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Vegetable peas (*Pisum sativum* L.) diversity: An analysis of available elite germplasm resources with relevance to crop improvement

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Abstract

Aim of study: To determine the amount of diversity in pea breeding materials with the objective to classify a set of potential parents carrying novel/economic variations that could be used in future breed pea varieties.

Area of study: ICAR–Indian Institute of Vegetable Research, Varanasi.

Material and methods: A total of 45 pea accessions were analysed for phenotypic and molecular diversity using 17 agro-morphological traits and 52 SSR markers.

Main results: All traits under investigation showed considerable genetic variation. The genotypes exhibited 6.7, 2.7 and 12-fold variation for traits viz., pods/plant, 10-pod weight and yield/plant, respectively. Among 52 SSR markers, 22 were found to be polymorphic. A total of 90 allelic variants were detected, with an average of 2.7 alleles/locus. PIC and D-values for markers AA135 (0.79 and 0.81) and PSMPSAD51 (0.7 and 0.74) were the highest, while AB40 (0.19 and 0.2) had the lowest. Two principal components PC1 and PC2 explained 46.96 and 23.96% of total variation, respectively. The clustering based on agro-morphological traits differentiated 45 individuals into three mega clusters, while SSR markers-based clustering classified these accessions into four groups.

Research highlights: Based on their uniqueness, we identified a set of genotypes (VRPD-2, VRPD-3, PC-531, 'Kashi Nandini', 'Kashi Udai', 'Kashi Mukti', 'Arkel', VRPE-101, 'Azad Pea-3', EC865944, VRPM-901 and VRP-500) harbouring genes for various economic traits. The findings presented here will be extremely useful to breeders who are working on improvement of peas through selective introgression breeding.

Additional key words: cluster analysis; PCA; SSR markers

Abbreviation used: AP-3 (Azad Pea-3); DTF (Days to 50% Flowering); FPP (Flower per Peduncle); MF (Multi-Flowered); PCA (Principal Component Analysis); PH (Plant Height); PIC (Polymorphic Information Content); PL (Pod Length); PPP (Pods per Plant); PW (Pod Width); SPP (Seeds per Pod); SSR (Simple Sequence Repeat); YPP (Yield per Plant); 100-GSW (100-Green Seed Weight).

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Introduction

Pea (*Pisum sativum* L., 2n=2x=14) is an Old World legume that was first cultivated 10,000 years ago (Zohary et al., 2012) and is the most important domesticated crop in the legume group. The cultivated varieties of *Pisum* are comprised of several morphologically distinct intraspecific forms, each developed for a specific end-use, such as human consumption as pulse and vegetable, livestock feed or ornamental gardening. According to FAO (http://www.fao.org/es/faodef/fdef04e.htm), it holds a status of primary pulse that serves as a valuable source of protein (17.58 to 28.67%; Weihai et al., 2017) for both man and animals. Furthermore, it is quite inexpensive and a readily available source of starch, fibers and sucrose. It is also enriched with various minerals, phytochemicals, antioxidants, flavonoids, tannins, and other phenolic compounds, all of which have significant health benefits (Kumari & Deka, 2021). Besides, peas are excellent plant species for biological nitrogen fixation and green manuring with its ability to enhance the productivity of its successor crop (Uhlarik et al., 2022).

The greater diversity in the *Pisum* gene pool has led its adaptation to a vast geographical area that includes Asia, Europe, Africa, the Americas and Oceania (Kreplak et al., 2019). World production of green peas in 2019 was 19.73 million tonnes and the major producers were China (11.38 million tonnes), India (5.56 million tonnes), France (0.28 million tonnes) and USA (0.23 million tonnes) that accounted for >85% of the total world production. Further, insight into data showed that although the area and production under green peas has become almost double from 1999 (1.5 million hectares and 11.39 million tonnes, respectively) to 2019 (2.51 million hectares and 19.73 million tonnes), a slight change has been observed in its productivity globally (7.6 to 7.8 tonnes/ha; http://www.fao.org/es/faodef/fdef04e.htm). It is widely recognized that during the domestication and crop improvement process, market-driven intense breeding for higher yield and uniformity has resulted into unwanted loss of genetic diversity of many crops, thus making them vulnerable to stresses. Further, self-pollinating crops such as pea end up with increased homozygosity and increasing loss of genetic variation (Cieslarová et al., 2011). Vegetable pea farming in India is presently dominated by few selected varieties: 'Arkel', 'Azad Pea-3' (AP-3), 'Kashi Udai', 'Kashi Nandini', 'Punjab-89', 'Arka Ajit' and 'Arka Karthik'. The large-scale seed multiplication of 'AP-3', 'Kashi Nandini' and 'Punjab-89' by the local farmers has again narrowed down the cultivation of local land races, resulting into considerable genetic loss.

While confronted with new challenges of crop genetic erosion, various biotic and abiotic stresses, productivity stagnation and issues of global climate change, pea breeding greatly relies on the exploitation of its genetic resources that could be part of cultivated gene pool or its wild relatives (Burstin et al., 2015). Moreover, understanding the detailed relationships between the genomes of modern cultivars, old cultivated landraces, ecotypes and wild relatives is critical because it may help breeders to develop climate resilient varieties with desirable alleles (Uhlarik et al., 2022). Additionally, genotypic characterization is also necessary for successful conservation, protection and utilization of naturally occurring variations in gene pool (Sharma et al., 2022). The current rate of genetic gain in peas could be doubled only through expansion of genetic diversity (Bari et al., 2021).

