



## Exploring the genetic diversity and population structure of Turkish common bean germplasm by the iPBS-retrotransposons markers

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### ABSTRACT

Present study was aimed to investigate the genetic diversity and population structure of Turkish common bean germplasm. A total of 96 bean genotypes were characterized with iPBS-retrotransposons that yielded a mean polymorphism information contents of 0.8. Mean gene diversity and Shannon information index were 0.14 and 0.25, respectively. Neighbor joining analysis divided the bean genotypes into two main group (A and B) according to their geographical regions, growth habits and seed size. Bingol-17, Sivas-14 and Hakkari-11 genotypes were found very distinct and can be used as candidate parents for the bean breeding. The higher efficiency and reproducibility of iPBS-retrotransposons was witnessed in common bean as compared to earlier studies using the same marker system. Results of this study will boost up the investigators for genotyping the larger germplasm of common bean with minimum laboratory infrastructure in developing and least developed countries.

**Key words:** Common bean, Genetic diversity, Genotyping, iPBS-retrotransposons, Landraces.

### INTRODUCTION

Common bean (*Phaseolus vulgaris* L,  $2n=2x=22$ ) is a self-pollinated, new world legume species and possess simplest non-duplicated genome (Nemli *et al.* 2015; Schmutz *et al.* 2014). Common bean has gained the interest of both farmers and consumers and represents 50% of grain legumes for direct human consumption in the developing countries (Devi *et al.* 2015; Razvi *et al.* 2018). Common bean is important source protein for poor families (Lima *et al.* 2012) and staple food for more than 200 million people of sub-Saharan Africa (Schmutz *et al.* 2014). Common bean was independently domesticated in north Mexico and Andes Mountains around 8000 years ago. Domestication resulted in the emergence of two gene pools; Mesoamerican and Andean gene pool (Bitocchi *et al.* 2013). It is believed that common beans was introduced in Europe in 16<sup>th</sup> and 17<sup>th</sup> century during the 1st voyage of Columbus (Gioia *et al.* 2013). From Europe, this crop was introduced to Ottoman Empire (present Turkey) in the 17<sup>th</sup> century (Bozoğlu and Sozen, 2011), allowing bean genotypes to spread around different regions of the world (Madakbaş *et al.* 2016).

Turkey is center of origin and diversification for different crop (Baloch *et al.* 2017). Turkey ranks third largest common bean producer having 813.000 tons annual production (FAO, 2013). Many local Turkish dishes are made through common bean and, therefore common bean has unique place in Turkish Agriculture (Nemli *et al.*, 2015; Erdinç *et al.*, 2017). Turkey is not the origin and

domestication center of common bean, however the previous studies indicated the presence of wide diversity in common bean landraces from Turkey (Bozoğlu and Sozen, 2011; Erdinç *et al.*, 2017; Sarıkamış *et al.*, 2009; Nemli *et al.*, 2014; 2015).

Different molecular markers have been utilized for assessing the diversity in common bean (Angioi *et al.*, 2010; Bitocchi *et al.*, 2013; Immaculee *et al.* 2015; Khaidizar *et al.* 2012; Mall and Chawla, 2015; Nemli *et al.*, 2015; Erdinç *et al.*, 2017). Retrotransposons are mobile genetic elements which are present in the interspersed repetitive genetic region and comprising major fractions of all eukaryotic genomes (Yaldiz *et al.*, 2018; Nadeem *et al.*, 2017). Retrotransposons are classified into two groups according to their structure and transposition cycle (Kalendar *et al.* 2010). Replication of retrotransposons generate the genetic diversity in eukaryotic genome. iPBS retrotransposons could serve as excellent source of dominant molecular markers for fingerprinting and studying the genetic diversity and relationship between individuals of wild accessions and cultivars in plants (Baloch *et al.*, 2015a). iPBS-retrotransposons markers have been studied for estimating the genetic diversity in different crops of Turkey (Baloch *et al.*, 2013; Baloch *et al.*, 2015a,b; Nemli *et al.*, 2015; Yaldiz *et al.*, 2018). In our previous studies, we observed that retrotransposons markers were very efficient for genetic diversity studies in term of number of total amplified and polymorphic bands. However Nemli *et al.* (2015) stated that

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efficiency of iPBS-retrotransposons in bean was conserved. Therefore we planned to this study to check the status of iPBS-retrotransposons markers in common bean for estimating the genetic diversity in Turkish common bean germplasm.

