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GENETIC DIVERSITY AND CLUSTER ANALYSIS OF FODDER COWPEA (*Vigna unguiculata* L. WALP.) GERMPLASM USING MAHALANOBIS D² STATISTICS

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ABSTRACT

Fodder cowpea [*Vigna unguiculata* (L.) Walp.] is an important forage legume valued for its rapid growth, high biomass production, nutritional quality, and adaptability to diverse agro-climatic conditions. The present study was conducted to assess the genetic diversity among 104 fodder cowpea genotypes comprising 100 germplasm accessions and four check varieties using Mahalanobis D² statistics. Based on genetic divergence, the genotypes were grouped into eleven distinct clusters following Tocher's method. Cluster IV was the largest, containing 16 genotypes, whereas Cluster VI contained only three genotypes. The highest intra cluster distance was recorded in Cluster VIII (217.67), indicating substantial variability among genotypes within the cluster. The maximum inter cluster distance was observed between Clusters III and XI (4939.95), followed by Clusters VII and XI (4857.77), suggesting the presence of wide genetic divergence among these groups. The large inter cluster distances indicate that genotypes from these clusters may serve as potential parents for hybridization programmes aimed at generating broad variability and superior recombinants. Based on cluster distribution, genetic divergence, and overall performance, genotypes EC-240988, IC-276936, IC-397908, IC-471384, EC-240693-1, IC-397907, and IC-399000 were identified as promising genetic resources for fodder cowpea improvement. The substantial genetic variability observed among the evaluated germplasm offers considerable scope for selection and utilization of diverse parents in breeding programmes for developing high-yielding and nutritionally superior fodder cowpea cultivars.

Keywords : Fodder cowpea, Genetic diversity, Mahalanobis D² analysis, Cluster analysis, Germplasm evaluation.

Introduction

Livestock plays a vital role in the agricultural economy of India by contributing substantially to food security, nutritional security, employment generation, and rural livelihoods. India possesses one of the largest livestock populations in the world, with more than 535 million livestock animals comprising cattle, buffalo, sheep, goats, and other domesticated species (Jadhav *et al.*, 2025). The livestock sector contributes significantly to the agricultural Gross Value Added

(GVA) and serves as a major source of income for small and marginal farmers.

Despite the vast livestock wealth, the availability of quality fodder remains a major constraint affecting livestock productivity in India. Recent estimates indicate that the country faces a shortage of approximately 11–32% in green fodder and about 23% in dry fodder, resulting in reduced animal productivity and increased feeding costs (ICAR-IGFRI, 2024). Furthermore, annual fodder demand in India is

estimated at nearly 1183 million tonnes, whereas the current supply is around 843 million tonnes, leading to an overall fodder deficit of about 29% (Jadhav *et al.*, 2025). Such deficits are expected to increase further due to rising livestock populations, shrinking grazing lands, and climate variability.

Projections suggest that by 2030, India may experience a green fodder deficit exceeding 33,500 million tonnes annually if appropriate interventions are not undertaken (Jadhav *et al.*, 2025). Similarly, earlier projections indicated green fodder deficits of nearly 65% and dry fodder deficits of about 25%, highlighting the urgent need for enhancing forage production through genetic improvement and efficient crop management strategies (Singh *et al.*, 2013).

Among forage legumes, cowpea [*Vigna unguiculata* (L.) Walp.] occupies a prominent position because of its rapid growth, high biomass production, superior nutritional quality, drought tolerance, and adaptability to diverse agro climatic conditions (Ehlers and Hall, 1997). The crop contains high crude protein content and serves as an excellent source of green fodder, hay, silage, and soil enrichment through biological nitrogen fixation. Its short duration and compatibility with multiple cropping systems make it highly suitable for sustainable fodder production under rainfed and semi arid environments.

The success of any crop improvement programme largely depends on the availability and utilization of genetically diverse germplasm. Genetic diversity provides opportunities for the identification of superior genotypes, broadening of the genetic base, and development of high yielding cultivars through hybridization. Assessment of genetic divergence

among genotypes therefore forms an essential component of plant breeding programmes.

