

Influence of leaf architecture on morpho-phenological and yield related traits of garden pea

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ABSTRACT

Plant leaf fixes the atmospheric carbon dioxide and controls the yield performance of a crop plant. Variation in leaf morphology correlates to the growth performances across the species. Garden pea (*Pisum sativum* **L. ssp.** *hortense***), having multi-foliate leaves, serves as a model for studying the leaf morphology due to the complex genetic and epigenetic regulation of the trait found in this species. We assessed the effect of three different leaf morphology- normal leaf (NL), semi-leafless (SL), and leafless (LL) on the morpho-phenological and yield-attributing traits in twenty genotypes of garden pea. It was found that leafless type had excellent standing ability and open canopy, while yield and yield related traits were highly correlated and superior in normal leaf type. The positive correlation between number of pods per plant and seeds per pod, pod length with pod yield indicates that these traits are the main components for yield contribution of three different leaf morphology of pea genotype. Principal component analysis (PCA) grouped the genotypes having a better standing ability, maturity time and yield performance in distinct class.**

Key words: Garden pea; leaf morphology; yield related traits; correlation; principal component analysis

INTRODUCTION

Garden pea (*Pisum sativum* L*.* spp. *hortense*) is one of the major winter vegetables and occupies 2.6 million ha around the globe, (Anon., 1). India is the second largest pea producing country with 17.0% of total world production after China (39.1%). Besides its significant value to food and nutritional security, garden pea has also been considered as an important model crop for studying the diverse leaf and canopy morphology in terms of leaf type, plant growth habit and indeterminacy/determinacy (Goldman *et al.,* 4 and Snoad, 11).

The wild/normal type mature pea is pinnately compound and consists of basal, foliaceous stipules, proximal leaflets and distal tendrils (Fig. 1) being represented by the genetic make-up *Af Tl St.* They typically exhibit the maximum yield potential, but affected under environmental limitation. Other types of pea are completely leafless (*af st Tl*), with reduced leaf and stipule due to the gene *af* and *st*. They have low plant growth rate, reduced leaf area and lower light interception than the conventional (standard) and semi-leafless types (Heath and Hebblethwaite, 6) which affects total pod yield significantly. Semileafless peas (*af ST Tl*), that have leaflets transformed into tendrils due the gene *af* and standard stipules (*ST*), counterbalance these disadvantages drawback

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due to the presence of developed normal stipules. Foliage type mutant (*Af St tl)* is a very rare mutant and high yielding. Notwithstanding, its acceptability is low due to lack of tendrils. Tendrils support the plants and reduced lodging as wind, winter rainfall and increasing pod weight usually results in lodging by harvest time. Reduced lodging may results in greater yield (Wehner *et al*., 13).

It was reported that pea yield is a function of plant population density, pod length, pod number per plant, seed number per pod, and weight of individual seeds (French, 3; Poggio *et al.*, 7) and average pod weight. Green pod yield and yield related traits are superior in normal leaf peas to other leaf types, therefore, in India, normal leaf garden peas are more popular and commercially grown in pea growing area. But in the era of climate change, drought resistant varieties are sustaining weather vagaries, leafless and semi-

Fig. 1. Different type of leaf morphology used in this study.

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leafless peas are more suitable because of the reduced total surface leaf area and better standing ability. Additionally lodging, blonding (yellow-shelled of pea due to less light interruption) and excess foliage are common in normal leaf morphology which is also a problem in processing type pea. Considering the advantages mentioned above for different pea foliage type, our objective in this study was to compare the performance of leafless and semi-leafless foliage type with the normal one to test whether any were superior to the normal type.

