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Biochemical characterization and variability in garden pea (*Pisum sativum* var. hortense) under cool hilly weather conditions

NIRMAL KUMAR HEDAU[1], RAMESH SINGH PAL[2], SALEJ SOOD[3], CHAUDHARI GANESH VASUDEO[4], LAKSHMI KANT[5] and ARUNAVA PATTANAYAK[6]

ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand 263 601

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ABSTRACT

Malnourishment is widespread and severe problems in most of the developing countries, and nutritionally rich food can address this issue efficiently by introducing nutritionally rich cultivars for cultivation. In this endeavor, 33 genotypes (30 agronomically superior advance lines and three cultivars) were analyzed for nutritional and essential agro-morphological attributes. This set of materials has shown wide significant variations for most of the nutritional attributes indicating significant levels of genetic diversity. Antioxidant metabolites (total carotenoids and total polyphenols) and total chlorophyll were positively correlated with each other. First, four principal components explained 70.47% of the total variation. Best-performing lines were marked for important nutritional and agro-morphological attributes and may be tested in the multi-location trial to be released as new nutritionally rich cultivars for on-farm production. Alternatively, these could also be of instant significance as a donor in the future breeding program.

Key words: Antioxidant properties, Biochemical characterization, Garden pea, Nutritional traits

Garden pea (*Pisum sativum* L.) is cultivated from the foothills to higher hills (northwestern Himalayan regions, temperate zone) and north Indian plains (subtropical zone) in different seasons (Hedau *et al.* 2015). Green peas are consumed as cooked and fresh, and generally marketed as fresh green pods throughout the year and across the world. Nutritionally, garden pea has its significance for higher proteins 7.2 g, fats 0.1 g, minerals 0.8 g, carbohydrates 15.8 g, calcium 20 mg, magnesium 34 mg, phosphorus 139 mg, copper 0.23 mg, sulphur 95 mg, iron 1.5 mg, riboflavin 0.01 mg, nicotinic acid 0.8 mg and vitamin C 9.0 mg/100g of edible portion (Sepehya *et al.* 2015). No significant amounts of toxicity or anti-metabolites in peas have been reported (Smart 1990). The higher amount of the phytic acid content present in food compounds leads to low bioavailability of iron, calcium and magnesium. Although detrimental effects of phytates have been reported, alleged beneficial effects have also emerged. Exploring bio-diversity to get better productivity and adaptation with the high nutritional value of vegetable crops, consumed as fresh, is of the chief significance in the current breeding programmes. The high carbohydrates, protein, better antioxidant properties with low phytic acid are the main nutritional parameters of green peas. The present study was undertaken to evaluate high yielding advanced lines developed at ICAR-VPKAS, Almora, Uttarakhand, India for the important nutritional and important agro-morphological attributes.

[1](e mail: hedaunirmal_2003@yahoo.co.in and Nirmal.Hedau@icar.gov.in); [2](e mail: Ramesh.Pal@icar.gov.in); [3](e mail: Salej.Sood@icar.gov.in); [4](e mail: Chaudhari.Ganesh@icar.gov.in); [5](e mail: Lakshmi.Kant@icar.gov.in); [6](e mail: director.vpkas@icar.gov.in).

MATERIALS AND METHODS

The present study, agronomically superior thirty advanced lines along with three released cultivars for the North-West Himalayan region, viz. VL *Ageti Matar* 7 (VL 7), *Vivek Matar* 10 (VM 10) and *Vivek Matar* 11 (VM 11) of garden pea were planted in a field experiment at ICAR-VPKAS, Experimental farm, Hawalbagh (29º36' N, 79º40' E and 1250 m above msl) under North-West Himalayan conditions. All standard recommended cultivation practices were followed with regard to nutrition supply, irrigation and plant protection measures during the entire growing season. Garden pea genotypes were evaluated in three replicates for the important nutritional attributes, viz. total chlorophyll (TChl), total carotenoids (TCar), total polyphenols (TPP), total carbohydrates (TCarbs), total sugar (TS), starch (ST), phytic acid (PA), total protein (TP) and important agro-morphological traits (days to first pod harvest (DTFPH), pod length (PL), shelling percentage (SP) and green-pod yield (PY)). Total chlorophyll and carotenoids were estimated in fresh green grain state and expressed on fresh weight basis, whereas other nutritional parameters were estimated on dry weight basis. Samples were dried in oven at 50±2°C and dried samples were milled to flour by using Newport

scientific super mill grinder with a 0.25 mm sieve. The samples were stored in airtight containers for further analysis and evaluated for other important nutritional parameters by following standard protocol(s)/procedures.

