Genetic diversity of hexaploid wheat and three Aegilops species using microsatellite markers

Naghavi MR [1], Aghaei MJ[2], Taleei AR[1], Omidi M[1] and Hassani ME[3]

[1]**Agronomy and Plant Breeding Dept., Agricultural College, University of Tehran, Karaj, Iran**
[2] **Seed and Plant Improvement Institute, National Plant Gene Bank of Iran, Mahdasht Road, Karaj, Iran**
[3] **Horticultural Dept., Agricultural College, University of Tehran, Karaj, Iran**

ABSTRACT

In this study a set of 21 simple microsatellite primers were used to determine the genetic relationship of the D-genome among 52 accessions of *T. aestivum* (AABBDD), *Ae. tauschii* (D^tD^t), *Ae. cylindrica* ($CC D^c D^c$) and *Ae. crassa* ($MM D^{cr1} D^{cr1}$), collected from 13 different sites in Iran. A total of 273 alleles were detected across all four species and the number of alleles per microsatellite marker varied from 3 to 27. The highest genetic diversity occurred in *Ae. tauschii* followed by *Ae. crassa,* and the genetic distance was the smallest between *Ae. tauschii* and *Ae. cylindrica*. Cluster analysis classified the four species in three groups, locating *Ae. tauschii* and *Ae. cylindrica* in the same cluster using the UPGMA method. It appeared that different genotypes of *Ae. tauschii* could be involved in the evolution of polyploid species. A high level of variation and also the highest number of unique alleles were observed within *Ae. crassa* accessions, indicating this species as a good potential source of novel genes for bread wheat improvement.

INTRODUCTION

Synthetic hexaploid wheat lines, produced by hybridization between commercial cultivars of *T. durum* or *T. aestivum* with *Ae. tauschii*, were used to improve the genetic base of hexaploid wheat (Kazi *et.al.,* 1996). Several of these lines and synthetic derivative cultivars are known to have important agronomical traits such as pest and disease resistance, environmental stress tolerance and adaptability to varied climatic conditions. The D genome is not limited to *T. aestivum* and *Ae. tauschii*. Two tetraploid wild species, *Ae. cylindrica* (2n=4x=28, CCDD) and *Ae. crassa* (2n=4x=28, MMDD), which are widely distributed in Iran, contain the D genome as well. However, there is no evidence that these species contributed to the D genome of hexaploid wheat. The distribution of *Ae. tauschii* is limited to Northern Iran, while the distribution of the other two tetraploid species is wider in Northern and Western Iran.

Ae. cylindrica growns in the most suitable areas north of Iran from Khorasan to Azarbaijan provinces, west of Iran from Azarbaijan to Lorestan provinces and in the center of Iran in such places as Semnan, Tehran, Ghazvin, Ghom, Markazi, Isfahan and Fars provinces. However, *Ae. crassa* is not widely distributed like *Ae. cylindrica*, but it is distributed in west of Iran, from Azarbaijan to Bushehr provinces in south, and central provinces such as Tehran, Ghazvin, Ghom, Markazi, Isfahan and Fars. *Ae. crassa* does not occur in Northern Iran, which is the main distribution area of *Ae. tauschii*. Introduction of different adaptability genes from these tetraploid species to bread wheat will produce new cultivars, with wider genetic base and adaptability, if the diversity of the D genome in these species is different to that in *Ae. tauchii* and *T. aestivum*.

Wide diversity within the D genome of *Ae. tauschii* populations in contrast with the D^t genome of *T. aestivum* was confirmed by an investigation with AFLP molecular markers (Dvorak *et al*., 1998, Lelly *et al*., 2000, Pestosova *et al*., 2000).

Genetic diversity in this species was evaluated using allozyme diversity (Watanabe *et al.* 1994), RAPD molecular markers (Goryunova *et al.* 2004), a combination of RAPD and AFLP molecular markers (Pester *et al.* 2003) and polymorphism for DNA sequences (Caldwell *et al.* 2004). Genetic diversity in the tetraploid species, *Ae. crassa*, was evaluated using isozyme markers (Nakai, 1989), SSR markers (Zhang and Devorak 1992; Naghavi et al. 2007) and *in situ* hybridization (Badaeva *et al.* 1998). The potential of introducing agronomic traits such as cytoplasmic male sterility (Murai *et al.* 1993), yield, seed quality and salinity tolerance (Liu *et al.* 2002) from *Ae. crassa* into wheat breeding programs has been demonstrated by various researchers.