MATERIALS AND METHODS

The experiment was conducted in research farm of the Division of Vegetable Science, Indian Agricultural Research Institute (IARI), New Delhi India for three consecutive years. New Delhi is located at an elevation of about 228 m above MSL, 20°40' north latitude and 77°13' east longitudes. The climate of New Delhi is typically sub-tropical with hot summers and cool winters. Twenty lines, fifteen from different places of India and six from the United States of America (Table 1), which included different leaf architecture, *viz.*, normal leaf (NL), semi-leafless (SL), and leafless (LL) were evaluated

for morpho-phenological and yield attributing traits in field condition in a randomized block design. Seeds were planted in first week of October in 5 m² plot with a spacing of 40 cm × 5 cm in three rows of 4.2 m length. The crop was fertilized with farmyard manure (10 tonnes/ha) and NPK (20:60:40 kg/ha), along with recommended cultural practices.

Data were recorded on eight traits *i.e.* days to 50% flowering $(D_{50}f)$, node bearing the first flower (NBF) recorded as the first flowering node from the base of the plant, plant height (cm) (PH) recorded as vine length from the soil surface to the tip of the main stem, number of pods per plant (NPP) as the total numbers of pods including all the pods with at least one seed on the main stem, pod length (cm) (PL), number of seeds per pod (NSP), shelling percent (SP) and green pod yield (q/ha) (YL).

Based on the phenotypic expression, two genotypes, Arkel (NL) and GP-6 (LL), having contrasting leaf morphology were selected for crossing to study the yield related in different generation. Arkel is an early maturing variety while GP-6 is a late bearing genotype. GP-6 was planted in the first week of October while Arkel was planted in the first week of November in order to achieve flowering

synchronization for making crosses. The F_1 seeds were collected and stored in a room at ambient condition. In next year, the F_1 s were grown during same time under almost same weather conditions and were manually selfed to obtain F_2 progenies. The final experiment for segregation study was laid out with thirty-five plants each of P_{1} and P_{2} , and thirty plants of F₁ and 208 of F₂ were grown in separate blocks in the following year. Further morpho-phenological and yield related traits were recorded for four generations $(P_1, P_2, F_1 \text{ and } F_2).$

Data with average of three replicates for each genotype were subjected to analysis of variance. Pearson correlation coefficients and level of significance were calculated, at p<0.05. Variability analysis for different traits was conducted using Principal Component Analysis (PCA), to evaluate the contribution of each quantitative character to the total variation of all genotypes. All statistical analysis was

carried out based on quantitative characters using R software 3.4.

RESULTS AND DISCUSSION

The ANOVA indicates highly significant differences (*P ≤* 0.01) for all the eight traits among the 20 garden pea genotypes (Table 2).

 $D_{\epsilon,0}$ was maximum for the LL genotype, GP-6 (103.26 days) followed by the SL plants ranging from 82.37 to 91.03 days, while NL plant took a smaller number of days to reach there (42.23-60.16 days). GP-17 line recorded shortest vegetative phase (D_{50} f in 42.23 days) and flowering duration indicating that this line follows an ephemeral life cycle which has a better water use efficiency. GP-6 (36.39 cm), which was LL, was found to be the shortest genotype under study. Wide range of variation was observed for different trait like NPP (9.4 to 18.36), PL (5.55 to 9.81 cm), NSP (4.07 to 8.85), SP (36.65 to 52.08) and YL (58.42 to

Table 2. Performance of twenty garden pea genotypes for various yield and yield related traits.

