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RESEARCH ARTICLE

Evaluation of Grass Pea (*Lathyrus sativus* L.) Landraces for Genetic Variability and Character Association for Growth, Yield and Quality Attributes

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Abstract

To study the genetic variability and character association for yield and quality traits of seeds and forage of grass peas, a field experiment was conducted during Rabi 2021–22 using local collections from Assam. Significantly, wider variability was observed for important traits such as days to maturity (111.6–130.67), green forage yield (41.27–20.67 g/plant), seed yield (3.09–5.67 g/plant), β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) and protein content in seeds (0.08–0.44% and 23.6–28.8%) and forage (0.07–0.37% and 13.3–20.1%), respectively. High heritability (>60%) and high genetic advance (>20%) were observed for leaf width, green forage yield, dry matter yield, crude protein content in leaves, and β-ODAP content in leaf and seed. β-ODAP content in leaf and seed has exhibited a negative correlation with green and dry forage yield/plant, number of primary branches, and seed weight. Therefore, genotypes with low β-ODAP content in forage and seeds can be developed through selection.

Keywords: Correlation, Landrace, Grass pea, β-ODAP, Genetic variability, Path.

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Introduction

Grass pea (*Lathyrus sativus* L.) is an important cool-season multipurpose legume crop cultivated mainly for its seeds and green fodder. It belongs to the family Fabaceae and the subfamily Faboideae with 2n = 14 diploid chromosomes. It is widely cultivated in India, Bangladesh, Nepal, Pakistan, and Ethiopia. The major grass pea or *khesari* cultivating states in India are Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Bihar, Orissa, West Bengal and Maharashtra (Mahata *et al*., 2018). Grass pea is considered a drought-tolerant, hardy crop that has the ability to grow in low-rainfall areas and in land with poor soil fertility (Palmer *et al*., 1989). It is resistant to various pests and diseases in comparison to other legumes (Yan *et al*., 2006). Grass pea can be used as a soil ameliorant due to its symbiotic nitrogen fixation (108–125 kg nitrogen ha^{-1}) with a mean value of 116 kg ha^{-1} (Peoples *et al*., 2008). Grass pea also fits well in the rice-based utera cropping system as it is adaptable to the heavy soil left after harvesting rice. The high proteinaceous tender leaves of grass pea cut at 50% flowering stage are good feed for domestic animals, whereas its seeds are used for human consumption as dal after splitting it or as pakoda prepared from its ground flour. Therefore, grass pea has immense potential to be utilized as a dual-purpose crop in low-input farming conditions.

Grass pea is a very nutritious crop as it contains 362.3 Kcal of energy per 100 g seed, 2.7% fat, 31.6% protein, 1.1% crude fiber, 51.8% nitrogen-free extract, and 2.2% ash content in its seeds (Rahman *et al*., 1974). Besides having a higher crude protein content (24–31%), it has a good amino acid profile to provide balanced nutrition to impoverished people (Hanbury *et al*., 2000). In spite of having so many advantages, the use of this crop is limited due to the presence of a neurotoxin, viz., β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) in different parts of the plant, including a higher content in seeds (Prakash *et al*., 1977; Jiao *et al*., 2006). β-ODAP is considered the causative factor for a neurodegenerative disorder called 'Neurolathyrism' in humans and animals that causes spastic paralysis and muscle atrophy of the lower limbs (Rao *et al*., 1964). The content of β-ODAP in grass pea germplasm varies from 0.02 to 2.59% (Kumar *et al*., 2011). Grass pea seeds containing less than 0.2% β-ODAP are deemed safe for ingestion by humans (Abd El-Moneim *et al*., 1999).

Low productivity and the presence of the neurotoxin β-ODAP are two major constraints for the cultivation of grass peas. In northeastern India, it is grown traditionally by the farmers using the local landraces, and there is no commercial cultivar that is higher in yield, suitable for livestock as well as pulse production, and low in neurotoxin content. Therefore, the development of grass pea varieties low in neurotoxin content, high green fodder, and high seed yield has become the main focus of plant breeders in the grass pea improvement program. The prospect of crop improvement in a particular crop species depends on the presence of genetic variability, as the effectiveness of the selection depends on the genetic base of the population. Among the multivariate analyses, the PCA has been found effective in identifying traits with high variability; correlations reveal the strength of the relationship between various yields and their components. Keeping the above facts in view, the present investigation was undertaken to study the genetic variability and character association with respect to growth, yield, and quality attributes among the local landraces of the grass pea.

Materials and Methods

Plant Materials

A total of 16 genotypes, including local checks (Prateek and Madhuri) of grass peas were collected from different parts of Assam for the evaluation trials. Prateek is a low β-ODAP containing grass pea variety with high-yielding ability, which is often used as a check-in grass pea germplasm evaluation programme in India. On the other hand, Madhuri is a high-yielding variety recommended for Assam, but it has a high concentration of β-ODAP (0.2%).

Crop Evaluation

The experiment was conducted in a randomized block design (RBD) with three replications at the Instructional cum Research Farm, Assam Agricultural University, Jorhat, during Rabi 2021-22 (26.046 ^{0}N latitude and 94.016 ^{0}E longitude with an altitude of 86.6 m above mean sea level). The seeds of each variety were sown in plots of 3×3 m^2 area by maintaining a spacing of 25 cm between rows and 15 cm between plants. The recommended package of practices was followed during crop production. The observations were recorded on five randomly selected plants from each replication for the following quantitative traits: days to 50% flowering, days to maturity, plant height (cm), number of leaves/plant, number of primary branches/plant, number of secondary branches/plant, leaf length (cm), leaf width (cm), green forage yield (g/plant), dry matter yield (g/plant), pods/plant, number of effective pods/ plant, pod length (cm), pod width (cm), number of seeds per pod, seed yield (g/plant), 100 seed weight (g).