Mahalanobis D² statistics has been widely employed as an efficient multivariate technique for estimating genetic divergence among genotypes based on multiple characters simultaneously (Mahalanobis, 1936). The technique facilitates the identification of genetically diverse parents capable of producing desirable recombinants and exploiting heterosis. Therefore, the present investigation was undertaken to assess the extent of genetic diversity among 104 fodder cowpea genotypes and to identify promising and genetically divergent parental lines for future breeding programmes.

Materials and Methods

Experimental Site

The present investigation was conducted during the *Kharif* season of 2022 at the Forage Research Block, Agricultural Research Institute (ARI), Rajendranagar, Hyderabad, Telangana, India. The experimental site is geographically located at 17°19' N latitude and 78°24' E longitude with an altitude of approximately 542 m above mean sea level. The region falls under the Southern Telangana Agro Climatic Zone and is characterized by a semi-arid tropical climate with sandy clay loam soils and moderate fertility.

Plant Material

The experimental material consisted of 104 fodder cowpea [*Vigna unguiculata* (L.) Walp.] genotypes, including 100 germplasm accessions obtained from the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, and four check varieties viz., BL-1, BL-2, BL-4 and Vijaya.

Table 1 : Genotypes list predominantly used in the investigation:

S No:	Genotype	Source	S No:	Genotype	Source
1	EC-2790	NBPGR-New Delhi	47	IC-626167	NBPGR-New Delhi
2	EC-4221	NBPGR-New Delhi	48	IC-276936	NBPGR-New Delhi
3	EC-107192	NBPGR-New Delhi	49	IC-284554	NBPGR-New Delhi
4	EC-240826	NBPGR-New Delhi	50	IC-319513	NBPGR-New Delhi
5	EC-240829	NBPGR-New Delhi	51	IC-331106	NBPGR-New Delhi
6	EC-240841	NBPGR-New Delhi	52	IC-334368	NBPGR-New Delhi
7	EC-240861	NBPGR-New Delhi	53	IC-353873	NBPGR-New Delhi
8	EC-240862	NBPGR-New Delhi	54	IC-372722	NBPGR-New Delhi
9	EC-240887	NBPGR-New Delhi	55	IC-372724	NBPGR-New Delhi
10	EC-240891	NBPGR-New Delhi	56	IC-397455	NBPGR-New Delhi
11	EC-240930	NBPGR-New Delhi	57	IC-397908	NBPGR-New Delhi
12	EC-240982	NBPGR-New Delhi	58	IC-399000	NBPGR-New Delhi
13	EC-240983	NBPGR-New Delhi	59	IC-401381	NBPGR-New Delhi
14	EC-240988	NBPGR-New Delhi	60	IC-410312	NBPGR-New Delhi
15	EC-240992	NBPGR-New Delhi	61	IC-420467	NBPGR-New Delhi