Populations of four species with the D genome from the National Plant Gene Bank of Iran were studied using microsatellite markers to evaluate genetic diversity among and within populations and to determine relationships between the D genome of these species.

MATERIALS AND METHODS

Fifty two accessions from four species, *T. aestivum*, *Ae. tauschii*, *Ae. cylindrica* and *Ae. crassa*, provided from the National Plant Gene Bank of Iran were used in this study. The accessions were collected from 13 different sites (for each species and from each site one accession was selected) in Iran. The sites were considered as centers of diversity for those species in Iran and usually most of the species are found to be prevalent there. The accessions were planted under

greenhouse conditions and genomic DNA was extracted from young leaves according to the method of Saghaei-Maroof et al. (1984). A total of 21 primer pairs (Roder et al. 1998), at least one primer pair from each arm of seven chromosomes of D genome, were selected for genotyping assays. The total number of alleles and polymorphic alleles were counted using PopGene 3.2. Polymorphism Information Content (PIC) was determined for each primer pair.

RESULTS AND DISCUSSION

A total of 272 alleles were detected by 21 primers. The number of alleles per primer ranged from 3 for WMS111 to 27 for WMS314 with an average number of 13 alleles per locus (Table 1). The lowest and highest PIC was 0.283 and 0.728 to WMS232 and WMS 311 respectively. Lelley et al. (2000) observed 90 different alleles for 14 microsatellite primers in Ae. tauschii populations. Their average of PIC was 0.68, which is a little larger than ours. Pestova et al. (2000) found 11-25 and an average of 18.8 alleles per microsatellite in evaluation of Ae. tauschii populations.

They observed 338 alleles for 18 primers, and the heterogeneity index varied from 0.75 to 0.95. In the evaluation of the genetic diversity of Iranian Ae. tauschii populations using 9 microsatellites, a total of 66 alleles and 4-12 alleles per primer was observed. The average of PIC was 0.659 (Saeidi et al. 2005). These results were similar to that of other researchers. Any disparities were mainly related to the differences in primers used and the very diverse material (genotypes) used. A total of 272 alleles were detected by 21 primers. The number of alleles per primer ranged from 3 for WMS111 to 27 for WMS314 with an average number of 13 alleles per locus.

The average of Nei's index of genetic diversity was 0.5008 among the four species. The highest diversity was 0.5571 in Ae. tauschii and the lowest diversity, 0.4697, was found in Ae. cylindrica (Table 2). In polyploid species that derived their D genome from Ae. tauschii the highest diversity was found in Ae. carassa and lowest diversity in Ae. cylindrica. The genetic diversity of T. aestivum was between that of Ae. carassa and Ae. cylindrical, but was more similar to Ae. cylindrica.

Genetic variability reported in D^t genome of bread wheat is much smaller than Ae. tauschii according to seed storage proteins, allozymes and DNA loci (Murphy et al.

1997). Low diversity in Ae. cylindrica populations were found based on variability of allozymes (Watanabe et al. 1994), C-banding (Badaeva et al. 2002), RAPD markers (Goryunova et al. 2004), a combination of RAPD and AFLP markers (Pester et al. 2003) and polymorphism for DNA sequences (Caldwell et al. 2004).

It is suggested that Ae. cylindrica is a relatively new tetraploid species (Gandhi et al. 2005). Some results suggested that D^t genome of bread wheat and D^c genome of Ae. cylindrica inherited from different biotype of Ae. tauschii (Badaeva et al. 2002; Caldwell et al. 2004). However, the relationship between Ae. cylindrica and subspecies of Ae. tauschii is not clearly understood (Gandhi et al. 2005). C-banding and in-situ hybridization studies on a tetraploid biotype of Ae. crassa suggested that the D^{cr1} genome of 4x Ae. crassa is highly modified compared with the D genome of the progenitor species Ae. tauschii (Badaeva et al. 1998).

Table1: List of 21 wheat microsatellites, the number of alleles and PIC

Microsatellites	Number of alleles	PIC
WMS484	19	0.664
WMS157	6	0.524
WMS469	14	0.633
WMS383	19	0.662
WMS337	14	0.644
WMS642	13	0.597
WMS190	23	0.574
WMS192	14	0.575
WMS194	18	0.605
WMS261	16	0.660
WMS314	27	0.715
WMS161	9	0.679
WMS295	10	0.688
WMS111	3	0.353
WMS212	11	0.649
WMS37	9	0.677
WMS311	21	0.728
WMS624	12	0.651
WMS232	4	0.283
WMS114	6	0.473
WMS33	5	0.298

Table 2- The Nei's index of genetic diversity for species

Species	No.	Genetic diversity Index
T. aestivum	13	0.4797
Ae. crassa	13	0.4966
Ae. cylindrica	13	0.4697
Ae. tauschii	13	0.5571

In conclusion, this study confirms the usefulness of SSR markers to study wheat genetic diversity. Additionally, the results obtained from this study could be useful for improving the understanding of diversity in and management of germplasm collections.