## MATERIALS AND METHODS

**Plant material and DNA extraction:** A total of 95 common bean genotypes including 76 populations collected from different geographical provinces of Turkey and 20 accessions obtained from United states department of agriculture (USDA) gene bank were used as plant material in the present study (Table 1 and Fig 1). Turkish populations were grown at experimental area of Abant Izzet Baysal University during 2016 using augmented block design maintaining 2m row length with 10 cm plant to plant distance. DNA was extracted from the young leaves of four weeks old seedling according to methodology of Doyle and Doyle (1990) with some modification of Baloch *et al.* (2016) and growth habit and seed weight data from Turkish populations was recorded by following the criteria of Sing *et al.* (1991) Some foreign bean accessions obtained from USDA were included in the study as control and their phenotypic data was not available.

**Retrotransposons analysis:** Seventy iPBS-retrotransposons primers developed by Kalendar *et al.* (2010) were screened on randomly selected 8 common bean genotypes to check the status of polymorphism. Out of these 70 primers, 10 iPBS retrotransposons primers producing sharp and clear bands were used for screening the whole set of genotypes (Table 2). PCR reaction was conducted in 25µl reaction mixture in Thermal cycle (T-100, Bio-Rad, USA), following the PCR condition of (Kalendar *et al.* 2010). PCR products were separated on 1.5% agarose gel in electrophoresis with 0.5 X TBE buffer for 2 h at 120 volts, stained with ethidium bromide and visualized under the UV Imager Gel Doc XR+ system (Bio-Rad, USA) light, and later photographed. A 100-bp (plus) ladder (Thermo Scientific) was used as a molecular weight marker.

**Bioinformatics analysis:** iPBS-retrotransposons is a dominant marker system and therefore scored in binary fashion as 1 (presence) and 0 (absence) for amplified bands respectively. Different diversity parameters such as Shannon information index (I), gene diversity (h) were also calculated using POPGENE program (Yeh *et al.* 2000). Polymorphism information contents (PIC) of the each iPBS retrotransposons primer was estimated using the formula of Hinze *et al.* (2015).  $PIC = 1 - \frac{p^2 + q^2}{2}$ , Where, p and q are the frequencies of presence versus absence bands of the both band of the iPBS primer. PIC was calculated for each band of the concerned iPBS-retrotransposons and was averaged for each primer. A pair wise genetic distance matrix between studied common bean accessions was calculated by applying Jaccard's coefficient (Jaccard, 1987) using R statistical

software. Neighbor joining cluster analysis was conducted using computer software program 'R'. The Bayesian clustering model was performed in 'STRUCTURE' program aiming to evaluate a comprehensive understanding about the genetic structure of collected common bean germplasm. We followed the criteria suggested by the Evanno *et al.* (2005) for the investigation of optimal number of clusters (number of K; number of subpopulations) and plotted the K against logarithm probability relative to standard deviation ( $\Delta K$ ).