Time to time, breeders have made efforts to study the diversity and evolution of pea, and a large number of polymorphic data points have been produced for each collection using morphological, cytological, biochemical markers and more recently through DNA-based technologies (Jing et al., 2010; Sanwal et al., 2021). A rich phenotypic diversity has been reported for various yield traits in peas (Kwon et al., 2012; Gixhari et al., 2014; Sanwal et al., 2021). Morphological traits represent the action of numerous genes and thus contain high information value but can be unreliable owing to a strong influence of the environment (Smykal et al., 2008b). In comparison, molecular markers can accurately score the underlying genetic variation and a wide range of markers based on polymorphism of DNA sequences such as RAPD and ISSR (Baranger et al., 2004; Tar'an et al., 2005), AFLP (Dyachenko et al., 2014), RBIP (Jing et al., 2010), SSR markers (Nisar et al., 2017; Sharma et al., 2022) and SNP (Bari et al., 2021; Uhlarik et al., 2022) have been used to dissect the variation present in *Pisum*. The SSRs have several advantages over other markers, including high polymorphism and abundance, codominant nature, relative ease of transfer and low cost of development (Izzah et al., 2013). Furthermore, it is important to determine how former as well as novel variations can be used to aid in the development of new varieties capable of responding to new environmental challenges. Thus, the current study aims to (i) assess the genetic diversity and relatedness of pea breeding material using agro-morphological traits at phenotypic and SSR markers at molecular level, and (ii) identify potential donors in order to target trait-specific pea breeding.

Material and methods

Location of study

The present investigation was undertaken at the experimental farm of ICAR–Indian Institute of Vegetable Research, Varanasi, India, which is located at 82°52′37″ E and 25°18′21″ N at an elevation of 83 m above mean sea level, during the winter season (Nov-March) of 2019-20. The site is agro-climatically representative of India's Middle Gangetic Plain Region, that has a humid subtropical climate with an annual average rainfall of 998 mm.

Plant material

To assess diversity, 45 pea genotypes were selected from 10 different centres across India (Table S1 [suppl]): 21 cultivars, 12 advanced breeding lines and 12 germplasm lines including exotic collections. Each genotype, in general, has its own breeding potential that, depending upon the need of hour can be a valuable resource for future breeding programs. As demand for trait-specific breeding lines has been raised, we summarized the special characteristics of these lines that have been previously reported (Table S1). These lines were diverse for one or more character such as maturity groups, flowering, pod or seed characters. All the accessions were grown under normal field conditions for their phenotypic evaluation. Each genotype was planted in Randomized Block Design with three replications. The genotypes were raised in plot consisting of 3 rows of 3-m length with spacing of 30 cm between rows and 10 cm between plants. The recommended package of practices was adopted to grow healthy crops. The mean meteorological data for the growth period is presented in the Fig. S1 [suppl].

Recording of observations

The 45 genotypes of peas were characterized for 17 agro-morphological traits. The molecular characterization of these accessions was also done by using SSR markers. Horticultural traits data were recorded on 10 competitive plants in each replication for nine traits: days to 50% flowering (DTF; No); plant height (PH; cm); pod length (PL; cm); pod width (PW; cm); pods per plant (PPP; No); 10-pod weight (10-PW; g); seeds per pod (SPP; No); 100-green seed weight (100-GSW; g) and yield per plant (YPP; g). Characterization for flower colour, maturity groups, PH, pod number/axil and plant anthocyanin pigmentation followed DUS descriptors of PPV & FRA (2007); seed shape as Tzitzikas et al. (2006); and mature seed colour was compared with RHS Colour Chart (1986).

DNA extraction, PCR, gel electrophoresis, allele calling and sizing

DNA was extracted from all the 45 pea genotypes individually by selecting young leaves at the 8-10 leaf stage. A standard CTAB method of DNA extraction with minor modification was used to isolate DNA from each pea accession (Doyle & Doyle, 1990). The isolated DNA was treated with RNase A (by adding 10 µL of RNase A to each DNA sample) for 60 min at 37°C. The DNA quality was checked by electrophoresis in 0.8% agarose gels using lambda DNA (50 ng) as a standard. A total of 52 SSR markers distributed across the genome were used to genotype the 45 pea accessions. All these markers were chosen from the mapping and diversity studies conducted by previous researchers (Ek et al., 2006; Gong et al., 2010; Ahmad et al., 2015). PCR amplifications were conducted in total volume of 25 µL comprising 1 µL of template DNA (25-35 ng), 0.4 µmol/L of each forward and reverse primers (Table S2) and 5 µL of 5× CTaq-& LOAD Mastermix (MP Biomedicals; 1.5 µmol/L dNTP final concentration). Amplifications were performed on a Thermocycler (Bio-Rad, Missisauga, ON, Canada) with the following profile: 95°C initial denaturation for 2 min, followed by 36 cycles of 30 s at 95°C, appropriate annealing temperature for 45 s and 1 min extension at 72°C. PCR products were resolved on 2.5% metaphor agarose gel stained with ethidium bromide in TBE buffer and analysed under UV light (Bio-Rad, gel analyser). To determine the size of each amplified product a 100 bp DNA ladder (Invitrogen, USA) was used. The amplified and not amplified products were scored '1' and '0', respectively, and allelic sizes were assigned to each product.

Diversity and population structure analysis

Phenotypic diversity analysis and principal component analysis (PCA) was constructed using the DARwin 6.0 program. For cluster analysis neighbor-joining approach was used. Based on SSR allelic data, the molecular-genetic relationship of 45 pea genotypes were determined using Nei's genetic distance (Nei, 1972). PowerMarker 3.51 (Liu & Spencer, 2004) was used to determine the major allele frequency, the number of alleles per locus, polymorphic information content (PIC), gene diversity (expected heterozygosity), and observed heterozygosity. A cladogram was constructed with MEGA 11 using N-J method. Robustness of the node of the cladogram was assessed from 1000 bootstrap replicates. Population structure was analysed using Structure 2.3.4 (Pritchard et al., 2000). The admixture model was used to investigate the structure of 45 individuals, and population numbers (k=2 to k=10) were accessed with a burn-in-period of 10,000 steps, followed by 100,000 Markov-chain Monte Carlo (MCMC) replicates with 10 iterations. The output was obtained from the online Structure Harvester program (Earl & VonHoldt, 2012) which showed the highest peak at k=2.

