Synthetic hexaploid wheat enhances variation and adaptive evolution of bread wheat in breeding processes

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Abstract Synthetic hexaploid wheat (SHW) that combines novel and elite genes from the tetraploid wheat Triticum turgidum L. and wild ancestor Aegilops tauschii Coss., has been used to genetically improve hexaploid common wheat. The abundant genetic diversity in SHW can effectively make breakthroughs in wheat genetic improvement through the inclusion of increased variation. In this paper, we reviewed the current advances in research and utilization of the primary SHW lines and SHW-derived wheat varieties that have enhanced evolution of modern wheat under conditions of natural and artificial selection in southwestern China. Using primary SHW lines, four high-yielding wheat varieties have been developed. In addition, using the SHW-derived varieties as breeding parents, 12 new wheat varieties were also developed. Results of genotype–phenotype and fingerprint analysis showed that the introgressed alleles from SHW lines have contributed a great number of elite characters to the new wheat varieties, and these elite characters include disease resistance, more spikes per plant, more grains per spike, larger grains, and higher grain-yield potential. We found that the primary SHW lines and SHW-derived varieties have identifiable effects to enhance genetic variation and adaptive evolution of modern hexaploid wheat, which significantly increased the grain yields of hexaploid wheat in recent years. These findings have significant implications in the breeding of high-yielding wheat varieties resistant to biotic and abiotic stresses using SHW as genetic resources.

Key words adaptive evolution, Aegilops tauschii, introgression, synthetic hexaploid wheat, wheat breeding.

China is the world’s largest bread wheat producer and consumer. Among the annual cereal production in China, wheat ranks as the second in terms of cultivation areas and the third in its total grain production. Diverse wheat varieties are grown in different regions, which can be divided into 10 major agro-ecological zones based on variety types, growing habits, climate conditions, and other environmental factors such as temperature, photoperiod, and growth cycle (He et al., 2001). The Southwestern Autumn-Sown Spring Wheat Zone is the third largest wheat production area in China and covers 2.2 million hectares of wheat cultivation land with an annual production of 7.26 million tons.

In the southwestern growing region, wheat is usually grown in rain-fed agro-ecosystems and experiences low photosynthetic radiation, in addition to high humidity and temperatures at the terminal growth stages of wheat, which can lead to serious insect pest, disease, and weed problems. Efforts to significantly increase yield in such a disadvantageous environment remain a great challenge for breeders. Therefore, continued studies to enhance wheat productivity are important for wheat breeders. The problem is that the frequent use of a few parental genotypes as genetic resources in modern wheat breeding programs and the monotonous planting of a limited number of wheat varieties across wide agro-ecosystems have caused serious genetic erosion in cultivated wheat (Porceddu et al., 1988; Nevo, 1995), which has significantly reduced genetic diversity and limits further improvement of wheat varieties. Exploiting new genetic variation in elite wheat genetic resources to produce ideal genotypes for breaking yield ceilings is a widespread ambition in wheat breeding efforts (Rajaram, 2002).

Hexaploid, or common, wheat (Triticum aestivum L., 2n = 6x = 42, AABBDD) is a globally important food crop and will become even more significant as the world’s population increases. Common wheat has evolved from two spontaneous hybridization events (McFadden & Sears, 1944; Feldman, 2001). Tetraploid T. turgidum L. wheat (2n = 4x = 28, AABB) hybridized with diploid Aegilops tauschii Coss. (2n = 2x = 14, DD) followed by spontaneous chromosome doubling is
the widely accepted pathway for the origin of common wheat (Feldman, 2001). The evolution of the polyploid Triticum wheats is distinctive in that domestication, natural hybridization, and allopolyploid speciation have all had significant impacts on their diversification (Yoshihiro, 2011). Following polyploidization, both genetic and epigenetic mechanisms may play an important role in altering gene expression (Soltis et al., 2004). However, it is broadly recognized that only a limited number of individuals of the donor species were involved in the origin and evolution of common wheat. Consequently, genetic diversity of common wheat is relatively narrow compared with that of its two donor species. Because of this evolutionary bottleneck, most of the genetic variation observed in tetraploid wheat and Ae. tauschii is not represented in the commonly available hexaploid germplasm.

