WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Agro Morphological Characterization and Diversity Assessment of Pea (*Pisum Sativum* L.) Germplasm Conserved in Genebank of Nepal

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DOI: https://doi.org/10.55248/gengpi.2023.4106

A B S T R A C T

A total 122 landraces collection collection of pea (Pisum sativum L.) germplasm accessions conserved in the Gene bank of Nepal was characterized for 8 quantitative and 11 qualitative traits to assess the magnitude of prevailing genetic variability at National Agriculture Genetic Resources Centre (Genebank), Khumaltar, Nepal during 2017. Shannon and Weaver diversity index (H') analysis revealed significant intra landrace diversity for both quantitative (0.783) and qualitative traits (0.536). The first two principle components with Eigen value >1 accounted for 66.3% of the entire variability for quantitative traits. For qualitative traits, four principle components with Eigen value >1 accounted for 61.6% of the entire variability. Four clusters were established with phenotypic similarities using Euclidean distance and average linkage methods. The landraces grouped into the cluster I were characterized by higher pod length, width and 100 seed weight. Pearson correlation analysis among seven quantitative traits showed highly significant positive correlation (r = 0.557***) between flowering days and maturity days. This variation indicated that there is a way to identify promising genotypes for pea breeding.

Keywords: Pea, phenotypic traits, Diversity index, quantitative trait, qualitative trait, pre breeding

Introduction

Pea (*Pisum sativum* L.) is one the world's oldest domesticated herb and the third most widely grown legume crop worldwide (Smykal et al, 2010). It is self-pollinated diploid with chromosome number 2n=14. It belongs to *Fabaceae* family which is largest family of flowering plants comprising of over 450 genera and 1200 species (Luitel et al, 2021).

Vavilov (1926) based on genetic diversity listed four different centres of origin for pea. Area comprising Central Asia, the Near East, Abyssinia and the Mediterranean is the center of origin of pea based on genetic diversity. This statement is supported by archaeological evidence which supports the existence of pea back to 10,000 B.C. in Near East and Central Asia.

Pea among other grain legumes accompanied cereals and formed important dietary. Pea is one the world's oldest domesticated winter vegetable and the third most widely grown legume crop worldwide (Smykal et al., 2010). In Nepal, pea is popular legume vegetable widely grown for fresh seeds, tender pods. As in other crops, the demand for high productivity has resulted limited number of high-yielding homogeneous varieties leading to genetic erosion. Few studies on the genotypes conserved in Genebanks states the existence of broad genetic diversity (Singh et al., 2013; Warkentin et al., 2015, In Nepal, pea is popular legume vegetable widely grown for fresh seeds, tender pods. It is grown in winter in terai and in summer in hills. In Nepal, garden pea is grown commonly in cool season for fresh green seeds, tender green pods, and dried seeds and consumed fresh or cooked(Ghale et al, 2004). According to a report (MoALD, 2021), pea is cultivated in 8,072 hectare (ha) of land with a total production 73,936 metric ton (mt) and productivity of 9.16/ha in Nepal. As in other crops, the demand for high productivity and homogeneity has resulted high-yielding varieties, homogeneous and limited number of varieties eventually leading to the loss of heterogeneous traditional local varieties (landraces) termed as genetic erosion. The loss of diversity due to genetic erosion has led to the narrowing of the genetic base which is the only basis for crop improvement in the future. However, the conservation efforts by Genebanks have contributed in prevention of the genetic erosion of the landraces.

Characterizing, assessing, using, and maintaining these resources is challenging because of the large number and heterogeneity of germplasm collections preserved at Genebanks. In order to diversify parental material for breeding and create a successful crop improvement program, a thorough analysis of the prevalent variety within the Genebank collection of germplasm is necessary (Kaur et al 2022). Few studies states the existence of broad genetic diversity in peas (Bhuvaneswari et al, 2017; Singh et al, 2017). This diversity has been conserved in gene banks and studied for the last 20 years (Singh et al, 2013; Warkentin et al, 2015). Morphological characterization is the first step in analyzing and describing germplasm (Bouhadida et al, 2013). Understanding morphological characteristics facilitates the pre breeding process of identification and selection of desirable attributes, design of new populations, gene transfer, and resistance to biotic and abiotic factors (Singh et al, 2014).

This study focused to investigate the agro morphological traits of the pea landrace conserved in National Genebank of Nepal to identify the elite lines for pre breeding.

2. Materials and methods

A total of 122 accessions were collected and conserved in the Nepal Genebank. These accessions were grown at Genebank, Khumaltar (N27.4 0 E085.2 0, 1360 m a.s.l.) during winter 2014 at the spacing of 60x60 cm with the recommended dose of fertilizer (15:40:10 kg NPK/ropani).Experiment was conducted in non-replicated design in rod row design for agro-morphological characterization. The phenotypic traits were recorded using a set of local descriptor developed with the reference of different scientific descriptors from NIAS Genebank, National Bureau of Plant Genetic Resources (NBPGR) and European Union descriptor (UPOV) (UPOV, 2003; Mahajan et al, 2000;NARO,1999).

