World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:3, No:1, 2009

Efficiency of Floristic and Molecular Markers to Determine Diversity in Iranian Populations of *T. boeoticum*

M. R. Naghavi, M. Maleki, and S. F. Tabatabaei

Abstract**—**In order to study floristic and molecular classification of common wild wheat *(Triticum boeoticum* Boiss.), an analysis was conducted on populations of the *Triticum boeoticum* collected from different regions of Iran. Considering all floristic compositions of habitats, six floristic groups (syntaxa) within the populations were identified. A high level of variation of *T. boeoticum* also detected using SSR markers. Our results showed that molecular method confirmed the grouping of floristic method. In other word, the results from our study indicate that floristic classification are still useful, efficient, and economic tools for characterizing the amount and distribution of genetic variation in natural populations of *T. boeoticum*. Nevertheless, molecular markers appear as useful and complementary techniques for identification and for evaluation of genetic diversity in studied populations.

Keywords**—***T. boeoticum*, diversity, floristic, SSRs.

I. Introduction

IRAN is not only one of the main sites of domestication of common and emmer wheat [1] but also a main center of distribution of wild wheats [2]. Therefore, it is supposed that the wild populations of *Triticum* species in this region contain high levels of genetic diversity. To date several studies have been conducted to reveal genetic diversity of wheat relatives in this region [3].

The genus *Triticum* comprises species of different ploidy levels from diploid (2n = 14) to hexaploid (2n = 42). *Triticum boeoticum* Boiss. with the genome A^bA^b (distributed mainly in West of Iran) has been reported as a valuable source of desirable genes conferring protein quality, amino-acid content or resistance [4].

Genetic diversity of a species within its floristic groups (e.g. its geographical populations) determines the rates of its adaptive evolution [5]. The floristic groups of *T. boeoticum* were determined according to the distribution of this species in geographical origins, along with its association with other species (principally *Aegilops* spp.). These associated compositions of species can be considered as the "richest wheat gene pool" that has been found in Iran [6].

In recent years, several molecular assays have been applied to assess genetic diversity among wheat cultivars [7]. Microsatellite or simple sequence repeats (SSRs) are highly mutable loci which may be present at many sites in a genome [8]. As the flanking sequence of these sites may be unique, primers can be designed to the flanking sequence (Jones et al., 1997). SSRs provide highly informative markers because they are co-dominant and generally have high polymorphic information content [9].

The goals of this study was to evaluate the genetic variability in a large collection of *T. boeoticum* populations sampled from different geographical regions of Iran using floristic and SSR markers.

II. Materials and Methods

Through the paths from Taleghan (located on the Northwest of Iran; habitat Nº 1) to Yasuj (located on the west of Iran; habitat Nº 100) one hundred habitats (populations) of the *T. boeoticum*, selected and marked (Fig. 1). According to the climatograms, these habitats were ordinated from cold to very cold; always in boundaries between arid to humid areas [10].

Fig. 1 The path and areas where the plant was studied in Iran

The habitats were generally in altitudes between about 1000 m to 2000 m above sea level.

Authors are with Agronomy and Plant Breeding Department, Agricultural College, University of Tehran, Karaj, Iran (phone: 0098-261-2246074; fax: 0098-261-2227605; e-mail: mnaghavi@ut.ac.ir).

The floristic compositions of the habitats were studied using the Braun-Blanquet [11] method. The collected data were firstly transformed by Neo-ZIGMA method [12], then analyzed and classified by anaphyto software [13].

Thirty six populations of *T. boeoticum* were randomly selected and genomic DNA was extracted from freshly collected leaves according to Saghai-Maroof et al. [14]. A total of 17 primer pairs (Röder et al. 1998), at least one primer pair from each of the seven A-genome chromosomes, were selected for genotyping. PCR was performed in 15 µl reactions, containing 40 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.3 µM of each specific primer, 200 µM of each dNTPs and one unit *Taq* DNA polymerase. Amplified PCR products were separated on 12% denaturing polyacrylamide gels using a vertical electrophoresis device, followed by silver staining. The bands were scored in binary notation, with 1 and 0 for presence and absence of bands, respectively. Binary matrix was used to estimate the genetic similarities between pairs, by employing Dice index [15]. These similarity coefficients were used to construct dendrogram using the unweighted pair group method with arithmetic averages (UPGMA), employing the NTSYS-PC version 2.02.

