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Relationships of *Aegilops tauschii* revealed by DNA fingerprints: The evidence for agriculture exchange between China and the West

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Abstract

Genetic diversity and relationships of wild goat grass (*Aegilops tauschii* Cosson) from Iran and Xinjiang, west China, as well as its weedy type from the Yellow River region of Shaanxi and Henan provinces in China were analyzed by simple sequence repeat (SSR) fingerprints. A high level of genetic diversity in *Aegilops tauschii* accessions from Iran was observed, and the richness of genetic diversity was followed by accessions from Shaanxi, Henan, and Xinjiang. The weedy type of *Aegilops tauschii* showed a close genetic relationship with the wild type from different regions in Iran. The results indicated that the weedy *Aegilops tauschii* found in the Yellow River region was most likely introduced from Iran—the diversity center of *Aegilops tauschii*. The weedy *Aegilops tauschii* populations found in the Yellow River region may be brought into the central part of China as a weed species together with common wheat (*Triticum aestivum* L.) from the West during various periods of time in history. This finding has provided strong evidence for the introduction of common wheat from the West into China *via* the Silk Road, and also demonstrated the important role of the Silk Road in the exchange of agriculture and other relevant technologies between China and the West.

Keywords: The Silk Road; *Aegilops tauschii*; Common wheat; SSR fingerprints; Genetic diversity; Affinity; Crop dispersion

1. Introduction

The Silk Road is a series of trade and cultural transmission routes that were central to cultural interaction through regions of the Asian continent connecting East and West Asia by linking traders, merchants, pilgrims, and urban dwellers from China to the Mediterranean Sea during various periods of time. Geographically, the Silk Road was interconnected series of ancient trade routes connecting Chang'an (today's Xi'an) to the West. Trade on the Silk Road was an important factor for the development of the great civilization of China, Japan, Egypt, Persia, Indian subcontinent, and Rome, and helped to lay the foundations for the modern world [1]. On the one hand, Chinese silk and culture were exported to the West, and on the other hand, advanced technologies, religions, cultures, economy, and other civilization activities were introduced to China *via* the Silk Road [1]. Historically, the trade route was initiated around 114 BC by the Han Dynasty, largely through the missions and explorations of Zhang Qian, although earlier trade across the continents had already existed. Till

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the Tang Dynasty, the Silk Road became most flourishing with the comprehensive exchange of religions, sciences, militaries, business trades, and agriculture [2]. However, during the Song and Yuan Dynasties, the economic center of China begun to move southward, and at the same time, business trades *via* sea routes become prevailing. Thereafter, the importance of the Silk Road started to decline. To the Ming Dynasty and Qing Dynasty, the Silk Road became stagnant.

According to the recent archaeological documentation, the trade and cultural transmission routes along the Silk Road already existed long before Zhang Qian, and they had played an important role in culture exchanges in the prehistorical periods. For example, the hexaploid common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) originated from the Fertile Crescent region in west Asia was introduced to the central part of China *via* the Silk Road about 4000 years ago [3,4]. To date, the routes and period of common wheat introduction to China is still in debating [2–4]. In this study, we analyzed genetic diversity and differentiation in the accessions of *Aegilops tauschii* Cosson collected in the fields of cultivated wheat as an accompanying weed, based on DNA fingerprints using genomic SSR molecular markers. The objective of this study was to explain the puzzle of how common wheat was introduced to China from a completely different angle.

