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Genetic Characterization and Population Structure of Pea (*Pisum sativum* L.) by Molecular Markers against Rust (*Uromyces viciae-fabae*) in Newly Developed Genotypes

Anmol Singh Yadav [1], Anil Kumar Singh [2], Ramesh Chand [1] and Shyam Saran Vaish [1,*]

[1] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India
[2] Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221 005, India
* Correspondence: ssvaishmyco@bhu.ac.in; Tel.: +91-945-136-1998

Abstract: The understanding of the genetic diversity of germplasm of any crop is necessary for genetic improvement. Pea (*Pisum sativum* L.) is a very important legume crop that provides protein and several essential vitamins, carbohydrates, and minerals. The genetic diversity and population structure of pea germplasm consisted of 115 entries of Australian accessions and 4 entries of Indian varieties used as checks with varying responses and severities of rust, which were analysed using 31 polymorphic SSR (Simple Sequence Repeats) markers. The combination of the markers revealed that 78 alleles were present at 32 loci. It was also observed that each marker had three alleles with an average PIC (Polymorphic Information Content) value of 0.272. The population structure analysis showed the genetic differentiation of the entries. The model-based population structure grouped the entries into three sub-populations of SP1, SP2, and SP3 having 37, 35, and 32 entries, respectively with 15 entries as admixtures. AMOVA (Analysis of Molecular Variance) disclosed that there was 56% variation among the individuals and 20% within the population. A mean fixation index (Fst) of 0.240 among the pea entries exhibited relatively significant variation in population. This study provides basic information to select parental lines for developing rust resistant varieties to meet the ultimate goal of sustainable agriculture.

Keywords: *Pisum sativum*; genetic diversity; population structure; SSR markers; AMOVA

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1. Introduction

Pea, *Pisum sativum* L. ($2n = 14$), is one of the most significant pulse crops grown around the world. It belongs to Fabaceae and has a genome size of about 4500 Mb [1]. Pea is a good source of protein with high levels of amino acids such as tryptophan and lysine in addition to vitamins, carbohydrates, and minerals [2]. Among the various biotic stresses to pea, rust (*Uromyces viciae-fabae*) is considered as one of the major diseases as it causes considerable yield losses ranging from 10 to 60 per cent [3].

The efforts have been made for the improvement of disease resistance and plant architecture, and mitigate lodging to increase the yield of pea to meet the global protein demand of a rising population under various breeding programs since three decades [4,5]. Crop species with a narrow genetic diversity are more sensitive to new diseases and other abiotic stresses, resulting in reduction in adaptability as well as yield. Hence, the wider genetic diversity of any germplasm is considered as a reason behind its beauty that is governed by gene diversity. The variants of high significance can be exploited for the development of desired crop varieties [6]. The necessity of collecting and exploiting genetic diversity for further progress has been acknowledged by several geneticists and breeders [7,8].

Since the use of traditional morphological or biochemical markers are not completely trustworthy because of environmental impact, morphological and molecular traits have

been found as key estimates for the evaluation of a germplasm. Seeds per pod, seed fresh weight and germination percentage have been used as morphological features to understand the diversity among pea genotypes [8,9].

The use of molecular markers is a precise and strong technique for assessing relationship among entries of a germplasm based on the genetic similarity estimates. The simple sequence repeats (SSR) markers owing to its abundance in the genome of plant species, high polymorphism, multi-allelic variation, co-dominance, high reproducibility and easy detection by polymerase chain reaction have been found very appropriate for understanding genetic diversity of pea and fiber crops [9–17]. They have been used for the identification of population structure and reconstruction of the evolutionary history of pea [18]. However, only a few studies on genetic diversity of pea using molecular tools have been done so far in India [8,18].

The identification of promising genotypes for various breeding purposes requires proper evaluation of genetic diversity and population structure of any germplasm to meet the ultimate goal of sustainable agriculture. Therefore, the present work is aimed to determine the genetic diversity and population structure of 119 entries of pea germplasm, consisting of 115 Australian accessions and 4 Indian varieties using the SSR markers to find out the amount of existing variation and its grouping based on their rust reactions.

