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Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines

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Abstract

Analysis of genetic variation is fundamental to plant breeding programs. The present study evaluated genetic diversity of thirty wheat cultivars and advanced breeding lines using phenological and agro-morphological characters and molecular markers (ISSRs) data. The field experiment was carried out in growing season of 2008-2009. The measured phenotypic traits (20 traits) illustrated significant differences among the wheat accessions. Variation for most of the traits was observed. The clustering pattern based on phenotypic data using WARD method assigned the wheat genotypes into four groups. The ten ISSR primers amplified a total of 86 bands in the set of thirty wheat accessions, of which 69 bands (80.2%) were polymorphic. The majority of the primers showed polymorphism information content (PIC) values close to the average (0.21-0.23), indicating diverse nature of the wheat accessions and/or highly informative ISSR markers used in this study. The genotyping data of the ISSR markers were used to assess genetic variation in the wheat accessions by CLINK- based dendrogram and principle coordinate analysis (PCoA). Both of the methods classified the 30 wheat accessions in five groups and presented similar grouping of the genotypes with some minor deviations. The results showed that the studied ISSR markers, provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. The analyzed wheat accessions showed a good level of genetic variability for both assessed quantitative and molecular characters. No correlation was found between variation measurements identified using molecular markers and quantitative traits. Molecular variation evaluated in this study in combination with agronomic and morphological characters of wheat can be useful in traditional and molecular breeding programs.

Keywords: Agr-omorphological and phonological traits, Genetic diversity, ISSR markers, Wheat.
Abbreviations: HEM, head emergence; HE, days to heading; FL, days to flowering; PM, physiological maturity date; GFP, grain filling period; AL, awn length; FLA, flag leaf area; LCC, leaf chlorophyll content; PL, peduncle length; PH, plant height; SPL, spike length; PL/PH, ratio of peduncle length to plant height; FSPD, fertile spikelets density; NSP, number of spikes per m^2; NSSP, number of seeds per spike; TSW, thousand seed weight; GY, grain yield; SY, straw yield; BY, Biological yield; HI, harvest index; TAB, total amplified bands; NMB, number of monomorphic bands; NPB, number of polymorphic bands; PPB, percentage of polymorphic bands; PIC, Polymorphism information content.

Introduction

Analysis of genetic relationships in crops is a prerequisite for crop breeding programs, as it serves to provide information about genetic variation (Mohammadi and Prasanna, 2003). Evaluation of genetic diversity using molecular markers is a cornerstone for understanding genome structure, the characterization and maintenance of genetic variation in plant germplasm, identifying genes underlying important traits, and devising optimal breeding strategies for crop improvement (Hayden et al., 2010). Lack of genetic diversity can potentially limit the ability of cropping systems to resist unknown or evolving pests, pathogens, or adverse environmental conditions. Morphological traits (syn. phenotypic traits) are commonly used to evaluate genetic variation because their measurements are simple Diversity analysis, based on morphological traits alone, may not be completely reliable because the traits are limited in number and influenced by environment (Fufa et al., 2005). Despite these limitations, phenotypic characters have been successfully used for genetic variation studies and cultivar development. Molecular diversity evaluated by using molecular markers is independent of the influence of environment and can be estimated by using DNA from any growth stage (Tatikonda et al., 2009). Molecular characterization is now the favored means to quantify variation within germplasm samples (Glaszmann et al., 2010). Molecular markers have clarified the structure of genetic diversity in a wide range of plant species. Molecular diversity studies evaluate all levels of genetic structure, ranging from relationships between species complex components to the origin of particular genotypes (Kilian et al., 2007). For these purposes, different marker systems such as AFLP (Altintas et al., 2008; Tatikonda et al., 2009), ISSR (Carvalho et al., 2009; Parvathaneni et al., 2011), RAPD (Kumar et al., 2009) and SSR (Pagnotta et al., 2009; Zarkti et al., 2010; Zaher et al., 2011) have been used. Inter simple sequence repeat (ISSR) technique is a PCR based method that is highly effective in plant fingerprinting and phylogenetic studies (Vaillancourt et al., 2008). ISSR analysis involves amplification of regions between adjacent and inversely oriented microsatellites using di, tri, tetra and

pentanucleotides SSR primers, with the advantage that knowledge of the DNA sequence of the target regions is not needed. This marker combines most of the benefits of AFLP and SSR analysis with the universality of RAPD (Pradeep Reddy, 2002; Carvalho et al., 2009). Our objectives of the present study were: (1) to determine the genetic diversity in wheat cultivars and breeding lines using phonological and agro-morphological traits and ISSR markers and (2) to compare the two methods of measuring diversity.

