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Microsatellite analysis of *Aegilops tauschii* germplasm

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Abstract The highly polymorphic diploid grass *Aegilops tauschii* is the D-genome donor to hexaploid wheat and represents a potential source for bread wheat improvement. In the present study microsatellite markers were used for germplasm analysis and estimation of the genetic relationship between 113 accessions of *Ae. tauschii* from the gene bank collection at IPK, Gatersleben. Eighteen microsatellite markers, developed from *Triticum aestivum* and *Ae. tauschii* sequences, were selected for the analysis. All microsatellite markers showed a high level of polymorphism. The number of alleles per microsatellite marker varied from 11 to 25 and a total of 338 alleles were detected. The number of alleles per locus in cultivated bread wheat germplasm had previously been found to be significantly lower. The highest levels of genetic diversity for microsatellite markers were found in accessions from the Caucasian countries (Georgia, Armenia and the Daghestan region of Russia) and the lowest in accessions from the Central Asian countries (Uzbekistan and Turkmenistan). Genetic dissimilarity values between accessions were used to produce a dendrogram of the relationships among the accessions. The result showed that all of the accessions could be distinguished and clustered into two large groups in accordance with their subspecies taxonomic classification. The pattern of clustering of the *Ae. tauschii* accessions is according to their geographic distribution. The data suggest that a relatively small number of microsatellites can be used to estimate genetic diversity in the germplasm of *Ae. tauschii* and confirm the good suitability of microsatellite markers for the analysis of germplasm collections.

Key words *Aegilops tauschii* · D genome · Microsatellite markers · Genetic diversity · Germplasm

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Introduction

The diploid grass *Aegilops tauschii* Coss. [syn. *Triticum tauschii* (Coss.) Schmal., *Aegilops squarrosa* auct. non L., 2n=14] is the D-genome donor to bread wheat (*Triticum aestivum* L., 2n=42, AABBDD). It has been found that the level of genetic variation in the D genome of wheat is limited. In contrast, the level of genetic variation in the present-day species of *Ae. tauschii* is extensive. *Ae. tauschii* contains more genetic variability for disease and insect resistance, isozymes, and seed storage protein than the D genome of *T. aestivum* (Hammer 1980; Lubbers et al. 1991) and represents a potential source for the improvement of bread wheat. *Ae. tauschii* includes two subspecies: ssp. *tauschii* and ssp. *strangulata* (Eig) Tzvel. It has been shown that ssp. *strangulata* is more closely related to bread wheat than ssp. *tauschii.*

The germplasm of *Ae. tauschii* species has been studied by morphological (Hammer 1978, 1980), physiological (Goncharov and Chikida 1995), seed storage protein (Konarev 1980), isozyme (Jaaska 1981; McIntyre 1988) and restriction fragment length polymorphism (RFLP) analyses (Lubbers 1991; Dvorak et al. 1998). The disadvantage of protein electrophoretic variants and morphological traits is the limited number of available alleles and markers. DNA-based markers have been applied successfully for the study of the *Ae. tauschii* gene pool (Lubbers et al. 1991; Dvorak et al. 1998) and the construction of linkage maps of this species (Gill at al. 1991; Lagudah et al. 1991). A higher level of RFLPs between *Ae. tauschii* accessions (60–75%) compared to hexaploid wheat (less than 10%) has been detected (Kam-Morgan et al. 1989).

Microsatellites or simple sequence repeats (SSRs) are tandem repeats of short (2–6 bp) DNA sequences. Microsatellites are found to be highly polymorphic in different animal and plant species. The analysis of microsatellites based on the polymerase chain reaction (PCR) is much easier to perform than RFLP analysis and is highly amenable to automation. Microsatellites are inherited in a codominant manner and, in most cases, they are chromosome-specific. SSRs have been successfully used for the construction of genetic linkage maps of wheat (Röder et al. 1998), for detecting genetic diversity (Fahima et al. 1998) and mapping agronomically important genes (Korzun et al. 1998). It has been shown that it is possible to distinguish closely related breeding material and to carry out phylogenetic studies by using only a small number of microsatellites (Plaschke et al. 1995; Struss and Plieske 1998).