All the chemicals and reagents were of analytical grade and double-distilled water was used throughout the analysis.

Total chlorophyll and carotenoids were estimated by the spectrophotometeric method (Nagata and Yamashita 1992). The total polyphenolic (TPP) compounds were determined by Folin Ciocalteu reagent (Singleton and Rossi 1965) and calculated from a standard calibration curve based on tannic acid (0–0.1 mg/mL), and the results were expressed as tannic acid equivalents mg per g dry weight (mg TAE/g DW). The nitrogen content was estimated by Kjeldhal method, based on the assumption that plant proteins contain 16 g/100 g nitrogen. Crude protein content was calculated using the formula, crude protein = nitrogen×6.25. Phytic acid contents of defatted legume flours were determined by the method of Haug and Lantzsch (1983). The phytic acid content was calculated from a calibration curve using phytate phosphorus salt in the range of 10–50 µg. Total sugar content and starch content were determined calorimetrically by the anthrone method (Thimmaiah 1999). Total carbohydrates content was estimated by Phenol–Sulfuric Acid Method (Dubois *et al.* 1956).

Data represent the mean of three replicate samples for each genotype. Analysis of variance (ANOVA) was performed using the Microsoft Excel. The genotypic and phenotypic coefficient of variation and heritability (broad sense) were calculated by standard statistical procedure (Burton and De Vane 1953, Johnson *et al.* 1955). The genotypic and phenotypic correlation coefficient was calculated as per the method of Singh and Choudhary (1979). The Principal Component Analysis based on Pearsons correlation matrix and cluster analysis were performed using a demo version of XLSTAT–Pro (Addinsoft). Correlation biplots of traits were generated on which genotypes were superimposed.

RESULTS AND DISCUSSION

The highly significant difference in mean squares inferred that there is inherent genetic variability among the genotypes with respect to all the attributes under study.

Frequency distribution for agro-morphological attributes

Significant variations were recorded in all four agro-morphological attributes, viz. days to first green pod harvest, i.e. earliness (127-138 days), pod length (7.2-10.3 cm), shelling percentage (45-60.2) and green pod yield (5.58-12.44 MT/ha), indicating the presence of considerable variability for these attributes (Table 1). Frequency distribution for green pod yield among the genotypes was classified into four groups with 2, 4, 10 and 17 genotypes in each group, respectively. The last frequency group, i.e. highest green pod yield ranged from 10.73- 12.44 MT/ha (Fig1c). Genotypes were also classified into four groups for other agro-morphological attributes, viz. earliness, pod length and shelling percentage. With regard to earliness, i.e. days to first pod harvest, the genotypes 2, 6, 23 and 2 were extra early, early, medium and late in maturity, respectively. In case of pod length and shelling percentage last two higher-frequency groups comprised 64 and 33% lines, respectively (Fig 1c).

Frequency distribution for nutritional attributes

Wide significant variations were observed for most of the attributes, viz. total chlorophyll (1.41-3.99 mg/100g), total carotenoids (4.92-13.23 mg/100g), total polyphenols (0.75-1.68 mg/100g), total carbohydrates (21.99-53.88