Results

Phenotypic traits

Analysis of variance showed significant differences between wheat accessions for all the traits except spike length (SPL), number of spikes per m^2 (NSP), biological yield (BY) and straw yield (SY) (data not shown). Mean values of phenological and agro-morphological traits measured are presented in Supplementary data. *Days to Head Emergence (HEM), Days to Heading* (HE) and *Days to Flowering* (FL). Genotypes 10, 13, and 14 were the earliest accessions, while number 17 was the latest. Days to flowering ranged from 175.7 to 187 days with an average of 180.2. On the average, heading date was 5 day after head emergence and flowering occurred 4 days after heading. *Physiological Maturity Date* (PM) and *Grain Filling Period* (GFP). Maturity time was similar among the most of genotypes even if, also for this trait, the differences among genotypes were significant. Grain filling period differed between 36.3 and 44.3 days. *Awn Length* (AL). One of the wheat accessions (Number 3) was awn-less. Genotype number 1 had the shortest awn with an overall mean of 0.51 cm. The awn length of most accessions was at interval of 5–6 cm. The Character was different among accessions ranging from 0 to 6.30. *Flag Leaf Area* (FLA). Flag leaf plays an important role during grain filling. Flag leaf area differed between 10.87 cm^2 (accession 2) and 26.34 cm^2 (M-81-13) with an average of 19.93 cm^2. Accession 2 had low number of seed per spike while M-81-13 had dense spike with higher number of seeds. *Leaf Chlorophyll Content* (LCC). The amount of leaf chlorophyll was similar among the genotypes with an overall mean of 53.56. Nevertheless, the differences among the genotypes were significant for this character. Pishgam, the new released and high yielding cultivar had the highest leaf chlorophyll content. *Peduncle length* (PL) and *Plant height* (PH). The awn-less wheat genotype (Number 3) had the shortest peduncle (29.55 cm). Plant height ranged from 74.3 to 120.1. The tallest genotype was HAMAM-4 (120.1 cm). *Spike Length* (SPL) and *Fertile Spikelets Density* (FSPD). Spike length was not significantly different among the genotypes. Spikelets density differed between 2.75 to 6.42. Shiraz had the densest spike. *Number of Spikes per m^2* (NSP). Since the seeds planted were equal in each experimental unit, the number of spikes per m^2 was influenced by seed germination rate and tillering potential. On average, advanced breeding lines produced a higher number of spikes in comparison with cultivars. *Number of Seeds per Spike* (NSSP). This character is one of the important components of grain yield. This character ranged from 21 in genotype 29 to 58.8 in Pishgam, the new released cultivar. *Thousand Seed Weight* (TSW). Seed weight is another important yield component. It differed from 32.23 g to 48.95 g with an overall mean of 38.59 g. Accession 5 was the best from this point of view, while had low number of seeds per spike. *Grain Yield* (GY), *Straw Yield* (SY), *Biological Yield* (BY) and *Harvest Index* (HI). Grain yield varied from 4.22 to 10.13 ton/ha. The most productive genotype was Pishgam while the least productive was HAMAM-4. Pishgam was one of the shortest genotypes and also had the highest biological yield and harvest index. On the other hand, HAMAM-4 was the tallest accession and possessed the lowest biological yield and harvest index.

Cluster analysis based on phenotypic data

The clustering pattern of the wheat genotypes based on phenotypic data using WARD method is depicted in Fig 1. The analysis assigned the genotypes into four groups. Group 1 included twelve accessions characterized by small plants, high number of seed per spike and higher grain yield. In the second cluster, four genotypes (numbers 4, 5, 12 and 17) grouped together which were tall and low-yielding. The rest of the genotypes could be separated into two clusters. The Third cluster comprised six genotypes. Breeding lines 1 and 3 were in a sub-cluster in this group. Accession 1 was awn-less and 3 had the shortest awn (0.51 cm), and in the fourth cluster the other genotypes were included.

