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Assessment of Genetic Diversity and Population Structure in Pea (*Pisum sativum* L.) Germplasm based on Morphological Traits and SSR Markers

Shalini Singh, B. Singh, V.R. Sharma[1], M. Kumar, U. Sirohi[2]

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ABSTRACT

Background: The study was undertaken to assess the genetic diversity and genetic structure among fifty-five pea accessions using morphological traits and SSR markers.

Methods: A total of 55 pea accessions were analyzed using eleven phenotypic traits and twenty SSR markers. The data obtained by morphological and molecular profiling was used for the analysis of genetic diversity and for the estimation of genetic diversity estimates, correlation, principal components analysis and population structure.

Result: This study reveals that majority of genetic variation was due to variation within population and were clustered into two distinct groups, which reveals a high admixture within individuals. Accessions *viz*., VRP-82, VRP-320, VRP-194, VRP-375, EC-97280 and EC-8724, showed great diversity as compared to the other accessions based on both morphological and molecular markers. These accessions may assist in developing and planning breeding strategies aimed to produce new varieties in the future.

Key words: Genetic diversity, Pea, Population structure, SSR markers.

INTRODUCTION

Garden pea (*Pisum sativum* L., 2n=14) is a widely cultivated vegetable crop native to Syria, Turkey, Israel and Ethiopia, for its versatile uses as pulses and livestock feed (Choudhury *et al*., 2007). In India, it is mostly grown in Uttar Pradesh, Madhya Pradesh, Bihar, Assam and Odisha with an area of 0.54 million hectares and an annual production of 5.4 million tonnes (NHB, 2019). It is grown for its tender green pods, dried seeds, canned, frozen or dehydrated form (Santalla *et al*., 2001).

Widespread cultivation of the crop, replacing landraces and traditional population, particularly the one resistant to biotic and abiotic stress, narrowed down the genetic base and has led to the loss of genetic variability. New breeding challenges imposed by global climate change and to meet the global demand, pea breeders have to undertake more efficient methods of selection and better take advantage of the large genetic diversity present in the gene pool using advanced methods. Phenotypic traits are widely used as the only accepted valid marker type for defining germplasm groups by the international union for the protection of new varieties of plants (UPOV) (Smykal *et al.,* 2008). Morphological characteristics represent the action of many genes but they can be unreliable due to the significant environmental influences. In contrast, molecular markers are useful to complement the morphological traits because they are independent of environmental effects and allow identification of the cultivar in the early stages of crop.

Among the various DNA markers available for molecular profiling of genotypes SSRs or microsatellites are highly reliable, accurate, co-dominant, generally highly polymorphic and also cost-effective (Jain *et al*., 2014). In pea, various genetic diversity studies were done using phenotypic traits (Uzma *et al*., 2018). Loridon *et al*. (2005) initially developed a set of SSRs for genetic diversity and linkage mapping of pea. Later, Rana *et al*. (2017) and Tahir *et al*. (2018) reported use of some polymorphic SSR markers for genetic diversity studies in pea. However, most of the studies involved accessions from the iso-climatic regions of the world.

In addition, a comprehensive study to evaluate the genetic diversity of the pea germplasms in sub-tropical conditions like Western Uttar Pradesh conditions has not been carried out in India and there is need to increase the production and productivity to meet rising population. Hence, the objective of the present study was to assess the level of genetic diversity within fifty-five collected pea accessions

Department of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110, Uttar Pradesh, India.
[1]CSIR-National Botanical Research Institute, Lucknow-226 001, Uttar Pradesh, India.
[2]Department of Agriculture Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250 110, Uttar Pradesh, India.

Corresponding Author: Shalini Singh, Department of Horticulture, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib-173 001, Himachal Pradesh, India.
Email: drshalinisinghhorti@gmail.com

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using morphological traits and SSR loci in Western Uttar Pradesh conditions.

MATERIALS AND METHODS

A total of fifty-five pea accessions were grown in randomized block design with three replications at Horticultural Research Center, SVPUAT, Meerut (U.P.), India (Table 1). Standard agronomic practices were followed to raise the crop. The traits *viz.,* DF-days to 50% flowering, PH- plant height (cm), NFFN-number of first fruiting node, LFFN-length of first fruiting node (cm), NPP-number of pods per plant, LP-length of pod (cm), WP-width of pod (cm), NSP-number of seeds per pod, GPY-green pod yield per plant (g), SW-shell weight per plant (g) and SY-seed yield per plant (g) were recorded for the five randomly selected plants in each replication. The mean values for each trait were analysed for descriptive statistics and genetic diversity analysis.