In the present report, we demonstrate the utility of microsatellite markers for germplasm analysis and the estimation of genetic relationships between 113 accessions of *Ae. tauschii* from the gene bank collection at IPK, Gatersleben. Microsatellite markers, developed on *T. aestivum* and *Ae. tauschii* sequences, were used to analyse the gene pool of *Ae. tauschii.*

Materials and methods

Plant material and DNA isolation

One hundred and thirteen *Ae. tauschii* accessions from the gene bank collection at IPK, Gatersleben, were analysed using microsatellite markers. The numbers of the accessions and their origin are shown in Fig. 3. DNA was isolated from 7-day old seedlings according to Anderson et al. (1992) and 10–20 green seedlings of each accession were pooled for the extraction of genomic DNA. In addition *T. aestivum* cv *Chinese Spring* was also included in the analysis. '*Chinese Spring*' DNA was extracted from whole seeds (Plaschke et al. 1995).

Microsatellite markers

Nine primer pairs representing wheat microsatellites (WMS) and nine primer pairs representing D-genome microsatellites (DMS) were chosen for the analysis. The microsatellite markers covered all chromosomes of *Ae. tauschii* except chromosome 6D (see Table 1). The isolation of wheat microsatellite markers was previously described by Röder et al. (1998). The D-genome microsatellites were obtained from *Ae. tauschii* ssp. *tauschii* (DMS 8, 19, 29, 34, 40, 43) and *Ae. tauschii* ssp. *strangulata* (DMS 61, 63, 111) by the same method. Chromosomal locations of the amplified loci, the number of alleles per locus and the average heterogeneity index for each locus are presented in Table 1. Primer sequences and PCR conditions for WMS markers except WMS 603 were published by Röder et al. (1998). Primer sequences of DMS markers, and for WMS 603, are available on request.

Polymerase chain reaction and fragment analysis

PCR reactions and fragment detection were performed as described by Röder et al. (1998). Fragment analysis was carried out using an automated laser fluorescence (ALF) sequencer (Pharmacia) and fragment sizes were calculated using the computer program Fragment Manager Version 1.2 (Pharmacia) by comparison with internal size standards.

Analysis of data

The area in which the *Ae. tauschii* Coss. accessions were collected was divided into five regions: I (Azerbaijan), II (Georgia, Armenia, Daghestan and the Caucasus), III (Tadjikistan, Kyrgyzstan, Kazakhstan and Central Asia), IV (Uzbekistan and Turkmenistan) and V (Iran and Afghanistan) (see Fig. 2). Genetic diversities (population heterogeneity indexes) for each locus were calculated for all accessions within groups and for two subspecies according to Nei and Roychoudhury (1974) and Lubbers et al. (1991). The average heterogeneity index is defined as the average level of genetic diversity over all loci.

Unique genotypes represent alleles occurring in a microsatellite locus only once among all accessions tested. The number of unique genotypes in different regions was culculated as the sum of unique alleles in all 8 microsatellite loci (see Table 2).

The presence or absence of each single fragment was coded by 1 or 0 respectively and scored in a binary data matrix. Genetic distance was calculated for each pair of lines using the percentage difference in the program NCLAS of the computer package SYN-TAX IV (Podani 1990). Cluster analysis was performed based on the unweighted pair-group method with arithmetic average (UPGMA).

Results

Microsatellite analysis

PCR reactions were performed with 113 *Ae. tauschii* accessions and *T. aestivum* cv *Chinese Spring.* The amplification products were highly polymorphic for all primer pairs (Fig. 1). A total of 188 alleles were detected for nine WMS and 150 alleles for nine DMS. The number of alleles per microsatellite marker varied from 11 to 25 (see Table 2). On average, 18.8 alleles per locus were observed. All microsatellite markers showed a high level of gene diversity and heterogeneity indexes varied from 0.75 to 0.95 (Table 1).

