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MOLECULAR CHARACTERIZATION OF COLD TOLERANT GERMPLASM OF PHASEOLUS BEANS WITH SEQUENCE RELATED AMPLIFIED POLYMORPHISM (SRAP) AND RETROTRANSPOSON-BASED INTERPRIMER BINDING SITES (iPBS) MARKERS

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ABSTRACT

In this study genetic diversity of 55 *Phaseolus sp.* beans selected for cold tolerance from different regions of Türkiye was investigated by using sequence related amplified polymorphism (SRAP) and retrotransposon-based interprimer binding sites (iPBS)markers. Four commercially registered cultivars, one accession of *Phaseolus coccineus* and a tepary bean *Phaseaolus acutifolius* species were included for comparison. Genomic DNA was isolated from young fresh leaves and PCR reaction was carried out using 30 SRAP (sequence related amplified polymorphism) and 12 iPBS (retrotransposon-based interprimer binding sites) primers. Similarity analyses were performed and dendrograms were produced according to the Unweighted Pair-Group Mean Arithmetic method (UPGMA). In PCR reactions, 331 total and 146 polymorphic bands were produced with 30 SRAP primer combinations. The number of polymorphic bands ranged between 1 and 12 with an average 4.86 polymorphic marker for each primer pair. Twelve iPBS primers produced 156 total bands and 72 of them were polymorphic. The highest polymorphism was obtained with SRAP primer combinations of Me8Em3 and Me7Em14 and iPBS primers 2270, 2394 and 2252. Characterization of germplasm with SRAP and iPBS primers was discussed in relation to cold tolerance, species, source, seed size, seed color and growth type. In conclusion, genetic variability of germplasm of 55 Phaseolus bean species, genotypes and cultivars selected for cold tolerance were effectively assessed by PCR based molecular techniques, SRAP and iPBS. High levels of polymorphism determined in the core collection may be used in breeding programs for the development of cold tolerant superior cultivars.

Key words: Phaseolus sp. Beans, Chilling Tolerance, Landraces, Genetic Diversity

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INTRODUCTION

Beans are the most important legume crop in the world grown for its fresh pods and dry seeds (Voysest and Dessert 1991; FAO, 2018). Although there has been a steady decrease in the acreage, Phaseolus bean is one of the most important legume species in Türkiye after chickpeas and lentils (FAO, 2018). Abiotic and biotic stress factors, competition by high value cash crops in limited irrigated lands and cheaper bean imports put pressure on small scale bean farms. Cold stress (chilling <10°C and freezing <0°C temperatures) is the most important stress factor limiting the productivity of the plant in the spring and in the autumn. Türkiye has a rich *Phaseolus spp* plant diversity (Khaidizar *et al.,* 2012). Utilization of rich bean biodiversity for development of tolerant cultivars may help safer bean production in the regions with different climatic and geographical conditions of the country. A large-scale germplasm screen by Kantar *et al* (2016) produced a core collection of cold tolerant beans. Investigation of genetic diversity of this cold tolerant core collection is expected to facilitate the initiation of a breeding program for the development of resilient bean cultivars that might outperform current cultivars in colder environments of early or late growth seasons. Various techniques were used for the molecular characterization of Phaseolus bean germplasm in previous studies (Nodari *et al.,* 1992). Allozymes (Santalla *et al.,* 2002), amplified fragment length polymorphism (AFLP) (Svetleva *et al.,* 2006; Kumar *et al.,* 2008), random amplified polymorphic DNA (RAPD) (Marotti *et al.,* 2007), retrotransposon-based interprimer binding sites (iPBSs) (Nemli *et al.,* 2015; Öztürk *et al.,* 2020), simple sequence repeats (SSR) (Guerra-Sanz 2004; Sarikamiş *et al.,* 2009; De Luca *et al.,* 2018), nuclear microsatellite loci (nSSRs) (De Luca *et al.,* 2018; Savic *et al.,* 2021), inter simple

sequence repeats (ISSRs) (Svetlava *et al.,* 2006; Marotti *et al.,* 2007; Cabral *et al.,* 2018), and sequence related amplified polymorphism (SRAP) (Ceylan *et al.,* 2014) have been used for the characterization of germplasm. SRAP is a simple and reproducible marker system used for the assessment of genetic diversity and phylogenetic studies in many crops including other legume species (Smutkupt *et al.,* 2006; Baloch *et al.,* 2010). iPBS is also a universal and nonspecific recent marker system that do not require prior sequence knowledge (Kalendar *et al.,* 2011), and has been used in the genetic diversity studies of many plant species including Phaseolus beans (Nemli *et al.,* 2015; Aydin and Baloch 2018). This study was carried out to investigate genetic diversity of the bean cold tolerant core collection using SRAP and iPBS transposon marker systems and to compare both marker systems for the molecular characterization of Phaseolus bean germplasm.

MATERIALS AND METHODS

A core collection of 55 *Phaseolus vulgaris* landraces including 4 cultivars selected for cold tolerance from previous studies were used as material (Table 1). The core collection included landraces of dry, snap and pole beans collected from different regions of Türkiye. Four commercially registered cultivars, one accession of *P. coccineus* and *P. acutifolius* from the United States Department of Agriculture's (USDA's) Agricultural Research Service (ARS), The Germplasm Resources Information Network (GRIN) were also included for comparison. Cold tolerant landraces collected from high altitudes were selected on the bases of visual Cold Damage Index (CDI) from controlled environment tests at seedling stage at 5 °C (4). The core collection mentioned included accessions with CDI below 3 (i.e. no or little visual chilling damage on the leaves) and susceptible accessions (i.e. CDI of 4-9) for comparison (Table 1).

Molecular characterization of the core collection was performed by using sequence related amplified polymorphism (SRAP) (Li and Quiros, 2001) and retrotransposon-based interprimer binding sites primers (iPBS) (Kalendar 2010) marker systems. SRAP is a PCR based molecular marker system working with forward and reverse primers. The primers consist of 17-18 nucleotides, 13-14 of which are core sequence with 3 being selective nucleotides (Li and Quiros 2001). iPBS markers was developed by (Kalendar 2010) based on location of specific inter primer binding sites of Long Terminal Repeat (LTR) retrotransposons. iPBS is a non-species specific, highly polymorphic and universal method for the determination of genetic polymorphisms (Kalender 2010; Kalendar *et al.,* 2011). The effectiveness of combined data of SRAP and iPBS was also assessed in comparison with SRAP and iPBS marker systems alone for genotyping the core collection.