Biochemical Analyses

The quality parameters, viz., β-ODAP and protein contents, were analyzed for both leaves and seeds. The β-ODAP content was estimated by following the spectrophotometric method as described by Rao (1978). Total nitrogen was estimated using the modified Kjeldahl method described by Scales and Harrison (1920). The nitrogen percentage was then multiplied by 6.25 to get the crude protein percentage.

Statistical Analyses

The mean data from each replication was used for further statistical analysis using the package "variability" of software R Studio version 4.3.1. The genetic parameters, viz., genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability, and genetic advance (GA), were estimated using the methodology as described by Burton and Devane (1953). The genotypic and phenotypic correlation coefficients for the traits were estimated as per the method of Al-Jibouri *et al*. (1958). Further, the path analysis was done following the procedure of Dewey and Lu (1959).

Results and Discussion

Genetic Variability for Growth, Yield and Quality Traits

The results of the present study have revealed the presence of substantial variation among the genotypes for all the traits except pod length and pod width (Table 1). Similar findings were also noticed by other researchers in grass pea (Singh and Roy, 2013; Jeberson *et al*., 2018; and Mahapatra *et al*., 2020; Tripathi *et al*., 2021). The poor variability for pod characters like length and width may be due to the narrow genetic base of the population. Abate *et al*., (2018) and Arslan *et al*., (2021) also observed non-significant variations for these traits.

Out of 21 traits, 19 were found to be significant and showed a considerable range of variation by the genotypes in their mean performances (Table 2). The green forage yield per plant varied from 11.27 to 20.67 g. The green forage yield of seven genotypes was observed to be above the

Table 1: Analysis of variance of grass pea genotypes for various quantitative characters

Source of variation	DF	Mean squares																			
		Days to 50% flowering	Days to maturity	Plant height (cm)	Leaves/ plant	Primary branches / plant	Secondary branches/ plant	Leaf length (cm)	Leaf width (cm)	Green forage yield (g/ plant)	Dry matter yield (g/ plant)	Pods/ plant	Effective pods/ plant	Pod length (cm)	Pod width (cm)	Seeds per pod	Seed yield (g/ plant)	100 seed weight (g)	Leaf β-ODAP %	Seed β-ODAP %	Leaf crude protein %	Seed crude protein %
Replications	2	0.270	10.940	4.020	0.180	0.020	0.020	0.140	0.000	0.340	0.110	0.530	1.080	0.080	0.010	0.006	0.018	0.17	0.000	0.000	0.040	0.060
Genotypes	15	88.550**	59.330**	16. 350*	147. 160**	0.610**	2.300**	0.700**	0.010**	19. 180**	2.350**	45. 930**	34.370**	0.040	0.010	0.210*	1. 210**	0.45*	0.025**	0.039**	14. 740**	7.610**
Error	30	2.410	5.650	7.830	19.890	0.200	0.760	0.100	0.000	1.410	0.390	12.790	9.800	0.030	0.005	0.090	0.380	0.19	0.000	0.000	0.055	0.040
C.V %		1.68	1.87	5.58	8.06	14.61	8.28	6.02	7.00	7.54	18.45	14.87	15.56	NS	NS	6.54	16.95	8.80	2.45	1.42	1.41	0.73

*,** Significant at 5% and 1% level of significance respectively

Table 2: Mean performance of grass pea genotypes for various quantitative traits

Name of the genotype	Days to 50% flowering	Days to maturity	Plant height (cm)	Leaves/ plant	Primary branches	Secondary branches	Leaf length (cm)	Leaf width (cm)	Green forage yield (g/plant)	Dry matter yield (g/plant)	Leaf crude protein (%)	Leaf β-ODAP %	Pods/ plant	Effective pods/ plant	Pod length (cm)	Pod width (cm)	Seeds per pod	Grain yield (g/plant)	100 seed weight (g)	Seed crude protein (%)	Seed β-ODAP (%)
JCL-21-N-4	93.67	128.00	52.91	52.60	3.33	10.13	5.38	0.61	16.93	3.40	19.95	0.097	27.47	24.07	3.43	1.14	4.73	4.34	5.32	28.18	0.176
JCL-21-N-3	92.67	130.00	50.82	62.03	3.27	11.33	5.32	0.60	15.60	3.30	18.67	0.228	35.53	30.20	3.41	1.08	4.60	5.67	4.87	27.59	0.291
JCL-21-N-2	94.00	129.67	50.05	56.33	3.00	10.93	5.38	0.56	15.33	3.93	15.40	0.190	29.13	23.13	3.69	1.16	4.67	3.96	4.80	25.02	0.208
JCL-21-N-1	92.33	128.00	53.59	65.20	3.67	11.47	5.09	0.57	20.67	6.07	19.19	0.071	27.80	23.87	3.44	1.09	4.73	4.51	5.44	27.71	0.095
JCL-21-N-6	94.33	129.00	52.75	63.47	3.00	11.00	5.09	0.58	13.33	2.80	16.98	0.349	26.07	21.40	3.39	1.15	4.87	3.42	4.95	26.48	0.417
JCL-21-N-5	93.33	130.33	51.65	52.07	3.40	12.27	4.76	0.54	18.00	3.53	16.10	0.195	27.27	22.33	3.31	1.11	4.53	4.05	4.61	25.38	0.236
JCL-21-M-6	93.33	126.67	45.43	44.53	2.07	9.93	5.05	0.48	12.80	2.53	15.23	0.259	25.27	20.67	3.30	1.14	4.47	3.63	4.73	24.85	0.373
JCL-21-M-5	95.00	128.33	51.39	44.20	2.27	9.20	5.03	0.48	11.27	2.27	13.30	0.303	23.80	20.47	3.25	1.11	4.33	3.61	4.83	23.92	0.394
JCL-19-M-1	90.67	124.33	48.03	52.27	3.07	10.53	4.44	0.41	17.50	3.33	18.49	0.084	22.93	20.40	3.48	1.07	4.33	3.95	5.12	27.07	0.109
JCL-19-M-2	92.67	127.00	51.08	48.20	3.33	10.40	5.69	0.47	13.13	2.70	13.48	0.241	18.27	15.07	3.27	1.09	4.33	3.30	4.58	24.50	0.274
JCL-10-3	94.00	129.00	48.55	49.73	2.80	9.93	5.61	0.64	13.07	3.40	20.13	0.371	26.73	21.73	3.53	1.17	4.33	3.52	4.75	28.82	0.441
JCL-10-2	94.00	130.67	49.10	52.13	2.60	9.80	4.67	0.63	15.00	2.80	17.15	0.250	24.00	17.60	3.51	1.17	4.40	3.87	5.09	26.83	0.328
JCL-10-4	92.67	128.00	51.66	63.87	3.67	9.87	5.63	0.59	18.20	4.33	16.63	0.151	20.53	19.60	3.45	1.15	4.13	3.09	4.64	26.08	0.170
JCL-10-1	93.67	127.33	50.41	54.87	2.73	11.80	6.05	0.47	17.03	3.07	16.16	0.202	22.67	18.00	3.42	1.08	4.60	3.16	4.77	25.67	0.254
Madhuri	94.00	126.00	46.78	61.33	3.13	9.60	5.44	0.52	15.27	3.60	15.11	0.181	27.33	23.40	3.36	1.13	4.47	4.31	4.70	24.33	0.200
Prateek	72.00	111.67	47.62	62.87	3.07	9.87	6.24	0.67	17.97	3.23	13.71	0.075	27.07	22.73	3.47	1.30	3.80	4.05	6.08	23.63	0.080
Mean	92.02	127.13	50.11	55.36	3.03	10.50	5.30	0.55	15.69	3.39	16.60	0.203	25.74	21.54	3.42	1.13	4.46	3.90	4.96	26.00	0.250
SE (m)	0.89	1.37	1.62	2.57	0.25	0.50	0.18	0.02	0.68	0.36	0.13	0.003	2.06	1.81	0.09	0.04	0.17	0.36	0.25	0.11	0.002
CD (5%)	2.70	4.14	4.87	7.76	0.77	1.51	0.56	0.07	2.06	1.09	0.41	0.009	6.22	5.45	NS	NS	0.51	1.08	0.76	0.33	0.006