2. Materials and Methods

2.1. Planting Materials and Experimental Design

One hundred and nineteen entries of pea germplasm were used for studies on genetic characterization and population structure (Table 1). The germplasm consisted of 115 Australian accessions obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and 4 Indian varieties as checks against rust. The experiment was conducted at the Agriculture Research Farm, Banaras Hindu University, Varanasi (25°15′20.3″ N; 82°59′10.3″ E) during 2016–2017 and 2017–2018.The planting of each of the entries of the germplasm was done in a single row of three meters with a row spacing of 40 cm and plant to plant of 15 cm within a row in a RBD (Randomised Block Design) with two replications.

Table 1. Showing the details of the germplasm used in the present studies.

Origin	Accession Number
Australia	EC865919, EC865920, EC865921, EC865922, EC865923, EC865924, EC865925, EC865926, EC865927, EC865928, EC865929, EC865930, EC865931, EC865932, EC865933, EC865934, EC865935, EC865936, EC865937, EC865938, EC865939, EC865940, EC865941, EC865942, EC865943, EC865944, EC865945, EC865946, EC865947, EC865948, EC865949, EC865950, EC865951, EC865952, EC865953, EC865954, EC865955, EC865956, EC865957, EC865958, EC865959, EC865960, EC865961, EC865962, EC865963, EC865964, EC865965, EC865966, EC865967, EC865968, EC865969, EC865970, EC865971, EC865972, EC865973, EC865974, EC865975, EC865976, EC865977, EC865978, EC865979, EC865980, EC865981, EC865982, EC865983, EC865984, EC865985, EC865986, EC865987, EC865988, EC865989, EC865990, EC865991, EC865992, EC865993, EC865994, EC865995, EC865996, EC865997, EC865998, EC865999, EC866000, EC866001, EC866002, EC866003, EC866004, EC866005, EC866006, EC866007, EC866008, EC866009, EC866010, EC866011, EC866012, EC866013, EC866014, EC866015, EC866016, EC866017, EC866018, EC866019, EC866020, EC866021, EC866022, EC866023, EC866024, EC866025, EC866026, EC866027, EC866028, EC866029, EC866030, EC866031, EC866032, EC866033
India	HUDP-15, HFP-8909, HFP-4, HFP-9907

2.2. DNA Extraction, Amplification and Electrophoresis

Genomic DNA of each of the entries of the test material was extracted from freshly growing leaves collected from 15-days-old plants by using the CTAB method [19]. This method was used following some minor modifications. Thirty-one SSR markers were used

to screen the test entries through polymerase chain reaction (Table 2). For the preparations of the master mix solution, 1.5 µL 10× reaction buffer, 0.2 µL dNTPs, 0.2 µL $MgCl_2$, 0.2 µL Taq polymerase, 0.6 µL of each forward and reverse primers, and 10.9 µL nuclease free water were mixed with 0.8 µL desired genomic DNA of each of the test entries. The polymerase chain reaction (PCR) amplification was carried out in a thermocycler (Eppendorf 5333 Master Cycler Gradient Thermal Cycle). The PCR was programmed for initial denaturation at 95 °C for 15 min, denaturation at 94 °C for 30 s, annealing according to primer for 1 min, extension at 72 °C for 2 min, and the cycle was repeated 40 times and the final extension for 5 min at 72 °C [20,21]. Thereafter, the amplified PCR product was resolved through electrophoresis in 2% (*w/v*) agarose gel containing 0.5 µg/mL ethidium bromide.

Table 2. Showing genetic diversity of the germplasm on the basis of polymorphic characteristics of SSR markers.

