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The Origin of Spelt and Free-Threshing Hexaploid Wheat

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Abstract

It is widely believed that hexaploid wheat originated via hybridization of hulled tetraploid emmer with *Aegilops tauschii* (genomes DD) and that the nascent hexaploid was spelt, from which free-threshing wheat evolved by mutations. To reassess the role of spelt in the evolution of *Triticum aestivum*, 4 disomic substitution lines of *Ae. tauschii* chromosome 2D in Chinese Spring wheat were developed and one of them was used to map the *Tg* locus, which controls glume tenacity in *Ae. tauschii*, relative to simple sequence repeat (SSR) and expressed sequence tag loci on wheat chromosome 2D. The segregation of SSR markers was used to assess the presence of *Tg* alleles in 11 accessions of spelt, both from Europe and from Asia. Ten of them had an inactive *tg* allele in the D genome and most had an active *Tg* allele in the B genome. This is consistent with spelt being derived from free-threshing hexaploid wheat by hybridization of free-threshing wheat with hulled emmer. It is proposed that the tetraploid parent of hexaploid wheat was not hulled emmer but a free-threshing form of tetraploid wheat.

Key words: *origin of wheat,* RFLP, *SNP,* spelta, *tenacious glume,* Triticum aestivum, wheat phylogeny

Wheat is one of the most ancient crops. Its domestication marks the transition from hunting–gathering to agrarian economy in western Asia, which marks the dawn of the evolution of western civilization. Today, wheat is 1 of 2 most important staples of humankind. Because of this significance of wheat, its origin and evolution has received extensive attention, and a great deal has been learned. There are 6 biological species of wheat at 3 ploidy levels: diploid (*Triticum monococcum*, genomes A^mA^m and *T. urartu*, genomes AA), tetraploid (*T. turgidum*, genomes BBAA and *T. timopheevii*, genomes GGAA), and hexaploid (*T. aestivum*, genomes BBAADD and *T. zhukovskyi*, $GGAAA^mA^m$). Genome relationships show that *T. monococcum*, *T. timopheevii*, and *T. zhukovskyi* form a separate lineage irrelevant to the evolution of the principal wheat lineage, which is formed by *T. urartu*, *T. turgidum*, and *T. aestivum*. *Triticum turgidum* evolved by hybridization of *T. urartu* with a close relative of *Aegilops speltoides* (genomes SS) (Sarkar and Stebbins 1956; Nishikawa 1983; Dvorak and Zhang 1990; Dvorak et al. 1993). The domestication of the resulting wild emmer (*T. turgidum* ssp *dicoccoides*) gave rise to domesticated emmer (*T. turgidum* ssp. *dicoccon*, genomes BBAA), from which free-threshing tetraploid wheat, such as durum (*T. turgidum* ssp. *durum*) evolved.

It has been known since the 1920s that the source of the BBAA genomes of hexaploid bread wheat (*T. aestivum* ssp. *aestivum)* was *T. turgidum* (Sax 1922; Kihara 1924), but the identity of the other parent remained unknown until the 1940s when Kihara (1944) and McFadden and Sears (1946) independently identified it as *Aegilops tauschii* (genomes DD). McFadden and Sears (1946) resynthesized hexaploid wheat as an amphiploid of wild or domesticated emmer with *Ae. tauschii.* The synthetic hexaploid wheat resembled spelt (*T. aestivum* ssp *spelta*, genomes BBAADD). They concluded therefore that spelt was the ancestral form of *T. aestivum* and that the free-threshing forms of *T. aestivum* evolved from spelt.

In spelt, glumes tenaciously enclose seeds, and strong mechanical force is needed to liberate seeds from glumes during threshing. The hulled trait is principally controlled by the *Tg* (tenacious glume) and *q* (speltoid) loci (for review of genetic control of the wheat free-threshing character, see Salamini et al. 2002), but other genes are undoubtedly involved (Simonetti et al. 1999). Because *Ae. tauschii* is hulled, it is natural to anticipate that the primitive hexaploid wheat was hulled and that free-threshing hexaploid wheat, such as common wheat (*T. aestivum* ssp. *aestivum*), club wheat (*T. aestivum* ssp. *compactum*), and the endemic Indian dwarf wheat (*T. aestivum* ssp. *sphaerococcum*) were derived from a hulled ancestor.

The *Tg* (=*Tg1*) locus is on the short arm of chromosome 2D in *Ae. tauschii* (Kerber and Rowland 1974; Nalam et al. 2007; Sood et al. 2009) and 2E in *Lophopyrum elongatum* (Dvorak and Chen 1984). *Tg* is also on chromosome 2S in *Ae. speltoides*, which is indicated by the presence of tenacious glumes in the disomic addition line bearing a pair of *Ae. speltoides* chromosome 2S added to the full BBAADD chromosome complement of common wheat cv "Chinese Spring" (henceforth CS) (Friebe et al. 2000). Consistent with this fact is the mapping of *Tg* on chromosome 2B (=*Tg2*) in wild emmer (*T. turgidum* ssp. *dicoccoides*, genomes BBAA) (Simonetti et al. 1999). The location of the *Tg* locus in the A genome of *T. aestivum* or that of *T. urartu* is unknown but a gene controlling soft glumes (*Sog*) was mapped on the short arm of chromosome $2A^m$ in *T. monococcum* (Taenzler et al. 2002; Sood et al. 2009). *Sog* was found to be proximal to the region where *Tg* should be located, and it is unlikely that *Sog* and *Tg* are orthologous (Sood et al. 2009). According to current knowledge, free-threshing hexaploid wheat must be simultaneously homozygous for inactive *tg* (or *sog*) alleles on 2A, 2B, and 2D. To discriminate between the orthologous *Tg* genes on wheat homoeologous chromosomes 2A, 2B, and 2D, we will use the standard wheat gene nomenclature for orthologous gene sets. We propose to rename the *Tg1* locus on 2D (Kerber and Rowland 1974) as *Tg-D1* because it was the first *Tg* gene discovered. Its putative orthologue on 2B (currently *Tg2*) will be renamed as *Tg-B1* and the putative orthologue on 2A (if it exists) as *Tg-A1*.

The free-threshing habit of *T. aestivum* also requires the presence of the dominant *Q* (square head) allele on the long arm of chromosome 5A (Mac Key 1954; Sears 1954; Muramatsu 1963). Wild and domesticated emmer and spelt have the recessive *q* allele causing speltoid spike morphology, tenacious glumes, and brittle rachis in hexaploid wheat (Mac Key 1954; Muramatsu 1986; Luo et al. 1999; Simons et al. 2006). An exception is the hulled domesticated emmer *T. turgidum* ssp *dicoccon* var *liguliforme*, which has the *Q* allele causing dense apical portion of the spike (Muramatsu 1985). The exceptional emmer with *Q* could be ancestral to free-threshing wheat or derived from it by hybridization. Because all cultivated forms of *T. turgidum* and *T. aestivum* ultimately descended from wild emmer, and because wild emmer harbors the *q* allele, *q* must be ancestral to *Q*, which originated by a dominant mutation.

Genetic relationships based on RFLP between hexaploid wheat and populations of *Ae. tauschii* defined by geography and their allegiance to either the tauschii or the strangulata gene pools (for description of the *Ae. tauschii* gene pools, see Dvorak et al. 1998b). These relationships point to a region from Transcaucasia to southwestern Caspian Iran as the birthplace of *T. aestivum* (Dvorak et al. 1998b). When McFadden and Sears (1946) formulated their hypothesis, spelt was known only in Europe, which conflicted with the hypothesized Asian origin of *T. aestivum* (Flaksberger 1930; Schiemann 1932). On these grounds and other evidence, the ancestral position of European spelt was questioned (Schiemann 1932; Mac Key 1966). The subsequent discovery of spelt cultivation in western Iran, Transcaucasia, Tajikistan, Afghanistan, and China (Kuckuck 1959; Dorofejev 1971) made opposition to the ancestral position of spelt in hexaploid wheat phylogeny on the basis of geography irrelevant, and the McFadden and Sears hypothesis was widely accepted.

Differences in spike disarticulation, allozyme polymorphism (Jaaska 1978), preliminary RFLP data (Dvorak and Luo 2001), and variation in the promoter region of HMW-glutenin genes (Blatter et al. 2002) suggested that European and Asian spelt may be polyphyletic. If that were indeed the case and if European spelt were indeed derived, the search for the ancestor of free-threshing wheat should focus on Asian spelt.

However, that avenue also runs into difficulties. The oldest archaeological sites with agriculture are in western Asia. However, free-threshing hexaploid wheat seems to precede spelt in the archaeological record in those sites (Nesbitt and Samuel 1996). The oldest remnants of non-hulled grains identified as hexaploid wheat come from Anatolia and are dated to the middle of the seventh millennium BC (Hillman 1978; de Moulins 1993), whereas the oldest remnants of spelt came from Transcaucasia and Kurdistan and are dated to the fifth millennium (Bakhteyev and Yanushevich 1980; Lisitsina 1984). Moreover, spelt remnants are sporadic in those strata. Nesbitt and Samuel (1996) persuasively argued that current day populations of spelt, irrespective whether European or Asian, are unlikely to descended directly from an representative of the original ancestor of free-threshing hexaploid wheat.

