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Genetic Diversity of Persian Wheat (*Triticum turgidum* ssp. *carthlicum*) Accessions by EST-SSR Markers

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ABSTRACT

To estimate the allelic variation at the expressed sequence tag simple sequence repeats (EST-SSR) among Persian wheat (*Triticum turgidum* ssp. *carthlicum* L.) accessions collected from fifteen countries and provide information for wheat breeding and improvement in southwest China, 87 Persian wheat accessions from fifteen countries were investigated by using EST-SSR markers. EST-SSRs were molecular markers belonging to the transcribed region of the genome. Therefore, any polymorphism detected by EST-SSRs might reflect better relationship among species or varieties. Fourteen primer pairs could successfully amplify the fragments in the 87 accessions, of which tri-nucleotide repeats were the dominant type. A total of 33 eSSR alleles were detected, and the number of alleles detected by single pair primers ranged from 3 to 7 per locus, with an average of 3.71. Clustering analysis suggested that most of the accessions with adjacent geographic origins had the tendency to cluster together. Therefore, when used in Persian wheat genetic analysis, EST-SSR markers not only act as genetic markers but also reveal differences in related gene expression.

Key words: Simple sequence repeat, genetic polymorphism, genetic diversity, clustering analysis, Persian wheat

INTRODUCTION

Since great number of alleles was lost through breeding and selection, more difficulties in wheat improvement have emerged for the modern agriculture system (Allard, 1996; Hoisington *et al*., 1999). The narrow genetic basis weakens the resistance of current wheat cultivars facing adverse factors and threatened further improvement for wheat (Wang *et al*., 2007). It is essential and urgent to exploit genetic resources from relatives of wheat with the richness of desirable genes.

Triticum turgidum ssp. *carthlicum* L. (AABB, 2n = 4x = 28), with the common name of Persian wheat, was early-maturing wheat with spring habit. And it is an important crop for human consumption. It is mainly grown in the Former Soviet Union Transcaucasia, Russian Federation Dagest, Georgia and northeast Turkey (Dong and Dian-Sheng, 1998). It has many beneficial traits, such as good resistance to powdery mildew dustbrand and stem rust, higher number of tillers and fertility, good fecundity, tolerance to low temperature and preharvest sprouting (Belay *et al*., 1994;

Raut *et al.*, 1984; Gol-denberg, 1984). With many beneficial traits and easiness of gene transferring to common wheat, *T. carthlicum* has been suggested as one of the most desirable donors for bread wheat improvement (Anamthawat Jonsson, 1996; Merker and Lantai, 1997; Pandey and Rao,1987; De Moraes *et al.*, 2000; Balatero and Darvey, 1993; De Pienaar and Sears, 1973). The genetic relationship between Persian wheat genotypes has been extensively studied based on morphology, agronomic traits, as well as enzymatic and molecular markers (Zhuang *et al.*, 2005).

Microsatellites, also described as simple sequence repeats (SSRs), are tandem repeated sequences comprised of mono-, di-, tri-, tetra-, penta-, or hexa-nucleotide units (Chambers and Macavoy, 2000; Ellegren, 2004) and ubiquitous in prokaryotes and eukaryotes (Powell *et al.*, 1996). EST-SSRs (expressed sequence tag simple sequence repeats) belong to the transcribed region of the genome and might be relatively well conserved. Therefore, any polymorphism detected by EST-SSRs might reflect better relationship between species or varieties. Evaluation of the germplasm with SSRs derived from ESTs might enhance the role of genetic markers by assaying variations in transcribed and known-function genes (Eujayl *et al.*, 2002). The controversy about the current status of the germplasm pool and the influence of breeding programs has not been solved for many years (Maccaferri *et al.*, 2003). Collection of germplasm is a continuous process requiring accurate monitoring of the status of the elite gene pool. For effective conservation and use of genetic resource, the evaluation of genetic variation within collections is crucial and could be dramatically enhanced by using molecular genotyping tools (Eujayl *et al.*, 2002).

The aim of this study was to estimate the allelic variations at the expressed sequences among Persian wheat accessions collected from fifteen countries based on EST-SSR markers and provide information for wheat breeding and improvement in Southwest China.