## RESULTS AND DISCUSSION

Within the investigated 96 population of common bean, 10 most polymorphic iPBS-retrotransposons primers were selected that resulted total of 96 bands. Of these, 69.79% bands showed polymorphism with an average of 9.6 bands per primer (Table 2). Highest number of total amplified bands (12) were observed with "iPBS2373 and iPBS2381" while lowest number of bands (6) were amplified by the primer iPBS2382. Fig 2 represent the genetic diversity profile of common bean genotypes using the iPBS-retrotransposons. Highest polymorphic bands (9) were produced by the three primers (iPBS2075, iPBS2081 and iPBS2241) while lowest (4) was observed by primer iPBS2381. Highest polymorphism of 90% was observed in primer "iPBS2075" while lowest (33.3%) was noted in iPBS2381 with a mean polymorphism of 71.2% (Table 2). Diversity observed in our study was higher than the previous studies conducted in Turkish bean using different molecular markers such as SRAP, POGP, cpSSR (Ceylan *et al.*, 2014). This clearly demonstrated that retrotransposons are highly polymorphic markers. However our results made disagreement with Nemli *et al.* (2015) that resulted in 180 bands with 47 iPBS-retrotransposons primers in 67 bean genotypes and they reported that efficiency of iPBS-retrotransposons primers is more conserved in bean compared with other crops such as cicer, lens, pea (Andeden *et al.*, 2013; Baloch *et al.*, 2015a,b). It could be seen that Nemli *et al.* (2015) used 53.19% primers having 18 nucleotides, while our all primers contains 12 nucleotides. 12 nucleotides makes these retrotransposons glorified RAPD markers. Therefore these retrotransposons primers should be verified in further studies. Gene diversity (h), shanon information index (I), and PIC were also calculated to check the informativeness of primers (Table 2). Gene diversity among iPBS retrotransposon varied between 0.07 (iPBS2080) and 0.28 (iPBS2241) with mean value of 0.14. Shanon information index ranged from 0.10 (iPBS2080) to 0.42 (iPBS2241) with an average of 0.25. PIC value ranged from 0.65 for primer iPBS2075 to 0.93 for primer iPBS2381 with an average of 0.80. All iPBS-retrotransposon in our study produced PIC greater than 0.6. For comparison, PIC value in our study was higher than the previous studies (Erdoğan *et al.* 2017; Nemli *et al.* 2015).