16	EC-244071	NBPGR-New Delhi	62	IC-546503	NBPGR-New Delhi
17	EC-244074	NBPGR-New Delhi	63	IC-546519	NBPGR-New Delhi
18	EC-244077	NBPGR-New Delhi	64	IC-39930	NBPGR-New Delhi
19	EC-4381	NBPGR-New Delhi	65	IC-202780	NBPGR-New Delhi
20	EC-4862	NBPGR-New Delhi	66	EC-43203	NBPGR-New Delhi
21	EC-244322	NBPGR-New Delhi	67	IC-202938	NBPGR-New Delhi
22	EC-244396	NBPGR-New Delhi	68	IC-209144	NBPGR-New Delhi
23	EC-244414	NBPGR-New Delhi	69	IC-209153	NBPGR-New Delhi
24	EC-5269	NBPGR-New Delhi	70	IC-257430	NBPGR-New Delhi
25	EC-16141	NBPGR-New Delhi	71	IC-259075	NBPGR-New Delhi
26	EC-528420	NBPGR-New Delhi	72	IC-397907	NBPGR-New Delhi
27	EC-528422	NBPGR-New Delhi	73	IC-471384	NBPGR-New Delhi
28	IC-202781	NBPGR-New Delhi	74	EC-724352	NBPGR-New Delhi
29	IC-202790	NBPGR-New Delhi	75	EC-724378	NBPGR-New Delhi
30	IC-202803	NBPGR-New Delhi	76	EC-724413	NBPGR-New Delhi
31	IC-202804	NBPGR-New Delhi	77	EC-725103	NBPGR-New Delhi
32	IC-430632	NBPGR-New Delhi	78	EC-472282	NBPGR-New Delhi
33	IC-437181	NBPGR-New Delhi	79	IC-202837	NBPGR-New Delhi
34	IC-471386	NBPGR-New Delhi	80	EC-96654	NBPGR-New Delhi
35	IC-471953	NBPGR-New Delhi	81	EC-98661	NBPGR-New Delhi
36	IC-488273	NBPGR-New Delhi	82	EC-367683	NBPGR-New Delhi
37	IC-519603	NBPGR-New Delhi	83	EC-724160	NBPGR-New Delhi
38	IC-519745	NBPGR-New Delhi	84	EC-240665	NBPGR-New Delhi
39	IC-560918	NBPGR-New Delhi	85	EC-724261	NBPGR-New Delhi
40	IC-561238	NBPGR-New Delhi	86	EC-240693-1	NBPGR-New Delhi
41	EC-18142	NBPGR-New Delhi	87	IC-372726	NBPGR-New Delhi
42	IC-569092	NBPGR-New Delhi	88	EC-724681	NBPGR-New Delhi
43	IC-596962	NBPGR-New Delhi	89	EC-724874	NBPGR-New Delhi
44	IC-601234	NBPGR-New Delhi	90	EC-240714	NBPGR-New Delhi
45	IC-610281	NBPGR-New Delhi	91	EC-738120	NBPGR-New Delhi
46	IC-625401	NBPGR-New Delhi	92	EC-240765	NBPGR-New Delhi
93	EC-244025	NBPGR-New Delhi	99	IC-259084	NBPGR-New Delhi
94	EC-738260	NBPGR-New Delhi	100	IC-259104	NBPGR-New Delhi
95	EC-244057	NBPGR-New Delhi	101	BL-1 (Check)	NBPGR-New Delhi
96	IC-39897	NBPGR-New Delhi	102	BL-2 (Check)	NBPGR-New Delhi
97	IC-622575	NBPGR-New Delhi	103	BL-4 (Check)	NBPGR-New Delhi
98	IC-257450	NBPGR-New Delhi	104	VIJAYA (Check)	NBPGR-New Delhi

Experimental Design and Crop Management

The experiment was laid out in an Augmented Complete Block Design (ACBD) following the procedure described by Federer (1956). The genotypes were evaluated in five blocks with repeated checks in each block. Each genotype was sown in a single row of 3 m length with a spacing of 30 cm between rows and 10 cm between plants. All recommended agronomic and plant protection practices for cowpea cultivation were followed throughout the crop growth period as per ICAR (2020) recommendations.

Observations Recorded

Observations were recorded on five randomly selected competitive plants from each genotype for fourteen agro-morphological, yield, and quality traits. The observations included:

1. Days to first flowering
2. Plant height (cm)
3. Number of primary branches per plant
4. Number of secondary branches per plant
5. Leaf length (cm)
6. Leaf width (cm)
7. Fresh leaf weight (g)
8. Fresh stem weight (g)
9. Leaf-to-stem ratio
10. Green fodder yield per plant (g)
11. Dry fodder yield per plant (g)
12. Dry matter content (%)
13. Crude protein content (%)
14. Crude fibre content (%)

The mean values of five plants were utilized for statistical analysis.

