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Research Paper

Characterization, evaluation and Identification of morphological markers in grasspea (*Lathyrus sativus* L.)

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ABSTRACT

A set of 400 grasspea germplasm lines was characterized and evaluated to identify unique genotypes with desirable agro-economic traits. Significant variability was observed for 10 quantitative and 6 qualitative traits. Several distinct genotypes were identified, including those with notable traits such as high leaflet number (7 per leaf), early flowering and maturity, anthocyanin pigmentation (dark purple aerial parts), leaf shape variation (narrow and broad leaves), flower colour (distinct red), pod and pod wing color (purple and red) and seed characteristics (extra-large size, green color and unique shapes like globular/spherical and square). These unique genotypes can serve as efficient morphological markers for identifying nutritionally improved grasspea varieties.

***Key words*:** Crop improvement, Germplasm, Traits, Selection, Unique genotypes.

INTRODUCTION

Grasspea (*Lathyrus sativus* L.) is an annual climate-smart grain legume used for food, feed, and sustainable crop production systems, particularly in drought-prone areas (Dixit *et al*. 2016). Grasspea is largely considered an "insurance crop" in areas that are prone to abiotic stresses. Because it can produce reliable yields when all other crops fail due to drought (Yan *et al*. 2006), excessive soil moisture, salinity, and low soil fertility. In India, it is cultivated up to 1300 m above sea level (Duke 1981), and in some parts of Ethiopia, it is also grown at a height of 2500–3000 m with annual rainfall of 1000 mm (Fikre *et al*. 2011b). Besides, grasspea is grown as a summer crop in the high altitudes of Kashmir and Nepal. However, it is grown as a winter crop at low altitudes in India and Bangladesh (Girma and Korbu 2012). Grasspea has spread over three continents and has adapted well to various harsh environments, from the highland volcanic soils of Ethiopia to heavy clay in the paddy rice fields of Bangladesh (Lambein *et al*. 2019).

Globally, the total acreage of grass pea was estimated at 1.50 million ha with an annual production of 1.20 million tons, mainly in South Asia and in East Africa Sammour 2014. In Ethiopia, the production of the crop has increased both in area coverage and production volume, from 1,85,490 tons in 2005 to 2,87,674 tons in 2015. The productivity per hectare has also risen from 1260 kg to 1808 kg in the same period (Central Statistical Authority of Ethiopia, 1990–2009). In Bangladesh, grasspea (*Khesari*) is cultivated on 239, 343 ha with a mean yield of 728 kg/ha both in dry areas and in flooded rice fields and it is often used as intercrop in wheat (*Triticum* sp.) or rice fields (Malek *et al*. 2000). In India, grasspea grown in about 0.28 million ha area and production is about 0.26 million tons with average productivity of 903 kg/ha. (DES, GoI, Min. of Agril. & FW (DA&FW, 2020-21). Major grasspea growing states are Chhattisgarh (0.15 mha), West Bengal (0.093 mha), and Bihar (0.052 mha). However, West Bengal ranked first in the production of grasspea (1.20 mt) followed by Chhattisgarh (0.96 mt) and Bihar (0.43 mt). Productivity recorded at the national level was 903 Kg/ha, which was found highest in West Bengal (1273 Kg/ha) followed by Bihar (1083 Kg/ha) and Chhattisgarh (630 Kg/ha) during Rabi 2020-21. The Indian grasspea farmers believe that it is a cash crop with an assured income.

Due to its importance as a survival food for some of the world's poorest people, *L. sativus* was listed among the crops included in the multilateral system of access and benefit sharing under the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2009). Information regarding different levels of diversity in *Lathyrus* germplasm would help to identify sources of broadening improved breeding pools (Patto and Rubiales 2014). Therefore, there is a need to collect, characterize, and conserve the genetic variation of grasspea to broaden the genetic base of grasspea for the development of improved cultivars.