Aegilops tauschii is one of the ancestral species that has donated the D genome to common wheat, and it serves as important genetic resources for wheat improvement [5,6]. Generally, there are two types of *Aegilops tauschii*, namely, the wild and weedy types. The wild type is distributed in natural habitats and reproduces naturally, whereas the weedy type occurs in agriculture ecosystems together with cultivated wheat as an accompanying weed. Therefore, the weedy type can only reproduce and disseminate under human's disturbance or assistance in agro-ecosystems. Since the weedy *Aegilops tauschii* is characterized by its fragile rachis, most spikes of the weedy *Aegilops tauschii* shatter at seed maturity and the shattered rachis fall into the soil of wheat fields before wheat harvesting. The shattered rachis (seeds) with strong dormancy can persist in soils till the next wheat growing season, while some non-shattered spikes will be harvested together with wheat and mixed in wheat grains. The weedy type of *Aegilops tauschii* remains and disseminates in agro-ecosystems by adaptation and growing together with wheat through generations. Therefore, it is only found in and/or near wheat farmlands. The wild type of *Aegilops tauschii* is distributed from the Mediterranean region, along the coastal areas of southeastern Turkey, to Iran, Syria, Russia (Caucasia, Turkmen, Pamirs-Artlett), Kazakhstan of Caspian, Afghanistan, Pakistan, and extending eastwards to the Yili Valley of Xinjiang in China [4–6]. Iran was commonly recognized as the centers of origin and genetic diversity for *Aegilops tauschii* [6].

Both wild and weedy types of *Aegilops tauschii* are found in China. The wild type of *Aegilops tauschii* populations is only found in the Yili Valley of Xinjiang, where is the eastern boundary of the wild populations of *Aegilops tauschii* in natural habitats for its world distribution [6–10]. Based on the records in literature including "*the Flora of China*", the weedy type of *Aegilops tauschii* from China was found in agro-ecosystems of the middle Yellow River region (e.g., Shaanxi and Henan) in the 1950's, occurring only as a weed species in wheat farmlands [11,12]. These weedy populations found in the middle Yellow River region are disjunctive distribution of the wild *Aegilops tauschii* populations occurring in the Yili Valley or other regions. It is difficult to believe that the weedy type of *Aegilops tauschii* was introduced from the West to central China by natural forces (e.g., seed or pollen dispersal), considering the fact that the natural dissemination of *Aegilops tauschii* through seed shattering is very limited. What is the reason for the weedy type of *Aegilops tauschii* that is distributed separately from its main distribution range and occurs sporadically in the middle Yellow River region? How and by what means was the weedy type of *Aegilops tauschii* introduced to the central part of China? These are interesting and important questions that should be addressed, not only for the archeological but also for the agricultural and biological point of views. Some authors reported genetic similarities of the weedy type occurring in the Yellow River region with the wild type found in the Yili Valley, through analyses using isozyme and gliadin electrophoreses [13,14]. However, there was no any explanation for the presence of the weedy *Aegilops tauschii* populations in the Yellow River region. We hypothetically propose that the weedy type of *Aegilops tauschii* found in the Yellow River region was introduced from west Asia into China (Chang'an) together with common wheat *via* the trade and cultural transmission along the Silk Road. Once introduced, the weedy type of *Aegilops tauschii* was spread to the nearby agricultural areas together with the further dissemination and cultivation of common wheat. Interestingly, the weedy *Aegilops tauschii* was only found in common wheat fields where primitive cultivation styles were still remained and traditional wheat landraces were cultivated.

In order to provide evidence to our hypothesis that the weed type of *Aegilops tauschii* was introduced to China along with the exchange of cultivated crops and agricultural technologies during the course of trade and cultural transmission between the ancient China and central/west Asia *via* the Silk Road, we used the simple sequence repeat (SSR) fingerprint technique to determine genetic diversity and relationships of the weedy and wild types of *Aegilops tauschii* collected from China and Iran—the center of *Aegilops tauschii* diversity. The primary objective of this study was to investigate genetic affinities of the weedy *Aegilops tauschii* populations from the Yellow River region with those from other regions, and to determine the possible dissemination route of the weedy *Aegilops tauschii*. The important role of the Silk Road in the exchange of agriculture and its relevant technologies between China and the

West was also discussed based on the evidence of DNA fingerprinting.