For genotyping, genomic DNA was isolated using CTAB extraction method (Doyle and Doyle, 1987) and was quantified using Bio-Rad's SpecTM Plus spectrophotometer. PCR amplification was performed using 20 SSR primer pairs (Table 2) and evaluated according to Kumar *et al.* (2019). A dendrogram was constructed based on the Jaccard's similarity matrix using the UPGMA clustering by using the software NTSYS-pc (Rohlf, 1998).

PIC-Polymorphism information content (Bostein *et al.*, 1980); Marker Index (MI) (Nagaraju *et al.*, 2001) and Rp-Resolving Power (Prevost and Wilkinson, 1999) was estimated. Furthermore, various other genetic diversity estimates (Huang *et al.*, 2021) were calculated using POPGENE v.1.32 (Yeh and Boyle, 1997). Principal Component Analysis (PCA) was done using R software (Venables and Ripley, 2002). Bayesian model-based clustering was also performed in STRUCTURE software, v.2.3.3. (Pritchard *et al.*, 2000) to identify the population structure of the germplasm using ancestry model with admixture and correlated allele frequency model was set according to Kumar *et al.* (2019). Analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992; Excoffier *et al.*, 1993) was performed to calculate variance components within and among the population with the help of GenALEx 6.5 (Peakall and Smouse, 2006). The mantel's test was done to correlate the morphological and molecular genetic matrices using XLStat Software (Smykal *et al.*, 2008).

RESULTS AND DISCUSSION

Based on phenotypic data, analysis of variance revealed that, all the character studied showed significant variation (Table 3). On the basis of Mahalanobis D^2 values, all the pea accessions were best grouped into 6 clusters (Rahman *et al.*, 2013). Comparative assessment of cluster means showed that for improving specific characters, the accessions should be selected from the cluster having high mean value for that particular character. This comparison indicates that clusters I and IV had better cluster means for most of the characters, therefore, these clusters might be considered better for selecting accessions as divergent

Table 1: List of pea accessions used and their source of collection.

Genotypes	Source
VRP-3	IIVR, Varanasi
VRP-13	IIVR, Varanasi
VRP-26	IIVR, Varanasi
VRP-194	IIVR, Varanasi
VRP-222	IIVR, Varanasi
VRP-375	IIVR, Varanasi
VRP-324	IIVR, Varanasi
VRP-115	IIVR, Varanasi
VRP-69	IIVR, Varanasi
VRP-313	IIVR, Varanasi
VRP-311	IIVR, Varanasi
VRP-73	IIVR, Varanasi
VRP-228	IIVR, Varanasi
VRP-321	IIVR, Varanasi
VRP-320	IIVR, Varanasi
VRP-355	IIVR, Varanasi
VRP-16	IIVR, Varanasi
VRP-22	IIVR, Varanasi
VRP-122	IIVR, Varanasi
VRP-383	IIVR, Varanasi
VRP-284	IIVR, Varanasi
VRP-65	IIVR, Varanasi
VRP-223	IIVR, Varanasi
VRP-402	IIVR, Varanasi
VRP-382	IIVR, Varanasi
VRP-176	IIVR, Varanasi
VRP-273	IIVR, Varanasi
VRP-327	IIVR, Varanasi
VRP-107	IIVR, Varanasi
VRP-156	IIVR, Varanasi
VRP-174	IIVR, Varanasi
VRP-95	IIVR, Varanasi
VRP-49	IIVR, Varanasi
VRP-276	IIVR, Varanasi
VRP-82	IIVR, Varanasi
VRP-145	IIVR, Varanasi
VRP-343	IIVR, Varanasi
VRP-131	IIVR, Varanasi
VRP-248	IIVR, Varanasi
VRP-64	IIVR, Varanasi
VRPM-15	IIVR, Varanasi
VP-233	IIVR, Varanasi
EC-97280	NBPGR, New Delhi
EC-8372	NBPGR, New Delhi
EC-8724	NBPGR, New Delhi
EC-71944	NBPGR, New Delhi
MO-23	IIVR, Varanasi
MO-19	IIVR, Varanasi
KS-228	IIVR, Varanasi
DPP-94/8-06	IIVR, Varanasi
Kashi Uday	IIVR, Varanasi
Kashi Mukti	IIVR, Varanasi
Kashi Shakti	IIVR, Varanasi
Kashi Samridhi	IIVR, Varanasi
Kashi Nandini	IIVR, Varanasi

Table 2: List of SSR primers with their sequence and annealing temperature.

