Turk J Biol
34 (2010) 271-279

doi:10.3906/biy-0809-24

Barley germplasms developed for scald disease resistance exhibited a high level of genetic diversity based on SRAP markers

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Received: 23.09.2008

Abstract: The objective of this study was to assess the genetic diversity and genetic relationships among 59 Turkish barley (*Hordeum vulgare* L.) germplasm lines (maintained for scald disease resistance breeding) using sequence-related amplified polymorphism (SRAP) markers. Seventeen SRAP primer combinations produced 83 polymorphic markers, with a mean polymorphism of 73.5%. The dendrogram created based on Nei's unweighted-pair group method using arithmetic average (UPGMA) indicated that there were 4 main clusters, which was supported by principle component analysis (PCA). Cluster I primarily included only scald-resistant germplasm lines, while clusters III and IV consisted of only scald-sensitive lines, but cluster II had both scald-resistant and scald-sensitive barley germplasm lines. The coefficients of genetic similarity among the genotypes ranged from 0.58 to 0.96, with a cophenetic correlation (r = 0.71) suggesting that the cluster analysis moderately represented the similarity matrix. The results indicate that a large amount of the genetic diversity present could be of great use in the development of future scald-resistant barley lines.

Key words: Barley, scald disease, SRAP, genetic diversity

SRAP belirteçlerinin yaprak lekesi hastalığına dirençlilik için geliştirilmiş arpa gen kaynaklarında ortaya çıkardığı yüksek genetik çeşitlilik

Özet: Bu çalışmanın amacı, yaprak lekesi hastalığına dayanıklılık için geliştirilmiş elli dokuz Türk arpa (*Hordeum vulgare* L.) gen kaynağında genetik çeşitliliğin ve aralarındaki genetik ilişkilerinin "Nükleotid dizi Bazlı Çoğaltılmış Bölge Polimorfizm" (SRAP) belirteçleri yardımıyla ortaya çıkarılmasıdır. Onyedi SRAP belirteç kombinasyonu toplam üretilen bantların % 73,5'ine tekabül eden 83 polimorfik bant üretmiştir. Nei'in Aritmetik Ortalamaya Dayalı Ağırlıksız Eşleştirme Metodu (UPGMA) ve Temel Bileşenler (PCA) analizi sonuçları tarafından desteklenen 4 ana grup ortaya çıkarılmıştır. Birinci grup sadece yaprak lekesi hastalığına dirençli hatların karışımından oluşurken, 3. ve 4. gruplarda sadece yaprak leke hastalığına hassas hatların var olduğu görülmüştür. İkinci grup ise hem dirençli hemde hassas hatları içermektedir. Genotipler arasındaki genetik benzerlik katsayıları 0,58 ile 0,96 arasında değişmekte olup, aralarındaki kofenetik korelasyon 0,71 olarak bulunmuştur. Bu durum, gruplamanın benzerlik matrisini orta derecede temsil ettiğini göstermektedir. Çalışmada ortaya çıkan sonuçlar, yüksek derecedeki genetik farklılığın gelecekteki yaprak lekesi hastalığına dirençli bireylerin geliştirilmesinde yararlı olabileceğini işaret etmektedir.

Anahtar sözcükler: Arpa, yaprak lekesi hastalığı, SRAP, genetik çeşitlilik

Introduction

Barley (*Hordeum vulgare* L.) is a widely variable species cultivated in nearly all regions of the world. It is primarily used for food, animal feed, and malt. The center of diversity for wild barley and the primary site of its domestication is considered to be the Fertile Crescent of the Near East (1-3). In Turkey over 3.6 million ha is planted with barley, mostly in central and southeast Anatolia, with an annual production of about 9 million metric tons (4).

Scald (*Rhyncosporium secalis* (Oudem) J. J. Davis) is one of the most important diseases of barley and is present in a large proportion of barley fields in Turkey (5). It causes up to 30% of yield loss and considerably reduces grain quality in barley fields (6). Given the significant reduction in barley production due to scald in Turkey, the Central Research Institute for Field Crops (CRIFC) in Ankara initiated a breeding program in 1983 to develop scald-resistant barley cultivars. Although this institute has a large number of germplasm lines in its collection, these sources cannot be used efficiently in barley breeding for resistance to scald disease due to the lack of adequate passport data for most of the germplasm lines.