2. Materials and methods

2.1. Plant materials

The *Aegilops tauschii* materials were collected from natural populations in central Asia (mostly in Iran) and in China (Xinjiang), the weedy populations were from the Yellow River region. A wheat cultivar, Chinese Spring (CS), from Sichuan Province in China was used as a control. All accessions (Table 1) were provided by the Crop Research Institute (CRI) of the Sichuan Academy of Agricultural Sciences (SAAS).

2.2. DNA extraction

Seed samples from all accessions were germinated in a growth chamber at 30 °C. The genomic DNA of 5 individuals per population was extracted at the three-leaf seedling stage [15]. The agarose gel electrophoresis and image acquisition and analysis were applied to determine the concentration of the extracted genomic DNA.

2.3. SSR analysis

Thirty-four SSR primer pairs developed from the D genome of common wheat distributed on different chromosome arms were used for analysis (Table 2). Of the 34 primer pairs, 18 were Xgwm primers according to Röder's classification [15], 16 were Xgdm primers according to Pestsova's classification [16]. The SSR markers were synthesized by the Takara Biotechnology Co. Ltd.

PCR was performed in a 25 μl reaction mixture following the protocol of Peng et al. [17], which contained PCR buffer (100 mM Tris–HCl pH 8.3), 1.5 mM $MgCl_2$, 0.2 mMdNTP, 50 ng microsatellite markers, 1 U Taq polymerase and 50–100 ng template DNA. Amplification on PTC-100^{TM} was carried out as follows: 3 min at 94 °C for denaturation, followed by 44 cycles of 1 min at 94 °C, 1 min at 60 °C, 2 min at 72 °C, 10 min at 72 °C for a final extension.

Table 1
Aegilops tauschii accessions and a common wheat cultivar used in this study with their accession code and origin

No[a]	Code of accessions[b]	Name or source	Origin
1	AS-77_h	Lushi goat grass	Henan, China
2	AS-78_h	Lushi goat grass	Henan, China
3	AS-79_h	Sanmen Gorge goat grass	Shaanxi, China
4	AS-80_h	Huixian goat grass	Henan, China
5	AS-82_h	Xinxiang goat grass	Henan, China
6	CD-21_s	Henan goat grass	Henan, China
7	AS-75_s	Xian goat grass	Shaanxi, China
8	AS-76_s	Dayan Pagoda goat grass	Shaanxi, China
9	CD-25_s	Wugong goat grass	Shaanxi, China
10	CD-27_s	Shaanxi goat grass	Shaanxi, China
11	AS-71_x	Gongnaisi goat grass	Xinjiang, China
12	AS-72_x	Yili goat grass	Xinjiang, China
13	AS-60_I	Middle East goat grass	Iran
14	AS-67_I	Iran goat grass	Iran
15	AS-68_I	Iranian goat grass	Iran
16	Ku2073_I	Japan	Iran
17	Ku2074_I	Japan	Iran
18	Ku2076_I	Japan3	Iran
19	TQ-02_I	Israel	Iran
20	TQ-08_I	Israel	Iran
21	TQ-13_I	Israel	Iran
22	TQ-17-1_I	Israel	Iran
23	TQ-18-12_I	Israel	Iran
24	TQ-22/2_I	Israel	Iran
25	TQ-38_I	Israel	Iran
26	TQ-56_I	Israel	Iran
27	TQ-81_I	Israel	Iran
28	TQ-90_I	Israel	Iran
29	TQ-16_I	Israel	Iran
30	SQ-214_I	CIMMYT	Iran
31	SQ-222_I	CIMMYT	Iran
32	CS	Chinese Spring	Sichuan Province, China

[a] Materials of No. 1–10 were weedy type of *Aegilops tauschii* and No. 11–31 were wild type of *Aegilops tauschii* from natural populations.
[b] AS, code for the Triticeae Research Institute of Sichuan Agricultural University; CD, code for the Crop Research Institute, Sichuan Academy of Agricultural Sciences; Ku, code for Kyoto University, Japan; TQ, code for the Weizmann Institute of Science, Israel; SQ, code for the International Maize and Wheat Improvement Center (CIMMYT), Mexico.