We report here a genetic study on the origin of spelt and its position in wheat phylogeny. We reason that if spelt is the ancestral hexaploid wheat and originated from hybridization of hulled domesticated emmer with hulled *Ae. tauschii* (McFadden and Sears 1946; Jaaska 1978; Porceddu and Lafiandra 1986; Kimber and Sears 1987), it should have the dominant *Tg-D1* allele on chromosome 2D because *Ae. tauschii* has the *Tg-D1* allele (Kerber and Rowland 1974). Spelt should also have either *Tg-A1* (or *Sog*) or *Tg-B1*, or both, depending on the genotype of emmer that was involved in hybridization with *Ae. tauschii*. However, if spelt is derived from hybridization of hulled emmer with free-threshing hexaploid wheat, it may have *Tg-A1* (or *Sog*) and/or *Tg-B1* alleles but must have the *tg-D1* allele, since the spelt 2D chromosome would have to come from the free-threshing hexaploid parent. Thus, the genotype of the locus in the D genome of spelt is critical for deciding which of the 2 scenarios is more likely.

Which of the 2 alternatives is true could be decided experimentally by comparing the *Tg* haplotypes in spelt and other forms of wheat. Unfortunately, the *Tg* locus is located in a region at which a chromosome homoeologous to rice chromosome Os7 was inserted into a chromosome homoeologous to rice chromosome Os4, thereby creating Triticeae chromosome 2 (Luo et al. 2009). This region shows poor synteny with the sequenced grass genomes (Pourkheirandish et al. 2007; Luo et al. 2009). This reality has so far stymied attempts to isolate *Tg*. We therefore use simple sequence repeat (SSR) markers linked to the *Tg-D1* locus to infer the D-genome genotype of spelt in this study.

Using this strategy, we inferred the *Tg-D1* genotypes in 11 accessions of spelt. We also used SNP and RFLP to examine the relationships of the A, B, and D genomes of spelt with other hexaploid and tetraploid wheats. The findings are used to infer the origin of spelt and suggest a model of the evolution of free-threshing hexaploid wheat.

Materials and Methods

Parental Lines and Phenotypic Evaluation of Segregating Progeny

Four European, 1 Ethiopian, and 6 Asian accessions of spelt were studied (Table 1). Each spelt was crossed with CS and F_2 families including the parental lines were planted in blocks of 3-m rows in the field. Plants were individually harvested and subjectively classified on the basis of glume tenacity, using CS and spelt parents as checks. Because glume tenacity is a quantitative trait based on at least 4 incompletely dominant genes, it is difficult to discriminate unequivocally between free-threshing and hulled plants. Two levels of rigor in allocating plants into the free-threshing class were used. The more rigorous classes, referred to as soft-1, were plants that were considered to be free threshing (although some may have been misclassified). The less rigorous class, referred to as soft-2, included the soft-1 plants and other phenotypically borderline plants. Plants that had clearly tenacious glumes were assigned into the tenacious-glume class. Other spike characteristics were recorded for each F_2 plant.

Glume tenacity was quantified in individual plants of some F_4 families. A single spike was wrapped in a small piece of cotton fabric, laid on a wooden board at a 45° angle relative to the axis of the board, and rolled over 3 times with a 15 kg PVC roller while the fabric including the spike was held immobile on the board. The spike material was shaken from the fabric into a pan, and the number of seeds still held in glumes was counted. The number of seeds in glumes was divided by the total number of seeds in the spike. This quotient is called glume tenacity and is zero if all seeds were liberated from glumes and 1.0 if none were. Glume tenacity was typically determined for 3–5 spikes per plant, and the quotients were averaged per plant. Mean glume-tenacity scores per plant were used as variables in statistical analyses of data.

Hypothesis and Its Statistical Tests

The primary hypothesis tested was that both spelt and CS have the recessive *tg-D1* allele on chromosome 2D. Because CS is free threshing, CS must be homozygous for the *tg* (or *sog*) alleles on 2A, 2B, and 2D and for *Q* on 5A. The free-threshing (soft-glumed) F_2 progeny should also be homozygous for these alleles. Such progeny is rare and is difficult to identify unequivocally, as pointed out above. The hypothesis was therefore tested by counting the numbers of spelt and CS alleles at SSR loci in soft-1, soft-2, and tenacious-glume phenotypic classes.

Consider, for example, spelt that is homozygous for the *Tg-D1* allele. The free-threshing F_2 plants must be homozygous for the *tg-D1* allele (and other soft-glume alleles on chromosomes 2A, 2B, and 5A). Because the source of the *tg-D1* allele was the CS parent, soft-glume classes are expected to be enriched for CS alleles at SSR loci linked to *tg-D1* on the 2D chromosome. The magnitude of the enrichment would depend on the strength of linkage between an SSR locus and *tg-D1*. The reverse is expected in the tenacious-glume F_2 phenotypic class. The class is expected to be enriched for spelt alleles at SSR loci linked to the *Tg* locus if spelt has the *Tg-D1* allele. However, because there can be up to 4 loci causing tenacious glume in F_2 plants, and because a single active allele is sufficient to produce the tenacious-glume phenotype, the tenacious-glume group would be only mildly enriched for the spelt SSR alleles.

Consider now a spelt that is homozygous for the *tg-D1* allele. Now the *tg-D1* allele can be contributed to the progeny equally by the CS and spelt parents. There will therefore be no enrichment for the CS alleles at SSR loci linked to *tg-D1* in F_2 in the soft-1 and soft-2 phenotypic classes. There will of course be also no enrichment for spelt SSR alleles in the tenacious-glume class. The counts of CS and spelt alleles at SSR loci linked to *tg-D1* are expected to

Table 1 Geographic origin of investigated accessions of spelt

Accession	Source	Origin
PI347850	NSGC, Aberdeen, Idaho	Switzerland
PI330558	NSGC, Aberdeen, Idaho	United Kingdom
PI347926	NSGC, Aberdeen, Idaho	Switzerland
PI355651	NSGC, Aberdeen, Idaho	Austria
PI297861	NSGC, Aberdeen, Idaho	Shewa, Ethiopia
PI367200	NSGC, Aberdeen, Idaho	Ghowr, Afghanistan
PI367199	NSGC, Aberdeen, Idaho	Harat, Afghanistan
417a	H. Kuckuck	Natch, Shahrkord, Iran
77d	H. Kuckuck	Esfahan, Iran
VIR 45366	Vavilov Inst. Plant Ind.	Azerbaijan
VIR 52442	Vavilov Inst. Plant Ind.	Tadjikistan

NSGC, National Small Grains Collection.

appear in a 1: 1 ratio in the soft-glume and tenacious-glume F_2 progeny in this case.

To test the hypothesis statistically, the counts of CS and spelt alleles at SSR loci on 2A, 2B, and 2D in the soft glume–1 and –2 F_2 phenotypic classes were compared with the counts of the same SSR alleles in the tenacious-glume class. The difference in the allele counts was statistically tested with 2 × 2 contingency table and Fisher's exact test using $\alpha = 0.05$.

In some crosses, glume tenacity was quantified in several F_4 families selected on the basis of prior information about the SSR genotypes and glume phenotypes in the $F_{2:3}$ generation. The SSR genotype of each F_4 plant was determined. Plants were then grown to maturity, and glume tenacity was quantified as described above. Plants within segregating families were grouped on the basis of their SSR genotypes. Glume-tenacity scores were used as variables in GLM (SAS version 9.1) in which the genotype was nested within family. The probability of differences among the means due to chance alone were computed with SAS version 9.1 using least square means of genotypes nested within family means on the basis of a priori hypotheses.

Disomic Substitutions of *Ae. tauschii* Chromosome 2D for the CS Homologue

Four disomic substitution (DS) lines with chromosome 2D replaced by *Ae. tauschii* chromosome 2D were developed in the CS genetic background. Synthetic wheats RL5402, RL5403, RL5405, and RL5406 were developed and supplied by E. R. Kerber, Agriculture Canada Rust Lab (RL), Winnipeg, Canada (Kerber and Rowland 1974). One parent of each synthetic was a tetraploid extraction from hexaploid Canadian cultivar Canthatch (TetraCanthatch) (Kerber 1964) and the other parent was an *Ae. tauschii* accession (Table 2). Each synthetic wheat was crossed as a male with CS monosomic 2D. Monosomic F_1 progeny were selected on the basis of root chromosome count and recurrently backcrossed to CS monosomic 2D, selecting monosomic progeny in each generation. After 6–7 backcrosses, monosomics were selfed, and 42-chromosome progeny was selected. Four different DS lines in which *Ae. tauschii* chromosome 2D replaced CS chromosome 2D were produced. The designations of the DS lines are in Table 2.