ISSR polymorphism

Fifteen ISSR primers were initially screened for their ability to produce polymorphic patterns across the thirty wheat genotypes. Ten primers which were repeatable and produced high resolution bands for all the genotypes were selected for evaluation of genetic diversity in the accessions (Table 2). The ten ISSR primers amplified a total of 86 bands in the set of thirty wheat accessions, of which 69 bands showed polymorphism and 17 bands were monomorphic. Number of bands varied from five (UBC-822) to twelve (UBC-834 and UBC 840). The percentage of polymorphic bands (PPB) ranged between 60 and 100 with an average of 80.2%. Mean numbers of bands and polymorphic bands per primer were 8.6 and 6.9, respectively (Table 2). The PIC values for the ten primers varied from 0.13 to 0.42 with an average of 0.22. More than half of the primers (6) showed PIC values between 0.21 and 0.23. The lowest and highest PIC indices were recorded for primer UBC-811 and UBC-815, respectively (Table 2).

Cluster analysis and PCoA based on genotypic data

Jaccard similarity matrix based on ISSR binary data was used to group the wheat accessions using the complete linkage (CLINK) method. Genetic similarity ranged between 0.48 and 0.91 (data not shown). The dendrogram classified the thirty wheat genotypes into five clusters (Figure 2). Cluster 1 included genotypes 1 and 23. Accessions 7, 19, 26, 25, 10, 30 and 29 were grouped in the second cluster. Cluster 3 contained five genotypes (8, 22, 28, 9 and 24). Cluster 4 contained a total of 13 genotypes (2, 3, 5, 15, 18, 6, 14, 12, 20, 16, 17, 21 and 4). Genotypes 11, 27 and 13 were grouped in cluster 5. Principle coordinate analysis results are illustrated in Figure 3. The thirty genotypes were grouped into five groups based on two-dimensional graph. Group 1 contained genotypes 1 and 23. Group 2 included 10 genotypes (7, 19, 20, 22, 28, 8, 26, 10, 30 and 29). The third group contained accessions 11, 27, 25, 9 and 24. Group 4 contained a total of 12 genotypes (2, 3, 5, 15, 18, 6, 14, 12, 16, 17, 21 and 4). Group 5 included only genotype 13.

Correlation analysis

The comparison between phenotypic traits and ISSR markers was carried out by Mantel correlation test. The correlation

Table 1. List, pedigree and some important character(s) of 30 wheat genotypes used in present study. Advanced lines are indicated by their pedigrees.

Genotype code	Name	Pedigree	Important character(s)
1		F103-L-1-12//PONY/OPATA	Short awn
		OR	High straw yield
2		F1.158/FDL//BLO/3/SH14414/CROW/4/C ICWH99381-0AP-0AP-OMAR-6MAR	
3		PYN/BAU//VORONA/HD2402	Awn-less, short peduncle
4	KATILA-13		High thousand seed weight
5		SARDARI-HD35/5/DMN//SUT/AG(ES86-7)/3/ ICWH99-0552-0AP-0AP-OMAR-3MAR	Drought resistance
6	Zarin		High potential yield
7		CA8055//KS82W409/STEPHENS	High straw yield
8	Bolani		Rust susceptible
9	Shahriar		Long awn
10	WS-82-9		Earliness, terminal drought resistance
11		SABALAN/4/VRZ/3/OR F1.148/TDL//BLO	Terminal drought resistance
12	HAMAM-4		Tallness, low grain yield
13		Atila2/PBW65	Earliness, long awn
14		KAUZ'S'/MACHETE	Earliness
15	M-79-7		High potential yield
16	pishtase		Terminal drought resistance
17		KAR-1//RMNF12-71/JUP'S'	Lateness, high straw yield
18	QAFZAH-25		Long awn
19	Marvdasht		High potential yield
20	Chamran		Long awn, high potential yield
21	M-81-13		Terminal drought resistance
22		TEVEE'S'//CROW/VEE'S'	Terminal drought resistance
23	M-83-17		Terminal drought resistance
24	M-83-6		Terminal drought resistance
25	M-82-6		Terminal drought resistance
26		Jcam/Emu"s"//dove"S"/3/Alvd/4/MV17/Attila	Dwarfness, earliness, high potential yield
27	Shiraz		Dense spike
28		STAR/SHUHA-4	Dwarfness
29	KATILA-1		Short spike
30	Pishgam		Dwarfness, high potential yield, drought resistance

