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EXPLORING THE VARIATION IN PHYSIOLOGICAL PARAMETERS AMONG DIFFERENT FIELD PEA GENOTYPES

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ABSTRACT

Field pea (*Pisum sativum* L.) is a significant pulse crop valued for its nutritional benefits. This study investigates the variation in physiological parameters such as leaf area, leaf area index (LAI), chlorophyll content and SPAD values among diverse field pea genotypes and their correlation with yield components. Using a randomized complete block design with three replications, the study assessed leaf area, chlorophyll content and SPAD values at various growth stages, alongside yield parameters including the number of pods per plant, number of seeds per pod and seed yield per plant. Results revealed that leaf area and LAI increased up to 60 DAS, with a subsequent decline at harvest. The genotype IPFD6-3 consistently exhibited superior leaf area and LAI. Chlorophyll content, measured as chlorophyll *a*, *b* and total chlorophyll, also varied significantly among genotypes, peaking at 60 DAS before declining. The highest chlorophyll content was observed in IC381455, correlating with better photosynthetic capacity. SPAD values, indicative of leaf nitrogen content, showed significant variations, with IC381455 performing best. Correlation analysis revealed that leaf area and chlorophyll content were strongly associated with yield components such as the number of pods per plant and the number of seeds per pod. Significant correlations were found between leaf area and total chlorophyll content ($r = 0.688$), as well as SPAD values ($r = 0.561$). Chlorophyll content was positively correlated with yield traits, emphasizing its role in enhancing reproductive development. While SPAD values correlated with yield components, the correlation with seed yield per plant was weaker ($r = 0.438$), indicating the influence of other factors. The findings underscore the importance of physiological parameters in determining field pea yield and suggest that optimizing leaf area and chlorophyll content can improve crop productivity.

Key words : Field pea, Leaf area, Chlorophyll content, SPAD values, Yield components.

Introduction

Field pea (*Pisum sativum* L.) is a crucial pulse crop extensively utilized in human nutrition. As a self-pollinated diploid (2n=14 chromosomes) belonging to the Fabaceae family, it features distinct green and yellow cotyledons. Originating from the Mediterranean region of Southern Europe and Western Asia, the field pea is characterized by its herbaceous, bushy, or climbing form, often glaucous in appearance. The plant's stems are weak, round and slender, ranging from 30 to 150 cm in length. Its leaves are alternate and pinnate, with 1-3 pairs of leaflets and a terminal branched tendril, while the leaflets themselves are ovate or elliptic. The inflorescence is a raceme that emerges from the axil of the leaf (Le *et al*., 2007). Globally, pea is the third most important pulse crop, following dry bean and chickpea, and in India, it ranks third among *rabi* pulses after chickpea and lentil. Nutritionally, field pea is rich in high-quality vegetable protein and contains all essential amino acids, making it a viable substitute for high-protein animal meat products in developing countries, including India. Fresh green peas are widely accepted as a nutritious vegetable (Singh *et* *al*., 2011).

Field pea thrives in a variety of soil types, from light sandy loams to heavy clays, but it does not tolerate saline or waterlogged conditions. As a winter crop, it requires a cool growing season with moderate temperatures throughout its lifecycle. High temperatures are more damaging to pea crops than frost and high humidity combined with cloudy weather can lead to fungal diseases such as damping-off and powdery mildew (Santalla *et al*., 2001). Seed and biomass yields in field pea are primarily influenced by cultivar, location and environmental conditions. The significant variation in pea seed quality within a year indicates the strong impact of environmental factors, agronomic practices and genetic factors (Kasturikrishna and Ahlawat, 1999). Yield variability and instability are major challenges in pea production, due to poor adaptability and low tolerance to biotic and abiotic stresses. Key yield-limiting factors include aphids, low-yielding local varieties, lodging, diseases (ascochyta blight, powdery mildew) and pod shattering. Abiotic stresses, such as high temperature and soil water deficit, are prevalent across growing areas and can significantly reduce yields (Kumar *et al*., 2003). The productivity differences among genotypes are assessed through the study of growth and yield parameters. Leaf area, as an indicator of photosynthetic capacity, influences the photosynthetic rate and thereby crop productivity (Wang *et al*.,, 1998). Variations in leaf area, chlorophyll content, and other related traits among genotypes and their relationship with yield, have been documented in several crops. This study aims to identify better-performing genotypes based on physiological parameters and to explore their correlation with yield.