Genotyping

Information about SNP at expressed sequence tag (EST) loci *XBE500206* (2A), *XBF201235* (2B and 2D), *XBE518440* (2B and 2D), *XBF428792* (2B), *XBE471132* (2D), *XBE489611* (2D), *XBE443339* (2D), *XBE404601* (2D), *XBE518440* (2D), *XB499561* (2D), *XBF291738* (2D), *XBE505206* (2D), *XBE471015* (2D), and *XBF428792* (2D) was downloaded from http://probes.pw.usda.gov:8080/snpworld/Search SNP database. The database also contains genome-specific primers (GSPs) for PCR amplification of these ESTs from polyploid wheat. SNPs and GSPs for EST loci not listed above were developed here using the strategy described by Akhunov et al. (2010).

SNPs were assayed in segregating populations as follows. The DNA targets were PCR amplified in a 20 µl PCR reaction containing 100 ng genomic DNA, 1× PCR buffer I (Life Technologies, Inc.) with a final concentration of 1.5 mM $MgCl_2$, 10 mM dNTPs, 50 pmol of each GSP primer, and 1 unit of *Taq* polymerase. The following amplification regime was used: 1 cycle of 94 °C for 5 min, 12 cycles of 94 °C for 30 s, 58–52 °C with −0.5 °C change each cycle for 30 s, and 72 °C for 3 min, 20 cycles of 94 °C 30 s, 52 °C for 30 s, and 72 °C for 3 min, then 1 cycle of 72 °C for 5 min, and finally a 10 °C steady-state for stand by. Amplicons were sized and checked for quality by 1% agarose gel electrophoresis. An aliquot of 5 µl from PCR reactions containing amplicons of high quality were treated with 2 units shrimp alkaline phosphatase (USB) and 10 units of exonuclease I (USB) in a 10 µl reaction. The 10 µl reaction was then diluted with 8 µl of water and an aliquot of 3 µl was taken for a Sanger sequencing reaction. The Sanger sequencing reaction contained 3.2 pmol primer, 1× sequencing buffer, and 2 µl Big Dye v3.1 (Life Technologies, Inc.). The reaction conditions were as recommended by Life Technologies, Inc. The sequencing reaction was precipitated and then dissolved in 12 µl of HiDi formamide (Life Technologies, Inc.) and sequenced with the ABI3730xl (Life Technologies, Inc.). Sequencing data were first processed with DNA Sequencing Analysis Software (Life Technologies, Inc.) and then with the pregap and gap programs in the Staden software package (http://staden.sourceforge.net/). The SNP genotype of a plant was inferred from the nucleotide sequence.

Microsatellite markers were amplified using fluorescent primers from genomic DNA in a 20 µl reaction. The reaction contained 100 ng genomic DNA, 1× PCR buffer I with a final concentration of 1.5 mM $MgCl_2$ (Life Technologies, Inc.), 10 mM dNTPs, 6.25 pmol of each primer, and 1 unit of *Taq* polymerase. Amplification regime was the same as above except for that an extension cycle at 72 °C was for 2 min rather than 3 min. Amplicons were diluted into HiDi formamide (Life Technologies, Inc.) with 0.5 µl of the PCR reaction mixed with 10 µl HiDi and 0.06

Table 2 DSs of *Aegilops tauschii* chromosome 2D for CS chromosome 2D

DS line	Line identification	Synthetic wheat	*Ae. tauschii* parent of synthetic wheat	Backcross generation
DSAt2D5402(CS2D)	Dv695, Dv696	RL5402	ssp. *tauschii* (RL5261)	BC_7
DSAt2D5403(CS2D)	Dv1179, Dv1180	RL5403	ssp. *anathera* (RL5266)	BC_6
DSAt2D5405(CS2D)	Dv1185-Dv1189	RL5405	ssp. *strangulata* (RL5288)	BC_6
DSAt2D5406(CS2D)	Dv1181	RL5406	ssp. *meyeri* (RL5289)	BC_6

µl ROX500 size standard (Life Technologies, Inc.). Diluted samples were denatured at 95 °C for 5 min and then immediately placed on ice. The amplicons were sized on an ABI3730XL (Life Technolgies, Inc.) using the default microsatellite module. Samples were scored by comparing the microsatellite profile of the sample with those of the parental spelt and CS.

Tg Mapping

CS was crossed with DSAt2D5403(CS2D), F_1 progeny was self-pollinated and 288 F_2 progeny and the parental lines were grown in the greenhouse. Each plant was classified into 1 of the 3 phenotypic classes of monohybrid segregation for an incompletely dominant gene on the basis of subjective scoring of glume tenacity. From 6 to 12 F_3 progeny were grown per each F_2 family to validate the F_2 inferences. Glumes of each F_3 plant were subjectively assigned into 1 of the 3 phenotypic classes. The $F_{2:3}$ phenotypic classification made it possible to assign each F_2 plant unequivocally to 1 of the 3 monohybrid genotypic classes.

DNAs were isolated from the F_2 plants as described previously (Dvorak et al. 2006), and each F_2 plant was genotyped with 9 SSR markers previously mapped on the short arm of wheat chromosome 2D. Marker number preceded by *gwm*, *barc*, and *wmc* were developed and mapped by Roder et al. (1998), Song et al. (2005), and Somers et al. (2004), respectively. Nine ESTs surrounding *Tg-D1* and harboring SNPs were mapped using GSPs for PCR amplification of DNA targets from F_2 plants and SNP genotyping strategy as described above. Eight of the EST loci were also mapped on the high-density *Ae. tauschii* EST genetic map (Luo et al. 2009). The 8 EST loci and 2 SSR loci served as anchors for the alignment of the wheat D-genome genetic map including the *Tg-D1* locus with the *Ae. tauschii* EST map reported by Luo et al. (2009). The alignment of the wheat 2D map with the *Ae. tauschii* genetic map was used to infer the *Tg-D1* region on the *Ae. tauschii* genetic map. Distances on the genetic maps were computed with JoinMap (Kyazma, Inc.).

To find the *Tg* region on chromosomes 2A and 2B relative to molecular markers, 96 F_2 plants from the cross *T. turgidum* ssp *durum* cv Langdon (LDN) × *T. turgidum* ssp *dicoccoides* PI428082 were genotyped with 6 2AS SSRs and 5 2BS SSRs and with 6 gene-based SNPs. The A- and B-genome GSPs and SNP detection by Sanger sequencing on ABI3730XL were used in mapping of the EST loci. The genetic map was constructed with JoinMap (Kyazma, Inc.).

Phylogenetic Analysis

Nucleotide sequences for 13 *T. aestivum* landraces and varieties selected to represent genetic diversity in *T. aestivum*, durum cv Altar, and 2 wild emmer accessions from the Dyiarbakir region in Turkey, the putative site of emmer domestication (Luo et al. 2007), and *Ae. tauschii* genomes present in synthetic wheats were downloaded from a database reported by Akhunov et al. (2010). In order to calculate the genetic distances, 131 A- and B-genome gene sequences shared by all 16 genetic stocks and 121 D-genome gene sequences shared by all 22 investigated genetic stocks were downloaded from the database (http://avena.pw.usda.gov/SNP/new/index.shtml) and aligned individually using the blastalign program (Belshaw and Katzourakis 2005). Sites that displayed nucleotide polymorphisms were extracted and concatenated. The concatenated sequences were used to estimate genetic distances among 16 genetic stocks in the A and B genomes and among 22 genetic stocks in the D genome based on Kimura's 2-parameter model (Kimura 1980) with the "dnadist" module in the Phylip package (Felsenstein 2005). Neighbor-joining (N-J) trees based on the estimated distance matrices were built using phylip. The phylogenetic trees were based on 100 bootstrapped samples. Bootstrap confidence of each node in the tree was estimated using 100 resampling replications.

Genetic distances for 172 accessions of *Ae. tauschii* and 178 accessions of *T. aestivum* (Supplementary Table 1) were computed using GDA (Lewis and Zaykin 1997) from RFLP data for 29 genes reported earlier (Dvorak et al. 1998b). Genetic distances were computed for individual accessions rather than for populations based on geographic distribution (*Ae. tauschii*) or subspecies (*T. aestivum*), as was done earlier (Dvorak et al. 1998b). N-J trees were built for all *Ae. tauschii* and wheat accessions or for wheat only.

Results

Synthetic Wheat and DS Lines and Their Glume Tenacity

Ae. tauschii 2D chromosomes present in synthetic hexaploid wheats RL5402, RL5403, RL5405, and RL5406 (Figure 1) were substituted for CS chromosome 2D by backcrossing each synthetic wheat 6 or 7 times to CS monosomic 2D (Figure 2 and Table 2). The glume tenacity of these DS lines was compared with that of spelt, parental synthetic wheats, TetraCanthatch (the tetraploid parent of the synthetics), and CS. The highest glume-tenacity score was in spelt (Table 3). Compared with spelt, glumes were softer in synthetic wheat and still softer in each DS line. The latter suggests that additional genes are likely present in the *Ae. tauschii* genome that affect glume tenacity in addition to the *Tg-D1* locus. TetraCanthatch has soft glumes and must therefore have the *Q* allele on chromosome 5A, inactive *tg* (or *sog*) allele on 2A and inactive *tg-B1* on 2B. Compared with CS, the spikes of DS lines were slightly narrower due to more acute angle of glumes to spike rachis caused by *Tg-D1* (Figure 2). Spike rachises in synthetic wheats were more fragile than in CS; DS lines were intermediate (Figures 1 and 2). However, even when all seeds were entirely mechanically liberated from hulls in synthetic wheat and DS lines, fragments of rachis still accompanied the chaff (Figures 1 and 2). In sterile spikes, rachis often remained intact after threshing (Figure 1).