Results and discussion

Agro-morphological characterization

The genotypes studied were found diverse for their flower colour (white, pink and purple); number of flowers per peduncle (1 to 5), seed shape (wrinkled, dented and round), mature seed coat colour and leaf types (normal and *afila*) etc. (Figs. 1, 2 and 3). Among the 45 genotypes, 39 (86%) had white, 4 had pink (8.8%) and 2 had purple (4.4%) flowers. Further, there was one single flowered genotype (VRPSel-17), seven multi-flowered (MF) pea

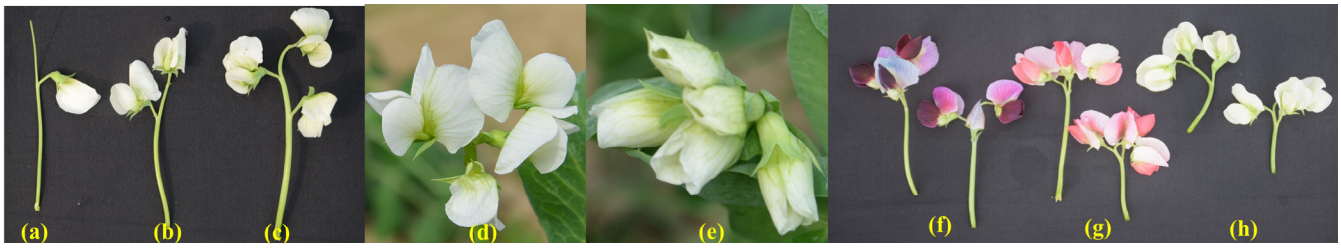

Figure 1. Flower number/peduncle and flower colour variations in studied genotypes of *Pisum*: (a) VRPSel-17. (b): PC-531. (c): VRP-500. (d) and (e): VRPM-901. (f): EC865944. (g): EC866019 and (h): VRPM-501

lines (VRP-500, VRPM-501, VRPM-502, VRPM-503, VRPM-901, EC865921 and EC865944), and the remaining accessions were in the double flowered group. The genotypes NDVP-250, HUDP-15, EC865921, EC865925, EC865943, EC865944 and EC866019 were leafless (*afila* type). All these genotypes also showed anthocyanin pigmentation, except NDVP-250 and HUDP-15. In addition, Kashmiria also had a pigmentation character.

Thus, grouping based on simple phenotypic traits is an important traditional approach that may play an essential role in genotype identification for any hybridization programs to manifest maximum heterosis and relatively better recombinant lines (Mohamed et al., 2019; Sharma et al., 2022). Ample variation for pea morphological traits has been reported in the studies conducted by Singh et al. (2014), Mohamed et al. (2019) and Sharma et al. (2022).

An analysis of variance (ANOVA) showed significant variation ($p<0.01$) for all the quantitative traits under study. The descriptive statistics and mean performance for these traits are presented in Tables S3 and S4, respectively. The genotypes showed a range of 32 to 85.3 days for DTF; 33.7 to 170.2 cm for PH; 6.1 to 9.2 cm for PL; 1.0 to1.8 cm for PW; 4.4 to 26.3 for PPP; 35 to 90.3 g for 10-PW; 4.9 to 9.2 for SPP; 26.7 to 57.3 g for 100-GWS and 22.3 to 165 g for YPP, respectively. A significant variation for PH (Mohamed et al., 2019; Bashir et al., 2019; Aman et al., 2021); PL (Aman et al., 2021; Kumar et al., 2021); PPP (Mohamed et al., 2019; Aman et al., 2021) and SPP (Kumar et al., 2021) has been previously reported for both Indian and exotic collections.

Among the other key economic traits, the genotypes showed 6.7, 2.7 and 12-fold variation for the PPP, 10-PW and YPP, respectively. The popular genotype 'Arkel' produced minimum number and short pods (PL=7.5 cm), having 100-GSW of 50 g with average pod yield of 22.3 g per plant. In contrast, VRPM-903 had a high average pod yield (165 g) that was primarily due to its high pod number. Among the 45 genotypes studied, 20 genotypes produced