Table 1 Chromosomal location, number of alleles and heterogeneity index of microsatellite markers. Chromosomal location of microsatellites marked with an asterisk was determined using nulli-tetrasomic lines of *Chinese Spring*

Marker	Location	No. of alleles per locus	Heterogeneity index
WMS 3	3D	12	0.78
WMS 102	2D	21	0.92
WMS 174	5D	25	0.91
WMS 190	5D	22	0.93
WMS 194	4D	17	0.92
WMS 261	2D	22	0.92
WMS 314	3D	24	0.95
WMS 437	7D	22	0.95
WMS 603	1D	23	0.91
DMS 8	3D	24	0.94
DMS 19	1D	24	0.93
DMS 29*	2D	11	0.82
DMS 34*	4D	20	0.91
DMS 40*	4D	13	0.76
DMS 43	5D	18	0.92
DMS 61	4D	11	0.75
DMS 63	5D	16	0.84
DMS 111	1D	14	0.80

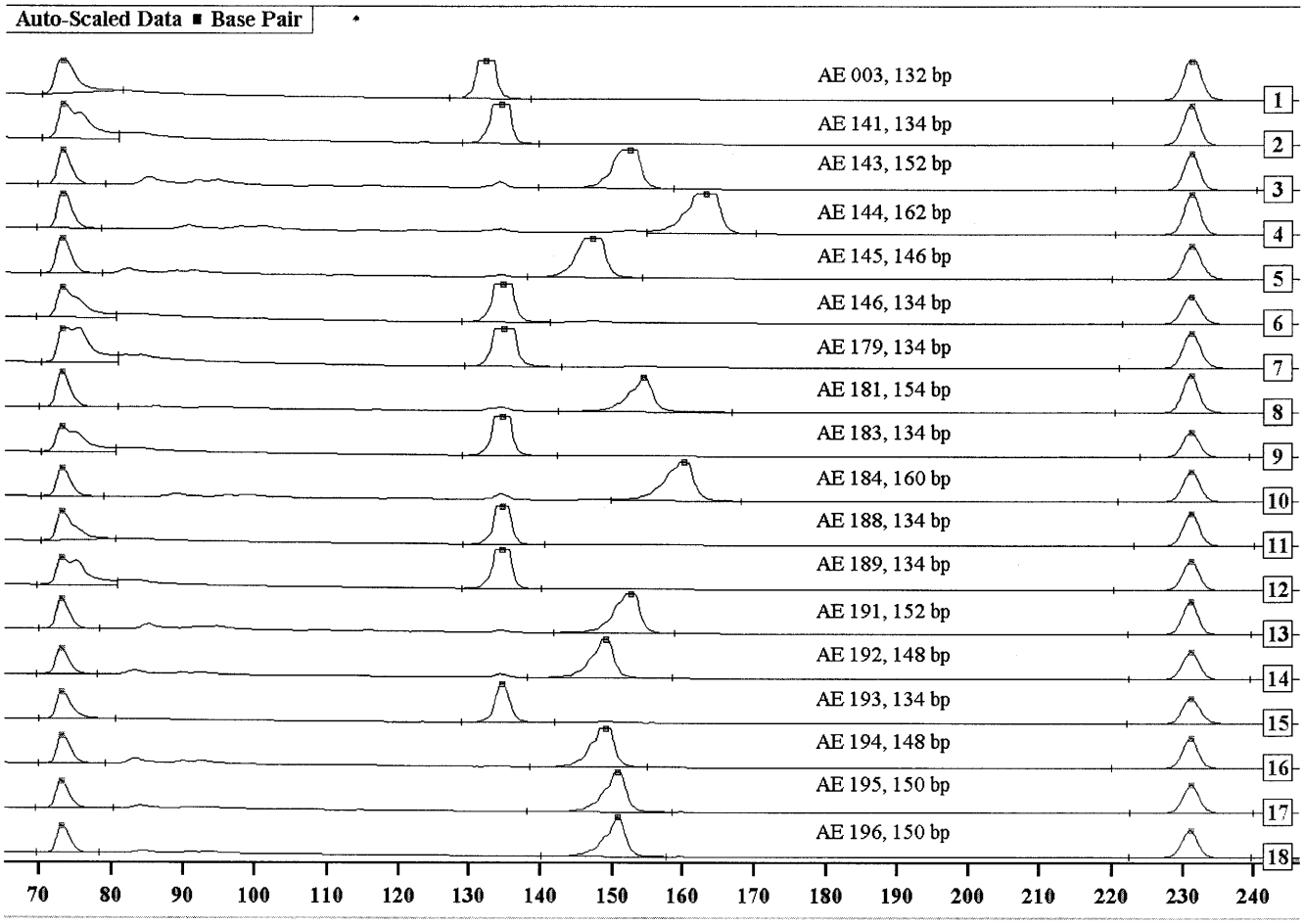

Fig. 1 High polymorphism of D-genome wheat microsatellite DMS 63 among *Ae. tauschii* accessions shown as a printout of the computer program Fragment Manager V1.2. The horizontal scale indicates the size in base pairs calculated from internal standards (73 bp and 231 bp). Accession numbers and the size of amplifying fragments are shown in each line. DMS 63 revealed nine alleles for 18 *Ae. tauschii* accessions

Most of the *Ae. tauschii* accessions were not homogeneous, 66% of accessions revealed two alleles per locus at least once. This is not surprising because DNA pools from 10 to 20 grains were studied.

Four alleles of '*Chinese Spring*' at microsatellite loci WMS 603, DMS 8, DMS 61 and DMS 111 were unique and were not found in *Ae. tauschii* accessions. '*Chinese Spring*' alleles at loci WMS 3 and WMS 174 were unique for *Ae. tauschii* and were correspondingly found in accessions AE 196 and AE 197. It is of interest that both of the accessions belong to ssp. *strangulata* and originated from Azerbaijan. All other alleles of '*Chinese Spring*' were more frequent in *Ae. tauschii*.