A total of 8 quantitative and 11 qualitative were measured. The observations were obtained from five plants per accession and five pods per plant per accession. Shannon–Weaver diversity indices (Shannon and Weaver, 1949) were calculated in order to estimate the phenotypic diversity for each qualitative trait with Microsoft Excel using the formula: Descriptive statistics, Shannon-Weaver diversity index (H') (Shannon and Weaver, 1964), and frequency distribution were employed to estimate and analyze the diversity via MS Excel. The coefficient of variation (CV) was calculated based on the formula CV (%) = (standard deviation/mean values) × 100. Mean values and standard deviation were calculated on the basis of the 5 individual plants or of the 5 randomly harvested pods per plant.

The Standardized Shannon-Weaver diversity index (H') (Shannon and Weaver. 1949) was calculated in Excel using the formula:

$\sum [(pi) \times \log (pi)]$

Where, H - Shannon diversity index

n - Individuals of a given type/species

N - Total number of individuals in a community

For the quantitative traits, accessions were divided into 10 phenotypic classes, <x-2.0sd, x-1.5sd, x-1.0sd, x-0.5s, x, x+0.5sd, x+0.5sd, x+1.0sd, x+1.5sd, >x+2.0sd, where x is average and SD is standard deviation. For qualitative traits, the frequency of the descriptive trait map was used for the calculation of Shannon and Weaver diversity indices. The diversity index was considered as low ($0.10 \leq H \leq 0.40$), intermediate ($0.40 \leq H \leq 0.60$) or high ($H \geq 0.60$) (Eticha et al, 2005).

The classifications of landraces on the basis of both quantitative traits were performed using multivariate principal component analysis (PCA) in MINITAB version 17 (Minitab, 2010). For systematic analysis, hierarchical clustering was performed using Euclidean distance and complete method. Distance between clusters were analyzed and reported as a dendrogram of Euclidean distances via MINITAB version 17.

3. Results

Phenotypic characterization based on observable characters is the pre requisite of all landrace populations to provide overall picture of the existing genetic diversity. This further supports the management, and conservation of genetic diversity (Manzano et al, 2001) and is very important for the selection of elite lines for pre breeding and development of varieties (Fraleigh, 1987; Smith et al,1991).Phenotypic characterization is used to analyze and document diversity within and between landraces based on their observable attributes (FAO, 2012). With the study of physical and biometric characters, conservation and utility of the particular landrace and the overall improvement can be properly implemented. Pea accessions of Nepal Genebank used for agro morphological study is presented in Annex 1**.**

Descriptive statistics and Shannon Weaver Diversity analysis

The descriptive statistics (average, range, and standard deviation) and Shannon-Weaver diversity indices (H') were used to measure the diversity of the accessions on quantitative characters presented in Table 1.

The coefficient of variance for all the genotypes were divided into three categories (above 20% was high, 10–20% was medium, and below 10% was low). Coefficient of variance of the 8 quantitative traits ranged from 4.84-54.68%. Highest CV (54.68) was observed in 100 seed weight followed by plant height (31.98), pod width (24.02), pod length (20.27) with CV value higher than 20. Flowering days (10.93), number of seed/pod (13.94) and maturity days (4.84) have CV value less than 20%. Days to seedling emergence were constant for all the accessions.

The higher Coefficient of variance for 100 seed weight, pod weight, pod length indicated the high intra varietal diversity in the pea germplasms. Therefore, a huge scope for the perfect selection of yield attributing traits existed based upon the phenotypic expression of these traits. The collections evaluated were not divergent for number of seed/pod, earliness encompassing flowering and maturity days with medium CV percentage. The zero CV for emergence revealed lack of variation within the germplasm.

Plant height (13.2-184.60cm) showed the highest diversity index 0.93 and days to maturity (115-164) showed the lowest diversity index 0.80. The yield attributing traits like flowering days (0.91), pod length (0.92), pod width (0.92), number of seeds/pod (0.91) and 100 seed weight (0.87) also showed high diversity index.

All accessions of pea showed good amount of intra varietal variation for 7 quantitative traits except the days to seedling emergence. The relatively higher diversity was seen for plant height compared to flowering days, pod length, pod width, number of seeds/pod. 100 seed weight and maturity days showed relatively less Shannon and diversity index. The evaluated pea germplasms possess good amount of variation between the populations for all yield attributing characters as they recorded Shannon Weaver diversity indices (H') above 0.6 as defined by Eticha et al, (2005).The high Shannon and diversity indices among the pea populations for qualitative traits tendril colour, tendril twinning, immature pod colour, flower colour , seed size, is confirmed by the result of this study as Eticha et al, 2005.The result obtained from this study revealed lack of variation in days to emergence among 122 pea genotypes which differs from the studies reporting high degree of genetic diversity for the same trait (Shah et al,2016, Azmat et al, 2011, Kumar et al, 2016). Days to emergence in most of the cases determine crop maturity. The genotypes which take lesser number of days to emergence usually mature earlier. Monpara and Dhameliya (2013) and Kosev (2013) reported that early maturing traits play vital role in the adaptation of genotype to environments and cropping systems and have positive impact on yield.

