Original Research Article

Estimation of genetic variability and ~~genetic~~ diversityof a~~n underutilized~~ wild legume *Vigna stipulacea*(Lam.)Kuntz to unravel its fodder potential

Comment [B1]: Uniform style of the JABB needs to be thoroughly followed, as mentioned in the given 'Author guidelines' and 'SDI-Paper-template' of the Journal.

ABSTRACT

In the context of climate change,a versatile wild legumelike *Vigna stipulacea*(Lam.) Kuntz, resistant to biotic & abiotic stresses would anticipate as an alternative to leguminous fodder. To figure out its potential for fodder yield, variability and diversity present in population was assessed by including thirty genotypes in randomized block design with three replications. High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was reported for number of secondary branches plant^{-1}, number of nodes plant^{-1}, internodal length, green fodder yield plot^{-1}, dry fodder yield plot^{-1}, leaf dry weight plant^{-1}, stem dry weight plant^{-1}, plant height, Leaf area index (LAI) and leaf stem ratio (LSR). As there was high GCV, direct selection of these characters is feasible. High heritability combined with high genetic advance was observed for number of secondary branches plant^{-1}, number of leaves plant^{-1}, number of nodes plant^{-1}, internodal length, green fodder yield plot^{-1}, dry fodder yield plot^{-1}, leaf dry weight plant^{-1}, stem dry weight plant^{-1}, seed yield plant^{-1}, plant height, LAI and LSR implying traits were controlled by additive gene action where direct selection is possible. Based on cluster analysis, genotypes were grouped into five clusters and highest inter-cluster distance was between clusters II & V implying greater divergence between them and can be considered as parents for hybridization. Cluster V & IV exhibited highest mean values for various traits contributing to fodder yield, genotypes belonged to these clusters can be utilized as parents for improving fodder yield of *Vigna stipulacea.*

Keywords: Analysis of variance, Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Heritability, Genetic Advance as per cent mean (GAM), Genetic divergence, Cluster analysis, Vigna stipulacea

Comment [B2]: Please revised the keywords as they are too many. It should be as-

Genetic variability, diversity, wild legume, fodder potential etc.

1. INTRODUCTION

Livestock sector plays a substantial part in rural livelihood and economy, contributing 6.0 % of total GVA NDDB, [1]. It transforms low-value, inedible or unpalatable material into milk, meat and eggs and contribute directly to food security. Besides food supply, it provides natural fertilizers, leather, traction and transportation, etc. and contribute to income security.

As it has considerable role in both food and income security of the nation, it deserves meticulous care and emphasis in terms of balanced nutrients for better health. The nutritive value of feed and fodder has a significant impact on livestock productivity. For better health and yield of milk, livestock need a balanced diet of three parts of green grass and one part of leguminous fodder [2][3].

Legumes belong to the family Fabaceae, the third-largest family of flowering plants [4], and only ten species are domesticated [5]which have recognized uses ranging from forage, green manure and cover crops in addition to their high protein. Besides its recognised uses, domesticated *Vigna* species due to their poor adaptability to stress, yield minimum. On the other side, wild *Vigna* species which are chief source of food, fodder and manure, possess remarkable characters of biotic and abiotic stress resistance [6]. Among 200 species of genus *Vigna*[7], it includes more than 100 wild species which do not even have common names [8] and are denoted as the under-exploited wild *Vigna* species, undomesticated *Vigna* species and wild *Vigna*[5]. These wild *Vigna* species are adapted to various habitats including harsh environments such as sandy beaches, acid soils, limestone rocks, deserts and wetlands and are capable to combat climate change. so, it is more appropriate to domesticate these wild species well adapted to environmental stress than improving stress resistance of the existing low level resistant domesticated species~~, due to the low levels of resistance to environmental stress~~. Tomooka *et al*. [8]reported that under harsh climatic and environmental conditions (biotic and abiotic stress), the domesticated *Vigna* species find difficult to resist and perform well while their wild under-exploited relatives thrive successfully.

Comment [B3]: Make a para

Comment [B4]: Please give reference, if possible.

Among the seven subgenera under the genus *Vigna*, the Asiatic one is Ceratotropis which includes 24 species of which *Vigna stipulacea* is an underutilised wild legume with good source of food and fodder, especially combat climate change regime of tropical habitats and biotic stress management [9]. It is locally known as minnipayaruwith a ~445.1 Mbp genome size and belongs to secondary gene pool of cultivated black gram and green gram. Seeds of this species are edible and primarily grown locally as pasture but often as food [10]. It inhabits parts of South Asia mostly lowland paddy fields prior to paddy cultivation which possess various favourable traits such as faster growth, shorter duration and broad resistance to various pests and diseases due to which it has potential for domestication as a new crop and the desirable traits present may be transmitted to the cultivated *Vigna* species for their improvement [11].

Wild *Vigna* species present a very wide range of untapped variability both in terms of important agronomic characteristics and genetic diversity which makes it an important source of information for crop improvement and an important food and animal feed source for the future [5]. The domestication, adoption and industrial utilization of the under-exploited wild *Vigna* species could be of utmost importance in contributing to achieving the FAO expectation to increase food production by 70 percent more by the year 2050 [5].