Genetic diversity of *Ae. tauschii* in different geographic regions

To estimate the genetic diversity of *Ae. tauschii* populations in different geographic regions all accessions were divided into groups according to their origin (Fig. 2, Table 2). It was impossible to make equal or near-equal groups according to the country of origin of the accessions because of the different numbers of accessions from the different countries. The five groups presented consist of accessions collected in one or several neighboring countries. The first and second groups represent accessions from the Caucasus, the third and fourth groups consist of accessions from Central Asia and the last group is compound and includes accessions from Iran and Afghanistan.

An average genetic diversity index was calculated for all groups over 18 microsatellite loci (Table 2). Group II (Armenia, Georgia, Daghestan and the Caucasus) revealed the highest level of gene diversity. Group V (Iran and Afghanistan) is also highly heterogeneous. Accessions growing in Uzbekistan and Turkmenistan (group IV) were the least variable. In general the accessions from Central Asia were less variable than the accessions from the Caucasus.

Unique genotypes (alleles) occurred in all regions (Table 2). In the Caucasian groups, the total number of unique genotypes over 18 microsatellite loci was higher than the number of accessions in the groups. A relatively high number of unique genotypes was also observed in group V (Iran and Afghanistan). This high value was due to the accessions from Iran where eight unique alleles were found in six accessions.