Tg Mapping

To map the *Tg-D1* locus relative to SSR and EST markers, DSAt2D5403(CS2D) was crossed with CS. A total of 288 F_2

Figure 1. Spikes of hexaploid amphiploids (synthetic wheats) produced by hybridization of a tetraploid with BBAA genomes extracted from cv "Canthatch" with 4 different accessions of *Aegilops tauschii* (Kerber and Rowland 1974). Fragments of rachises found in chaff after complete mechanical threshing are to the right of spikes. The complete rachis to the right of the spike RL5402 resulted from threshing of a sterile spike.

progeny plus the parental lines were grown in the greenhouse and assigned into 1 of the 3 phenotypic classes for monohybrid segregation for an incompletely dominant gene on the basis of subjective scoring of glume tenacity. An F_3 family was grown per each F_2 plant and used for the validation of the F_2 genotypes. The *Tg-D1* locus was located 43.5 cM from the end of the 2DS map. The locus was flanked by the SSR marker *Xwmc112*, which was 3.1 cM distal to *Tg-D1*, and EST marker *XCA658378*, which was 4.2 cM proximal to *Tg-D1* (Figure 3).

Eight of the EST loci harboring SNP markers and 3 SSR loci were then mapped on the comparative *Ae. tauschii* genetic map (Luo et al. 2009). Using shared markers between the wheat 2D map and *Ae. tauschii* map, the *Tg-D1* locus was inferred to be in a 9.7-cM interval between *Xwmc112* on the distal side and the EST locus *XBG263347* on the proximal side (Figure 3).

To infer the approximate location of the *Tg* region on chromosomes 2A and 2B relative to molecular markers, 96 F_2 plants from the cross LDN × *T. turgidum* ssp *dicoccoides* PI428082 were genotyped with 6 2AS SSRs and 4 2BS SSRs and with 6 gene-based SNPs (Figure 3). A putative *Tg-A1* region was inferred to be in a 35.3-cM interval on chromosome 2A between SSR markers *Xbarc212* and

Figure 2. Spikes of CS and DS lines in which CS chromosome 2D was replaced by *Aegilops tauschii* chromosome 2D from synthetic wheats in Figure 1. Note large rachis fragments in chaff after full mechanical threshing.

Table 3 Tenacity scores in spelt, synthetic wheat (RL), DS lines, the wheat parent of synthetic wheats (TetraCanthatch), and CS

Line	*N*	Tenacity score[a]
Spelt PI347850	1	1.000a
Spelt PI330558	1	1.000a
Spelt PI347926	1	1.000a
Spelt PI355651	1	1.000a
Spelt PI297861	1	1.000a
Spelt PI367200	1	1.000a
Spelt PI367199	1	0.980a
Spelt 417a	1	1.000a
Spelt 77d	1	1.000a
Spelt VIR 45366	3	0.966a
Spelt VIR 52442	3	1.000a
RL5402	6	0.859b
RL5403	21	0.882b
RL5405	11	0.890b
RL5406	1	0.817b
DSAt2D5402(CS2D)	7	0.435d
DSAt2D5403(CS2D)	3	0.271e
DSAt2D5406(CS2D)	9	0.653c
TetraCanthatch	1	0.264ef
CS	18	0.124f

[a] Means followed by the same letter are not significantly different at the 5% probability level.

Xgwm359. Using *Xwmc177* shared with a map reported by Sood et al. (2009), the *Sog* region was inferred to be in a 18.5-cM region proximal to *Xwmc177*. On chromosome 2B, the *Tg* region was inferred to be in a 15.8-cM interval between comparative SNP markers *XCJ600989* and *XBE518440* (Figure 3).

In addition to mapped markers, Figure 3 shows the approximate location of SSR markers *Xwmc407* (2A), *Xgwm445* (2A), *Xgwm210* (2B), and *Xwmc25* (2B). These markers were used in several segregating populations from crosses of spelt with CS but were not polymorphic between LDN and PI428082. Their approximate locations were inferred from their locations relative to other SSR markers on maps reported by Somers et al. (2004).

Spelt *Tg* Loci

Eleven accessions of spelt were crossed with CS and F_2 progeny was grown in the field. F_2 plants were subjectively classified into soft-1, soft-2, and tenacious-glume classes. F_3 progeny of each F_2 plant was grown and individually genotyped with 2A, 2B, and 2D SSR markers.

Spelt PI347850

SSR locus *Xwmc25* on chromosome 2B showed a significant excess of CS alleles in the soft-1 F_2 class compared with the tenacious-glume class (Supplementary Table 2). An excess of CS alleles was also observed at the *Xgwm359* SSR locus on chromosome 2A. However, an excess of CS alleles was also observed in the tenacious-glume class suggesting a segregation distortion favoring the CS chromosome 2A and making data for 2A inconclusive. No difference was observed between the soft and the tenacious-glume classes in the counts of spelt and CS alleles at the *Xwmc112* and

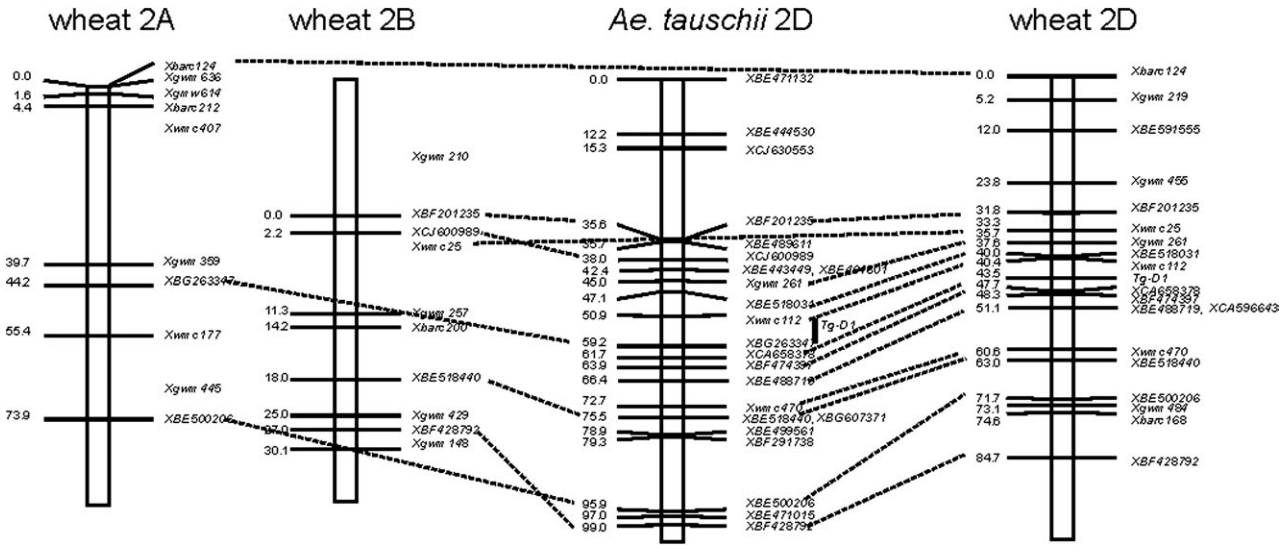

Figure 3. Comparative map of wheat chromosome 2D based on the DSAt5403(CS2D) × CS mapping population. Map of *Aegilops tauschii* chromosome 2D is based on mapping population AL8/78 × AS75 (Luo et al. 2009) and maps of tetraploid wheat chromosomes 2A and 2B are based on the mapping population durum cv "Langdon" × wild emmer PI428082. Loci designated by 2 capital letters are EST loci mapped with SNP markers and loci designated with 3 lowercase letters are SSRs. The *Tg-D1* locus was mapped on the wheat 2D map, and shared markers were used to infer the location of the locus in the *Ae. tauschii* genome and the A and B genomes of tetraploid wheat. The location of markers accompanied by cM (to the left of a chromosome) was determined here. The location of markers not accompanied by cM is based on comparison with wheat SSR consensus map (Somers et al. 2004).

Xgwm261 SSR loci on chromosome 2D (Supplementary Table 2).

To obtain additional data, several contrasting F_4 families were grown, and glume tenacity was quantitatively assessed as described in Materials and Methods; 0.0 was a fully free-threshing score and 1.0 was fully hulled score. Substitution of the spelt allele for the CS allele at the *Xwmc112* locus on chromosome 2D did not significantly affect glume tenacity, suggesting that PI347850 and CS had the *tg-D1* allele on chromosome 2D (Supplementary Table 3). Substitution of the spelt allele at the *Xbarc200* locus on chromosome 2B for the CS allele resulted in a significant increase in glume tenacity, indicating that PI347850 had the *Tg-B1* allele, whereas CS had the *tg-B1* allele on 2B. No inference could be made about the glume-tenacity genotype on spelt chromosome 2A due to lack of polymorphism. The F_4 data substantiated that PI347850 had the *Tg-B1* allele on chromosome 2B and showed that it had the inactive *tg-D1* allele on chromosome 2D.