Hence, the present investigation was undertaken with the objectives of determining the amount of untapped genetic variability and genetic diversity present among*Vigna stipulacea* genotypes and use that information in crop improvement of *Vigna stipulacea* or related wild and domesticated *Vigna* species for fodder yield.

2. MATERIALS AND METHODS

2.1 Plant Materials

Thirty genotypes of *Vigna stipulacea*which, ~~were~~ collected from various locations were accessed from ICAR-NBPGR (National Bureau of Plant Genetic Resources), New Delhi and~~formed the material of the study. The genotypes~~ were used as materials for the study. The list of the genotypes is given ~~are listed~~ in the Table 1[12].

2.2 Experimental Design

Experiment was performed during *Rabi* 2021-2022 in Randomized Block Design (RBD) with three replications at the Department of Plant Breeding and Genetics, College of Agriculture, Vellayani, Kerala Agricultural Universitylocated at a latitude of 8.440° N and longitude of 76.9888° E with an altitude of 29 m above MSL(mean sea level). Seeds were first scarified using sand paper and then sown in field at a spacing of 30 x 15 cm in a plot size of 3m^2. At 50 percent flowering stage,observations were recorded from five randomly selected representative plants and evaluated for characters such as number of secondary branches per plant, number of leaves per plant, number of nodes per plant, internodal length (cm), days to 50 percent flowering, green fodder yield per plot (g), dry fodder yield per plot (g), leaf dry weight per plant (g), stem dry weight per plant (g), number of seeds per pod, 100 seed weight (g), seed yield per plant, plant height (cm), days to maturity, leaf area index (LAI) and leaf stem ratio (LSR).

Table 1. List of accessions of *Vigna stipulacea* used for the study

Comment [B5]: bold

Treatment	Accession name	Institute	District	State
T1	IC 331457	NBPGR*	Bilaspur	Chhattisgarh
T2	IC 121435	NBPGR	-	-
T3	IC 261384	NBPGR	Kurnool	Andhra Pradesh
T4	IC 550551	NBPGR	Srikakulum	Andhra Pradesh
T5	IC 550545	NBPGR	Srikakulum	Andhra Pradesh
T6	IC 553557	NBPGR	West Godavari	Andhra Pradesh
T7	IC 524667	NBPGR	Cuddapah	Andhra Pradesh
T8	IC 553494	NBPGR	Kurnool	Andhra Pradesh
T9	IC 622865	NBPGR	Bhopal	Madhya Pradesh
T10	IC 261321	NBPGR	Kurnool	Andhra Pradesh
T11	IC 550531	NBPGR	Vizianagaram	Andhra Pradesh
T12	IC 622860	NBPGR	Rewa	Madhya Pradesh
T13	IC 610275	NBPGR	Kurnool	Andhra Pradesh
T14	IC 625694	NBPGR	Idukki	Kerala
T15	IC 553525	NBPGR	Prakasam	Andhra Pradesh
T16	IC 521215	NBPGR	Ramnathpuram	Tamil Nadu
T17	IC 553520	NBPGR	Prakasam	Andhra Pradesh
T18	IC 331436	NBPGR	Khurda	Odisha
T19	IC 550548	NBPGR	Srikakulam	Andhra Pradesh
T20	IC 553516	NBPGR	Prakasam	Andhra Pradesh
T21	IC 417392	NBPGR	Coimbatore	Tamil Nadu

T22	IC 331453	NBPGR	Raipur	Chhattisgarh
T23	IC 331454	NBPGR	Raipur	Chhattisgarh
T24	IC 305192	NBPGR	Kurnool	Andhra Pradesh
T25	IC 553510	NBPGR	Prakasam	Andhra Pradesh
T26	IC 550536	NBPGR	Vizianagaram	Andhra Pradesh
T27	IC 550532	NBPGR	Vizianagaram	Andhra Pradesh
T28	IC 553538	NBPGR	Krishna	Andhra Pradesh
T29	IC 550538	NBPGR	Vizianagaram	Andhra Pradesh
T30	IC 553554	NBPGR	West Godavari	Andhra Pradesh

*NBPGR=?

2.3 Statistical Analysis

Significant differences between genotypes for various characters was tested by using analysis of variancefor replicated data applying the method suggested by Panse and Sukhatme[13] by using “GRAPES” (General R based Analysis Platform Empowered by Statistics) software of Kerala Agricultural University [14].The mean values were compared at a *p*< 0.05 significance level.

Genetic parameters such as coefficient of variation, heritability and genetic advance as percent of mean were analysed and estimated using “GRAPES” software of Kerala Agricultural University [14]. Range of coefficient of variation was classified according to the scale given by Sivasubramanian and Menon [15]and the obtained values of heritability and genetic advance as percent of mean for each trait was classified according to the scale put forthe by Johnson *et al*. [16].