No	SSR Marker	No of Alleles (Na)	Major Allele Frequency (A)	Gene Diversity (H_e)	Heterozygosity (H_o)	PIC *
1	AA135	2	0.588	0.484	0.000	0.367
2	AA176	2	0.907	0.167	0.000	0.153
3	AA206	2	0.991	0.016	0.000	0.016
4	AA345-145	2	0.932	0.125	0.000	0.117
5	AA372.1	2	0.705	0.415	0.000	0.329
6	AA416	2	0.563	0.492	0.000	0.371
7	AA446	2	0.806	0.311	0.000	0.263
8	AA505	2	0.529	0.498	0.000	0.374
9	AA57	4	0.617	0.505	0.697	0.419
10	AA61	2	0.983	0.033	0.000	0.032
11	AA98	4	0.714	0.412	0.563	0.334
12	AB24	2	0.949	0.095	0.000	0.091
13	AB60	2	0.655	0.451	0.000	0.349
14	AD146	2	0.789	0.331	0.000	0.276
15	AD147	2	0.991	0.016	0.000	0.016
16	AD237	4	0.403	0.657	0.000	0.584
17	AD70290	2	0.991	0.016	0.000	0.016
18	B14	2	0.537	0.497	0.000	0.373
19	B16	9	0.420	0.630	0.352	0.555
20	P46	2	0.806	0.311	0.000	0.263
21	PSAB60	2	0.529	0.498	0.000	0.374
22	S244	2	0.857	0.244	0.000	0.214
23	CAASESP527	2	0.882	0.207	0.000	0.186
24	S144	2	0.974	0.049	0.000	0.047
24	S85	3	0.680	0.439	0.000	0.350
26	B179	2	0.899	0.181	0.000	0.164
27	CAASESP1173	2	0.907	0.167	0.000	0.153
28	P282	3	0.672	0.494	0.000	0.443
29	CAASESP524	2	0.781	0.341	0.000	0.283
30	CAASESP1193	2	0.672	0.440	0.000	0.343
31	P255	3	0.420	0.653	0.000	0.579
	Mean	3	0.747	0.328	0.052	0.272
	Max.	9	0.991	0.657	0.697	0.584
	Min.	2	0.403	0.016	0.000	0.016

*, denotes Polymorphic Information Content.

The gel was visualized and documented through a gel documentation system (Protein Simple Alpha Imager HP system). A DNA ladder of 100 bp was used for identifying the band size of amplified products. The polymorphic bands were evaluated as binary data based on whether each amplicon was present (1) or absent (0). Thereafter, the obtained similarity matrix was subjected to the UPGMA (Unweighted Pair Group Method with an Arithmetical Mean) by DARwin software [22]. The un-rooted phylogenetic tree was prepared according to the scale and the *p*-distance method was used to compute the distances.

2.3. Population Structure and Gene Flow

Population structure was evaluated by the Structure v 2.3.4 software using the data acquired from SSR profiling [23]. The optimum number of population (K) was selected by testing K = 1 to K = 10 using five independent runs of 100,000 burns in period length followed by 100,000 MCMC (Markov Chain Monte Carlo) replication. Further, the population structure was envisaged using the online tool structure harvester. The K value was estimated by the log probability LnP (D) based on in its rate change between successive Ks.

2.4. Genetic Diversity Analysis and Analysis of Molecular Variance (AMOVA)

In order to evaluate the genetic diversity, gene diversity (expected heterozygosity), observed heterozygosity, major allele frequency, and polymorphic information content, PIC for each SSR locus was obtained by using Power marker software 3.25 [24]. The number of sub-populations obtained through analysis in Structure v 2.3.4 software was used for AMOVA (Genetic Diversity Analysis and Analysis of Molecular Variance) as well as for Nei's genetic distance analysis in GenAlEx v6.503 [25]. The fixation index (Fst) and gene flow of the population were derived from AMOVA.

3. Results

3.1. Polymorphic Levels of Simple Sequence Repeats Loci

The existing genetic diversity of the present pea germplasm was understood by assessing the polymorphic level of SSR (Simple Sequence Repeats) loci on the basis of the different parameters, viz., number of alleles per locus, major allele frequency, gene diversity, heterozygosity, and polymorphic information content (Table 2). A total of 78 alleles, detected from 31 SSR loci, were amplified among the 119 pea entries. The number of alleles counted for 32 loci varied from two to nine with an average of three alleles for each SSR marker. The lowest number of alleles per locus was observed from AA135, AA176, AA206, AA345 AA372.1, AA416, AA446, AA505, AA61, AB24, AB60, AD146, A147, AD70290, B-14, P46, PSAB 60, S244, S144, B179, CAASESPS527, CAASESPS1173, CAASESPS524, and CAASESPS1193 markers. The maximum number of alleles was nine and they were located at the B16 locus. The PIC (Polymorphic Information Content) value of the SSR markers ranged from 0.016 to 0.584 with an average of 0.272. Three markers such as AD237, B16, and P255, showed a PIC value of >0.5 indicating a higher polymorphism. Further, they were more informative of all the SSRs used. The observed heterozygosity values varied from 0.0 to 0.697 with a mean of 0.052. The gene diversity ranged from 0.016 to 0.65 with a mean of 0.32 (Table 2). Markers P255 and AA206 had the highest and the lowest gene diversity, respectively. The major allele frequency varied from 0.40 to 0.99 with a mean of 0.74 (Table 2). Markers AD237 and AA206 showed the lowest and the highest major allele frequency, respectively. The PIC values and diversity score of most of the SSR markers disclosed enough variability to distinguish all the 119 entries. The marker AD237 located on chromosome number VII that exhibited the highest gene diversity (0.657) and PIC (0.585) was followed by P255 with 0.653 and 0.579 values for gene diversity and PIC, respectively.

