

Morphometric and amplified fragment length polymorphism marker analysis in some landrace wheat (*Triticum aestivum*) genotypes collected from north-west Iran

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Abstract

Wheat (*Triticum aestivum*) is one of the most important food staples in Iran. Understanding of the genetic variability among landrace wheat germplasm is important for breeding. The aim of this work was to characterize sixty landrace wheat accessions collected from north-west Iran, and to assess mutual genetic distances by use of morphometric and AFLP markers. In morphometric trait assay, results of variance analysis and descriptive statistics indicated that the genotypes differed significantly for all of the traits, and indicated considerable diversity. A dendrogram was constructed from morphological traits. The genotypes were grouped into two clusters, between which the mean Euclidian distance was 4.77. Twelve AFLP primer pairs detected 128 polymorphic bands among the sixty genotypes. The mean polymorphism rate based on AFLP data was 31%; however, wide polymorphism among primer pairs was observed (22 to 40%). Polymorphic information content, calculated to assess the informativeness of each marker ranged from 0.28 to 0.4, with a mean of 0.37. Five distinct clusters were obtained by using the un-weighted neighbour-joining method with arithmetic means analysis. In this study, the landrace genotypes did not demonstrate significant association between agronomic performance and genetic diversity determined using AFLPs. Clearly the study showed that there are genotypes with similar agronomic performance and different genetic constitutions. This information can be used to protect intellectual property and for breeding and selection programmes.

Key words: AFLP, diversity, landraces, morphometric, wheat.

Abbreviations: AFLP, amplified fragment length analysis; GCV, genotypic coefficient of variation; PCV, phenotypic coefficient of variation; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment-length polymorphism; UNJ, unweighted neighbour-joining; UPGMA, arithmetic means analysis; PIC, polymorphic information content; STS, sequence tagged-site.

Introduction

Wheat (*Triticum aestivum* L.) is a staple food and one of the important agricultural crops. It constitutes the basis for human nutrition and is of enormous economic importance in both Iran and worldwide. Wheat is used mainly for human consumption and supports nearly 35% of the world population (Schuster et al. 2009). Information on germplasm diversity and genetic structure of landrace gene pools is critical in wheat breeding (Altintas et al. 2008). Narrow genetic background has restricted development of improved varieties of crop that are tolerant to biotic and abiotic stresses. Early farmers grew genetic blended cultivars (landraces) on a very large scale and therefore extended genotypes possess effective genetic makeup against various plant diseases. Today it is recognized that the use of genetically different varieties is an effective strategy to minimize genetic vulnerability (Smale et al. 2002). Depending on geographical regions, landraces have specific genetic background that can be used in genetic

research programmes (Allard 1996). In addition, landraces are important genetic resources that improve gene pools of modern cultivars by introducing new alleles (Feldman, Sears 1981; Nevo, Payne 1987).

Modern plant breeding systems have narrowed the genetic base of cultivated crops. This has promoted the search for new gene pool resources that might be used in plant breeding programmes. Genetic diversity analysis can be conducted using different methods, such as descriptor lists of morphological characters and molecular methods. This is of significance for the production of food for the increasing world population (Fu, Somers 2009). Genetic similarity can be evaluated by pedigree analysis (Barrett et al. 1998) or by assessing morphological (Schut et al. 1997) and biochemical traits (Metakovsky, Branlard 1998) or more recently, by DNA markers (Barrett et al. 1998; Pagnotta et al. 2005).

Although several types of molecular markers have been employed to evaluate genetic diversity in wheat, such as random amplified polymorphic DNA (RAPD) (Joshi,

Nguyen 1993), restriction fragment-length polymorphism (RFLP) (Siedler et al. 1994; Kim, Ward 1997), amplified fragment-length polymorphism (AFLP) (Altintas et al. 2008; Talebi et al. 2012), sequence tagged-site (STS) (Chen et al. 1994) and inter-simple sequence repeat (ISSR) (Fatehi et al. 2011), AFLPs are considered to be a robust marker system that is reproducible and can be used to generate a large number of data points in a single analysis (Powell et al. 1996; Tohme et al. 1996). So far, the genetic diversity of Iranian landrace wheat accessions is not well understood. This study was designed to assess genetic diversity and population structure of Iranian wheat landrace accessions at the molecular level using morphological and AFLP markers and to determine relationship between genetic diversity and geographical sources of origin.