Table 1. Descriptive statistics and Shannon– Weaver diversity index of quantitative traits

Traits	Minimum	Maximum	Mean	CV %	SD	H'
Emergence days (No.)	10.00	10.00	10±0	0.00	0.00	0.00
Flowering days (No.)	76.00	129.00	94.85±0.94	10.93	10.36	0.91
Pod length (cm)	3.20	7.94	5.34±0.10	20.27	1.08	0.92
Pod width (cm)	0.91	9.11	5.74±0.12	24.02	1.38	0.92
Maturity days (No.)	115.00	164.00	149.66±0.66	4.84	7.25	0.80
Plant height (cm)	13.20	184.60	98.87±2.86	31.98	31.62	0.93
Number of seeds/pod	3.00	8.00	5.65±0.07	13.94	0.79	0.91
100 Seed Weight (g)	3.20	28.50	10.79±0.53	54.68	5.90	0.87
Mean						0.783
SD						0.319

CV=Coefficient of variation, SD=Standard deviation, H' =Shannon-Weaver diversity indices

Shannon –Weaver diversity analysis for qualitative traits are presented in Table 2. The diversity index ranged from 0.23(pod texture) up to 1.30 (pod curvature). Seven qualitative traits tendril colour (0.97), tendril twinning (0.94), immature pod colour (0.68), flower colour (0.62), seed size (0.678), seed colour (0.679) have high diversity index (H ≥0.60).

Table 2. Shannon–Weaver diversity index, descriptor states and frequency of qualitative traits

Traits	Rank	Phenotypic Class	Frequency	Proportion (%)	H’
Foliage Colour	1	Yellowish green	47	38.5246	0.481
	2	Green	69	56.5574	
	3	Blue green	6	4.91803	
Tendril Colour	1	Pale green	27	22.1312	0.969231
	2	Green	43	35.2459	
	3	Purplish green	52	42.6230	
Leaf size	1	Very small	6	4.91803	0.248591
	3	Small	28	22.9508	
	5	Medium	46	37.7049	
	7	Large	38	31.1475	
	9	Very large	4	3.27869	
Flower Colour	1	White	52	42.6230	0.620986
	2	Creamish white	70	57.3771	
	3	Cream	0	0	
Immature pod Colour	1	Light green	11	9.01639	0.682505
	3	Slight light green	50	40.9836	
	5	Green	56	45.9016	
	7	Slight dark green	3	2.45902	
	9	Dark green	2	1.63934	
Twining tendril	3	Low	22	18.0327	0.94295
	5	Intermediate	55	45.0819	

	7	High	45	36.8852	
Growth pattern	1	Flat	0	0	0
	2	Erect	122	100	
Pod texture	1	Rough	115	94.2623	0.231215
	2	Smooth	2	1.6393	
	3	Tuberculate	5	4.09836	
Pod curvature	1	Absent	8	6.55738	1.301009
	3	Weak	57	46.7213	
	5	Medium	51	41.8033	
	7	Strong	6	4.91803	
	9	Very Strong	0	0	
Seed size	1	Small	34	27.8689	0.67818
	2	Medium	43	35.2459	
	3	Large	45	36.8853	
Seed Colour	1	Cream	37	30.3279	0.679853
	2	Light yellow	39	31.9672	
	3	Whitish green	46	37.7049	
	4	Green	0	0	
	99	Others	0	0	
Seed wrinkle	1	Absent	0	0	0
	9	Present	2	100	
Mean					0.536
SD					0.399

The mean Shannon-Weaver diversity indices (H') for quantitative characters is 0.783 which is relatively higher than thequalitative characters with 0.536. All the plants of the accessions were erect and the seeds were wrinkled with no variation observed.

The estimate of H' considers both richness and evenness of the phenotypic classes of the traits Yadav et al., 2018). Study of Shannon diversity index has been widely used in the estimation of existing diversity in germplasm collection of different crops like barley (Tolbert et al, 1979).Mean Shannon-Weaver diversity indices (H') for quantitative characters is relatively higher than qualitative characters indicating the accessions vary between populations in quantitative traits and suggests the possibility of selection of elite lines based on requirement and breeding objective. The lack of variation for few characters as plant growth habit, seed wrinkling and days to emergence revealed no genotype and environment interaction. Based on the variation in seed size, the population can be grouped into small seed and big seed groups and can be utilized as an important character for pre breeding.

Principal Component Analysis

PCA is an important tool to identify the important traits which have greater impact on the total variables and each coefficient of the vectors indicate the degree of contribution of every original variable with which each principal component is associated (Sanni, Fawole, Guei, Ojo, & Somado, 2008). The first three principal components are stated to be most often the most important in revealing the variation patterns among the different genotypes and the characters associated in differentiating various genotypes (Clifford and Stephenson, 1975; Guei, Sanni, Abamu, and Fawole, 2005). The criterion of was chosen to determine the cutoff limit for the coefficients of the proper vectors. According to Raji (2002), coefficients greater than 0.3 (regardless the direction positive or negative) as having a large enough effect are considered important, while traits having a coefficient less than 0.3 are considered having least effect on the overall variation observed which was adopted in the present study(Yadav, 2018).