Materials and Methods

The experiment was conducted using a randomized complete block design (RCBD) with three replications. The gross plot size was 3 m x 2.25 m (6.75 m²) and the net plot size was 2.4 m x 2.15 m (5.16 m²), with inter-row spacing of 45 cm and intra-row spacing of 10 cm. Fertilization involved applying 20:40:60 kg NPK per hectare in the form of urea, single super phosphate, and muriate of potash as a basal dose at sowing. Seeds of field pea genotypes, sourced from AICRP College of Agriculture, Vijayapur, University of Agricultural Sciences, Dharwad were dibbled at a depth of 5.0 cm on October 21, 2021. Aftercare included irrigation at critical growth stages, earthing up at 30 days after sowing and maintaining weed-free plots through inter culture and hand weeding. Recommended fungicidal and insecticidal sprays were applied to protect the crop from diseases and pests. Harvesting occurred at physiological maturity, indicated by yellowing pods. The harvested pods were sundried for a day, manually threshed, cleaned and dried until the moisture content reached 13 percent, after which the net plot yield was recorded for all plots.

Collection of experimental data

Leaf area per plant (dm² plant⁻¹) : Leaf area were determined by using disc method on dry weight basis (Vivekananda *et al*., 1972).

$$\text{Leaf area (LA)} = \frac{\text{Weight of dry leaves per plant (g)} \times \text{Area of disc (dm}^2\text{)}}{\text{Weight of disc (g)}}$$

Leaf area index (LAI) : The leaf area index was worked by using the formula given by Watson (1952).

$$\text{LAI} = \frac{\text{Leaf area per plant (dm}^2\text{)}}{\text{Land area occupied by the plant (dm}^2\text{)}}$$

SPAD values: The chlorophyll content of green leaves can now be measured using a SPAD (Soil Plant Analysis Development) chlorophyll meter, which provides a reading indicative of leaf greenness. This reading varies with changes in the leaf's nitrogen content (Sheshshayee *et al*., 2016).

Estimation of chlorophyll Content : The chlorophyll content was measured at 30 and 60 DAS using the method described by Shoaf and Lixm (1976). Fresh leaf tissue (100 mg) was cut into small pieces and incubated in 10 ml of dimethyl sulfoxide (DMSO) in the dark for 24 hours. After incubation, the sample was placed in a boiling water bath for five minutes. The optical density was then measured at 663 nm and 645 nm using a UV-VIS spectrophotometer. If the volume decreased during boiling, it was adjusted back to 10 ml with DMSO. Chlorophyll-a, chlorophyll-b, and total chlorophyll contents were calculated using the formulae provided below and expressed in milligrams per gram of fresh weight of the sample (mg g^{-1} fr. wt.).

$$\text{Chlorophyll 'a'} = (12.7\ A663)+(2.69\ A645) \times \frac{V}{1000 \times a \times W}$$

$$\text{Chlorophyll 'b'} = (22.9\ A645)+(4.68\ A663) \times \frac{V}{1000 \times a \times W}$$

$$\text{Total Chlorophyll} = (20.2\ A645)+(8.02\ A663) \times \frac{V}{1000 \times a \times W}$$

Where, A = Absorbance at specific wave length (645, 663 nm), V = Final volume of the chlorophyll extract (ml), W = Fresh weight of the sample (g), a = Path length of light (l)

Number of Pods and Seeds per plant : The number of pods produced per plant was counted and recorded at harvest in tagged plants. Additionally, the total number of seeds per pod in tagged plants was counted, and the mean number of seeds per pod was calculated.

Seed yield per plant (g) : The seeds separated from each plant were weighed and seed yield per plant were expressed in gram using electronic balance.

Statistical analysis : Fischer's method of analysis of variance (ANOVA) was used to analyze the data and interpret the results, following the recommendations of Panse and Sukhatme (1967). The significance level for the F and t tests was set at P=0.05. Critical difference (CD) values were calculated at the 5 percent probability level wherever the F test was significant.

Correlation Plot Analysis : A correlation plot was created using R software. The dataset was imported and a correlation matrix was computed with the cor() function. The correlation plot was generated using the corrplot() function from the corrplot package.