Materials and methods

Plant materials and field evaluation

Seed samples of 60 landraces of wheat (*Triticum aestivum* L.) were collected from agricultural fields of different geographical location in North-west Iran (Fig 1). Genetic variation of plants sown from the seeds was studied both at morphological and molecular level. The landraces were collected on the basis of morphological characteristics including plant height, seed color, seed size etc. and information from farmers cultivating them. Cultivation was conducted in the experimental field of Islamic Azad University of Sanandaj, in Kurdistan province (Northwest of Iran) in 2009 to 2010. Seeds were hand sown with each genotype in two rows of 4.0 m, and row-to-row distance of 0.25 m. The experiment was laid out in randomized complete block design with three replications. Plots were irrigated at tillering, joining, flowering and grain filling stages. Fertilizer was applied before sowing (50 kg N ha⁻¹ and 30 kg P ha⁻¹) and at stem elongation (50 kg N ha⁻¹). The total dry weight and grain yield (g m⁻²) were measured

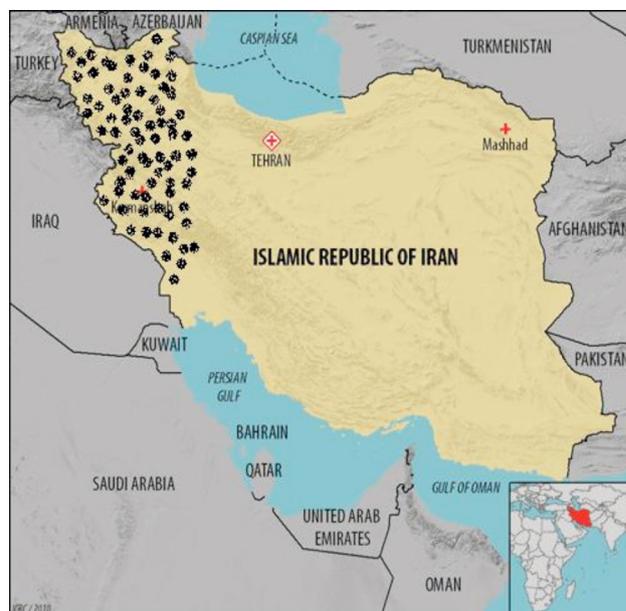


Fig. 1. Map of Iran showing the approximate areas where 60 landrace bread wheat accessions used in this study were collected.

by harvesting each plot at crop maturity. Ten plants were randomly chosen from each plot to measure other yield traits.

DNA isolation and AFLP analysis

DNA was extracted from 2 g young leaves collected from 10-day-old seedlings (bulked samples consisted of randomly selected 5 to 10 plants from each accession) using the CTAB method according to Lassner et al. (1989) with modification described by Torres et al. (1993). A 200 ng sample genomic DNA of each accession was digested simultaneously with 10 units each of *Mse*I and *Sse*8387I (Vos et al. 1995) (Table 1) at 37 °C for 4 h. Following digestion, *Sse*8387I and *Mse*I adapters (Cinagene, Iran) were ligated to restricted fragments at 20 °C for 2 h and the

Table 1. Oligonucleotide sequences used in the AFLP analysis

Type	Oligonucleotide	Sequence
Adapters	<i>Sse</i> 8387I adapter	5/-TCGTAGACTGCGTACATGCA-3/
		5/-TGTACGCAGTCTAC-3/
	<i>Mse</i> I adapter	5/ GAC GAT GAG TCC TGA G 3/
		5/ TAC TCA GGA CTC AT 3/
Non-selective primers	<i>Sse</i> 8387I-00	5/ GACTGCGTACATGCAGG3/
	<i>Mse</i> I-00	5/ GAT GAG TCC TGA GTA A 3/
Selective primers	<i>Sse</i> -AC (S12)	5/ GACTGCGTACATGCAGGAC3/
	<i>Sse</i> -AT (S14)	5/ GACTGCGTACATGCAGGAT3/
	<i>Sse</i> -CG (S23)	5/ GACTGCGTACATGCAGGCG3/
	<i>Sse</i> -CT (S24)	5/ GACTGCGTACATGCAGGCT3/
	<i>Mse</i> -GT (M22)	5/ GATGAGTCTGAGTAAGT3/
	<i>Mse</i> -TC (M24)	5/ GATGAGTCTGAGTAATC3/
	<i>Mse</i> -AT (M14)	5/ GATGAGTCTGAGTAAAT3/