DNA Isolation. Seeds from 55 genotypes were germinated and fresh leaves were harvested from each genotype at 2-3 leaf stage. Genomic DNA was isolated from young fresh leaves using the Gene Jet Genomic DNA Purification Kit (Thermo Scientific). Quality of DNA evaluated by agarose gel electrophoresis.

SRAP Analysis. A total of 30 SRAP primer combinations were tested in this work (Table 2). The thermal cycling profile used for SRAP PCR reactions was 96°C for 5 min, followed by five cycles at 94°C for 1 min, 35°C for 1 min and 72°C for 2 min, 30 cycles at 94°C for one min, 50°C for 1 min and 72°C for 2 min and a final extension at 72°C for 10 min. DNA amplification was performed in a 15 µL reaction volume containing 1.0 µL 10x PCR buffer, 20 ng genomic DNA, 0.2 µM of each primer, 2.5 mM MgCl2, 100 µM each dNTP and 1.0 U/µL of Taq DNA polymerase (Thermo Scientific). The amplification products were separated by electrophoresis on a 2 % (w/v) agarose gel. The polymorphic bands were scored as either present (1) or absent (0).

iPBS Analysis. iPBSs based molecular characterization of 55 genotypes was carried out using 12 iPBS primers (Table 3). Thermal cycling profile used was 94°C for 5 min followed by 35 cycles at 94°C for 30 sec 55°C for 45 sec and 72°C for 1 min and a final extension at 72°C for 1 min. DNA amplification was in 15 µl total volume containing 20-30 ng of template DNA, 200 µM of each dNTP, 3 mM $MgCl_2$, 0.2 µM of each primer, and 1 U Taq polymerase.. The amplification products were separated by electrophoresis on a 2% (w/v) agarose gel. The polymorphic bands were scored as either present (1) or absent (0).

Data Analysis. Data obtained for each primer pair were analyzed using NTSYS Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1 Exeter Software, Setauket, N.Y., USA, (Rohlf 2000) and dendrograms were created by UPGMA grouping in SHAN module based on the similarity matrix according to (Dice 1945). Furthermore, Eigen values were calculated, Principal Component Analysis (PCA) was performed and graphs showing inter varietal distances in two dimensional matrices were generated. Correlations between marker systems were estimated by Mantel Test (Mantel 1967). The PIC value for each locus was calculated as proposed by (Roldàn-Ruiz *et al.,* 2000).

RESULTS AND DISCUSSION

SRAP Analysis. Bean germplasm of 55 genotypes were screened with 30 SRAP primer combinations and 331 total and 146 polymorphic bands were produced (Table 2). The number of polymorphic bands ranged from 1 to

12 with an average 4.86 bands per primer pairs. The highest band number was achieved with primer combination of Me8Em3, and band size ranged from 150 to 1500. (Table 2). The highest polymorphic information content values were obtained with primer combinations of Me13Em2 (0.43), Me6Em9 (0.41), Me6Em6 (0.41), Me8Em2 (0.40) and Me12Em1 (0.40), and average polymorphic information content was 0.31 (Table 2). Similarity matrix showed that Accession Number (AN) 65 and AN 68 were the genetically closest genotypes (Similarity Index of 96.6%) followed by AN 65 and BT-67 (SI 95.9 %) and AN 68 and BT-67 (SI 93.8 %) (data not presented). The most distant genotypes with SI of 25.3 % were HK-60 and HK-37 followed by HK-60 and BT-16 (SI 28.1 %) and HK-60 and AN 62 (SI 8.8 %) (data not presented). On the bases of cluster analyses with 146 markers, similarity index of the core collection ranged from 0.42 to 0.97 (Figure 1). The core collection of 55 genotypes formed two main groups as A and B and each main group formed two subgroups (A-1, A-2 and B-1, B-2). Majority of genotypes (48) clustered within group A and remaining 7 genotypes clustered in group B. HK-26 and HK-37 collected from Hakkari province were clustered very close in the dendrogram and placed in sub-group A-2. They were green beans with a similar seed shape and seed color whereas HK-26 was climbing and HK-37 bush type.

iPBS Analysis. PCR reactions of 55 genotypes with 12 iPBS-retrotransposon primers produced 156 total and 72 polymorphic bands with 48 % polymorphism rate. iPBS primer 2270 produced the highest polymorphic band number (12) and highest % polymorphism (0.92) followed by primer 2394 (9 and 0.60 respectively) and iPBS2252 (9 and 0.69 respectively) (Table 3). Polymorphic information content (PIC) values varied between 0.30 (iPBS2230 and iPBS 2253) and 0.43 (iPBS 2238) and iPBS 2389) with an average PIC value of 0.36. Öztürk *et al* (2020) found average PIC value of 0.33 in their study with 71 local bean genotypes and reported the lowest PIC value as 0.19 with iPBS2077 and the highest PIC value as 0.42 for iPBS primers 2381, 2385, and 2389.

Table 1. Phaseolus bean landraces/cultivars, sources, growth type, seed colour, 100 grain weight (GW), and cold damage index (CDI) used for molecular characterization.