**Table 1:** Passport Data of 96 common bean genotypes used in this study.

Genotype	Growth Habit	Seed weight (g)	Country	Collection region	Altitude	Coordinates
Tokat-18	NA	NA	Turkey	Tokat	1425 m	37° 02'169/ 40° 30'227
Bitlis-14	C	31.62	Turkey	Bitlis	1522 m	38° 11'967 / 42° 20'644
Elazığ-25	C	31.87	Turkey	Elazığ	1313 m	38° 21'174 / 39° 22'660
Muş-39	P	28.39	Turkey	Muş	1280 m	39° 05'383 / 41° 30'168
Muş-10	P	40.87	Turkey	Muş	1489 m	39° 06'752 / 42° 08'046
Bitlis-115	C	31.39	Turkey	Bitlis	2002 m	38° 29'645 / 42° 04'575
Samsun-32	NA	NA	Turkey	Samsun	240 m	36° 27'455 / 41° 12'501
Samsun-23	NA	NA	Turkey	Samsun	600 m	35° 52'406 / 41° 04'470
Samsun-29	NA	NA	Turkey	Samsun	600 m	36° 27'455 / 41° 12'501
Hakkari-44	P	46.73	Turkey	Hakkâri	1764 m	35° 59'410 / 41° 04'404
Göksun	I.B	33.18	Turkey	K.Maraş	1350 m	36° 29'377 / 38° 01'140
Tokat-22	NA	NA	Turkey	Tokat	1415 m	37° 05'502 / 40° 27'299
Muş-15	C	37.75	Turkey	Muş	1514 m	39° 10'268 / 42° 05'099
Muş-01	P	31.2	Turkey	Muş	1607 m	39° 05'869 / 42° 38'738
Tokat-13	NA	NA	Turkey	Tokat	-	-
Van-13	C	32.45	Turkey	Van	1702 m	38° 05'736 / 43° 15'575
Bingöl-60	C	36.16	Turkey	Bingöl	1500 m	39° 27'172 / 40° 30'366
Hakkari-37	C	35.59	Turkey	Hakkâri	2054 m	37° 36'246 / 43° 42'370
Hakkari-43	C	49.6	Turkey	Hakkâri	1764 m	37° 33'418 / 43° 37'329
Akman98	I.B	28.68	Turkey	Ticari çeşit	-	-
Bingöl-45	C	37.58	Turkey	Bingöl	1500 m	39° 26'0208 / 40° 32'428
Elazığ-42	NA	NA	Turkey	Elazığ	877 m	38° 41'578 / 39° 53'162
Van-47	I.B	48.21	Turkey	Van	1689 m	39° 00'036 / 43° 21'362
Van-36	NA	NA	Turkey	Van	1876 m	37° 49'064 / 44° 06'905
Hakkari-11	C	28.67	Turkey	Hakkâri	2097 m	37° 36'332 / 43° 42'526
Göynük	I.B	47.96	Turkey	Ticari çeşit	NA	NA
Sivas-43	NA	NA	Turkey	Sivas	NA	38° 06'133 / 39° 21'041
Bitlis-105	C	36.16	Turkey	Bitlis	1728 m	38° 27'483 / 42° 26'602
Muş-50	P	25.62	Turkey	Muş	1489 m	39° 06'752 / 42° 08'046
Bingöl-52	C	26.84	Turkey	Bingöl	1500 m	39° 27'455 / 41° 12'501
Samsun-26	NA	NA	Turkey	Samsun	600 m	36° 05'214 / 41° 02'046
Sivas-14	NA	NA	Turkey	Sivas	NA	38° 24'508 / 40° 05'392
Tokat-15	NA	NA	Turkey	Tokat	NA	35° 57'505 / 40° 13'516
Sivas-36	NA	NA	Turkey	Sivas	NA	37° 52'040 / 39° 05'575
Bitlis-120	I.B	20.13	Turkey	Bitlis	1543 m	38° 17'889 / 42° 15'891
Malatya-45	C	38.23	Turkey	Malatya	1158 m	38° 14'905 / 37° 55'605
Muş-07	C	36.86	Turkey	Muş	1550 m	39° 03'619 / 42° 19'105
Tokat-14	NA	NA	Turkey	Tokat	NA	37° 12'093 / 40° 16'398
Niğde-dernason	P	41.29	Turkey	Ticari çeşit	NA	NA
Tokat-03	NA	NA	Turkey	Tokat	NA	35° 53'564 / 40° 28'471
Muş-53	I.B	43.18	Turkey	Muş	1369 m	38° 38'595 / 41° 44'016
Sivas-66	NA	NA	Turkey	Sivas	NA	36° 14'182 / 39° 11'055
Bitlis-111	C	44.11	Turkey	Bitlis	1689 m	38° 29'404 / 42° 32'232
Bingöl-07	I.B	37.22	Turkey	Bingöl	1154 m	39° 03'502 / 40° 45'401
Tokat-66	NA	NA	Turkey	Tokat	NA	36° 51'400 / 40° 26'521
Tunceli-11	NA	NA	Turkey	Tunceli	NA	39° 28'052 / 38° 57'164
Tokat-21	NA	NA	Turkey	Tokat	NA	37° 13'405 / 40° 32'433
Samsun-24	NA	NA	Turkey	Samsun	NA	35° 52'407 / 41° 04'472
Sivas-63	NA	NA	Turkey	Sivas	NA	37° 38'308 / 39° 29'348
Bitlis-66	C	39.92	Turkey	Bitlis	1459 m	38° 26'765 / 41° 51'660
Bitlis-79	C	33.31	Turkey	Bitlis	1423 m	38° 28'878 / 41° 43'845
Malatya-13	C	16.43	Turkey	Malatya	1369 m	37° 59'707 / 38° 01'503
Muş-11	NA	NA	Turkey	Muş	1489 m	39° 06'752 / 42° 08'046
Tokat-04	NA	NA	Turkey	Tokat	-	36° 07'454 / 40° 16'531
Tokat-05	NA	NA	Turkey	Tokat	-	36° 12'302 / 40° 19'473
Sivas-59	NA	NA	Turkey	Sivas	-	36° 41'496 / 39° 48'160
	NA	NA				

Table 1 continue.....