Genetic divergence was evaluated using D^2statistics method suggested by Mahalanobis[17] which was elaborated by Rao [18]. The contribution of individual trait to genetic divergence was evaluated using the method suggested by Singh and Chaudhary [19], Nadarajan and Gunasekaran [20]. Data recorded for various characters from representative samples was evaluated using Indo stat software by following Tochers method.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

The utmost requirement for any effective breeding programme is the availability of considerable variation in the germplasm. Therefore, assessing the genetic variability existing in its genetic makeup is crucial. Analysis of variance is a powerful tool that partition genetic variability from total variability and helps a breeder on deciding further breeding programme. The analysis of variance for various characters of *V. stipulacea* *are* mentioned in Table 2. Analysis of variance revealed that the mean sum of squares due to genotypes is significant for all characters considered except number of seeds per pod among all accessions. Gore [21] observed significant differences among 94 accessions of *Vigna stipulacea* and *Vigna trilobata* for most of the characters such as plant height, number of days to 50 percent flowering, days to maturity and number of seeds per pod which is in line with the present study except for number of seeds per pod. The reason for non-significant variation for number of seeds per pod in the present study might be due to the fact that either the number of accessions studied were low or the wild materials have less seed yield potentiality and variability. Result was in accordance with the reports of Venkateswarlu[22],Sathya and Jayamani [23] and Sineka *et al.*[24]in *Vigna radiata.*

3.2 Genetic Parameters

3.2.1 Coefficient of variation

The values of PCV and GCV ~~were~~ are given in the Table 3~~.~~ and represented graphically in the Figure 1. Biometrical characters exhibited high PCVthan GCV for all the traits considered except for plant heightimplying the presence of environmental effects on expression of these characters. However, environmental influence was not observed for ~~character~~ plant height as the values of PCV and GCV were same. Similar results were observed in *Vigna mungo*[25], *Vigna unguiculata*[26], *Vigna subterrenean*[27] and *Vigna mungo* [28].

Comment [B6]: omit

A high range of GCV and PCV was reported for number of secondary branches plant per plant, number of nodes per plant, internode length (cm), green fodder yield per plot (g), dry fodder yield per plot (g), leaf dry weight per plant (g), stem dry weight per plant, seed yield per plant (g), plant height (cm), leaf area index and leaf stem ratio (LSR). As GCV is high, direct selection of these characters are feasible and results ~~were~~ are in accordance with Venkatesan *et al.*[29] in *Vigna mungo* and Praveena [30] and Varanya*et al.*[31] in *Vigna unguiculata.*

Similarly, Phogat *et al.*[32] and Singh *et al.*[33] got high PCV and GCV for green fodder yield per plant and dry matter yield per plant. For leaf dry weight per plant and stem dry weight per plant, Malarvizhi *et al.*[34] and Singh *et al.*[35] obtained comparable results. High value of PCV and GCV was reported by Thorat and Gadewar[36] for leaf area index also.

Days to 50 percent flowering andnumber of seeds per pod exhibited low GCV and moderate PCV. Low GCV was observed by Varanya*et al.*[31], Phogat *et al.* [32] and Praveena [30] in *Vigna unguiculata* for days to 50 percent flowering which implies that ~~these~~ this character~~s~~ had less genetic variability. Number of leaves per plant and 100-seed weight had medium range of PCV and GCV and similar results of PCV were observed for 100-seed weight by Bandi *et al.*[37]. Days to maturity had low GCV and PCV and similar results were obtained by Bandi *et al.*[37] in *Vigna mungo* andSineka *et al.*[24] in *Vigna radiata*.

3.2.2 Heritability and genetic advance as percent mean (GAM)

In any crop improvement effectiveness of selection is affected by heritability and genetic advance because high heritability combined with high genetic advance associates with additive gene action which directly increase the heritability of a character by partitioning environmental effect from total variability. The values of heritability and genetic advance as percent of mean ~~were~~ are mentioned in Table 4~~. And~~ and represented graphically in Figure 2. High range of heritability combined with high genetic advance was observed for number of secondary branches per plant, number of leaves per plant, number of nodes per plant, internodal length, green fodder yield per plot, dry fodder yield per plot, leaf dry weight per plant, stem dry weight per plant, seed yield per plant, plant height, leaf area index and leaf stem ratio which implies that these characters were controlled by additive gene action where direct selection is feasible and can be used as good selection criteria for crop improvement in *V. stipulacea* to be utilized for fodder purpose. Similar results were obtained by Onwubiko [27] in *Vigna subterrenean* for number of branches per plant, number of nodes and internodal length, Malarvizhi *et al.* [34] for number of branches per plant, number of leaves per plant, dry weight of leaves, dry weight of stem, dry matter yield, plant height , Kumar *et al.*[38] for seed yield per plant in *Vigna unguiculata* and Varanya*et al.*[31] for number of leaves per plant, LAI, green fodder yield per plant, stem dry weight per plant, leaf dry weight per plant, plant height and seed yield per plant in *Vigna unguiculata*.

Table 2. Analysis of variance of sixteen characters of *Vigna stipulacea*.