PCA is used to study the dispersal of accessions was based on the Principal component-1 and Principal component-2 of quantitative traits representing the existing phenotypic variation within the collection (Fig 1). The first two principle components with Eigen value >1 accounted for 66.3% of the entire variability (Table 3). The first principal component accounted for 41.7 % of the total variance mainly influenced by the traits like 100 seed weight (0.53%), pod width (0.54%) and pod length (0.53) with positive loading contributing to yield parameters in agreement with findings of Gixhari et al, (2014). Similarly, in the second principal component accounting for 24.6 % of the total variation, flowering days (0.63%) with positive loading, maturity days (0.58%) and plant height (0.41%) with negative loading are important characters with major contribution. The remaining third (14.1%), fourth (0.8 %), fifth (0.5 %), sixth (0.3%), seventh (0.1%) accounted for quantitative traits (Table 3). The scatter plot of the first two principal components in Figure 1 accounts for 66.3% of cumulative variance.

In this study, pod length, pod width, days to flowering and plant height explained most of the variation. Gixhari et al, (2014). Umar et al. (2014) study observed that weight pod-1, seed weight, pod weight, seed weight pod-1, number of pods plant-1 and total pod number, pod thickness and pod length dominated the first principal component and contributed to the 40.29% of the total variation. Similarly, Esposito et al. (2009) reported that the first two components explained 67.7% and 69.8 % of variability.

Table 3. Principal component analysis and Eigen analysis of the correlation matrix for quantitative traits

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	2.9208	1.7220	0.9838	0.6072	0.4090	0.2416	0.1157
Proportion	0.417	0.246	0.141	0.087	0.058	0.035	0.017
Cumulative	0.417	0.663	0.804	0.891	0.949	0.983	1.000
Flowering Days	0.027	0.635	-0.306	0.193	0.679	-0.044	-0.047
Pod length	0.531	-0.089	-0.146	-0.307	0.014	-0.348	-0.688
Pod width	0.540	-0.086	0.103	-0.184	0.181	-0.387	0.688
Maturing Days	0.196	0.586	-0.286	0.097	-0.709	-0.091	0.124
Plant Height	0.252	-0.418	-0.372	0.788	-0.014	-0.042	-0.003
No of seed/pod	-0.207	-0.249	-0.809	-0.441	0.022	0.106	0.188
100 Seed Weight	0.530	0.030	0.023	-0.096	0.045	0.840	0.019

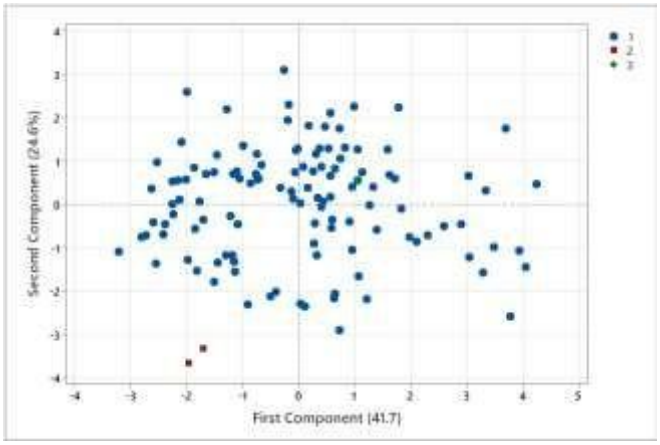

Fig. 1 Scatter plots based on first two principal component for quantitative traits

For qualitative traits, four principle components with Eigen value >1 accounted for 61.6% of the entire variability (Table 4). The first principal component accounted for 27.9 % of the total variance mainly influenced by the traits like flower colour (48.2%), tendril colour (37.5%), seed size (38.8%), seed colour (34.3%) with positive loading and leaf size (0.439%) with negative loading (Roseroet al, 2021, Devi et al, 2021). Similarly, in the second principal component accounting for 12.8 % of the total variation, immature pod colour (39.7%) positive loading, pod texture (0.63%), seed wrinkle (43.9%) with negative loading, are important characters with major contribution. The scatter plot of the first two principal components in Figure 2 accounts for 4.07% of cumulative variance.

Table 4. Principal component analysis and Eigen analysis of the correlation matrix