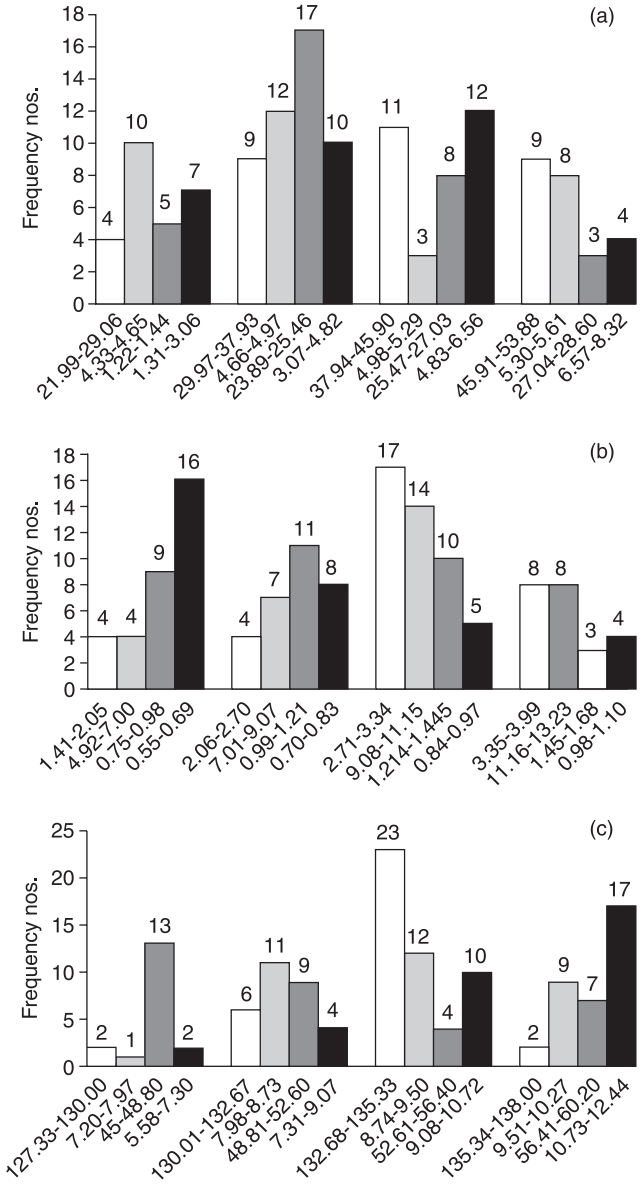

Fig 1 Frequency distribution for (a) Total carbohydrates (□), Total sugar (▒), Total protein (▓) and starch (■); (b) Total chlorophyll (□), Total carotenoids (▒), Total polyphenols (▓) and phytic acid (■); (c) Days to first fruit harvest (□), Pod length (▒), Shelling percentage (▓) and green pod yield (■) of 33 garden pea genotypes.

mg/100g), total sugar (4.33-5.61 mg/100g), starch (1.31-8.32 mg/100g), phytic acid (0.55-1.10 mg/100g) and total protein (22.31-28.60%), showing substantial variability for nutritional attributes (Table 1). All the nutritional attributes were classified into four groups. Last frequency group showing highest values comprised 9, 8, 3 and 4 genotypes for total carbohydrates, total sugar, total protein and starch, respectively (Fig 1a). Last two groups possessing the higher amount of antioxidant metabolites (total carotenoids and total polyphenols) and total chlorophyll, comprised about 40-75% of total genotypes (Fig 1b). In case of phytic acid content, more than 50 per cent genotypes fall in first frequency group (lower values) ranged from 0.55 to 0.69 mg per 100 g which is an acceptable range with respect to bioavailability of nutrients (Fig 1b).

Genetic variability

The mean squares and genetic parameter estimates for the 12 attributes are mentioned in Table 1. The analysis of variance showed that the mean squares for the genotypes were highly significant for all the attributes under study. The variance components showed the higher phenotypic variance than the genotypic variance in most of the traits studied. The phenotypic variance was divided into heritable (genotypic variance) and non heritable (environmental variance) components. The magnitude of the genotypic variance for all the traits was higher than the environmental variance (Table 1). The findings are similar to the earlier reports (Jaiswal *et al.* 2015, Singh 1985, Tiwari and Lavanya 2012) in pea, (Ubi *et al.* 2001, Omoigui *et al.* 2006) in cowpea and (Hedau *et al.* 2008[a,b]) in tomato and capsicum. The bare minimum differences in GCV and PCV coupled with low ECV for the traits studied implied that the traits are mostly presided over by genetic factors with the modest role of environment in the phenotypic expression of the traits. Hence, selection for these traits on the basis of the phenotypic value will be highly effective. This variability could form the basis to proceed further in genetic improvement for these nutritional quality traits through hybridization and selection.

Broad sense heritability estimates were generally high ranging from 93.41-99.72% for all the nutritional attributes except total protein (69.84%). Among agro-morphological attributes, 89.71, 91.71, 98.83 and 99.91% heritability (broad sense) were found for green pod yield, days to first fruit harvest, pod length and shelling percentage, respectively as reported earlier (Singh 1985, Tiwari and Lavanya 2012). However, these heritability estimates along with genetic advance will be more sensible in predicting the ensuing effect for the selection of the best individuals from a population (Ubi *et al.* 2001). With respect to nutritional attributes, genetic advance in per cent of mean was recorded maximum for starch content followed by total carotenoids, total chlorophyll, phytic acid, total carbohydrates, total polyphenols, total sugar and total protein. However, among agro-morphological traits, green pod yield, shelling percentage, pod length and days to first fruit harvest had shown 27.81, 19.82, 16.96 and 2.72% genetic advance, respectively.