Results and Discussion

Leaf Area and Leaf Area Index (LAI)

Leaf area is a crucial morphological character in field pea, significantly contributing to yield by reflecting the plant's photosynthetic capacity. Maintenance of leaf area is vital for yield formation, as it provides a good estimate of the plant's ability to photosynthe size. Observations showed that the Leaf Area Index (LAI) and leaf area increased up to 60 days after sowing (DAS) before decreasing sharply, indicating a decline in the plant's capacity to produce and maintain assimilates. The leaf area (dm^2 $plant^{-1}$) at various stages significantly differed among the genotypes at all growth stages (Table 1). Leaf area increased up to 60 DAS, with a slight decrease observed at harvest. At 30 DAS, the genotype IPFD6-3 recorded the highest leaf area (4.52 dm^2), which was on par with genotypes HUP-2 and P-725. The lowest leaf area was recorded in DWD local (2.99 dm^2) and Nippani local-2 (3.05 dm^2). At 60 DAS, IPFD6-3 again showed the maximum leaf area (5.77 dm^2), while DWD Local (4.24 dm^2) and Nippani local-2 (4.30 dm^2) had the minimum. At harvest, IPFD6-3 maintained the highest leaf area (4.25 dm^2), on par with HUP-2 and P-725, with DWD Local (2.72 dm^2) and Nippani local-2 (2.78 dm^2) recording the minimum. These results align with Gupta *et al*. (1986), who indicated a positive correlation between LAI and yield in field pea. Genotypes with higher seed yield exhibited significantly higher leaf area and LAI, confirming the positive association of leaf characteristics with seed yield (Mishra *et al*., 2009). Despite a decline in leaf area and LAI at later stages, maintaining higher values of these parameters is desirable for supporting

Table 1 : Genotypic variation in leaf area (dm^2 $plant^{-1}$) at different growth stages in field pea.

S. no.	Genotypes	Leaf area (dm^2plant^{-1})			Leaf area index		
		Days after sowing			Days after sowing		
		30	60	At harvest	30	60	At harvest
1.	IPF4-9	3.15	4.40	2.88	0.698	0.976	0.638
2.	IPF99-25	3.25	4.50	2.98	0.722	1.000	0.662
3.	KPMR-400	3.36	4.61	3.09	0.747	1.024	0.687
4.	IC381455	3.73	4.98	3.46	0.829	1.107	0.769
5.	IC208399	3.64	4.89	3.37	0.809	1.087	0.749
6.	EC292167	3.59	4.84	3.32	0.798	1.076	0.738
7.	EC598851	3.42	4.67	3.15	0.760	1.038	0.700
8.	P725	3.97	5.22	3.70	0.882	1.160	0.822
9.	P744	3.91	5.16	3.64	0.869	1.147	0.809
10.	HUP-2	4.07	5.32	3.80	0.904	1.182	0.844
11.	IPFD6-3	4.52	5.77	4.25	1.004	1.282	0.944
12.	TRCP-8	3.12	4.37	2.85	0.693	0.971	0.633
13.	DMR-7	3.14	4.39	2.87	0.694	0.972	0.634
14.	RACHANA	3.10	4.35	2.83	0.689	0.967	0.629
15.	Nippani local-2	3.05	4.30	2.78	0.669	0.947	0.609
16.	DWD Local	2.99	4.24	2.72	0.664	0.942	0.604
	Mean	**3.50**	**4.77**	**3.25**	**0.782**	**1.060**	**0.722**
	S.Em. ±	**0.11**	**0.15**	**0.10**	**0.024**	**0.033**	**0.022**
	CD at 5%	**0.30**	**0.43**	**0.29**	**0.070**	**0.095**	**0.065**

Table 2 : Genotypic variation in Chlorophyll *a*, Chlorophyll *b* and Total chlorophyll content in leaf (mg g^{-1} fr.wt.) at different growth stages in field pea.

S. no.	Genotypes	Chlorophyll '*a*' content		Chlorophyll '*b*' content		Total chlorophyll content	
		30DAS	60DAS	30DAS	60DAS	30DAS	60DAS
1.	IPF4-9	1.49	1.67	0.51	0.73	1.94	2.33
2.	IPF99-25	1.45	1.63	0.49	0.70	1.93	2.32
3.	KPMR-400	1.46	1.64	0.53	0.74	2.02	2.40
4.	IC381455	1.81	1.96	0.79	0.98	2.60	2.93
5.	IC208399	1.35	1.55	0.45	0.65	1.87	2.25
6.	EC292167	1.34	1.52	0.43	0.64	1.85	2.22
7.	EC598851	1.59	1.75	0.61	0.82	2.09	2.48
8.	P725	1.57	1.76	0.48	0.66	2.18	2.57
9.	P744	1.69	1.87	0.66	0.87	2.35	2.72
10.	HUP-2	1.62	1.76	0.63	0.84	2.34	2.68
11.	IPFD6-3	1.78	1.95	0.75	0.95	2.53	2.91
12.	TRCP-8	1.33	1.51	0.41	0.58	1.82	2.21
13.	DMR-7	1.54	1.72	0.55	0.76	1.88	2.26
14.	RACHANA	1.72	1.88	0.72	0.93	2.35	2.74
15.	Nippanilocal-2	1.29	1.45	0.38	0.57	1.67	2.03
16.	DWDLocal	1.42	1.60	0.48	0.69	1.91	2.30
	Mean	1.528	1.701	0.554	0.757	2.083	2.459
	S.Em. ±	**0.046**	**0.051**	**0.016**	**0.022**	**0.062**	**0.074**
	CD at 5%	**0.132**	**0.147**	**0.046**	**0.063**	**0.179**	**0.213**

