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Diversity and Relationships among Durum Wheat Landraces (Subconvars) by SRAP and Phenotypic Marker Polymorphism

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Abstract: Germplasm characterization is essential and molecular markers provide valuable information for breeding programs. Sequence-Related Amplified Polymorphism (SRAP) and phenotypic markers were studied to determine diversity and relationships among 40 subconvares of *Triticum durum* landraces from the region of North West of Iran and Azerbaijan. The 12 combinations of forward and reverse SRAP primers were used to evaluate the 38 landraces and two cultivars and produced 65 scorable markers, of which 56.730% was polymorphic for all 40 genotypes. As to phenotypic markers, 27 quantitative traits were evaluated in field with 4 replications, 22 of them (81%) were found to be polymorphic. The UPGMA (UN weighted-Pair Group Method Arithmetic average) dendrogram based on the 27 phenotypic markers distinguished all genotypes, but failed to detect any geographic association in durum landraces. The UPGMA dendrogram based on the 12 combinations of SRAP markers distinguished landraces, it was concurs with subconvars grouping characteristic (traits of major gene). SRAP markers are useful for studying diversity and relationships among and have potential in marker-aided selection, linkage mapping and evolutionary studies.

Key words: Durum wheat, SRAP, phenotypic marker, genetic diversity, UPGMA

INTRODUCTION

Landraces or traditional cultivars are locally adapted diverse populations, which are the result of natural selection and farmers' cultivation methods. They have contributed genetic material to many breeding programs and constitute important plant genetic resources. Landraces have attracted the scientific interest because of the presence of genetic variability in well-adapted backgrounds. Considerable concerns have emerged, however, because of the quick rate at which landraces disappear due to their replacement by commercial cultivars (Esquinas-Alcazar, 1993). The estimation of genetic diversity of landraces is necessary because it determines significance of the gene pool and the manner in which it can be utilized. Measures of genetic diversity based on the molecular markers can be used on the predicted progeny performance in various crops (Autrique *et al*., 1996) and the molecular methods can be employed to better characterize genetic reassures. The genetic variability in agronomic traits and morphological characteristics of various durum wheat landraces have been studied (Jaradat, 1991; Autrique *et al*., 1996; Pecetti *et al*., 2001). Biochemical markers such as isozymes and seed storage proteins Payne (1987), DNA-based markers, such as Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphism (AFLPs) and Random Amplified Polymorphic DNA (RAPD) have been used to evaluate the genetic diversity among durum wheat landraces (Mantzavinou *et al*., 2005; Pujar *et al*., 1999; Pecetti *et al*., 2001; Autrique *et al*., 1996). Other molecular markers, such as microsatellites or Simple Sequence Repeats (SSRs) and AFLPs have also been used to study genetic relationships among durum wheat cultivars (Dograr *et al*., 2000; Soleimani *et al*., 2002). Among all these molecular markers, RAPDs and SRAPs, which are simple, user friendly, cost-and time-effective (Williams *et al*., 1990; Gulsen *et al*., 2007; Fufa *et al*., 2005), have been used successfully for the evaluation of plant genetic resources in wheat genotypes plant materials (Mukhtar *et al*., 2002; Mantazavinou *et al*., 2005). Sequence-Related Amplified Polymorphism (SRAP) markers were recognized as a new and useful molecular marker system for mapping and gene tagging in Brassica (Li and Quiros, 2001). SRAP markers are PCR_based markers that amplify open reading frames and produce a number of co-dominant markers per amplification. SRAPs use forward and reverse primers, 17 or 18 nucleotides long and primers consist of a core sequence of 13 or 14 bases,