Correlation

The nature and magnitude of both genotypic and phenotypic correlation coefficients between the nutritional traits were estimated and presented in Table 2. The genotypic correlation coefficients were higher than their corresponding phenotypic correlation in most of the nutritional attributes indicated that the association was mainly due to genetic factors. The correlation between agro-morphological attributes (days to first fruit harvest, i.e. earliness, pod length,

Table 1 Range, mean, variance, coefficient of variation, heritability (broad sense) and genetic advancement for different attributes in garden pea lines

Trait	Range	Mean	MS[a]	CD (P=0.05)	PV[b]	GV[c]	EV[d]	PCV[e] (%)	GCV[f] (%)	ECV[g] (%)	H^2B^h (%)	GA[i] (%) mean
TChl (mg/100g)	1.41-3.99	3.00	1.26	0.12	0.425	0.420	0.005	21.73	21.59	2.43	98.77	44.21
TCar (mg/100g)	4.92-13.23	9.82	14.08	0.37	4.727	4.672	0.055	22.14	22.01	2.34	98.88	45.09
TPP (mg/100g)	0.75-1.68	1.15	0.16	0.10	0.054	0.051	0.004	20.27	19.59	5.20	93.41	39.00
TCarbs (mg/100g)	21.99-53.88	39.54	196.94	2.72	67.509	64.681	2.829	20.78	20.34	4.25	95.81	41.02
TS (mg/100g)	4.33-5.61	4.89	0.43	0.11	0.147	0.143	0.005	7.85	7.72	1.42	96.70	15.64
ST (mg/100g)	1.31-8.32	4.59	8.47	0.14	2.825	2.818	0.008	36.62	36.57	1.93	99.72	75.23
PA (mg/100g)	0.55-1.10	0.74	0.07	0.05	0.024	0.023	0.001	20.77	20.42	3.68	96.69	41.37
TP (%)	22.31-28.60	25.11	5.21	1.31	2.173	1.520	0.653	5.87	4.91	3.22	69.84	8.45
DTFPH (Days)	127.33-138.00	131.94	10.20	0.88	3.610	3.315	0.295	1.44	1.38	0.40	91.71	2.72
PL (cm)	7.20-10.27	9.08	1.70	0.13	0.572	0.565	0.007	8.33	8.28	0.90	98.83	16.96
SP (%)	45.00-60.20	51.62	74.10	0.24	24.715	24.693	0.022	9.63	9.63	0.29	99.91	19.82
PY (MT/ha)	55.78-124.35	103.29	674.91	8.06	241.652	216.644	25.008	15.05	14.25	4.83	89.71	27.81

[a] = mean squares, [b] = phenotypic variance, [c] = genotypic variance, [d] = environmental variance, [e] = phenotypic coefficient of variation, [f] = genotypic coefficient of variation, [g] = environmental coefficient of variation, [h] = broad sense heritability, [i] = Genetic advance in % of mean.

Table 2 Genotypic (r_g) and Phenotypic (r_P) correlation coefficient among different nutritional attributes in garden pea lines

Variables		TChl	TCar	TPP	TCarbs	TS	ST	PA	TP
TChl	r_g		0.9997**	0.3491*	-0.0131	0.0435	0.0345	-0.1490	0.1339
	r_P		0.9867**	0.3388*	-0.0085	0.0445	0.0343	-0.1474	0.1086
TCar	r_g			0.3426*	-0.0342	0.0145	0.0871	-0.1774	0.1406
	r_P			0.3293*	-0.0360	0.0178	0.0867	-0.1710	0.1020
TPP	r_g				0.0085	0.1663	0.0008	0.0933	0.2802
	r_P				0.0050	0.1544	0.0007	0.0889	0.2303
TCarbs	r_g					0.9409**	0.0396	0.2863	-0.0274
	r_P					0.9109**	0.0364	0.2743	-0.0170
TS	r_g						-0.0018	0.1557	0.0742
	r_P						-0.0017	0.1529	0.0307
ST	r_g							-0.3919*	-0.2305
	r_P							-0.3845*	-0.1870
PA	r_g								0.0933
	r_P								0.0722
TP	r_g								
	r_P								