Spelt PI330558

Marker *Xwmc25* on chromosome 2B showed a statistically significant excess of CS alleles over the spelt alleles in the soft-1 group compared with the tenacious-glume group (Supplementary Table 2), suggesting that PI330558 had the *Tg-B1* allele on chromosome 2B. No significant differences were found in CS and spelt allele counts at SSR loci on 2A and 2D suggesting that PI330558 had the *tg-A1* (or *sog*) and *tg-D1* alleles on chromosomes 2A and 2D, respectively.

Spelt PI347926

Only the B-genome and D-genome SSR markers were polymorphic. A significant excess of CS alleles was observed in the soft-2 class compared with the tenacious-glume class at the *Xwmc25* locus on 2B. The soft-1 class also showed an excess of CS alleles over the spelt alleles, but the number of genes in that class was insufficient for the test to be statistically significant. The data indicated that PI347926 had the *Tg-B1* allele on chromosome 2B. The counts of CS and spelt alleles at the 2 SSR loci on 2D did not differ between the soft-glume and the tenacious-glume classes suggesting that PI347926 and CS had the *tg-D1* allele on 2D.

Spelt PI355651

Strong segregation distortion favoring the CS chromosome was observed at the end of chromosome 2B. While the more proximal SSR locus *Xwmc25* segregated in the expected 1:1 ratio ($P = 0.57$), the more distal locus *Xgwm210* showed an excess of CS alleles in all 3 classes, including the tenacious-glume class in which the spelt allele should had been preferred ($P = 0.02$). Segregation distortion precluded determining the 2B glume genotype. No segregation distortion accompanied loci on chromosomes 2A and 2D: *Xwmc112* ($P = 0.232$) and *Xgwm261* ($P = 0.239$). Data for both chromosomes showed that the spelt and CS chromosomes 2A and 2D harbored the same alleles.

Spelt PI297861

Segregation distortion favoring CS SSR alleles was observed at all 3 studied chromosomes; *Xbarc124* on 2A ($P = 0.04$), *Xwmc200* on 2B ($P = 0.05$), and *Xwmc112* ($P < 0.01$) and *Xgwm261* ($P < 0.01$) on 2D. No conclusion could therefore be made about the glume genotype on the basis of F_2 data.

Glume tenacity was quantified in several segregating F_4 families. The substitution of the spelt allele at the *wmc112* SSR locus on 2D for the CS allele did not significantly change glume tenacity (Supplementary Table 3). Due to the small number of plants in family 30-7NT-1, the substitution of 2B *Xbarc200* allele, although greatly increasing glume tenacity, was not significant. However, the same allele substitution in 2 nonsegregating F_4 families resulted in a statistically significant increase in glume tenacity. These data suggest that PI297861 harbored the *Tg-B1* and *tg-D1* allele. No inference could be made about the 2A chromosome.

Spelt PI367200

Allele counts in soft-1 or soft-2 classes differed significantly from those in the tenacious-glume class at none of the investigated SSR loci (Supplementary Table 2). PI367200 appears to have the *tg* allele on 2A, 2B, and 2D.

Spelt PI367199

Allele counts differed significantly in both soft-1 and soft-2 classes from those in the tenacious-glume class at SSR loci *Xwmc636* and *Xgwm148* on 2A and 2B, respectively, but not at the *Xwmc112* locus on chromosome 2D (Supplementary Table 2). PI367199 has the dominant *Tg-A1* (or *Sog*) allele on 2A, the *Tg-B1* allele on 2B but the *tg-D1* allele on 2D.

Spelt 417a

Allele counts differed significantly in both soft-1 and soft-2 classes from that in the tenacious-glume class at the SSR locus *Xbarc200* on 2B, suggesting that Iranian spelt 417a had the dominant *Tg-B1* allele (Supplementary Table 2). No difference between classes was observed in allele counts at SSR loci on chromosomes 2A and 2D suggesting that spelt 417a harbors the *tg-A1* (or *sog*) and *tg-D1* alleles.

The substitution of the spelt allele for the CS allele at the 2A SSR locus *Xgwm445* had no significant effect on glume tenacity in F_4 families (Supplementary Table 3). Likewise, no significant difference was observed when the CS SSR allele at the *Xwmc112* locus on chromosome 2D was replaced by the spelt allele. The substitution of the spelt allele at the *barc200* SSR locus on chromosome 2B significantly increased glume tenacity in segregating F_4 families 211-2F-1 and 211-2F-1:8. Data obtained in F_4 corroborated F_2 data.

Spelt 77d

Allele counts differed significantly in soft-2 glume class from that in the tenacious-glume class at the SSR locus *Xbarc200* on 2B. However, it was the spelt allele that showed an elevated frequency compared with the CS allele. This difference was undoubtedly an artifact because it suggested

that spelt had a soft-glume allele and the CS had a tenacious-glume allele (Supplementary Table 2). At the *Xwmc112* SSR on 2D, soft-1 and soft-2 glume classes showed a significant increase in the CS allele count compared with that of the spelt allele (Supplementary Table 2). Iranian spelt 77d therefore appears to have the recessive *tg-A1* (or *sog*) allele on 2A, *Tg-B1* on 2B, and *Tg-D1* on 2D.

Spelt VIR 45366

Only SSR markers on 2A and 2D were studied (Supplementary Table 2). Significant differences between CS and spelt allele counts at the SSR locus *Xgwm636* on 2A were observed between the soft-1 and the soft-2 glume classes on one hand and the tenacious-glume class on the other hand. The remarkable enrichment of the classes for the CS *Xgwm636* allele was to some extent caused by segregation distortion favoring the CS 2A chromosome. The tenacious-glume class was also significantly enriched for the CS *Xgwm636* allele compared with the expected 1 spelt:1 CS allele ratio ($P = 0.03$). No segregation distortion and no enrichment for CS alleles were observed at the *Xwmc261* locus on chromosome 2D. These data suggest that Azerbaijan spelt VIR 45366 harbors the dominant *Tg-A1* and recessive *tg-D1* allele, whereas the genotype at the 2B locus is unknown.

Spelt VIR 52442

No statistically significant differences between the phenotypic classes were observed at investigated SSR loci on chromosomes 2A and 2D (Supplementary Table 2), indicating that VIR 52442 had the *tg-A1* (or *sog*) allele and *tg-D1* allele. A significant difference was observed between the soft-2 glume and the tenacious-glume classes at the *Xbarc200* locus on chromosome 2B, suggesting that VIR 52442 has the dominant *Tg-B1* allele.

Spelt Origin

Four European spelt accessions, Ethiopian spelt accession PI297861, and 5 of the 6 Asian spelt accessions had the *tg-D1* allele on chromosome 2D (Table 4), which was consistent with the hypothesis that one parent of spelt was free-threshing hexaploid wheat. The fact that 8 spelt accessions had the *Tg-B1* allele on chromosome 2B is consistent with the hypothesis that the other parent was hulled tetraploid wheat.

The presence of the *Tg-A1* allele on chromosome 2A was detected in only 2 accessions. Although the remaining 8 accessions did not have the *Tg-A1* allele, they could have had the *Sog* allele that would almost certainly segregate independently of SSR markers used.

Five of the 11 F_2 progenies segregated plants with compact spikes (Table 4). Segregation was investigated in the cross CS × PI330558 (Table 5). Compact spike morphology cosegregated with the spelt SSR alleles *Xwmc112* and *Xgwm261* on chromosome 2D showing that the gene controlling compact spike morphology was on the spelt chromosome 2D. The linkage of the compact spike phenotype with the spelt allele at *Xwmc112* was tighter than that with spelt allele *Xgwm261*. Because *Xwmc112* is proximal to *Xgwm261* (Figure 3), the gene controlling compact spike must be proximal to these 2 genes, which agrees with the location of the wheat *C* gene controlling compact spike morphology in wheat (Unrau 1950; Worland and Law 1986; Johnson et al. 2008). The *C* allele must be hypostatic to some other gene in spelt because PI330558 has lax spikes typical for spelt. The presence of the *C* allele on spelt chromosome 2D showed that the free-threshing parent of European spelt PI330558 was club wheat (*T. aestivum* ssp *compactum*).

One Asian spelt accession, Iranian spelt 77d, had the *Tg-D1* allele. This outcome is consistent with the ancestral position of spelt in *T. aestivum* evolution. Because only a single accession had this attribute, it was important to consider other alternatives, such as the possibility that Iranian spelt originated independently from the rest of hexaploid wheat.