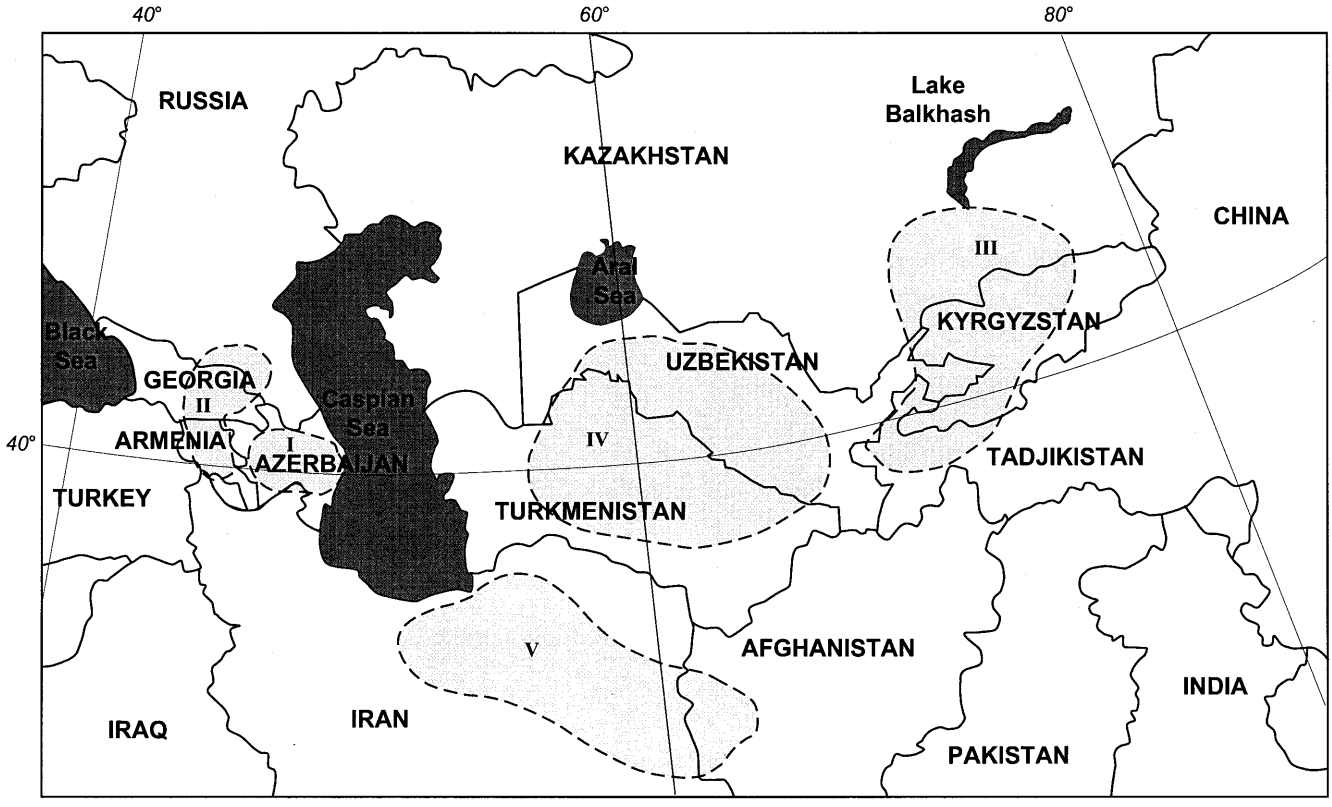

Fig. 2 Geographic regions in which *Ae. tauschii* accessions were collected. *I* – Azerbaijan, *II* – Georgia, Armenia, Daghestan and the Caucasus, *III* – Tadjikistan, Kyrgyzstan, Kazakhstan and Central Asia, *IV* – Uzbekistan and Turkmenistan and *V* – Iran and Afghanistan

Table 2 Number of accessions, number of unique genotypes and average heterogeneity index for the five geographic regions into which *Ae.tauschii* accessions were classified and for subspecies of *Ae.tauschii*

Classification	Geographic origin	No. of accessions	No. of unique genotypes	Average heterogeneity index
Region				
I	Azerbaijan	39	42	0.83
II	Armenia, Georgia, the Caucasus, Daghestan	21	24	0.94
III	Tadjikistan, Kyrgyzstan, Kazakhstan, Central Asia	16	3	0.82
IV	Turkmenistan Uzbekistan	23	13	0.79
V	Iran, Afghanistan	14	11	0.86
Taxonomic group				
ssp. *tauschii*		65	87	0.82
ssp. *strangulata*		48	78	0.83

A similar estimation of the average heterogeneity indexes and the number of unique genotypes was made for the different subspecies of *Ae. tauschii*. There was no significant difference in the genetic diversity of ssp. *tauschii* and ssp. *strangulata* (Table 2). Almost complete allelic differentiation between the two subspecies was found for a number of loci. Different alleles were fixed in the two subspecies at the DMS 29, DMS 40, DMS 61, DMS 63 and WMS 603 loci.