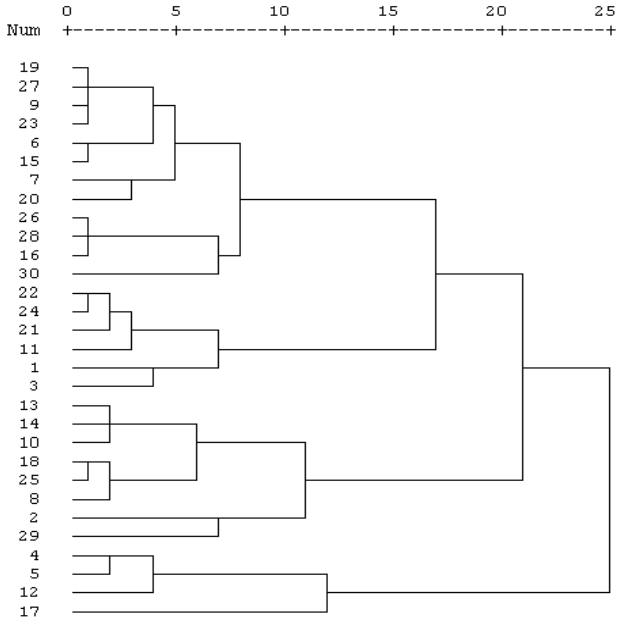

Fig1. Ward dendrogram of 30 wheat cultivars and advanced breeding lines based phenotypic data, showing relationships among the genotypes.

between the two measurements was 0.049 and it was not significant, showing that they were not related.

Discussion

Phenological and agro-morphological traits measured (20 traits) depicted significant differences among the wheat accessions. Variation for most of the traits was observed. Agronomic, morphological and phonological traits are very important for grouping wheat genetic resources, and also are essential and useful for plant breeders seeking to improve existing germplasm by introducing novel genetic variation for certain traits into the breeding populations (Lage et al., 2003; Pagnotta et al., 2005; Salem et al., 2008; Pagnotta et al., 2009; Zarkti et al,. 2010). Therefore, these characteristics have good potential in order to select and to conserve genotypes. Our study by using ISSR markers revealed a high level of genetic diversity among the wheat accessions. The markers detected 6.9 polymorphic bands per primer with an average polymorphism of 80.2%. Variable efficiencies of different marker systems for detecting DNA polymorphism in wheat have been reported. Joshi and Nguyen (1993) observed 1.8 polymorphic bands per RAPD primer among 15 wheat cultivars, while SSRs with 6.2 alleles/bands were more polymorphic (Plaschke et al., 1995). Nagaoka and Ogihara (1997) detected 3.7 polymorphisms per ISSR primer, while Carvalho et al. (2009) reported 12.9 polymorphic bands per primer using 18 ISSR primers in 48 wheat accessions. We detected a high level of polymorphism among the wheat genotypes using ISSRs, indicating high efficiency of the marker technique to reveal genetic diversity in the case of wheat. The lowest polymorphism value (57.1%) was obtained with the UBC-876 primer [$(GATA)_2(GACA)_2$] (Table 2). Primers based on more infrequent tetranucleotide SSRs amplified few bands in rice (Blair et al., 1999), while detected more polymorphism in Dent and Popcorn (Kantety et al., 1995). The ISSR primers with dinucleotide motifs (GA)n, (CT)n and (AG)n produced a high level of polymorphism (Table 2). These results are in agreement with those of Carvalho et al. (2009) who reported that dinucleotide primers were more suitable for amplifying ISSRs in bread and durum wheat. SSRs seems to be randomly distributed in the genome, and $(GA)_n$ dinucleotide repeats are most abundant in plant species (Wang et al., 1994; Steinkellner et al., 1997).The PIC values differed between 0.13 and 0.42 with an average of 0.22. The majority of the primers (6) showed PIC values close to the average (0.21-0.23) (Table 2). The moderate values of PIC for the ISSR primers could be attributed to the diverse nature of the wheat accessions and/or highly informative ISSR markers used in this study. The PIC index has been used extensively in many genetic diversity studies (Tatikonda et al., 2009; Talebi et al., 2010; Thudi et al., 2010). The genotyping data for all the ISSR markers were used to assess the genetic variation in wheat genotypes by CLINK- based dendrogram and principle coordinate analysis. The results of the two methods were comparable. Both of the two methods classified the 30 wheat accessions in 5 groups and presented similar grouping of the genotypes with some minor disagreements. The obtained clusters/groups were not in accordance with the known geographical location. The fact that there was no correlation between variation measurements identified in wheat using molecular markers and quantitative traits are in agreement with previous studies. Esmaeilzadeh Moghaddam et al. (2005) reported low and non significant correlations (-0.23 and -0.25) for AFLP and agronomic characters in wheat lines evaluated in Iran and Mexico, respectively. Rana et al. (2005) reported a correlation of 0.04 between morphological traits and AFLP markers in cotton. Garcia et al. (2007) found no relationship (r =0.025) between RAPD and morphological characters in perennial dalligrass. However, Autrique et al. (1996) in their genetic diversity study in durum wheat using RFLP and agronomic traits calculated a moderate correlation (0.47) which was a result of using wider range of genotypes representing more than one ecotype.The low or no correspondence between variation measurements based on molecular markers and agro-morphological characters should not be considered a limitation of these systems. The disparity of the two measurements indicates that germplasm classification and selection for crossing in plant breeding programs should not be relied on only one variation measurement. Semagn (2002) stated two reasons for the general lack of correlation between molecular and morphological variation: 1) Molecular markers cover a large proportion of the genome, including coding and non-coding regions, and 2) molecular markers are less subjected to artificial selection compared with morphological markers. Correspondence between molecular and agronomic diversity might be improved by analyzing more morphological and DNA markers (Martinez et al., 2005).