REFERENCES

1. Badaeva E.D., Friebe B., Zoshchuk S.A., Zelenin A.V., Gill B.S. (1998) Molecular cytogenetic analysis of tetraploid and hexaploid *Aegilops crassa*. *Chromosome Research*, 6, 629-637.
2. Caldwell K., Dvorak J., Lagudah E.S., Akhunov E., Luo M-C., Wolters P., Powell W. (2004) Sequence polymorphism in polyploid wheat and their D-genome diploid ancestor. *Genetics*, 167, 941–947.
3. Dvorak J., Luo M.C., Yang Z.L., Zhang H.B. (1998) The structure of the *Aegilops tauschii* genepool and the evolution of hexaploid wheat. *Theoretical and Applied Genetics,* 97, 657-670.
4. Gandilyan P.A., Jaaska V.E. (1980) A stable introgressive hybrid from hybridization between *Aegilops cylindrica* host and *Triticum aestivum* L. *Genetika,* 16, 1052–1058.
5. Goryunova S.V., Kochieva E.Z., Chikida N.N., Pukhalskyi V.A. (2004) Phylogenetic relationships and intraspecific variation of D-genome *Aegilops* L. as revealed by RAPD analysis. *Russian Journal of Genetic,* 40, 515–523.
6. Lelley T., Stachel M., Grausgruber H., Vollmann J. (2000) Analysis of relationships between *Aegilops tauschii* and the D-genome of wheat utilizing microsatellites. *Genome,* 43, 661–668.
7. Liu C.G., Wu Y.W., Hou H., Zhang C., Zhang Y. (2002) Value and utilization of alloplasmic common wheats with *Aegilops crassa* cytoplasm. *Plant Breeding*, 121, 407-410.
8. Mujeeb-Kazi A., Rosas V., Roldan S. (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. x *T. tauschii*; 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement. *Genetic Resources and Crop Evolution*, 43, 129–134.
9. Murai K., Tsunewaki K. (1993) Photoperiod-sensitive cytoplasmic male sterility in wheat with *Ae. crassa* cytoplasm. *Euphytica,* 67, 41-48.
10. Murphy J.P., Griffey C.A., Finney P.L., Leath S. (1997) Agronomic and grain quality evaluations of *Triticum aestivum* x *Aegilops tauschii* backcross populations. *Crop Science*, 37, 1960–1965.
11. Naghavi M.R., Mardi M., Pirseyedi S.M., Kazemi M., Potki P., Ghaffari M.R. (2007) Comparison of genetic variation among accessions of *Aegilops tauschii* using AFLP and SSR markers. *Genetic Resources and Crop Evolution*, 54, 237-240.
12. Nakai Y. (1989) D-genome donors for *Aegilops crassa* (MMDD, MMDDDD) and *Ae. vavilovii* (DDMMSS) deduced from esterase analysis by isoelectric focusing. *Japanese journal of Genetic*, 57, 349-360.
13. Pester T.A., Ward S.M., Fenwick A.L., Westra P., Nissen S.J. (2003) Genetic diversity of jointed goatgrass (*Aegilops cylindrica*) determined with RAPD and AFLP markers. *Weed Science,* 51, 287–293.
14. Pestsova E., Korzun V., Goncharov N.P., Hammer K., Ganal M.W., Röder M.S. (2000) Microsatellite analysis of *Aegilops tauschii* germplasm. *Theoretical and Applied Genetics,* 101, 100–106.
15. Saghai-Maroof M.A., Soliman K., Jorgensen R.A., Allard R.W. (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences,* 81, 8014-8018.
16. Watanabe N., Mastui K., Furuta Y. (1994) Uniformity of the alpha-amylase isozymes of *Aegilops cylindrica* introduced into North America: comparisons with ancestral Eurasian accessions. In: Wang K, Jensen B, Jaussi C (eds) Proc. 2nd Int. Wheat Symp. Utah State University, Logan, USA.
17. Zhang H.B., Dvorak J. (1992) The genome origin and evaluation of hexaploid *Triticum crassum* and *Triticum syriacum* determined from variation in repeated nucleotide sequences. *Genome*, 35, 509-515.