Molecular techniques are useful for fingerprinting and establishing genetic relatedness among breeding materials, such as germplasm collections. Molecular markers are widely used in cereal breeding, phylogenetic studies, comparative genomics, and gene and quantitative trait loci (QTLs) mapping (7,8). SRAP (sequence-related amplified polymorphism) markers are recognized as a useful and primarily dominant molecular marker system. SRAP, a PCR-based marker, is based on 2-primer amplifications in which the primers are 17 or 18 nucleotides long, and has been used for mapping and gene tagging in *Brassica* (9). It has also been employed to assess genetic diversity and phenetic relationships among a broad range of cool- and warm-season turf grass species (10), and squash (*Cucurbita moschata*) (11), as well as genetic relationships among subpopulations of single species (12,13). SRAP has proven to be more informative than AFLP (amplified fragment length polymorphism), RAPD (rapid amplified polymorphic DNA), ISSR (inter simple sequence repeat), and SSR (simple sequence repeat) markers (11,13,14).

The aim of the present study was to determine the magnitude of genetic diversity and genetic relationships among 59 Turkish barley germplasm lines maintained for scald disease resistance breeding programs, based on SRAP markers.

Materials and methods

To assess the genetic diversity and relationships among scald-resistant and scald-sensitive barley germplasm lines maintained for scald disease breeding activities, 59 Turkish barley lines (24 scald disease germplasm-resistant lines [R] and 35 scald-sensitive germplasm lines [S]) in the CRIFC's germplasm collection (Table 1) were screened using 17 SRAP primer combinations (Table 2). The symbol "/" in the germplasm lines in Table 1 represents the cross of 2 lines observed on both sides, while the symbol "//" indicates the second cross in which one of the individual-lines was produced from the former one. For instance, the germplasm line that was produced from the A and B was crossed with the C, and could be presented as C// A / B in Table 1.

Plant material

CRIFC has a large number of barley germplasm lines and frequently screens them for scald resistance genes that could be incorporated into the ongoing barley-breeding program. The procedures used to evaluate the barley germplasm with respect to scald disease (*Rhyncosporium secalis*) were described in detail elsewhere (15). Scald evaluation scores ranged from 0 to 4; 0 indicating no visible symptoms (highly resistant) and 4 indicating total collapse of a plant (highly susceptible). In all, 59 germplasm lines (35 susceptible lines with scald scores of 3 or 4, and 24 resistant lines with scald scores of 0 or 1) were selected according to the their parental origin (Table 1), and only 1 line that shared the same parental origin was included in the study.

DNA extraction, selection of molecular markers, and optimization of polymerase chain reaction (PCR) conditions

Total DNA was extracted from 10-15 mg of young leaf tissue from 1 seedling for each line using the CTAB method (16) and screened using 17 SRAP primers (Table 2). PCR was performed in a total

Table 1. Scald resistant and sensitive Turkish barley germplasm lines used in the study.

