Assessing wheat genetic diversity using quality traits, amplified fragment length polymorphisms, simple sequence repeats and proteome analysis

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ABSTRACT

The genetic diversity among 10 Iranian bread wheat (Triticum aestivum) genotypes was analysed using 12 quality traits, 320 amplified fragment length polymorphisms (AFLP) polymorphic fragments, 491 simple sequence repeats (SSR) alleles and 294 proteome markers. The results revealed that the genotypes differed for quality traits, AFLP, SSR and proteome markers. The average genetic diversity based on quality traits (0.684 with a range of 0.266–0.997) was higher than AFLP (0.502 with a range of 0.328–0.717), SSR (0.503 with a range of 0.409–0.595) and proteome (0.464 with a range of 0.264–0.870) markers. Although there were apparent similarities between the groupings of particular genotypes, the overall correspondence between the distance matrices appeared to be rather low. In this study the cluster analysis based on AFLP data showed the closest agreement with the region of origin or pedigree information of each genotype. In addition to the genetic diversity assessment, specific proteins with known function were detected uniquely for the studied genotypes. Our results suggest that the classification based on quality traits and genotypic markers of these wheat genotypes will be useful for wheat breeders to plan crosses for positive traits.

INTRODUCTION

Knowledge of genetic diversity among adapted cultivars or elite breeding materials has a significant impact on the improvement of crop plants and this information has been successfully used for efficient germplasm management and genotype selection for different breeding purposes. Genetic diversity has been evaluated in wheat and its relatives using morphological data, protein variation and DNA markers. However, it is important to determine whether different diversity estimation methods provide similar information concerning the degree of variation among wheat genotypes. The major goal of the present study was assessing genetic diversity in ten Iranian bread wheat genotypes with different levels of salt and drought tolerance by means of quality traits and SSR, AFLP and proteome markers.

MATERIALS AND METHODS

Plant Materials and quality traits: Ten spring wheat genotypes (*Triticum aestivum* L.) with different levels of salt and drought tolerance were chosen for this study (Table 1). Twelve quality traits were recorded in triplicate and the data means were used for the analyses.

DNA Marker analysis: Total genomic DNA was isolated using a CTAB method. The AFLP analysis was performed based on the method described by Vos *et al.* (1995) using twenty one *Eco*RI+NNN/*Mse*I+NNN primer combinations. Sixty-six SSR primer pairs were used based on Röder *et al.* (1998). Amplification reaction products were separated on a 5% denaturing polyacrylamide gel. The resulting images were scored manually.

Proteome analysis: Protein extraction was applied according to Finnie *et al.* (2002). Two-dimensional electrophoresis analysis was performed. The CBB stained protein spots were excised and analyzed using Applied Biosystems 4700 Proteomics Analyzer at the Protein and Proteomics Centre in the University of Singapore (Mass Spectrometry Services, Protein and Proteomics Centre, Department of Biological sciences). Protein digestion, desalting and concentration of samples were carried out using Montage® In-Gel Digestion Kits (Millipore and Applied Biosystems, Foster City, CA). Protein identification was performed by searching in a non-redundant protein sequence database program using Mascot server (http://www.matrixscience.com).

Data analyses: Data from quality traits and proteome analysis were standardized and used to estimate the distance matrix. Cluster analysis was performed using the unweighted pair grouping method of arithmetic averages (UPGMA). Correlation between all the matrices was tested using Mantel's non-parametric test. The support values for the degree of confidence at the nodes of the AFLP, SSR and AFLP+SSR dendrograms were analyzed by 1000 bootstrap resampling using PHYLIP 3.57c computer software.

RESULTS

Quality traits analyses: Distance estimates based on 12 quality traits ranged from 0.266 to 0.997 with an average of 0.684. Cluster analysis based on the quality data assigned the genotype into two groups. The first cluster includes Bafgy and Mahuti, originating from the center of Iran (Yazd), as well as Roshan and Shole. The genotypes in the first cluster had low to moderate means for all traits. The rest of the genotypes were assigned to group 2; Khazar and Bulani showed the highest similarity.