Primer	Sequences	Annealing temp (°C)
PEA-01	F: 5′GAACTAGAGCTGATAGCATGT3′	67
	R: 5′GCATGCAAAAGAACGAAACAGG3′	
PEA-02	F: 5′GACATTGCCAATAACTGG3′	65
	R: 5′GGTTCTGTTCTCAATACAAG3′	
PEA-03	F: 5′GATGTGATAGGCCTAGAACAAGC3′	69
	R: 5′CAGTCACACACTACAAGAGATC3′	
PEA-04	F: 5′GTGGCTGATCCTGTCAACAA3′	66
	R: 5′CAACAACCAAGAGCAAAGAAAA3′	
PEA-05	F: 5′CCCAGTGAAGAAGGTCAACA3′	62
	R: 5′CAATGGTGGCAAATAGGAAA3′	
PEA-07	F: 5′CCATTTCTGGTTATGAAACCG3′	65
	R: 5′CTGTTCCTCATTTTCAGTGGG3′	
PEA-09	F: 5′CTGGAATTCTTGCGGTTTAAC3′	67
	R: 5′CGTTTTGGTTACGATCGAGCAT3′	
PEA-11	F: 5′CACACGATAAGAGCATCTGC3′	58
	R: 5′GCTTGAGTTGCTTGCCAGCC3′	
PEA-12	F: 5′TGGATTGGATTGGATGATGA3′	63
	R: 5′TGGAGCCCTTAGTCCACAAC3′	
PEA-14	F: 5′CCAAGAAAGGCTTATCAACAGG3′	68
	R: 5′TGCTTGTGTCAAGTGATCAGTG3′	
PEA-15	F: 5′AATTTGAAAGAGGCGGATGTG3′	67
	R: 5′ACTTCTCTCCAACATCCAACGA3′	
PEA-18	F: 5′TGTAGAAGCATAAGAGCGGGTG3′	68
	R: 5′TGCAACGCT CTT GTT GAT GATT3′	
PEA-19	F: 5′TAGTTTTGAACTTTGGCCGTAT3′	69
	R: 5′CACACCCTAATCTAGGCTATCC3′	
PEA-20	F: 5′CAATCGATCAGACAGTCCCCTA3′	66
	R: 5′AAGCTCACCTGGTTATGTCCCT3′	
PEA-21	F: 5′TGTGGGGCTTGTTACACTGA3′	65
	R: 5′AGCTACCATAACAGACAAAACC3′	
PEA-23	F: 5′TTCCAACCATGGAAGCTTTT3′	62
	R: 5′TTCTTCGTCGGGTACAGTGA3′	
PEA-26	F: 5′AGCTCTTTCTTCCACCACCA3′	58
	R: 5′AGCTCTTTCTTCCACCACCA3′	
PEA-27	F: 5′TTTAGCACAGAACAGCGTAGT3′	64
	R: 5′TAACGCCCTTGAGAATTTCG3′	
PEA-28	F: 5′AAATGGCCGTTTTATGATCG3′	61
	R: 5′CGGAGCTGAACCTTCTGGTA3′	
PEA-29	F: 5′GCTACTGGAGGAGGCTTTCA3′	58
	R: 5′GCCTTCTACACAACGGCTTC3′	

parents (Table 4). The similar results are exhibited with the findings Shrivastava *et al.* (2012).

The twenty primers which generated clear and distinct polymorphic alleles used in this study produced a total of 40 alleles. The effective number of alleles produced per locus ranged from 1 to 2 with an average of 1.5 bands per locus (Fig 1). Similar reports were presented by Handerson *et al.* (2014) and Rana *et al.*, (2017). Further, the most informative locus under this study was PEA-03 with PIC value of (0.996). A marker with PIC value greater than 0.5 is considered to be a very informative (Prakash *et al.*, 2016). Few studies also suggested that a greater number of accessions and primer pairs need for improving allelic richness (Negisho *et al.,* 2017). The highest (0.50) and lowest (0.03) expected heterozygosity (He) values obtained were with PEA-14 and PEA-11, 26, 27 respectively. Whereas, highest observed heterozygosity and Shannon's information index was obtained with PEA-14. It formed the average Shannon information index (I) value of 0.44, which was lower as compared to studies of Rana *et al.*, (2017). Observed heterozygosity (H_o) was ranged from 0.0 to 0.92 in primers PEA-3 and PEA-14, respectively. Expected heterozygosity (H_e) was ranged from 0.03 to 0.5 in primers PEA-29 and PEA-1 (Table 5).