Table 4 Summary of phenotypic segregation in the F_2 generation and inferred *tenacious-glume* locus genotypes in spelt

Spelt	Origin	Segregating spike phenotypes	Genome		
			A	B	D
PI347850	Switzerland	S, Q, C[a]	*tg1?*	*Tg1*	*tg1*
PI330558	United Kingdom	S, Q, C	*tg1?*	*Tg1*	*tg1*
PI347926	Switzerland	S, Q, C	?	*Tg1*	*tg1*
PI355651	Austria	S, Q	*tg1?*	?	*tg1*
PI297861	Ethiopia	S, Q	*tg1?*	*Tg1*	*tg1*
PI367200	Afghanistan	S, Q	*tg1?*	*tg1*	*tg1*
PI367199	Afghanistan	S, Q, C	*Tg1*	*Tg1*	*tg1*
417a	Iran	S, Q	*tg1?*	*Tg1*	*tg1*
77d	Iran	S, Q	*tg1?*	*tg1*	*Tg1*
VIR 45366	Azerbaijan	S, Q, C	*Tg1*	?	*tg1*
VIR 52442	Tadjikistan	S, Q	*tg1?*	*Tg1*	*tg1*

[a] S, Q, and C stand for spelt-like, square-head, and compact spike morphology, respectively.

The Phylogeny of *Ae. tauschi* and *T. aestivum*

RFLP and SNP data reported earlier (Dvorak et al. 1998b; Akhunov et al. 2010) were employed to examine the position of Iranian spelt within *T. aestivum*. Nei's genetic distances between single accessions were computed from RFLP at 29

Table 5 Cosegregation of compact spike with SSR alleles in F_2 plants from the cross CS × spelt PI330558

Chromosome	Locus	Spike density	Spelt SSR allele	CS SSR allele	*P* value
2A	*wmc407*	Compact	21	27	1.0000
		Noncompact	14	16	
2B	*barc200*	Compact	20	30	0.6910
		Noncompact	14	18	
2D	*gwm261*	Compact	28	18	0.0128
		Noncompact	24	42	
2D	*wmc112*	Compact	33	15	0.0001
		Noncompact	20	46	

loci in 172 accessions of *Ae. tauschii* and the D genomes of 178 accessions of *T. aestivum*, and a N-J tree was built. Several primitive hexaploid wheat subspecies were included in addition to spelt: Macha wheat (*T. aestivum* ssp *macha*), which is an endemic Georgian wheat with brittle rachis, Yunan wheat (*T. aestivum* ssp *yunanense*), which is spelt-like wheat grown along the Lujiang and Lanchangijang rivers in Yunan, China, Tibeatan wheat (*T. aestivum* ssp *tibetanum*), which is feral wheat with brittle rachis in Tibet, and *T. aestivum* ssp. *vavilovi*, which is a spelt-like wheat from Transcaucasia.

The *Ae. tauschii* accessions (Supplementary Table 1) clustered into 2 distinct branches consisting largely of either strangulata gene pool accessions, affiliated with *Ae. tauschii* ssp *strangulata*, or tauschii gene pool accessions, affiliated with *Ae. tauschii* ssp *tauschii*. The virtual absence of genotypes intermediate between the 2 clusters (Supplementary Figure 1) suggested that there is little gene flow between the pools, even when they are sympatric, such as in Transcaucasia. The strangulata gene pool cluster comprised accessions from Transcaucasia (Armenia, Azerbaijan, and Nakhichevan) and southwestern and southeastern Caspian Iran. The tauschii gene pool cluster consisted of accessions from Turkey, Transcaucasia, Iran, Turkmenistan, Afghanistan, Pakistan, and China.

All 178 *T. aestivum* accessions (Supplementary Table 1) formed a single cluster showing that the *T. aestivum* D genome is monophyletic and that all subspecies of *T. aestivum* including European and Asian spelt share the same D genome. The wheat branch was embedded within the strangulata gene pool cluster. Strangulata gene pool accessions from southwestern Caspian Iran collected in the vicinity of Rasht formed a branch that was closer to the wheat branch than other branches within the strangulata gene pool (Supplementary Figure 1).

To visualize the relationships of individual accessions within the *T. aestivum* D-genome branch, only genetic distances between wheat accessions were used to build a D-genome N-J tree (Supplementary Figure 2). Accession AL8/78 of *Ae. tauschii* (boxed in Supplementary Figure 2) was used as an outgroup. Asian spelt, including Iranian accessions, formed a branch that was closest to the *Ae. tauschii* root. The majority of Chinese landraces, including Yunan wheat and Tibetan wheat, formed a cluster in the center of the tree. Modern cultivars were scattered throughout the tree. European spelt appeared to be largely monophyletic and formed a separate branch in the tree, confirming separate origins of Asian and European spelt.

An N-J tree was also constructed from the nucleotide sequences of 121 D-genome genes in 13 accessions of *T. aestivum*, including Iranian spelt 405a, and 9 randomly selected accessions of *Ae. tauschii* from the strangulata and tauschii gene pools (Akhunov et al. 2010). The accessions were selected so as to represent genetic diversity in *T. aestivum* as reflected in N-J tree constructed for 476 *T. aestivum* lines genotyped at 153 RFLP loci (Akhunov et al. 2010). A total of 2458 polymorphic sites were analyzed. The *Ae. tauschii* genomes present in synthetic wheats RL5405 (*Ae. tauschii* ssp. *strangulata*) and RL5406 (*Ae. tauschii* ssp. *meyeri*) were members of the strangulata gene pool cluster (Figure 4). Like in the tree based on RFLP, the strangulata gene pool cluster and wheat cluster formed sister branches. The *Ae. tauschii* genomes present in synthetic wheats RL5402 and RL5403 were members of the tauschii gene pool cluster, which formed a separate branch of the N-J tree. If Iranian spelt 405a originated by independent hybridization of tetraploid wheat with *Ae. tauschii* in Iran, its D genome would branch off at the base of the wheat branch, which was not observed. Wheat accessions formed a monophyletic branch, and the most basal position in the wheat branch was held by Chinese landraces with very high bootstrap confidence. The branch containing the Iranian spelt 405a was embedded in the wheat branch and clustered with 2 bread wheat landraces collected in southeastern Turkey and Anatolia.

To compare the D-genome relationships with those in the A and B genomes, an N-J tree was built for the 13 *T. aestivum* accessions and 2 accessions of wild emmer from the Diyarbakir area in Turkey, the putative geographic area of emmer domestication (Ozkan et al. 2005; Luo et al. 2007), and 'Altar' durum. The nucleotide sequences of 131 genes in the A and B genomes reported by Akhunov et al. (2010) were used, and 5144 polymorphic sites were analyzed. Although the exact topology of the tree based on the A- and B-genome genes differed from that based on the D-genome genes (Figure 4), the wheat accessions, including the Iranian spelt, formed a single monophyletic branch with 100% bootstrap confidence. The branch was a sister branch to the tetraploid wheat branch including wild emmer and durum. Iranian spelt 405a was again embedded within the *T. aestivum* branch and with CS was most distant from tetraploid wheat accessions. These data show that all 3 genomes of Iranian spelt 405a are part of the monophyletic wheat lineage.

Discussion

Tg Mapping and Genotyping

Tg-D1 was mapped 3.1 cM proximal to SSR marker *Xwmc112* and 4.2 cM distal to EST *X*CA658378. The location of the gene relative to the SSR loci on the *Ae. tauschii* chromosome 2D agreed with that reported by other investigators (Nalam et al. 2007; Sood et al. 2009). Because SSR markers are poorly transferable between wheat genomes, gene-based SNP markers were included on the 2D map and their synteny across the 3 wheat genomes was used to predict the putative location of the *Tg* locus relative to SSRs in the A and B genomes.

The use of segregation of linked SSR markers rather than the *Tg-D1* haplotype itself introduced some degree of uncertainty in the inferences because the confidence in an inference depended largely on the strength of the linkage. Confidence was high in genotyping *Tg-D1* because the SSRs were only several cM away from the *Tg-D1* locus and because the *Tg-D1* locus was mapped on the basis of segregation of the glume phenotype in the DSAt2D5403(CS2D) × CS mapping population. In the B genome, the location of *Tg-B1*