III. Results and Discussion

Considering all floristic compositions of habitats, six floristic groups (syntaxa) within the populations were identified. The distribution of the 100 populations (habitats) in six groups did not follow an exact geographical order (in each group there were similar habitats that had closer endogenic vegetation). Each group could also be identified by the special structural pattern of wild wheat spikelet [6].

TABLE I
Location, Repeat and Number of Alleles of Sequence Repeat (SSR) Markers Used in this Study

Marker no.	Marker name	Location	Repeat	Number of alleles
1	GWM130	7A	((GT)22	7
2	GWM155	3A	(CT)19	4
3	GWM156	5A	(GT)14	10
4	GWM160	4A	(GA)21	11
5	GWM164	1A	(CT)16	5
6	GWM165	4A	(GA)20	9
7	GWM265	2A	(GT)23	9
8	GWM293	5A	(CA)24	9
9	GWM304	5A	(CT)22	5
10	GWM33	1A-1B-1D	(GA)19	9
11	GWM334	6A	(GA)19	8
12	GWM350	7A	(GT)14	6
13	GWM397	4A	(CT)21	3
14	GWM427	6A	(CA)31, (CA)22	12
15	GWM617	2A	(GA)23	17
16	GWM635	7A, 7D	(CA)10 (GA)14	14
17	GWM674	3A	(CT)16CCC(GT)4	7
Average				8.5

Floristc groups of the west of Iran appeared to be more homogenous (lower in diversity) and therefore to be larger than those of the northwest of Iran.

Amplification of the SSR markers was performed using 17 primer pairs that produced 147 reproducible fragments, 145 of which were polymorphic (99%) (Table 1). The number of alleles per locus ranged from 3 to 17, with an average of 8.5 alleles per locus. The highest allele number was obtained at the *Xgwm617* locus. The genetic similarity values determined from the 145 polymorphic SSR bands had a mean of 0.39 and ranged from 0.04 to 0.73.

A sample of 36 populations of *T. boeoticum* was clustered in two main groups using SSR molecular analysis. The high level of variation of *T. boeoticum* detected by SSRs was consistent with the previous reports using AFLP markers [9]. This reflects probably both varietal differences and influence of climatic conditions as it was proposed also by Pagnotta et al. [16]. Other effects (e.g. accidental seed transfer with crops) could contribute to the spreading of genotypes to more distant regions [4].

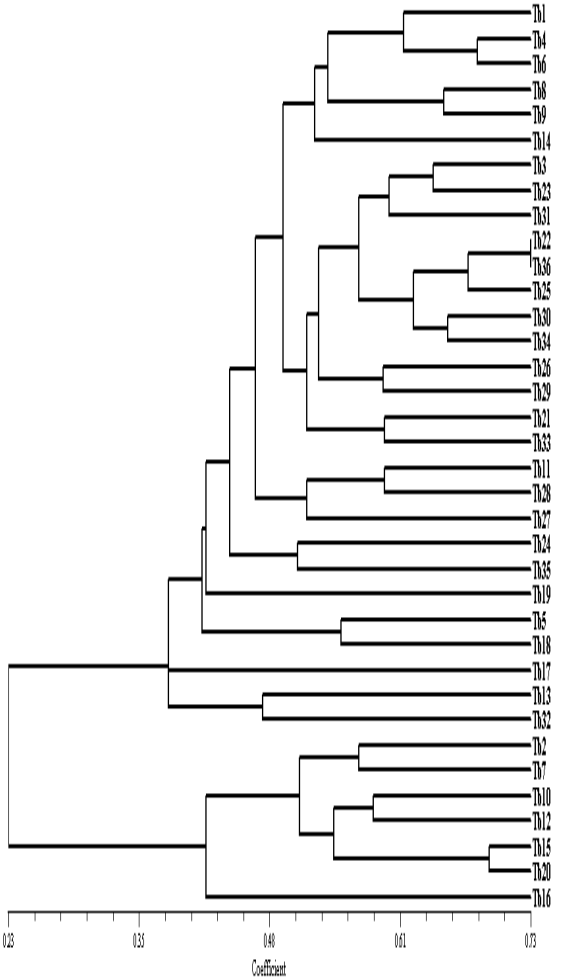

Fig. 2 Dendrogram showing the relationships among populations of *T. boeoticum* based on SSR markers

These level of variation showed that sampling natural populations of *T. boeoticum* and evaluation of sampled materials could bring to light more desirable genes [3]. Relative genetic distances between *T. boeoticum* populations,

expressed by the dendrogram, were relatively high for most groups. The habitats of wild wheats in the west of Iran (east of Fertile Crescent) are potentially the ideal areas to explore the suitable genes for further transferring into the cultivated wheat [17].