Comment [B7]: Omit end fullstop

Sl. No.	Source of variation	Mean square			CD(5%)	CV
		Genotypes	Replication	Error		
	Degrees of freedom	29	2	58		
1	Number of secondary branches per plant	16.28*	15.24	1.64	2.09	12.051
2	Number of leaves per plant	36.73*	11.43	3.77	3.172	9.316
3	Number of nodes per plant	7.19*	3.633	0.50	1.15	14.463
4	Internode length (cm)	1.98*	0.121	0.03	0.286	11.33
5	Days to 50 percent flowering	20.92*	0.844	8.95	4.889	8.525
6	Green fodder yield per plot (g)	165703.76*	34.133	6202.10	128.714	13.856
7	Dry fodder yield per plot (g)	3019.84*	648.68	59.95	12.66	11.058
8	Leaf dry weight per plant (g)	2.46*	0.420	0.07	0.437	14.244
9	Stem dry weight per plant (g)	1.57*	0.070	0.05	0.372	13.99
10	Number of seeds per pod (g)	3.97^{NS}	0.311	3.39	-	14.488
11	100 seed weight (g)	0.07*	0.012	0.02	0.218	10.479
12	Seed yield per plant (g)	2.89*	0.170	0.13	0.588	14.164
13	Plant height (cm)	117.45*	410.800	16.66	6.671	11.809
14	Days to maturity	33.420*	14.233	3.498	3.057	3.411
15	Leaf Area Index (LAI)	3.831*	0.102	0.126	0.58	17.93
16	Leaf Stem Ratio (LSR)	0.31*	0.058	0.012	0.182	10.286

** Significant at 5%, NS – Non-significant*

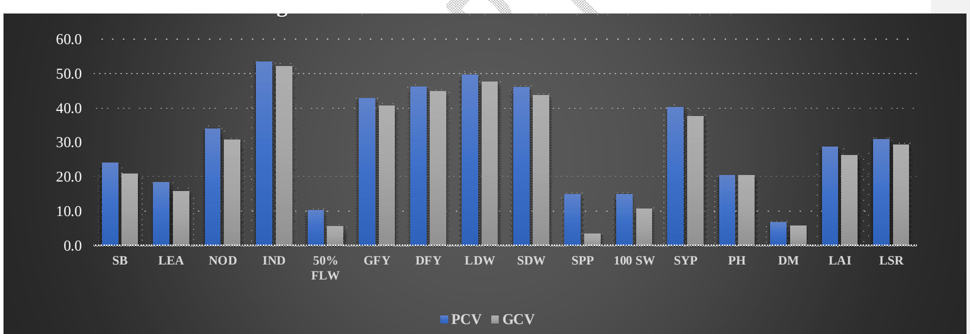
Table 3. Components of variance for sixteen characters in *Vigna stipulacea*.

Comment [B8]: bold

Comment [B9]: Omit end fullstop.

Sl. No.	Characters	Mean ± SE	PCV (%)	GCV (%)
1	Number of secondary branches per plant	10.61 ± 0.738	24.06	20.82
2	Number of leaves per plant	20.83 ± 1.120	18.44	15.91
3	Number of nodes per plant	4.87 ± 0.406	33.92	30.69

4	Internode length (cm)	1.56 ± 0.101	53.35	52.12
5	Days to 50 percent flowering	35.09 ± 1.727	10.25	5.69
6	Green fodder yield per plot (g)	568.37 ± 45.47	42.87	40.57
7	Dry fodder yield per plot (g)	70.02 ± 4.470	46.20	44.86
8	Leaf dry weight per plant (g)	1.88 ± 0.154	49.69	47.62
9	Stem dry weight per plant (g)	1.63 ± 0.131	45.95	43.76
10	Number of seeds per pod (g)	12.71 ± 1.063	14.89	3.46
11	100 seed weight (g)	1.27 ± 0.077	14.99	10.65
12	Seed yield per plant (g)	2.54 ± 0.208	40.30	37.72
13	Plant height (cm)	34.57 ± 2.357	20.51	20.51
14	Days to maturity	54.77 ± 1.08	6.70	5.77
15	Leaf Area Index (LAI)	1.978 ± 0.20	28.78	26.37
16	Leaf Stem Ratio (LSR)	1.081 ± 0.064	30.97	29.26



SB - No. of secondary branches	**50% FLW - Days to 50% flowering**	**SDW - Stem dry weight per plant (g)**	**PH - Plant height (cm)**
LEA - No. of leaves per plant	**GFY - Green fodder yield per plot (g)**	**SPP - Number of seeds per pod (g)**	**DM - Days to maturity**
NOD - No. of nodes per plant	**DFY - Dry fodder yield per plot (g)**	**SW - 100 seed weight (g)**	**LAI - Leaf Area Index**
IND - Internode length (cm)	**LDW - Leaf dry weight per plant (g)**	**SYP - Seed yield per plant (g)**	**LSR - Leaf Stem Ratio**