3.2. Genetic Relationship among Pea Germplasm Population

The changing block colors within the entries shows the changes in allele size and the different color blocks in the bar plot represent the genetic diversity among the present population (Figure 1a). Based on the ΔK (Delta K) values, the population structure of 119 entries was determined and the ΔK was 3. The population structure with SSRs yielded into a dramatic peak of the probability following the adjustment of the number of populations to three. Further, it helped to divide these pea entries into three sub-populations. By matching the LnP(D) and Evanno's ΔK values by cumulative K from 2 to 10, we observed an increase in LnP(D) values up to K = 3 with the highest log probability score at the same position (Figure 1a). It was also noticed that the population of 119 entries contains 104 pure entries and 15 admixtures. Out of the 104 pure entries, 37 entries belonged to sub

population-1 (SP1), 35 to sub population-2 (SP2), and 32 to sub population-3 (SP3) which are shown in green, blue, and red, respectively (Figure 1b).

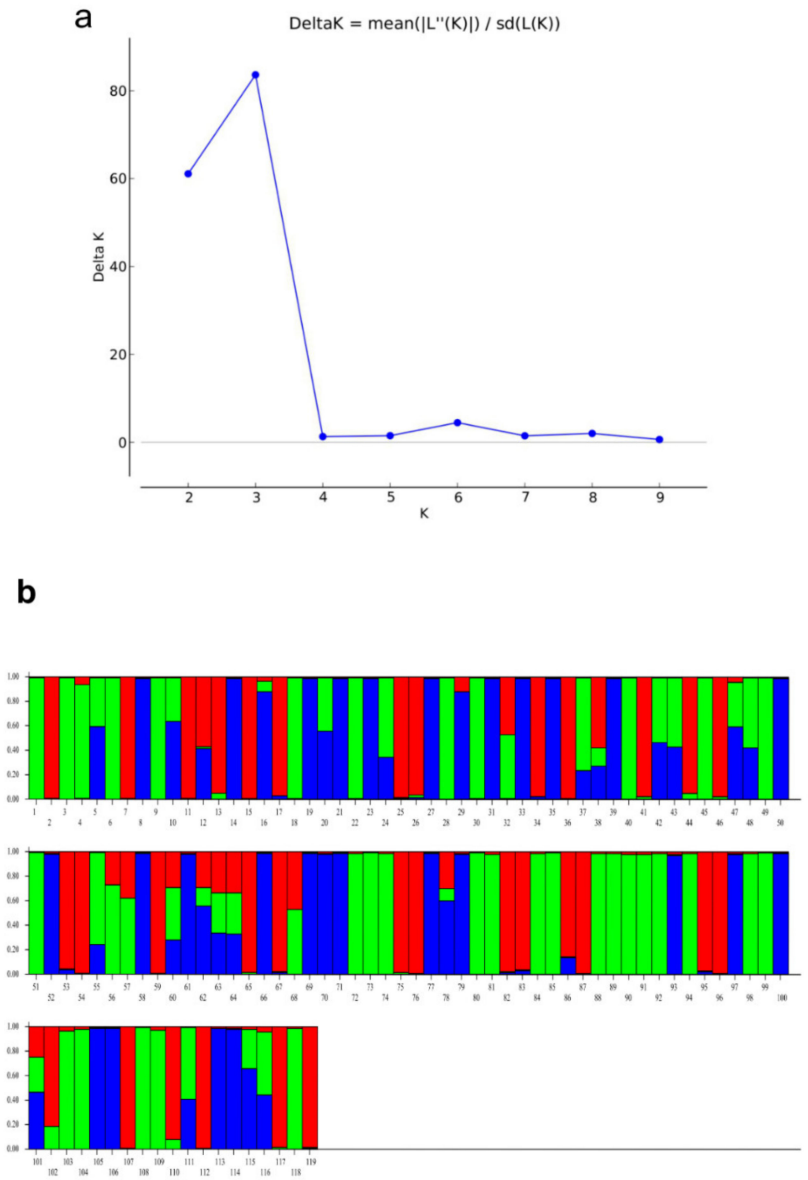

Figure 1. Population structure analysis of pea germplasm (119 entries) based on 31 SSR markers using STRUCTURE harvester 2.3. (**a**) Graphical plot of ΔK values showing the maximum value at K = 3; (**b**) Distribution of 119 pea genotypes into three different sub-populations; sub population-1 (SP1), sub population-2 (SP2), and sub population-3 (SP3) shown in green, blue, and red color, respectively.

The UPGMA (Unweighted Pair Group Method with an Arithmetical Mean) analysis based on the genetic dissimilarity using the neighbour-joining method with DARwin categorized the pea germplasm into three groups (Figure 2). Group I consisted of 41 entries with 33 and 8 entries in Sub-groups Ia and Ib, respectively; whereas, Group II had 72 entries that were further clustered into three Sub-groups namely IIa, IIb, and IIc with 6, 23, and 43 entries, respectively. The Group III had six entries with no further Sub-group. Out of the four Indian varieties used as differentials, the variety HFP-4 belonged to Group I, and two varieties (HFP-8909 and HFP-9907) were to Group III; however, the variety HUDP-15 was found at the admixture group of both Australian and Indian entries (Figure 1b). The area under disease progress curve (AUDPC) for pea rust disclosed that the five accessions, i.e., EC865975, EC865921, EC865951, EC865929, and

EC866033, were distributed over all the three groups and exhibited lower AUDPC (Area Under Disease Progress Curve) values varying from 292 to 351 (Table 3).