Seed Sources Code	Seed Sources (registered cultivars or used as parental materials)	Seed Sources Codes	Seed Sources (registered cultivars or used as parental materials)
R1	Rabur / Luther	**S1**	73th/105//e10bulkci7321/3/Cwb117-5-9-5
R2	Ne 76148 / Wbcbpi 388643	**S2**	Roho//Alger/Ceres362-1-1/3/Alpha/Durra
R3	Yea 761 - 3 / Yea 741 - 2	**S3**	Cwb117-9-7/3 / Roho // Alger / Ceres362-1-1
R4	324 p.k - 5 / Tuil 10	**S4**	Viringa's'/3 / 4679 / 105 // 132th
R5	Yea 171 - 3 / Yea 50.1	**S5**	Anteres / Ky63-1294 // Cwb117-77-9-7
R7	3896/1-15/3/3896/28//284/28/4/Einbull//ci7321	**S7**	Cwb117-5-9-5/3 / Roho / Masurka // Icb-103020
R8	Yea 1139 /Yea 605.5	**S8**	Cwb117-5-9-5 // Rhn-03 / Lignee640
R9	132th / 22 / 3 / Tokak p386 / P49 -10	**S9**	Chicm/An57//Albert/3/Icb-102379/4/Gkomega
R11	Yea 324/ Yea 68.3	**S10**	Radical / Birgit // Pamir-163
R12	Yea 762 - 2 / Tokak	**S11**	Tokak / 4857 // Yea2049-3-1-1
R13	Yea 1727 / Yea 6055	**S12**	Alpha / 2104
R14	3896/1-15/3/3896/28//284/28/4/Einbull//ci7321	**S13**	1993-94(iwfbsp)
R20	3896/1-15/3/3896/28//284/28/4/Einbull//ci7321	**S14**	Eskişehir / Övd st 3760
R22	Yea 454 - 1 / 5054	**S15**	Yea557.6 / Yea422.1 // 80-5042
R25	Yea 762 - 2 / Yea 605 - 5	**S16**	Yea605.20
R29	Flamenco / Wm	**S17**	Tokak /3/ Alpha // Sul / Nacta
R30	H272/bgs/3/mzg/gva//pi002917/4/deiralla10//mzg/dl71	**S18**	4857 / Viringa"s"
R32	Cwb22 - 6 - 13 / Icb - 102411	**S19**	111th / p12-119 // 4857
R33	Tryll / Hudson // Obruk - 86	**S20**	Platen672 // 353th / p12-119
R34	Bastion m	**S21**	Antares/Ky63-1294/3/Roho//Alger/Ceres362-1-1
R35	Avd-121 / Bülbül-89	**S23**	Tokak / Pamir-175
R37	4814/3/3896 / gzk // 132th	**S24**	Pamir-159 / Wkn185-82
R39	Wbelt-39 / Tokak	**S25**	Pamir-010 / Pamir-159
R40	Wysor	**S26**	Roho / Masurka // Obruk
		S28	274 Eskişehir / Ovd
		S29	Antares / Ky63-1264 // Lignee 131
		S30	Yea276/132th//5053/3/Coss/Owb71080-44-1h
		S31	2925/1 // 1246 / 78/3 / Yea475-4/4 / Tarm-92
		S32	5807 / 4857
		S33	Tokak / Cwb117-77-9-7
		S34	Sonja / Ms // p12222 / Scio /4 / Tokak
		S35	Cum-50 / 700.1
		S36	11Eskişehir / Ovd
		S37	Pamir-42 / Bülbül
		S40	Belts-60-1807/Henry//Sussex/3/2/Barsoy/4 /b/a/5/k-247/240113/Vavilon

Table 2. The forward and reverse sequences and primer combinations of sequence-related amplified polymorphism (SRAP) for the study.

Codes	Primer Sequences	Primer combinations
Forward	***Primers***	*Me1 - Em1*
Me1	TGA GTC CAA ACC GGA TA	*Me1 - Em2*
Me2	TGA GTC CAA ACC GGA GC	*Me1 - Em5*
Me3	TGA GTC CAA ACC GGA AT	*Me1 - Em6*
Me4	TGA GTC CAA ACC GGA CC	*Me1 - Em7*
Reverse	***Primers***	*Me1 - Em12*
Em1	GAC TGC GTA CGA ATT AAT	*Me1 - Em13*
Em2	GAC TGC GTA CGA ATT TGC	*Me1 - Em16*
Em3	GAC TGC GTA CGA ATT GAC	*Me2 - Em2*
Em5	GAC TGC GTA CGA ATT AAC	*Me2 - Em16*
Em6	GAC TGC GTA CGA ATT GCA	*Me3 - Em3*
Em7	GAC TGC GTA CGA ATT CAA	*Me3 - Em 12*
Em9	GAC TGC GTA CGA ATT CAG	*Me3 - Em16*
Em12	GAC TGC GTA CGA ATT CTC	*Me4 - Em1*
Em13	GAC TGC GTA CGA ATT CTG	*Me4 - Em3*
Em14	GAC TGC GTA CGA ATT CTT	*Me4 - Em9*
Em16	GAC TGC GTA CGA ATT GTC	*Me4 - Em14*