Genotypes	D_{50} f	NBF	NPP	NSP	PH	PL	SP	YL
AP ₃	47.38 ^{efg}	8.97fghi	16.34abcd	7.76 abc	52.46 ^{def}	8.39 ^{cd}	44.18bc	76.81fgh
Arka Ajit	58.56 ^d	9.38 ^{fgh}	13.97 ^{de}	7.55^{bc}	53.95^{de}	7.44 ^{ef}	39.65 ^{def}	77.67 ^{fgh}
Arkel	51.23 ^{ef}	9.62 ^{fgh}	17.28 abc	8.08 abc	48.99 ^{ef}	8.26 _{cde}	48.14^{ab}	80.30 ^{efg}
GP 17	42.239	8.39hi	16.51 abcd	7.84 ^{abc}	45.16 ^f	7.47 ^{ef}	50.90 ^a	88.97 ^{de}
GP 473	60.16 ^d	8.78ghi	18.36a	8.28ab	59.53^{cd}	9.81a	50.14a	104.18 ^a
GP-943	53.52^{de}	10.85 def	15.36bcde	7.96 abc	63.13°	7.87def	43.98bcd	99.66 ^{ab}
GP-48	53.29def	9.88fgh	14.47 ^{cde}	7.69abc	51.86 ^{ef}	7.36 ^f	41.75 ^{cdef}	81.87 ^{ef}
GP-904	48.90 ^{efg}	9.87 ^{fgh}	16.63 abcd	8.37 ^{ab}	65.40 ^c	9.52^{ab}	41.79cdef	92.54bcd
GP-906	53.84 ^{de}	12.39 _{bcde}	14.46 ^{cde}	8.26 ^{ab}	59.74 ^{cd}	7.80def	43.02 ^{cde}	98.19abc
Pusa Pragati	46.18 ^{fg}	8.82ghi	18.19ab	8.85^{a}	49.39ef	8.72bc	52.08 ^a	78.35fgh
$VL-10$	47.56 ^{efg}	8.95 ^{ghi}	16.69 abc	8.04^{ab}	73.46 ^b	7.96 ^{def}	43.04cde	90.67 ^{cd}
$VL-7$	48.61 ^{efg}	8.28hi	16.41abcd	8.37 ^{ab}	48.46 ^{ef}	7.46 ^{ef}	50.21a	83.47def
VRP-6	48.16 ^{efg}	10.49 ^{efg}	16.52 abcd	8.05 abc	47.94 ^{ef}	8.27 ^{cde}	42.17cdef	77.00fgh
EC-677211	83.93bc	15.71a	9.40 ^f	5.33 ^d	91.03 ^a	5.899	39.16 ^{ef}	63.19 ^{jk}
EC-677212	84.37bc	13.17^{bc}	9.50 ^f	5.00 ^d	87.67 ^a	6.039	39.84 ^{cdef}	65.67 ^{ijk}
EC-677213	85.67bc	12.61bcd	12.63^e	5.00 ^d	90.10 ^a	5.569	39.99 ^{cdef}	71.67 ^{ghij}
EC-677214	82.45 ^c	14.10^{ab}	12.77e	5.33^{d}	86.73a	6.049	40.30 ^{cdef}	71.00hij
EC-677215	82.37 ^c	12.90^{bc}	12.90°	5.33^{d}	85.43a	6.279	37.98 ^f	74.33fghi
EC-677216	91.03 ^b	11.87 ^{cde}	13.10^e	5.67 ^d	89.77 ^a	5.719	39.33 ^{ef}	79.67fgh
GP ₆	103.26°	7.30 ⁱ	10.08f	4.07e	36.39 ^g	7.77 def	36.66 ^f	58.42 ^k
General Mean	62.87	10.54	14.86	7.23	64.76	7.50	43.35	81.16
p-Value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
CV(%)	7.00	11.38	11.92	9.97	6.91	6.88	6.29	6.84

Day to 50% flowering (Days)(D_{En}f), Node bearing first flower (NBF), Plant height (cm) (PH), Number of pods per plant (NPP), pod length (cm) (PL), number of seeds per pod (NSP), shelling percent (SP) and green pod yield (q/ha) (YL). Same letters indicate no significant difference, different letters indicate significant difference at p=<0.0001.

104.18 q/ha). There was a clear difference between three different leaf forms with respect to different agronomic traits. LL genotype, GP-6 took maximum D_{50} f followed by SL genotypes. Yield associated traits like NPP, PL, NSP were also inferior in LL and SL as compared to normal leafy lines. Correlation study showed that the pod yield was positively associated with NPP, PL, NSP and SP but negatively associated with D_{50} f, NBF and PH (Fig. 2).