The genetic resources of T. turgidum and Ae. tauschii can be used as the sources of elite genes for the development of modern common wheat. To enhance the effectiveness of transferring elite genes from the two ancestral species to common wheat, scientists have replicated the pathway of natural origin of hexaploid wheat (SHW) from crosses between T. turgidum and Ae. tauschii with various sources (Xu & Dong, 1989; Lan & Yen, 1992; Mujeeb-Kazi et al., 1996). Over 1000 SHW lines have been produced from crosses of more than 600 Ae. tauschii accessions stored at the International Maize and Wheat Improvement Center (CIMMYT; Mexico City, Mexico) (Mujeeb-Kazi et al., 1996; van Ginkel & Ogbonnaya, 2007). Chinese scientists have shown intense interest in SHW lines produced by CIMMYT since the beginning of the 1990s, and consequently more than 200 such lines have been introduced into China (Yang et al., 2009). In recent years, about 300 new SHW lines involving T. turgidum landraces and wild Ae. tauschii accessions have been developed by Sichuan Agricultural University (Zhang et al., 2010; Luo et al., 2012; Hao et al., 2013) and Sichuan Academy of Agricultural Sciences, which has provided an important set of genetic resources for wheat breeding.

Synthetic hexaploid wheat lines have shown outstanding resistance to diseases and pests, tolerance to environmental stresses, in addition to their desirable quantitative traits. However, SHW lines also have a large number of unfavorable traits, such as late maturity, taller plants, and difficulty in threshing. Therefore, developing new commercial genotypes using SHW lines is difficult. To overcome the unfavorable traits of SHW lines, a breeding schedule was designed for our program to exploit the desired traits in SHW lines (Fig. 1) including five aspects. These are: (i) screening and identifying SHW lines for targeted traits, such as high resistance to stripe rust, good quality high molecular weight glutenin subunits (HMW-GS), and resistances to pre-harvest sprouting; (ii) selecting elite Sichuan varieties with early maturity and dwarf or semi-dwarf stature as agronomic parents; (iii) using SHW lines with targeted traits to cross and backcross with agronomic parents; (iv) planting BC1F2 populations in stripe rust nurseries to select

1 Breakthroughs in high-yielding wheat varieties by using primary SHW lines

1996.4-5 Syn-769 X SW-3243
1997.4-5 F1 X Chuan-6415
1997.8-9 BC1F1
1998.4-6 BC1F2 individual selection
1998.8-9 BC1F3 individual selection
1999.5-6 BC1F4
Advanced lines for yield trial (99-1572)
Chuanmai-42 tested in Provincial yield trial during 2001-2003 and National yield trial during 2002-2004
Released by Sichuan government in 2003 and by MOA in 2004
Note: 99-1572 was the entry No. of Chuanmai-42 before released

Fig. 1. Breeding procedure of wheat variety Chuanmai-42 released by the Sichuan Government and the Ministry of Agriculture, China (MOA) in 2004.
Table 1: Chinese wheat varieties derived from primary synthetic hexaploid wheat lines

| Variety | Pedigree | Year released |
|---------|----------|---------------|
| Chuanmai-38 | Syn-769/SW-3243//Chuan-6415 | 2003† |
| Chuanmai-42 | Syn-769/SW-3243//Chuan-6415 | 2003†, 2004† |
| Chuanmai-43 | Syn-769/SW-3243//Chuan-6415 | 2004†, 2006† |
| Chuanmai-47 | Syn-768/MY-26/SW-26/3/MY-26 | 2005† |

† Variety released by Sichuan Province; ‡ Variety released by Chinese Ministry of Agriculture.

Table 2: Yields of four synthetic derivatives in Sichuan Provincial and Chinese national yield trials

| Variety | Yield (t/ha) | % of check | Checks | Data source | Year |
|---------|-------------|------------|--------|-------------|------|
| Chuanmai-42 | 6.22 | 170† | Chuanmai-28 | SPYT | 2002 |
| | 6.05 | 123 | Chuanmai-107 | SPYT | 2003 |
| | 5.35 | 116 | Chuanmai-107 | NYT | 2003 |
| | 6.09 | 116 | Chuanmai-107 | NYT | 2004 |
| Chuanmai-43 | 5.63 | 116 | Chuanmai-107 | SPYT | 2003 |
| | 5.27 | 119 | Chuanmai-107 | SPYT | 2004 |
| | 5.75 | 111 | Chuanmai-107 | NYT | 2004 |
| | 6.07 | 115 | Chuanmai-107 | NYT | 2005 |
| Chuanmai-38 | 5.48 | 136† | Chuanmai-28 | SPYT | 2002 |
| | 5.15 | 105 | Chuanmai-107 | SPYT | 2003 |
| Chuanmai-47 | 5.15 | 112 | Chuanmai-107 | SPYT | 2004 |
| | 5.28 | 105 | Chuanmai-107 | SPYT | 2005 |