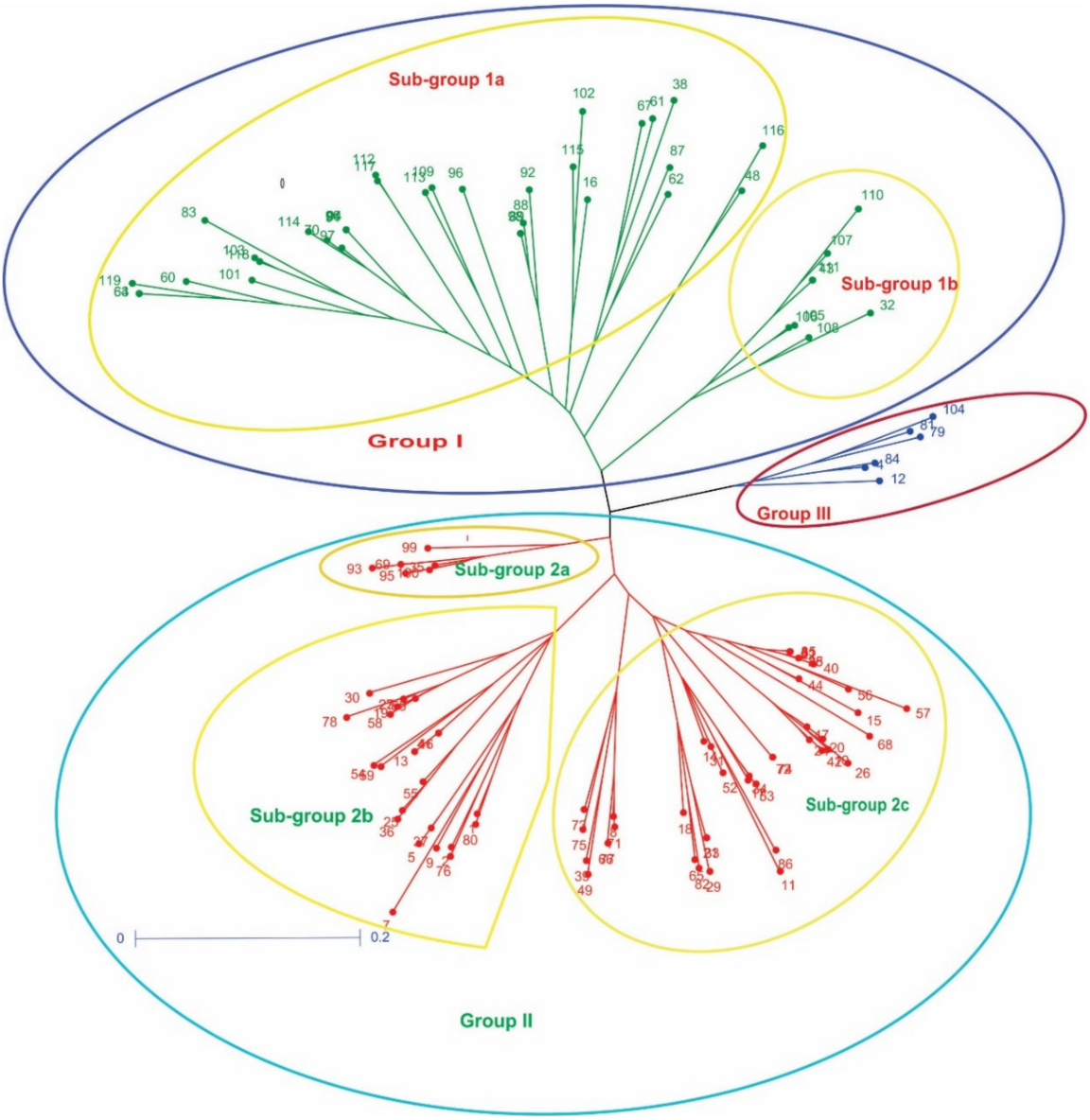

Figure 2. Illustrating the Unrooted Neighbour joining (U-NJ) tree of pea germplasm (119 entries) prepared using DARwin software.

The analysis of molecular variance showed 24% variation among the population and 56% among the individuals; whereas, the variation within the individuals was 20%. The calculation of Wright's F statistic of all SSR loci exhibited an inbreeding coefficient (Fis) of 0.733; however, the Fit coefficient was 0.797. The determination of the mean fixation index (Fst) for the polymorphic loci across all the entries revealed that it was 0.240. The low haploid Nm of 0.791 indicated good gene exchange among the populations. Further, the analysis demonstrated low and high genetic differentiation among sub-populations and within sub-populations, respectively.

Table 3. Depicting the development of pea rust (*Uromyces viciae-fabae*).

Entry No.	Accession No.	AUDPC	Entry No.	Accession No.	AUDPC	Entry No.	Accession No.	AUDPC
1	EC865919	592	41	EC865959	872	81	EC865999	745
2	EC865920	700	42	EC865960	1091	82	EC866000	547
3	EC865921	334	43	EC865961	560	83	EC866001	481
4	EC865922	834	44	EC865962	476	84	EC866002	543
5	EC865923	723	45	EC865963	575	85	EC866003	876
6	EC865924	698	46	EC865964	843	86	EC866004	679
7	EC865925	463	47	EC865965	546	87	EC866005	881
8	EC865926	537	48	EC865966	628	88	EC866006	845
9	EC865927	794	49	EC865967	527	89	EC866007	507
10	EC865928	682	50	EC865968	491	90	EC866008	530