Table 1 continue.....

Hakkari-63	C	18	Turkey	Hakkari	1955 m	37° 35208 / 43° 04488
Bingöl-61	C	28.06	Turkey	Bingöl	-	40° 28482 / 39° 27181
Muş-51	C	40.6	Turkey	Muş	1463 m	38° 13447 / 42° 10513
Sivas-44	I.B	59.81	Turkey	Sivas		37° 59066 / 39° 12411
Bitlis-114	C	33.3	Turkey	Bitlis	1459 m	38° 26765 / 42° 51660
Van-28	NA	NA	Turkey	Van	2072 m	38° 08452 / 44° 12332
Karacaşehir	P	22.09	Turkey	Ticari çeşit	-	-
Tokat-87	NA	NA	Turkey	Tokat	-	36° 45566 / 40° 41567
Muş-18	C	42.37	Turkey	Muş	1293 m	39° 40424 / 41° 58975
Hakkari-69	C	28	Turkey	Hakkari	1915 m	37° 32928 / 44° 08427
Bitlis-121	C	50.82	Turkey	Bitlis	1459 m	38° 26765 / 41° 51660
Van-33	C	33.73	Turkey	Van	2005 m	37° 47409 / 44° 07448
Hakkari-71	C	43.25	Turkey	Hakkari	1724 m	37° 25223 / 44° 29056
Malatya-71	C	43.51	Turkey	Malatya	1465 m	38° 05052 / 37° 57494
Hakkari-13	C	38.08	Turkey	Hakkari	2097 m	37° 29370 / 43° 38184
Muş-43	C	46.43	Turkey	Muş	1577 m	39° 12636 / 41° 23917
Elazığ-2	C	57.5	Turkey	Elazığ	877 m	38° 41578 / 39° 53162
Hakkari-79	NA	NA	Turkey	Hakkari	1137 m	37° 29096 / 43° 37693
Van-37	C	29.21	Turkey	Van	2244 m	38° 01147 / 43° 39146
Sivas-01	NA	NA	Turkey	Sivas	-	38° 05251 / 40° 09476
K.maraş-91	NA	NA	Turkey	K.maraş	-	37° 11306 / 38° 12226
Hakkari-76	C	29.1	Turkey	Hakkari	1135 m	37° 29773 / 43° 34389
Muş-02	C	62.5	Turkey	Muş	1550 m	39° 03619 / 42° 19105
Bitlis-71	C	27.29	Turkey	Bitlis	1197 m	38° 29116 / 41° 47168
Bingöl-11	I.B	34.5	Turkey	Bingöl	1542 m	39° 04033 / 40° 48557
Tokat-07	NA	NA	Turkey	Tokat	-	35° 36455 / 40° 09451
Bitlis-90	C	21.6	Turkey	Bitlis	1655 m	38° 32695 / 42° 06804
*Kolombiya-1 (619392)	NA	NA	NA	Kolombiya	NA	NA NA
*Hindistan-1 (629386)	NA	NA	NA	Hindistan	NA	NA NA
*Kolombiya-2 (619385)	NA	NA	NA	Kolombiya	NA	NA NA
*Kolombiya-3 (619396)	NA	NA	NA	Kolombiya	NA	NA NA
*Amerika-1 (632432)	NA	NA	NA	Amerika	NA	NA NA
*Kolombiya-4 (619388)	NA	NA	NA	Kolombiya	NA	NA NA
*Kolombiya-5 (619393)	NA	NA	NA	Kolombiya	NA	NA NA
*Brezilya-1 (562689)	NA	NA	NA	Brezilya	NA	NA NA
*Kolombiya-6 (619389)	NA	NA	NA	Kolombiya	NA	NA NA
Kolombiya-7 (619395)	NA	NA	NA	Kolombiya	NA	NA NA
*Kolombiya-8 (619391)	NA	NA	NA	Kolombiya	NA	NA NA
*Kolombiya-9 (619394)	NA	NA	NA	Kolombiya	NA	NA NA