MATERIALS AND METHODS

Materials: The materials were kindly provided by Dr. Bockelaman (American National Plant Germplasm System) and the germplasm lab of the Triticeae Research Institute, Sichuan Agricultural University. A total of 87 Persian wheat accessions were collected from 15 countries (Table 1). The materials spanned most of the ecological range of Persian wheat in the world were selected to evaluate genetic diversity based on the adaptability for the environment in southwest China.

Methods

Genomic DNA extraction: Genomic DNA was extracted from bulk sampling of a minimum of ten individual plants for all accessions, following the procedure described by Sharp *et al.* (1988). Primers (Table 2) derived from bread and durum wheat EST sequences, were retrieved from the Triticeae EST-SSR coordination project published by Peng and Lapitan (2005), Eujayl *et al.* (2002), Nicot *et al.* (2004), Chen *et al.* (2005) and Yang *et al.* (2005).

PCR reaction: PCR (Polymerase Chain Reaction) reactions contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM primer and 25 ng template DNA in a reaction volume of 25 μL. The amplification program consisted of the following cycles: 94°C for 4 min.

Table 1: Materials detected in this research

Origin	Accession No.	No
Turkey	PI470729, PI470730, PI470731, PI470732, PI470733, PI470734, PI532475, PI532476, PI532477, PI532478, PI532479, PI532480, PI532481, PI532482, PI532483, PI532484, PI532485, PI532487, PI532488, PI532489, PI532490, PI532491, PI532492, PI532493, PI532494, PI532495, PI532496, PI532497, PI532498, PI532499, PI532500, PI573178, PI573179, PI573180, PI573181, PI573182	36
Georgia	PI61102, PI78812, PI94748, PI94749, PI94750, PI94751, PI94752, PI94753, PI94754, PI94755, PI115816, PI115817, PI251914, PI352278, PI352282, PI499972, PI585017, PI585018	18
England	PI532513, PI532514, PI532515, PI532516, PI532517	5
Former Soviet Union	PI352279, PI352280, PI352281, PI532501	4
Iran	PI283887, PI283888, PI283889, PI283890	4
Albania	AS2265, AS2266, AS2267, AS2268	4
Canada	PI532505, PI532509, PI532510	3
Poland	PI286070, PI286071, PI532504	3
United States	PI532502, PI532506, PI532507	3
Russian Federation	CItr7665, PI341800	2
Iraq	PI70738	1
China	PI168672	1
Hungary	PI272521	1
Armenia	PI349040	1
Ethiopia	PI387696	1

40 cycles of 94°C for 1 min, 55 to 63°C (depending on the primer sets) for 1 min and 72°C for 1 min and a final extension at 72°C for 10 min. Amplification products were separated by electrophoresis in 6% polyacrylamide gel and silver stained (Tixier *et al*., 1997).

Genetic diversity and clustering analysis: Bands were scored as 1 for present and 0 for absent. Population genetic analysis was carried out using POPGENE version 1.31 (Yeh and Boyle, 1997), based on the model for codominant markers with diploid individuals. The raw data matrix was subjected to the calculation of the genetic similarity coefficient (GS) between accessions: GS = 2Nij/(Ni+Nj), where Nij is the number of alleles in common between accessions i and j and Ni and Nj are the total numbers of alleles observed for accessions i and j, respectively. The dendrogram was constructed based on the genetic similarity coefficient using the UPGMA (unweighted pair group with arithmetic average) method with the computer software NTSYS-pc (Rohlf, 1990).

RESULTS

EST-SSR polymorphisms: Genetic variations in the 87 Persian wheat accessions were detected by 14 EST-SSR primers on all seven wheat homologous chromosome groups (Table 2). The 14 EST-SSR loci consisted of 4 SSR types, 1 di-, 8 tri-, 1 tetra-, and 2 penta-nucleotide repeats (the remaining 2 types were not reported). A total of 33 EST-SSR alleles were detected and the number of alleles detected on a single locus ranged from 3 (BE473227, SWES178, SWES19, SWES209, BE424205, SWES3 and GPW7055) to 7 (CAU12), with an average of 3.71. The average PIC value of each locus was 0.483, with the highest of 0.714 for SWES86 and the lowest of 0.150 for BE424205 and SWES19.

Genetic distance: The genetic distances (GD=1-GS) were calculated for paired comparisons of all 87 accessions. Higher genetic variations in the 87 Persian wheat accessions were observed.