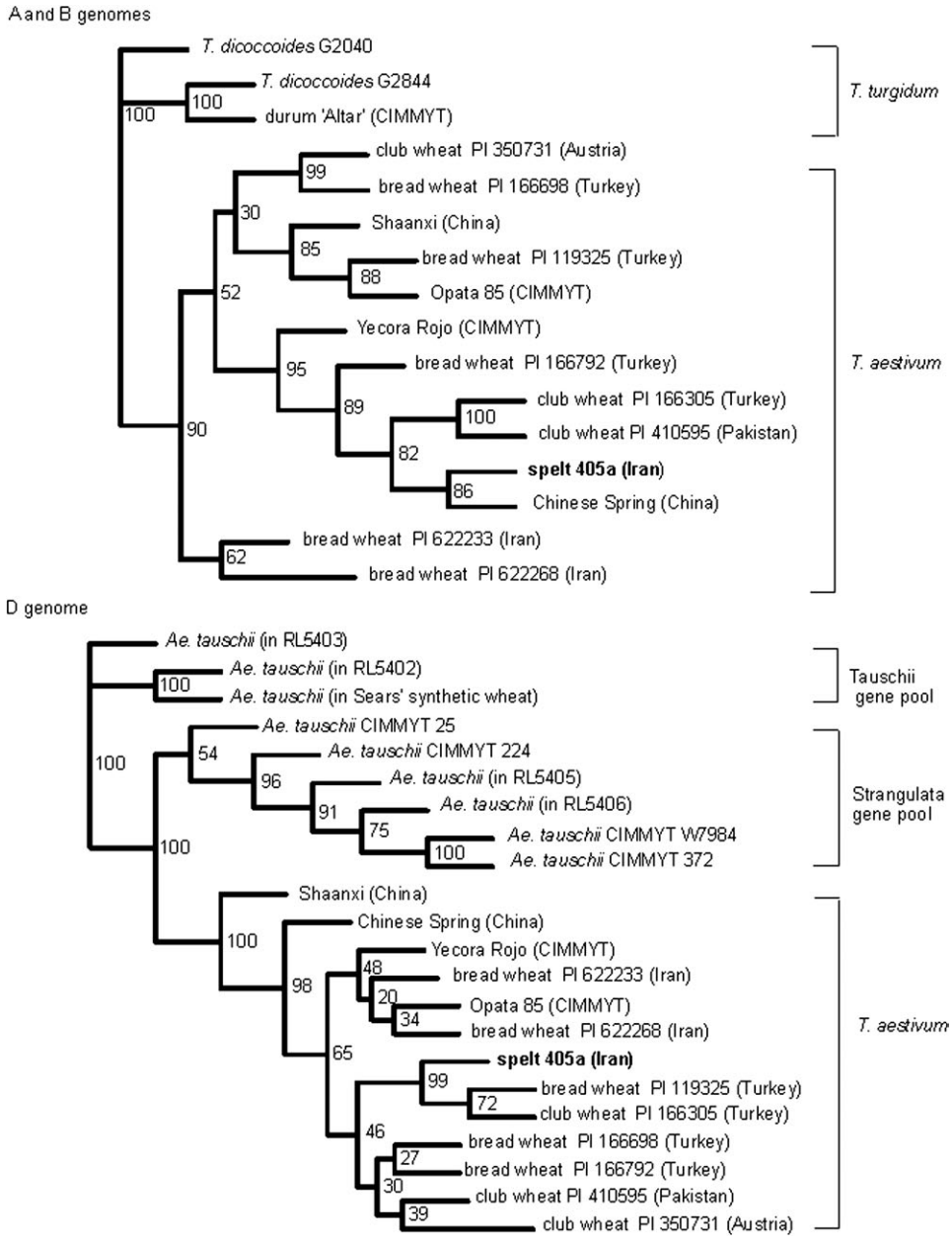

Figure 4. Unrooted consensus trees of 13 accessions of *Triticum aestivum* including Iranian spelt (bold), 9 accessions of *Aegilops tauschii*, 2 accessions of wild emmer from southeastern Turkey, and one accession of durum, based on nucleotide sequences of 131 A- and B-genome genes and 121 D-genome genes. Bootstrap confidence is indicated at nodes.

was inferred on the basis of marker synteny of chromosome 2B with chromosome 2D. The locus is expected to be within a 16-cM interval between *Xwmc25* and *XBE518440*. Because the locus was segregating in most of the crosses of spelt × CS, confidence in the existence of the *Tg-B1* on the spelt chromosome was also high. In contrast, the existence of the *Tg-A1* gene was questionable. The *Sog* locus controlling glume softness was mapped on chromosome $2A^m$ in *T. monococcum*, 75cM proximal to the distal SSR *Xbarc124* (Sood et al. 2009) and proximal to the *Tg* region (Sood et al. 2009). It is possible that the same locus exists in the wheat A genome. The locus would segregate independently of the 2A SSR markers used here. Because of this uncertainty, the inferred glume-tenacity genotypes on spelt chromosome 2A must be treated with caution and await for detailed mapping of genes affecting glume tenacity on the 2A chromosome of wild and domesticated emmer. Surprisingly, linkage was detected between the distal SSR *Xgwm636* and the glume tenacity in spelt accessions PI367199 and VIR 45366 even though the SSR marker should have shown free recombination with *Sog*.

The Origin of Spelt

Ten of the 11 accessions of spelt studied had the inactive *tg-D1* allele on chromosome 2D, which is consistent with spelt being derived from free-threshing wheat by hybridization. The fact that 8 of 10 accessions of spelt had at least one active *Tg* allele in the A or B genome suggests that the other parent was hulled, i.e., domesticated or wild emmer. This finding is consistent with suggestions that European spelt was derived from hybridization of hulled emmer with free-threshing hexaploid wheat (Schiemann 1932; Mac Key 1966).

Additional evidence for the derived origin of European spelt was more recently provided by the distribution of the *a* and *p* B-genome γ-gliadin alleles in tetraploid and hexaploid wheat (von Buren 2001). The *GAG56B a* allele was exclusively found in European spelt and durum, whereas the *p*-type alleles were present in bread wheat. The dichotomy of the A and B genomes of durum and emmer on one hand and those of *T. aestivum* on the other hand was observed also here. The wheat phylogenetic tree based on sequences of 131 genes showed that the A and B genomes of durum are closer to those of emmer than to those of *T. aestivum*. The proximity of the A and B genomes of European spelt to those of emmer and durum is consistent with the origin of European spelt via hybridization involving emmer and argues against the ancestral position of European spelt in the phylogeny of bread wheat.

Half of the accessions of European and Asian spelt segregated compact spikes. The genetic cause of spike compactness was examined in spelt PI330558 and was found to be due to the *C* gene on spelt chromosome 2D. At least for some of the spelt, the free-threshing hexaploid parent was therefore club wheat, which substantiated the hypothesis that, at least some accessions of European spelt originated from hybridization of club wheat with emmer (Schiemann 1932; Mac Key 1966). Because the *C* allele for compact spikes is dominant over the lax spike allele, and because spelt PI330558 had lax spike morphology, *C* must be hypostatic to some other gene in spelt, possibly *q*.

Current data suggest that free-threshing wheat was an ancestor of not only European spelt but also of some of the Asian forms of spelt although the exact role free-threshing wheat has played is debatable. It is theoretically possible that spelt is indeed ancestral and originally had the *Tg-D1* allele, but the allele was replaced by the *tg-D1* allele as a consequence of subsequent hybridization of spelt with free-threshing hexaploid wheat. The following reasoning makes this scenario unlikely. The substitution of the spelt *q* gene for *Q* in CS resulted in partially tenacious glumes (Luo et al. 1999). Likewise, substitution of a *Tg* gene for *tg*, as in the DSAt2D (CS2D) lines, also resulted in partially tenacious glumes. Therefore, if spelt originated as McFadden and Sears (1946) imagined, spelt genotype would be *Tg-A1/Tg-A1* (or *Sog/Sog*); *Tg-B1/Tg-B1*;*Tg-D1/Tg-D1*;*qq*. If such spelt hybridizes with free-threshing wheat, all 4 loci would segregate, and progeny with tenacious glumes could be produced by any combination of the 4 active alleles. The likelihood of the *Tg-D1* allele to persist in the progeny would be the same as that of *Tg-B1*. That was not observed. Most accessions of spelt had *Tg-B1* but only one had *Tg-D1* ($P < 0.01$).

If spelt was ancestral but was later subjected to recurrent hybridization with sympatric free-threshing wheat leading to the near fixation of the *tg-D1* allele in spelt, all 3 spelt genomes would be related to sympatric free-threshing wheat and spelt accessions would therefore be scattered across the *T. aestivum* phylogenetic tree. This pattern was observed for modern wheat cultivars but not for spelt. Of 53 accessions of European spelt, 45 formed a single monophyletic branch in the phylogenetic tree of hexaploid wheat based on D-genome RFLP. At locus *Xpsr901* on 2D, 41 of 50 ($f = 0.82$) European spelt accessions shared an otherwise rare ($f = 0.03$) *b* allele, which was shared with *Ae. tauschii* (Dvorak et al. 1998b). The *Xpsr901* frequencies mimic the distribution of γ-gliadin alleles in European spelt and bread wheat (von Buren 2001). Most accessions of Asian spelt also appeared to be related to each other and were allocated to the same branch in the D-genome N-J tree. The branch was separate from a branch formed by the European accessions of spelt, indicating that Asian and European accessions of spelt were polyphyletic. Spelt polyphylesis has been suggested also on other grounds (Jaaska 1978; Blatter et al. 2002). However, because all spelt share the same basic D-genome genotype, indicated by monophylesis of the wheat branch in the phylogenetic tree, and by having the same *Nor-D1*a rDNA haplotype (Dvorak et al. 1998b), they all were almost certainly derived from a common hexaploid ancestor. Subsequent gene flow from *Ae. tauschii* expanded the diversity of wheat D genome as evidenced by shared polymorphism in *T. aestivum* D genome and *Ae. tauschii* (Dvorak et al. 1998a, 1998b; Talbert et al. 1998; Blatter et al. 2002; Caldwell et al. 2004).