average (>15.69 g/plant) of all the genotypes. The highest green forage yield was found in JCL-21-N-1 with 15.02% more yield than Prateek and 35.36% more yield than Madhuri. JCL-10-4 followed it with 1.28% more yield than Prateek and 19.19% more yield than that of Madhuri in the present study. The variation in dry matter yield/plant was from 2.27 g to 6.07 g. The range of seed yield/plant and 100 seed weight was 3.09 to 5.67 g and 4.61 to 6.08 g, respectively. Among the 16 genotypes, the seed yield of eight genotypes was above average (>3.90 g/plant). Out of them, the highest seed yield was recorded from JCL-21-N-3 followed by JCL-21-N-1. The seed yield of genotype JCL-21-N-3 was 40 and 31.55% higher over the check Prateek and Madhuri, respectively. On the other hand, the yield of the second-best genotype JCL-21-N-1 was 11.36% higher than Prateek and 4.64% higher than Madhuri's. JCL-21-N-1 was the only genotype to perform well for both fodder and seed yield. Comparatively, higher seed yield and seed weight were observed by Tadesse and Bekele (2003) in Ethiopian landraces of the grass pea.

Days to maturity varied from 111.67 to 130.67 days. Prateek was observed to be the earliest among all the genotypes to reach 50% flowering as well as pod maturity stage. Among the landraces, JCL-19-M-1 was the early genotype with 124.33 days to maturity.

For quality traits, among the genotypes, high variation was observed for β-ODAP content in leaves (0.071–0.371%) and seeds (0.080–0.441%). A similar range of β-ODAP concentration in seed was also observed by Parihar *et al.* (2006) and Hanbury *et al.* (2000). Since the critical limit of β-ODAP level in grass pea grains for human consumption is 0.2%, we always choose genotypes with low (<0.2%) β-ODAP content (Abd El-Moneim *et al*., 1999; Yan *et al*., 2006). Among the genotypes under study, the concentration of β-ODAP in leaves was found to be below average (<0.203%) in nine genotypes. The lowest β-ODAP content in leaves was observed in JCL-21-N-1 followed by Prateek, JCL-19-M-1 and JCL-21-N-4. However, only JCL-21-N-1 had 5.63% lower β-ODAP content as compared to check variety Prateek (0.075%). Thus, these superior genotypes could be considered a good source for grass pea improvement for fodder purposes.

The β-ODAP content in seed was below average (<0.250%) in nine genotypes, but no genotype was observed with seed β-ODAP content less than the check variety Prateek (0.080%). However, four genotypes had seed β-ODAP content less than that of the local check variety Madhuri (0.200%). They were JCL-21-N-1, JCL-19-M-1, JCL-10-4 and JCL-21-N-4 with 52.5, 45.5, 15 and 12% less β-ODAP concentration, respectively, than that of Madhuri. These four genotypes were found safe for human consumption as they contain less than 0.2% β-ODAP and can be considered to promote commercial production.