Table 2: Chromosome locations, number of alleles, repeat motif and PIC values of EST-SSR markers

Locus	Chromosome location	Number of alleles	Repeat motif	PIC value
BE473227	6B	3	AGC	0.495
SWES178	2B	3	TTTTG	0.260
SWES19	7B	3	CAG	0.150
SWES179	2B	4	GAG	0.230
SWES209	7B	3	CAA	0.359
BE424205	2B	3	AAC	0.150
SWES18	1A	4	TGA	0.698
BE399069	3B	4	TA	0.658
SWES86	1A	4	ACC	0.714
SWES3	3B	3	TATCG	0.590
SWES207	5B	4	ACAG	0.517
GPW7055	4A	3	CAA	0.598
CAU12	7B	7	AGC	0.695
CAU5	3B	4	TTTTG	0.650

The value of GD varied from 0.059 to 0.800, with the mean of 0.398. The genetic distance between accessions PI532484 (from Turkey) and PI286070 (from Poland) was the highest (GD = 0.800), whereas accessions PI94750 and PI352278 (both from Georgia) was the lowest (GD = 0.059). From all the GD data, it was found that there was usually lower genetic distance among the accessions located close regions, vice versa.

Clustering analysis: To visualize genetic relationships between the 87 Persian wheat accessions, a dendrogram was constructed (Fig. 1). Each genotype had a unique banding profile and all genotypes were clustered to four major groups at the average GS value of 0.602. Group I included 4 genotypes from Turkey, 3 genotypes from Former Soviet and 1 genotype from Iraq; Group II included 3 genotypes all from Turkey. Group III consisted of 38 genotypes from 8 countries, they are all genotypes from United States, most of the genotypes from Turkey and others were from Russian, Armenia, Former Soviet, Canada, China and Albania. Group IV possessed the remaining 38 genotypes, including all the genotypes from Iran, Georgia, Poland, England and Hungry, besides, the other genotypes were from Albania, Canada, Russian and Turkey.

According to the dendrogram, it was noteworthy that the accessions from Iran, Georgia, Poland, England, United States and most accessions from Turkey were clustered together, respectively. It was suggested that most of the accessions with adjacent geographic origins had the tendency to cluster together.

DISCUSSION

Microsatellite markers are becoming the markers of choice due to the level of polymorphism as well as higher reliability (Plaschke *et al.*, 1995; Fu *et al.*, 2005). In this study, we detected relatively higher plymorphism in Persian wheat accessions compared with that in durum wheat (Wang *et al.*, 2007). The PIC value of markers indicates the usefulness of DNA markers for gene mapping, molecular breeding and germplasm evaluation(Peng and Lapitan, 2005). The average PIC value of each locus was 0.483 in Persian wheat and 0.459 in durum wheat, with the highest of 0.714 and the lowest of 0.150 in the former while with the highest of 0.838 and the lowest of 0