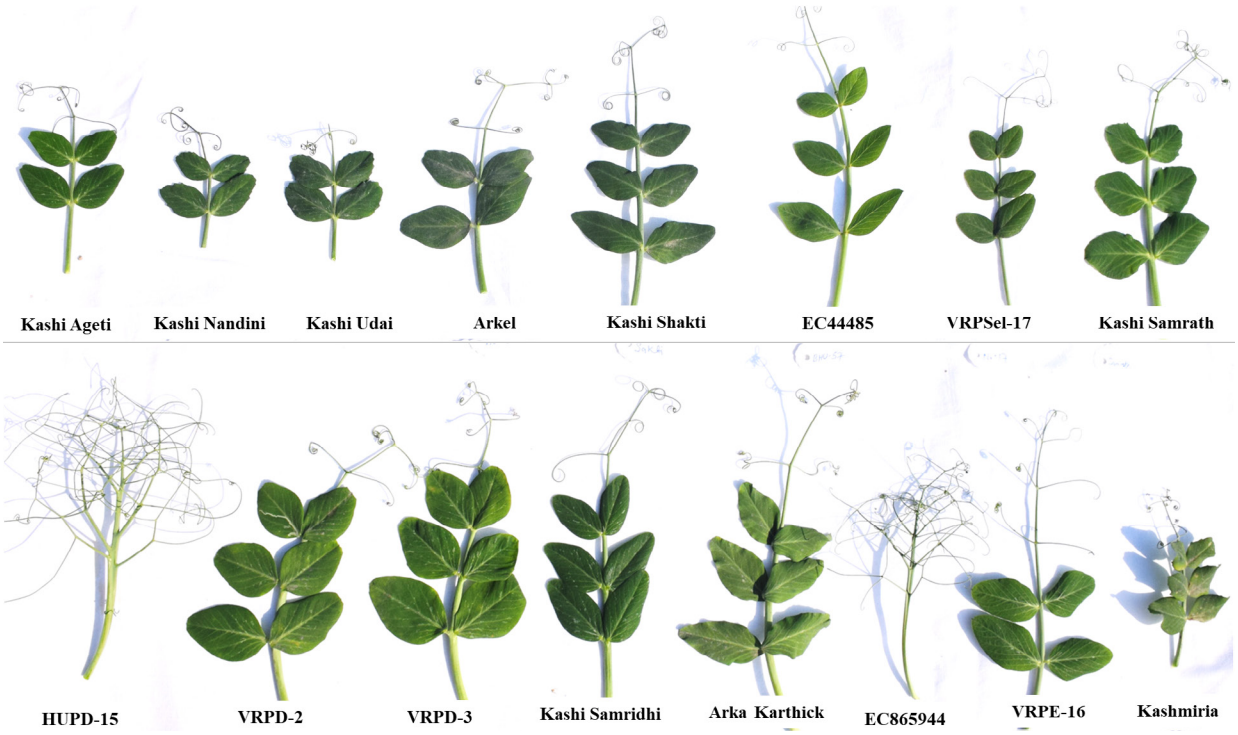

Figure 2. Leaf variation in some of the selected genotypes of *Pisum.*

higher YPP while 25 genotypes lower YPP than the mean value (60.1 g). The traits PPP and PL correlated positively with the YPP (Table S5).

The selected set of genotypes represents four groups for anthesis: five genotypes belong to extra-early (DTF ≤ 40 days); four to early (DTF: 40-50); 25 to medium (DTF: 50-80) and 11 to late maturing groups (DTF≥70 days). The early-flowering genotypes are preferred in peas by the farmers to fetch good income and as a catch crop between the main season crops.

Additionally, those cultivars also escape the major diseases *viz*., pea powdery mildew (Devi et al., 2022) and rust before the onset of favourable conditions for disease.

The genotypes in the present study start flowering as early as in 30 days of sowing. Kumar et al. (2021) showed DTF variation of 47.50 to 80.40 in pea genotypes, while Bari et al. (2021) found a variation of 60-84 days in a USDA collection of 482 peas. Singh et al. (2014), by studying 35 released vegetable peas varieties of India, reported that a total of 18 were extra-early and early-flowering and 16 were mid-flowering, which suggests that breeders in the country have focused on development of early and mid-varieties of vegetable peas. It is commonly observed that when the same genotypes are grown in hilly regions, flowering takes longer to initiate (Devi et al., 2021).

Molecular characterization

We assayed 52 SSR primer pairs, but only 31 amplified properly (Table 1; Fig. S2), and those amplifying ambiguous, unclear, and faint bands were not considered (21 markers). Further, 22 of the 31 markers were found to be polymorphic, indicating that the level of genomic variations between the 45 accessions was 70.96%. Previous studies found that the level of variation within pea genotypes ranged from 38.46% (Prakash et al., 2015) to 82.35% (Singh et al., 2021). The polymorphic alleles identified in this study indicated considerable diversity among the accessions. The number of alleles per locus ranged from 1 to 6, with amplification of a total of 90 alleles, with a mean of 2.91 alleles per locus. The marker AA135 had the highest number of alleles (six), followed by PSMPSAD51 (five) (Table 1). The PIC and gene diversity (D-values) for each marker revealed the informativeness in resolving the diversity among the accessions (Singh et al., 2021). PIC and D-values were highest for markers AA135 and PSMPSAD51 and lowest for marker AB40. The average PIC value in the present study was 0.46 which is very close to the value (0.44) reported by Mohamed et al. (2019) in local accessions of Southern Tunisia, while higher than value (0.36) reported by Sharma et al. (2022) in 56 Indian accessions. Other studies reported average PIC values of 0.52 (Loridon et al., 2005), 0.89 (Smykal et al., 2008a) and 0.62 (Smykal et al., 2008b).

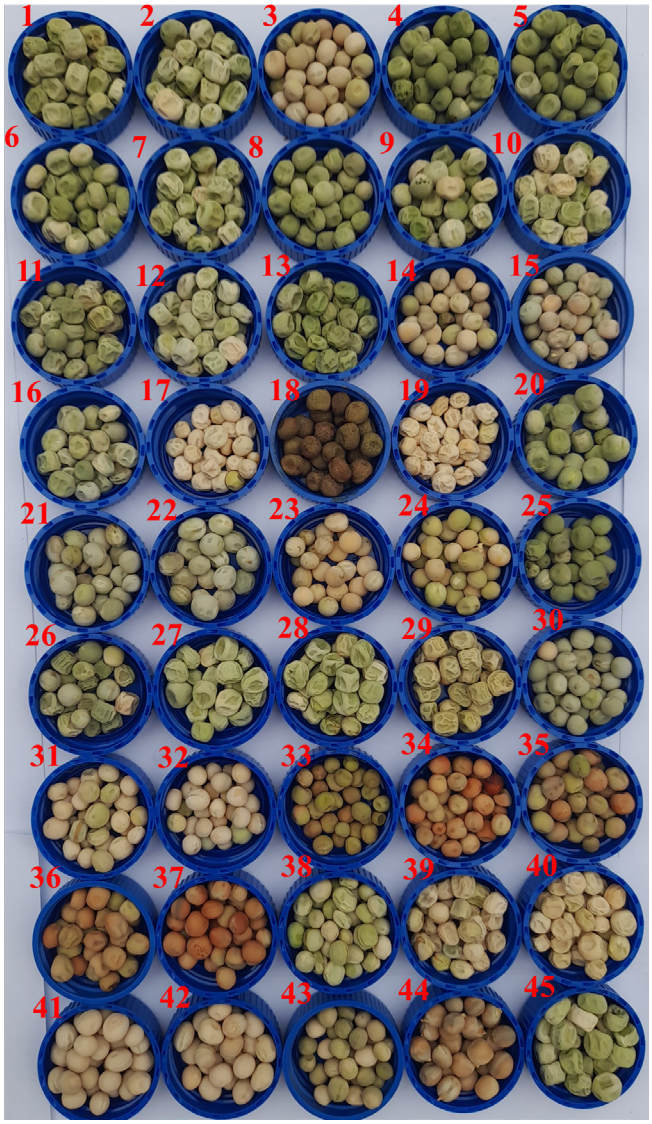

Figure 3. Seed coat variations in 45 accessions of peas. The number 1-45 represents the genotypes as described in Table S1 [suppl]

Further, the allelic size of many of these markers was in the range of other reports (Gong et al., 2010; Prakash et al., 2015; Nisar et al., 2017; Sharma et al., 2020). The range of number of alleles per locus (2-6) found was closely associated to earlier reports of 2-4 (Sharma et al., 2020) and 2-5 (Nisar et al., 2017), while this range was higher (2-8) in Prakash et al. (2015). The average number of alleles per primer pair in the present study (2.97) was close to those found by Mohamed et al. (2019) and Sharma et al. (2020), 2.88 and 2.2, respectively.