volume of 20 mL and consisted of 5 ng/μL of genomic DNA, 1 × PCR buffer ($MgCl_2$ free), 2.5 mM $MgCl_2$, 250 mM dNTP, 0.25 μM of each primer, and 0.5 units *Taq* DNA polymerase, using the following PCR cycle program: 5 cycles at 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 1 min, and then 35 cycles at 94 °C for 1 min, 50 °C for 1 min, and 72°C for 1 min, and a 5-min cycle for extension at 72 °C. Between 7 and 10 mL of each sample was loaded on 5% polyacrylamide (19:1 acrylamide:bisacrylamide) gel with 1× TBE, which were then run under 250 V for 2 h. Gels were stained with ethidium bromide and visualized under UV light using a Vilber Lourmat gel visualization system (Cedex, France).

Scoring gels and data analysis

Polymorphic bands were considered binary characters and scored as '1' for presence and '0' for absence for each germplasm line. Genetic diversity parameters, including the percentage of polymorphic bands, the observed number of alleles (n_a), the effective number of alleles (n_e), Nei's gene diversity (h), total genetic diversity (H_t), genetic diversity within groups (resistant vs. sensitive) of germplasm lines (H_s), and the coefficient of genetic differentiation (G_{st}) among the groups of germplasm lines were calculated with standard errors (±SE) using the POPGENE 32 computer program (17).

Molecular genetic similarity (GS*ij*) was calculated for SRAPs as the simple matching coefficient (SM) for qualitative data (GSij = m/n), where m is the number of SRAP band variants in common between germplasm lines i and j, and n is the total number of polymorphic SRAP bands compared between i and j. Then the obtained genetic similarity matrix was used to construct a dendrogram using Nei's UPGMA method (unweighted pair-group method using arithmetic average) (18) to determine the genetic relationships among the germplasm lines. The goodness-of-fit of the dendrogram to the original genetic similarity matrix was calculated by computing the cophenetic value, using the COPH (cophenetic) and MXCOMP (matrix comparison) modules of the numerical taxonomy multivariate analysis system (NTSYS-pc) (19). PCA was also performed with the use of the DCENTER and EIGEN procedures of the NTSYS-pc software package. This multivariate approach was chosen to provide information complementary to the cluster analysis, because cluster analysis is more sensitive to closely related individuals, whereas PCA is more informative regarding distances between major groups (20).

Results

In total, 17 different combinations of SRAP primers were employed; 4 forward primers and 11 reverse primers were used. All of the combinations of primers revealed polymorphism among the tested germplasm lines. The amplified fragments ranged in size from 50 to 500 bp (Figure 1). The mean number of DNA band products per primer was 5.0 (range: 1-10). Primer combinations of *Me1-Em2*, *Me4-Em1*, and *Me4-Em14* had a high number of polymorphic bands (10, 10, and 8, respectively). In all, 83 polymorphic markers (bands) were identified using the 17 primers and mean polymorphism was 73.5%. Fourteen of these 83 markers that belonged to primer combinations *Me1-Em1*, *Me3-Em12*, *Me1-Em7*, *Me1-Em2*, *Me2-Em16*, *Me4-Em1*, and *Me4-Em9* produced remarkable differences in frequency between scald-resistant and scald-sensitive germplasms. Furthermore, 2 markers that belonged to *Me1-Em2* and *Me4-Em1*, and 1 marker that belonged to *Me2-Em16* were observed only in scald-sensitive germplasms (Table 3).