† The yield of the control variety (Chuanmai-28) was very low due to high susceptibility to stripe rust, and the yield increasing percentages of Chuanmai-42 and Chuanmai-38 over Chuanmai-28 were extremely high in 2002. NYT, National Yield Trial; SPYT, Sichuan Provincial Yield Trial.
Zhang et al., 2006a, 2006b). Further molecular mapping proved that all the four varieties had the same allele derived from the durum wheat donors (Li et al., 2006, 2007). The stripe rust resistance gene from Chuanmai-42, designated YrCH42, was located near the centromere of chromosome 1B and flanked by nine SSR markers (Xwmc626, Xgwm273, Xgwm11, Xgwm18, Xbarc137, Xbarc187, Xgwm498, Xbarc240, and Xwmc216). YrCH42 was most closely linked to Xgwm498 and Xbarc187, with genetic distances of 1.6 and 2.3 cM, respectively (Li et al., 2006). YrCH42 is therefore amenable to marker-assisted selection in wheat breeding programs. Seedling tests with 26 Puccinia striiformis f. sp. tritici isolates and allelic tests indicated that YrCH42, Yr24, and Yr26 are likely to be the same stripe rust resistance gene (Li et al., 2006).

2.3 Genetic effects of an introgressed high-yielding locus in Chuanmai-42 from the DD donor of SHW lines

A total of 1029 SSR markers were used to reveal the introgression loci in Chuanmai-42 by comparing this variety with its SHW and common wheat parents (Li et al., 2011). One hundred and twenty-seven recombinant inbred lines (RILs, F8) derived from Chuanmai-42 and Chuannong-16 were used to evaluate the genetic effects of the introgression loci from SHW on yield-related traits in wheat across six environments in Sichuan Province from 2006 to 2009. One locus tightly linked to SSR marker Xbarc1183 was significantly associated with grain yield and the Chuanmai-42-type allele increasing the yield potential was derived from the SHW according to pedigree analysis (Li et al., 2011). The average grain yield of RILs with this SHW allele was 8.92% higher than that of RILs with Chuannong-16 (non-SHW derivation) allele (Li et al., 2011). Moreover, the SHW allele also increased tiller numbers per plant, spike numbers, grain numbers, and harvest index (Li et al., 2011). Xbarc1183 was located on the long arm of chromosome 4D, according to a scan of two telosomic chromosomes of Chinese Spring and substitution lines of D genome in Longdon durum, indicating that the D genome of SHW from wild wheat grass Aegilops tauschii provided not only biotic/abiotic stress resistance genes but also elite yield-related genes. Most varieties derived from Chuanmai-42 inherited the SHW allele on Xbarc1183. This SHW allele would be quickly fixed on the future Chuanmai-42-derived variety population and lead to a rapid decreasing of genetic diversity on this genome when underlying the artificial selection on the wheat breeding. So, the genomic region linked to Xbarc1183 in Chuanmai-42 provided by SHW is a candidate locus that could play an extremely important role in the further development of high-yield wheat breeding.