No	Source	Grow Type and Seed Colour	100 GW (g)	CDI	No	Source	Grow Type and Seed Colour	100 GW (g)	CDI
AN-58	Erz*	Bush, White	34.7	5	**BT-67**	Bitlis	Climbing, Brown	71.4	3
AN-61	Erc*	Climbing, White	27.7	9	**BT-69**	Bitlis	Bush, Pinto	31.4	3
AN-62	Ard*	Climbing, White	29.8	8	**BT-75**	Bitlis	Climbing, Variegated Camel	62.6	3
AN-65	Erz	Bush, White	32.0	6	**EL-30**	Elazığ	Bush, White	43.4	3
AN-68	Erz	Bush, White	57.0	2	**HK-22**	Hakkari	Climbing, Camel	42.9	3
AN-75	Erz	Climbing, White	42.9	2	**HK-26**	Hakkari	Climbing, Brown	33.2	3
AN-80	Erz	Bush, White	32.2	5	**HK-37**	Hakkari	Bush, Light Brown	39.9	3
AN-102	Erz	Bush, White	42.8	2	**HK-44**	Hakkari	Climbing, White	41.5	3
AN-110	Erz	Climbing, Biege	32.9	7	**HK-51**	Hakkari	Climbing, Camel	55.4	3
AN-115	Erz	Climbing, White	32.6	6	**HK-54**	Hakkari	Bush, White	27.8	3
AN-123	Erc	Bush, White	35.9	1	**HK-57**	Hakkari	Bush, white	33.2	2
AN-127	Erz	Bush, Pinto	-	9	**HK-59**	Hakkari	Climbing, White	64.6	2
AN-201	Erz	Climbing, Brown	26.6	2	**HK-60**	Hakkari	Climbing, White	118.4	2
AN-238	Erz	Bush, White	42.1	2	**IR-1**	Iran	Bush, Pinto	39.3	3
AN-247	Kars	Bush, White	85.6	2	**ML-49**	Malatya	Climbing, White	35.2	2
AN-303	Erz	Bush, cream-biege	78.2	2	**ML-6**	Malatya	Climbing, Dark Brown	54.8	3
AN-323	Erz	Semi-climbing, White	46.2	2	**MŞ-52**	Muş	Climbing, Brown variegated	64.9	3
AN-329	Erz	Climbing, White, P.coccineus	89.7	2	**PI-476860**	ARS-GRIN*	Beige with brown Specs	36.6	6
AN-338	Erz	Bush, Beige	31.2	6	**Erz- 2**	Erc	Bush, White	40.1	1
AN-390	Erz	Climbing, White	48.9	2	**TR- 64907**	AARI*	No data	-	3
AN-395	Erz	Climbing, White	31.9	2	**TR- 64995**	AARI	Climbing, Pinto	42.2	1
BN-56	Bingöl	Climbing, White	38.9	1	**PI-477039**	ARS-GRIN	*P. acutifolius (wild tepary)*	4.4	4
BN-8	Bingöl	Semi-climbing, camel	41.8	2	**HNGL**	BL*	Bush, Pinto	37.8	5
BT-106	Bitlis	Semi-climbing, Camel	29.6	2	**CYRL**	BL	Bush, white	47.0	6
BT-124	Bitlis	Climbing, Brown	78.8	2	**Göynük**	Cv*	Bush, White	53.5	3
BT-16	Bitlis	Bush, White	51.3	4	**Önceler**	Cv	Bush, Pinto	36.1	3
BT-52	Bitlis	Climbing, Camel	40.8	2	**Kantar-05**	Cv	Bush, Pinto	36.4	5
					Elkoca-05	Cv	Bush, white	47.4	7

*Erz=Erz, Erc=Erzincan, Ard=Ardahan, ARS-GRIN= United States Department of Agriculture's (USDA's) Agricultural Research Service (ARS), The Germplasm Resources Information Network (GRIN), AARI= Aegean Agricultural Research Institute, İzmir, BL= breeders Line, Cv=Commercial Cultivar.

CYRL and Elkoca-05 were the closest genotypes with Similarity Index of 97.2 % followed by AN 390 and AN 323 (SI 95.8 %) and HNGL and Elkoca-05 (SI 94.4 %) (data not presented). AN 329 and AN 110 were genetically the most distant genotypes from other cultivars and landraces (SI 22.2 %) followed by genotypes of AN 329 and TR-64907 (SI 25.0 %) and AN 247 and HK-57 (27.8 %) (data not presented).

Dendrogram with 55 genotypes produced two main groups (A and B) that each group subdivided into two sub-groups (A-1, A-2 and B-1 B-2) (Figure 2). Forty-eight genotypes clustered in group A whereas 7 genotypes clustered in group B. CYRL and Elkoca-05 genotypes in A-1 at the genetic similarity index of 0.97. HK-26 and HK-37 from Hakkari in subgroup A-2 had similar seed characteristics despite their difference in growth type (climbing and bush type respectively). Sub-group B-2 contained landraces of HK-60, HK-59, AN 247, AN 75, AN 303 and AN 329 whereas sub-group B-1 consisted of only *P. acutifolius* species PI-477039 (Figure 2).

Combined Data Analysis. Data from both SRAP and iPBS marker systems were combined to investigate whether combined data would yield extra information for the characterization of bean germplasm. For this aim, a total of 218 markers were used. Similarity matrix showed the closest genotypes were AN 68 and AN 65 with an average similarity index (SI) value of 96.7% followed by the second closest genotype/cultivar of CYRL and Elkoca-05 (SI 95.8 %) and HNGL (SI 94.5 %) (data not presented). Genetically most distant genotypes were AN 75 and BT-16 (SI 41.3 %) followed by the second most distant genotypes of AN 61 and HK-60 (SI 42.3%) and AN 61 and HK-59 (SI 42.9 %) (data not presented).

Similarity Index Values of the genotypes ranged between 0.50 and 0.97 in the cluster analysis with 218 polymorphic markers of SRAP and iPBS-retrotransposon data (Figure 3). In the dendrogram generated, 55 genotypes separated into two main groups (A and B) and each group further extended into two sub-groups (A-1, A-2 and B-1, B-2). Majority of genotypes (48) were clustered in group A and 7 genotypes were clustered in group B (Figure 3).

Principle Component Analysis (PCA) were performed based on Eigen values and two-dimensional graph was produced. The first three Eigen values explained 45, 78 % of total variance (Table 4). Two-dimensional graph from PCA showed that genotypes formed three clearly distinguishable groups (Figure 4). As a result of PCA analysis PI-477039 clearly separated from other genotypes, not included in any group and place alone in the two-dimensional graph. The chilling tolerant landraces collected from high altitude provinces such as HK-59 HK-60 (collected from Hakkari), 247 (Kars), 75, 303, 329 (Erzurum) located together at the top of the two-dimensional graph (Figure 4).

Mantel test on the bases of Similarity Matrix based on data from SRAP and iPBS marker systems had high correlation coefficients ($r = 0.85$, $P < 0.0001$).

Sequence related amplified polymorphism (SRAP) and retrotransposon-based interprimer binding sites (iPBS) showed high levels of polymorphism in the core collection studied. Ceylan *et al.,* (2014) tested 13 SRAP primer combinations in the assessment of genetic diversity of 14 nationally registered common bean cultivars. They obtained polymorphism ratio of 64 % and concluded that SRAP primers were efficient in the assessment of genetic diversity of bean genotypes. In current study, 30 SRAP primer combinations were used in genetic assessment of more diverse germplasm of 55 different Phaseolus species, landraces and cultivars. Mean polymorphism ratio in our study was 43%. Primer combination Me8Em3 gave the highest polymorphic band number (12), followed by Me8Em2 (11). The highest polymorphism (0.86 %) was obtained with Me8Em3 primer combination followed by Me7Em14 (0.83 %). This study and Ceylan *et al.,* (2014) both included bean cultivars of Göynük and Önceler in the germplasm list that may give valuable information for the comparison of the results of SRAP marker test. Dendrograms produced with SRAP and iPBS markers listed Göynük and Önceler in the same sub-group with a Similarity Index of 0.89 and 0.83 respectively in our study and Ceylan *et al.,* (2014) using SRAP marker system listed Göynük and Önceler in the same sub-group as well.