Table 2
The 34 microsatellite (SSR) primer pairs used in analysis with their name and location on chromosomes, *PIC* and Shannon index

Name and the location[a]	*PIC*	Shannon index (*I*)
Xgdm33-1D (S)	0.768	1.651
Xgwm106-1D (S)	0.615	0.999
Xgwm642-1D (L)	0.561	1.066
Xgdm126-1D (L)	0.705	1.366
Xgdm111-1D (L)	0.490	0.769
Xgwm261-2D (S)	0.555	1.041
Xgdm210-2D (S)	0.482	0.692
Xgwm102-2D (S)	0.617	1.208
Xgwm157-2D (L)	0.432	0.743
Xgdm6-2D (L)	0.754	1.502
Xgwm382-2D (L)	0.816	1.737
Xgwm114-3D (S)	0.818	1.813
Xgwm183-3D (S)	0.486	0.961
Xgdm128-3D (L)	0.409	0.786
Xgwm383-3D (L)	0.446	1.070
Xgdm38-3D (L)	0.779	0.810
Xgdm129-4D (S)	0.627	1.287
Xgdm165-4D (L)	0.555	1.041
Xgwm194-4D (L)	0.373	0.761
Xgwm609-4D (L)	0.271	0.512
Xgwm192-5D (S)	0.604	1.104
Xgwm205-5D (S)	0.490	0.769
Xgwm583-5D (L)	0.609	1.295
Xgdm116-5D (L)	0.531	0.900
Xgdm118-5D (L)	0.492	0.685
Xgwm469-6D (S)	0.539	0.922
Xgdm108-6D (S)	0.607	1.015
Xgdm14-6D (S)	0.277	0.582
Xgwm55-6D (L)	0.447	0.723
Xgdm98-6D (L)	0.662	1.260
Xgdm130-7D (S)	0.443	0.787
Xgwm44-7D (C)	0.421	0.798
Xgdm46-7D (L)	0.498	1.021
Xgwm428-7D (L)	0.578	1.034

The annealing temperature of all microsatellite primer pairs is 60 °C.
[a] (S) Short chromosome arms, (L) long chromosome arms, (C) the centromere.

2.4. Denaturing polyacrylamide gel electrophoresis

The amplified DNA products were loaded on 6% denaturing polyacrylamide gels and separated by electrophoresis for 2 hours at 200 to 350 V. The banding patterns were visualized after the silver staining. The banding patterns on the gels were documented by photographing with a digital camera.

2.5. Data analysis

SSR markers are co-dominant, therefore, different bands under the same primer pairs represent different alleles at a particular locus. The banding patterns were scored as genotypes and represented by capital letters. The homozygous genotypes were recorded as AA, BB, or CC, and the heterozygous genotypes were recorded as AB, AC, BC, etc. The output of the scored banding patterns of all the Aegilops. *tauschii* accessions was transformed into a data matrix that was subjected to the analysis by the software package PopGene (ver. 1.31) [18]. The genetic similarity coefficient and Shannon index were analyzed. $PIC = 1-\Sigma p_i^2$ was calculated to reflect polymorphism informative content (*PIC*) [19], where p_i stands for the frequency of the *i*th allele at a particular SSR locus of the Aegilops *tauschii* accessions. An arbitrary standard based on the *PIC* value was set to determine genetic diversity: *PIC* value <0.25 was as lower level of genetic diversity and *PIC* value >0.50 as higher level of genetic diversity [20]. $I = -\Sigma p_i \ln p_i$ was adopted to reflect Shannon index, where p_i stands for the frequency of the *i*th allele at a particular SSR locus. Cluster analysis was conducted based on the matrix of similarity coefficients of genomic SSR in all the Aegilops *tauschii* accessions, using the sequential agglomerative hieratical nested cluster analysis (SAHN) and unweighted pair-group method arithmetic average (UPGMA) methods installed in the software package NTSYS-pc [21].