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at the 5', CCGG in the forward primer and AATT in the reverse primer, targeting open reading frames in genomic sequences. This core sequence is followed by three selective nucleotides at the 3' end of each primer. SRAP markers are more consistent and repeatable than RAPDs and are less labor-intensive and time-consuming to produce than amplified-fragment length polymorphism techniques (Welsh and McClelland, 1990; Li and Quiros, 2001; Ferriol *et al*., 2003; Budak *et al*., 2004a-c; Gulsen *et al*., 2005, 2007). The SRAP marker technique that uses facile sequencing of selected bands is a simple and reliable method. Further, it targets coding sequences in the genome and results in a moderate number of co-dominant markers (Li and Quiros, 2001). SRAP markers possess multiloci and multi-allelic features, which make them potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting genotypes. However, SRAP markers may not be randomly distributed across the genome (Li and Quiros, 2001). Limited information is available on the chromosomal locations of SRAP markers (Fufa *et al*., 2005), their linkage with plant traits and the potential of SRAP markers for genetic diversity studies in wheat. Therefore, SRAP markers were employed to examine their potential for genetic diversity analysis in durum wheat.

The objectives of this study, were to evaluate SRAP and phenotypic markers to determine diversities and relationships among subconvars of Durum Wheat landraces in North West of Iran and the Republic of Azerbaijan.

MATERIALS AND METHODS

Plant materials: Subconvars of durum wheat were defined and identified by Dorofeev *et al*. (1979), which was based on spike characteristics. In this study, two cultivars and 38 subconvars of durum wheat landraces from North West of Iran and Republic of Azerbaijan were evaluated (Table 1). For SRAP marker study seeds of one Spike were germinated and its leaf was used, while seeds from each genotype were used for phenotypic marker study.

Phenotypic traits: The seeds were planted on the 22 of October, 2005 in the Agricultural research station of AZAD University in Ardabil city, located at 38°5' North latitude, 48°17' East longitude was 20×5 cm. Experimental design was (RCBD) with 4 replications. Fourty durum wheat genotypes were evaluated for 27 morphological traits including phonological (days to tiller, stems elongation, heading, pollination and physiological maturating) morphological (plant height, length of spike, seed density in spike and total number of tillers), yield and yield components (1000 kernels weight, number of seeds in spike, number of fertile tiller and harvest index) and flour quality (Hectoliter Weight, Suni Bug Damaged Kernals, Yellow Berries, Protein percent, Sedimentation Test, Hardness Index, Wet Gluten, Gluten Elasticity, Gluten Index, Dry Gluten, Semolina Percent, Disc Pressure Test and Quality Marker) characteristics.

DNA extraction and SRAP amplification conditions: Total genomic DNA was isolated from young frozen leaf tissue of individual genotypes using the method of Dellaporta *et al*. (1983). Twelve different combinations were employed using four forward and four reverse primers (Table 2). Each 25 μL reaction mixture was consisted of 0.4 μM of each primer, 200 μM of each dNTPs, 2.5 μL 10× PCR Buffer, 1.5 mM $MgCl_2$ as a final concentration and 1, unit of Taq polymerase (Qiagen),

Table 1: The genetic material (Durum wheat landraces) used

Origin	Country	Subconvar	Origin	Country	Subconvar
Nexcevan1	Azerbaijan	Melanopus	Uromiyey1	Iran	Niloticum
Nexcevan2	Azerbaijan	Africanum	Shamaxi3	Azerbaijan	Apulicum
Hasanbarog	Iran	Boeuffi	Noran1	Iran	Niloticum
Noran	Iran	Boeuffi	Xanlar	Azerbaijan	Apulicum
-	Iran	Zardak (cultivar)	Langan1	Iran	Hodieform
Nexcevan3	Azerbaijan	Leocomelan	Quba	Azerbaijan	Alboprovniale
-	Iran	Seimare (cultivar)	Nexcevan9	Azerbaijan	Leocomelan
Shamaxi1	Azerbaijan	Hordieforme	Shamaxi4	Azerbaijan	Erythromelan
Uromiyey2	Iran	Africanum	Ahar1	Iran	Hordeiforme
Nexcevan4	Azerbaijan	Hordieforme	Langan4	Iran	Hordeiforme
Kordestan	Iran	Africanum	Ardabil1	Iran	Hordieforme
Shamaxi2	Azerbaijan	Murciense	Ardabil2	Iran	Nilticum
Nexcevan6	Azerbaijan	Africanum	Tabriz1	Iran	Leucrum
Nexcevan7	Azerbaijan	Murciense	Langan2	Iran	Hordieforme
Nexcevan8	Azerbaijan	Leucruum	Shamaxi5	Azerbaijan	Boeuffi
Tabriz2	Iran	Boeuffi	Tabriz2	Iran	Africanum
Xanlar	Azerbaijan	Leucrum	Nexcevan10	Azerbaijan	Boeuffi
Lerik	Azerbaijan	Leocomelan	Ahar2	Iran	Niloticum
Qax	Azerbaijan	Leucruum	Goliblagh	Iran	Boeuffi
langan3	Iran	Leocumelan	Nexcevan11	Azerbaijan	Nilticum