MATERIALS AND METHODS

A set of 400 grasspea germplasm lines (local germplasm collection) was characterized and evaluated over the years. The experiment was laid out in an augmented block design using three checks, namely, Ratan, Prateek, and Mahateora. The experiment was conducted at the Research Farm of IIPR-RS, Bhopal, during Rabi 2019-20 to 2023-24. Climatic conditions of the experimental site are categorized as semi-arid with cold winters followed by hot summers. The soil composition of the experimental site was clay loam with low content of organic matter (nitrogen and phosphorus). The number of blocks was 20, having 24 germplasm lines in each block. Each germplasm line was sown in two rows with a row length of 4 m. Spacing was kept 30 cm apart and plant-to-plant distance was 15 cm. Standard agronomic practices were followed to raise a good crop. Data was recorded on five randomly selected plants for 10 quantitative traits [Plant height (cm), No. of primary branch/ plant, Leaflets/leaf, Days to Flower initiation, Days to 50% Flowering, Days to Maturity, No. of Pods/plant, No. of seeds/pod, 100 seed weight (gm) and Yield/ plant] and 6 qualitative traits (Arial plant parts colour, leaf type, Flower colour, Pod colour, Seed coat colour, Seed size and seed shape). The collected data was subjected to statistical analysis using MS excel (2016) and presented in Table 1.

RESULTS AND DISCUSSION

A wide magnitude of variations was exhibited for various agro-morphological traits in grasspea (Table 1 & Figure 1, 2, 3, 4, 5, 6). The mean and range of nine quantitative traits is given in Table 1. Promising genotypes (named as Indian Pulse Lathyrus i.e. IPLa is selection from local germplasm) were selected for quantitative agro-economic traits (Table 2). Based on the number of days to flower initiation, the earliest-flowering (40 days) genotypes were IPLa 59, IPLa 68, IPLa256, IPLa 416, IPLa 436 and IPLa 442, for days to 50% flowering, IPLa 59, IPLa 442, IPLa 52, IPLa 447 (47 days), IPLa 68 and IPLa 416 (48 days); for the maximum branches (5) per plant were IPLa 308, IPLa 306, IPLa 278, IPLa 314, IPLa 195, IPLa 384; for the smallest plant height were IPLa 56 and IPLa 150 (20.0 cm), and for the tallest plant height, genotype IPLa 429 (69.0 cm) was selected. The heaviest pod bearing (75 pods per plant) genotype was IPLa42, whereas the highest number of seeds per pod (6) was IPLa 396 with regard to days to 80% maturity, the earliest-maturing genotype was IPLa 419 (96 days). The highest grain yield per plant (> 27.5 g) was noted for IPLa 42. The 100-seed weight of large-seeded genotypes was IPLa 456 (18.1 gm), IPLa430 (15.8 gm) and IPLa569 (14.2 gm). Qualitative traits were also characterized as per the descriptors of IPGRI, Rome, and NBPGR, New Delhi, India. Further, Phenotypic markers, such as foliage, flower, seed,fruit color, and leaf form, are critical for differentiating among various germplasm resources.

Table 1. Variability for agro-economic traits in grasspea

S.No.	Character	Range	Mean	S E
1.	Days to Flower initiation	40−69	47.28	±0.20
2.	Days to 50 % flowering	46−77	56.24	±0.22
3.	Days to maturity	96−145	124.49	±0.26
4.	No. of pods/plant	9−75	29.12	±0.40
5.	Plant height (cm)	20.3−69.0	44.31	±0.37
6.	No. of primary branches/plant	2−5	2.99	±0.01
7.	No. of seeds/pod	1 − 5	3.42	±0.02
8.	100 seed weight (gm)	4.0−18.1	7.79	±0.07
9.	Yield/ plant (gm)	6.3−27.5	14.68	±0.18

Days to 50% flowering

Genotypes, namely IPLa 59 (46 days), IPLa 442 (47 days), IPLa 52 (47 days), IPLa 417 (47 days) observed a little early as compared to check varieties Mahateora (51 days), Prateek (52 days), and Ratan (53 days) (Table 2). The small-seeded lakhori types (South-East Asian) are usually much earlier flowering than the large-seeded lakh types (European). Sarwar *et al*. (1995) reported a range of 43 to 88 days in 1072 accessions collected in Bangladesh.

Days to maturity

Genotypes, IPLa 419 (96 days) was found earliest as compared to check varieties Mahateora (117 days), Prateek (122 days) and Ratan (123 days) (Table 2). Similarly, assessment of 732 lines in Canada gave a range of 97 to 121 days, which was similar to that found in India. In Australia, it was found a range of 137 to 148 days with maturity being rated as when the pods had all turned a golden brown, and the leaves were still on the plant (Ramya *et al.* 2024).