I.B: Indeterminate bush, C: Climber, P: Prostrate, \*: USDA genotypes, NA: not available

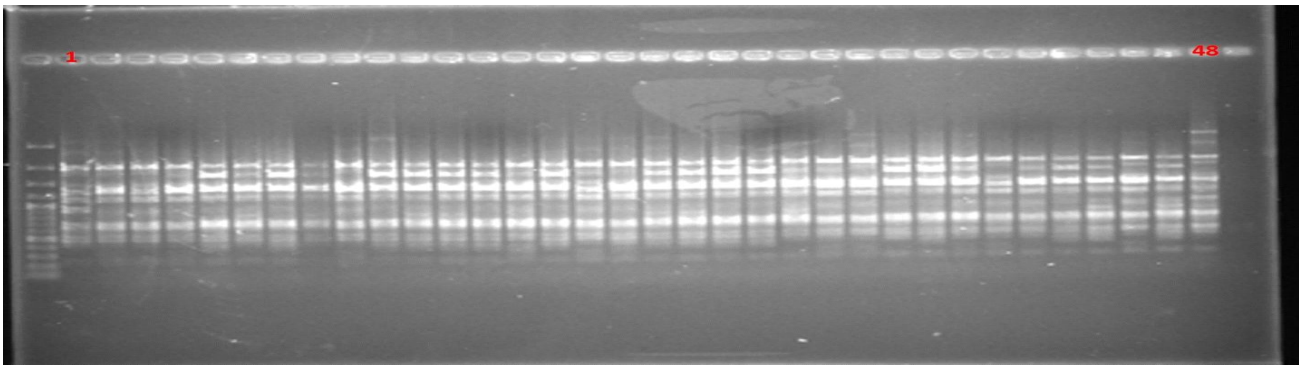
**Table 2:** Informations about the retrotransposons primers used in the diversity studies.

Marker name	sequence	Amplified bands			Diversity parameters			
		At (°C)	TB	PB	P%	h	I	PIC
iPBS2080	CAGACGGCGCCA	63	9	5	55,6	0,07	0,10	0,79
iPBS2075	CTCATGATGCCA	50	10	9	90	0,10	0,19	0,65
iPBS2081	GCAACGGCGCCA	65	11	9	81,8	0,27	0,41	0,82
iPBS2382	TGTTGGCTTCCA	52	6	5	83,3	0,16	0,24	0,90
iPBS2386	CTGATCAACCCA	52	8	7	87,5	0,13	0,16	0,76
iPBS2241	ACCTAGCTCATC	55	11	9	81,8	0,28	0,42	0,78
iPBS2373	CCCAGCAAACCA	55	12	7	58,3	0,23	0,33	0,88
iPBS2380	CAACCTGATCCA	50	9	6	66,6	0,08	0,15	0,69
iPBS2381	GTCCATCTTCCA	50	12	4	33,3	0,16	0,23	0,93
iPBS2393	TACGGTACGCCA	50	8	6	75	0,18	0,27	0,78
Total			96	67				
Average			9,6	6,7	71,32	0,14	0,25	0,80

At: Annealing temperature, TB: total amplified bands, PB: polymorphic bands, P%: polymorphism percentage h: gene diversity, I: Shannon information index, PIC: polymorphism information contents.