the increasing demand for assimilates during seed development.

The data on LAI is presented in Table 1. The mean LAI ranged from 0.782 at 30 DAS to 1.060 at 60 DAS, decreasing to 0.722 at harvest. Across all genotypes, LAI increased up to 60 DAS before decreasing. At 30 DAS, IPFD6-3 exhibited the maximum LAI (1.004), while DWD local had the minimum (0.664). At 60 DAS, the maximum LAI was recorded by IPFD6-3 (1.282) and HUP-2 (1.182), with the minimum observed in DWD Local (0.942) and Nippani local-2 (0.947). At harvest, IPFD6-3 maintained the highest LAI (0.944), on par with HUP-2 and P-725, whereas the lowest LAI was recorded in DWD Local (0.604), on par with Nippani local-2 and Rachana. These findings suggest that genotypes with higher LAI and leaf area sustain a more active assimilatory surface, crucial for meeting the source demands of developing sinks at later stages.

Variation in Chlorophyll content across Growing Stages in Pea genotypes

The chlorophyll *a*, chlorophyll *b*, and total chlorophyll content values varied significantly among pea genotypes at different growth stages. This variation is largely due to physiological changes occurring throughout the plant's life cycle. Chlorophyll content typically peaks during periods of vigorous vegetative growth when photosynthesis is most active. As the plant approaches maturity, chlorophyll content often declines due to aging, reduced photosynthetic activity and environmental stress. Additionally, chlorophyll synthesis and maintenance are influenced by nutrient availability, particularly nitrogen, which is essential for chlorophyll production. These changes in chlorophyll content reflect the plant's adaptation to its developmental and environmental conditions. Hsiao *et al*. (2016) observed that chlorophyll content decreases with plant age and stress, emphasizing the dynamic nature of chlorophyll synthesis and degradation. The data on chlorophyll *a* (Table 2) revealed significant differences among genotypes at all stages of crop growth. Chlorophyll *a* content generally increased from 30 days after sowing (DAS) to 60 DAS. At 30 DAS, IC381455 recorded the highest chlorophyll *a* content (1.81 mg g^{-1} fresh weight), while Nippani local-2 had the lowest (1.29 mg g^{-1} fresh weight). At 60 DAS, IC381455 again exhibited the highest chlorophyll *a* content (1.96 mg g^{-1} fresh weight), with Nippani local-2 showing the lowest (1.45 mg g^{-1} fresh weight).

Similarly, chlorophyll *b* content showed a slight increase from 30 DAS to 60 DAS. At 30 DAS, IC381455 had the maximum chlorophyll *b* content (0.79 mg g^{-1} fresh weight), while Nippani local-2 had the minimum (0.38 mg g^{-1} fresh weight) (Table 2). By 60 DAS, IC381455 maintained the highest chlorophyll *b* content (0.98 mg g^{-1} fresh weight), whereas Nippani local-2 recorded the lowest (0.57 mg g^{-1} fresh weight). Total leaf chlorophyll content also increased slightly from 30 DAS to 60 DAS (Table 2). At 30 DAS, IC381455 exhibited the highest total chlorophyll content (2.60 mg g^{-1} fresh weight), while Nippani local-2 had the lowest (1.67 mg g^{-1} fresh weight). At 60 DAS, IC381455 continued to show the highest total chlorophyll content (2.93 mg g^{-1} fresh weight), with Nippani local-2 recording the lowest (2.03 mg g^{-1} fresh weight). These results underscore the importance of maintaining higher chlorophyll content, especially at later stages of crop growth, as it contributes significantly to yield determination in field pea. Higher chlorophyll content helps sustain leaf activity for longer periods, thereby supporting the photosynthesis needed for developing pods. Mishra *et al.* (2009) also reported that chlorophyll *a* and chlorophyll *b* contents in green gram peaked up to 45 DAS and declined significantly by 60 DAS.