Principal components analysis (PCA) and cluster analysis

A cladogram of test genotypes was constructed using 16 traits (Table S1; excluding mature seed colour; Table S4) for relationship based upon agro-morphological traits

Table 1. Level of polymorphism and PIC for 22 SSR markers.

Sr. No.	Marker	Type	Ta (°C)	LG	Allele No	Size (bp)	Gene diversity (D)	PIC
1	AA135	SSR	50	VII	6.0	300-370	0.81	0.79
2	AA163.2	SSR	50	V	3.0	275-300	0.46	0.40
3	AA339	SSR	50	VII	3.0	300-325	0.43	0.36
4	AB140	SSR	50	III	3.0	350-400	0.49	0.42
5	AB40	SSR	50	II	3.0	300-325	0.20	0.19
6	AD270	SSR	50	III	3.0	250-275	0.43	0.39
7	AD56	SSR	50	VII	4.0	175-225	0.50	0.47
8	AD61	SSR	50	III	3.0	120-150	0.64	0.56
9	AD73	SSR	50	III	4.0	225-300	0.62	0.57
10	AnMtL6	SSR	62	III	3.0	280-300	0.31	0.28
11	c5DNAmet	SSR	65	VI	4.0	275-320	0.63	0.57
12	Fw_Trap__220	SCAR	55	III	4.0	175-225	0.38	0.36
13	Fw_Trap_340	SCAR	55	III	4.0	150-450	0.55	0.47
14	P1188	EST-SSR	65	-	3.0	150-175	0.27	0.26
15	P636	EST-SSR	65	-	4.0	200-250	0.73	0.68
16	P664	EST-SSR	65	-	4.0	200-300	0.61	0.54
17	PEA120	EST-SSR	55	-	4.0	150-200	0.62	0.55
18	PSAC75	SSR	52	V, VI, I	4.0	175-225	0.33	0.31
19	PSGAPA1	SSR	50	V	4.0	100-150	0.59	0.54
20	PSMPA6	SSR	50	VI	2.0	150-200	0.50	0.38
21	PSMPD23	SSR	50	-	4.0	50-100	0.60	0.52
22	PSMPSAD51	SSR	70	VI	5.0	250-550	0.74	0.70

LG: linkage groups. (-) indicate no information. PIC: polymorphic information content. Ta: annealing temperature.

and 31 primer pairs for relationship based on SSRs amplification. Broadly, the results showed independent relationship between diversity and the geographic distribution of evaluated genotypes. The results of PCA and cluster analysis are presented below.

Based on agro-morphological traits

Based on 16 agro-morphological traits, the PCA analysis showed two principal components PC1 and PC2 having eigenvalues 4.23 and 2.16 respectively, which explained 46.96% and 23.96% of the total variation (Table S6). Characters like 10-PW (0.91), 100-GSW (0.90), SPP (0.78), PL (0.77) and PW (0.50) showed positive loadings and explained the maximum variance in the first principal component (PC1). Similarly, YPP (0.96), PPP (0.85), PL (0.44), PH (0.37), DTF (0.27) and PW (0.25) had highest positive loadings and explained the highest variance in the PC2. Thus, these traits were the most effective to discriminate genotypes under investigation. In the study conducted on the garden pea, Devi et al. (2021) reported 74.88% of variation by the significant principal components. Also, Esposito et al. (2007) found that the first two components showed 69.8% of phenotypic variation in pea genotypes. Similar findings regarding loading of traits were also reported by Arif et al. (2020). As per the dispersion on PCA diagram, two broad groups and four subgroups of genotypes could be identified (Fig. 4). The genotypes VRPSel-17, IC-296678, 'Kashi Samrath', 'Arka Ajit', EC865944, VRP-500 and VRPM-901 were located distinct on the graph showing their diverse genetic make-up for one or more traits. Umar et al. (2014), studying pea genotypes from different origins, reported that pod length and width explained the maximum variation and were responsible for grouping of cultivars of different origins. The present study also reports ample variation for the pod characteristics.

Furthermore, clustering using neighbor-joining approach accessed three mega clusters (I, II and III). The mega cluster I could be divided into four sub-clusters, IA, IB, IC and ID (Fig. 5). Cluster IA included 18 genotypes (40%), followed by cluster ID with 17 genotypes (37.7%) and cluster IC with 10 genotypes (22.2%). The MF genotype VRPM-901 placed separately from rest of the genotypes, showing its unique identity (Figs. 4 and 5, cluster III). Incidentally, most of the early maturing genotypes viz., 'Kashi Udai' (Arkel × FC-1), 'Kashi Nandini' (P 1542 × VT-2-1), 'Kashi Mukti' (Cross No. 7 × PM-5), 'Kashi Ageti' (PM-5 × MG'), VRPE-16 (Azad P-5 or KS-225 ×