Lower estimates obtained for all the diversity parameters indicate low allelic richness in the analysed germplasm, which implies more number of germplasm were need to be included for improving allelic richness. Furthermore, Rp varied between 0.51 to 3.20 and the value of MI ranged between 0.08 to 2.97, which shows informative and polymorphic content of primers for assessment of diversity.

Jaccard's similarity index was ranged from 0.589 between accessions 'EC-97280 and VRP-320' (distant) to 0.974 between accessions 'VRP-49 and VRP- 95' (closest). Cluster analysis based UPGMA dendrogram (Fig 2) outlined by SSR primer pairs grouped the 55 pea accessions into four clusters. PCA showed similarities in grouping with UPGMA and showed the 76% of variability based on first 4 components. PC1 showed 64% of total variability with Eigen value of 35.43. Whereas, the components namely, PC2, PC3 and PC4 revealed 4.5%, 4.3% and 3.38% of variability, respectively (Fig 3). The results agreed with other studies which indicate that these characteristics contribute maximally to a genetic divergence of pea (Cupic *et al.*, 2009; Suman *et al.*, 2019; Singh *et al.*, 2021).

Based on population structure analysis, the ΔK value observed maximum at Δk = 2 which implies all accessions grouped into two groups that shows highly admixture

Table 3: Descriptive statistics of morphological data.

Statistics	DF (50%)	PH	NFFN	LFFN	NPP	LP	WP	NSP	GPY	SW	SY
Minimum	59.67	29.67	4.42	12.52	3.42	5.11	1.01	3.75	10.81	3.15	5.29
Maximum	67.67	177.81	12.50	42.85	22.84	9.07	1.88	7.33	62.52	33.24	30.59
Median	63.00	69.16	9.08	30.43	8.33	7.33	1.32	5.58	23.85	12.76	11.39
Mean	63.18	74.40	8.85	29.31	9.57	7.25	1.31	5.44	27.46	14.18	13.37
Variance (n-1)	5.51	560.19	4.04	45.00	23.03	0.91	0.03	0.72	169.51	48.52	47.53
Standard deviation (n-1)	2.35	23.67	2.01	6.71	4.80	0.95	0.17	0.85	13.02	6.97	6.89

population (Fig 4). This also showed that there is no correspondence of genotypic matrix with the geographical location. Similar type of finding where reported by Jain *et al*., (2014); Rana *et al*., (2017) and Tahir *et al*., (2018). Whereas, in case of AMOVA analysis, it was observed that the genetic diversity of present population is mainly due to within population almost about 73% and the variation due among population is only 24%. The variance among the population

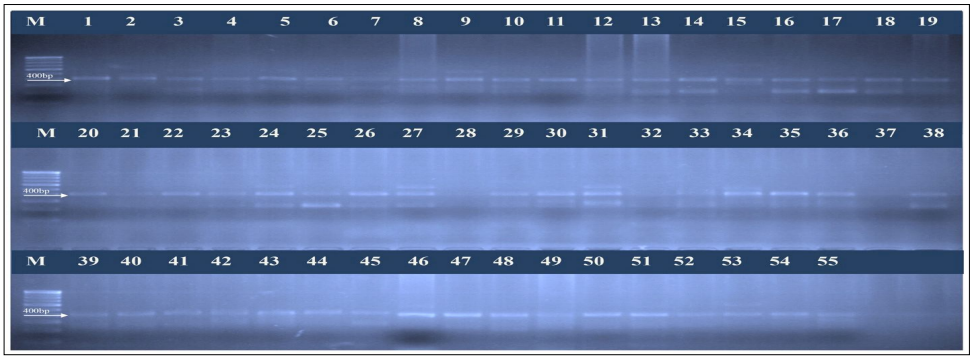

Fig 1: SSR profiling pattern of 55 accessions of pea with PEA-05 primer (M-100 bp molecular marker).

Table 4: Cluster wise mean values of 11 morphological traits in pea (*Pisum sativum* L.).