Variation in SPAD Values among Pea Genotypes

The SPAD (Soil Plant Analysis Development) values, which measure leaf greenness and are indicative of chlorophyll content and nitrogen status, exhibited significant variation among all pea genotypes at different growth stages. At 30 days after sowing (DAS), the genotype IC381455 registered the highest SPAD value (31.67), while Nippani local-2 recorded the lowest (22.40). At 60 DAS, IC381455 again had the maximum SPAD value (47.61), which was on par with IPFD6-3 and Rachana, whereas Nippani local-2 had the minimum value (38.44) (Table 3). These differences can be attributed to the genetic diversity among genotypes, which affects their ability to absorb and utilize nitrogen efficiently, thereby influencing chlorophyll synthesis. Sheshshayee *et al.* (2016) noted that SPAD readings vary with changes in leaf nitrogen content, supporting this explanation. Thus, the varying capacities of different genotypes for nitrogen uptake and assimilation result in significant differences in chlorophyll content and SPAD values.

Yield and Yield Components in Pea genotypes

Grain yield in peas is primarily determined by plant growth, development and various yield components. The assessment of yield levels and their components provides insights into the efficiency of different pea varieties. Variations in seed yield and its components are largely attributable to differences in growth factors, such as total dry matter production and its allocation to reproductive parts. Seed yield results from complex physiological, biochemical and physicochemical interactions within the plant, with dry matter production in reproductive parts being a crucial determinant.

Number of Pods per plant

The number of pods per plant varied significantly among the genotypes (Table 3). The genotype IC381455 produced the highest number of pods per plant (20.60), followed by IPFD6-3 (19.80) and Rachana (18.40). The variation in the number of pods per plant can be attributed to genetic differences among the genotypes, which affect their flowering potential and pod-setting capacity. This result is consistent with the findings of Moot and Neil (2015), who also reported significant differences in pod yield among pea genotypes.

Number of Seeds per Pod

Significant differences were observed in the number of seeds per pod among the genotypes (Table 3). IC381455 had the highest number of seeds per pod (6.60), closely followed by IPFD6-3 (5.80) and Rachana (5.50). This variation is primarily due to genetic factors influencing seed development and pod formation. Saket *et al.* (2017) also reported similar variations in pod yield and seed number among different pea genotypes, highlighting the role of genetic makeup in seed production.

Seed Yield per Plant (g)

Seed yield per plant showed significant variation among the genotypes (Table 3). IC381455 achieved the highest seed yield per plant (9.18 g), with IPFD6-3 (8.05 g) and Rachana (8.00 g) also performing well. The lowest seed yield per plant was recorded in Nippani local-2 (4.54 g). The variation in seed yield is influenced by the genotype's capacity for dry matter accumulation and its efficient allocation to reproductive parts. This finding aligns with previous studies that highlighted differences in seed yield among pea varieties due to genetic and environmental factors (Saket *et al.*, 2017).

Correlation between physiological and yield parameters in field pea genotypes

The analysis of correlations between physiological parameters and yield components in field pea genotypes revealed significant relationships that underscore the impact of physiological traits on yield. Leaf area (LA) at different growth stages (30 DAS, 60 DAS, and harvest) demonstrated robust correlations with total chlorophyll content (TCC) and SPAD values. Specifically, LA at 30 DAS was positively correlated with TCC ($r = 0.688$) and SPAD values ($r = 0.561$), indicating that a larger leaf area supports higher chlorophyll content and better nitrogen status (Fig. 1). This trend persisted through 60

Table 3 : Genotypic variation in SPAD values, yield and yield components in field pea.