Materials and methods

Plant materials and site description

Thirty wheat genotypes were planted in early November, growing season of 2008-2009 at research farm of Razi University, Kermanshah (latitude 34°20´ N, longtitude 46°20´ E, altitude 1351.6 m above sea level), Iran (Table 1). The seeds were provided by Dry land Agricultural Research Institute, and Agricultural and Natural Resources Research Center, Kermanshah, Iran. Kermanshah is located in west of Iran and has a mean annual temperature of 13.8°C and has annual rainfall of 478 mm. The soil texture of the research farm was sandy-loam. The experiment was carried out in a randomized complete block design (RCBD) with three replications. Seeds were pretreated with Mancozeb to minimize the probability of seed- and soil-borne diseases. The seeds were sown in five 3 m long rows, spaced 20 cm apart in end of November. The final stand density was set to be 400 plants per m^2.

Measurement of phenological and agro-morphological traits

The central three rows were used for measurements to avoid border effects. Ten random plants from each plot were selected and the different traits were measured. The plants in 1 m^2 in each plot were harvested to calculate yield and yield components. The phonological traits recorded in this study were days to head emergence (HEM), days to heading (HE), days to flowering (FL), physiological maturity date (PM), and grain filling period (GFP). The traits used for determining agro-morphological characters were awn length (AL), flag leaf area (FLA), leaf chlorophyll content (LCC), peduncle length (PL), plant height (PH), spike length (SPL), ratio of peduncle length to plant height (PL/PH), fertile spikelets density (FSPD), number of spikes per m^2 (NSP), number of seeds per spike (NSSP), thousand seed weight (TSW), grain yield (GY), straw yield (SY), Biological yield (BY), and harvest index (HI).

DNA extraction and ISSR amplification

Wheat young leaves were harvested from all genotypes and used for DNA isolation using the CTAB method described by Murray and Thompson (1980). Fifteen ISSR markers were used for screening all the genotypes and revealing genetic

Table 2. Features of ISSR markers used for analysis of genetic diversity of wheat genotypes and the amplified products