Statistical Analysis

Genetic divergence among the genotypes was estimated using Mahalanobis D² statistics (Mahalanobis, 1936), which provides a generalized measure of distance among genotypes based on multiple traits simultaneously. The genotypes were grouped into clusters using Tocher's method as described by Rao (1952). Average intra-cluster and inter-cluster distances were calculated to assess the magnitude of genetic diversity among the genotypes. The interpretation of genetic divergence and selection of diverse parents were carried out according to the principles outlined by Singh and Chaudhary (1979). Genotypes belonging to clusters separated by large inter-cluster distances were considered genetically

divergent and suitable for utilization in hybridization programmes.

Results and Discussion

Genetic Divergence Analysis

The assessment of genetic diversity among the 104 fodder cowpea genotypes using Mahalanobis D² statistics revealed the existence of substantial genetic variability within the experimental material. The genotypes were grouped into eleven distinct clusters following Tocher's method, indicating the presence of considerable genetic divergence among the germplasm accessions. The occurrence of a large number of clusters suggested the availability of a broad genetic base that could be effectively utilized in fodder cowpea improvement programmes.

Grouping of genotypes

Table 2 : Distribution of 104 fodder cowpea genotypes into different clusters based on Mahalanobis D² analysis studied during *kharif*,2022

Clusters	No. of genotypes	Name of the genotypes
Cluster I	14	EC-2790, EC-240829, EC-240887, EC-16141, IC-437181, IC-471386, IC-519745, IC-39930, IC-202780, EC-43203, IC-259075, IC-471384, EC-724874, EC-244057.
Cluster II	9	EC-244074, EC-244077, EC-4381, EC-18142, IC-420467, IC-546503, BL-1, BL-2, BL-4.
Cluster III	5	IC-319513, IC-331106, EC-240693-1, IC-372726, EC-244025.
Cluster IV	16	EC-4221, EC-240826, EC-240861, EC-240982, EC-240983, EC-240988, IC-202781, IC-202790, IC-202803, IC-488273, IC-284554, IC-353873, IC-397908, IC-399000, EC-472282, IC-257450.
Cluster V	13	EC-4862, EC-244322, EC-244396, EC-244414, IC-561238, IC-569092, IC-596962, IC-601234, IC-610281, IC-202938, EC-96654, EC-98661, IC-259104.
Cluster VI	3	EC-724413, EC-725103, EC-240765.
Cluster VII	7	EC-240891, EC-244071, IC-471953, IC-546519, IC-743025, EC-367683, EC-724160.
Cluster VIII	6	IC-626167, IC-334368, IC-410312, EC-724352, EC-738120, IC-622575.
Cluster IX	13	EC-107192, EC-240841, EC-240992, EC-5269, IC-430632, IC-519603, IC-560918, IC-202837, EC-240665, EC-724261, EC-724681, EC-738260, IC-39897.
Cluster X	9	IC-625401, IC-372722, IC-372724, IC-397455, IC-401381, IC-209144, EC-724378, EC-240714, VIJAYA.
Cluster XI	9	EC-240862, EC-240930, EC-528420, EC-528422, IC-202804, IC-276936, IC-209153, IC-397907, IC-259084.

Among the eleven clusters formed through Mahalanobis D² analysis, Cluster IV accommodated the highest number of genotypes (16), followed by Clusters I, V, and IX with 14, 13, and 13 genotypes, respectively. In contrast, Cluster VI contained only three genotypes and represented the smallest group. The distribution of genotypes across clusters demonstrated that accessions from different geographical regions were frequently grouped together, whereas those originating from the same region were often placed in separate clusters. This finding suggests that geographical origin does not necessarily reflect the extent of genetic divergence among fodder cowpea genotypes. Similar results have been reported by

Vamshi *et al.* (2022), Barik *et al.* (2024), and Borah and Khan (2023), who observed a lack of correspondence between geographical distribution and genetic diversity in cowpea germplasm. The observed pattern of clustering may be attributed to factors such as selection pressure, genetic drift, germplasm exchange, and environmental adaptation, which contribute to the accumulation of genetic variability independent of geographical boundaries (Kandel *et al.*, 2023; Barathi *et al.*, 2023). These results highlight the importance of assessing genetic divergence using multivariate statistical approaches rather than relying solely on geographical origin for parent selection in breeding programmes.