S. no.	Genotypes	SPAD Values		Number of pods plants^{-1}	Number of seeds pods^{-1}	Seed yield plant^{-1} (g)
		30 DAS	60 DAS			
1.	IPF4-9	27.31	43.34	13.00	4.50	7.45
2.	IPF99-25	26.53	42.56	16.80	4.80	6.59
3.	KPMR-400	27.50	43.52	16.60	4.60	7.08
4.	IC381455	31.67	47.61	20.60	6.60	9.18
5.	IC208399	26.08	42.02	12.80	4.00	5.41
6.	EC292167	25.32	41.35	12.40	3.80	5.15
7.	EC598851	28.91	44.96	17.20	5.00	6.05
8.	P725	28.55	44.58	16.20	5.00	5.96
9.	P744	30.60	46.67	17.60	5.30	7.80
10.	HUP-2	29.66	45.66	17.30	5.20	7.73
11.	IPFD6-3	31.42	47.41	19.80	5.80	8.05
12.	TRCP-8	24.96	40.99	11.20	3.60	5.10
13.	DMR-7	27.20	43.32	13.80	4.20	5.50
14.	RACHANA	31.12	47.18	18.40	5.50	8.00
15.	Nippanilocal-2	22.40	38.44	10.80	3.40	4.54
16.	DWDLocal	27.52	43.55	16.00	4.20	6.12
	Mean	**27.92**	**43.95**	**15.66**	**4.72**	**6.61**
	S.Em. ±	**0.84**	**1.34**	**0.46**	**0.14**	**0.19**
	CD at 5%	**2.42**	**3.86**	**1.33**	**0.40**	**0.56**

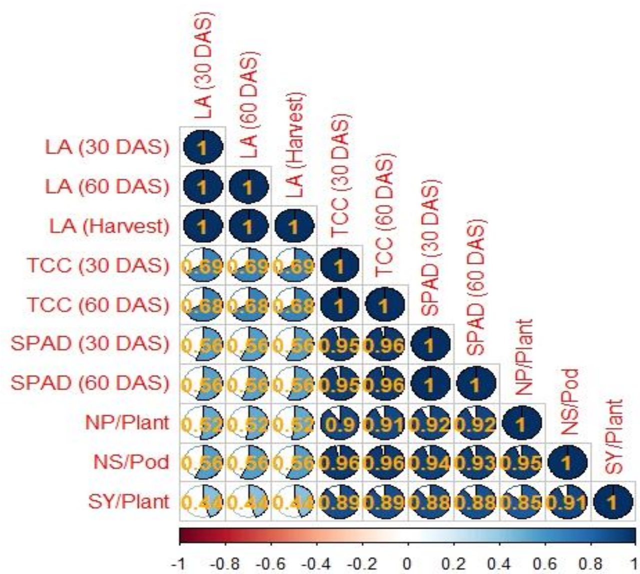

Fig. 1 : Correlation between Leaf area (LA), Total chlorophyll content (TCC), SPAD values and yield parameters in field pea genotypes.

DAS and at harvest, suggesting that increased leaf area enhances photosynthetic capacity and overall plant health, which are crucial for achieving higher yields. Total chlorophyll content (TCC) at 30 DAS and 60 DAS was strongly correlated with SPAD values (r = 0.949 and r = 0.96, respectively), highlighting the role of chlorophyll in determining nitrogen levels and plant vigor. TCC also showed significant positive correlations with yield components such as the number of pods per plant (NP/Plant) and number of seeds per pod (NS/Pod), with correlations of r = 0.903 and r = 0.958, respectively. These correlations suggest that higher chlorophyll content facilitates better reproductive development, supporting the finding that chlorophyll content is a critical determinant of yield. This aligns with recent research by Mamedov *et al.* (2021), which emphasized the importance of chlorophyll in improving yield traits.

SPAD values, reflecting leaf nitrogen content and greenness, were significantly correlated with NP/Plant (r = 0.918) and NS/Pod (r = 0.936), further reinforcing the link between leaf nutrient status and yield potential (Fig. 1). However, the correlation between SPAD values and seed yield per plant (SY/Plant) was weaker (r = 0.438), suggesting that while physiological traits like chlorophyll content and leaf area are crucial for determining plant health and reproductive success, other factors, including environmental conditions and crop management practices, also influence seed yield. This observation is supported by recent findings from Nadeem *et al.* (2023), who highlighted that while physiological parameters are important, their impact on yield is mediated by a complex interplay of genetic and environmental factors.

Conclusion

This study highlights significant variation in physiological parameters among diverse field pea genotypes, revealing their critical role in influencing yield components. The genotypes IPFD6-3 and IC381455 consistently demonstrated superior leaf area, leaf area index, and chlorophyll content, which positively correlated with yield parameters such as the number of pods per plant and seeds per pod. High leaf area and chlorophyll content were associated with better photosynthetic capacity and nitrogen utilization, essential for optimal plant growth and yield. Although SPAD values showed strong correlations with physiological traits, their weaker association with seed yield per plant underscores the complexity of yield determination, which is also influenced by environmental factors and crop management. These findings provide valuable insights for breeding programs aiming to enhance field pea productivity by focusing on genotypes with optimal physiological traits.

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Conflict of interest

Authors have declared that no conflict of interest exist.

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