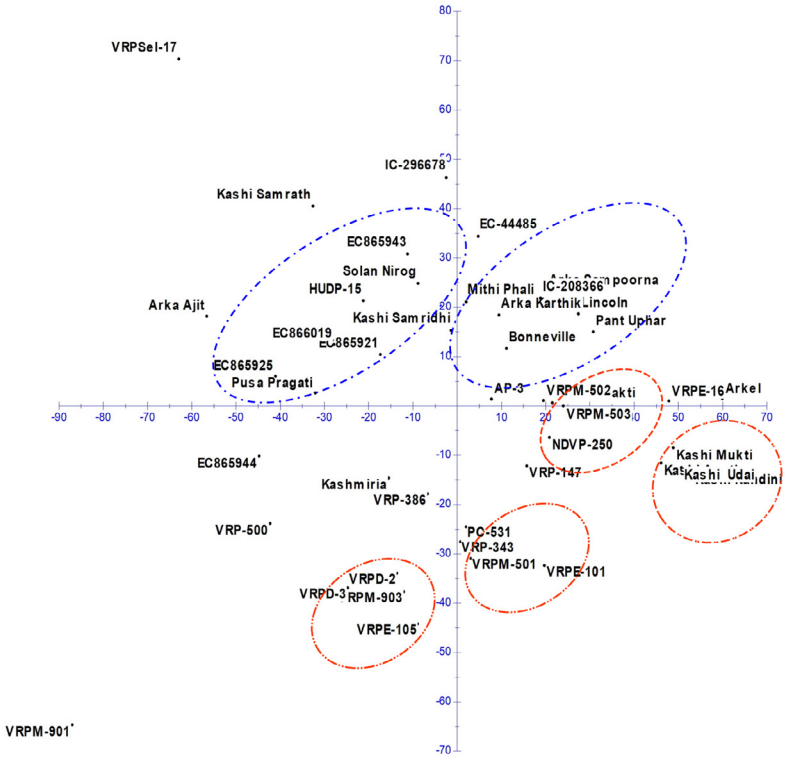

Figure 4. Distribution of pea genotypes into different quadrants based on the first two components.

Ageta) and 'Arkel' (introduced from UK), were clustered together in Cluster IB, showing their similar background for this trait. The cluster IA mainly consisted of genotypes with average pod yield between 31 to 62 g, performed below the population mean. In these genotypes, the pod number ranged from 7.2 (EC-44485) to 12.8 (EC865943) with a mean value of 9.06 (Table S4). The edible podded lines VRPD-2 and VRPD-3 developed at ICAR-IIVR were grouped in sub-cluster ID, while 'Arka Sampoorna' and 'Mithi Phali' were in sub-cluster IA (Fig.5). Besides the higher yield, which is mainly contributed by their longer, broader and heavier pods, both the VRPD-2 and VRPD-3 have the additional trait of powdery mildew resistance under field conditions. As a result, they can be used as potential parents in a breeding program devoted specifically to the development of parchment free cultivars.

The genotypes VRPSel-17 (Cluster II) and VRPM-901 (Cluster III) showed most separated clustering, owing to their unique flowering behaviours of single flower per node and multi flowers per node, respectively. The cluster IC contained the *afila* and pulse type genotypes (EC865921, EC865944, EC865925, EC866019 and Kashmiria). The highest yielding cultivars (pod yield ≥ 90 g/plant) from mid and late maturity group (VRPM-903, VRPE-105, VRPD-2, VRPD-3, VRP-500, VRPM-901 and EC865944) were included in three different sub-groups IC, ID and III, indicating their distinct make up for the trait under consideration.

During the cluster analysis of 160 vegetable pea genotypes, Sanwal et al. (2021) reported a total of 14 clusters based on nine morphological traits. They reported non-random grouping of genotypes of different origins in the clusters. In the present study, a non-random association of genotypes in a single cluster could be seen. However, the genotypes in this study were grouped in the same sub-cluster showing similarity in their morphology such as earliness, pod length and width. The random distribution of genotypes of diverse origin in a single cluster indicates that the divergence in pea is not related to the geographical origin. The tendency of genotypes occurring in clusters cutting across the geographical boundaries demonstrates that geographical isolation need not necessary to be related to diversity and was at random (Gatti et al., 2011). Such parallelism between geographical distribution and diversity might be due to some forces other than geographical distance like genetic architecture of population, heterogeneity, history of selection, or proximity of development of traits (Sureja & Sharma, 2001).

Based on SSR markers derived from pea genome

A cladogram based on molecular data from 31 SSR markers (Fig. 6; Table S6) grouped the 45 accessions into four clusters, having clusters II and IV 22 and 20 genotypes respectively, while cluster I contained EC865921 and 'Arka Karthik', and cluster III the genotype 'Lincoln', separated from the rest of the genotypes (Fig. 6). Similar clustering of VRPM-501, VRPM-502, and VRPM-901 could be traced back to their common ancestry (Table S1).

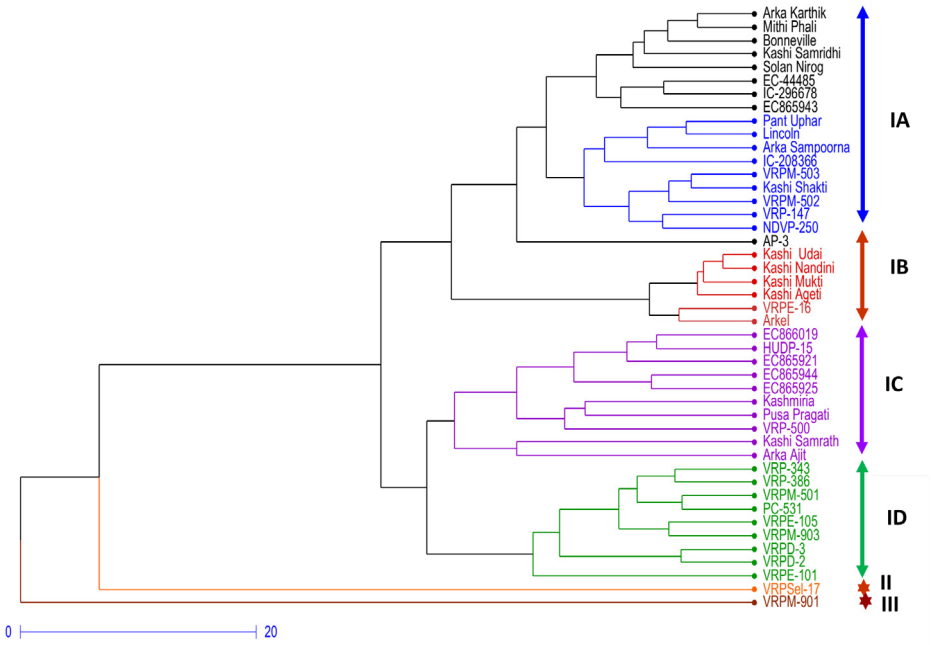

Figure 5. Cladogram of the 45 pea accessions, showing the relationship based on the genetic similarity matrix data of agro-morphological traits.