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12
Eigenvalue	3.3442	1.5382	1.3486	1.1660	0.9946	0.8511	0.7370	0.5811	0.5467	0.4427	0.3068	0.1429
Proportion	0.279	0.128	0.112	0.097	0.083	0.071	0.061	0.048	0.046	0.037	0.026	0.012
Cumulative	0.279	0.407	0.519	0.616	0.699	0.770	0.832	0.880	0.926	0.963	0.988	1.000
Foliage Cl	-0.030	0.172	-0.096	0.789	0.169	-0.253	0.161	0.287	0.207	-0.293	0.004	0.085
Tendril Cl	0.375	0.112	-0.374	0.154	0.112	-0.139	-0.214	0.235	-0.282	0.541	0.008	-0.422
Leaf size	-0.439	-0.136	-0.039	0.178	-0.165	0.144	-0.192	0.127	-0.066	0.264	0.748	0.146
Flower CL	0.482	-0.196	-0.147	0.042	0.037	0.022	-0.209	0.025	-0.090	0.078	-0.017	0.804
Imm pod CL	0.221	0.397	0.374	-0.093	-0.171	0.212	-0.273	0.368	0.575	0.174	0.021	0.005
Twining tendril	-0.110	0.111	-0.419	-0.039	-0.673	-0.440	-0.184	-0.205	0.246	-0.016	-0.114	0.046
Gr pattern	0.033	-0.133	-0.653	-0.162	0.035	0.532	0.062	0.253	0.254	-0.313	0.033	-0.112
Pod texture	-0.046	-0.642	0.048	-0.090	0.046	-0.277	0.334	0.262	0.416	0.363	-0.102	-0.023
Pod curvature	0.275	0.208	0.013	0.088	-0.432	0.217	0.747	-0.043	-0.133	0.187	0.151	0.063
Seed size	0.388	-0.007	0.050	-0.336	0.060	-0.446	0.067	0.134	0.001	-0.407	0.571	-0.133
Seed Cl	0.343	-0.253	0.054	0.326	0.043	0.179	-0.122	-0.653	0.356	0.019	0.208	-0.251
Seed wrinkle	0.161	-0.439	0.278	0.221	-0.503	0.142	-0.215	0.297	-0.303	-0.292	-0.149	-0.219

Yield attributing quantitative traits such as 100 seed weight, pod width, pod length and earliness are the traits for grouping the pea accessions (Gixhari et al, 2014, Devi et al, 2021, Roseroet al, 2021). As, the first two principal components largely discriminate the pea accessions, they can serve as an important traits for the characterization of these accessions.

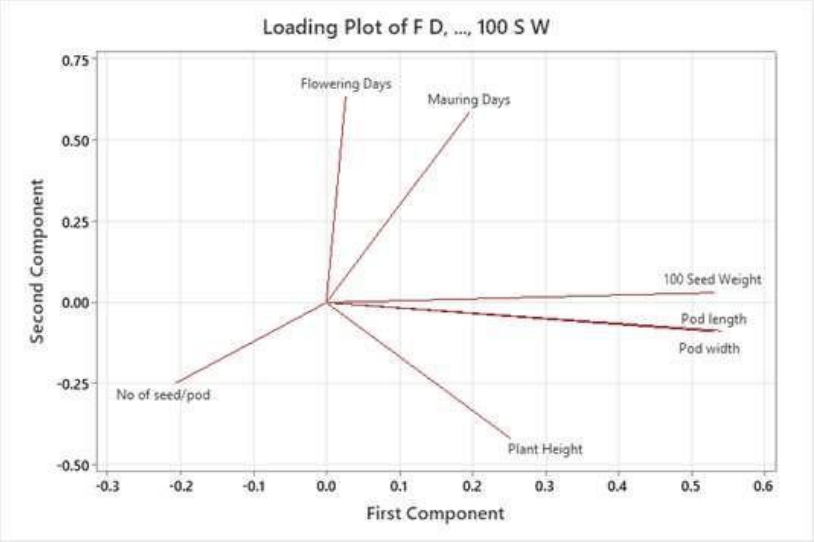

Fig. 2 Loading plot of agro-morphological traits of eight pea genotypes included in yield trial

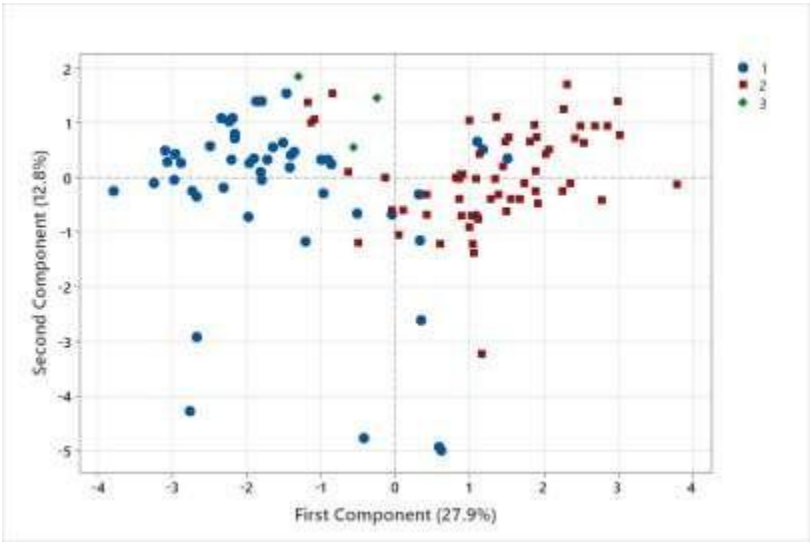

Fig. 3 Scatter plots based on first two principal component for qualitative traits

Result of this study revealed the dominant influence of the qualitative traits like flower colour, tendril colour, seed size, seed colour with positive loading (Rosero et al, 2021, Devi et al, 2021) and leaf size with negative loading in the first principal component. The genotypes in cluster I stood out taller with higher number of seed weight which is preferred market trait (Rosero et al, 2021).