PCR reactions with 12 iPBS-retrotransposon primers with 55 genotypes produced 156 total band number, 72 polymorphic bands, 48 % polymorphism and 36 % polymorphic information content. Nemli *et al.,* (2015) obtained 180 polymorphic band using 83 combinations for the characterization of 67 local green bean accessions, landraces and cultivars with 0.73 polymorphic information content and the highest polymorphic band number with iPBS primer 2394 (12), 2252 (8) and 2395 (8). In the current study, iPBS primers 2270, 2394 and 2252 gave the highest polymorphic band number. Therefore, iPBS primers 2270, 2394 and 2252 used in both studies appeared to be effective in assessing genetic diversity. Aydın and Baloch, (2019) characterized 96 common bean germplasm with iPBS-retrotansposons and their dendrogram divided the bean genotypes into two main group (A and B) according to their geographical regions, growth habits and seed size. They reported higher efficiency and reproducibility of iPBS-retrotransposons witnessed in common bean and

recommended it for genotyping the larger germplasm of common beans.

Table 2. Number of Primers, Primer Combinations, Total Band Number, Polymorphic Band Number, % Polymorphism and Polymorphic Information Content obtained using SRAP primers with 55 bean landraces and cultivars.

Primer Combinations	Primer Primer sequence (First Line Forward and second line reverse)	Total Band Number	Polimorphic Band Number	% Polymorphism	Polymorphic Information Content
Me6Em6	TGAGTCCAAACCGGACA GACTGCGTACGAATTGCA	16	10	0, 63	0, 41
Me8Em2	TGAGTCCAAACCGGACT GACTGCGTACGAATTTGC	15	11	0, 73	0, 40
Me7Em14	TGAGTCCAAACCGGACG GACTGCGTACGAATTCTT	6	5	0, 83	0, 19
Me8Em3	TGAGTCCAAACCGGACT GACTGCGTACGAATTGAC	14	12	0, 86	0, 38
Me8Em10	TGAGTCCAAACCGGACT GACTGCGTACGAATTCAT	14	10	0, 71	0, 38
Me6Em7	TGAGTCCAAACCGGACA GACTGCGTACGAATTCAA	5	1	0, 20	0, 17
Me11Em15	TGAGTCCAAACCGGAAC GACTGCGTACGAATTGAT	11	7	0, 64	0, 32
Me11Em16	TGAGTCCAAACCGGAAC GACTGCGTACGAATTGTC	12	7	0, 58	0, 34
Me12Em1	TGAGTCCAAACCGGAGA GACTGCGTACGAATTAAT	15	8	0, 53	0, 40
Me12Em2	TGAGTCCAAACCGGAGA GACTGCGTACGAATTTGC	11	2	0, 18	0, 32
Me12Em5	TGAGTCCAAACCGGAGA GACTGCGTACGAATTAAC	5	3	0, 60	0, 17
Me12Em6	TGAGTCCAAACCGGAGA GACTGCGTACGAATTGCA	10	3	0, 30	0, 30
Me13Em2	TGAGTCCAAACCGGAAG GACTGCGTACGAATTTGC	17	6	0, 35	0, 43
Me13Em9	TGAGTCCAAACCGGAAG GACTGCGTACGAATTCAG	12	7	0, 58	0, 34
Me5Em17	TGAGTCCAAACCGGAAG GACTGCGTACGAATTCCA	10	4	0, 40	0, 30
Me5 Em 19	TGAGTCCAAACCGGAAG GACTGCGTACGAATTCGA	7	2	0, 29	0, 22
Me8 Em 6	TGAGTCCAAACCGGACT GACTGCGTACGAATTGCA	10	4	0, 40	0, 30
Me9 Em 6	TGAGTCCAAACCGGAGG GACTGCGTACGAATTGCA	5	3	0, 60	0, 17
Me6 Em 9	TGAGTCCAAACCGGACA GACTGCGTACGAATTCAG	16	4	0, 25	0, 41
Me1 Em 6	TGAGTCCAAACCGGATA GACTGCGTACGAATTGCA	11	5	0, 45	0, 32
Me1 Em 9	TGAGTCCAAACCGGATA GACTGCGTACGAATTCAG	12	3	0, 25	0, 34
Me2 Em8	TGAGTCCAAACCGGAGC GACTGCGTACGAATTCAC	11	3	0, 27	0, 32
Me3 Em 16	TGAGTCCAAACCGGAAT GACTGCGTACGAATTGTC	12	6	0, 50	0, 34
Me4 Em 6	TGAGTCCAAACCGGACC GACTGCGTACGAATTGCA	10	1	0, 10	0, 30

Me10 Em 15	TGAGTCCAAACCGGAAA GACTGCGTACGAATTGAT	13	5	0, 38	0, 36
Me10 Em 2	TGAGTCCAAACCGGAAA GACTGCGTACGAATTTGC	13	4	0, 31	0, 36
Me10 Em 5	TGAGTCCAAACCGGAAA GACTGCGTACGAATTAAC	11	2	0, 18	0, 32
Me10 Em 6	TGAGTCCAAACCGGAAA GACTGCGTACGAATTGCA	11	3	0, 27	0, 32
Me11 Em 3	TGAGTCCAAACCGGAAC GACTGCGTACGAATTGAC	7	3	0, 43	0, 22
Me12 Em 4	TGAGTCCAAACCGGAGA GACTGCGTACGAATTTGA	9	2	0, 22	0, 27
Total/Mean		331	146	0, 43	0, 31

Table 3. Number of Primers, Primer Codes, Total Band Number, Polymorphic Band Number, % Polymorphism and Polymorphic Information Content obtained using iPBS-retrotransposon primers with 55 genotypes.