*, ** Significant at P=0.05 and P=0.01 level, respectively.

shelling percentage and green pod yield) is not estimated due to non fulfilment of normal distribution of the sample. This may be due to the fact that all the advanced lines were selected for desired value of agro-morphological traits. The positive significant correlation was found among total chlorophyll, total carotenoids and total polyphenols and also between total sugar and total carbohydrates contents in fresh green grain pods. However negative correlation was found between phytic acid and starch content in fresh green grain. Pea starch is advantageous in nutritional point of view due to its considerable resistant starch content (Polesi *et al.* 2011). However, higher amount of the phytic acid content leads to low bioavailability of iron, calcium and magnesium in food compounds. Therefore, high total carotenoids, total sugar and starch would be suitable selection criteria for the genetic improvement in nutritional quality attributes except total protein in fresh green grain.

Cluster analysis

Hierarchical cluster analysis was used to see patterns of clustering between the garden pea genotypes. The data matrix included as objects each of the 12 attributes analysed for the 33 genotypes. Pearson correlation was used as similarity criterion and furthest neighbour as a clustering method (Fig 2). Using similarity level, garden pea genotypes were classified into three groups. The dendrogram of 33 genotypes showed three clusters (Fig 2). Cluster 1 consisted of 22 genotypes, all of which were advanced breeding lines and released varieties developed at the Institute. Cluster 2 comprised eight genotypes derived from crosses where VM 11 was used as one of the parents. Three genotypes (VP 1331, VP 1332 and VP 1323) formed cluster 3, which are all elite fixed breeding lines, indicating the low level of genetic diversity. The clustering pattern observed in the present study clearly indicated that the variables included in the study were sufficient and the parental genotypes used to generate the garden pea breeding lines were diverse. Further, based on nutritional parameters the genotypes from different clusters can be used in breeding programme to generate new variants.

Principal Component Analysis

Variation and association present in the genotypes were also explained by the principal components analysis taking

Table 3 Squared cosines of the variables

Parameter	Factors			
	F1	F2	F3	F4
TChl	0.682	0.118	0.021	0.052
TCar	0.708	0.103	0.011	0.046
TPP	0.133	0.179	0.053	0.139
TCarbs	0.116	0.733	0.062	0.003
TS	0.040	0.772	0.045	0.000
ST	0.036	0.003	0.623	0.016
PA	0.197	0.117	0.282	0.000
TP	0.031	0.023	0.266	0.339
DTFPH	0.185	0.000	0.070	0.383
PL	0.479	0.085	0.071	0.006
SP	0.486	0.033	0.043	0.150
PY	0.425	0.069	0.020	0.003

Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

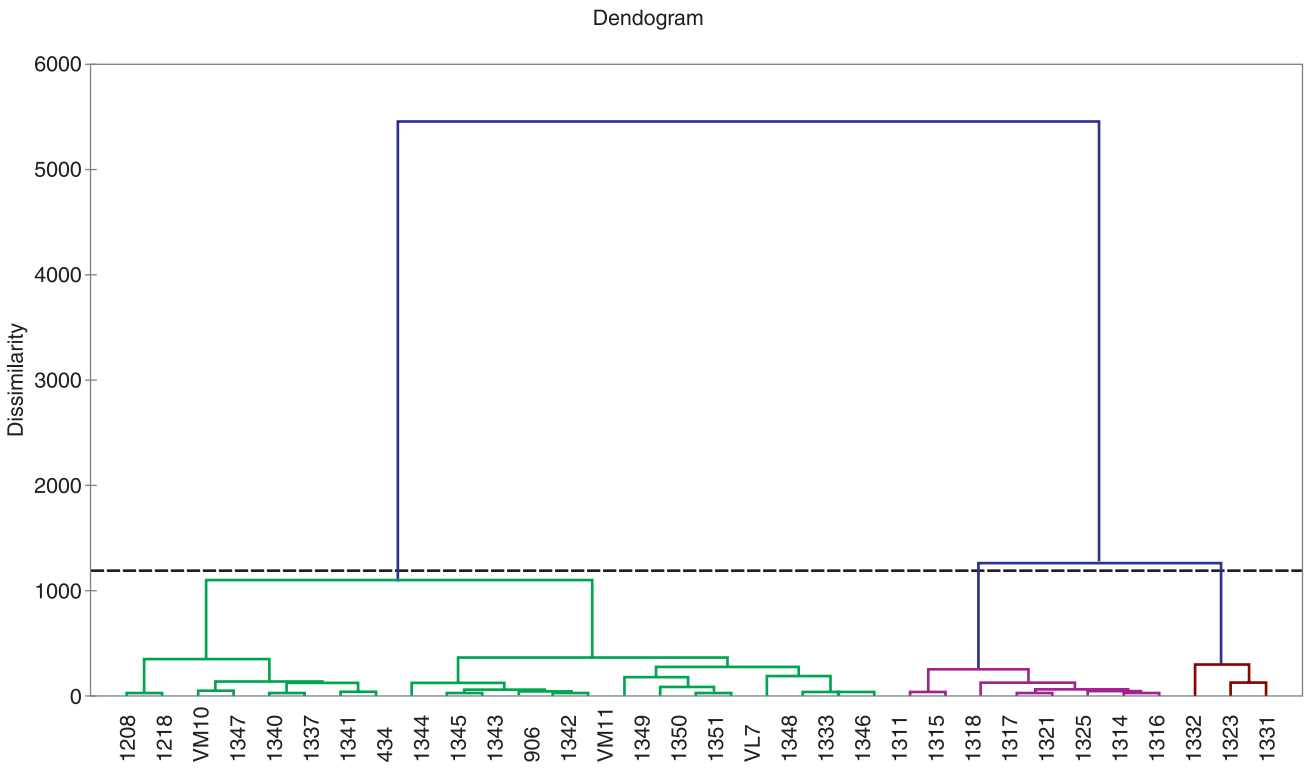

Fig 2 Dendogram showing the relationship among 33 garden pea genotypes based on the eight quality and four agro-morphological attributes.

eigenvalues greater than unity that explained 70.47% of the variance. Best-performing genotypes were marked for important nutritional and agro-morphological traits studied. Squared cosines of the variables (Table 3) showed that Factor 1 is related mainly to Tchl and TCar, Factor 2 to Tcarbs and TS, Factor 3 to ST and PA, and Factor 4 to TP. Principal component analysis indicated strong positive correlations between TChl and TCar as seen from the plot of Factor 1 (F1) and Factor 2 (F2) which described 47.95% of the total variation (Fig 3). Positively significant correlation was also