Similarly, a wide range of variability was also observed for crude protein content in leaves (13.30–20.12%) and seeds (23.62–28.82%). The crude protein content in leaves was found to be above average (>16.60%) in eight genotypes. It was highest in the genotype JCL-10-3, followed by JCL-21-N-4 and JCL-21-N-1. The genotype JCL-10-3 had 46.82% more protein content in its leaf than that of Prateek and 33.22% more than that of Madhuri. In seed also, the crude protein content was above average (>26.00%) in eight genotypes, with the maximum protein content in JCL-10-3 followed by JCL-21-N-4 and JCL-21-N-1. The genotype JCL-10-3 had 21.96% more seed protein content than that of Prateek and 18.45% more than that of Madhuri. In contrast to our germplasm, Rajendran *et al*. (2019) observed the highest range of seed protein content (28.82–27.2%) in the set of germplasm from the ICARDA and β-ODAP content in seeds from the sets of germplasm from Ethiopia (0.32–0.47%) and Pakistan (0.38–0.53%). Moreover, genotype JCL-21-N-1, possessing good fodder and seed-yielding ability also had a high amount of crude protein content and low neurotoxin content in its leaves and seeds. This promising genotype could be a good source for future grass pea improvement programs.

The estimates of genetic parameters are presented in Table 3. Among the characters studied, β-ODAP content in leaf, followed by β-ODAP content in seed and dry matter yield/plant exhibited higher GCV and PCV. It will offer a good scope of selection for these three characters. High GCV and PCV for β-ODAP content were also reported by Sharma *et al*. (2001) and Bhosle *et al*. (2008). Lyngdoh (2018) reported high GCV and PCV for β-ODAP content and dry matter yield/plant. The high magnitude of PCV was observed for seed yield/plant, number of pods/plant, and effective pods/plant, though GCV for these characters was found to be moderate, and there was a large difference between PCV and GCV, indicating a higher effect of environment on these characters. A moderate level of GCV and PCV was observed for leaves per plant, primary branches/plant, green forage yield/plant, leaf width, and leaf crude protein, which suggests the possibility of further improvement out of the variability for these traits. Low GCV and PCV were observed for days to 50% flowering, days to maturity, plant height, leaf width, seeds per pod, and crude protein content in the seed, which was in congruence with the findings of Bhosle *et al*. (2008), Parihar *et al*. (2015), Basaran *et al*. (2013), Jeberson *et al*. (2018) and Ranjithkumar *et al*. (2020). However, secondary branches per plant, leaf length, and 100 seed weight showed low GCV with moderate PCV.

Heritability (broad sense) estimates give information on the degree of genetic determination out of the total variation observed for a character (Singh, 2002). High heritability was recorded for β-ODAP content in leaf and seed, crude protein content in leaf and seed, days to 50% flowering, green forage yield per plant, leaf width, days to maturity, number of leaves /plant, leaf length, and dry matter yield/plant. Similar findings for β-ODAP content,

Table 3: Genetic variability parameters for growth, yield and quality traits in grass pea genotypes

Traits	*Range*	*Mean ± SEM*	*GCV (%)*	*PCV (%)*	h^2_{bs}	*GA (% of mean)*
Days to 50% flowering (DAS)	72.0-95.0	92.02 ± 0.89	5.82	6.06	92.28	11.52
Days to maturity (DAS)	111.67-130.67	127.13 ± 1.37	3.33	3.82	76.01	5.98
Plant height (cm)	45.43-53.59	50.11 ± 1.62	3.36	6.52	26.63	3.58
Leaves per plant	44.20-65.20	55.36 ± 2.57	11.77	14.26	68.08	20.00
Primary branches	2.07-3.67	3.03 ± 0.25	12.26	19.07	41.32	16.23
Secondary branches	9.20-12.27	10.50 ± 0.50	6.81	10.72	40.32	8.91
Green forage yield (g/plant)	11.27-20.67	15.69 ± 0.68	15.51	17.25	80.87	28.73
Dry matter yield (g/plant)	2.27-6.07	3.39 ± 0.36	23.77	30.09	62.40	38.68
Leaf length (cm)	4.44-6.24	5.30 ± 0.18	8.40	10.34	66.08	14.07
Leaf width (cm)	0.41-0.67	0.55 ± 0.02	12.68	14.49	76.63	22.87
Pods per plant	18.27-35.53	25.74 ± 2.06	13.82	20.30	46.35	19.38
No. of effective pods per plant	15.07-30.20	21.54 ± 1.81	14.22	21.08	45.52	19.77
No of seeds per pod	3.80-4.87	4.46 ± 0.17	4.44	7.90	31.56	5.14
Seed yield (g/plant)	3.09-5.67	3.90 ± 0.36	14.34	22.21	41.70	19.07
100 seed weight (g)	4.61-6.08	4.96 ± 0.25	5.98	10.63	31.58	6.92
Leaf β-ODAP%	0.071-0.371	0.203 ± 0.003	45.75	45.82	99.71	94.11
Seed β-ODAP%	0.080-0.441	0.253 ± 0.002	45.31	45.33	99.90	93.29
Leaf Crude protein%	13.30-20.12	16.60 ± 0.13	13.32	13.40	98.89	27.29
Seed Crude protein%	23.62-28.82	26.00 ± 0.11	6.11	6.15	98.59	12.49

green forage yield, and dry matter yield were reported by Pandey *et al.* (1997) in grass peas and Kumar *et al.* (2002) in cowpeas. This suggests that there is a low influence of the environment on the phenotypic expression of these characters. The moderate heritability was exhibited by primary branches per plant, secondary branches per plant, number of pods per plant, number of effective pods per plant, number of seeds per pod, seed yield per plant, and 100 seed weight, whereas plant height showed low heritability. Mahapatra *et al.* (2020) reported high heritability for days to 50% flowering and days to maturity, while moderate heritability was reported for branches per plant and 100 seed weight. A similar finding for days to maturity and seeds per pod was also reported by Jeberson *et al.* (2018). Moderate heritability for primary and secondary branches per plant and low heritability for plant height were in agreement with Ranjithkumar *et al.* (2020).