It was also observed that D_{50} f, NBF, and PH were negatively associated with pod forming traits such as NPP, PL, NSP and SP. This is due to the fact that the dry matter distribution is the consequence of assimilates flowing from source organs to sink organs via a transport path. It is mostly governed by the vigour (competitive ability to attract assimilates) of the sink organs, as vegetative development continues throughout the production of reproductive structures in indeterminate type peas. The presence of two sinks that can potentially draw assimilates and their competition result in poor pod development and filling. The connection between yield related features and pod yield demonstrated a clear demarcation of lines based on their leaf and yield attributes using principal component analysis (Fig. 3a, b).

The first principal component (PC), with an eigen value of 5.85, accounted for 73.2 percent of the overall variability of the data set. The eigen value of the second PC was 1.09, and it was responsible for 13.6 percent of the variation. The remaining six generated PCs (*i.e.* PC3 to PC8) yielded progressively smaller eigen values and did not explain significant variability in the data set (˂13.2% total). The sample score plot for PC1 vs. PC2 is shown in Fig. 3a, b. Furthermore, a particular characteristic or line is from the origin, the more observations diverge (differ), and the more characteristics or lines cluster into discrete groups

Fig. 2. Correlation presented in red and blue colour represents the relationship between variables and the colour intensity showed correlation magnitude between the observed variables.

and are positioned in distinct quadrants, the more they diverge from each other. Days to 50% flowering (D50f), node bearing first flower (NBF), and plant height (PH) from pod length (PL), number of pods per plant (NPP), are all positioned in the opposite direction (obtuse angle to straight line) (Figure 3a), indicating a significant negative correlation between plant growth related traits and pod formation traits, which agreed with the negative association obtained through correlation analysis (Table 3.). Almost all semi-leafless lines were found on the other side of the cluster of lines with typical leaves, pods, and yield characteristics (Fig. 3b). Also, leafless pea line were positioned in the negative side of PC1, at

Fig. 3. Principal component analysis (PCA) plots. (a) Loading plots for different morpho-phenology and yield related traits on PC1 and PC2 and (b) PCA scores plot for different garden pea advanced breeding lines.

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an obtuse angle with semi-leafless and normal, and more related to trait days to 50% flowering. This indicates that leafless pea is low yielding and has later maturing growth habit. Despite its unfavorable yield and maturity, leafless type may be useful in situation where excellent standing ability and

an open top are high priorities. To combine, high yielding capacity of Arkel (NL type) and excellent standing ability of GP-6 in one line, a cross was made (Fig. 4).

The yield performance of F_1 generation raised by crossing normal and leafless line and $F₂$ from

Fig. 4. Flow chart of crossing between Normal leaves (Arkel as Female parents) and Leafless (GP-6 as male parents) lines.

the selected F_1 plant on the basis of phenotypic performance of the cross progeny showed better standing ability and almost similar yield to normal type. It is important here to mention that there is an issue to reduced cross-compatibility between Arkel and GP-6 as very few seeds were formed per pod. For better understanding of the trend of different trait and comparison with both parents, mean data were recorded on $\mathsf{P}_{_{1}},\mathsf{P}_{_{2}},\mathsf{F}_{_{1}},$ and $\mathsf{F}_{_{2}}$ for Arkel × GP-6 cross combination and are presented in the Box and whisker plot (Fig. 5).

Most of the growth related and yield attributing traits of segregating generation were found intermediate between both parents. There were significant differences among these generations $(P_1,$ P_2 , F_1 and F_2) for all quantitative characters. Arkel is a very popular genotype with superior horticultural traits than GP-6. Traits related to earliness of genotypes like days to 50% flowering and node bearing first flower the F_1 and F_2 were between parental values but improve standing ability than Arkel. In both segregating generation, we found that number of pods per plant, pod length, seed number per pod, shelling percentage of F_2 were higher than GP-6 (LL type parent line). The findings will aid breeders in combining superior standing ability and high yielding characteristics in a single genotype, allowing them to achieve the aim of long-term performance in lowyielding environments.