Table 2: Primer sequences used for SRAP analysis

Name	Noclutid sequences
Forward primer:	
ME1	TGA GTC CAA ACC GGA TA
ME2	TGA GTC CAA ACC GGA GC
ME7	TGA GTC CAA ACC GGA CG
Me8	TGA GTC CAA ACC GGA CT
Reverse primer:	
EM2	GAC TGC GTA CGA ATT TGA
EM3	GAC TGC GTA CGA ATT GAC
EM5	GAC TGC GTA CGA ATT AAC
EM6	GAC TGC GTA CGA ATT GCA

25 ng DNA templates. Eppendorf Mstercycler Gradiant was used and cycling parameters included: one cycle of 5 min at 94°C, 5 cycles of three steps: 1 min of denaturation at 94°C, 1 min at 35°C and 2 min elongation at 72°C in the following 30 cycles the annealing temperature was increased to 50°C, with a final elongation step of 5 min at 72°C. PCR products were separated on 12% polyacrylamide gels at 400 V for 11 h and gels were dried overnight.

Data analysis: Presence or absence of each SRAP was coded as 1 and 0, respectively. Where, 1 indicated the presence of specific allele and 0 indicated, its absence. The distance matrix and dendrograms were constructed using the numerical taxonomy multivariate analysis system (NTSYS-pc ver. 2.1) software package. Genetic polymorphism, a similarity matrix and Nei's gene diversity index were used to compute Neil's standard genetic distance coefficients (Nei and Li, 1979) and to construct an Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) dendrogram (Sneath and Sokal, 1973) using SPSS (ver. 10.0) software package.

In order to see how well a cluster analysis represents the distance matrix, COPH module was used to transform the tree matrix to a matrix of ultrametric distances (a matrix of distances implied by the cluster analysis). Finally, MXCOMP module was used to compare these ultra-metric distances and distance matrix produced for UPGMA analysis.

RESULTS AND DISCUSSION

Analysis of the two cultivars and 38 subconvars of durum wheat landrace from Iran and the republic of Azerbaijan with 12 SRAP primer combinations identified a total of 65 reproducible fragments (Table 3).

Among them 56.37% were polymorphic, ranging in size from 150-653 bp. The number of fragments detected by an individual primer combination ranged from 1-12, with an average of 8.

Based on the percentage of polymorphic fragments, different levels of polymorphism ranged from 25% (ME1/EM2) to 100% (ME7/EM5). This finding concurs with the work done by Ferriol *et al.* (2003) in the *Cucurbita pepo* collection

Table 3: Primer (forward and reveres) combinations used for SRAP analysis

Primer combinations	Total fragment	Polymorphism (%)	Gene diversity
ME1-EM2	3.00	25.00	0.1
ME2-EM6	6.00	74.00	0.21
ME8-EM3	4.00	48.00	0.28
ME7-EM6	8.00	77.50	0.28
ME7-EM5	12.00	100.00	0.30
ME2-EM2	6.00	65.50	0.26
ME6-EM2	6.00	58.00	0.24
ME1-EM5	3.00	35.00	0.25
ME8-EM5	5.00	40.00	0.2
ME7-EM2	4.00	45.5	0.25
ME2-EM3	3.00	43.00	0.25
ME7-EM3	5.00	50.00	0.21
Total	65.00	-	-
Mean	5.41	56.37	0.24
SD	2.57	21.09	5,248E-02