The studies of heritability and genetic advance can decipher the mode of gene action involved in controlling the traits. Johnson *et al.* (1955) reported that estimates of heritability along with genetic gain would be more rewarding than heritability alone in predicting the resulting effect of selection to identify the best individual. In the present study, high heritability coupled with high genetic advance was recorded for β-ODAP content in leaf and seed, crude protein content in leaf, green forage yield/plant, dry matter yield/plant, and leaf width. This indicated a significant contribution of additive gene action to the expression of these traits, thus providing a good opportunity for the selection of these traits. Similar findings were also reported by Pandey *et al.* (1997) and Lyngdoh (2018) for β-ODAP content, green forage yield, and dry matter yield in grass peas. High heritability coupled with moderate genetic advance was observed for leaves/plants, days to 50% flowering, and leaf length. Besides that, moderate heritability coupled with moderate genetic advance was observed for the number of primary branches/plant, pods per plant, effective pods/plant, seed yield/plant, and crude protein content in seed. These results indicated the

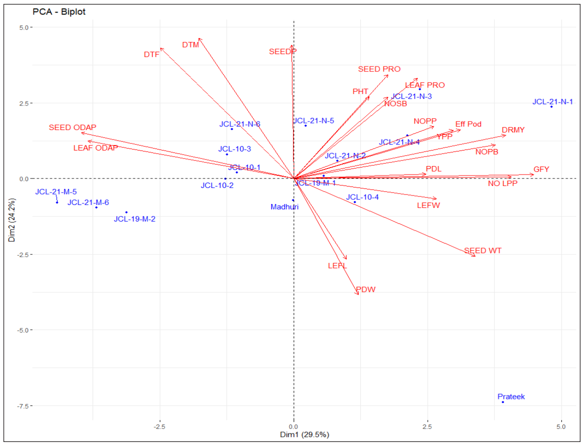

Figure 1: Principal component analysis (PCA) biplot representing the distribution of genotypes and their interrelationship with quantitative traits (PC1 and PC2)

involvement of both additive and dominant gene action in controlling the expression of these traits. Further, days to maturity, number of secondary branches/plant, seeds per pod, and 100 seed weight exhibited high to moderate heritability coupled with low genetic advance, suggesting that the expression of these traits was preponderantly under the influence of non-additive gene action. The high to moderate heritability observed for these traits was an indication of high environmental influence, due to which simple selection would not be effective. In addition, low heritability as well as low genetic advance was observed for plant height, which indicates the predominance of non-additive gene action for the trait plant height. As suggested by Singh and Narayanan (1993), selection would not be effective for the traits controlled by non-additive gene action; rather, heterosis breeding for such traits would be useful. Similar findings for days to maturity, days to 50% flowering, primary branches/plant, seed yield/plant, number of seeds/pod, 100 seed weight, and plant height were also reported by other researchers (Wutletaw and Endashaw, 2003; Abate *et al*., 2018; Jeberson *et al*., 2018; Mahapatra *et al*., 2020; and Ranjithkumar *et al*., 2020).

Principal Component Analysis

PCA is one of the most widely used methods of multivariate analysis to analyze the genetic variability in a given population. PCA is a measure of how important an impact a certain trait has in explaining total variability, and each coefficient of proper vectors indicates the degree of contribution of every original variable with which each principal component is associated (Sanni *et al*., 2012). In the present study, the first five principal components exhibited eigenvalues >1.00 and contributed to 81.98% cumulative variance among the traits studied (Table 4). According to various reports, the first three components (PC1, PC2 and PC3) and associated traits are the most reliable to explain the variation present in the population. In the present study, the first three PC contributed 66.90% of the total variations. Similar findings have also been observed by Tripathi *et al*., (2021) in grass pea.

The first principal component (PC1) showed 29.53% of the total variability. The higher absolute values in the principal components indicate a higher contribution of traits towards the divergence. Among the traits green forage yield (0.85), number of leaves (0.77), dry matter yield (0.75), number of primary branches (0.71), 100 seed weight (0.64), number of effective pods (0.59) and seed yield/plant (0.57) have contributed maximum and positive to the genetic divergence in PC1, while the characters seed β-ODAP content (-0.75) and leaf β-ODAP content (-0.73) exhibited the maximum negative contribution to the first principal component. The second component (PC2) contributed 24.22% to the total variance. The major characters contributing to the second component include days to maturity (0.88), number of seeds/plant (0.84), days to flowering (0.83), seed crude protein content (0.65), leaf crude protein content (0.63), plant height (0.51) and number of secondary branches/plant (0.51). Principal component 3 (PC3) exhibited 13.15% of the total variance. The characters, viz., number of pods/plant (0.66), leaf width (0.61), number of effective pods/plant (0.53), seed β-ODAP content (0.50) and leaf β-ODAP content (0.47) showed the maximum positive contribution to the genetic variance of PC3.

The biplot diagram (Figure 1) for principal components represents the distribution and diversity for both traits and genotypes. The PCA biplots differentiated the genotypes by traits. Genotypes that are closer to the origin and closer to each other are said to have more similarity, while genotypes that are apart from each other are more divergent. PC1 differentiated the genotypes for days to flowering, maturity, and β-ODAP contents in leaves and seeds. The divergent parents from the biplot can be selected for the further improvement program, like Prateek for seed weight and JCL-21-N-1 for growth and yield attributes with low β-ODAP content.

Correlation and Path Analysis

Yield is a complex trait and dependent on many other quantitative related traits. The interrelationship among yield and its component traits can be determined through correlation analysis. On the other hand, path coefficient analysis helps in partitioning the correlation coefficients into direct and indirect effects, which enables the identification of the cause of the association of yield attributes with yield. Two separate correlation and path analyses were performed to study the relationship between green forage yield as well as seed yield and its attributes.