**Fig 1:** Collection site of Turkish common bean genotypes used in this study.

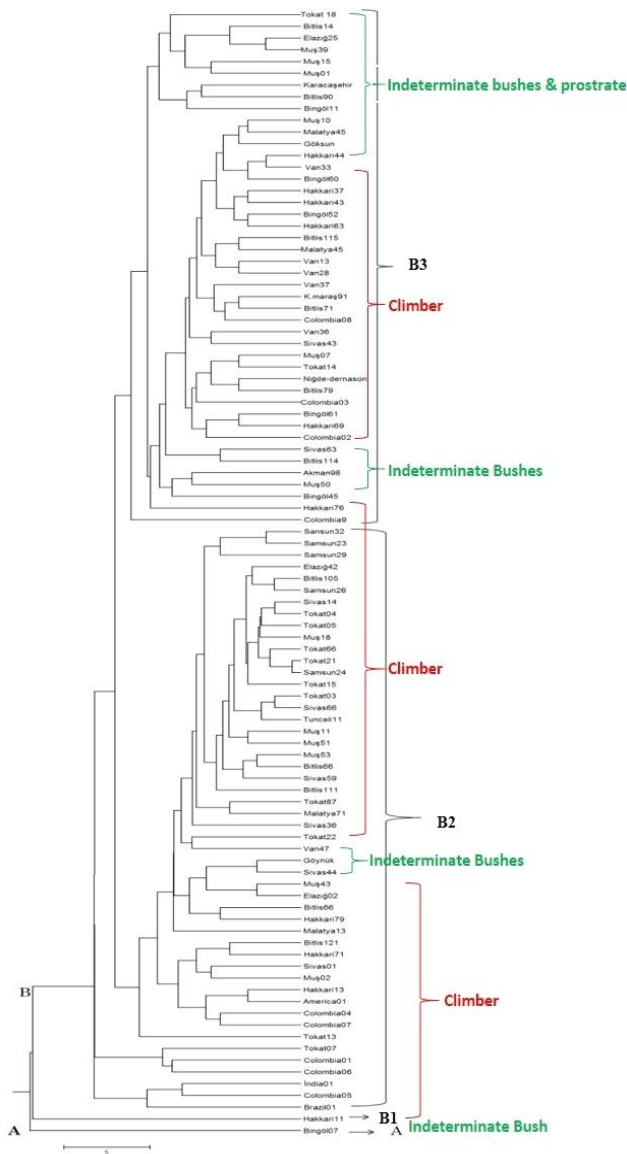


**Fig 2:** Genetic diversity profile of common bean genotypes using the iPBS-retrotransposons.

Jaccard genetic distance coefficient was calculated and highest genetic similarity was observed between pair of bean landrace Tokat21-Samsun24. While lowest genetic similarity was found between Bingöl7-Sivas14. As both of these genotypes reflected minimum similarity, these genotypes can be used as candidate parents of common bean in Turkey. Neighbor joining analysis divided all common bean landraces into two main clusters (Fig 3) based on the genetic similarity among the pair of bean landraces according to geographical regions and growth habit. Cluster A harbored only one landrace “Bingöl7” having indeterminate bushy growth habit. Group B was subdivided into three subgroups, B1, B2 and B3. Subgroup B1 contained only one landrace Hakkari-11 with climber growth habit. Group B2 contained a total of 52.08% of the common bean genotypes having climber growth habit. Genotypes from Samsun, Tokat, Sivas, Malatya, Tunceli and Elazığ were clustered together in B2, showing that genotypes from eastern part of central turkey to central area of the black sea region clustered together. Growth habit also played a key role in the clustering of genotypes and 94% genotypes clustered in the group B2 belongs to climber growth and thus expressing the dominance of climber growth habit in these regions, while remaining genotypes in subgroup B2 contained indeterminate bush type growth habit. Subgroup B3, clustered a total of 45.83% common bean genotypes used in this study and mostly genotypes belongs to Hakkari, Van, Bitlis, Bingöl, Muş, K.