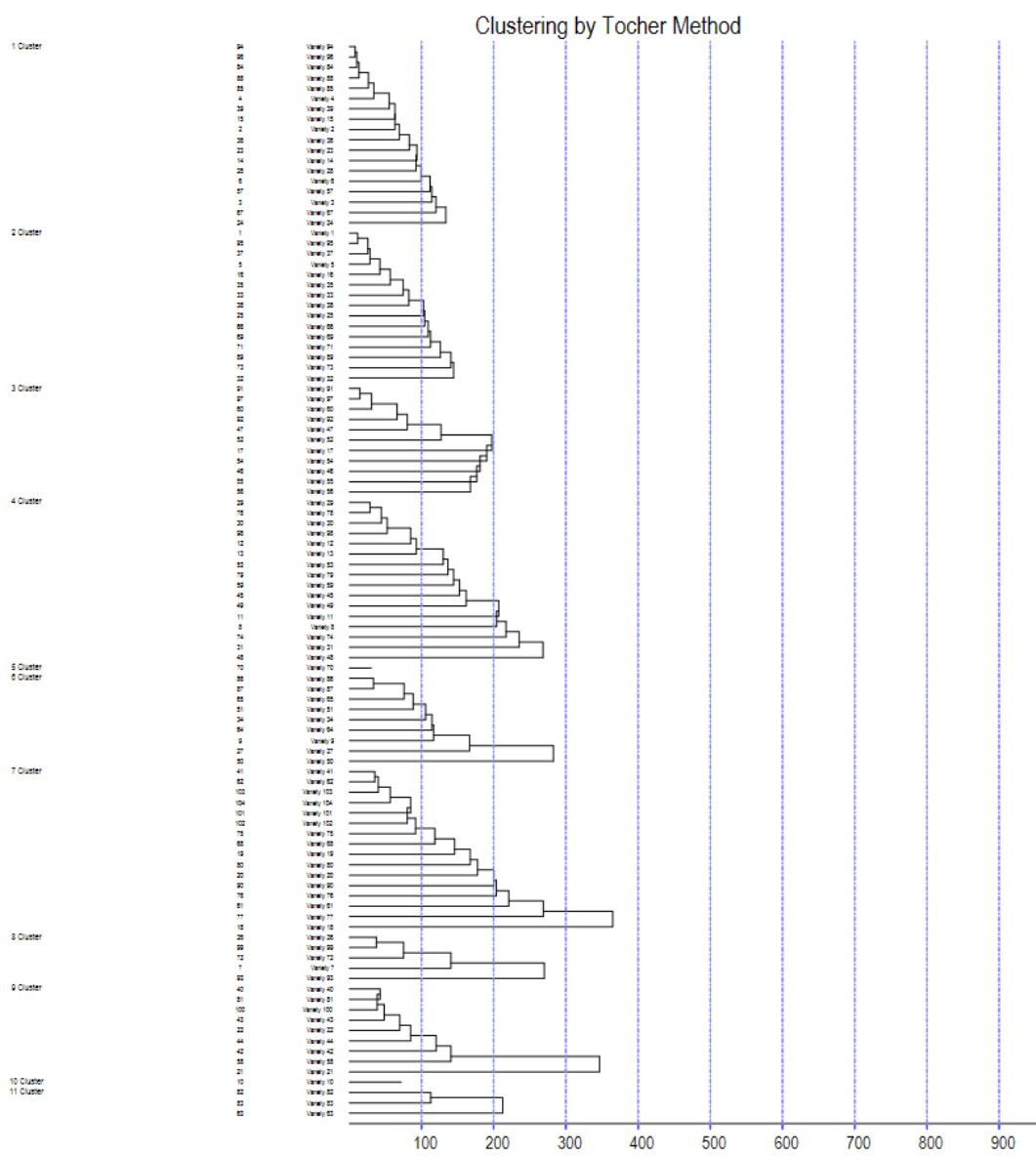


Fig. 1 : Clustering Pattern of 104 Genotypes by Tocher's Method

Intra and Inter Cluster Distances

The average intra and inter cluster distances among the eleven clusters are presented in Table 3