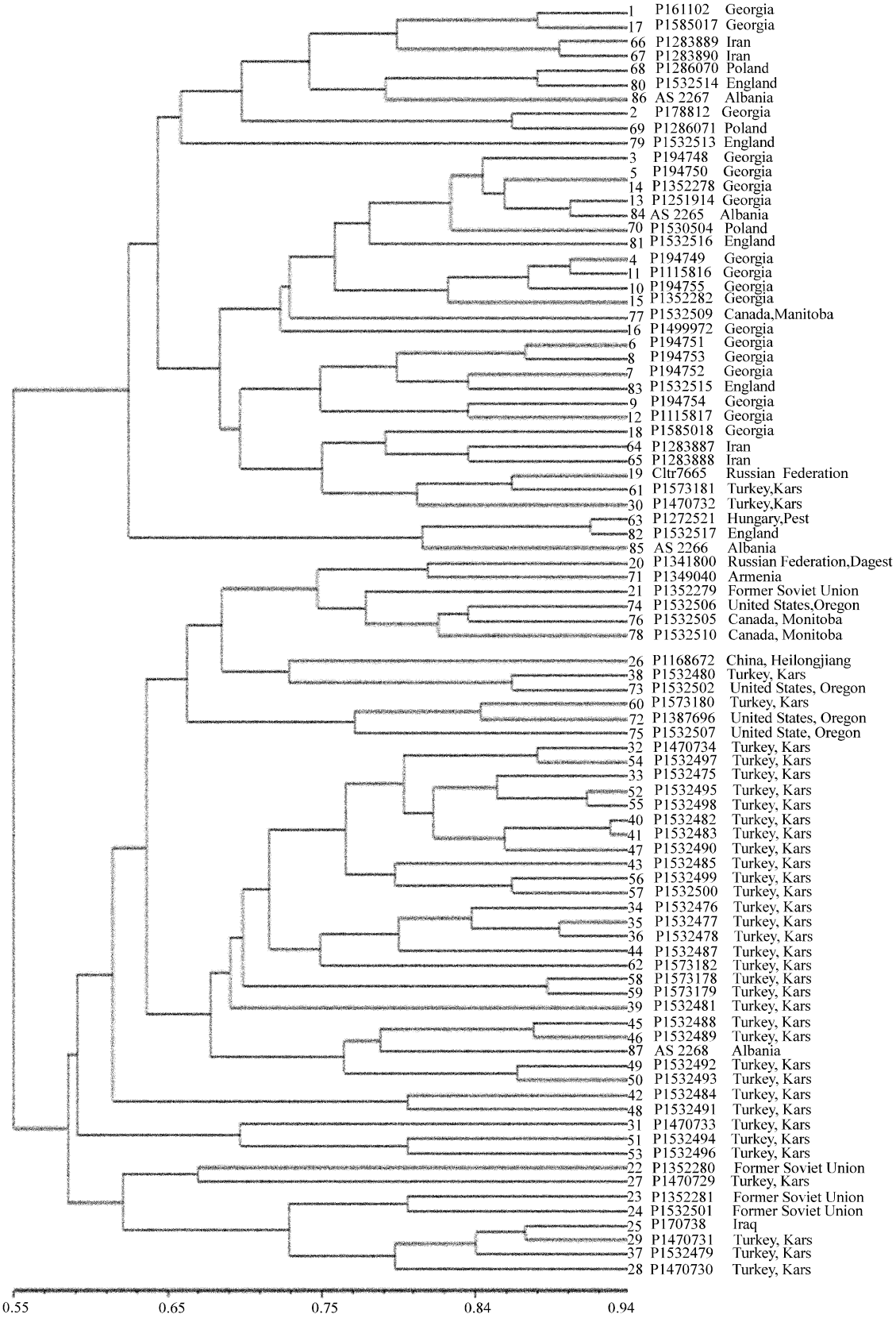

Fig. 1: Geographic distributions of the tested accessions of Persian wheat

in the latter, respectively. It might be the reason that Persian wheat collected in this study was from more extensive range of Geographic origin than that in Wang *et al.* (2007). Another reason could be that different markers were used in the two experiments.

In the previous research, high variations of storage protein and agronomic traits were observed in the Persian wheat accessions (Zhuang *et al.*, 2007). In this study, high EST-SSR polymorphism were detected among 87 Persian wheat. While the EST-SSR, derived from the coding region could more directly and effectively reflect the genetic information and evaluate the genetic diversity. Different genetic markers only can interprete different genetic information of the Persian wheat. Therefore, we should combine all the research to make better use of Persian wheat.

It was found that the estimate of genetic distance was geographically independent when studying the relationship of the allozyme genetic distance and geographic distance in wild emmer wheat populations (Fahima *et al.*, 1999, 2002). It was also proved that the genetic differentiation between populations was independent of geographical distances between the sites of collection by Mantel test ($r = 0.104$, $p = 0.809$) (Mantel, 1967). While, some studies showed that genetic distance had no significant correlation with geographic distance in different *dicoccoides* wheat populations (Nevo *et al.*, 1982) and the Ethiopian tetraploid wheat (Yifru *et al.*, 2006). Even though, in this study, we also found that some accessions located far from each other, had higher genetic distance, and vise versa. For example, the accession PI532484 (from Turkey, 39°55'45", N, 32°51'12", E) and PI286070 (from Poland, 52°14'42", N, 21°00'43", E) was the farest from each other with the GD of 0.800, but the accessions PI94750 and PI352278 (both of them from Georgia) with the GD of 0.059.

CONCLUSION

This research could provide some good information for persia wheat conservation and utilization. Meanwhile, EST-SSR, derived from the coding region, could more directly reflect influences imposed by breeding efforts on the elite germplasm. Therefore, more attention should be focused on the establishment of a genotype database using convenient and effective indicators such as EST-SSR, and hence monitoring the dynamic change in the gene pools and as a reference forgermplasm management and breeding strategies.

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