Origin of Free-Threshing Hexaploid Wheat

If hexaploid wheat originated by hybridization of domesticated emmer with *Ae. tauschii*, as is widely believed, the transformation of such wheat to free-threshing wheat would require recessive mutations at the *Tg* loci and *Sog* if present in wheat and a dominant mutation at the *q* locus. The accumulation of up to 4 mutations, particularly, the dominant mutation of *q* to *Q*, and their fixation in the population of hexaploid wheat would take time, which makes the absence of spelt before the appearance of free-threshing wheat in the archaeological sites perplexing. If, however, the tetraploid ancestor of hexaploid wheat was already free-threshing, only a single recessive mutation, from *Tg-D1* to *tg-D1*, would be needed to make the primitive hexaploid free-threshing. In that case, little time would be needed for the emergence of fully free-threshing wheat, which is consistent with the archaeological record.

If the tetraploid ancestor of hexaploid wheat were free-threshing, the resulting hexaploid might have been similar to the synthetic wheats shown in Figure 1; they were produced from crosses of a free-threshing tetraploid extracted from the A and B genomes of *T. aestivum*, with *Ae. tauschii*. Using the properties of the synthetic wheats as an approximation

of nascent hexaploid wheat, the following characteristics would set the hexaploid apart from spelt. 1) Because of the presence of *Q* on 5A, the glumes of hexaploid wheat ancestor would be less tenacious than those of spelt and spikes would be more compact than those of spelt because *q* increases glume tenacity and brings about lax spike morphology. 2) Fragments of rachis of such primitive wheat would persist in chaff even after full mechanical liberation of seeds from glumes, as shown in Figure 1. 3) The seeds of the nascent hexaploid would be round and similar to those of *T. aestivum* ssp *aestivum* and would differ from the seeds of spelt, which are long and often have a keel reminiscent of seeds of emmer (Figure 5). The roundish shape of seeds characteristic of free-threshing hexaploid wheat is controlled to a large extent by genes in the A and B genomes. This is shown by the shape of seeds in tetraploid *T. turgidum* ssp *carthlicum*, which are virtually indistinguishable from those of bread wheat. Search for D-genome germplasm in 14 *T. turgidum* ssp *carthlicum* accessions with 29 RFLP loci evenly distributed across the D genome failed to reveal any D-genome germplasm in the genome of *T. turgidum* ssp *carthlicum* (Dvorak J and Luo MC, unpublished data).

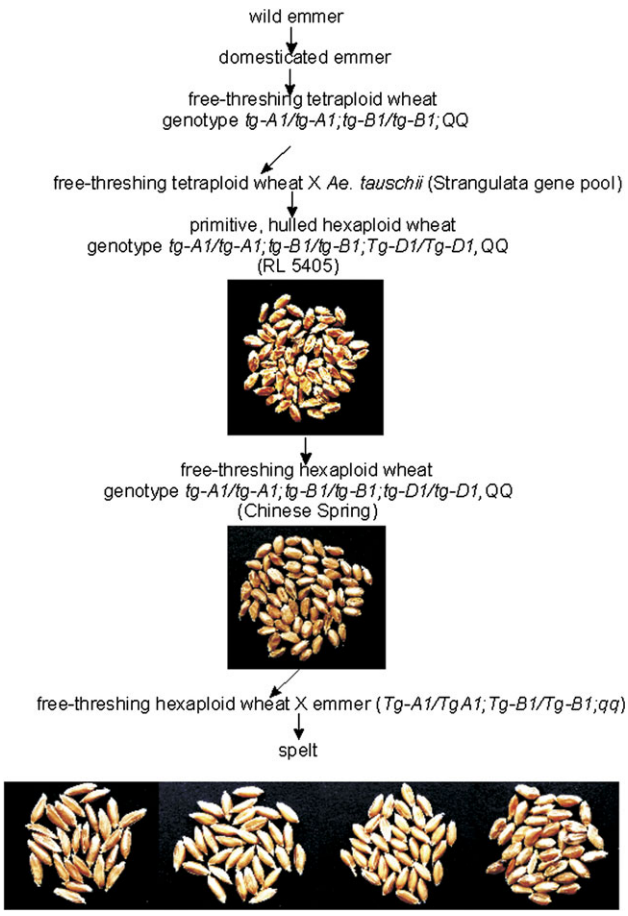

Figure 5. A model of evolution of free-threshing wheat and spelt (except for those from Iran). Seed sources are in parentheses except for those of spelt that are from left to right: PI347926 (Switzerland), PI221419 (former Yugoslavia), PI330558 (United Kingdom), and PI367199 (Afghanistan).

The descent of hexaploid wheat from free-threshing tetraploid wheat is also more consistent with the origin and distribution of the *Q* gene. The *Q* allele is essential for the square-head spike morphology of hexaploid wheat and its free-threshing. Since *Q* originated only once, it could have originated either at the tetraploid or at the hexaploid level (Simons et al. 2006). If it originated at the hexaploid level (spelt being ancestral), free-threshing hexaploid wheat would have to precede free-threshing tetraploid wheat and *Q* would have to migrate from hexaploid wheat to tetraploid wheat to become fixed in all free-threshing tetraploid lineages. If mutation of *q* into *Q* took place at the tetraploid level, the nascent hexaploid would have *Q*, which would be immediately fixed in the hexaploid population. It was suggested before that most free-threshing wheat in archaeological sites in western Asia was tetraploid (Zohary and Hopf 1994). Kislev (1979/1980) draw attention to the existence of extinct, free-threshing wheat with small, round seeds, he called *T. parvicoccum*, in sites across Middle East and Balkans and speculated that it was tetraploid.

The following scenario of the evolution of free-threshing hexaploid wheat is proposed here (Figure 5). Free-threshing syndrome is postulated to have originated at the tetraploid level. Free-threshing tetraploid wheat hybridized with strangulata gene pool in southwestern Caspian Iran or Transcaucasia. A mutation from *Tg-D1* to *tg-D1* converted the ancestral hexaploid wheat into a fully free-threshing form. Seeds of the ancestral hexaploid and those of its free-threshing hexaploid descendants were shorter than those of spelt and resembled those of bread wheat (Figure 5). In this scenario, the primitive hexaploid wheat is expected to be hulled due to the *Tg-D1* allele on chromosome 2D but to have the *Q* gene on 5A. Of all of the forms of spelt studied to date, only Iranian spelt has shown these attributes. Iranian spelt, 77d collected near Esfahan (I250 in Supplementary Figure 2), had the *Tg-D1* allele and is a member of the monophyletic branch of *T. aestivum* (present data and Dvorak et al. 1998a, 1998b). Another Iranian spelt, 407a (=I252 in Supplementary Figure 2), had the *Q* allele, characteristic of free-threshing wheat, instead of the *q* allele expected for spelt (Luo et al. 1999).

Since all accessions of Iranian spelt belong to the monophyletic *T. aestivum* population, the presence of characteristics expected in the primitive hexaploid wheat among Iranian spelt, such as *Tg-D1* and *Q*, suggests that Iranian spelt populations are remnants of the missing link between free-threshing tetraploid wheat and free-threshing hexaploid wheat. However, *Tg-B1* was also detected in Iranian spelt, suggesting that the tetraploid parent of Iranian spelt was emmer. While *Tg-D1* and *Q* are consistent, *Tg-B1* conflicts with what is suggested here for the missing link.

A number of lines of evidence show that most of the nucleotide diversity in hexaploid wheat was contributed by hybridization (Akhunov et al. 2010; Dvorak et al. 2011). Because hybridization of hexaploid wheat with *Ae. tauschii* is difficult, gene flow from *Ae. tauschii* into *T. aestivum* must have taken place principally via triploid hybrids or hexaploid amphiploids from hybridization of tetraploid wheat with *Ae. tauschii* (Dvorak et al. 1998b). The structure of nucleotide

diversity in *T. aestivum* shows that these events lagged greatly behind the expansion of the cultivation of hexaploid wheat resulting in the impoverishment of D-genome diversity (Akhunov et al. 2010). For this same reason, however, the D genome is more informative about the structure of the gene pool of hexaploid wheat at the dawn of its evolution than the A and B genomes. The phylogenetic tree based on nucleotide sequences of 121 D-genome genes including 2458 polymorphic sites, placed both Chinese landraces at the basal position in the tree. The basal position of Chinese wheat in wheat evolution is consistent with the antiquity of Chinese wheat and the idea that it conserved the gene pool of young *T. aestivum* as consequence of migrating outside of the geographic distribution of tetraploid wheat and *Ae. tauschii*, which severed gene flow from the ancestors into the hexaploid gene pool (Dvorak et al. 2006). If Iranian spelt is ancestral to all hexaploid wheat, its D genome should have the same basal position in the tree as Chinese wheat. Spelt 405a (=I249 in Supplementary Figure 2), collected 21 km from spelt accession 407a in north western Iran (Kuckuck 1964) was included into a phylogenetic tree built from nucleotide sequences of 121 D-genome genes. The spelt clustered with Turkish bread and club wheat. This fact and the observation that genotypes of different accessions of Iranian spelt differ from each other suggest that, if Iranian spelt population is the ancestor of *T. aestivum*, it was very likely subsequently hybridized with other wheat.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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