Table 4: Number of total bands and number of polimorphysim band on the subconvars of *T. durum*

Subconvars	No. of total bands	No. of polimorphysim band
T.durum v. *boeuffi*	1-5	0-4
T.durum v. *africanum*	2-7	1-3
T.durum v. *erythromelan*	2-10	1-8
T.durum v. *apulicum*	2-7	3-4
T.durum v. *leucomelan*	2-12	1-5
T.durum v. *Hordeiforme*	5-12	5-12
T.durum v. *melanopus*	3-6	1-3
T.durum v. *leucurum*	2-8	3-8
T.durum v. *niloticum*	3-10	2-6

Gene diversity ranged from 0.1 (ME1/EM2) to 0.3 (ME7/EM5), with an average of 0.23 per primer combination (Table 3). Ferriol *et al.* (2003) also reported that ME1/EM2 and ME8/EM3 combined primer had the lowest genetic diversity in *C. orifera* sp. The highest average number of polymorphic bands was found among different subconvars in hordeiforme and lowest number of polymorphic bands in Boeuffi and Albiproviancale subconvars (Table 4). Fufa *et al.* (2005) in a study of genetic diversity in 30 Red winter cultivars by SRAP DNA marker reported that 23 SRAP markers produced 468 amplified fragments with an average genetic diversity of 0.418, which ranged from 0.1-0.9. They also, showed the diversity estimates for SRAP marker from 0.11-0.677 with average value of 0.357. As such, the SRAP markers provided more conservative estimates of genetic diversity than SSR markers. Also, SRAP Markers were used for the evaluation of genetic diversity in *Buffalograss* (Budak *et al*., 2004a-c), *Okra* Germplasm (Gulsen *et al*., 2007), Collection germplasm of *Cucurbit pepo* (Ferriol *et al*., 2003) and *Brasica* (Li and Quires, 2001).

The analysis of genetic similarity coefficients (Nei and Li, 1979) for pairs of genotypes (subconvars) showed (no reported) that the mean value of genetic

similarity was 0.73 with a range from 0.01-0.97. Subconvars of leucumelam landraces from Naxcivan and Lerik (Azerbaijan) and Langan3 (Iran) regions were the least related to any other subconvars used in this study. The genetic similarity coefficients among those that subconvars and other genotypes ranged from 0.01-0.34, with the average of 0.302. The subconvars of albiprovienciale (Quba) from Azerbaijan and Africanum (Tabriz 2) from Iran were the highest related to other genotypes, which ranged from 0.380-0.889 with the average of 0.77.

Cluster analysis based on SRAPs markers: A cluster analysis was performed using the Nei and Li (1979) genetic diversity and UPGMA method. The dendrogram grouped the different accessions in two major clusters (Fig. 1). The cluster 1 included the subconvars of Leucumelan, Boeuffi, Apulicum and Mursiens, while cluster 2 included other subconvars of *T. durum* landraces such as Hordeiforme, Leucurum, Niloticum, Africanum, Erythromelan and Albiprovincial. In the within-group on the cluster 1 there is high similarity among the subconvars of Landrace from different regions. This grouping concurs with subconvars grouping of landrace durum wheat by Dorofeev *et al.* (1979) methods. This might be due to the fact that the grouping done by Dorofeev *et al.* (1979) was based on the major gene effects.

In this study, there was no correlation between genetic diversity and geographical distribution.