Genetic distance

Pairwise comparisons were made between all accessions and the average dissimilarity values were calculated based on WMS- and DMS-derived data. The smallest genetic distance (0.081) was between accessions AE 230 and AE 231 which differ only at two loci. These accessions were collected in the neighboring Caucasian countries of Armenia and Azerbaijan. It is interesting that accession AE 230 belongs to ssp. *strangulata* while accession AE 231 has been classified

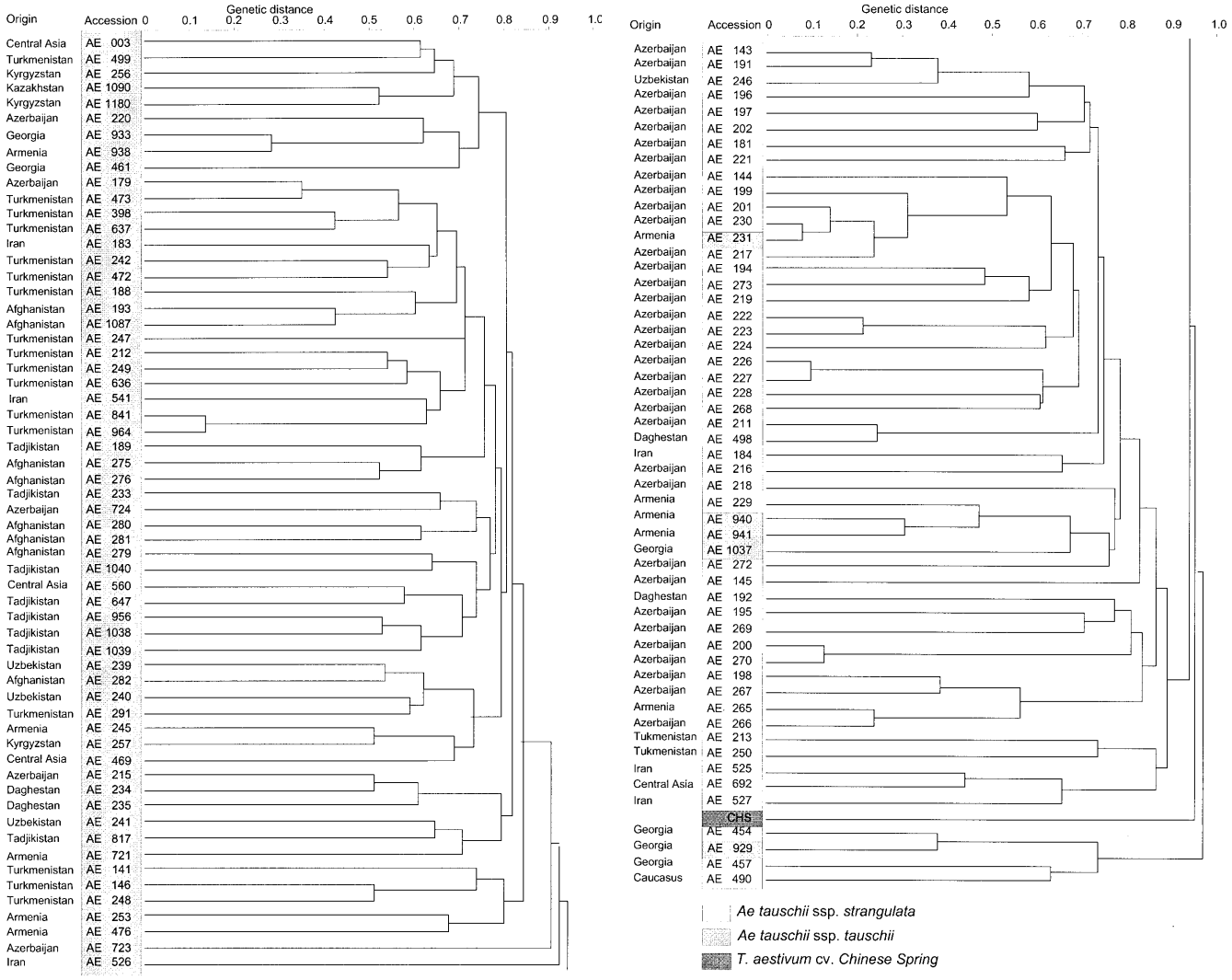

Fig. 3 Dendrogram of 113 *Ae. tauschii* accessions (AE 003–AE 1087) and *T. aestivum* cv *Chinese Spring* (*CHS*), based on the genetic distance (percentage difference) calculated from data of 18 microsatellites, using UPGMA as the clustering method

as ssp. *tauschii*. The largest genetic distance (1.0) was observed between many accessions since they had no common fragments.

Genetic dissimilarity values between accessions were used to produce a dendrogram of the relationships among the accessions (Fig. 3). The result shows that all accessions could be distinguished. The accessions are clustered in two large groups. Only four accessions from the Caucasus (including three from Georgia) are grouped separately. The first cluster contains only ssp. *tauschii* accessions. An accession AE 541 which has been described as intermediate between subspecies (Hammer 1980) belongs to ssp. *tauschii* based on the microsatellite data. The second main cluster corresponds to ssp. *strangulata*. This cluster includes four exceptional accessions that belong morphologically to ssp. *tauschii*. Three of these cluster together (AE 940, AE 941 and AE 1037), representing an atypical ssp. *tauschii* characterized by shorter spikelets. The fourth is accession AE 231, closely related to AE 230.

'*Chinese Spring*' is located separately from any of the two clusters in the dendrogram. Average disimilarity values calculated between '*Chinese Spring*' and ssp. *strangulata* and ssp. *tauschii* were comparable, 0.96 and 0.95 respectively.