Table 2. Identification of promising grasspea genotypes forquantitative traits

Character	Promising germplasm lines	M	P	R
Days to Flower Initiation	IPLa 68 (40), IPLa 436 (40), IPLa 59 (40), IPLa 442 (40), IPLa 416 (40), IPLa 256 (40),	42	43	45
Days to 50 % Flowering	**IPLa 59 (46),** IPLa 442 (47), IPLa 52 (47), IPLa 417 (47),IPLa 68 (48), IPLa 416 (48)	51	52	53
Days to maturity	**IPLa 419 (96)**	117	122	123
No. of pods/plant	**IPLa 42 (**75), IPLa 395 (53), IPLa 58 (51), IPLa 259 (51), IPLa 407 (51)	26	30	25
Plant Height (cm)	IPLa 429 (69.0), IPLa 271 (63.0), IPLa 56 (20.0), IPLa 150 (20.3),	47.6	47.1	45.1
No. of Primary Branches/plant	IPLa 308 (5), IPLa 306 (5), IPLa 278 (5), IPLa 314 (4), IPLa 195 (4), IPLa 384 (4)	3	3	3
No. of seeds/pod	**IPLa 396 (5)**, IPLa 509 (4), IPLa 468 (4), IPLa 377 (4), IPLa 129 (4), IPLa 39 (4)	4	3	4
100 Seed weight (gm)	**IPLa 456 (18.1), IPLa 430 (15.8), IPLa 366 (13.4),** IPLa 532 (11.8), IPLa 419 (11.6), IPLa 448 (11.4)	8.2	8.3	8.1
Grain yield/plant (gm)	**IPLa 163 (27.5**), IPLa 334 (25.6), IPLa 530 (25.0)	11.4	10.4	10.2

M: Mahateora; P: Prateek & R: Ratan are the check varieties

Plant height

Genotype IPLa 429 (69.0 cm) was identified for the tallest plant height as compared to the check varieties Mahateora (47.6 cm), Prateek (47.1 cm), and Ratan (45.1 cm) (Table 2). Whereas, IPLa 56 has shortest plant height (20.0 cm). In general, plant height has been found to vary widely. Indian collections varied from 15 to 68 cm, while in the Canadian collection, it ranged from 24.5 to 172 cm. The large-seeded lines from Europe and the Mediterranean region usually produce taller plants with larger biomass than lines from South East Asia (Campbell 1997).

Primary branches per plant

Genotypes, IPLa 308, IPLa 306, and IPLa 278 (5 branches/plant), were selected for a high number of primary branches per plant as compared to check varieties Mahateora, Ratan, and Prateek, all having three primary branches per plant (Table 2). Accessions from France had 5.2 primary branches, while those from Bangladesh had 5.7, from Ethiopia 5.0, from Cyprus 5.5, from Afghanistan 5.0, from Germany 5.0, and from the previous USSR 5.5, and most studies showed high correlation of branches per plant and grain yield (Campbell 1997).

Significant number of leaflets per leaf (3-7)

Unique genotypes, namely, IPLa 215-1 (7 leaflets with no tendrils), IPLa 464 (6 leaflets with no tendrils), IPLa 181 (5 leaflets/leaf), IPLa 332 (4 leaflets/leaf) and IPLa 62 (3 leaflets/leaf), have been identified. This large number of leaflets in a leaf could be introgressed in to low ODAP lines to use as an efficient morphological marker for easy identification of low ODAP elite lines (Figure 1). Such genotypes may be interesting for breeders as the presence of additional pair of leaflets may increase the photosynthetic efficiency of the plant, enable further increases in biomass at lower densities and increase the leaf area index of a crop so that dense canopy may help in suppressing the emerging weeds (Ambrose 2004). Similarly, a pea mutant developed at the Institute of Forage Crops in Pleven has four pairs of leaflets on every node;it was found interesting for forage production (Ellis *et al*. 2009). Pea is closely related to grasspea, it has a unique place among the economically important legume species due to its variable leaf morphology. The tendril-less phenotype of the mutant in sweet pea (*Lathyrus odoratus*) was also identified (Punnett**,** 1923), where tendrils were transformed into leaflets as in the case of garden pea (*Pisum sativum*). The conversion of tendrils into leaflets in mutants demonstrates that the tendril is a modified leaflet, inhibited from completing laminar development (Julie Hofer *et al*. 2009).