Table 2. Estimation of components of variance in 60 wheat landrace genotypes for six agronomic characteristics evaluated in the field. CV=coefficient of variation; *, **=significantly at 0.05 and 0.01 level, respectively.

Component of variance	df	Plant height	Mass of 1 000 seeds	Number of seeds per spike	Harvest index	Biomass	Yield
Replication	2	0.007	24.22**	0.34**	1.17	34.07	8.16
Genotypes	59	0.008	19.51**	0.31**	2.19*	234.07*	42.19*
Error	118	0.006	1.15	0.02	0.67	80.77	10.71
CV (%)		10.51	12.91	16.21	11.88	21.12	30.11

digested fragments were preamplified using 20 cycles of 94 °C for 30 s, 60 °C for 1 min and 72 °C for 1 min according to the manufacturer's instructions.

Selective amplification was then performed according to the manufacturer's instructions (Cinagene, Iran). Diluted preselective PCR product samples (3.75 µL) were used as DNA templates for selective amplifications. The PCR selective amplification was carried out in the same manner as PCR preamplification. For selective amplification, the following cycle profile was used with 12 primer combinations: 2 min at 94 °C; 10 cycles: 30 s at 94 °C, 30 s at 63 °C (touchdown 1 °C per cycle to 54 °C), 2 min at 72 °C and 23 cycles: 30 s at 94 °C, 30 s at 54 °C, 2 min at 72 °C. All amplifications were performed in a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA). The AFLP Sse8387I primers were 5' end-labeled with the fluorescent dye IRD-800 or IRD-700 (Fermentas, Germany). A DNA analyzer system (LI-COR 4200 DNA analyzer; Lincoln, NE, USA) was used to detect the amplified fragments as described by Mardi et al. (2005). The resulting images were printed and manually and independently scored.

Data analysis

For morphological traits, variance analysis for grain yield and other related characteristics was performed after verifying the homogeneity of trait variance error using the Bartlett's test. Least significant difference (LSD) values were calculated at the 5% probability level. SAS (SAS Institute, Inc., 1996) procedures and programmes were used for these calculations. Correlations of genotypic and phenotypic traits was determined according to the method described by Kwon and Torrie (1964). DNA bands obtained with AFLP markers were scored visually for the presence

(1) and absence (0) of bands for all 60 accessions. DARwin version 5.0 was used to calculate pair-wise genetic distances and to construct a dissimilarity matrix (Perrier et al. 2003). The dissimilarity matrix obtained was subjected to cluster analysis using the unweighted neighbour-joining (UNJ) method (Gascuel 1997), followed by bootstrap analysis with 1 000 permutations to obtain a dendrogram for the 60 accessions (Perrier et al. 2003).

Results

Diversity of morphological traits

Results of analysis of variance and descriptive statistics indicated that the genotypes differed significantly for all traits, indicating considerable diversity, which could be utilized in developing high yielding cultivars through selection breeding (Table 2 and 3). The variability between genotypes was high for number of seeds per spike, thousand seed weight, yield, harvest index and plant biomass, while plant height did not significantly differ between genotypes (Table 2). This indicated that differences existed between the accessions for many traits. For each of the traits evaluated, descriptive statistics, including range mean, standard deviation, phenotypic and genotypic coefficients of variation (PCV and GCV) and broad sense heritability are summarized in Table 3.