Cluster analysis based on phenotypic markers: Twenty five phenotypic markers were scored for the 40 genotype of *T. durum* landrace and 81.0% of them were found to be polymorphic. The ratio of polymorphic phenotypic marker

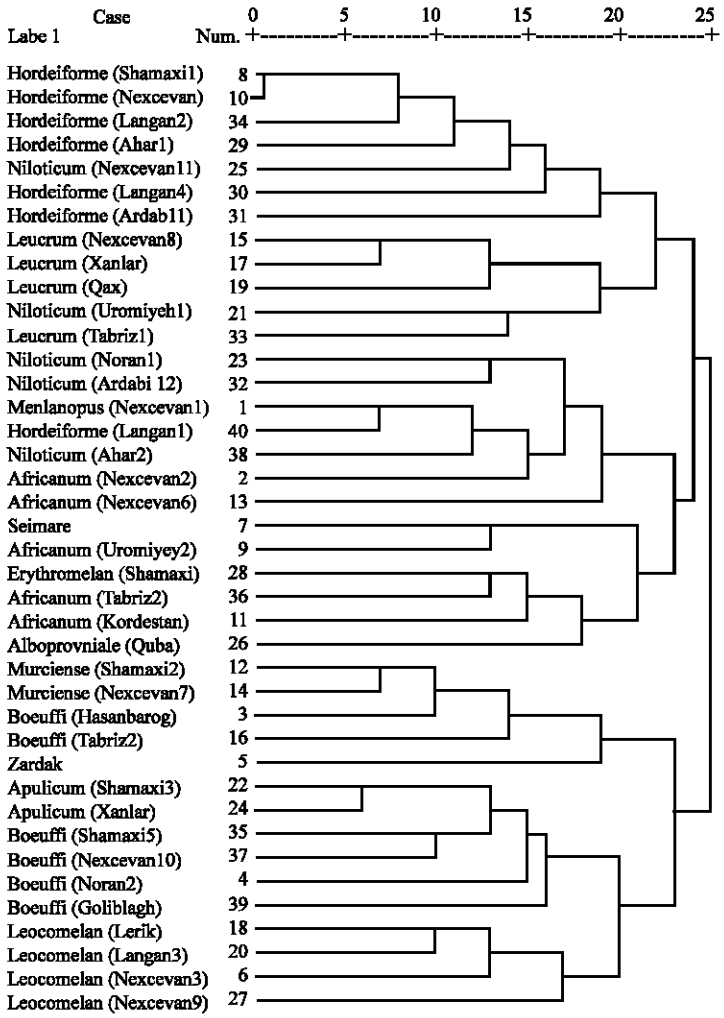

Fig 1: An Unweighted Pair-Group Method with Arithmetic Average (UPGMA) Dendrogram of genetic relationships among 40 subconvars of durum wheat calculated on the basis analysis by means of 12 SRAP primer combinations