Primer Code	Sequences	Total band Number	Polimorphic Band Number	% Polimorphism	Polymorphic Information Content
2075	CTCATGATGCCA	11	3	0, 27	0, 32
2077	CTCACGATGCCA	14	8	0, 57	0, 38
2228	CATTGGCTCTTGATACCA	12	3	0, 25	0, 34
2252	TCATGGCTCATGATACCA	13	9	0, 69	0, 36
2253	TCGAGGCTCTAGATACCA	10	7	0, 70	0, 30
2270	ACCTGGCGTGCCA	13	12	0, 92	0, 36
2380	CAACCTGATCCA	16	6	0, 38	0, 41
2394	GAGCCTAGGCCA	15	9	0, 60	0, 40
2230	TCTAGGCGTCTGATACCA	10	3	0, 30	0, 30
2238	ACCTAGCTCATGATGCCA	17	5	0, 29	0, 43
2276	ACCTCTGATACCA	14	3	0, 21	0, 38
2374	CCCAGCAAACCA	11	4	0, 36	0, 32
Total/Mean		156	72	0.48	0.36

Table 4. Eigen Values obtained with the combined data from SRAP and iPBS analysis.

Genotype No	Eigen Values	% Rate	Cumulative Rate	Genotype no	Eigen Values	% Rate	Cumulative Rate
ÇYRL	6, 78	24, 92	24, 92	HK-60	0, 22	0, 83	88, 73
Elkoca-05	4, 45	16, 34	41, 26	61	0, 22	0, 81	89, 54
Erz -2	1, 23	4, 52	45, 78	62	0, 20	0, 74	90, 28
HNGL	1, 02	3, 73	49, 51	65	0, 19	0, 71	90, 99
Göynük	0, 93	3, 42	52, 94	TR-64907	0, 19	0, 68	91, 67
Kantar-05	0, 85	3, 10	56, 04	BT-67	0, 18	0, 65	92, 32
Önceler	0, 70	2, 58	58, 62	68	0, 17	0, 63	92, 95
IR-1	0, 69	2, 52	61, 14	BT-69	0, 16	0, 59	93, 54
ML-6	0, 62	2, 26	63, 40	75	0, 15	0, 57	94, 10
BN-8	0, 56	2, 07	65, 47	BT-75	0, 15	0, 54	94, 65
PI-476860	0, 53	1, 93	67, 40	80	0, 14	0, 50	95, 15
BT-16	0, 50	1, 85	69, 25	102	0, 13	0, 48	95, 63
HK-22	0, 49	1, 78	71, 04	BT-106	0, 13	0, 46	96, 10
HK-26	0, 46	1, 67	72, 71	110	0, 12	0, 45	96, 54
PI-477039	0, 43	1, 56	74, 27	115	0, 11	0, 42	96, 96
EL-30	0, 40	1, 46	75, 73	123	0, 11	0, 40	97, 36
HK-37	0, 40	1, 45	77, 19	BT-124	0, 10	0, 38	97, 74

HK-44	0, 36	1, 31	78, 50	127	0, 09	0, 34	98, 08
ML-49	0, 34	1, 25	79, 75	201	0, 09	0, 32	98, 41
HK-51	0, 32	1, 18	80, 92	238	0, 08	0, 28	98, 69
BT-52	0, 31	1, 13	82, 06	247	0, 07	0, 27	98, 96
MŞ-52	0, 30	1, 09	83, 14	303	0, 07	0, 25	99, 20
HK-54	0, 28	1, 02	84, 16	323	0, 06	0, 23	99, 43
BN-56	0, 27	0, 98	85, 14	329	0, 06	0, 21	99, 64
HK-57	0, 26	0, 95	86, 09	338	0, 04	0, 15	99, 79
AN-58	0, 25	0, 93	87, 03	390	0, 04	0, 13	99, 92
HK-59	0, 24	0, 88	87, 90	395	0, 02	0, 08	> 100%
				TR-64995	0, 00	0, 00	> 100%