Most of the vegetable-type genotypes originating from India grouped together in one mega cluster (II), except Kashmiria which is pulse-type. The analysis of the population structure divided the 45 pea accessions into two groups with few admixtures (Fig. 7). This support our cluster analysis which grouped most of the genotypes into two groups. Kumari et al. (2013), using 28 pea genotypes and 32 SSR markers, grouped them in two clusters, and one into two sub-clusters. Ram (2021) studied the diversity using SSR markers, in which 24 genotypes were grouped into two clusters based on 11 polymorphic makers; similar to our study, he also reported that the 'AP-3' and 'Arkel' were grouped in the same cluster. Thus, the SSR markers are reliable for accessing diversity due to its reproducibility across the laboratories. The information about diversity can be used to predict the progeny performance.

Potential genotypes to be utilized in pea breeding programs

The genotypes under study showed considerable variation for phenotypic appearance. The importance of simple morphological traits in breeding programs cannot be ignored as these traits are directly or indirectly correlated to many other economical traits. One important example of such variation is the flower and seed coat colour, and their possible correlation to antioxidant activities. According to Devi et al. (2019), coloured genotypes of peas differing in maturity types were positively correlated with total phenol content and total flavonoids content. They also reported that the purple flowered and dark seed coat coloured genotypes EC-9485 and VRP-233 had the highest total phenol (128.63 and 104.00 mg GAE/100 g) and flavonoid contents (45.84 and 36.84 mg CE/100 g) respectively, along with strong antioxidant potential, when compared to white flowered and light seed-coat coloured genotypes. Thus, selection of dark coloured pea genotypes may result in considerable genetic improvement for antioxidant compounds in the segregating progenies. Furthermore, knowledge of gene action controlling various economic traits helps in the selection of parents, as well suggests the appropriate breeding procedure to be used for their genetic improvement (Sharma & Sharma, 2013). Devi et al. (2018a) also reported significant genotypic and phenotypic coefficient of variations, heritability and genetic advance for total phenolics, total flavonoids, cupric ion reducing antioxidant capacity (CUPRAC) and ferric reducing antioxidant power (FRAP) activities. This suggests that selection on these traits can be used to isolate more promising lines, indicating the role of additive gene action in the inheritance of these traits, being likely to respond to selection. However, direct selection for a genotype with high pod yield, phenolics and flavonoids contents is difficult to achieve, due to negative association of the above traits. Furthermore, genotypes with high phenolics contents and high yield (VRPD-2, VRPD-3 and PC 531) could be used in crossing programs to incorporate better antioxidant potential along with higher yield through pedigree breeding and selection from segregating populations.

Earliness is a highly desirable trait in vegetable peas owing to its high marketable price early in the season. In addition, the early-maturing varieties could escape the devastating disease of powdery mildew (Devi et al., 2022), rust, as well as the effect of high temperatures in the late

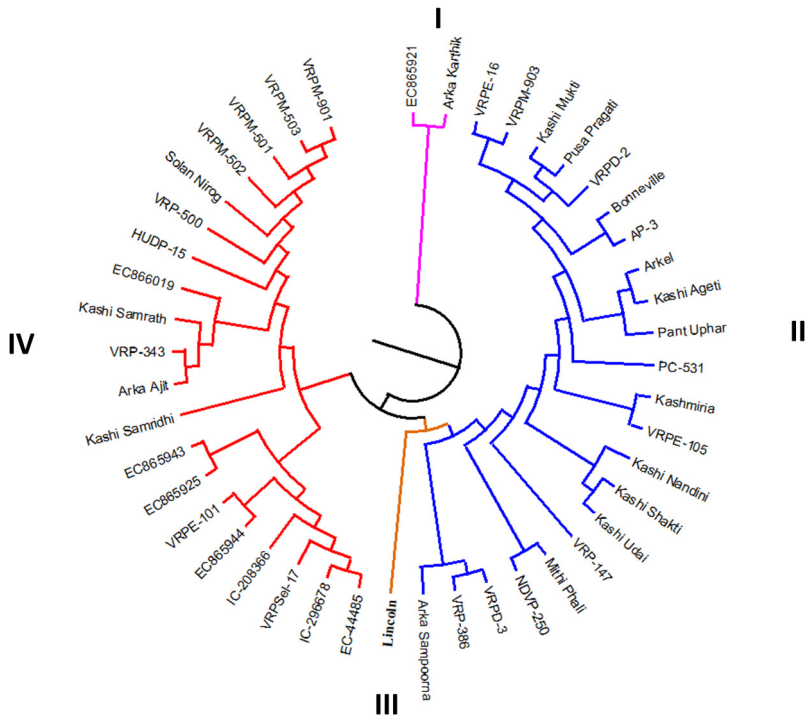

Figure 6. Cladogram based on molecular marker data from 31 SSR markers (see Table S6), showing the relationships among the 45 pea genotypes under study.

seasons under north Indian conditions. The trait had a high heritability which is governed by dominant genes (Mohan et al., 2013). 'Kashi Nandini' was found to be earliest in flowering as compared to late flowering genotype EC866019, with nearly 2.7-fold variation for DTF (Table S4). Further, it is noteworthy that due to simple inheritance of earliness and their wider adaptation, the varieties 'Kashi Nandini', 'Kashi Udai' and 'Arkel' are quite popular in the pea growing belt of India, as the pods are ready in early December, and farmers can raise the succeeding wheat crop. Thus, all the five lines carrying genes for earliness (Tables S1 and S4) could be potential parents for breeding early maturing varieties.