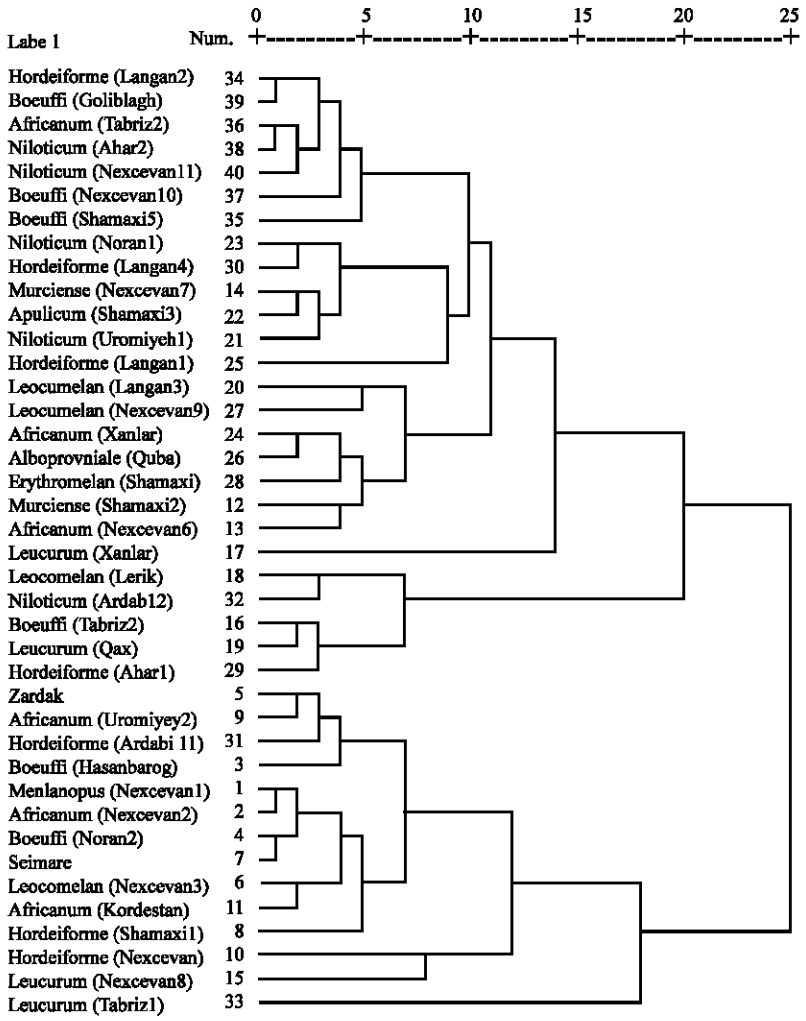

Fig 2: An Unweighted Pair-Group Method with Arithmetic Average (UPGMA) Dendrogram based on dissimilarity matrix costracted from the 27 phenotypic markers scored for 40 subconvars of durum wheat from Iran and Azerbaijan

(81.0%) is higher than that of polymorphic molecular markers (56.37%) in the same landrace. This might be due to the quantitative nature of most phenotypic markers, such as plant height, HI, seed weight, length of spike and number of seeds per spike, in which more genes are involved compared to qualitative traits of molecular markers (Allard, 1999; Gulsen *et al*., 2007). The cluster Dendrogram based on phenotypic marker data successfully distinguished all 40 landraces from one another (Fig. 2).

Molecular and phenotypic marker-based analysis produced two different clustering patterns in this study. This may be caused by quantitative control of phenotypic traits studied and/or fluctuations in environmental conditions, having potential effect on phenotypic performances (Gulsen *et al*., 2007). This is due to the fact that the estimated distance based on phonotypical traits and SRAP markes had a low non significant correlation (r = 0.12).

Fufa *et al*. (2005) also, reported non significant correlation coefficients among distance estimates of morphological and SRAP markers. Because they beloved the SRAP markers tended to have low correlation with the other genetic diversity estimates, they may provide different and unique insights into genetic diversity.

Few studies of SRAP markers have been reported in wheat. SRAP markers may be used for improving cultivars, understanding relationships, establishing germplasm collections and integrating markers into genetic linkage maps. Therefore, the use of these markers is commended for the investigation of genetic diversity of durum wheat landraces because SRAP markers were

replicable and they correlated with major gene traits, for example with the grouping indicators of Dorofeev *et al.* (1979).

CONCLUSION

The 12 combinations of forward and reverse SRAP primers were used to evaluate the 38 landraces and two cultivars and produced 65 scorable markers, of which 56.730% was polymorphic for all 40 genotypes. As to phenotypic markers, 27 quantitative traits were evaluated in field with 4 replications, 22 of them (81%) were found to be polymorphic. The UPGMA (UN weighted-pair group method arithmetic average) dendrogram based on the 27 phenotypic markers distinguished all genotypes, but failed to detect any geographic association in durum landraces. The UPGMA dendrogram based on the 12 combinations of SRAP markers distinguished landraces, it was concurs with subconvars grouping characteristic (traits of major gene). SRAP markers are useful for studying diversity and relationships among and have potential in marker-aided selection, linkage mapping and evolutionary studies.

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