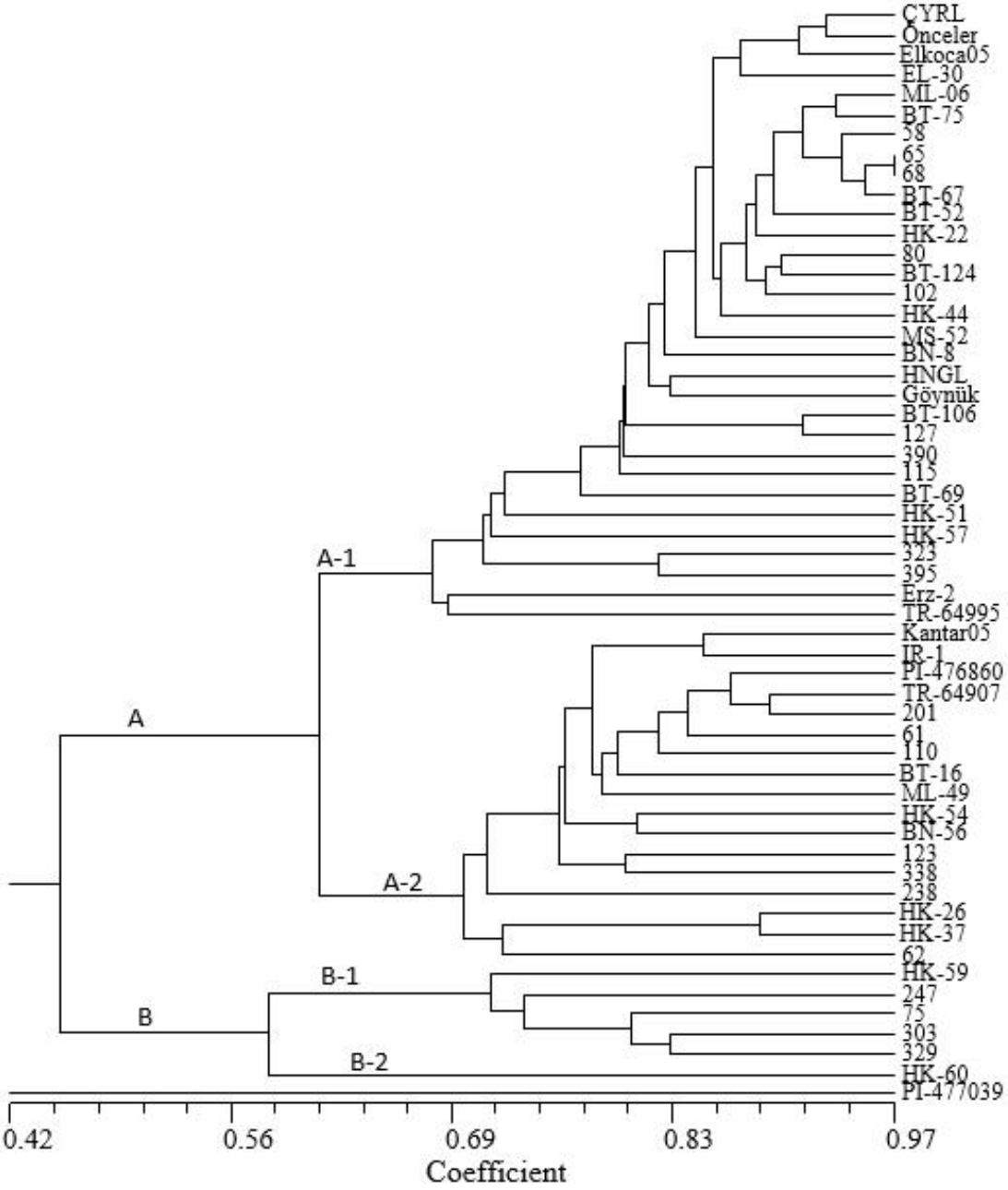

Figure 1. Dendrogram produced according to the unweighted pair-group mean arithmetic method (UPGMA) using sequence related amplified polymorphism (SRAP).

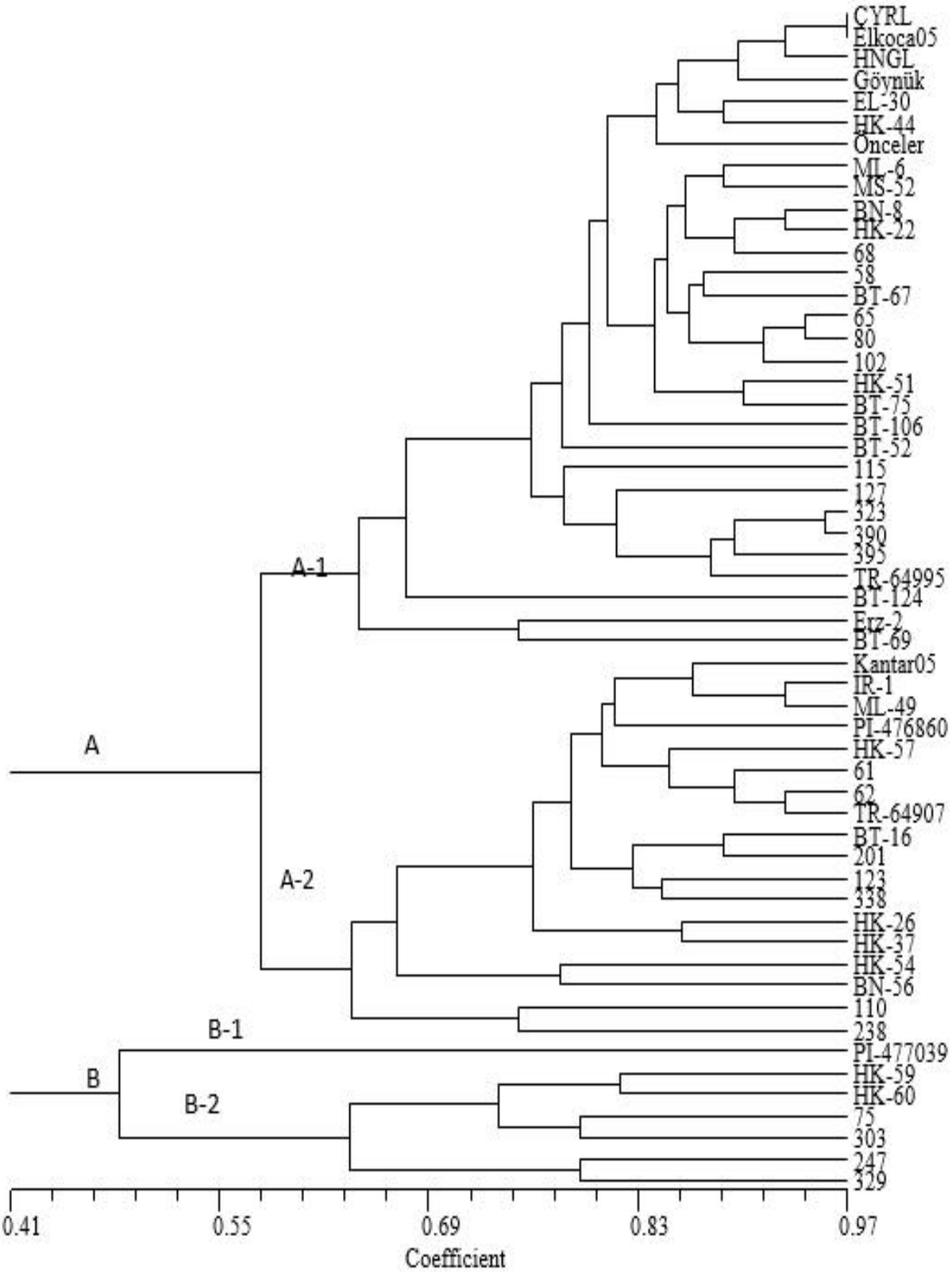

Figure 2. Dendrogram produced according to the unweighted pair-group mean arithmetic method (UPGMA) using iPBS-Retrotransposon Markers.