Correlation and Path Coefficient Analysis for Green Forage Yield with its Component Characters

Green forage yield/plant was positively correlated with leaves/plant, number of primary branches/plant, number of secondary branches/plant, and dry matter yield/plant at both genotypic and phenotypic levels (Table 5). A considerable amount of phenotypic correlation was observed between green forage yield/plant and crude protein content in leaves. Selection of promising genotypes for these traits would result in an improvement in green forage yield/plant. Similar findings were also reported by Mihailovic *et al*. (2013) and Lyngdoh (2018). On the other hand, β-ODAP content in leaves was found to be negatively correlated with green forage yield/plant at genotypic as well as phenotypic levels. This suggested that improvement in green forage yield would also result in the development of genotypes with low neurotoxin content in their leaves. For the selection of genotypes, a negative correlation of β-ODAP content in leaves with any other character is considered useful. In this study, a significant negative correlation of

Table 4: Principal component analysis in grass pea for growth, yield and quality traits

Traits	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*
Days to 50% flowering (DAS)	-0.47	0.83	-0.11	0.09	-0.04
Days to maturity (DAS)	-0.34	0.88	-0.00	0.15	0.04
Primary branches	0.71	0.21	-0.40	0.17	0.24
Secondary branches	0.33	0.51	-0.29	-0.14	0.31
Leaves per plant	0.77	0.01	-0.02	0.13	0.36
Plant height (cm)	0.27	0.51	-0.28	0.29	0.41
Green forage yield (g/plant)	0.85	0.02	-0.43	0.02	-0.11
Dry matter yield (g/plant)	0.75	0.27	-0.29	0.17	-0.03
Leaf length (cm)	0.19	-0.51	0.04	0.28	0.51
Leaf width (cm)	0.51	-0.13	0.61	0.46	0.19
Number of pods per plant	0.50	0.33	0.66	-0.40	0.15
Number of effective pods per plant	0.59	0.31	0.53	-0.43	0.14
Pod length (cm)	0.47	0.03	0.27	0.42	-0.30
Pod width (cm)	0.23	-0.73	0.47	0.31	0.07
Number of seeds per pod	-0.01	0.84	0.03	-0.07	0.15
Seed yield per plant (g)	0.57	0.30	0.40	-0.58	-0.02
100 seed weight (g)	0.64	-0.49	0.19	-0.02	-0.20
Leaf β-ODAP (%) content	-0.73	0.24	0.47	0.24	0.28
Seed β-ODAP (%) content	-0.75	0.29	0.50	0.18	0.18
Leaf Crude protein (%) content	0.44	0.63	0.23	0.29	-0.41
Seed Crude protein (%) content	0.33	0.65	0.24	0.38	-0.37
Eigenvalue	6.20	5.09	2.76	1.78	1.39
Variance (%)	29.53	24.22	13.15	8.48	6.60
Cumulative variance (%)	29.53	53.75	66.90	75.38	81.98

Table 5: Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation between green forage yield and its component traits

	D50%F	*DM*	*PH*	*Leaves/pl.*	*PB*	*SB*	*LL*	*LW*	*Leaf β-ODAP*	*Leaf CP*	*DMY*	*GFY*
D50% F		1.000**	0.458	-0.338	-0.146	0.167	-0.490	-0.392	0.476	0.288	-0.024	-0.344
DM	0.858**		0.599*	-0.289	0.018	0.401	-0.548*	-0.215	0.498*	0.385	0.026	-0.271
PH	0.149	0.266		0.349	0.755**	0.851**	-0.150	0.184	-0.092	0.398	0.533*	0.404*
Leaves/pl.	-0.281	-0.126	0.244		0.878**	0.454	0.286	0.521*	-0.423	0.241	0.730**	0.694**
PB	-0.074	-0.035	0.425**	0.444**		0.566*	0.191	0.345	-0.656**	0.396	0.878**	0.943**
SB	0.114	0.149	0.166	0.093	0.265		-0.018	-0.140	-0.173	0.323	0.471**	0.624**
LL	-0.439**	-0.357*	0.034	0.274	0.097	-0.141		0.302	-0.085	-0.287	-0.010	0.043
LW	-0.323*	-0.061	0.118	0.369**	0.135	-0.108	0.325*		0.018	0.310	0.254	0.163
Leaf β-ODAP	0.460**	0.435**	-0.046	-0.359*	-0.421**	-0.123	-0.065	0.025		-0.098	-0.618*	-0.870**
Leaf CP	0.281	0.330*	0.181	0.175	0.242	0.186	-0.232	0.268	-0.095		0.481	0.359
DMY	-0.023	0.084	0.258	0.488**	0.526**	0.226	0.070	0.189	-0.480**	0.370**		0.913**
GFY	-0.330	-0.200	0.179	0.501**	0.454**	0.324*	0.101	0.202	-0.781**	0.327*	0.573**	

*Significant at 5% probability level, **Significant at 1% probability level

D50%F= Days to 50% flowering
DM = Days to maturity
PH = plant height (cm)
Leaf CP = Crude protein content in leaf (%)
Leaves/pl. = number of leaves per plant
PB = Primary branches per plant
SB = Secondary branches per plant
DMY = Dry matter yield per plant (g)
LL = Leaf length (cm)
LW = Leaf width (cm)
Leaf β-ODAP = β-ODAP content in leaf (%)
GFY = Green forage yield per plant (g)

leaf β-ODAP content with dry matter yield per plant and primary branches/plant was observed at the genotypic and phenotypic levels. Basaran *et al.* (2013) have also reported a significantly negative correlation of β-ODAP content with yield and yield attributes of grass peas and mentioned that this may help to develop a variety higher in yield with low β-ODAP content.