Cluster Analysis

Four clusters were established with the (Fig. 4), with phenotypic similarities using Euclidean distance and average linkage methods.

The first cluster include 65 genotypes with 53.3% of total population (Table 5). This group in particular stood out for taller plants 112.98 cm (average 87.87) and 100 seed weight 13.69 g (10.78 g). This trait is important yield attributing parameter and higher seed weight is also market preferred trait (Rosero et al, 2021).Second cluster is made up of 14 genotypes with 11.5% of the studied population corresponding to attributes pertaining to yield attributing parameters as pod length 5.8 cm(5.3), pod width 5.9cm (5.7) and number of seeds/pod 6 (5). However, the genotypes are late maturing 159 days (150).

Third cluster consists of two genotypes with (1.6%) and pertaining the early maturing traits. For the early maturing for pre breeding, the two genotypes in this cluster should be selected. The fourth cluster with 41 genotypes (33.6%) genotypes are less plant heights 81.8 cm(98.8) as shown in table 5.

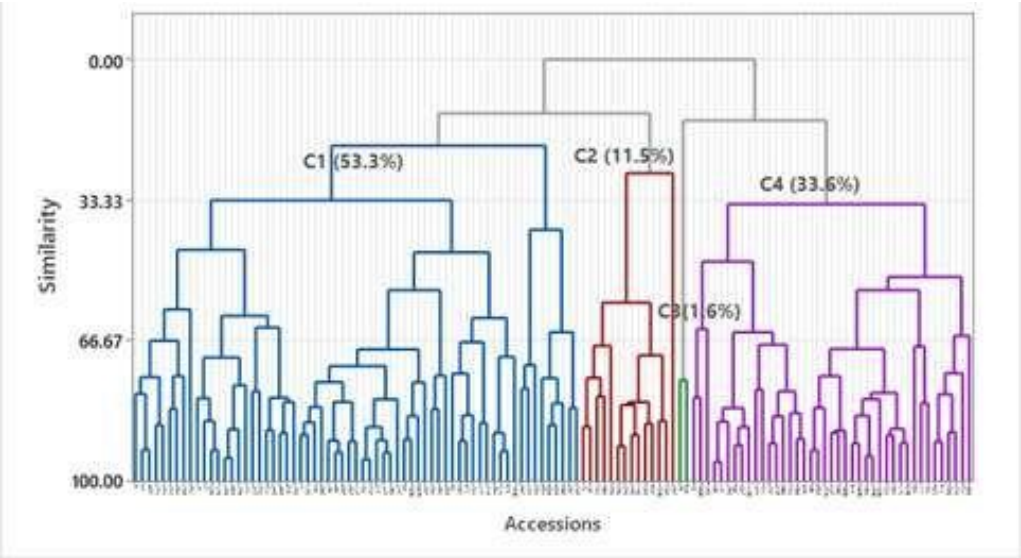

Fig. 4 UPGMA clustering Pea based on Euclidean distance for quantitative traits

Table 5. Grouping of pea landraces by Euclidean UPGMA method

Cluster	I		II	III	IV	
Number of accession /cluster	65		14	2	41	
Genotypes	CO 2028	CO 2190	CO 2033	CO2030	S_23	CO 2200
	CO 2029	CO 2189	S_65	CO1153	S_30	CO 2197
	CO 2931	CO 2188	C0 1795		S_86	CO 1142
	CO 2032	CO 2186	CO 1790		S_166	CO 1135
	S_1	CO 2181	D_217		S_16	CO 1136
	S_25	CO 2206	D_135		S_250	CO 1137
	S_31	CO 2205	D_205		S_263	CO 1138
	S_36	CO 2204	D_215		D_45	CO 1145
	S_50	CO 2203	Acc_11128		CO 1798	CO 241
	S_71	CO2201	CO 245		CO 1791	Acc_11126
	S_175	CO 2199	D_142		CO 2187	Acc_11124
	S_188	CO2198	Acc_9144		CO 2185	CO 242
	S_196	CO 1800	Acc_9151		CO 2184	CO 246
	S_254	CO 1801	Acc_9110		CO 2182	CO 243
	S_260	CO 1139			CO 2209	Acc_7845
	D_16	CO 1140			CO 2208	Acc_7847
	D_27	CO 1143			CO 2207	Acc_8374
	D_40	CO 1144			CO 2202	Acc_8377
	D_57	CO 1146			Acc_8376	Acc_7588
	D_79	CO 1147			Acc_9207	Acc_6116
	D_91	CO 1148			Acc_6118	
	D_116	CO 1149				
	D_1799	CO 1150				
	D_1797	CO 1151				
	D_1796	CO 1152				
	CO 1794	CO 1154				
	CO 1792	Acc_8357				
	CO 2196	Acc_6130				
	CO 2195	Acc_6131				
	CO_1793	Acc_6132				
	CO_2194	Acc_6115				
	CO_2193					
	CO_2192					
	CO_2191					