According to Nei (21), the proportion of polymorphic loci is not a good measure of genetic variation. A more appropriate measure of genetic variation is average heterozygosity or gene diversity (h). In the present study Nei's (21) genetic diversity of the scald-sensitive germplasms (0.333 ± 0.03) was slightly higher than that of the scald-resistant germplasms (0.316 ± 0.04) (Table 4). Total genetic

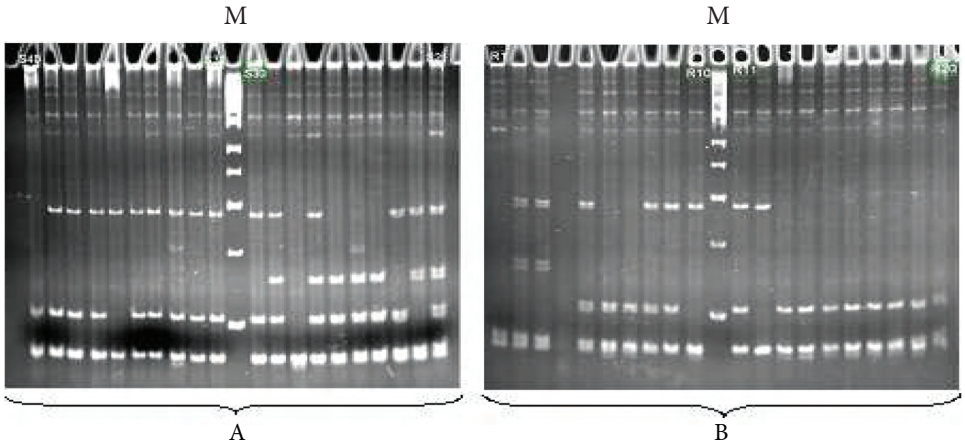

Figure 1. SRAP profiles of sensitive (A) and resistant (B) Turkish barley germplasm lines with *Me3 - Em3* primer combination (M = marker).

Table 3. Proportions of observed frequency of SRAP markers within scald resistant and sensitive germplasm lines.

Primer combination	Marker length (bp)	Frequency of the markers (%)	
		Resistant	Sensitive
Me1 - Em1	500	67	14
Me3 - Em 12	250	75	23
Me1 - Em7	200	33	77
Me1 - Em2	100	0	46
	200	0	31
	300	33	60
	350	33	60
Me2 - Em16	140	0	34
	220	25	57
Me4 - Em1	180	0	14
	220	0	54
	300	17	43
Me4 - Em9	450	17	66
	430	17	77

Table 4. Population genetic diversity parameters estimated with the POPGENE computer program (17) for resistant and sensitive Turkish barley germplasm lines.

	N	*na*	*ne*	*h*	*# of Polymorphic loci (P)*	*% of P*	*Ht*	*Hs*	*Gst*
Scald Resistant Germplasm Line Collection	23	1.916 ± 0.06	1.55 ± 0.07	0.316 ± 0.04	76	91.6	0.278 ± 0.006	-	
Scald Sensitive Germplasm Line Collection	34	1.976 ± 0.03	1.568 ± 0.05	0.333 ± 0.03	81	97.6	0.301 ± 0.004	-	
Overall	57	2.000	1.61 ± 0.04	0.348 ± 0.02	83	100	0.347 ± 0.002	0.324 ± 0.002	0.065

n_a = observed number of alleles; n_e = effective number of alleles (24); h = Nei's gene diversity (25); I = Shannon's information diversity index (26).

diversity (H_t) was 0.350 ± 0.002 and the genetic diversity within populations (H_s) was 0.325 ± 0.002. The coefficient of genetic differentiation (G_{st}) between scald-resistant and scald-sensitive germplasm line groups was low (0.065) (Table 4).

The phylogenetic dendrogram constructed with SRAP data and the UPGMA clustering algorithm indicates that the germplasm lines R1 and S10 spanned the extremes of the dendrogram, with all other germplasm lines distributed in between them, grouping the germplasm lines into 4 main clusters. While cluster I was characterized by only scald-resistant barley germplasm lines, clusters III and IV included only scald-sensitive lines, except the scald-resistant R29 line, whereas cluster II included both scald-resistant and scald-sensitive lines. The germplasm lines R1 and S28 were not included in any cluster group. Analysis also showed that the S13 and S14 germplasm lines are almost identical, exhibiting 96% similarity. In general, the genetic similarity coefficient of all the lines ranged from 0.58 to 0.96 (Figure 2). Cophenetic correlation between ultrametric similarities of the tree and the similarity matrix was moderate (r = 0.71), suggesting that the cluster analysis moderately represented the similarity matrix.