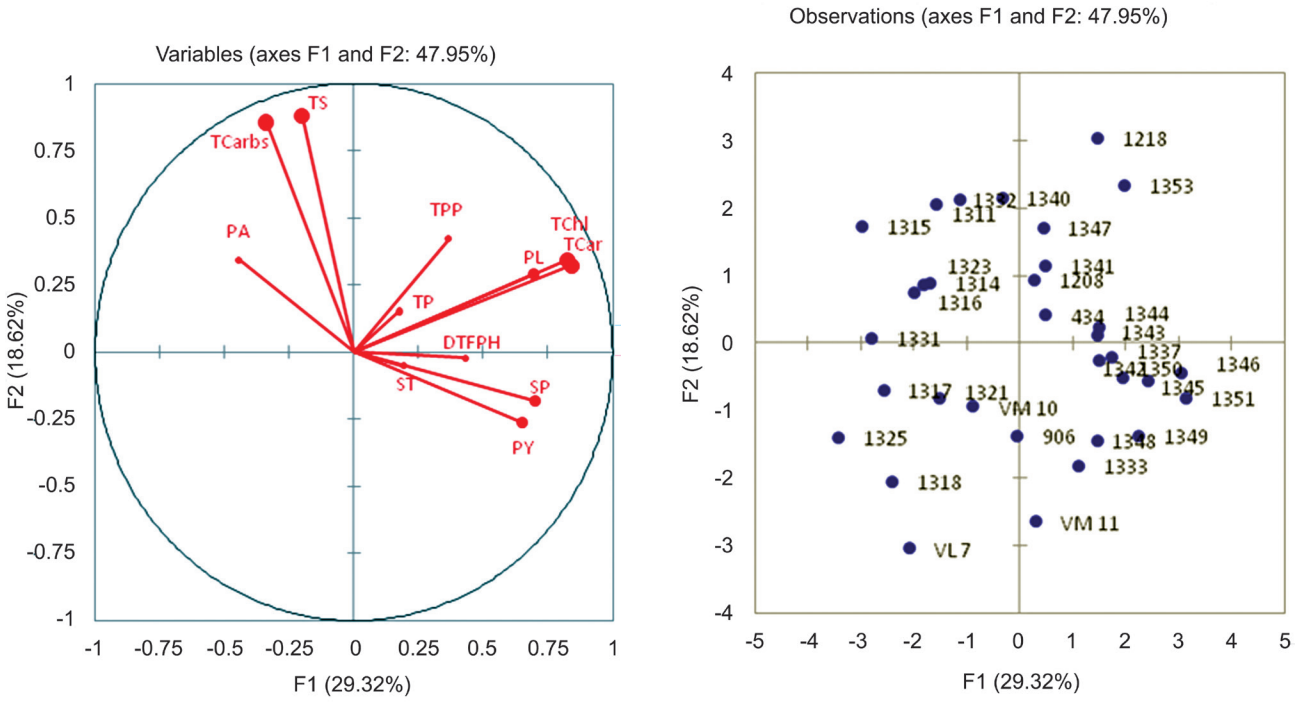

Fig 3. F1:F2 plot showing relationship among traits (TChl, TCar, TCarbs and TS) and lines.

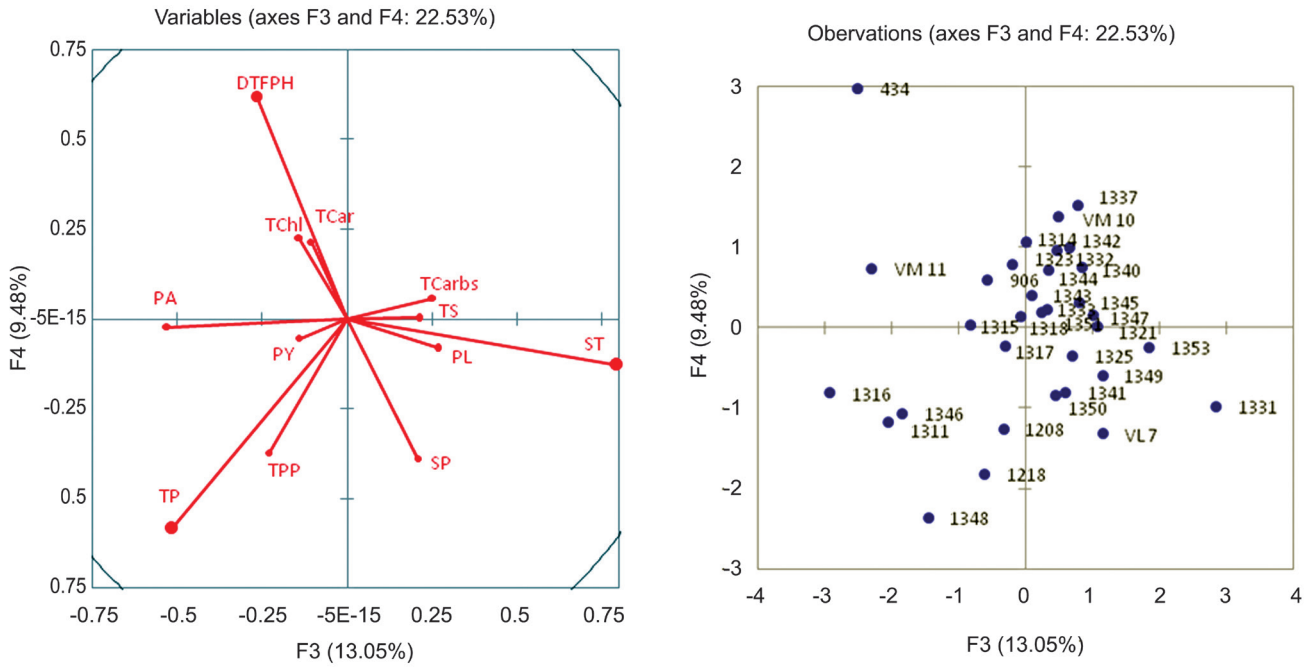

Fig 4. F3:F4 plot showing relationship of ST, PA and TP with other traits and lines.