Table 6. Cluster means for pea landraces

Traits	Cluster I	Cluster II	Cluster III	Cluster IV
Flowering days	91.831± 10.4 (76-118)	111.357± 9.762 (86-129)	85.000± 7.318 (83-87)	94.488± 9.825 (81-108)
Pod length	5.902±1.083 (4.46-7.92)	5.829±1.004 (4.3-7.94)	4.710± 0.906 (4.6-4.8)	4.314±1.098 (3.2-5.02)
Pod width	6.575±1.383398(4.76-9.11)	5.952±1.290 (4.37-7.3)	5.785±1.271 (5.23-6.23)	4.349±1.404 (0.92-5.62)
Maturity days	149.415±7.263 (137-158)	158.571± 7.368 (152-164)	116.000± 6.884 (115-117)	148.634±6.579 (137-158)
Plant height	112.985±31.734 (39-185)	83.614±27.907 (13.2-116.2)	96.0±25.394 (71.4-120.6)	81.854±30.148 (43.4-146.88)
Number of seeds/pod	5.345±0.792 (3-7)	5.900±0.7124 (5-7)	6.300±0.720 (6-7)	6.010±0.706 (5-7)
100 seed weight	13.698±5.932 (5.3-28.5)	11.216±5.205 (8.23-15)	6.200±4.922 (6.1-6.3)	6.244±5.955 (3.2-20.5)

*value in parenthesis shows the range of the concerned trait.

Correlation Analysis

Correlation analysis studies the relationship between yield and its parameters. Pearson correlation analysis among seven quantitative traits presented in Table 7 showed highly significant positive correlation between flowering days and maturity days. The association between these explains the need to consider early flowering trait for early maturing of the genotype. The pod length and pod width plant height and pod length and pod length with 100 seed weight has significant positive relation which further supplements the result by the cluster analysis in Fig 2 and Table 6. Number of seed and pod width is significantly negatively correlated and the increase in pod width will not increase the seed number. Increasing the number of seeds per pod, the number of pods per plant and seed weight could be reduced as the Table 7 reveals the significant negative correlation between number of seeds per pod and seed weight. (Tiemerman et al, 2005).

Table 7. Pearson's correlation analysis between seven quantitative traits

Traits	Flowering Days	Pod length	Pod width	Maturity Days	Plant Height	No of seed/pod
Pod length	-0.037					
Pod width	-0.055	0.849**				
Maturing days	0.557**	0.231	0.148			
Plant Height	-0.236	0.365**	0.336**	-0.122		
No of seed/pod	-0.093	-0.108	-0.315**	-0.174	0.111	
100 seed weight	0.059	0.760**	0.771**	0.291	0.304**	-0.304**

*, **, Significantly different from zero at 5 and 1 % level.

Knowledge of correlations among different traits is essential to design an effective breeding strategy for any crop (Mazid et al, 2013). Correlation analysis studies the relationship between yield and its components. Pearson correlation analysis among seven quantitative traits presented in Table 7 showed highly significant positive correlation between flowering days and maturity days. The simple explanation for this result would be that, the earlier the genotype flowers, the early will be the maturity. The association between these explains the need to consider early flowering trait for early maturing of the genotype. The pod length and pod width plant height and pod length and pod length with 100 seed weight has significant positive relation which further supplements the result by the cluster analysis in Fig 2 and Table 5. Number of seed and pod width is significantly negatively correlated and the increase in pod width will not increase the seed number. Increasing the number of seeds per pod, the number of pods per plant and seed weight could be reduced as the Table 6 reveals the significant negative correlation between number of seeds per pod and seed weight. (Tiemerman et al, 2005).

Similar trend of correlation among these characters for garden pea has also been previously reported by Rahman et al. (2019). The negative correlation between number of seeds per pod with 100 seed weight was observed in these genotypes which as reported by Bashir et al. (2014). They also observed positive association among pod length, pod width, seeds pod-1 and green pod yield similar to this study. Similarly, Rahman et al. (2019) also observed positive correlation of pod yield with plant height, pod length, pods plant-1 and seeds pod-1. Green pod yield manifested significantly positive correlation with plant height, pods plant-1, pod length, 100-green seed weight and 100-green pod weight (Table 6), indicating the importance of these traits in the improvement of green pod yield of garden pea. Similar trend was reported by Singh et al. (2019). The positive correlation between days to flowering and maturity suggests that these traits can be advantageously used for selecting early genotypes. The expression of seed weight is governed by additive and

non-additive genetic effects however the selection genotypes with more pods per plant and a greater number of seeds per pod could improve yield (Brijendra et al, 2013; Iqbal et al, 2017; Kumar et al, 2017; Gupta et al, 1984).

CONCLUSION

The collections from Genebank requires a good phenotyping and evaluation to identify the elite lines for pre breeding. Based on the phenotypic traits, the study demonstrated the existence of a high amount of agro morphological diversity in pea genotypes. This variation indicated that there is a way to identify promising genotypes based on the traits. However, for pre breeding with the defined breeding objective, the genotype selection should be carried out based on the cluster analysis result from this study. The result of this study is based on one year and the interaction between environment and genotype is not taken into consideration. However, the preliminary result of the experiment related to morphological trait is very important to characterize the existing genotypes, as it helped to estimate variability existing in the landraces which were due to genotype character.