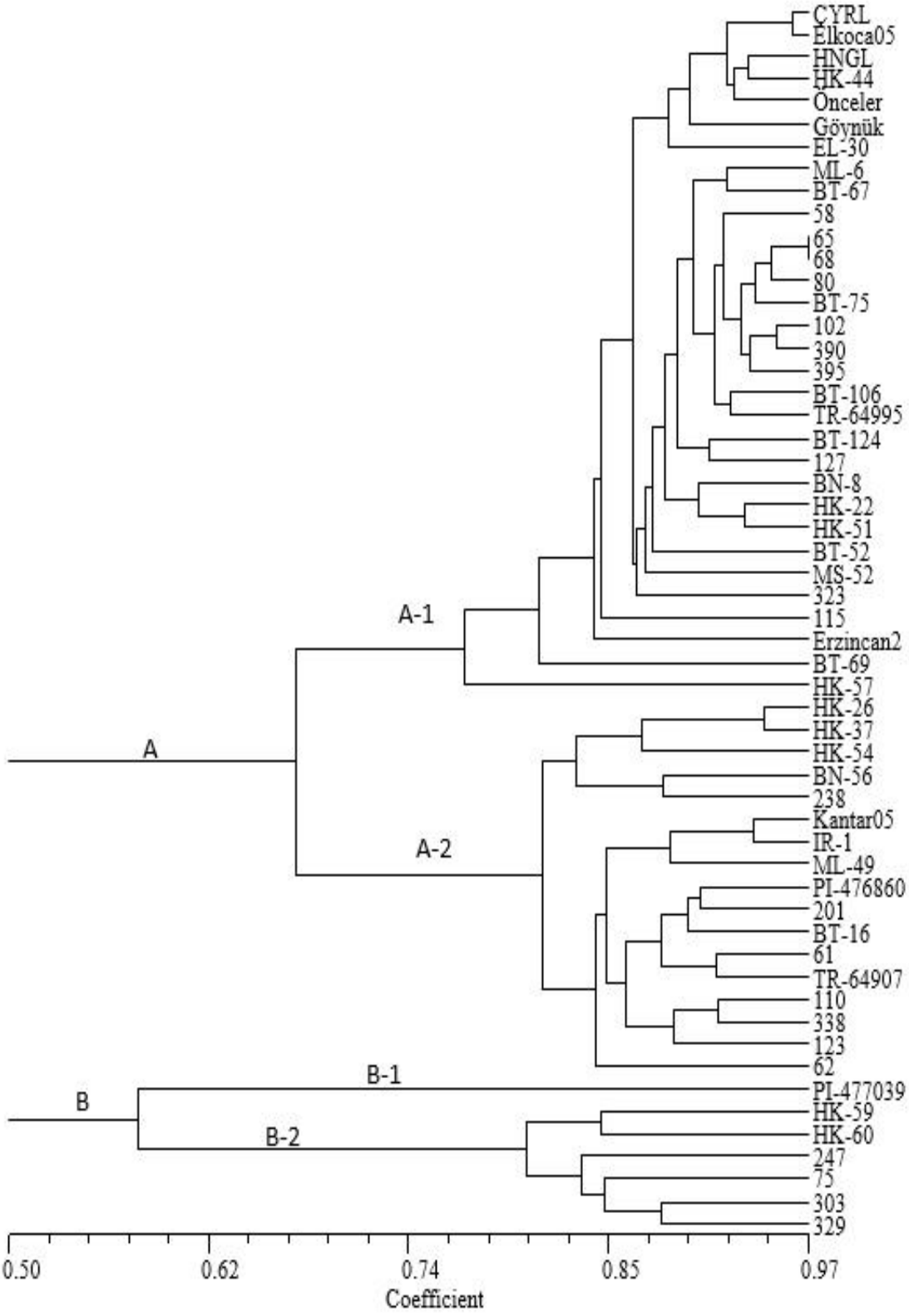

Figure 3 Dendrogram produced according to the unweighted pair-group mean arithmetic method (UPGMA) using both SRAP and iPBS Markers.

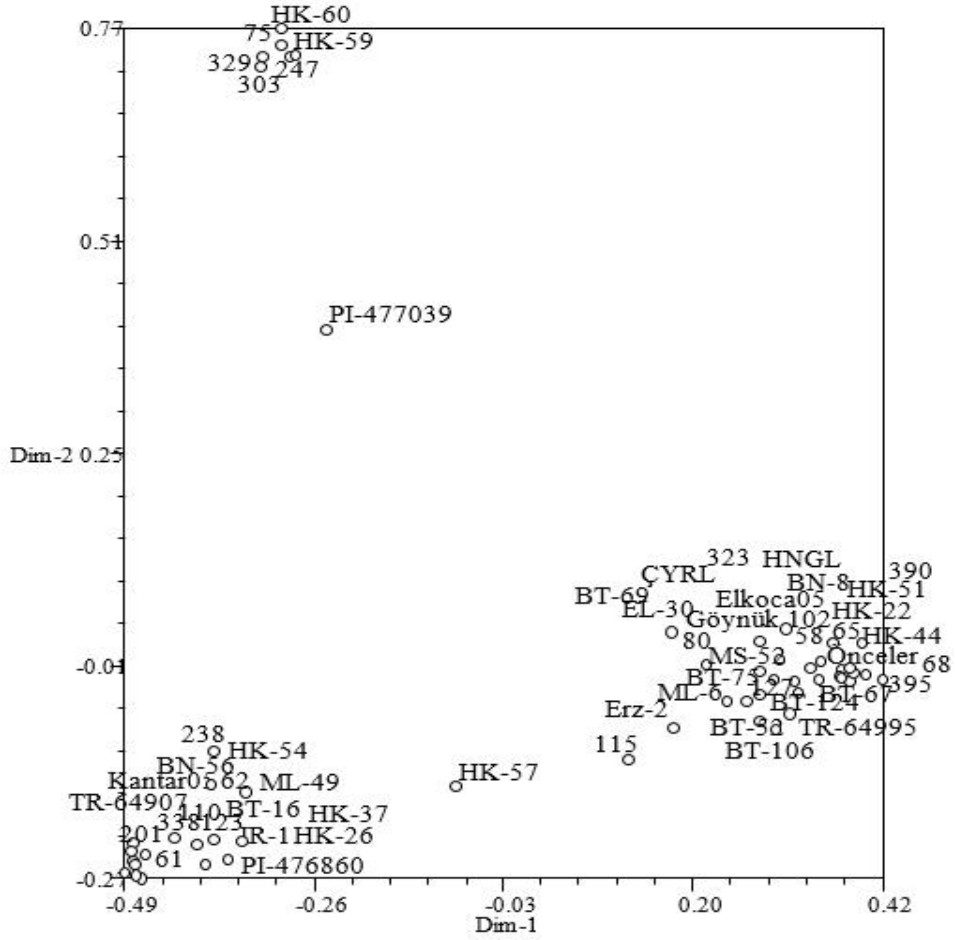

Figure 4. Distribution of 55 genotypes in two-dimensional graph from PCA Analysis based on combined data

Khaidizar *et al.,* (2012) investigated genetic diversity of 38 common bean landraces including 12 nationally registered cultivars using Simple Sequence Repeats (SSR). Their list of landraces and cultivars also included some of the cultivars tested in our study (Göynük, Önceler, Kantar-05, AN-338, AN-303, AN-115 and AN-58). In their UPGMA dendrogram, Göynük and Önceler were clustered in the same group and sub-group in line with our results and data of Ceylan *et al.,* (2014).