Fig.~~-ure~~ 1. PCV and GCV of selected characters

Comment [B10]: bold

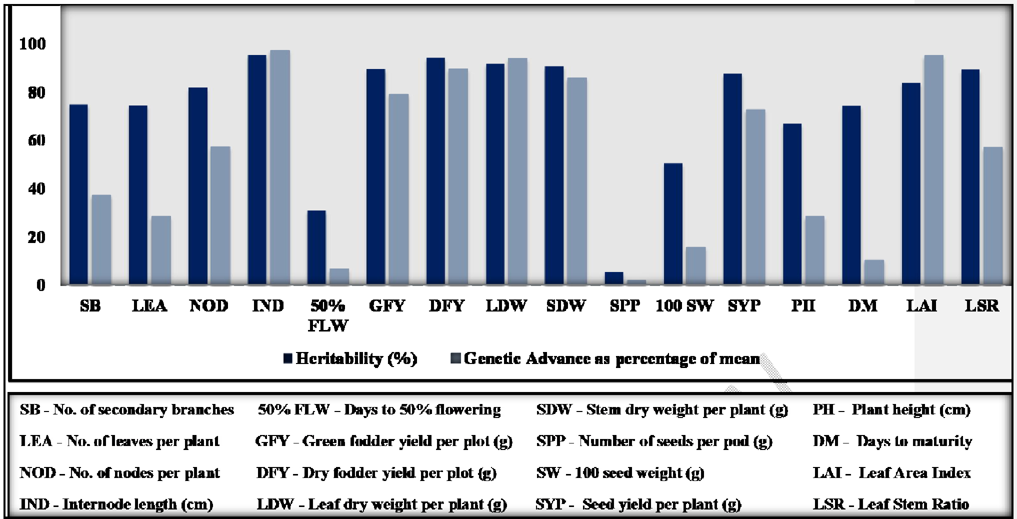


Fig. ~~ure~~ **2.** Heritability and genetic advance as per cent mean (GAM) of selected characters

Table 4. Heritability and genetic advance of sixteen characters in *Vigna stipulacea*.

Comment [B11]: bold and omit end fullstop.

Sl. No.	Characters	Heritability (percent)	Genetic advance as percent of mean (GAM)
1	Number of secondary branches per plant	74.90	37.12
2	Number of leaves per plant	74.50	28.29
3	Number of nodes per plant	81.80	57.19
4	Internode length (cm)	95.40	97.32
5	Days to 50 percent flowering	30.80	6.51
6	Green fodder yield per plot (g)	89.60	79.09
7	Dry fodder yield per plot (g)	94.30	89.72
8	Leaf dry weight per plant (g)	91.80	94.00
9	Stem dry weight per plant (g)	90.70	85.82
10	Number of seeds per pod (g)	5.40	1.65

11	100 seed weight (g)	50.50	15.59
12	Seed yield per plant (g)	87.60	72.74
13	Plant height (cm)	66.80	28.24
14	Days to maturity	74.20	10.23
15	Leaf Area Index (LAI)	83.90	95.06
16	Leaf Stem Ratio (LSR)	89.30	56.96

3.3 Cluster Analysis

Genetic diversity is an essential prerequisite for crop improvement programme because when genetically varied parents are crossed it may foster heterosis or produce superior recombinants due to accumulation of gene combinations from both parents. Genetic diversity associated with germplasm is abundant and often utilised for developing several improved crop ~~types~~ varieties. Extent of genetic diversity present among thirty genotypes under study was estimated with Mahalanobis D^2 statistics using Tochers method. All sixteen characters were considered for cluster analysis and given in Table 5. All the genotypes were grouped into five clusters indicating the existence of a substantial genetic variance for the traits being evaluated. Similar divergence analysis was done by Janjal and Mehta [39] who grouped forty-seven genotypes into 9 clusters in rice bean (*Vigna umbellata*), Praveena *et al*. [30] grouped thirty fodder cowpea (*Vigna unguiculata*) genotypes into 11 clusters, Sahoo *et al*.[40]grouped fifty genotypes into 12 clusters in moth bean (*Vigna aconitifolia*) which were used for identifying genetically distant parents for hybridization studies. Grouping of genotypes into different clusters revealed cluster I was the largest with seventeen genotypes followed by cluster II with seven genotypes, cluster III with four genotypes and cluster IV & cluster V with one genotype each. Five cluster groups including various genotypes was represented by a dendrogram and shown in Fig. 3.

Comment [B12]: Put these reference here. Rest remain same.

3.3.1 Intra and inter cluster distances

Average inter and intra cluster D^2 values of five clusters ~~were~~ are given in Table 6. The inter-cluster D^2 values were higher than the intra-cluster D^2 values implying that there is substantial genetic variation between cluster genotypes for the attributes in question. The ~~maximum and minimum~~ intra-cluster D^2 values varied from 13.24 ~~(cluster II)~~ to 12.19 ~~(cluster III)~~. The ~~H~~highest intra-cluster distance was observed in cluster II (13.24) followed by cluster I (12.27) and cluster III (12.19). Cluster IV and cluster V intra cluster D^2 values were zero as they consist single genotype.

The inter-cluster D^2 values varied from 25.17 to 16.93. The highest inter-cluster D^2 values among genotypes was observed between clusters II and V (25.17) followed by clusters I and IV (22.84), I and V (22.51), I and II (19.82), III and IV (19.72), II and III (19.51), III and V (19.31), I and III (19.21), II and IV (17.63) lowest inter-cluster D^2 values was observed between cluster IV and V (16.93). Fig. 4. represents the intra and inter cluster genetic distances among the genotypes.

The genotypes in cluster II had the highest intra-cluster distance, suggesting that genotypes of this clusters differ in terms of morphological traits and performance. Maximum inter-cluster distance observed for clusters II and V (25.17) shows that the genotypes belonged to these clusters vary greatly and might be exploited in a hybridization strategy to produce superior recombinants in the segregating generations. Low levels of intra-cluster distances were noticed between cluster IV and V (16.93)indicatingof little genetic diversity within the group. Thus, choosing parents from this cluster may be discouraged.