Primer	Sequence (3'-5')	TAB[a]	NMB[b]	NPB[C]	PPB[d]	PIC[e]
UBC-811	$(GA)_8C$	7	2	5	71.4	0.13
UBC-814	$(CT)_8A$	8	2	6	75	0.22
UBC-815	$(CT)_8G$	8	0	8	100	0.42
UBC-822	$(TC)_8A$	5	2	3	60	0.23
UBC-826	$(AC)_8C$	7	1	6	85.7	0.21
UBC-834	$(AG)_8TT$	12	2	10	83.3	0.23
UBC-840	$(GA)_8TT$	12	0	12	100	0.22
UBC-845	$(CT)_8TT$	10	2	8	80	0.22
UBC-852	$(TC)_8AA$	10	3	7	70	0.15
UBC-876	$(GATA)_2$ $(GACA)_2$	7	3	4	57.1	0.18
Total		86	17	69		
Minimum		5	0	3	57.1	0.13
Maximum		12	3	12	100	0.42
Average		8.6	1.7	6.9	80.2	0.22

[a] Total amplified bands, No. of monomorphic bands,[c] No. of polymorphic bands,,[d] Percentage of polymorphic bands
[e] Polymorphism information content.

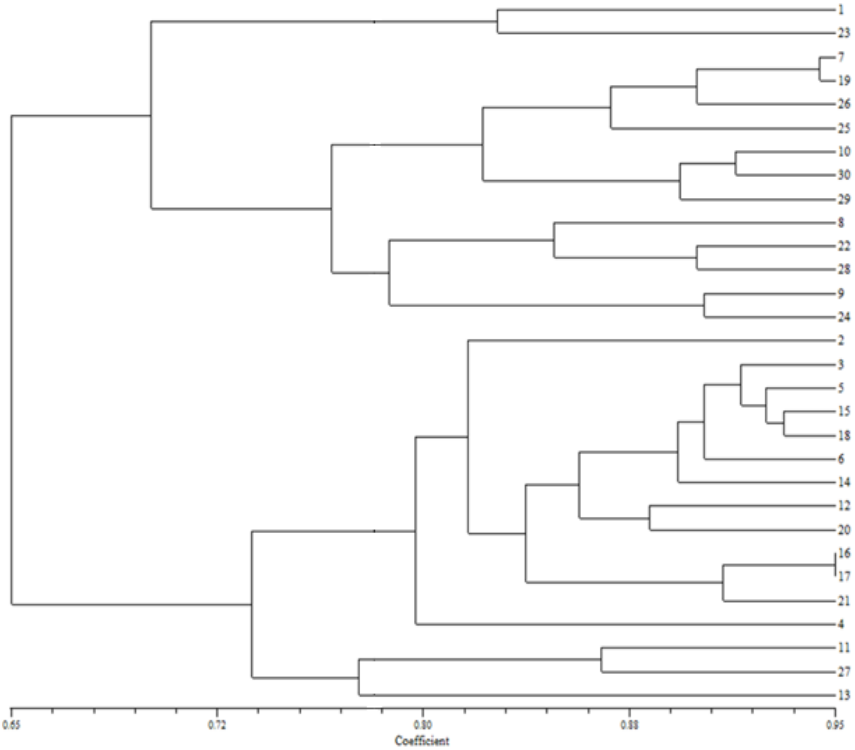

Fig 2. CLINK Dendrogram showing the genetic relationships among 30 wheat cultivars and advanced breeding lines based on ISSR marker data.

diversity. PCR amplification was conducted according to Williams et al. (1990) with the exception that the reactions were performed in a volume of 25 µl in a CPRBETT Research thermocycler. Amplified PCR products were run in 1.2% agarose gels. Gels were stained with ethidium bromide and visualized with a UV transilluminator. Out of 15 ISSR primers, 10 produced high resolution bands for all samples and were used for data analysis (Table 2).

Statistical Analysis

Analysis of variance appropriate to RCBD was carried out using SAS. Least significant difference (LSD) test was used for the mean comparisons. Cluster analysis was conducted using Ward method based on distance matrix obtained for 20 quantitative phenotypic traits by SPSS software. ISSR markers were scored for the presence (1) or absence (0) of amplified bands for each of 30 samples. The ISSR binary data matrix was used to calculate Jaccard's similarity coefficient. Cluster analysis was performed via complete linkage method using NTSYS-pc software version 2.02 (Rohlf, 2000). Principle coordinate analysis (PCoA) was also carried out by this software. For each ISSR marker, total amplified bands, number of monomorphic bands, number of polymorphic bands, percentage of polymorphic bands (PPB) and polymorphism information content (PIC) were recorded. PIC was calculated according to the formula of Anderson et al. (1993), as PIC = $1-\sum p_i^2$, where p_i is the frequency of the *i*th allele of the locus in the set of thirty wheat genotypes. Comparison between the ISSR-based Jaccard's similarity matrix and quantitative traits -based distance matrix was performed by Mantel correlation test (Garcia et al., 2007) using XLSTAT software.