3. Results

3.1. Genetic diversity detected by SSR makers

Different Aegilops *tauschii* accessions showed a relatively high level of genetic diversity as revealed by the SSR fingerprints (Fig. 1). The *PIC* values of the analyzed SSR loci varied from 0.27 to 0.82, with an average of 0.55, in all the accessions (Table 2). Following the Botstein standard of *PIC* [20], the average *PIC* value of 34 primer pairs was all higher than 0.25—the value that indicates low level of genetic diversity. Of the 34 *PIC* values, 14 varied between 0.25 and 0.50, indicating a moderate level of genetic diversity in theses loci, and 19 (55.90% of 34 markers) were higher than 0.50, indicating a high level of genetic diversity in these loci. In general, the results showed that 31 Aegilops *tauschii* accessions had a higher level of genetic diversity. *PIC* values of the primer pairs Xgdm33-1D(S), Xgdm126-1D (L), Xgdm6-2D (L), Xgwm382-2D(L), Xgwm114-3D(S), and Xgdm38-3D(L) were higher than 0.70, indicating a very high level of genetic diversity, whereas those of Xgwm609-4D (L), Xgwm194-4D (L), and Xgdm14-6D (S) were lower than 0.40, suggesting a relatively moderate level of genetic diversity. The values of Shannon index (*I*) were higher than 1.5 for the loci distrib-

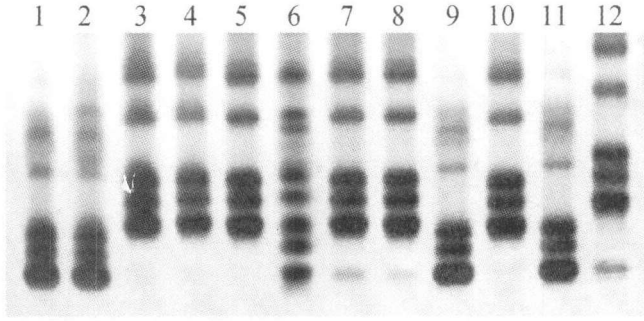

Fig. 1. Electrophoretic results of the amplified products of Xgwm106-1D (S) detected by PAGE. Lanes 1–11 are AS-79_h, AS-80_h, AS-82_h, CD-21_s, AS-75_s, AS-76_s, CD-25_s, CD-27_s, AS-71_x, AS-72_x, and AS-60_I, respectively, and lane 12 is Chinese Spring (CS). The codes refer to those in Table 1.

uted on chromosomes 1D, 2D, and 3D, whereas that of Xgwm609-4D(L) was only 0.512 (the lowest in all accessions) (Table 2). These results indicated that chromosomes 1D, 2DL and 3D of Aegilops *tauschii* have a relatively high level of genetic diversity at SSR loci, but chromosome 4D has relatively low genetic diversity.