The dendrogram shows that the pattern of clustering of the *Ae. tauschii* accessions is related to their geographic distribution. Most of the ssp. *strangulata* accessions present in the gene bank at IPK, Gatersleben, originated from the Caucasus. Only a few ssp. *strangulata* accessions were from Iran and Turkmenistan. The origin of most of the ssp. *tauschii* accessions was Central Asia. Therefore the first cluster represents a majority of Central Asian accessions and the second cluster includes a majority of Caucasian accessions. The accessions from one country and from neighboring countries tend to cluster together. There is obvious clustering of the accessions from Azerbaijan, Turkmenistan, Afghanistan and Tadjikistan. Accessions from Iran are spread throughout the dendrogram.

Comparison of data derived from wheat microsatellites (WMS) and *Ae. tauschii* microsatellites (DMS)

Out of 18 microsatellites, nine WMS were obtained from *T. aestivum* and nine DMS were obtained from *Ae. tauschii*. It is of interest to investigate whether there is any difference in the calculated genetic distances between accessions when microsatellites from different sources were used.

Genetic dissimilarities were calculated and two dendrograms were constructed based on each set of markers separately. Both dendrograms were similar to each other and showed a clustering pattern similar to that obtained with all 18 microsatellites. Two large clusters could always be distinguished. The nine DMS markers were sufficient to divide the *Ae. tauschii* accessions into subspecies. Most of the accessions in the DMS-derived dendrogram could be distinguished, only two pairs of accessions were identical.

All genotypes were differentiated by the WMS markers. The subspecies were separated with the exception of one group of four ssp. *strangulata* accessions which grouped to the main 'ssp. *tauschii*' cluster. Obviously, more than nine, but less than 18 microsatellites are enough for the subspecies differentiation of *Ae. tauschii*.