Pods per plant

Genotypes, IPLa 42 (75), IPLa 395 (53), IPLa 58 (51), IPLa 259 (51), and IPLa 407 (51) were identified for the maximum number of pods per plant (Table 2). This character varies in our collections in India from 2.4 to 59. There is a direct association with branches per plant having higher pod numbers. The larger-seeded types also have more pods per plant as they normally have increased plant height because of a correlation between large seeds, pods per plant, and plant height. This is also associated with late maturity and increased plant biomass (Campbell 1997).

Seeds per pod

Genotypes, IPLa 273 and IPLa 345, were selected for a higher number of seeds per pod (5 to 6 seeds per pod) (Table 2). The small-seeded lines normally had a higher number of seeds per pod than the large-seeded lines. Results reported from

Fig. 1: Variation for number of leaflets per leaf in grasspea (3-7 leaflets/leaf) a. Normal, b. IPLa 62 (3), c. IPLa 332 (4), d. IPLa 181 (5), e. IPLa 464 (6), f. IPLa 215 (7)

Nepal showed a range of 2 to 5 seeds (Yadov 1995). It is reported that the European lines normally have fewer seeds per pod than those from Southeast Asia. It would appear that this characteristic requires more detailed examination, as seeds per pod normally have a high correlation with yield.As increased seed size is usually also highly correlated with higher yield, plant breeders might want to consider increasing seeds per pod in large-seeded types as an effective means of increasing yield (Campbell 1997).

Extra-large seed

Six genotypes viz., PLa 456 (18.1 gm), IPLa 568 (18.0 gm), IPLa 745 (15.8 gm), IPLa 430 (15.8 gm), IPLa 569 (14.2 gm) and IPLa 366 (13.4 gm), were identified for large seed size based on 100-seed weight (Table 2. Preference for larger seeds is common in all grain legumes such as grasspea, lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), faba bean (*Vicia faba*) and is a product of human selection (Patto and Rubiales 2014). The grasspea lines of Mediterranean or European origin were consistently higher yielding, with much larger seeds and later phenology as compared to coloured-seeded with relatively small seeds, originating mainly from Asia and Ethiopia (Przybylska *et al.* (2000).

Qualitative traits

Aerial plant colour (Anthocyanin pigmentation)

The peculiar genotypes, IPLa 509 and IPLA 129, were identified for anthocyanine-rich whole plant parts (Table 3, Figure 2). Anthocyanins represent the red, purple, and blue pigments in many flowers, fruits, vegetables, and cereals. They are also recognized as important health-promoting components in the human diet with protective effects (due to powerful anti-oxidant properties of anthocyanin pigment) against many chronic diseases, including cardiovascular diseases, obesity, and cancer (Cappellini *et al*. 2021).

Table 3. Identification of promising grasspea genotypes for important qualitative traits

Character	Promising germplasm lines	Mahateora	Prateek	Ratan
Arial plant parts colour	IPLa 129 and IPLa 509 (Anthocyanin pigment with purple colour)	Green	Green	Green
Leaf type	IPLa 332 (Narrow leaf) IPLa 532(broader leaf) IPLa 448 (broader leaf)	Normal	Normal	Normal
Leaf shape	IPLa332 (Linear- lanceolate) IPLa532, IPLa 448 (Lanceolate-obovate)	Lanceolate	Linear	Linear -lanceolate
Flower colour	IPLa 62 (red)	pink	blue	blue
Pod colour	IPLa 509 (red), IPLa 129 (purple)	green	green	green
Pod wings colour and shape	IPLa 509 (red and wavy)	Green and normal	Green and normal	Green and normal
Seed colour	IPLa 273, IPLa 345, IPLa 400 (Green colour)	brown	gray	gray
Seed shape	Globular/spherical/Square	Triangular	irregular	Irregular

Fig. 2: High anthocyanin pigmented grasspea: a. IPLa 509 b. IPLa 129 c. Normal plant type.

Leaf type (Narrow and broad leaf)

Specific genotypes, namely, IPLa 332 for narrow leaf (linear-lanceolate) and IPLa 532 and IPLa 448 for broader leaf (lanceolate-obovate) type, were selected as useful morphological markers (Table 3, Figure 3). Broad leaves are typically flat and broad, creating a large surface area to capture lots of sunlight and undergo photosynthesis. These leaves do an excellent job of catching sunlight and creating energy. However, they can't survive a cold winter, so many of these plants lose their leaves in the harsh climatic conditions. However, thin leaves have much smaller surface area than broadleaves, that the amount of photosynthesis the plant can undertake Julie Hofer *et al*. (2009).