11	EC865929	344	51	EC865969	544	91	EC866009	744
12	EC865930	469	52	EC865970	871	92	EC866010	562
13	EC865931	824	53	EC865971	850	93	EC866011	872
14	EC865932	833	54	EC865972	794	94	EC866012	436
15	EC865933	799	55	EC865973	756	95	EC866013	796
16	EC865934	538	56	EC865974	849	96	EC866014	539
17	EC865935	540	57	EC865975	292	97	EC866015	845
18	EC865936	536	58	EC865976	782	98	EC866016	861
19	EC865937	868	59	EC865977	839	99	EC866017	878
20	EC865938	826	60	EC865978	459	100	EC866018	853
21	EC865939	572	61	EC865979	896	101	EC866019	746
22	EC865940	762	62	EC865980	873	102	EC866020	650
23	EC865941	723	63	EC865981	860	103	EC866021	548
24	EC865942	676	64	EC865982	842	104	EC866022	843
25	EC865943	452	65	EC865983	869	105	EC866023	527
26	EC865944	389	66	EC865984	550	106	EC866024	576
27	EC865945	871	67	EC865985	861	107	EC866025	588
28	EC865946	706	68	EC865986	861	108	EC866026	383
29	EC865947	852	69	EC865987	546	109	EC866027	916
30	EC865948	881	70	EC865988	524	110	EC866028	382
31	EC865949	876	71	EC865989	656	111	EC866029	857
32	EC865950	886	72	EC865990	910	112	EC866030	853
33	EC865951	344	73	EC865991	549	113	EC866031	345
34	EC865952	883	74	EC865992	565	114	EC866032	383
35	EC865953	825	75	EC865993	550	115	EC866033	351
36	EC865954	915	76	EC865994	492	116 *	HUDP-15	371
37	EC865955	757	77	EC865995	542	117 *	HFP-8909	851
38	EC865956	569	78	EC865996	866	118 *	HFP-4	1136
39	EC865957	849	79	EC865997	566	119 *	HFP-9907	514
40	EC865958	860	80	EC865998	864			