3.2. Cluster analysis

A dendrogram was constructed based on the cluster analysis of genetic similarity coefficients of genomic SSR fingerprints in all the accessions using the UPGMA method (Fig. 2). All the Aegilops *tauschii* and wheat accessions were clustered into three major groups. The Group I included the wheat cultivar, Chinese Spring (CS), and one Aegilops *tauschii* accession from Iran (SQ-222_I); Group II included nine wild Aegilops *tauschii* accessions from Iran; Group III encompassed other nine wild Aegilops *tauschii* accessions from Iran, two wild accessions from Xinjiang, and all the weedy accessions from the Yellow River region. This result in general suggested that: (1) the wheat cultivar (CS) and an Aegilops *tauschii* accession from Iran were closely related (with 100% of similarity coefficient), supporting the previous conclusion that Aegilops *tauschii* is one of the ancestral species of common wheat; (2) Aegilops *tauschii* accessions from different localities were considerably differentiated, and some accessions from Iran and Xinjiang shared a close genetic affinity; (3) the weedy Aegilops *tauschii* accessions from the Yellow River region (Shaanxi and Henan) possessed a close genetic relationship with some wild accessions from Iran, and they were closely related to each other (Fig. 2). Further analysis demonstrated significant genetic differentiation between the weedy accessions from Shaanxi and Henan within Group III, where two subgroups (Shaanxi subgroup and Henan subgroup) were clearly identified based on genetic differentiation of the accessions. The Shaanxi subgroup included all the accessions from Shaanxi, whereas the Henan subgroup included all accessions from Henan and one accession from Shaanxi. These results evidently indicated that the weedy Aegilops *tauschii* accessions should have been introduced directly to Chang'an (Xían) – the ancient Chinese capital that was the eastern ending point of the Silk Road, from the West (Iran) as a weed mixed with common wheat. After its introduction, the weedy *Aegilops tauschii* was spread out from Chang'an to other areas along the Yellow River region, together with the dispersion and cultivation of the cultivated wheat. Considerable genetic differentiations between the weedy Aegilops *tauschii* populations distributed in Shaanxi and Henan were developed after a long period of geographic isolation. In addition, cluster analysis indicated that genetic relationship between the weedy Aegilops *tauschii* accessions from Shaanxi and wild accessions from Iran was closer than that between accessions from Shaanxi and Xinjiang, of which the two wild accessions from the Yili Valley were separated in the two subgroups. The wild accession (AS-72_x) collected from Xinjiang showed the closest relationship with that from Iran, and this result was in a complete concordance with the reality that Xinjiang is the eastern boundary of the natural distribution for wild Aegilops *tauschii*.

4. Discussion

Our data from the SSR fingerprints clearly showed abundant genetic diversity in the wild Aegilops *tauschii*

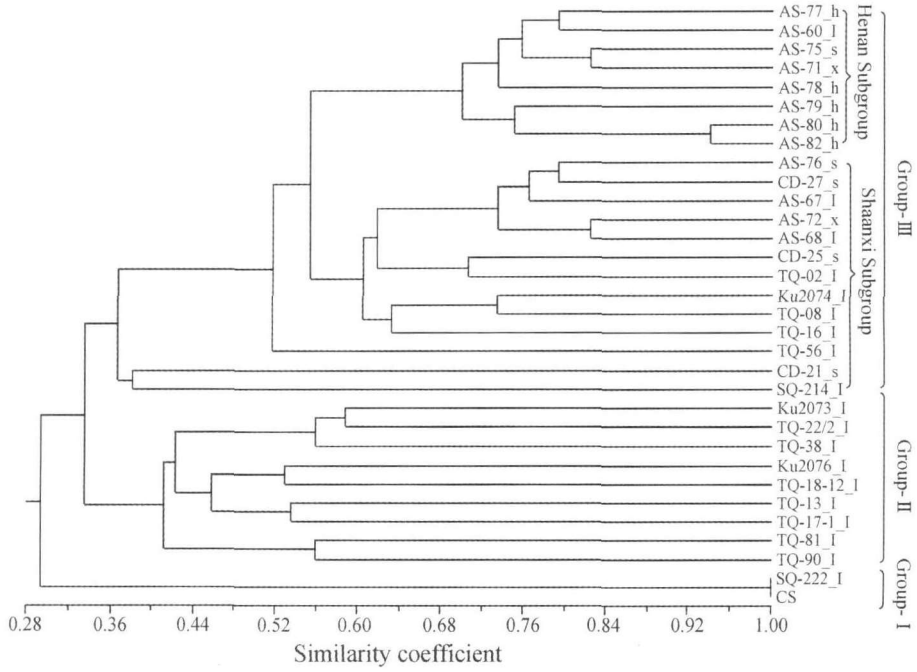

Fig. 2. A UPGMA dendrogram of the 31 *Aegilops tauschii* accessions and the wheat cultivar Chinese Spring (CS) constructed based on genetic similarity coefficients of the genomic SSR fingerprints.