Among traits, grain yield (g m^{-2}) ranged from 40.63 to 143.77 with a mean value of 65.43 g m^{-2} . High differences between the maximum and minimum mean values were found for all other traits. These results showed the adaptability of tested genotypes to semi-arid environments of north-west of Iran. Across the traits studied, PCV ranged from 0.61% for harvest index to 24.5% for grain yield (Table

Table 3. Summary of descriptive statistics: variance analysis, genetic variation (GV), phenotypic variation (PV) and broad-sense heritability (H^2_{bs}) components in wheat genotypes

Trait	Max	Min	Mean	SE of mean	GCV (%)	PCV (%)	H^2_{bs}
Plant height	110.51	65.70	84.70	9.77	13.21	17.78	0.49
Harvest index	0.53	0.25	0.41	0.07	0.37	0.61	0.61
Yield (g m^{-2})	143.77	40.63	65.43	20.21	16.44	24.50	0.27
Biomass (g m^{-2})	2270.86	877.44	1574.13	219.27	6.21	8.91	0.27
Thousand seed mass (g)	51	27.50	42.08	4.06	8.92	13.23	0.42
Number of seeds per spike	60.80	15.22	30.12	7.71	5.63	8.16	0.58

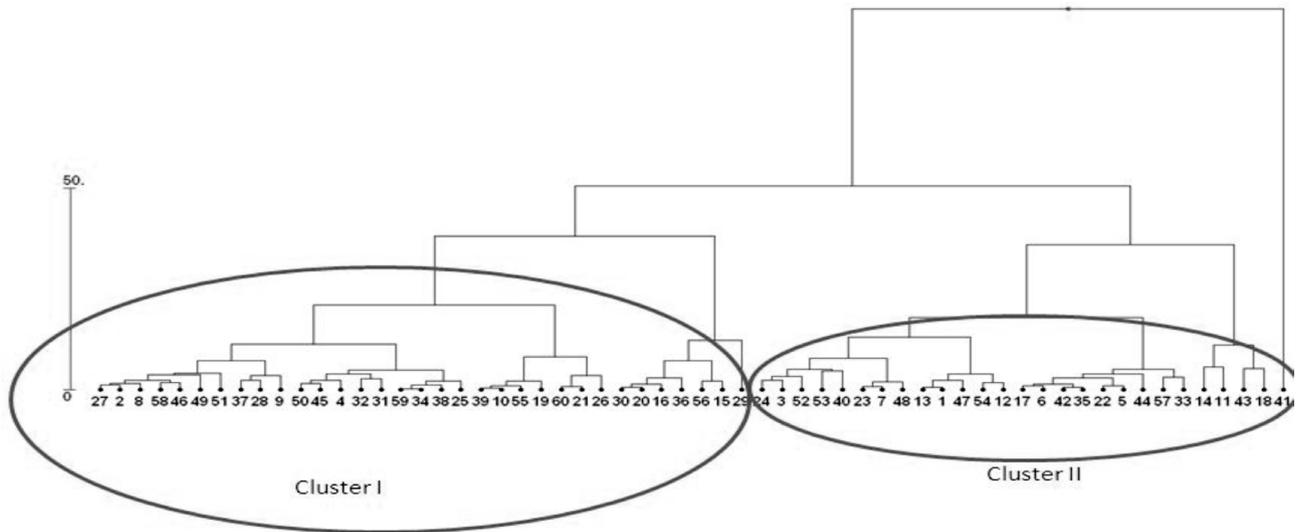


Fig. 2. Unweighted neighbour-joining dendrogram of 60 landrace wheat genotypes based on genetic distances computed from morphometric traits.

3). The GCV values were the lowest (<2%) for harvest index and highest (16.44%) for seed yield. In contrast, H^2 estimates were comparatively low for seed yield and biomass (0.27). Among the agronomic and morphologic traits, selecting genotypes using 1000-seed weight, number of seeds per spike and harvest index was improved yield. Due to higher heritability estimates, great benefits from selection might be expected for all the traits studied. The present results are in accordance with those previously reported by Ahmadzade et al. (2011), Talebi et al. (2012) and Maniee et al. (2009). The higher values of heritability for number of seeds per plant, harvest index and 1000-seeds weight indicate that these characters can be used as genetic parameters for improvement and selection of high yielding genotypes. These results were in accordance with the findings of

Kahrizi et al. (2010) and Khayatnejad et al. (2010).