*, Denotes differential; AUDPC, stands for area under disease progress curve.

4. Discussion

In the present work, the average PIC value of 0.272 justifies enough allelic variation in the population for studies on genetic diversity. The observed variation in their values may be due to genotypic variation. Similar results were obtained by Ram et al. [26] who studied 24 pea genotypes for genetic diversity by using the SSR markers and detected 2.91 alleles per locus with a mean PIC value of 0.39. However, the varying PIC values have also been obtained by previous workers owing to variation in the number of SSR markers and number of genotypes used in their studies [1,7,8,18,27–30]. Mohamed et al. [30] evaluated 12 pea local lines and found an average of 0.44 PIC value per locus. Jain et al. [1] studied 96 cultivars of pea using 31 SSR markers and found the PIC values that varied from 0.01–0.56 among the SSR markers. Haliloglu et al. [31] evaluated 62 forage pea specimens collected from the north-eastern Anatolia region of Turkey by using the 28 SSR markers and found an average PIC value of 0.41 that ranged from 0.03–0.70. Sharma et al. [32] evaluated 40 pea genotypes using

24 EST-SSR markers and noticed an average PIC value of 0.349 that ranged from 0.095–0.500. Although the higher PIC value was reported by Singh et al. [18], they characterized 47 garden peas by using 34 SSR markers and found a 0.55 PIC value. Similarly, Bouhadida et al. [27] evaluated 19 pea accessions by using eight SSR markers and observed a PIC value of 0.62, and Duque-Zapata et al. [28] studied 50 pea accessions using 16 polymorphic SSR markers and obtained an average PIC value of 0.62.On the other hand, Kimaro et al. [17] evaluated the genetic diversity of 48 pigeon pea genotypes using 33 SSR markers and found an average PIC value of 0.46.The present study also justifies that the test markers, due to their polymorphic nature, may be used for the categorization of the germplasm.The inclusion of some trait-linked SSR markers and the removal of the monomorphic and spurious bands from the analysis may have contributed to the lower number of alleles in the current study. Haliloglu et al. [31] observed that the number of alleles (Na) per primer varied from 2 to 4 with a mean of 2.89 alleles per locus. Teshome et al. [33] found 13 out of 15 EST SSR markers were polymorphic and observed a total of 37 alleles in 46 pea accessions. The study also revealed an average number of alleles per locus was 3.1. Kimaro et al. [17] noticed a total of 155 alleles at 33 loci and detected an average of 4.78 alleles per marker. Out of the studied SSR markers, four markers, namely AA146, AA416, AA446, and AA505, have also been used by Rai et al. [34] and Singh et al. [20]. Further, they reported that these markers to be linked to Quantitative Trait Loci (QTLs) responsible for rust resistance.

The gene diversity (He) for the SSR loci ranged from 0.016–0.657 with a mean of 0.328. A similar range of gene diversity of 0.03–0.62 was reported by Jain et al. [1]. Handerson et al. [29] and Ram et al. [26] have found an average of 0.46 gene diversity in their studies. The major allele frequency was 0.747 which indicates that the alleles' distribution in the pea germplasm was averagely common. Similarly, an average of 0.65 and 0.66 major allele frequency was reported by Mohamed et al. [30] and Ram et al. [26], respectively. In the present study, the observed heterozygosity (Ho) values ranged from 0.00 to 0.697 with a mean of 0.052. It is obvious that in a self-pollinated species, the observed heterozygosity is found to be very low (on average, 6%) [29,35]. Similarly, in our study we have also found a low averaged Ho of 5.20%.