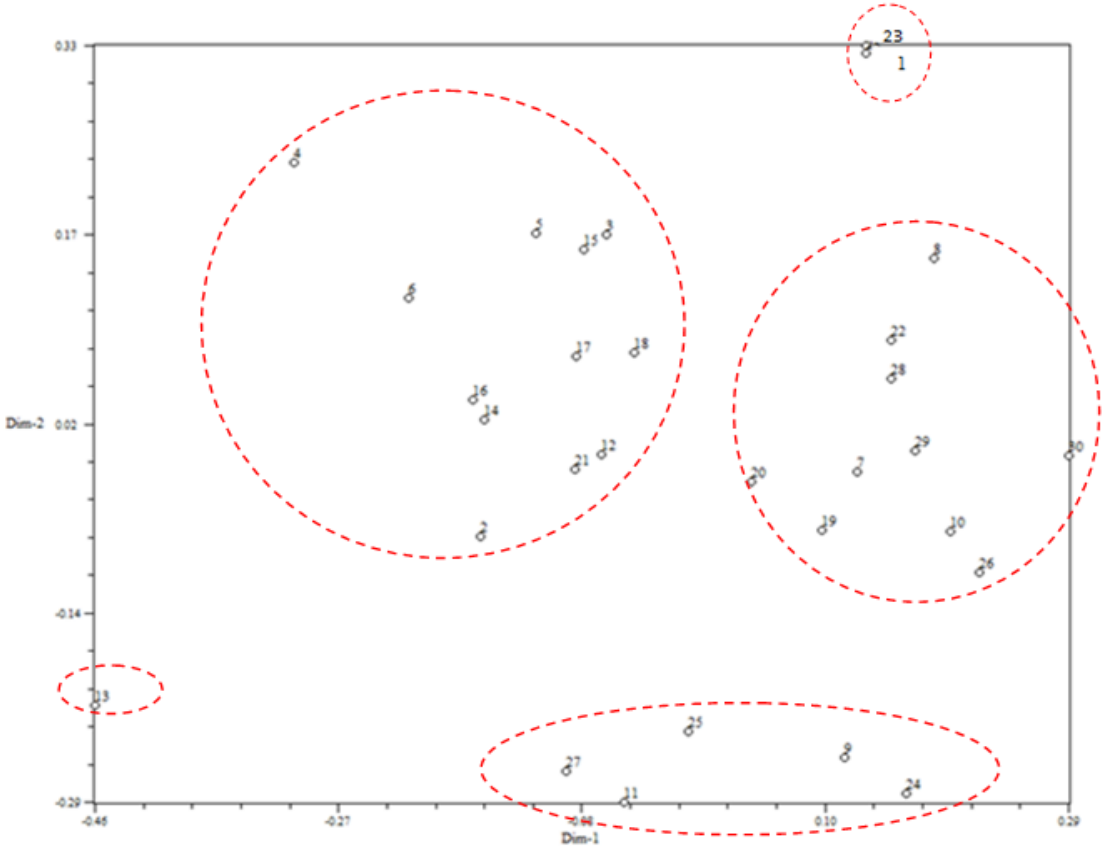

Fig 3. Scatter plot of wheat genotypes using principle coordinate analysis based on ISSR data, showing patterns of relationship among the genotypes. The numbers plotted represents individual genotypes.

Conclusion

Knowledge of the level of genetic variation among accessions is prerequisite for germplasm conservation and breeding programs. The analyzed wheat accessions showed a good amount of genetic variability for evaluated quantitative characters. The ISSR markers evaluated in our research provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity in wheat. No correlation was found between the variation measurements identified using molecular markers and quantitative traits. Molecular diversity assessed in this study in combination with the phenological and agro morphological traits can be useful in traditional and molecular wheat breeding programs.

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