A dendrogram was constructed from a morphological dissimilarity trait matrix. The genotypes were grouped into two clusters, located at mean Euclidian distance value of 4.77. The first cluster contained 33 genotypes and remained genotypes grouped in the second cluster (Fig. 2). The first cluster included three sub clusters: the first sub cluster contains genotypes with pair dissimilarity values ranging from 0.13 to 0.45. These genotypes had a higher grain yield and thousand seed weight compare to others. The second and third sub-clusters contained genotypes with moderately high yield and biomass production. Most of these genotypes originated from the Kurdistan and Kermanshah provinces located in western Iran. The second cluster also included three sub clusters: the first was comprised of genotypes

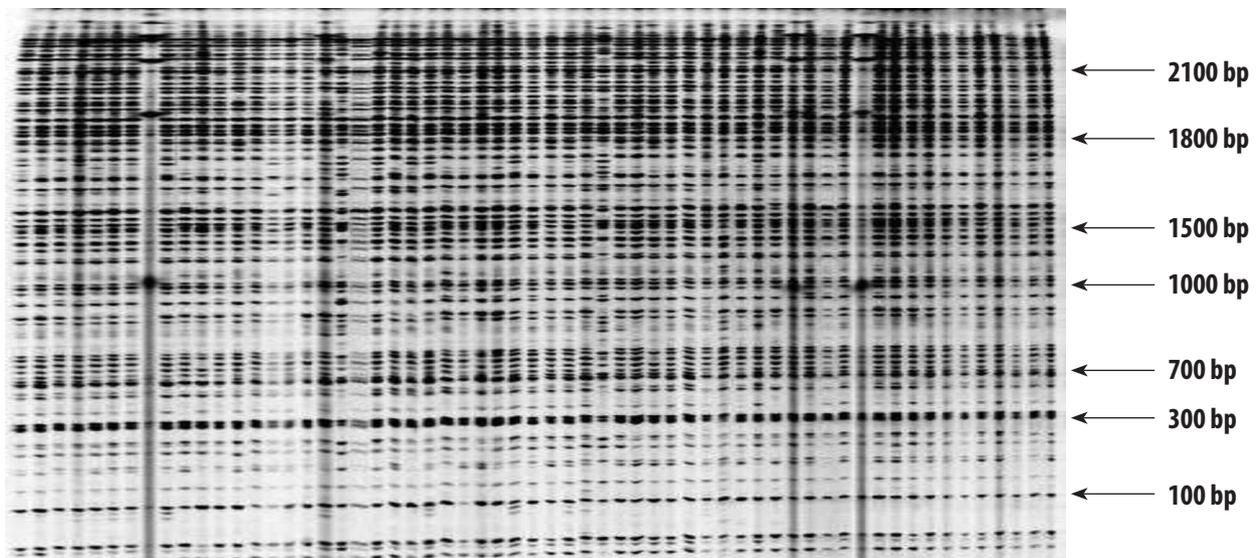


Fig. 3. Amplification profile for 60 landrace wheat accessions obtained using a AFLP primer pair (Mgt/Scg).

Table 4. Characteristics of the amplification products obtained with 12 AFLP primer pairs used to analyze the genetic diversity of 60 landrace wheat genotypes

No	Primer Combination	Total number of amplification products	Number of polymorphic bands	Percent of polymorphism	PIC value
1	S12/M22	45	16	35	0.44
2	S12/M24	42	13	30	0.29
3	S12/M14	51	12	23	0.34
4	S14/M22	39	10	25	0.28
5	S14/M24	44	12	27	0.32
6	S14/M14	37	15	40	0.40
7	S23/M22	39	14	35	0.39
8	S23/M24	49	11	22	0.41
9	S23/M14	51	17	33	0.48
10	S24/M22	43	13	30	0.40
11	S24/M24	44	14	32	0.41
12	S24/M14	37	15	40	0.44

mostly from north-west Iran (Azerbaijan province) and compared to the other two sub clusters, showed relatively higher yield. Interestingly, genotype number 41, collected from Mahabad (Azerbaijan province), grouped separately and was not included in a cluster.

AFLP diversity

The 12 AFLP primer combination pairs used in this study generated 128 polymorphic (mean = 10.6 per primer pair) and 392 monomorphic bands across the 60 landrace wheat genotypes. Figure 3 shows a representative amplification pattern obtained using S23/M22 primer combinations. These results suggested the efficiency of the AFLP primer combinations used and confirmed that the AFLP technique successfully generated polymorphic markers. The mean

polymorphism rate was 31%, but a wide polymorphism range among primer pairs was observed (22 to 40%) (Table 4).