PCA was performed based on the genetic similarity matrix in order to better understand the relationships between the germplasm lines. The first 2 Eigen vectors accounted for 66.8% of the total variation; the 1st and 2nd components accounted for 51.3% and 15.5%, respectively. PCA and UPGMA analysis yielded similar groupings (Figure 3).

Discussion

This is the first study to assess the outcome of barley germplasm collection and maintenance for scald-resistant breeding, with respect to the magnitude of genetic diversity and changes in genetic similarity or differentiation among the germplasm lines that occurred over time due to breeding activities.

The percentage of polymorphic loci estimated using SRAP markers was high for both scald-resistant (91.6%), and scald-sensitive (97.6%) germplasm lines. These results were expected, because specific resistant germplasms are continuously selected and bred by agriculturists. However, Nei's genetic diversity (*h*) showed that there was no significant difference between these 2 types of germplasms, suggesting that the difference between polymorphic loci might not be reflected in the existing diversity. Moreover, the coefficient of genetic differentiation (*Gst* = 0.065), which can be interpreted as 93.5% of the total genetic variation, was within germplasm groups (resistant or sensitive), while 6.5% was between these groups. A previous study using AFLP analysis reported that 32% of total genetic variation was within Turkish wild barley populations (22), which is considerably lower

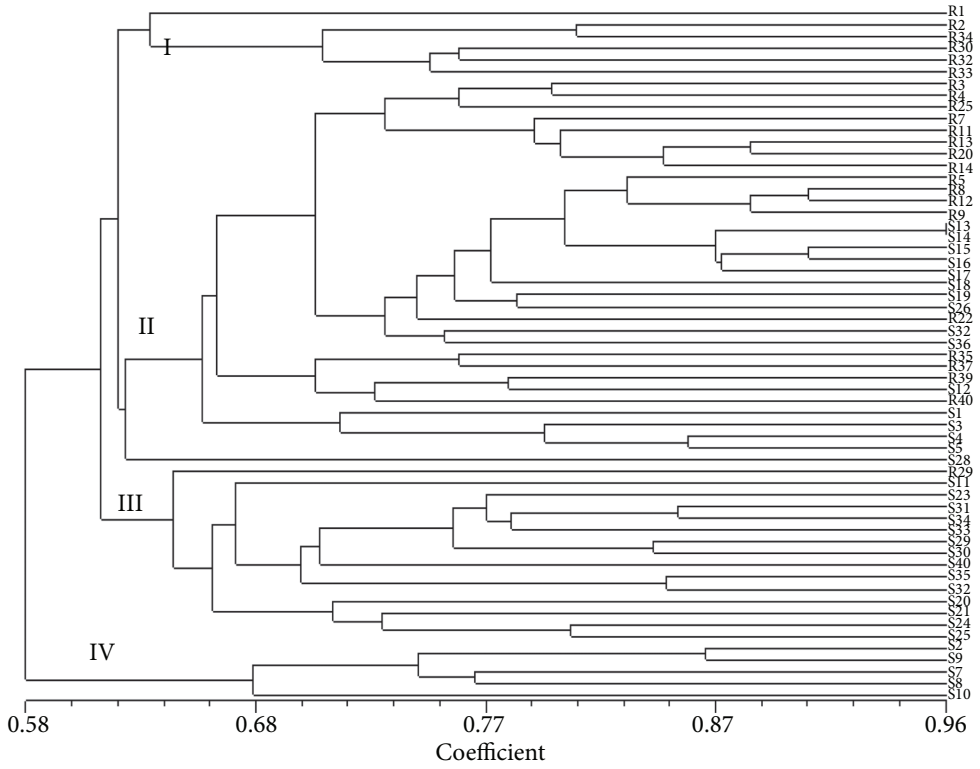

Figure 2. The UPGMA dendrogram based on the similarity matrix constructed from the 83 SRAP markers amplified for 59 Turkish barley lines. Cluster numbers are given in roman numbers. For germplasm line codes, please refer to Table 1.