Table 3 : Average intra and inter cluster distances among clusters in fodder cowpea

[illegible]

The average intra and inter cluster distances among the eleven clusters are presented in Table 3. The intra cluster D² values ranged from 116.58 to 217.67, indicating varying degrees of genetic variability among genotypes within individual clusters. The highest intra cluster distance was recorded in Cluster VIII (217.67), followed by Clusters VII (215.34), IV (199.78), VI (181.34), III (176.12), and IX (174.95). The relatively large intra cluster distances observed in these clusters suggest the presence of substantial genetic variation among their constituent genotypes, thereby providing opportunities for the selection of superior lines with desirable trait combinations.

The inter cluster distances revealed a wide range of genetic divergence among the clusters. The maximum inter cluster distance was observed between Clusters III and XI (4939.95), followed by Clusters VII and XI (4857.77), VII and VI (3872.35), V and VII (3338.51), III and V (3235.55), and III and VI (3217.90). The large genetic distances among these clusters indicate the presence of highly diverse genotypes, suggesting that crosses involving parents selected from such clusters may generate broad variability and increase the likelihood of obtaining desirable transgressive segregants in subsequent generations. Similar findings were reported by Vamshi *et al.* (2022), Borah and Khan (2023), and Barik *et al.* (2024), who emphasized the importance of utilizing

genetically divergent parents to enhance breeding efficiency and recover superior recombinants.

In contrast, the minimum inter cluster distance was observed between Clusters V and X (150.28), followed by Clusters II and V (199.06) and Clusters II and X (225.08), indicating a close genetic relationship among the genotypes grouped within these clusters. Such low inter cluster distances suggest limited genetic divergence and comparatively lower prospects for generating novel variability through hybridization. Similar observations regarding the significance of inter cluster distance in parent selection have been reported in fodder cowpea, vegetable cowpea, and other forage crops.

Overall, the results demonstrated that geographical origin alone may not serve as a reliable criterion for parent selection. Instead, genotypes belonging to clusters separated by greater inter cluster distances should be prioritized in breeding programmes to maximize heterosis, broaden the genetic base, and enhance the chances of recovering superior segregants. Comparable conclusions were drawn by Borah and Khan (2023) and Vamshi *et al.* (2022) in their studies on genetic divergence in fodder cowpea.

Cluster Mean Performance

The cluster mean values for different agro morphological, fodder yield and quality traits are presented in Table 4.

Table 4 : Cluster mean values for agro morphological, fodder yield and quality traits in fodder cowpea

	DFF	PH	NPB	NSB	LL	LW	FLW	FSW	L/ S	GFY	DFY	DMC	CP	CF
Cluster I	54.59	37.98	8.93	7.12	9.78	6.75	48.30	65.06	0.74	113.35	22.01	19.36	15.98	28.22
Cluster II	53.60	35.34	8.67	5.69	8.69	6.26	43.28	59.70	0.72	102.98	19.69	19.08	15.83	27.88
Cluster III	53.73	26.20	8.32	5.90	7.67	5.49	61.68	76.77	0.80	138.45	28.89	20.79	17.70	30.14
Cluster IV	54.75	30.43	8.58	6.46	8.56	6.11	54.43	70.00	0.77	124.43	24.08	19.41	16.98	28.41
Cluster V	57.00	39.40	7.20	9.40	9.40	5.50	38.70	56.40	0.68	95.10	17.90	18.80	15.30	29.30
Cluster VI	53.44	23.74	7.60	5.04	7.33	5.23	38.79	55.69	0.69	94.48	17.61	18.57	15.46	26.99
Cluster VII	52.44	43.47	8.75	6.68	9.19	5.94	62.48	77.54	0.8	140.03	29.98	21.28	17.51	30.82
Cluster VIII	53.40	21.88	7.28	4.96	6.98	4.92	47.64	63.80	0.74	111.42	21.10	17.58	15.72	26.84
Cluster IX	53.44	48.77	9.00	7.27	10.32	5.74	52.44	67.79	0.77	120.14	24.00	19.93	16.87	28.73
Cluster X	53.00	46.40	9.40	3.40	10.00	6.40	42.20	56.60	0.74	98.80	18.10	18.30	14.30	25.30
Cluster XI	53.33	45.90	9.33	6.47	10.63	5.63	34.30	51.23	0.66	85.53	16.30	19.03	14.70	27.93