accessions collected from Iran. The weedy type of Aegilops *tauschii* accessions collected from the Yellow River region (Shaanxi and Henan) also had moderate genetic diversity. The abundant genetic diversity among the Aegilops *tauschii* samples collected from a wide range of areas served as a solid foundation for the study of genetic relationships among the wild and weedy types of Aegilops *tauschii*. Our data further demonstrated that wild Aegilops *tauschii* accessions collected from Xinjiang of China were closely related to those from some areas in Iran, suggesting that Aegilops *tauschii* occurring in Xinjiang is a portion of the wild Aegilops *tauschii* gene pool in the world. Aegilops *tauschii* is an annual plant species, of which the wild type is widely distributed in Mediterranean and Central Asia, and the weedy type appears as a weed in agricultural fields of many wheat cultivation regions. Why is Aegilops *tauschii* found in the Yellow River region (Shaanxi and Henan) of China only as weedy populations? Why there were no natural populations with the capacity of self-reproduction found there? Considering that the Yellow River region is geographically isolated from the range of natural distribution of Aegilops *tauschii*, then in which way this species is disseminated to the central part of China?

The plausible explanation for the disjunctive distribution of Aegilops *tauschii* in the Yellow River region of China is by human's activity, through which the long-distance dispersion (e.g., moving crops around) becomes possible. There are two types of Aegilops *tauschii*: the wild and weedy types. The former develops into self-reproducible populations occurring in natural habitats, but the latter can only survive under the human disturbed agro-ecosystems as weedy populations in wheat farmlands. The Aegilops *tauschii* accessions found in the Yellow River region belong to the typical weedy type. Aegilops *tauschii* is a strict self-pollinating species, and the dispersion of its seeds relies essentially on shattering of spikes that fall on the grand at seed maturity, therefore, the distance of seed movement is considerably limited. This species is characterized by having fragile rachis, enclosed seeds with hulled glumes, and strong seed dormancy, and these characters promoted the persistence of Aegilops *tauschii* as an accompanying weed in the fields and the adjacent areas of cultivated wheat farmland. The Yellow River region was the cradle of Chinese civilization and the centers of agriculture, economy, and culture, as well as the focal point for exchange between China and the West, which provided a prerequisite for the introduction (with wheat) and propagation of Aegilops *tauschii*, as a weed of wheat crop.

The SSR fingerprint analysis indicated that the weedy type of Aegilops *tauschii* found in the Yellow River region possesses a relatively high genetic affinity with the wild Aegilops *tauschii* accessions collected from different localities in Iran. To date, many weedy populations of Aegilops *tauschii* are still found in the Middle East region, including Iran. This indicates that weedy type of Aegilops *tauschii* found in the Yellow River region is originated from Iran or the neighboring areas. The weedy populations found in the Yellow River region of China are beyond the natural distribution range of wild Aegilops *tauschii*. It is therefore extremely difficult to explain the presence of weedy Aegilops *tauschii* in central China through natural seed dispersal. The only way to interpret the presence of the weedy Aegilops *tauschii* populations in central China is the human activity-mediated long distance dispersal. The existence of the Silk Road as important trade and cultural transmission routes is well known for a long historical time [1,2]. Therefore, the West-and-China communication *via* the Silk Road has played an essential role in the exchange of agriculture and its relevant technologies, through which the weedy type of Aegilops *tauschii* was introduced to central China together with common wheat. It is well known that the hexaploid common wheat was originated from Central Asia, from where this crop species was disseminated and cultivated over the world [22,23]. Therefore, the introduction of common wheat and its accompanying weed Aegilops *tauschii* to China from the West, as a part of agricultural exchanges, *via* the Silk Road provides reasonable explanation to the occurrence of this species in wheat cultivation areas in central China. That can also explain the reason why the weedy type found in China still maintained a close relationship with the wild type of Aegilops *tauschii* from its center of origin. It is deduced that the civilized ancient China and Persia maintained intimate and frequent exchanges for trade, culture, and scientific technology at about 4000 years ago. Agriculture should have played a predominantly important role in the society at that time, and naturally agriculture and its related technologies became the important part of the exchange. If this inference is rational, the mystery concerning how and from where common wheat (as well as its accompanying weeds) was introduced to China from the West gains an excellent explanation from the new evidence of DNA fingerprints.