maraş (South East Anatolian region of Turkey). Subgroup B3 reflected a good admixture of growth habit by clustering all three growth habits; indeterminate bushes, prostrate and climber and all these growth habits had been universally accepted and reported (Singh *et al.* 1991). Here also climber growth habit dominated (61.36%) as compared to the indeterminate and prostrate genotypes (38.64%). Genotypes from Van, Muş, Bitlis and Niğde province contained climber growth habit, while some genotypes from Bingöl, Muş and Tokat provinces clustered together on the basis of similar indeterminate to prostrate growth habit. It was observed that the mixing of some genotypes belonging to one region with other region and from one group to other group and seed size was one of the important factor. For example mostly genotypes belonging to Muş province of Turkey clustered in the subgroup 2, however some genotypes (Mus-1, 7, 10, 50) from the same province grouped in the subgroup B3 with genotypes having 100 seed weight more than 40g, possibly explaining the role of growth habit and seed size in addition to geographical provenance in clustering the bean accessions.

Structure analysis divided the bean accessions into two different subpopulations (Fig 4). Highest  $\Delta K$  (Fig 5) value was observed at K2 ( $\Delta K=13.262209$ ) illustrating that bean germplasm in Turkey was originated/introduced from two different subpopulation. The landraces in A group have very big seed size while the landraces in the

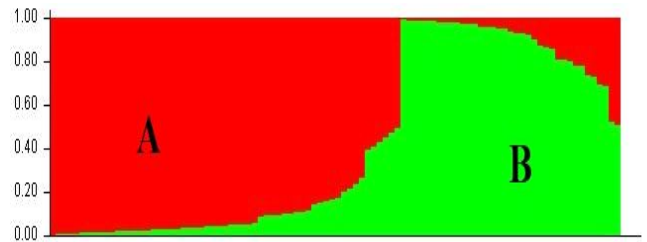


**Fig 3:** Neighbor joining clustering of 96 common bean genotypes using the iPBS-retrotransposons.

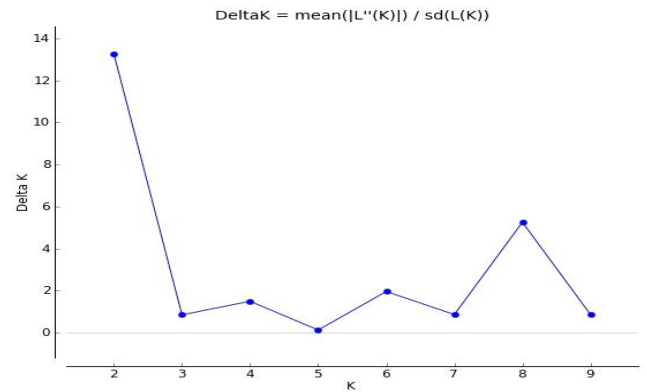
group B have small medium seed size which showed that seed size was the main source of variations and clustering of the landraces was based on their seed structure. Singh *et al.* (1991) also reported that bean germplasm of Andean origin have bigger seed size while bean from Mesoamerica have smaller seed size. Most of the bean genotypes used in this study have bigger seed size and probably they belong to Andean gene pool. Some genotypes from Colombia and

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**Fig 4:** Population structure of 96 common bean genotypes.



**Fig 5:** Delta K value representing the optimum numbers of populations obtained in this study.

Brazil were used as comparison and these genotypes are originated near Andean region and it could be concluded that genotypes used in this study belongs to Andean gene pool.

**CONCLUSION**

This study comprehensively explained the genetic diversity and population structure of Turkish common bean using iPBS-retrotransposons markers. Some candidate genotypes were noticed as putative parents for bean breeding and iPBS-retrotransposons markers was also found efficient and reproductive for common bean fingerprinting. Results of this study will boost up the investigators for genotyping the larger germplasm of common bean with minimum laboratory infrastructure in developing and least developed countries.

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