As shown in Table 6, the maximum positive direct effect on green forage yield/plant was exhibited by plant height, followed by dry matter yield/plant, crude protein content in leaves, and number of leaves/plant. Out of these, only dry matter yield and number of leaves per plant showed a significant and positive correlation with green forage yield per plant. Thus, direct selection for these two traits would be helpful in enhancing green forage yield. The direct contribution of plant height and number of leaves/plants to green forage yield was also reported by Singh and Roy (2013). The positive direct effect of leaf β-ODAP content on green forage yield was nullified by the high negative indirect effect of leaf β-ODAP *via* days to maturity and dry matter yield/plant, which ultimately resulted in the negative correlation between leaf β-ODAP and green forage yield/plant. The number of primary and secondary branches/plants showed a negative direct effect on green forage yield/plant. The positive correlation between these two traits and green forage yield was due to high positive indirect effects *via* plant height and dry matter yield/plant. The residual value was 0.043 which indicated that only a small proportion of the variation in green forage yield was contributed by some unknown variables not included in this study.

Correlation and Path Coefficient Analysis for Seed Yield with its Component Characters

Among the traits, a strong positive correlation for seed yield was observed with the number of pods/plants and the number of effective pods/plants at both phenotypic and genotypic levels (Table 7). Therefore, the selection of promising genotypes for these two traits would simultaneously improve the seed yield/plant. These findings also concurred with those of other researchers (Mahapatra *et al*., 2020; Jeberson *et al*., 2018; and Ranjithkumar *et al*., 2020). The characters that showed a significant negative correlation with seed β-ODAP content were primary branches/plant, green forage yield/plant, and 100 seed weight. The significant negative correlation between seed β-ODAP content and 100 seed weight was earlier reported by Quader (1985) and Das and Kundagrami (2002). On the other hand, β-ODAP content in seeds had a significant and positive correlation with days to 50% flowering and maturity, which was also reported by Talukdar (2009). These findings suggest that selection for early flowering and early maturity, along with the enhancement of primary branches/plant, seed size, and green forage yield, would pave the way for the development of low neurotoxin-containing varieties. Crude protein content in seeds showed a significant and positive correlation with the number of seeds per pod. Thus, protein content in seeds would increase with the increase in the number of seeds/pod.

The direct and indirect effects of different yield attributes on seed yield are presented in Table 8. The characteristics that exhibited a positive direct effect on seed yield/plant were the number of seeds/pod, number of effective pods/plant, number of primary and secondary branches/plant, and crude protein content in the seed. However, only the number of effective pods/plants had a significant positive association with seed yield/plant. Therefore, direct selection for a high number of effective pods/plants would pave the way to a high seed yield/plant. Similar findings were reported by Ranjithkumar *et al*. (2020) and Mahapatra *et al*. (2020). A positive indirect effect nullified the negative direct effect of pods/plant on seed yield via the number of effective pods/plant and the number of seeds/pods, which

Table 6: Direct (diagonal) and indirect effects of component characters on green forage yield per plant at genotypic level

	D50%F	*DM*	*PH*	*Leaves/ pl.*	*PB*	*SB*	*LL*	*LW*	*Leaf β-ODAP*	*Leaf CP*	*DMY*	*r with GFY*
D50%F	0.1171	-2.3039	0.8962	-0.0432	0.0431	-0.0988	0.2065	0.3741	0.3111	0.1711	-0.0174	-0.3440
DM	0.1171	-2.3041	1.1705	-0.0369	-0.0053	-0.2377	0.2461	0.2057	0.3252	0.2288	0.0193	-0.2713
PH	0.0537	-1.3789	1.9559	0.0446	-0.2227	-0.5043	0.0632	-0.1754	-0.0601	0.2369	0.3912	0.4041
Leaves/pl.	-0.0396	0.6665	0.6828	0.1276	-0.2587	-0.2689	-0.1205	-0.4965	-0.2779	0.1431	0.5357	0.6938
PB	-0.0171	-0.0410	1.4775	0.1121	-0.2947	-0.3354	-0.0806	-0.3294	-0.4286	0.2357	0.6444	0.9428
SB	0.0195	-0.9242	1.6642	0.0579	-0.1668	-0.5927	0.0074	0.1341	-0.1129	0.1919	0.3457	0.6241
LL	-0.0574	1.3461	-0.2936	0.0365	-0.0564	0.0104	-0.4212	-0.2884	-0.0554	-0.1707	-0.0073	0.0427
LW	-0.0460	0.4978	0.3603	0.0666	-0.1019	0.0835	-0.1276	-0.9523	0.0121	0.1842	0.1868	0.1633
Leaf β-ODAP	0.0558	-1.1472	-0.1801	-0.0543	0.1935	0.1025	0.0357	-0.0176	0.6530	-0.0584	-0.4532	-0.8704
Leaf CP	0.0337	-0.8866	0.7792	0.0307	-0.1168	-0.1912	0.1209	-0.2949	-0.0641	0.5947	0.3530	0.3586
DMY	-0.0028	-0.0607	1.0427	0.0932	-0.2588	-0.2792	0.0042	-0.2424	-0.4033	0.2861	0.7339	0.9128

Residual = 0.043

Table 7: Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation between seed yield and its component traits