2.4 Genetic effects of the 1BS chromosome arm from the AABB donors of SHW lines on main agronomic traits in Chuanmai-42

Chuanmai-42 is a non-1BL/1RS wheat variety with high-yield potential and good resistance to stripe rust. The 1BS chromosome arm of Chuanmai-42 was confirmed to be from its SHW parent Syn-769 and carried a stripe rust resistance gene, YrCH42 (Li et al., 2006, 2011). To understand the genetic effects of the 1BS and 1RS chromosome arms on yield-related traits in wheat, 127 RILs (F8) derived from Chuanmai-42 and Chuannong-16 (1BL/1RS translocation variety) were evaluated in Sichuan Province (Li et al., 2009). A total of 16 traits of Chuanmai-42 and Chuannong-16 and the RIL population, such as spike numbers, grain numbers per spike, thousand-grain weight, and grain yield, were investigated. The 1BS chromosome arm lines derived from Chuanmai-42 and the 1RS chromosome arm lines derived from Chuannong-16 were significantly different in six traits. The 1BS chromosome arm increased the ratio of spikes to summit population and harvest index, whereas the 1RS chromosome arm only positively affected tiller number per plant and spikelet number per spike. The average grain yield of RILs with the 1BS chromosome arm was 2.91% higher than that of RILs with the 1RS chromosome arm. Because the 1RS chromosome arm contains the Sec-1 gene, which significantly degrades the processing quality of wheat, and its rust resistance genes are ineffective against rust races in China, the 1RS chromosome arm should be replaced with the 1BS chromosome arm of Chuanmai-42.

3 Significant contribution of SHW-derived varieties Chuanmai-42 and -43 to the evolution of modern wheat

3.1 Contribution of quantitative trait loci to greater yield potential in Chuanmai-42

In our previous study, quantitative trait loci (QTLs) for greater yield potential in Chuanmai-42 were identified and found to be mainly located on Chromosomes 1B, 3A, 4A, 4D, and 5B. To validate the genetic contributions of the allelic variation, 18 derived lines from the RIL populations of Chuanmai-42/Chuannong-16, including Chuanmai-104, Chuanmai-64, eight high-yield derivatives, and eight low-yield derivatives, were used to dissect the genetic contributions of these QTLs to yield potential. The frequency of Chuanmai-42...
alleles was more than 55% in Chuanmai-104, Chuanmai-64, and eight high-yield derivatives, while it was less than 50% in most low-yield derivatives. In the yield-related genomic regions on chromosomes 1B and 4D, Chuanmai-104, Chuanmai-64, and the high-yield derivatives inherited the QTL alleles increasing grain number per spike and spike number per plant from Chuanmai-42 (Li et al., 2014). Moreover, the QTL alleles of Chuanmai-42 that were transferred to the two new varieties Chuanmai-104 and Chuanmai-64 on chromosomes 4A and 3A produced higher grain number per spike and thousand-grain weight, respectively. The transfer frequency of Chuanmai-42 alleles to the high-yield derivatives was higher than to the low-yield derivatives, and the desirable QTL alleles of Chuanmai-42 mainly contributed the higher grain number per spike in Chuanmai-104, Chuanmai-64, and the eight high-yield lines. The QTLs for large spikes and higher thousand-grain weight in Chuanmai-42 played an important role in the high-yielding potential of its derivatives.

3.2 Physiological characterization of high-yielding potential of SHW-derived varieties

The high-yielding potential of SHW or SHW-derived lines was positively correlated with improved physiological traits such as higher biomass yield, higher maximum photosynthetic rate (Rees et al., 1994; del Blanco et al., 2001), and lower canopy temperature (Rattey & Shorter, 2010). Comparison of canopy photosynthesis rate and the accumulation and portioning of dry matter between three SHW-derived varieties and five local elite non-SHW-derived varieties was carried out in rain-fed environments with high humidity and low photosynthetic active radiation in Sichuan (Tang et al., 2014). Their results showed that the SHW-derived varieties showed stronger vigor in early growth stages and accumulated more aboveground dry matter and higher spike dry weight at anthesis than the non-SHW-derived varieties. Furthermore, at maturity, less dry matter was partitioned to non-grain organs such as leaves and rachis in the SHW-derived varieties and resulted in higher harvest indexes.

Although no significant difference in canopy parameters were found among varieties at anthesis, SHW-derived varieties had lower extinction coefficients at 20 days after anthesis (around the middle of grain filling), and this was positively correlated with higher canopy photosynthesis at 0 and 20 days after anthesis. The SHW-derived varieties had higher chlorophyll contents in flag and penultimate leaves and better performance under adverse environments than non-synthetic-derived varieties at most times after anthesis (Tang et al., 2014). The SHW-derived lines also had characteristics of CIMMYT’s wheat for irrigated environment, as shown by the intermediate, dynamic canopy type, early maturity, good chlorophyll retention capacity, rust resistance, and high yield. Grain yield was positively correlated with most of the physiological traits, and synthetic-derived lines yielded significantly more (11.50% on average) than non-SHW-derived varieties. The greater early growth vigor, more accumulated dry matter, good chlorophyll retention capacity, and higher capacity of canopy photosynthesis were the physiological bases of high-yield potential in SHW-derived varieties.