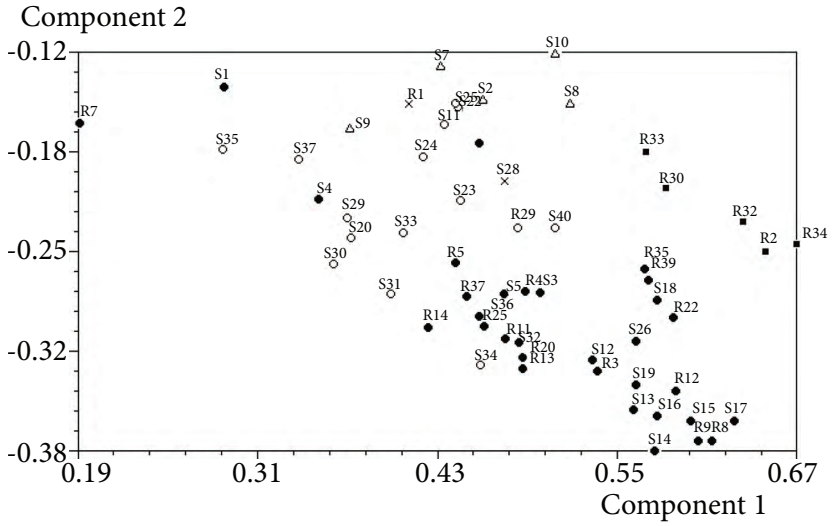

Figure 3. The principle component analysis (PCA) of 59 Turkish barley germplasm lines.▪: Cluster number I, ◆: II. ◇: III, Δ: IV, ×: not clustered. Cluster numbers are given.

than the present study's findings. Although the genetic diversity of wild populations was expected to be higher, the observed high genetic variation within the germplasm groups could have been due to SRAP's high power of generating polymorphism and the breeding activities among the germplasm lines. As reported by Budak et al. (14), among the tested markers (SRAP, SSR, ISSR, and RAPD), the SRAP system appeared to have greater discriminating power in buffalo grass (*Buchloe dactyloides* (Nutt.) Englem), which is a native C4 grass species.

Moreover, Li-Wang et al. (23) reported that in radish cultivars the average number of polymorphic bands detected by each SRAP primer combination was much higher than that revealed by RAPD and ISSR primers, further indicating that SRAP is a more efficient technique due to its capacity to reveal more informative bands. The results of the current study also indicate that the SRAP marker system is suitable for molecular characterization and assessing genetic diversity in barley. The differences in the frequency of 14 markers in the scald-resistant and scald-sensitive germplasms in the present study, especially markers observed only in scald-sensitive germplasms, imply that these markers could be related to sensitivity to scald. To clarify this issue, however, further studies with the pedigreed barley materials are needed.

The dendrogram based on SRAP marker data successfully discriminated all the barley germplasm lines for scald breeding, except for 2 lines (S13 and S14). Although these 2 lines have a very high similarity coefficient (96%), their parental origins were labeled as different from each other using CRIFC. These 2 lines may have been mislabeled by the breeders, even though they may have shared the same parentage. UPGMA analysis and PCA successfully discriminated the scald-resistant and scald-sensitive germplasm lines. Cluster I contained only scald-resistant lines, and clusters III and IV included only scald-sensitive lines, except for line R29; however, cluster II contained both scald-resistant and scald-sensitive barley lines. All these lines have been cultivated in Turkey for a long time, suggesting that the genotypes of these scald-resistant and scald-sensitive lines may have been blended during this period, resulting in the dendrogramic structure observed in the present study.

UPGMA analysis also showed that genetic variation within cluster I was 0.72, which is higher than that of clusters III (0.64) and IV (0.68). This difference could be due to the fact that scald-resistant lines are continuously selected by breeders to combat the disease; therefore, their similarity coefficient value was higher compared to that of the scald-sensitive lines. Despite continuous selection activity, both the high percentage of polymorphism obtained with SRAP markers (73.5%) and the low similarity coefficient revealed by UPGMA analysis suggest that there is a large degree of polymorphism present in Turkish barley lines, which could be a great asset for accelerating scald disease resistance breeding without causing genetic erosion in the future.

Acknowledgements

We are grateful to I. Sayım and Z. Mert for kindly providing the Turkish barley germplasm lines and scald disease data for the study.

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