3.3.2 Cluster means

Cluster means of sixteen characters showed that Cluster V had highest mean values for number of leaves per plant, number of per plant and plant height. Cluster IV had highest mean values for number of secondary branches per plant, internodal length, green fodder yield per plot, stem dry nodes per plant, green fodder yield per plot, dry fodder yield per plot, leaf dry weight per plant, 100 seed weight, seed yield per plant, plant height. Cluster III had highest mean value for days to 50 percent flowering, days to maturity, leaf area index and leaf stem ratio. Cluster II had highest mean value for number of seeds per pod. Cluster means of various characters ~~were~~ are given in Table 7.

Through cluster means, it is inferred that cluster V genotypes can be selected for improving number of leaves per plant, number of nodes per plant, green fodder yield per plot, dry fodder yield per plot, leaf dry weight per plant and plant height. Cluster IV is a better contributor for number of secondary branches per plant, internodal length, green fodder yield per plot, stem dry weight per plant, 100-seed weight, seed yield per plant, plant height. Since genotypes belonged to cluster V and cluster IV had highest mean values for major fodder yield and yield attributing traits, consequently these genotypes can be utilized as parents in breeding programmes for improving fodder yield of *Vigna stipulacea*.

3.3.3 Per cent contribution of individual character to total divergence

From percentage contribution analysis, it was concluded that among all the characters studied seed yield per plant (26.67%) contributed majorly towards total divergence followed by leaf stem ratio (20.92%), internodal length (20.00%),leaf area index (8.97%), green fodder yield per plot (6.90%), number of nodes per plant(5.98%), dry fodder yield per plot (5.52%), stem dry weight per plant (2.07%), 100 seed weight (1.38%), number of secondary branches per plant(0.92%), days to 50% flowering(0.23%), number of leaves per plant (0.23%), plant height (0.23%). Among all the characters studied leaf dry weight per plant, number of seeds per pod and days to maturity showed least contribution towards total divergence. ~~Similar divergence analysis was done by Janjal and Mehta [39] who grouped forty-seven genotypes into 9 clusters in rice bean (*Vigna umbellata*), Praveena *et al*. [30] grouped thirty fodder cowpea (*Vigna unguiculata*) genotypes into 11 clusters, Sahoo *et al*.[40]grouped fifty genotypes into 12 clusters in moth bean (*Vigna aconitifolia*) which were used for identifying genetically distant parents for hybridization studies.~~

Comment [B13]: Omit these reference here and put these in 3.3 as shown. Please see 3.3.

Table 5. Grouping of *Vigna stipulacea* genotypes into different clusters

Comment [B14]: bold

Cluster number	Number of genotypes in each cluster	Accessions in each cluster
I	17	T3 (IC 261384), T6 (IC 553557), T21 (IC 417392), T20 (IC 553516), T8 (IC 553494), T16 (IC 521215), T14 (IC 625694), T9 (IC 622865), T17 (IC 553520), T18 (IC

		331436), T13 (IC 610275), T30 (IC 553554), T11 (IC 550531), T12 (IC 622860), T23 (IC 331454), T22 (IC 331453), T19 (IC 550548)
II	7	T2 (IC 121435), T7 (IC 524667), T10 (IC 261321), T4 (IC 550551), T5 (IC 550545), T24 (IC 305192), T27 (IC 550532)
III	4	T26 (IC 550536), T29 (IC 550538), T15 (IC 553525), T28 (IC 553538)
IV	1	T1 (IC 331457)
V	1	T25 (IC 553510)

Table 6. **Average intra and inter-cluster distances**

Comment [B15]: bold

	I	II	III	IV	V
I	**12.27**				
II	19.82	**13.24**			
III	19.21	19.51	**12.19**		
IV	22.84	17.63	19.72	**0.00**	
V	22.51	25.17	19.31	16.93	**0.00**

Table 7. **Mean values and percentage contribution of individual characters towards total divergence**

Comment [B16]: bold

Sl. No.	Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Percent contribution (%)
1	Number of secondary branches per plant	10.96	9.57	9.33	**14.67**	13	0.92
2	Number of leaves per plant	20.41	19.43	21.17	27.00	**30.33**	0.23
3	Number of nodes per plant	3.98	5.67	6.25	6.67	**7.00**	5.98
4	Internodal length (cm)	0.96	2.25	2.35	**3.00**	1.83	20.00

5	Days to 50% flowering	34.16	35.24	**38.67**	35.67	35.00	0.23
6	Green fodder yield per plot (g)	447.08	588.19	883.33	**900.00**	**900.00**	6.90
7	Dry fodder yield per plot (g)	52.86	77.86	87.25	150	**158**	5.52
8	Leaf dry weight per plant (g)	1.35	2.10	2.44	4	**4.80**	**0.00**
9	Stem dry weight per plant (g)	1.31	1.79	1.88	**3.47**	3.10	2.07
10	Number of seeds per pod	12.10	**13.86**	13	13.67	13	**0.00**
11	100 seed weight (g)	1.23	1.33	1.21	**1.60**	1.40	1.38
12	Seed yield per plant	1.98	3.78	2.32	**3.90**	3.00	**26.67**
13	Plant height (cm)	31.92	37.43	36.75	**42.67**	**42.67**	0.23
14	Days to maturity	54.20	55.29	**58.33**	56.00	55.00	**0.00**
15	Leaf area index	0.06	0.12	**0.16**	0.15	0.14	8.97
16	Leaf stem ratio	1.00	0.96	**1.57**	0.93	1.51	20.92

