Breed. 4 (2006) 772–778.
[56] D. Mares, K. Mrva, Genetic variation for quality traits in synthetic wheat germplasm, Aust. J. Agric. Res. 59 (2008) 406–412.
[57] A. Kunert, A.A. Naz, O. Dedeck, K. Pillen, J. León, AB-QTL analysis in winter wheat: I. Synthetic hexaploid wheat (*T. turgidum* ssp. *dicoccoides* × *T. tauschii*) as a source of favourable alleles for milling and baking quality traits, Theor. Appl. Genet. 115 (2007) 683–695.
[58] P.R. Ryan, H. Raman, S. Gupta, T. Sasaki, Y. Yamamoto, E. Delhaize, The multiple origins of aluminum resistance in hexaploid wheat include *Aegilops tauschii* and more recent *cis* mutations to *TaALMT1*, Plant J. 64 (2010) 446–455.
[59] T.S. Cox, R.G. Sears, R.K. Bequette, Use of winter wheat × *Triticum tauschii* backcross populations for germplasm evaluation, Theor. Appl. Genet. 90 (1995) 571–577.
[60] T.S. Cox, R.G. Sears, R.K. Bequette, T.J. Martin, Germplasm enhancement in winter wheat × *Triticum tauschii* backcross populations, Crop Sci. 35 (1995) 913–919.
[61] G.L. Brown-Guedira, M. Guedira, A.K. Fritz, T.J. Martin, O.K. Chung, G.L. Lockhart, B.W. Seabourn, B.S. Gill, T.S. Cox, Notice of release of KS04WGRC49 hard winter wheat germ plasm with unique glutenin and gliadin proteins, Annu. Wheat Newslett. 51 (2005) 190.
[62] E.G. Pestsova, A. Börner, M.S. Röder, Development and QTL assessment of *Triticum aestivum–Aegilops tauschii* introgression lines, Theor. Appl. Genet. 112 (2006) 634–647.
[63] G.P. Pradhan, P.V. Vara Prasad, A.K. Fritz, M.B. Kirkham, B.S. Gill, Effects of drought and high temperature stress on synthetic hexaploid wheat, Funct. Plant Biol. 39 (2012) 190–198.
[64] M.E. Sorrells, J.P. Gustafson, D. Somers, S. Chao, D. Benscher, G. Brown-Guedira, E. Huttner, A. Kilian, P.E. McGuire, K. Ross, J. Tanaka, P. Wenzl, K. Williams, C.O. Qualset, Reconstruction of the synthetic W7984 × Opata M85 wheat reference population, Genome 54 (2011) 875–882.
[65] M. Inagaki, B. Humeid, S. Tawkaz, A. Amri, Some constraints on interspecific crossing of durum wheat with *Aegilops tauschii* accessions screened under water-deficit stress, J. Plant Breed. Genet. 2 (2014) 7–14.
[66] P. He, B.R. Friebe, B.S. Gill, J.M. Zhou, Allopolyploidy alters gene expression in the highly stable hexaploid wheat, Plant Mol. Biol. 52 (2003) 401–414.
[67] L.R. Kong, Y.C. Dong, J.Z. Jia, Chromosome configurations and fertility characteristics of hybrids between *Triticum aestivum* L. and *Aegilops tauschii* (Coss.) Schmal, Acta Biol. Exp. Sin. 30 (1997) 35–43 (in Chinese with English abstract).
[68] T.S. Cox, L.G. Harrell, P. Chen, B.S. Gill, Reproductive behavior of hexaploid/diploid wheat hybrids, Plant Breed. 107 (1991) 105–118.
[69] A.K. Fritz, T.S. Cox, B.S. Gill, R.G. Sears, Molecular marker-facilitated analysis of introgression in winter wheat × *Triticum tauschii* populations, Crop Sci. 35 (1995) 1691–1695.
[70] J.Z. Jia, S.C. Zhao, X.Y. Kong, Y.R. Li, G.Y. Zhao, W.M. He, R. Appels, M. Pfeifer, Y. Tao, X.Y. Zhang, R.L. Jing, C. Zhang, Y.Z. Ma, L.F. Gao, C. Gao, M. Spannagl, K.F.X. Mayer, D. Li, S.K. Pan, F.Y. Zheng, Q. Hu, X.C. Xia, J.W. Li, Q.S. Liang, J. Chen, T. Wicker, C.Y. Gou, H.H. Kuang, G.Y. He, Y.D. Luo, B. Keller, Q.J. Xia, P. Lu, J.Y. Wang, H.F. Zou, R.Z. Zhang, J.Y. Xu, J.L. Gao, C. Middleton, Z.W. Quan, G.M. Liu, J. Wang, International Wheat Genome Sequencing Consortium, H.M. Yang, X. Liu, Z.H. He, L. Mao, J. Wang, *Aegilops tauschii* draft genome sequence reveals a gene repertoire for wheat adaptation, Nature 496 (2013) 91–95.
[71] J. Lang, H.Q. Zhang, H. Yang, Research advances in application of *Aegilops tauschii* on wheat genetics and breeding, J. China Agric. Univ. 19 (2014) 269–274 (in Chinese with English abstract).
[72] M.K. Das, G.H. Bai, A. Mujeeb-Kazi, S. Rajaram, Genetic diversity among synthetic hexaploid wheat accessions (*Triticum aestivum*) with resistance to several fungal diseases, Genet. Resour. Crop. Evol. 63 (2016) 1285–1296.
[73] B.S. Gill, SNPing *Aegilops tauschii* genetic diversity and the birthplace of bread wheat, New Phytol. 198 (2013) 641–642.