Plant height is considered as highly heritable polygenic character (Mohan et al., 2013). The current study reports significant variation among genotypes for this trait, and were categorized as short (19 genotypes), medium (11 genotypes) and tall (15 genotypes). Many studies have reported high heritability and high genetic advance for this trait, which could easily be transferred to their progenies (Singh et al., 2010). In general, all short type genotypes were early-flowering, mid-season were medium in height and late-flowering types were of taller growing habit. However, it is important to highlight that taller varieties are less desirable in peas, both for grain and vegetable peas, because they are prone to logging, and logging resistance has been kept as a major breeding goal in *Pisum* breeding. On the other hand, performance of short stature varieties reduces drastically when subjected to environmental variations other than the optimum. Thus, the genotypes with medium growth habit are the best while looking for the future trait of interest. Accordingly, the genotypes AP-' among early and PC-531, VRP-500, VRPM-903 and VRPE-105 could be the best suitable for high yield with intermediate plant height. Further, it is interesting to note that taller cultivars have high-biomass architecture with profuse foliage and are prone to numerous diseases; yet, they are chosen by farmers owing to their long production season (Checa et al., 2020). However, staking and trailing in these cultivars could surge the production cost by 52%. The *afila* trait could reduce the production cost as interlocking of plants through the growing tendrils could impart self-staking. Such cultivars can also avoid birds menace as they act as natural nets. Additionally, this trait have been reported to harbour minimum foliar diseases, high productivity and high water use efficiency (Ondrej et al., 2011; Checa et al., 2020). The genotype EC865944 had high pod yield (100.7 g) along with resistance to powdery mildew and can be utilized for introgression of the *afila* gene.

Flowers have considerable agricultural and economic impact, since much of the human and livestock food is the product of flowers (Stewart et al., 2016). Traits such as number of flowers per peduncle and number of flowers per plant, are the key yield attributing traits that should be targeted through various breeding programs. The pea improvement would benefit from a breeding plan that

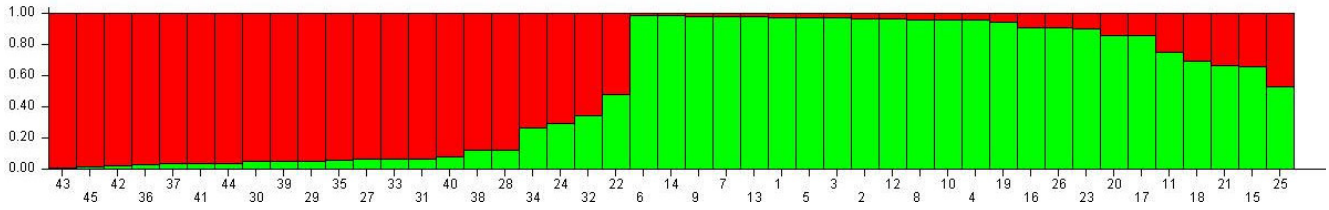

Figure 7. Population structure (Q) modelled with k =2. The 45 accessions were divided into two groups comprising 24 (green) and 21 (red) accessions, respectively. Numbers below the figure represent the accession numbers given in Table S1.

includes more flowers per peduncle, pods per peduncle, more flowering nodes, more seed per leaf, and so on. Our previous studies have highlighted the importance of MF genotypes in yield enhancement in vegetable pea mainly attributed by above mentioned traits (Devi et al., 2018b; Mishra et al., 2020). For the utilization of MF in breeding programs, the trait is reported to be controlled by two genes with epistatic interaction and inhibitory gene actions (Devi et al., 2021). The simple pedigree method of breeding along with selection for the multi-flowering in the segregating generation (F_3 onward) will be suitable for breeding MF genotypes. In addition to the genic interactions, several inhibitory genes also play major role in the expression of this trait; thus, selection of MF plants in every generation followed by progeny row evaluation becomes essential for increasing the frequency of MF in subsequent generations (Devi et al., 2021). The MF genotypes VRPM-901 and VRP-500 could be used to introgress the MF trait into single and double podded pea cultivars. This study also identified one unique genotype VRPSel-17 which has a single flower on all its flowering nodes with consistent flowering behaviour of single FPP over the years, and due to its uniqueness, it can be used in genetic studies including flower architecture.

Conclusion

The 45 accessions of *Pisum* showed considerable diversity for 17 agro-morphological traits. Among the various economic traits, the genotypes showed 6.7, 2.7 and 12-fold variation for the PPP, 10-PW and YPP, respectively. Principal component analysis has identified few characters (PPP, 10-PW, PL, 100-GSW, SPP and YPP) which play a prominent role in classifying the variation existing in the germplasm set. At molecular level, two markers (AA135 and PSMPSAD51) were found highly polymorphic with the highest PIC and D-values. The agro-morphological and molecular studies divided these accessions into three and four mega clusters, respectively. The present study facilitated the identification of potential accessions harbouring novel, favourable alleles for various economically important traits. The genotypes VRPD-2, VRPD-3 and PC-531 could be utilized in crossing programs to incorporate better antioxidant potential along with higher yields; 'Kashi Nandini', 'Kashi Udai', 'Kashi Mukti', 'Arkel' and VRPE-101 for earliness; AP-3, PC-531, VRP-500, VRPM-903 and VRPE-105 for high yield with intermediate plant height; VRPD-2 and VRPD-3 for parchment free genotypes; EC865944 to introgress the *afila* gene; and VRPM-901 and VRP-500 to introgress the MF trait into single and double podded pea cultivars. The results and derived information of this study could be utilized across the pea breeding community to develop high yielding modern cultivars in both vegetable and grain peas.

Authors' contributions

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Formal analysis: V. Sagar
Funding acquisition: Not applicable
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Resources: J. Devi
Software: Not applicable
Supervision: J. Devi, P. M. Singh, T. K. Behera
Validation: J. Devi, V. Sagar
Visualization: J. Devi, R. K. Dubey, V. Sagar
Writing – original draft: J. Devi, R. K. Dubey, V. Sagar
Writing – review & editing: J. Devi, P. M. Singh, T. K. Behera

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