The arrangement of leaves and supporting structure (branches, tendrils, stems) across the species are well adapted for intercepting light, higher

Fig. 5. Box and whisker plots of parents (Arkel (P1) –NL type and GP-6 (P2)-LL type) and segregating generation (F₁) and F_2) for comparison of morpho-phenological and yield attributing traits in different generation.

leaf area means high light interception. It results into more photosynthesis and ultimately high yield potential. In garden pea, total area of leaf *i.e* the combined areas of the leaflets, stipules, petioles, and tendrils accounts for most of the photosynthetic surface of the plant. Various studies reported that normal leaflets and stipule size exhibit highest yield potential in garden pea (Baigorri *et al*., 2; Wehner *et al*., 13). We also found normal leaf (NL) pea lines (GP-473) produced highest yield. However SL lines (EC-677215 and EC-677216) produced similar yield to normal leaf type peas. It was reported by Wehner *et al.* (13) that SL type pea averaged only 62% of total area. While GP-6, a LL type peas has smallest leaf area, 24% of the area of normal type. GP-6 has dwarf plant type and it is very desirable plant type in garden pea from commercial point of view as it does not require staking and results in saving resources both in terms of money and labour (Shubha *et al*., 9). An extra early NL type pea line (GP-17) was also identified in this study, which first flowered in within 30 day of sowing and 50% flowered in less than 45 days of sowing. Further, if we compare other yield related quality traits like number of pods per plant, pod length, number of seeds per pod and shelling percentage of lines, the NL pea lines were found higher. However, under stressed condition like prolong drought, NL cultivars do not perform well due to yield reduction (Stelling, 12). Similarly, under stressed condition, LL type lines restrict leaf growth and light interception, which negatively affects seed yield but on contrary, SL type lines counterbalance the negative effect and make-up yield potential due to developed stipules (Baigorri *et al*., 2). Additionally, the water use by SL lines is less than that of NL type pea lines because of reduced leaf surface area (Harvey, 5). It was also reported by Baigorri *et al.* (2) that in drought condition, SL type of lines maintain a similar percent allocation of dry matter into pod and seeds as in normal watered condition. These types of low water requiring lines producing similar amount of dry matter can be recommended in drought prevailing area. SL and LL type lines have reduced leaf surface; therefore, pest and disease attack are comparatively less than in NL type lines. Interestingly leaf minor and powdery mildew (devastating disease of pea prevailing in dry weather particularly at the time of pod maturity) was affected less in SL and LL type lines than NL, although we did not scale the severity of disease incidence in different leaf type. Further, in another study with the same set of genotypes for Fusarium wilt resistance by the authors in which, most of the semi leafless and leafless lines were found resistant to Fusarium wilt (Shubha *et al.*, 10). A comparison of important traits observed in three different leaves mention in (Table 3).

Plant population density, pod number per plant, seed number per pod, and individual seed weight all influence pea yield (French, 3; Poggio *et al*., 7). In segregating generation, we found that all traits related to pod quality (pod length, number of pods per plant, shelling percentage, seed number per pod) were higher than GP-6 (LL type parent line). Earliness is a highly desirable quality in vegetables in the sense that the prevailing prices in the market are invariably high early in the season. Days to flowering and node bearing first flower depict the earliness of a particular genotype (Sharma *et al.*, 8). We also found F₂ plants took lesser number of days to flower than GP-6 parent line and also flowered in lower node than Arkel parent line. Results indicate that crossing of NL and LL type pea lines combine desirable attributes. Therefore, breeding in this direction would help in achieving line(s) having high yield potential and short stature and superior combining ability. Moreover, the information about better standing ability, open canopy along with high yield potential of genotypes become essential for area having lower harvestable pod yields, higher yield variability and a reduction in suitable pea growing areas.

AUTHORS' CONTRIBUTION

Conceptualization of research (K. Shubha and ShriDhar); Designing of the experiments (K. Shubha and ShriDhar) Contribution of experimental materials (ShriDhar) Execution of field/lab experiments and data collection (K. Shubha) Analysis of data and interpretation (Raman R. K. and K. Shubha) Preparation of the manuscript (K. Shubha, Mukherjee A, and Maity A.).

DECLARATION

The authors declare no conflict of interest

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