Flower colour (distinct red colour flower)

A remarkable genotype IPLa 62 is identified for red flower colour (Table 3, Figure 4). The flower colour in *L. sativus* can be blue, pink, red, white, or various combinations of these colours. The white-flowered types are generally found in the Mediterranean region. In India, Nepal and Pakistan, the flower colour is blue, with a very few plants having red flowers. The flower colour is closely correlated to seed coat colour, with white flowers producing white seeds and coloured flowers producing coloured seeds. Flower color diversity may also be related to functional differences in pollinator attraction and success (Ioannis *et al*. 2024). A white or cream colour was associated with

a. Narrow leaf (IPLa 332) **b. Broader leaf (IPLa 532)** **c. Broader leaf (IPLa 448)** **d. Normal leaf**

Fig. 3: Variation for leaf shape and size in grasspea.

a b c d e f

Fig. 4: Unique genotypes for flower, pod and pod wing colour in grasspea:

a low tannin level in the seeds, while the colourful blooming accessions had higher tannin contents (Eugeniusz *et al*. 2010).

Pod colour (Anthocyanin rich grasspea pods)

Two distinct genotypes, IPLa 129 and IPLa 509, were identified for purple and red coloured pods and pod wings, respectively (Table 3, Figure 4). Color is an essential agronomic trait, and the consumption of high anthocyanin-containing vegetables in the daily diet does provide benefits to human health. The significant differences in the compositions and contents of anthocyanins are responsible for the pod color ranging from brick-red to purplish-red. Anthocyanins are modified by hydroxylation, methylation, glycosylation, and acylation. This adds versatility to the colors and stability of anthocyanins.As the number of hydroxyl groups in the B-ring increases, the color of the anthocyanin becomes bluer. Methylation, on the other hand, leads to a red shift in the color of anthocyanins (Zhang *et al*. 2021).

Seed colour and shape

Visual quality of grasspea grains (*Lathyrus sativus* L.) is one of the most important determinants of the market value. Seed coat color, seed shape and seed size are the major components of physical seed quality of grasspea and are considered important breeding objectives. Colors of the seed coat, hilum ring, and corona are important, along with susceptibility to postharvest darkening, which decreases seed value (RieSadohara *et al.* 2022). Deshpande and Campbell (1992) reported that the extent of tanninsin the seed is highly correlated with seed coat colour hence, white colour seed coat have low levels of tannin.

Green seed coat colour

Two genotypes namely IPLa 273, IPLa 345 and IPLa 400, were identified for green seed coat colour (Table 3, Figure 5). Greenish gray seed colourin grasspea was also reported by (Polignano 2005). It is also noticed that developmental, environmental, and genetic factors affecting seed color.

a. IPLa 273 (Green seed) **b. IPLa 345 (Green seed)** **c. IPLa 400 (Green seed)** **d. Normal Seed Colour**

Fig. 5: Green seeded genotypes compared to normal seed colour

Fig. 6: Variability for seed shape: a. Mahateora (normal irregular), b. IPLa 401 (globular), c. IPLa 835 (square), d. IPLa 793 (almost globular), e. IPLa 830 (globular), f. IPLa 129 (Globular)

Seed shape variants

Three grasspea genotypes, namely IPLa 401, IPLa 793, IPLa 830 and IPLa 129 identified for globular seed shape and very similar to the pea seed shape. However, IPLa 835 was identified for square shape seed (Table 3, Figure 6). Seed shape is an important agronomic trait with continuous variation among genotypes (Singh *et al*. 2024).

CONCLUSION

It is concluded that the grasspea germplasm lines exhibited a high magnitude of variation for key morphological traits. Specific genotypes possessing narrow and broad leaves, a higher number of leaflets per leaf, anthocyanin-rich purple and red pods, six seeds per pod, green seed coat colour, globular seed shape, and extra-large seed size were identified and selected, and being validated. These traits can serve as effective and easily recognizable morphological markers for use as donor characters in breeding programmes aimed at introgressing them into low-ODAP lines, thereby enabling effortless visual identification of elite, nutritionally improved grasspea lines under field conditions.

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