Phaseolus Species. Both marker systems effectively characterized *Phaseolus vulgaris* landraces and cultivars. SRAP and iPBS markers systems and combined data produced similar UPGMA dendrogram (Figure 1, 2 and 3). SRAP marker system separated wild tepary bean, *P.acutifolius* PI-477039 from other landraces and placed alone in the bottom of the dendrogram (Figure 1).

Geographical Location. HK-59, HK-60, 75, 303, 75, 247 and 329 formed a distinct cluster in the dendrograms obtained with both SRAP and iPBS markers. These landraces were cold tolerant genotypes collected from usually higher elevation provinces of Erzurum, Hakkari and Kars including *P. coccineus* landrace 329. Most distant cultivars and landraces CYRL, Önceler, Elkoca-05 El-30 Ml-6 and BT-75 were clustered in group A-1. They were registered cultivars and were collected from the landraces of low elevation provinces (Table 1). There are, however, other cultivars from high elevation provinces and sources of high and low cold tolerance in group A-1 (Table 1, Figure 1, 2 and 3).

Seed Size and Seed Color. 100 Grain Weight of Phaseolus beans ranged between 26.6 g (AN 201) and 118.4 g (HK-60) with an average of 46.1 g (Table 1). Apart from small seeded wild tepary bean, large seeded genotypes were listed in group B whereas small or medium seeded landraces and cultivars listed in the most distant group A-1 and A-2 subgroups in the UPGMA dendrograms produced by SRAP, iPBS and combined data (Figure 1, 2 and 3). White seed color is dominant in the germplasm followed by brown/camel and variegated

seed color (Table 2). In group B genotypes, all six landraces had white seed color whereas cultivars and landraces with various seed color remained in group A-1 and A-2 in all 3 dendrograms (Figure 1, 2 and 3). Group B-2 landraces had white seed coat color and large 100 seed weight e.g. HK-60 (118.4 g), HK-59 (64.6 g), AN-247 (85.6 g), AN-75 (42.9 g), AN-303 (78.2 g) and AN-329 (89.7 g) although all the genotypes were Andean types with 100 seed weight above 25.0 g (Figure 3; Table 1).

Plant Growth Type. The genotypes were not grouped according to their growth patterns (bush or climbing) in all three dendrograms formed based on SRAP, iPBS and combined data. Bush and climbing type genotypes were randomly clustered in the groups. The most distant genotypes CYRL, Önceler, Elkoca-05 and EL-30 were bush types, but ML-6 and BT-75 in contrary were climbing types in group A-1 (Figure, 1, 2 and 3; Table 3).

Cold Tolerance. Phaseolus bean germplasm of 55 genotypes tested in this study included the core collection of *Phaseolus vulgaris* genotypes and cultivars selected in previous experiments that may be used in breeding programs for cold tolerance. Some genotypes with medium cold tolerance and some susceptible landraces as well as different species of *Phaseolus cocccines* and *Phaseolus acutifolius* were also included in the germplasm for the sake of scientific comparisons. The core collection contained landraces from high elevation areas of Türkiye as well 3 accessions of foreign origin. Assessment of genetic variability, genotyping and preservation of core collection are of importance for the development of cold tolerant superior cultivars. Of the lines tested, HK-59, 247, 75, 303, 329 and HK-60 were chilling tolerant landraces (CDI 2) (Table 1) and placed in Group B (B-1 and B-2) cluster together showed the same pattern in all 3 UPGMA dendrograms produced by SRAP, iPBS and combined data (Figure 1, 2 and 3). Chilling susceptible cultivars of CYRL, Önceler and Elkoca-05 (CDI of 5, 3, and 7 respectively) clustered together in the top of the three dendrograms (group A) obtained with SRAP, iPBS and combined data. However in group A-2 chilling tolerant and susceptible landraces and cultivars were clustered together (Figure 1, 2 and 3). Germplasm core collection tested here had predominantly larger seeded laundresses and cultivars (100 grain weight over 25.5 g), which are regarded of Andean origin. Apart from 4.4 g of PI-477039 (wild tepary, *P. acutifolius*), 100 grain weight of Phaseolus beans ranged between 26.6 g (AN 201) and 118.4 g (HK-60) with an average of 46.1 g. Both SRAP and iPBS marker systems distinguished larger seeded genotypes in one distinct subgroup with exception of AN 75 of relatively medium 100 grain weight (42.9 g). Larger seeded landraces of HK-59, 247, 75, 303, 329 and HK-60 were chilling tolerant landraces (CDI 2) collected from usually higher elevations as opposed to chilling susceptible cultivars of CYRL, Önceler and Elkoca-05 (CDI of 5, 3, and 7 respectively) clustered together in the most distant groups. However, chilling tolerant landraces with smaller 100 grain weights were present in the germplasm (e.g. Erz-2 with CDI=1 and grain weight of 39.3 g and BT-106 with CDI=2 and grain weight of 29.6 g) did not group with those chilling tolerant genotypes with larger seed. Landraces adapted to colder climatic conditions at higher elevations may be used as a source of cold tolerance. Tomato (Patterson *et al.,* 1978) and *Festuca indigesta* (Angosto and Matilla 1994) accessions collected from higher elevations were reported to be more cold tolerant. In chickpeas, accessions from higher elevations germinated better at cold temperatures and were more cold tolerant (Mayer and Poljakoff-Mayber 1989). In our experiment, Phaseolus sp. accessions from higher elevation areas appear to be more chilling tolerant which may warrant further studies. Cold tolerant genotypes probably adapted to colder and unstable climatic conditions in highland regions. No consistent and meaningful clustering appeared with regard to seed color and growth types of the germplasms tested in both marker systems.

Conclusion: In conclusion, genetic variability of germplasm of 55 Phaseolus bean genotypes including *P. vulgaris*, *P. cocccines* and *P. acutifolius* selected for cold tolerance were effectively assessed by PCR based molecular techniques, namely Sequence Related Polymorphism (SRAP) and retrotransposon-based inter primer binding sites (iPBS). High levels of polymorphism were present within the germplasm studied. Characterization of germplasm with SRAP and iPBS primers was discussed in relation to cold tolerance, species, source, seed size, seed color and growth type. High levels of polymorphism determined in the core collection may be used in the breeding programs for development of superior cold tolerant cultivars. SRAP and iPBS marker system alone or together may also be employed in the assessment of bean germplasm.

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