In general, every population can be assessed based on its geographical distribution, but it is also frequently based on additional factors such as the phenotype, behavior, and ecology of the individuals collected. In this investigation, the ΔK value was found to be 3 clustering the 119 pea entries into three genetically distinct groups. This was also confirmed by UPGMA analysis. The finding indicates that there is no correlation between genetic diversity of a germplasm and the place of origin. The conducted work helped to differentiate the germplasm into three groups on the basis of their genetic diversity. Similarly, Mohamed et al. [30] concluded that the place of origin does not represent a major reason for differentiation following grouping of 12 pea accessions into three sub-groups. Rana et al. [7] identified three groups for 151 pea accessions collected from the different parts of the world. Ahmad et al. [36] found four groups for 34 pea genotypes of different origins. However, Ferradini et al. [37] noticed two peaks at delta K graph, i.e.,K = 2. Hanci and Cebeci [38] evaluated wild pea accessions, local varieties, and commercial pea varieties. They observed two major groups among all the 15 accessions. Similarly, Duque-Zapata et al. [28] have also seen two clusters among 50 pea accessions. Bouhadida et al. [27] reported two groups among 19 pea accessions by using the eight simple sequence repeats (SSR) markers. Ram et al. [26] concluded two major clusters with sub-cluster 1 and sub-cluster 2 with a total of 11 and 10 pea lines, respectively. Singh et al. [18] also identified two major groups I and II among 47 garden pea genotypes representing dwarf and tall, respectively. Haliloglu et al. [31] found three clusters of 61 forage pea land races through UPGMA analysis.

In this study, three Indian varieties formed the group with Australian accessions which indicates that they have similar genetic constitution. This may be possible due to a gene flow between the populations. The admixture found in one Indian variety may be due to having one parent each from Australian and Indian lines. Further, it also suggests that the ancestors from various distant geographical places exchanged lineages of distinct gene pools or accessions representing diverse gene pools during the cultivation at an early

stage of pea domestication [39]. Similarly, Rana et al. [7] also observed similarities and dissimilarities between pea accessions of various countries and found three major groups. However, Ahmad et al. [36] reported four population structure groups corresponding to patterns of geographical distribution.

Five genotypes distributed in all the three groups showing moderately resistant reactions against rust disclosed enough diversity among them. It significantly indicates that there may be a horizontal distribution of rust resistant genes. Hence, these accessions can be used as breeding material to develop a new rust resistant variety through gene pyramiding.

The analysis of molecular variance of the population revealed a high genetic variance among the individuals (56%). This genetic differentiation among the individuals in the population may be due to the inclusion of genotypes from the different places of origin for rust resistance. Ferradini et al. [37] made a brief account of pea genotypes and found 68% genetic diversity among individuals. Alike, Scaerano et al. [35] found 68% genetic difference among the landraces and 32% within the landraces of common bean. Mohamed et al. [30] also reported genetic diversity among and within the local pea accessions of 90% and 10%, respectively.

A low percent of variance (20%) within the individuals was observed indicating a high purity of germplasm without any mixture. However, the genetic variance explained among the population was low (24%) regardless of their geographical distance that may be due to an increase in the spread of alleles among various populations. Similar low variance among the population was also reported by Ram et al. [26] and Ferradini et al. [37].

The Wright's F Statistic used demonstrated a deviation from the Hardy–Weinberg law. However, there was a low fixation index (Fst = 0.240) of alleles which might be attributed to a lot of variation among the individual genotypes within the groups. This high variability within the groups was most likely to be attributed to the fact that the entries were heterogeneous pure lines or homozygous, particularly at all loci, but with genetic constitutions that differed from one another. Jain et al. [1] reported Fst values ranging from 0.11 to 0.19 in four sub-populations of pea genotypes indicating low to high genetic differentiation. Tahir et al. [40] found Fst values of Subgroup 1 and 2 were 0.0478 and 0.267, respectively.

5. Conclusions

The analysis of genetic diversity of pea germplasm using 31 SSR markers infers considerable diversity and divides the germplasm into three groups. Further, it discloses that there are five accessions having resistance to rust. The used SSR markers could be used as a potential tool for germplasm characterization and its utilization in pea breeding program. Eventually, it would not be an exaggeration to state that the present outputs would be helpful in achieving the ultimate goal of global zero hunger, also known as Sustainable Development Goal-2 (SDG2), to mitigate hunger, achieve food security with improved nutrition, and promote sustainable agriculture.

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