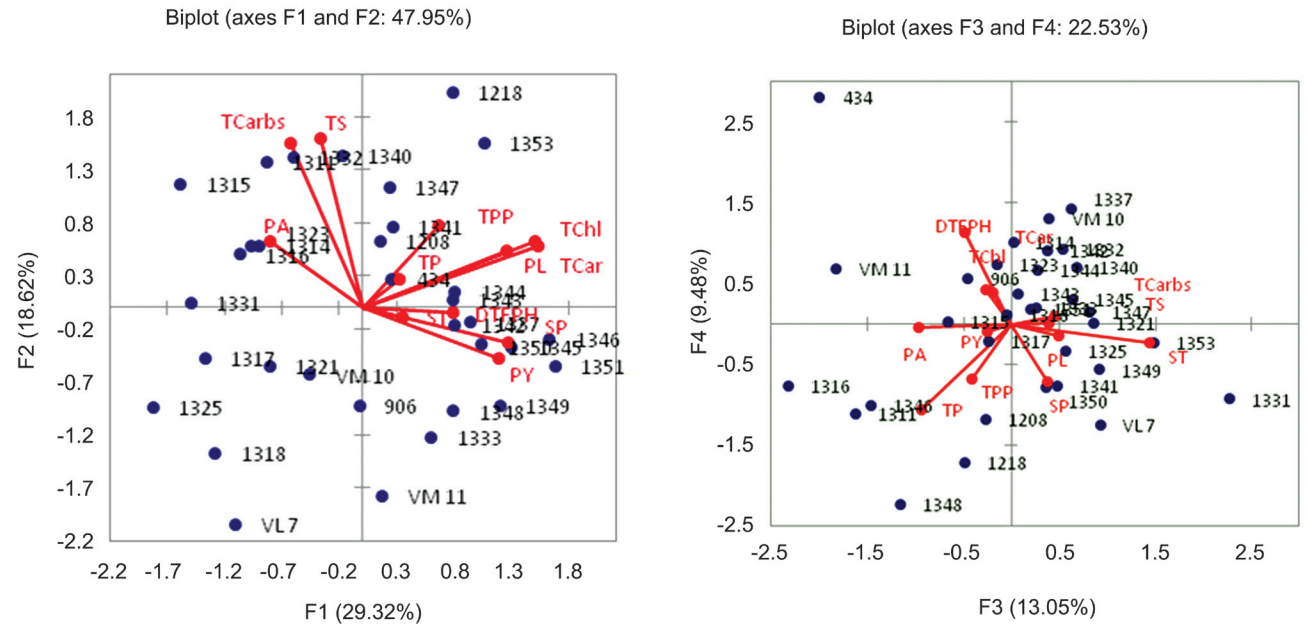

Fig 5. Biplots (F1:F2 and F3:F4) showing relationship among traits and lines.

observed between TS and Tcarbs. This plot, however, failed to explain status of ST, PA and TP which can be viewed on F3: F4 plot (Fig 4). The plot F3: F4 indicated that PA is negatively correlated with the ST, as may be seen from Table 3. Superimposing the genotypes (lines) on the four traits biplots (Fig 5, F1:F2) indicated that VP 1340, VP 1218, VP 1315, VP 1331 and VP 1332 are exceptional for Tcarbs and TS. With respect to Tchl and TCar, lines VP 1346, VP1345, VP1337, VP1351, VP1353 and VP 1218 were found unique. VP 1349, VP 1350, VP 1343, VP 1208 and VP 1218 were found outstanding for high green pod yield and high shelling percentage. Overlaying genotypes on three traits biplots (Fig 5, F3:F4) showed that VP 1346, VP 1348 and VP 1218 are rich in TP content. VP 906, VP 1342, VP 1331 VL7 and VM 10 were shown lower values for PA content. VP 1353 and VP 1321 showed better ST content in peas (Table 3).

Identified promising lines for the important nutritional traits like VP 1346 (chlorophyll, carotenoid and protein); VP 1341 (Polyphenols); VP 1340 (carbohydrates and sugar) and VP 906, VP 1331 and VP 1342 (low phytic acid) could be of instant significance for further use in breeding programme.

Lines, viz. VP 1349, VP 1208 and VP 1218 showed the high green pod yield with reasonably better nutritional attributes, therefore, may be future wonder varieties. Further, some of the promising lines may also be used as donor in future breeding programmes.

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