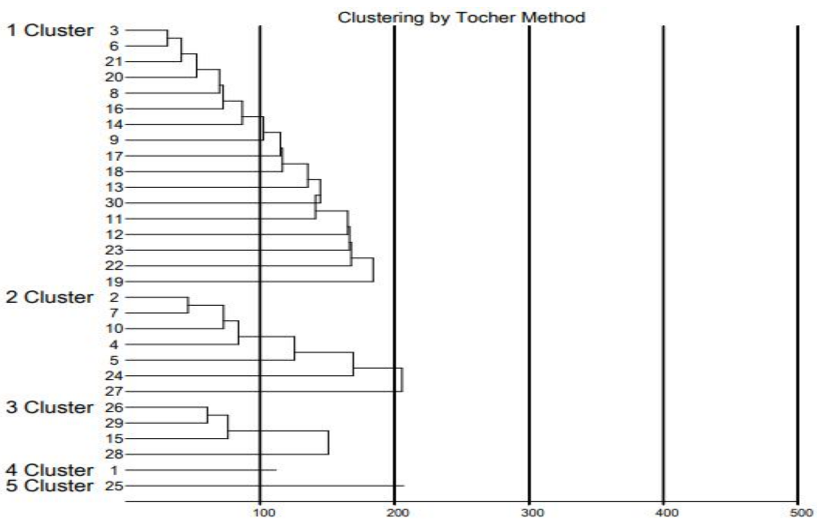
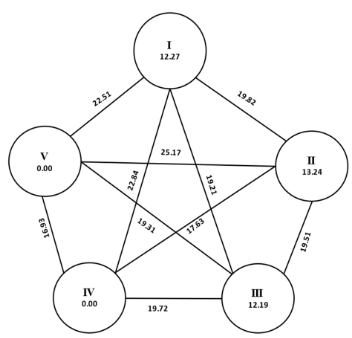


Figure 3. **Dendrogram showing pattern of clustering of thirty genotypes of *Vigna stipulacea***



Cluster diagram (not the scale)

Comment [B17]: bold

Comment [B18]: Please make the figure more visible and large as readable like below-

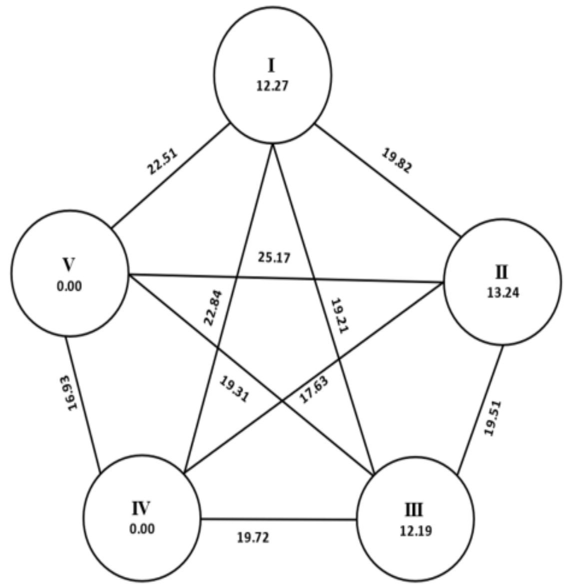


Figure 4. Cluster diagram showing intra and inter cluster distances

Comment [B19]: bold

Conclusion

Studies on the genetic variability and diversity of the wild and domesticated wild relatives of the *Vigna* species are essential to provide useful information that will aid to improve the cultivated varieties or to domesticate the wild ones. Analysis of variance revealed that all the characters except number of primary branches per plant and number of seeds per pod differed significantly and had considerable variation among genotypes. A high range of GCV and PCV was reported for most of traits and direct selection of those characters is feasible. High range of heritability combined with high genetic advance was observed for most of the fodder yielding and yield attributing characters which implies that these characters were controlled by additive gene action where direct selection is feasible and can be used as good selection criteria for crop improvement of *V. stipulacea* to be utilized for fodder purpose. Genotypes of clusters II and V can be used as parents for crossing in hybridization programmes and aid in exploiting heterosis as they had maximum inter-cluster distance. Genotypes belonged to cluster V and cluster IV had highest mean values for major fodder yield and yield attributing traits, hence can be utilized as parents in breeding programmes for improving fodder yield of *Vigna stipulacea*.

Comment [B20]: Not present in Table 2. Why?

If not given in Table 2, then please omit, as in text of 3.1

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text to image generators have been used during writing or editing of manuscript.