Clusters	DF (50%)	PH	NFFN	LFFN	NPP	LP	WP	NSP	GPY	SW	SY
I	62.43	60.97*	7.10*	22.47*	15.52**	7.60	1.33	5.33	47.52**	23.54**	24.57**
II	63.70	64.89	8.00	25.63	7.84	6.52	1.15*	4.87*	19.01	9.92	9.25
III	62.19	69.45	8.66	28.14	5.78*	7.50	1.47**	5.47	17.60*	8.90	8.50
IV	66.25**	139.61**	8.27	35.01**	6.05	6.48*	1.22	6.27**	15.29	8.39*	6.93*
V	62.07*	73.40	10.35	34.54	9.73	8.06**	1.34	5.97	34.42	17.82	16.82
VI	65.08	79.18	10.72**	34.67	12.26	6.61	1.19	5.06	26.56	14.84	11.47

Table 5: Primer name, molecular weight range, PIC, RP, MI, polymorphic and monomorphic bands, polymorphism % and genetic diversity estimates of SSR primers.

Primer name	Molecular weight range (bp)	PIC	RP	MI	na	ne	I	Obs_Hom	Obs_Het	Exp_Hom	Exp_Het	Nei
PEA-01	200-500	0.990	2.10	2.97	2.000	1.994	0.692	0.746	0.255	0.497	0.503	0.499
PEA-02	100-300	0.980	1.66	1.96	2.000	1.948	0.680	0.236	0.764	0.509	0.491	0.487
PEA-03	360	0.996	0.51	0.99	2.000	1.612	0.567	1.000	0.000	0.617	0.383	0.380
PEA-04	800	0.376	1.78	0.38	2.000	1.241	0.345	1.000	0.000	0.804	0.196	0.194
PEA-05	200-400	0.977	2.28	2.93	2.000	1.984	0.689	0.382	0.618	0.500	0.500	0.496
PEA-07	120	0.770	1.38	0.77	2.000	1.746	0.618	1.000	0.000	0.569	0.431	0.427
PEA-09	60	0.590	1.60	0.59	2.000	1.471	0.500	1.000	0.000	0.677	0.323	0.320
PEA-11	210	0.080	1.96	0.08	2.000	1.037	0.091	1.000	0.000	0.964	0.036	0.036
PEA-12	230	0.190	1.90	0.19	2.000	1.075	0.156	1.000	0.000	0.929	0.071	0.070
PEA-14	200-400	0.894	2.00	1.78	2.000	2.000	0.693	0.073	0.927	0.495	0.505	0.500
PEA-15	50-400	0.769	3.20	1.54	2.000	1.471	0.500	0.600	0.400	0.677	0.323	0.320
PEA-18	480	0.985	0.70	0.99	2.000	1.826	0.645	1.000	0.000	0.544	0.456	0.452
PEA-19	220	0.686	1.49	0.69	2.000	1.612	0.567	1.000	0.000	0.617	0.383	0.380
PEA-20	200-300	0.968	1.85	1.94	2.000	1.990	0.691	0.146	0.855	0.498	0.502	0.497
PEA-21	100-300	0.875	2.56	1.75	2.000	1.862	0.656	0.382	0.618	0.533	0.467	0.463
PEA-23	290	0.343	1.80	0.34	2.000	1.198	0.305	1.000	0.000	0.833	0.167	0.165
PEA-26	250	0.078	1.96	0.08	2.000	1.037	0.091	1.000	0.000	0.964	0.036	0.036
PEA-27	270	0.078	1.96	0.08	2.000	1.037	0.091	1.000	0.000	0.964	0.036	0.036
PEA-28	240	0.135	1.93	0.14	2.000	1.075	0.156	1.000	0.000	0.929	0.071	0.070
PEA-29	260	0.078	1.96	0.08	2.000	1.037	0.091	1.000	0.000	0.964	0.036	0.036
Average		0.59			2.000	1.513	0.441	0.778	0.222	0.704	0.296	0.293

na= Observed number of alleles; ne = Effective number of alleles; I: Shannon's Information index; Observed (Obs) and Expected (Exp) homozygosity (Hom) and heterozygosity (het); Nei: Nei's (1973) expected heterozygosity.