AFLP and SSR analyses: AFLP analysis of the 10 wheat genotypes based on twenty one primer combinations revealed a total of 320 polymorphic amplified DNA fragments. The fragments generated by AFLP primer combinations ranged from 35 (E-AGG/ M-GAG) to 4 (E-ACT/ M-CAT). Estimates of genetic diversity based on AFLP data varied from 0.328 to 0.717 with an average of 0.502. Grouping based on AFLP data showed relative agreement with genotypes region of origin (Fig. 1). In this grouping, two genotypes, Mahuti and Bafgy originating from the center of Iran were closely clustered together. Arvand and Roshan, two salt-tolerant genotypes, were also grouped together, with very high bootstrap value. Falat, a variety from CIMMYT materials, was separated from the others genotypes.

Sixty-six wheat SSR loci produced a total of 491 alleles across all the genotypes. The number of alleles per locus ranged from 1 to 17, with an average of 7.44 alleles per locus. Pairwise genetic distance between genotypes based on the SSR data varied from 0.409 to 0.595 with an average distance of 0.503. The cluster analysis revealed a relatively different grouping pattern in comparison to AFLP data (Fig. 2). In the resulting dendogram, Bafgy and Roshan were assigned to the same cluster and Arvand was separated from Roshan and grouped with Khazar. In both data, Falat was distinct from the other genotypes.

AFLP and SSR combined data analysis showed a different grouping pattern compared to the individual methods. Based on this grouping, Khazar was differentiated from the others and Kavir and Falat along with Bulani and Sholeh showed very close relationships. Bafgy and Arvand were grouped together with Mahuti and Roshan.

Proteomics analyses: Based on comparative analysis of 2-D maps of 10 wheat genotypes, 241 out of 294 protein spots were detected in all the genotypes (Fig. 3). Among these protein spots, the expression level of 177 showed significant quantitative changes ($P<0.01$) in at least one genotype compared to the others. We identified 13 common and eight genotype specific protein spots using MALDI TOF-TOF mass spectrometry (MS). The majority of the identified proteins were involved in metabolism, energy, protein destination and storage, and plant defence mechanisms. Pairwise, the genetic distance estimates based on 294 protein markers ranged from 0.264 to 0.870 with an average value of 0.464. There was no general concordance between the groupings obtained from proteome and other data sets (Fig. 4). However, a high similarity was found between Arvand and Roshan genotypes in the proteome data, which was in keeping with the AFLP based grouping (0.461). The Afgani landrace from Afghanistan was completely separated from the other genotypes.

Mantel's test: The correspondence of genetic relationships revealed by different types of molecular and morphological data was tested using Mantel test. The result showed no significant correlations between genetic distances of data set except between AFLP and AFLP+SSR.

DISCUSSION

The knowledge about the genetic relationships of genotypes provides useful information to address breeding programs and germplasm resource management**.** In this study, quality data analysis of the bread wheat genotypes was coupled with molecular analyses (AFLP, SSR and proteomes markers) to investigate the genetic relationships among ten Iranian bread wheat genotypes. The genotypes showed diverse quality traits and distinct AFLP, SSR and proteome patterns. The range of genetic distance based on quality traits was, on average, higher than AFLP, SSR and proteome markers which may reflect the influence of the environment on the performances of the materials.

In the present study AFLPs provided a better view of genetic relationships among the genotypes than the other markers. The grouping generated by AFLP data showed a certain agreement with the regions of origin of the samples (Bafgy and Mahuti come from central Iran) or the pedigree (Roshan and Arvand share a common ancestor). However, it may be postulated that SSR markers, due to their more rapid evolution than other marker types, may provide a more accurate measurement of similarity in highly related wheat lines. In conclusion, any one of these methods might be used to study genetic diversity and genotype grouping, but each approach would provide different information. The choice of genetic diversity analysis method depends largely upon the scope and the tools available to the researcher. Compared to molecular markers, the strong environmental evidence on quality characteristics makes these traits relatively less reliable and inefficient for precise discrimination of closely related genotypes and the analysis of their genetic relationships. However, phenotypic traits are useful for preliminary, fast, simple, and inexpensive genotype identification and can be used as a general approach for assessing genetic diversity among different cultivars (Marti *et al.,* 2007).

Our results suggest that molecular approaches along with quality studies may be used to evaluate genetic diversity and assess the genetic relationships between bread wheat genotypes with high accuracy. Therefore, the classification obtained for these Iranian wheat genotypes, based on quality traits and molecular markers will be a useful tool to Iranian breeders to plan crosses for positive agronomic characters by choosing genotypes with appropriate diversity.