In conclusion the present electrophoresis study of molecular analysis was well in match with conventional characterization of wild wheat populations. In other word, the results from our study indicate that floristic classification are still useful, efficient, and economic tools for characterizing the amount and distribution of genetic variation in natural populations of *T. boeoticum*.

REFERENCES

[1] N.I. Vavilov, "Studies on the origin of cultivated plants". Inst Appl Bot Plant Breed, Leningrad. 1926.

[2] G. Kimber, and M. Feldman, "Wild Wheat: An Introduction". Special Report No. 353, University of Missouri, Columbia. 1987.

[3] M. Moghaddam, , B. Ehdaie, and G. Waines, "Genetic diversity in populations of wild diploid wheat Triticum urartu Thum. ex. Gandil. revealed by isozyme markers".' Genetic Resources and Crop Evolution, vol 47, pp. 323–334.

[4] J. Ovesna,, L. Kucera, R. Bockova and V. Holubec,. 'Characterisation of powdery mildew resistance donors within Triticum boeoticum accessions using RAPDs.' Czech Journal of Genetics and Plant Breeding, Vol. 38, pp. 117–124. 2000

[5] P.K. Gupta, H.S. Balyan, P.C. Sharma and B. Ramesh, 'Microsatellites in plants: a new class of molecular markers.' Current Science, Vol. 70, pp. 45-54. 1996.

[6] S.M. Fakhre-Tabatabaei, and T. Ramak-Massoumi, "Triticum boeoticum ssp. Thaoudar existed in Iran" Cereal Research Communication Vo. 29, pp. 121-126. 2001

[7] M.R. Naghavi,, M. Mardi, H.A. Ramshini, & B. Fazelinasab, 'Comparative analyses of the genetic diversity among bread wheat genotypes based on RAPD and SSR markers.' Iranian Journal of Biotechnology Vol, 2, pp.195-202, 2004.

[8] M. Morgante,, A. Pfeiffer, I. Jurman, G. Paglia and A.M. Olivieri " Isolation of microsatellite markers in plants". In: Karp A, Isaac PG, Ingram DS (eds) Molecular tools for screening biodiversity, Plants and animals. Chapman and Hall, London. 1998.

[9] M.R. Naghavi, M. Mardi, S.M. Pirseyedi, M. Kazemi, P. Potki and M.R. Ghaffari (2006). 'Comparison of genetic variation among accessions of Aegilops tauschii using AFLP and SSR markers.' Genetic Resources and Crop Evolution Vol. 54, pp. 237-250, 2006.

[10] S. Bazgir, (2000) 'Agriclimatological zoning of wheat in dryland farming.' MS diss., University of Tehran.

[11] J. Braun Blanquet, Plant sociology. Kelttz Scientific Books, West Germany. 1983.

[12] M. Atri, 'A presentation of some aspects of the application of neosigmatist method.' Iranian Journal of Biology, Vol. 12, pp. 1, 1996

[13] J.P. Briane, Anaphyto-ver. 95, Laboratorie de systematique et ecologie vegetale Bat. 362, Universite Paris XI, Orsay 91405. 1995.

[14] M.A. Saghai-Maroof, K. Soliman, R.A. Jorgensen and R.W. Allard, 'Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics.' Proceedings of the National Academy of Sciences of the United States of AME 81: 8014-8018. 1984.

[15] M. Nie, and W.H. Li, 'Mathematical model for studying genetic variation in terms of restriction endonucleases.'Proceedings of the National Academy of Sciences of the United States of AME, Vol 76, pp. 5269–5273. 1979

[16] M.A. Pagnotta M.A., E. Nevo, A. Beles and E. Porceddu , 'Wheat storage proteins: glutenin diversity in wild emmer, T. dicoccoides, in Israel and Turkey. 2 DNA diversity detected by PCR.' Theoritical Applied Genetics, Vol. 91, pp. 409–414. 1995.

[17] M.W. van Slageren, "Wild Wheats: a monograph of Aegilops L. and Amblyopyrum (Jaub. & Spach) Eig (Poaceae)", Wageningen Agricultural University Papers 94–7, Wageningen, the Netherlands. 1994.