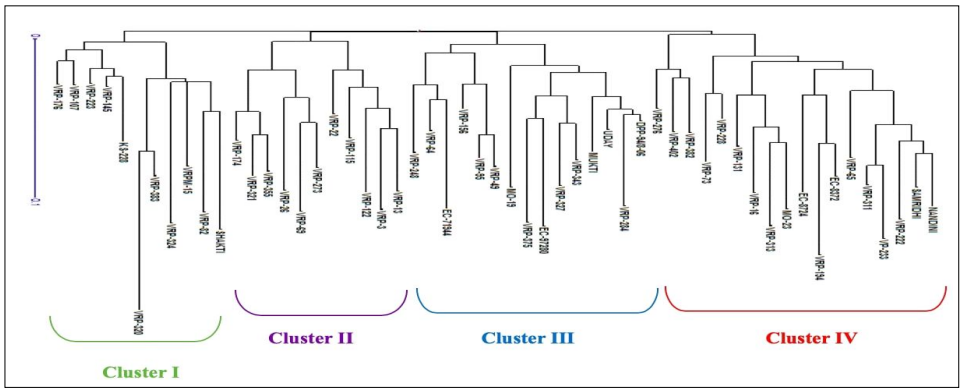

Fig 2: Dendrogram represents clustering of 55 pea accessions constructed using UPGMA based on Jaccard's similarity coefficient obtained from SSR analysis.

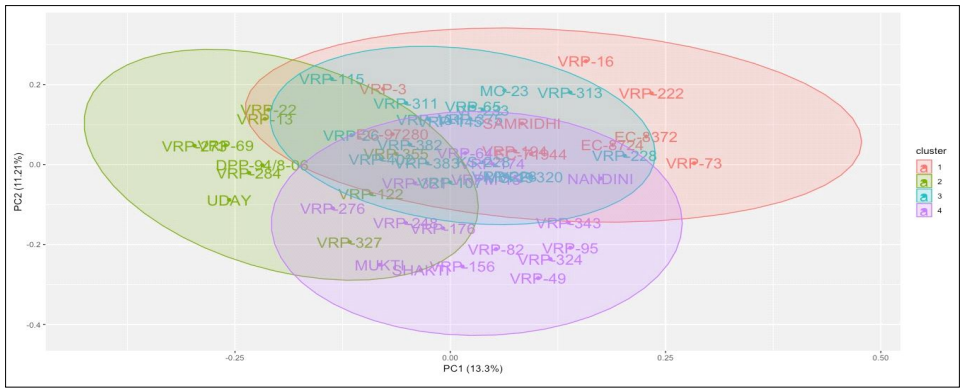

Fig 3: 2-Dimensional principal component analysis based on SSR markers in pea.

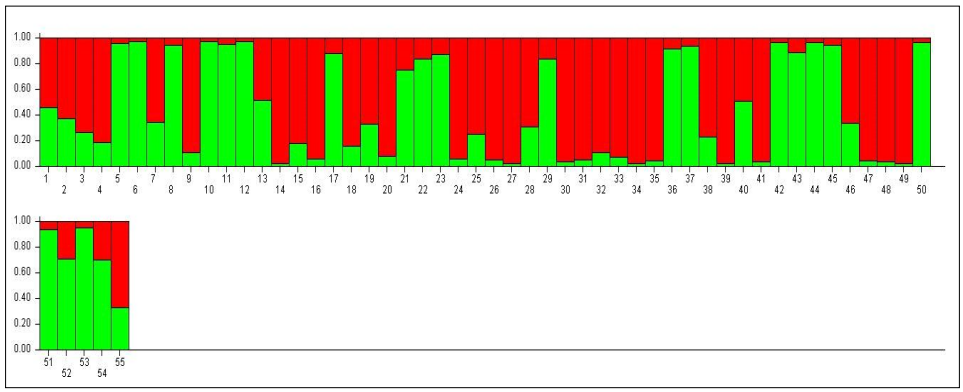

Fig 4: Population structure of pea accessions using SSR markers.

is low. But all the total comparison, however, showed significant variation between accessions because of the presence of a large number and differences in the frequency of unique and rare alleles between accessions. Similar results were obtained by Rana *et al*., (2017). Based on the Mantel's test between the morphological and molecular matric indicated the low to medium positive significant correlation. Similar type of low to medium correlation between the matrices was obtained by Smykal *et al*., (2008) and Handerson *et al*. (2014) in pea. The accessions of both phylogenic trees with low correlation showed the environmental effect on the accessions. Thus, despite its self-pollinating nature, the present study further reveals that pea germplasm is widely intermixed, resulting in greater diversity that breeders can study and utilize to develop new, improved pea varieties.

CONCLUSION

Accessions *viz*., VRP-82, VRP-320, VRP-194, VRP-375, EC-97280 and EC-8724, showed great diversity as compared to the other accessions based on both morphological and molecular markers. These accessions may assist in developing and planning breeding strategies aimed to produce new varieties in the future. Further for better

resolution of genetic diversity of the pea germplasm can be exploited by the use of higher number of polymorphic markers and more number of accessions.

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