The cluster mean values revealed considerable variation among the eleven clusters for all the agro morphological, fodder yield and quality traits studied, indicating the presence of substantial genetic diversity among the evaluated fodder cowpea genotypes. Cluster VII recorded the highest mean values for fresh leaf weight (62.48 g), fresh stem weight (77.54 g), green fodder yield per plant (140.03 g), dry fodder yield per plant (29.98 g), dry matter content (21.28%) and crude fibre content (30.82%), suggesting its potential

importance as a source of superior fodder yield and biomass production. Cluster III exhibited high values for leaf to stem ratio (0.80), dry matter content (20.79%) and crude protein content (17.70%), indicating its usefulness in improving fodder quality traits.

Cluster IX recorded the highest plant height (48.77 cm), whereas Cluster XI showed the highest leaf length (10.63 cm). Cluster X possessed the highest number of primary branches per plant (9.40), while

Cluster V exhibited the highest number of secondary branches per plant (9.40). The superior performance of different clusters for specific traits indicates the unequal distribution of favourable alleles among the clusters.

The simultaneous consideration of cluster mean performance and inter cluster distances provides an effective basis for parent selection. Genotypes belonging to clusters with high mean performance and wide genetic divergence may be utilized in hybridization programmes to generate broad variability and recover superior transgressive segregants. Therefore, crosses involving genotypes from highly divergent clusters such as III, VII and XI may prove beneficial for developing high yielding and nutritionally superior fodder cowpea cultivars. Similar observations were reported by Vamshi *et al.* (2022), Borah and Khan (2023), Barik *et al.* (2024) and Kandel *et al.* (2023).

Identification of Promising Genotypes

Based on genetic divergence, cluster distribution, and overall mean performance, several genotypes were identified as promising candidates for future fodder cowpea improvement programmes. Genotypes EC-240988, IC-276936, IC-397908, IC-471384, EC-240693-1, IC-397907, and IC-399000 exhibited superior performance for important fodder yield and quality traits and were distributed across genetically diverse clusters. The identification of such genotypes is particularly important, as the selection of parents based on both genetic divergence and agronomic performance has been recognized as an effective breeding strategy in self pollinated crops, including cowpea and blackgram (Singh and Chaudhary, 1979; Borah and Khan, 2023; Barathi *et al.*, 2023).

The substantial genetic diversity observed among the evaluated germplasm suggests considerable potential for genetic improvement through hybridization and selection. Genotypes belonging to highly divergent clusters are expected to generate greater variability and facilitate the recovery of superior recombinants in subsequent generations. Therefore, the promising genotypes identified in the present study may serve as valuable parental resources for broadening the genetic base of fodder cowpea and developing high-yielding cultivars with enhanced biomass production and improved fodder quality for sustainable forage production systems.

Conclusion

The present study revealed substantial genetic diversity among 104 fodder cowpea genotypes, which were grouped into eleven distinct clusters based on

Mahalanobis D² statistics. The maximum inter cluster distance was observed between Clusters III and XI, indicating the presence of highly divergent genotypes suitable for hybridization. Genotypes such as EC-240988, IC-276936, IC-397908, IC-471384, EC-240693-1, IC-397907 and IC-399000 were identified as promising genetic resources for fodder yield and quality improvement. Therefore, these diverse genotypes may be effectively utilized in future breeding programmes for developing superior fodder cowpea cultivars with enhanced productivity and nutritional quality.

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