Table 1 Pedigree/origin of 10 spring wheat genotypes

Genotype	Origin	Pedigree
Shole	Iraq	Landrace
Khazar	Iran- Gorgan	p4160(F3*Nr69)LR64
Arvand	Iran- Khozestan	Rsh(Mt-Ky*My48
Falat	Iran- Mecsice	Kvz/Buho,,s,,//Kal/Bb=seri82
Kavir	Iran- Zabole	Stm/3/Kal/V534/Jit716
Mahuti	Iran- Yazd	Landrace
Afghani	Afghanistan	Landrace
Roshan	Iran	Rsh*2/10120
Bafgy	Iran- Yazd	Landrace
Bulani	Iran- Cistan	Landrace

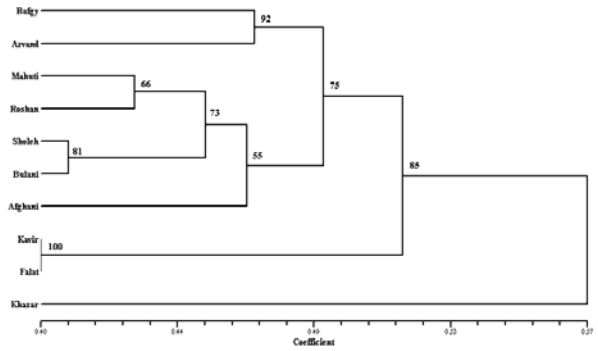

Figure 1 UPGMA dendrogram of 10 wheat genomes based on genetic distances computed from AFLP and SSR markers. Numbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses.

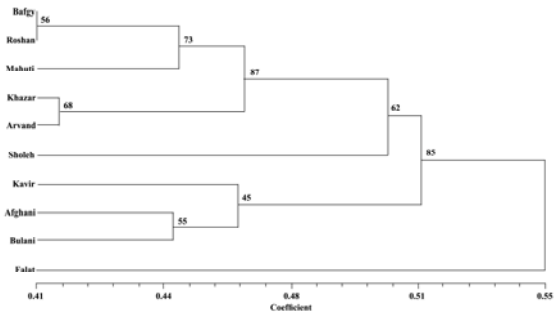

Figure 2 UPGMA dendrogram of 10 wheat genomes based on genetic distances computed from SSR markers. Numbers on the branches are bootstrap values (%) obtained from 1000 replicate analyses.

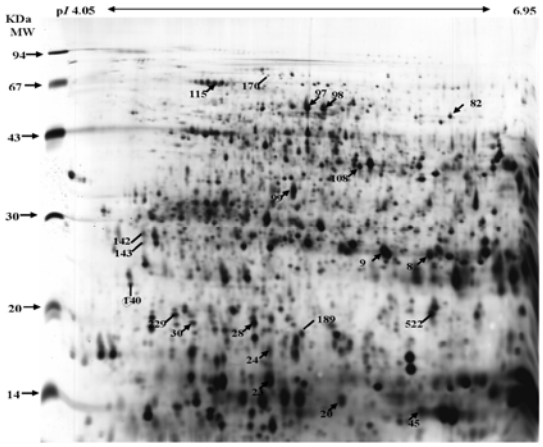

Figure 3 2-D gel analyses of proteins extracted from grains of Khazar-1 genotypes harvested under well-watered conditions. In the first dimension (IEF), 120 µg of protein was loaded on an 18 cm IPG strip with a linear gradient of pH 4-7. In the second dimension, 12% SDS-PAGE gels were used. Proteins were visualized by silver staining. Arrows represent proteins identified by mass spectrometry.

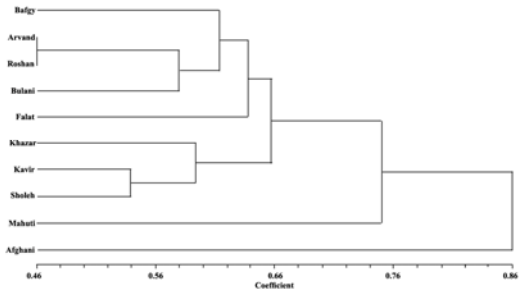

Figure 4 UPGMA dendrogram of 10 wheat genomes based on genetic distances computed from proteomes.

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