3.3 Contribution of SHW-derived varieties to evolution of modern wheat for yield improvement

In the Sichuan Basin, climatic characteristics such as relatively high temperature in early spring, low photosynthetic irradiation, and high humidity, have hindered wheat production. The shorter tillering stage and longer spike differentiation period encourage more florets per spike but also cause fewer tillers and lower spike capacity, resulting in unstable grain yield. Increasing the grain number per spike and spike number per unit area, maintaining high biomass, improving the harvest index, shortening the growth period, and improving grain and biological productivity will be important ways to enhance wheat yield potential in the Sichuan Basin (Tang et al., 2014).

Synthetic hexaploid wheats and SHW-derived varieties have higher spike numbers, higher grain number per spike, larger grains, and resistance to stripe rust. By using primary SHWs, four wheat varieties with high-yielding potential have been developed (Table 2). Furthermore, 12 new wheat varieties were also developed using SHW-derived varieties (Chuanmai-42 and -43) as parents (Table 3). The grain yield of new variety Chuanmai-104 was higher than that of its parent Chuanmai-42 by 8.4% in the national yield trial (Table 3).

Table 4 shows the average grain yield of Sichuan released wheat varieties in provincial or national trials since the 1990s. The grain yield of Sichuan wheat varieties has crossed two thresholds. The first was that the average grain yield of varieties released from 2001 to 2005 was 0.53 t/ha higher than that of varieties released from 1991 to 2000. This improvement occurred because primary SHW-derived varieties such as Chuanmai-42 with high-yield potential and disease resistance began to be released in 2003. The second threshold was that the average grain yield of varieties released from 2011 to 2013 was 0.45 t/ha higher than that of varieties released from 2006 to 2010.
A group of varieties derived from Chuanmai-42 that had grain yields of 6 t/ha, such as Chuanmai-104, Chuanmai-64, and Shumai-969, began to be released in 2011. Among them, Shumai-969, developed by Sichuan Agricultural University, is twice derived from SHWs (Table 3). In the pedigree of Shumai-969, the newly SHW parent SHW-L1 came from Chinese *Triticum turgidum* Lanmai (AS2255) and Middle Eastern *Aegilops tauschii* (AS60) (Zhang et al., 2004). Dr. Liu Dengcai of Sichuan Agricultural University successfully developed Shumai-969 with a new HMW-GS composition (Dx3.1 and Dy1) for good quality and a new *Ppd-D1* allele from AS60 for early heading, using SHW-L1 crossed with a Sichuan variety SW-8188 (Chuanmai-36) and topcrossed with Chuanmai-42 (Xiang et al., 2009; Chen et al., 2012; Huang et al., 2012). Therefore, the primary SHWs and SHW-derived varieties have played critical roles in the improvement of yield potential in modern wheat in Sichuan.

### 4 Summary

Synthetic hexaploid wheat produced by crossing tetraploid wheat (AABB) with wild *Aegilops tauschii* (DD) possesses rich genetic diversity and elite genes for the improvement of common wheat. Wheat varieties developed involving CIMMYT-developed SHW lines have been commercially released in China in a relatively short period of breeding. To date, 17 wheat varieties of high-yielding potential have been developed from primary SHW lines and/or SHW-derived varieties. Genotype-phenotype and fingerprint analysis showed that alleles corresponding to disease resistance, higher spike numbers per plant, higher grain numbers per spike, larger grains, and high grain yield potential have been introgressed into the newly developed wheat varieties. The recent advances in wheat breeding by the use of SHW as genetic resources in China have provided valuable information with great promise for common wheat improvement worldwide. We concluded that the primary SHW and SHW-derived varieties have great potential for enhancing the grain yield of modern common wheat. Synthetic hexaploid wheat lines developed by CIMMYT and Chinese scientists are important genetic resources for wheat breeding in the future.

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