The polymorphism information content (PIC) value of different primer combinations ranged between 0.28 to 0.48 (Table 4). The lowest and the highest PIC values were recorded for primer S23/M14 and S14/M22, respectively. In addition, the proportion of missing values had no influence on these quality parameters. PIC values and marker indices were almost identical to those published by Bohn et al. (1999) and Manifesto et al. (2001) in wheat.

The average genetic distance, based on F_{st} values among 60 genotypes, was 0.46, and ranged from 0.23 to 0.82. To evaluate phylogenetic relationships between landrace wheat accessions a dendrogram based on 163 loci AFLPs

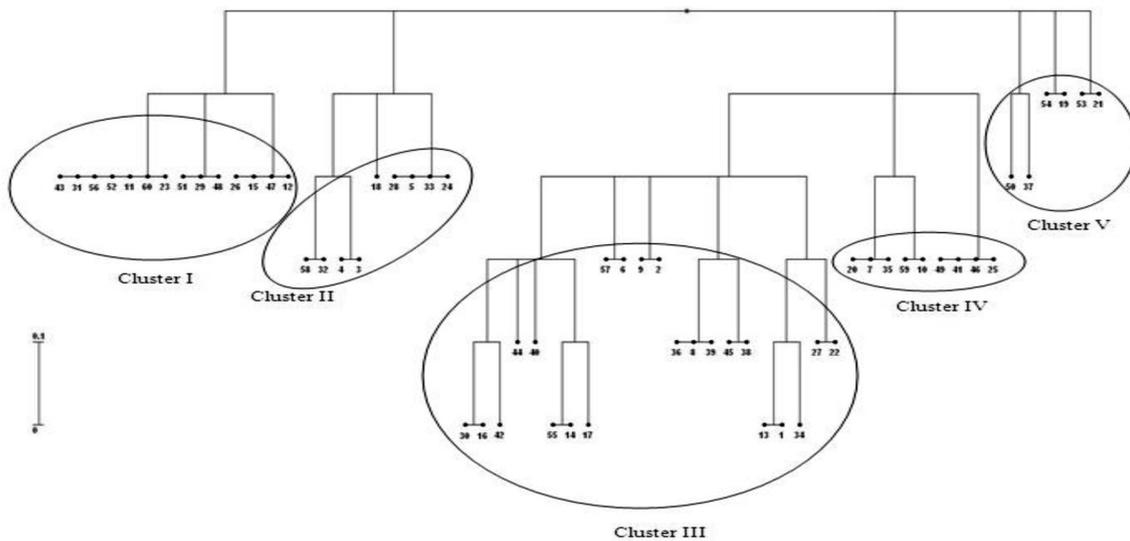


Fig. 4. Unweighted neighbour-joining dendrogram of 60 landrace wheat genotypes, based on genetic distances computed from AFLP markers.

was constructed using UPGMA (Fig 4). According to the constructed trees, bread wheat accessions fell into five major categories. The first and second clusters included genotypes with medium yield and mainly collected from north-west Iran. The third cluster of genotypes, which were mostly collected from western Iran, showed higher yield compared to other genotypes in field assay. The fourth and fifth cluster did not distinguish genotypes based on their geographical collection site and mainly were comprised of genotypes with tall height and low productivity.

Discussion

Efficient utilization of the genetic potential held in the germplasm collections requires, among other things, better knowledge of the genetic diversity present in the collected material. Knowledge of the extent of plant trait variability and the association of specific traits with geographic origin not only facilitates breeding programmes but also helps to define the needs and locations for future collections of germplasm. The principal aim of this study was to provide additional and valid molecular markers suitable for the examination of genetic diversity and to assess phylogenetic relationships in the complex of 60 landrace bread wheat accessions, to obtain useful molecular data for the local gene pool. The AFLP analysis appeared to be efficient for the detection of the polymorphic molecular markers in this population complex and was shown to be an attractive approach to achieve this objective. A total of 163 polymorphic AFLP fragments were scored from twelve of the used primer combinations. This number of AFLPs is greater compared to other types of molecular markers used in previous study on wheat, such as ISSR (Fatehi et al. 2011) and RAPD (Sun et al. 2003).