	D50%F	*DM*	*PH*	*PB*	*SB*	*GFY*	*Pods/pl.*	*Eff. pods*	*SPP*	*100SW*	*Seed β-ODAP*	*Seed CP*	*SY*
D50%F		1.000**	0.458	-0.146	0.167	-0.344	-0.086	-0.109	0.926**	-1.058**	0.512*	0.346	-0.116
DM	0.858**		0.599*	0.018	0.401	-0.271	0.053	-0.007	0.908**	-1.052**	0.525*	0.464	-0.032
PH	0.149	0.266		0.755**	0.851**	0.404	0.061	0.123	0.666**	-0.035	-0.093	0.480	0.121
PB	-0.074	-0.035	0.425**		0.566*	0.943**	0.044	0.310	0.260	0.208	-0.761**	0.354	0.408
SB	0.114	0.149	0.166	0.265		0.624**	0.251	0.179	0.859**	-0.069	0.213	0.279	0.314
GFY	-0.330*	-0.200	0.179	0.454**	0.324*		0.207	0.310	-0.111	0.611*	-0.884**	0.247	0.372
Pods/pl.	-0.046	0.110	-0.007	0.139	0.328*	0.062		1.060**	0.672**	0.337	-0.023	0.363	0.984**
Eff. pods	-0.090	0.028	0.078	0.214	0.261	0.178	0.834**		0.511*	0.356	-0.197	0.379	0.927**
SPP	0.493**	0.527**	0.370**	-0.062	0.288	0.049	0.100	0.086		-0.684**	0.319	0.550*	0.439
100SW	-0.547**	-0.437**	0.021	0.072	-0.151	0.315*	0.146	0.164	-0.053		-0.696**	0.048	0.375
Seed β-ODAP	0.493**	0.461**	-0.054	-0.496**	-0.144	-0.797**	-0.013	-0.135	0.178	-0.392**		0.082	-0.331
Seed CP	0.333**	0.393**	0.230	0.213	0.180	0.216	0.219	0.235	0.317*	0.051	0.081		0.358
SY	-0.089	0.081	0.009	0.158	0.152	0.186	0.737**	0.805**	0.088	0.245	-0.214	0.210	

*Significant at 5% probability level, **Significant at 1% probability level
Pods/pl. = number of pods per plant Eff. Pods = number of effective pods per plant SPP= number of seeds per pod
100SW= 100 seed weight (g) Seed β-ODAP = β-ODAP content in seed (%) SY = Seed yield per plant (g)
Seed CP = Crude protein content in seed (%)

Table 8: Direct (diagonal) and indirect effects of component traits on seed yield per plant at genotypic level

	DTF	*DM*	*PH*	*PB*	*SB*	*GFY*	*Pods/pl.*	*Eff. pods*	*SPP*	*100SW*	*Seed β-ODAP*	*Seed CP*	*r with SY*
DTF	0.0117	-0.8783	-0.1759	-0.0633	0.0292	0.1467	0.0172	-0.0749	0.6930	0.1680	-0.0830	0.0933	-0.1163
DM	0.0117	-0.8784	-0.2297	0.0077	0.0704	0.1157	-0.0107	-0.0049	0.6792	0.1670	-0.0851	0.1251	-0.0320
PH	0.0054	-0.5257	-0.3838	0.3272	0.1492	-0.1723	-0.0123	0.0850	0.4982	0.0056	0.0151	0.1295	0.1210
PB	-0.0017	-0.0156	-0.2900	0.4331	0.0993	-0.4019	-0.0088	0.2133	0.1945	-0.0330	0.1233	0.0955	0.4079
SB	0.0020	-0.3523	-0.3266	0.2451	0.1754	-0.2661	-0.0502	0.1235	0.6428	0.0109	0.0345	0.0752	0.3142
GFY	-0.0040	0.2383	-0.1551	0.4083	0.1095	-0.4263	-0.0414	0.2135	-0.0833	-0.0971	0.1433	0.0665	0.3722
Pods/pl.	-0.0010	-0.0467	-0.0235	0.0190	0.0439	-0.0881	-0.2004	0.7303	0.5027	-0.0534	0.0037	0.0979	0.9842
Eff. pods	-0.0013	0.0062	-0.0474	0.1341	0.0315	-0.1322	-0.2125	0.6889	0.3824	-0.0566	0.0319	0.1023	0.9273
SPP	0.0108	-0.7974	-0.2556	0.1126	0.1507	0.0474	-0.1347	0.3521	0.7482	0.1087	-0.0518	0.1483	0.4393
100SW	-0.0124	0.9237	0.0135	0.0901	-0.0120	-0.2606	-0.0675	0.2455	-0.5120	-0.1588	0.1128	0.0131	0.3754
Seed β-ODAP	0.0060	-0.4612	0.0357	-0.3293	-0.0373	0.3767	0.0045	-0.1353	0.2391	0.1105	-0.1621	0.0221	-0.3307
Seed CP	0.0040	-0.4078	-0.1843	0.1535	0.0489	-0.1052	-0.0728	0.2613	0.4114	-0.0077	-0.0133	0.2696	0.3577

Residual = 0.086

resulted in a positive correlation between pods/plant and seed yield. Days to maturity, plant height, green forage yield per plant, 100 seed weight, and β-ODAP content in seed showed a negative direct effect on seed yield. Similar findings were reported by Kour and Agarwal (2016) and Jeberson *et al*. (2018). The residual value was 0.086, which signified that a small proportion of the variation in seed yield was due to some unknown variables that were not included in this study.

Conclusion

Among the genotypes, except pod length and width, ample variation has been observed for fodder, seed yield, and quality attributing traits, which suggests the scope for developing improved varieties of grass pea. Among the genotypes, JCL-21-N-1 exhibiting high fodder and seed yield along with low β-ODAP content, could be used as a dual-purpose grass pea variety for Assam. High GCV and PCV were observed for β-ODAP content in leaf and seed and dry matter yield per plant. High heritability coupled with high genetic advance was recorded for β-ODAP content in leaf and seed, crude protein content in leaf, green forage yield per plant, dry matter yield per plant, and leaf width. Selection based on these traits would be effective in bringing improvement in the studied grass pea genotypes. The principal component analyses have shown wider variations and can be used to identify the parameters contributing to variability and the selection of suitable genotypes for selection and crop improvement. Among the characters studied, β-ODAP content in leaves, number of leaves per plant, and dry matter yield per plant were highly heritable and had exhibited a strong direct effect on green forage yield, with a significant correlation indicating the importance of these characteristics for improving green forage yield. In the case of seed yield components, the number of effective pods per plant had moderate heritability, high positive direct and indirect effects on seed yield, and a significant positive correlation. Therefore, selection for these traits would be effective for improving seed yield.

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