Discussion

Microsatellite analysis of the *Ae. tauschii* collection revealed extensive polymorphism between the different genotypes. The mean number of alleles per microsatellite locus was 18.8. The number of alleles per locus in cultivated bread wheat germplasm had been found to be significantly lower (Plaschke et al. 1995). Microsatellite marker WMS 261 revealed 22 different alleles in *Ae. tauschii* germplasm, while a screen of over 100 varieties of wheat from different parts of the world showed that three allelic variants of WMS 261 were widespread and only three additional rare alleles were found (Korzun et al. 1998). This greater microsatellite polymorphism parallels the greater genetic diversity in *Ae. tauschii.*

The object of our study was to estimate the genetic diversity of an *Ae. tauschii* collection in the gene bank at IPK (Gatersleben). This collection does not cover all areas of the species and mostly includes accessions from the Caucasus and from Central Asia. The highest level of gene diversity in *Ae. tauschii* populations (0.94) was found in a group of accessions from Georgia, Armenia and Daghestan. The gene diversity of Iranian populations (after elimination of the Afghanistan accessions from group V) was also high and had a value of 0.92. A high level of gene diversity of *Ae. tauschii* in the Caucasus and Iran was also reported by Lubbers et al. (1991) and Dvorak et al. (1998) based on RFLP data.

Dvorak et al. (1998) suggested that the principal area of origin of *T. aestivum* is the Caucasus, and Armenia in particular, but the south-western coastal area of the Caspian Sea and a corridor between the two areas may have played a role as well. Our finding of '*Chinese Spring*' microsatellite alleles in *Ae. tauschii* ssp. *strangulata* accessions from Azerbaijan confirms the possibility of *T. aestivum* originating in the Caucasian region.

Ae. tauschii grows across a very large area of southwest Asia. Subspecies *tauschii* is distributed from eastern Turkey to western China and Pakistan, whereas ssp. *strangulata* occurs in two unlinked regions, the southeastern Caspian Sea and the Caucasus. Most of the ssp. *strangulata* accessions analysed were from the Caucasus and most of the ssp. *tauschii* accessions originated from Central Asia. Contrary to RFLP (Lubbers et al. 1991) and seed storage protein data (Konarev, 1980), there was no significant difference in the genetic diversity of the two subspecies based on microsatellite data (Table 2). In both *Ae. tauschii* subspecies the mean genetic diversity was very high.

The results suggest that microsatellite markers are valuable for the estimation of the genetic diversity of *Ae. tauschii* germplasm. The dendrogram obtained from microsatellite-derived data allows all accessions to be distinguished and clearly divides *Ae. tauschii* into two subspecies.

The second cluster of the dendrogram includes four exceptional accessions that have been classified as ssp. *tauschii*. According to the microsatellite data obtained, accessions AE 231, AE 940, AE 941 and AE 1037 should however belong to ssp. *strangulata*. *Ae*. *tauschii* is an autogamous species. The cross-pollination rate of *Ae*. *tauschii* varies with the year and is usually less than 10%. Hybridizations and morphologically intermediate forms between ssp. *tauschii* and ssp. *strangulata* have been reported by Hammer and Knüpffer (1979) and Hammer (1980). Dvorak et al. (1998) provided evidence for gene migration between the two subspecies in Iran and to a lower extent in the Caucasus. In this report all atypical ssp. *tauschii* accessions in the cluster 'strangulata' originated from Armenia and Georgia. These accessions may be a result of gene migration from ssp. *tauschii* to ssp. *strangulata* in the Caucasian region. Four accessions from the same region are not within the two main clusters of the dendrogram. The reason for the uniqueness of these accessions needs further investigation. Obviously these exceptional accessions contribute significantly to the high level of polymorphism in *Ae. tauschii* populations in the Caucasus.

Microsatellites developed from *T. aestivum* sequences revealed an even higher level of polymorphism in *Ae. tauschii* accessions than microsatellites obtained from *Ae. tauschii* sequences. The mean heterogeneity index for WMS was 0.91 compared to 0.85 for DMS. Therefore it is clear that there is no problem in using wheat microsatellite markers in related species such as *Ae. tauschii*. There was no significant difference in the dendrograms obtained from each set of markers. Microsatellite markers both from hexaploid wheat and *Ae. tauschii* can be applied successfully for the study of genetic diversity in, and the subspecies identification of, *Ae. tauschii* accessions.

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