Is it possible that the weedy type of Aegilops *tauschii* found in the Yellow River region was introduced from Xinjiang? Previous investigations showed that Xinjiang was not the origin center of common wheat. The wild type of Aegilops *tauschii* is only distributed in the Yili Valley in Xinjiang as natural populations at the altitudes of 600—1500 m, and it can form predominant populations in the springs when favorite rainfall and climate conditions arrived in the valley [7–10]. However, the weedy type of Aegilops *tauschii* has never been found in Xinjiang. Considering the fact that no weedy type of Aegilops *tauschii* was found in the Yili Valley region, in addition to the special dispersion (seed shattering) of the wild type of Aegilops *tauschii*, we strongly suggest that the weedy type of Aegilops *tauschii* found in the Yellow River region has no directly linkage with the wild type occurring in the Yili Valley.

The SSR fingerprint further indicated that there was considerable genetic differentiation among the weedy Aegilops *tauschii* accessions from Shaanxi and Henan provinces. Interestingly, most of the accessions from Shaanxi were clustered into one subgroup (Shaanxi subgroup),

whereas all of the accessions from Henan were clustered into another subgroup (Henan subgroup). This indicated that the initial introduction of the weedy type of Aegilops *tauschii via* the Silk Road arrived in the Shaanxi where the capital of ancient capital Chang'an was located. With the gradual introduction of wheat cultivation, the weedy Aegilops *tauschii* spread to the neighbouring areas. The wheat cultivation-dissemination process might have taken a long period of time, and as a consequence, evident genetic differentiation had occurred among the accompanying weedy accessions of Aegilops *tauschii* from Shaanxi and Henan. Some new genotypes that varied significantly from the primary accessions had generated in the accessions from Henan. Obviously, most of the weedy accessions from Shaanxi Province still maintained a relatively close relationship with the wild types of Aegilops *tauschii* from Iran. This can be reflected by the clustering of some Iran wild accessions into the Shaanxi subgroup in the dendrogram (Fig. 2). However, all the weedy accessions from Henan and one accession from Shaanxi displayed obvious genetic differentiation compared with the wild types from their center of origin — Iran. Consequently, none of the wild accessions from Iran was included in the Henan subgroup (Fig. 2).

The Silk Road served as the major transmission routes promoted exchanges of trade and culture between east and west Asia, as well as east Asia and Europe in a historical period of time, which provided the prerequisite for introducing cultivated wheat and its weeds to China. As a consequence, weedy Aegilops *tauschii* found in China may be initially brought into central China from its diversity center Iran *via* the Silk Road. Considering that the Yili Valley of Xinjiang was the only passage of the northern route of the Silk Road, we believe that the introduction route of common wheat and its mixed weedy Aegilops *tauschii* might have taken place through the middle or south passages to central China, instead of passing through the north passage. There might be another possibility that the introduction of wheat and weedy Aegilops *tauschii* from Iran might have taken place through the Eurasian Prairies and eastward to the Great Bend of the Yellow River (Hetao areas), and then extended southward to central China. No matter which introduction route of common wheat and weedy Aegilops *tauschii* to China is the actual route, this study indicated evidently that the weedy type of *Aegilops tauschii* found in China was originated somewhere in Iran — the diversity center of Aegilops *tauschii*, and was introduced to the central part of ancient China together with the introduction of cultivated common wheat. The above conclusion has provided a new thought for the further investigation of origin, dispersal, and evolution of common wheat grown in China.

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