The high level of polymorphism observed in this study is in accordance with the research of Altintas et al. (2008), Eivazi et al. (2008) and Shoaib and Arabi (2006). The relationship observed using molecular markers may provide information on the history and biology of genotypes, but it does not necessarily reflect that observed for agronomic traits (Metais et al. 2000). Previous studies have shown that the estimated genetic divergence from morpho-agronomical traits is not completely similar to AFLP-based results (Altintas et al. 2008). We found that there was no relationship between morphological divergence and geographical origin as accessions from one region occurred in more than one cluster.

Accessions from different geographical origins often appeared unique clusters in one part of the dendrogram, suggesting that the morphological variation is determined not only by environmental differences but also by genetic factors. The genotypes showed diverse phenotypic characteristics and distinct AFLP patterns. The range of genetic distance based on AFLP was on average higher than phenotypic data, which may reflect the influence of

the environment on plant performance. Polymorphisms produced by AFLP markers in anonymous regions are randomly distributed over the whole genome. The differences between phenotypic traits and AFLP profiles are the result of different gene expression of functional genomic regions controlling various traits. Therefore, DNA markers and phenotypic data will not necessarily yield closely matching results (Vollmann et al. 2005; Eivazi et al. 2008). There are several reasons for this, which may apply also to the AFLP technique, such as sensitivity of the working conditions and equipment used for molecular analysis. Another problem might arise from overestimation of genetic similarity, as fragments with the same molecular weight could have different origin. Martinez et al. (2005) considered that the correspondence between different methods might be improved by analyzing more morphological characters and AFLP markers.

The present study showed existence of high genetic diversity in Iranian landrace wheat germplasm accessions. The magnitude and pattern of genetic variation detected in this study can be useful for germplasm management and use in breeding programmes (Tanya et al. 2011). The exploitation of crosses between genetically distant parents and those from diverse local sources may produce higher heterosis, better genetic recombination and segregation in their progenies, and result in varieties with a broad genetic base (Chahal, Gosal 2002). The selection of genotypes in this study was primarily based on different geographic origin and also considered the important role of north-western of Iran in wheat production for poor farmers. Therefore, we believe that there is a need for molecular marker studies to complement study of morphological traits in the field. This would reduce the amount of material for study as well as the costs of experiments. It is expected that when diverse genotypes are involved in breeding programmes, as a result of reshuffling of alleles due to recombination, there are better chances for the appearance of transgressive segregation with beneficial traits that can be selected to extract high yielding lines with desirable trait combinations.

Further, large genetic variation exists between wheat genotypes, which can be used efficiently for gene tagging and genome mapping of crosses to introgress the favorable traits, such as high yield potential, disease and insect resistance, into the cultivated genotypes. The huge diversity contained in wheat germplasm collected and maintained mainly in the Iran, has not been exploited effectively in cultivar development. The main reason for this might be the large numbers of accessions, which makes it difficult and time consuming to evaluate material for all useful yield traits in field trials and to choose the most promising ones. Development of wheat core collections based on species could facilitate utilization of the huge diversity stored in genebanks in Iran. Results of the present study indicate that extent of genetic variability in the Iranian wheat gene pool seems to have remained quite constant. This constancy

should be considered of qualitative relevance, as it indicates that the landrace genotype pool has been enriched by material different from the native and locally adapted germplasm, which resulted in broadening of the genetic background in different regions from north-west of Iran. Therefore, the classification obtained for these Iranian wheat genotypes, based on morphological traits and molecular markers will be a useful tool for Iranian breeders to plan crosses for positive agronomic characters by choosing genotypes with appropriate diversity. The previous crossing programs in most research centers of Iran have been based on only phenotypic characters. The current study confirmed the importance of molecular studies (inexpensive, fast and informative markers) alongside morphological data, to determine genetic variation among genotypes with the aim to select diverse parents in new crossing programmes.

Acknowledgements

Authors would like to acknowledge the Research and Innovation Deputy of Islamic Azad University, Sanandaj Branch for providing the financial support.

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