Understanding of the interaction of the traits among themselves and with the economic yield is of great use for plant breeders. Correlation studies provide information on the nature and extent of association between any two traits which makes it possible for genetic improvement in one trait that could subsequently improve the other trait of a pair. Designating a germplasm subset based exclusively on agro morphological data will allow Genebanks to develop a core set as representative as possible of the available genetic diversity.

With this study, CO2198 for more seed weight and CO2030 for early maturity could be identified as elite genotypes. The promising genotype requires further morphological and molecular evaluation for identification of elite lines. The promising landraces needs to be maintained as core collection of garlic germplasms for Nepal Genebank.

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Appendix A: List and code of entries used for evaluation

SN	Entries	Code
1	Reg .N. CO 2028	CO 2028
2	CO 2029	CO 2029
3	CO 2931	CO 2931
4	CO 2032	CO 2032
5	CO 2033	CO 2033
6	CO 2030	CO 2030
7	Surkhet - 1	S_1
8	S - 23	S_23
9	S - 25	S_25
10	S - 30	S_30
11	S - 31	S_31
12	S - 36	S_36
13	S - 50	S_50
14	S - 65	S_65
15	S - 71	S_71
16	S - 86	S_86
17	S - 166	S_166
18	S - 16	S_16
19	S - 175	S_175
20	S - 188	S_188
21	S - 196	S_196
22	S - 250	S_250
23	S - 254	S_254

24	S - 260	S_260
25	S - 263	S_263
26	Dailakh - 16	D_16
27	D - 27	D_27
28	D - 40	D_40
29	D - 45	D_45
30	D - 57	D_57
31	D - 79	D_79
32	D - 91	D_91
33	D -116	D_116
34	CO 1799	CO 1799
35	CO 1798	CO 1798
36	CO 1797	CO 1797
37	CO 1796	CO 1796
38	C0 1795	C0 1795
39	CO 1794	CO 1794
40	CO 1792	CO 1792
41	CO 1791	CO 1791
42	CO 1790	CO 1790
43	CO 2196	CO 2196
44	CO 2195	CO 2195
45	CO 1793	CO 1793
46	CO 2194	CO 2194
47	CO 2193	CO 2193
48	CO 2192	CO 2192
49	CO 2191	CO 2191
50	CO 2190	CO 2190
51	CO 2189	CO 2189
52	CO 2188	CO 2188
53	CO 2187	CO 2187
54	CO 2186	CO 2186
55	CO 2185	CO 2185
56	CO 2184	CO 2184
57	CO 2182	CO 2182
58	CO 2181	CO 2181
59	CO 2209	CO 2209
60	CO 2208	CO 2208
61	CO 2207	CO 2207
62	CO 2206	CO 2206
63	CO 2205	CO 2205
64	CO 2204	CO 2204
65	CO 2203	CO 2203
66	CO 2202	CO 2202
67	CO 2201	CO 2201
68	CO 2200	CO 2200
69	CO 2199	CO 2199

70	CO 2198	CO 2198
71	CO 2197	CO 2197
72	CO 1800	CO 1800
73	CO 1801	CO 1801
74	CO 1142	CO 1142
75	CO 1135	CO 1135
76	CO 1136	CO 1136
77	CO 1137	CO 1137
78	CO 1138	CO 1138
79	CO 1139	CO 1139
80	CO 1140	CO 1140
81	CO 1143	CO 1143
82	CO 1144	CO 1144
83	CO 1145	CO 1145
84	CO 1146	CO 1146
85	CO 1147	CO 1147
86	CO 1148	CO 1148
87	CO 1149	CO 1149
88	CO 1150	CO 1150
89	CO 1151	CO 1151
90	CO 1152	CO 1152
91	CO 1153	CO 1153
92	Dailakh - 217	D_217
93	D - 135	D_135
94	D - 205	D_205
95	D - 215	D_215
96	CO 241	CO 241
97	Acc.N. 11128	Acc_11128
98	Acc.N 11126	Acc_11126
99	Acc.N. 11124	Acc_11124
100	CO 242	CO 242
101	CO 246	CO 246
102	CO 1154	CO 1154
103	CO 245	CO 245
104	CO 243	CO 243
105	Acc.N. 7845	Acc_7845
106	Acc.N. 7847	Acc_7847
107	Acc.N. 8374	Acc_8374
108	Acc.N. 8375	Acc_8375
109	Acc.N .8376	Acc_8376
110	Acc.N. 8377	Acc_8377
111	Dailakh - 142	D_142
112	Acc.N. 7588	Acc_7588
113	Acc.N. 9144	Acc_9144
114	Acc.N. 9207	Acc_9207
115	Acc.N. 9151	Acc_9151

116	Acc.N. 9110	Acc_9110
117	Acc.N. 6130	Acc_6130
118	Acc.N. 6131	Acc_6131
119	Acc.N. 6132	Acc_6132
120	Acc.N. 6115	Acc_6115
121	Acc.N. 6116	Acc_6116
122	Acc.N. 6118	Acc_6118