REFERENCES

1. NDDB [National Dairy Development Board]. Share of agriculture and livestock sector in GDP. 2023. Available: https://www.nddb.coop/information/stats/GD Pcontrib
2. Sollenberger J, Dunn K, Brewer K, Marshall JG. Measuring the value and impact of health sciences libraries: planning an update and replication of the rochester study. J. Med. Lib. Assoc. 2009; 97: 308- 312.
3. Vendramini JMB, Arthington JD, Adesogan AT. Effects of incorporating cowpea in a subtropical grass pasture on forage production and quality and the performance of cows and calves. J. Br. Grassl. Soc. 2012; 67(1): 129–135.
4. Bhat R, Karim AA. Exploring the nutritional potential of wild and underutilized legumes. Compr. Rev. Food Sci. Food Saf. 2009; 8: 305–331.
5. Harouna DV, Venkataramana PB, Ndakidemi PA, Matemu AO. Under-exploited wild *Vigna* species potentials in human and animal nutrition: A review. Global food security. 2018; 18:1-1.
6. Popoola JO, Aremu BR, Daramola FY, Ejoh AS, Adegbite AE. Morphometric analysis of some species in the genus *Vigna* (L.) Walp: implication for utilization for genetic improvement.J. of Biol. Sci. 2015; 15(4): 156-166.
7. Pratap A, Malviya N, Tomar R, Gupta DS, Jitendra K. *Vigna*. In: Pratap A, Kumar J. (eds), Alien Gene Transfer in Crop Plants: Achievements and Impacts. Springer Science+Business Media, LLC: Berlin/Heidelberg, Germany, 2014; pp.163–189.
8. Tomooka N, Naito K, Kaga A, Sakai H, Isemura T, Ogiso-Tanaka E, Iseki K, Takahashi Y. Evolution, domestication and neo-domestication of the genus *Vigna*. Plant Genet. Resour. Characterisation Util. 2014; 12: 168–171.
9. Tripathi K, Gore PG, Rajpoot SK, Singh N, Gupta V. Minni Payaru [*Vigna stipulacea* (Lam.) Kuntz.]: an underutilized ancient legume of India. Indian Journal of Traditional Knowledge. 2021; 20(4):1084-7.
10. Takahashi Y, Sakai H, Ariga H, Teramoto S, Shimada TL, Eun H, Muto C, Naito K Tomooka N. Domesticating *Vigna stipulacea*: chromosome-level genome assembly reveals VsPSAT1 as a candidate gene decreasing hard-seededness. Frontiers in Plant Science, 2023; 14: 1119625.
11. Takahashi Y, Sakai H, Yoshitsu Y, Muto C, Anai T, Pandiyan M, Senthil N, Tomooka N, Naito K. Domesticating *Vigna stipulacea*: a potential legume crop with broad resistance to biotic stresses. Frontiers in Plant Science. 2019; 10: 1607.
12. Gore PG, Gupta V, Singh R, Tripathi K, Kumar R, Kumari G, Madhavan L, Dikshit HK, Venkateswaran K, Pandey A, Singh N. Insights into the genetic diversity of an underutilized Indian legume, *Vigna stipulacea* (Lam.) Kuntz., using morphological traits and microsatellite markers. Plos one. 2022;17(1): e0262634.
13. Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers. 3rd edn., ICAR, New Delhi, 1978; p.34
14. Gopinath PP, Adarsh VS, Joseph B, Prasad R. GRAPES (General R-shiny based Analysis Platform Empowered by Statistics). 2020; Available: https://www.kaugrapes.com/home.version 1.0.0. DOI: 10.5281/zenodo.4923220
15. Sivasubramanian S, Menon M. Heterosis and inbreeding depression in rice. Madras Agric.J. 1973; 60: 1139p.
16. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. Agron. J. 1955; 47: 314 – 318.

Comment [B21]: Uniform style of the JABB needs to be thoroughly followed, as mentioned in the given 'Author guidelines' and 'SDI-Paper-template' of the Journal.

Comment [B22]: Berlin/Heidelberg, Germany: Springer Science+Business Media, LLC; 2014, pp.163-189.

Comment [B23]: 1087? or 1097? write like 1084-87.

Comment [B24]: New Delhi: ICAR; 1978, p.34.

17. Mahalanobis, P. C. Study on the generalized distance in statistics. Proceedings of the National Institute of Sciences of India, Calcutta. 1936; 2: 49-55.
18. Rao CR. Advanced statistical methods in biometrical research, 1st Edn. Johan Wiley and Sons, New York, 1952.
19. Singh, R. K. and Chaudhary, B. D. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India, 1977.
20. Nadarajan N, Gunasekaran M. Quantitative genetics and biometrical techniques in plant breeding. Kalyani publishers, New Delhi, 2012; pp.182- 193.
21. Gore, PG. Inter and intra-specific variations in two closely related wild *Vigna* species: *V. trilobata* (L.) Verdc. and *V. stipulacea* (Lam.) Kuntz. Ph. D (Ag.) thesis, Indian Agricultural Research Institute, New Delhi; 2019.
22. Venkateswarlu, O. Correlation and path analysis in green gram. Legume Res. 2001; 24 (2): 115-117.
23. Sathya M, Jayamani P. Heterosis and combining ability studies in greengram. Journal of Food Legumes. 2011; 24(4): 282-7.
24. Sineka T, Murugan E, Sheeba A, Hemalatha G, Vanniarajan C. Genetic relatedness and variability studies in greengram (*Vigna radiata* (L.) Wilczek). Electronic Journal of Plant Breeding. 2021; 12(4): 1157-62.
25. Konda CR, Salimath PM, Mishra MN. Genetic variability studies for productivity and its components in blackgram [*Vigna munga*(L.) Hepper]. Legume Research-An International Journal. 2009; 32(1): 59-61.
26. Khan H, Viswanatha KP, Sowmya HC. Study of genetic variability parameters in cowpea (*Vigna unguiculata* L. walp.) germplasm lines. The Bioscan. 2015; 10(2):747-750.
27. Onwubiko NC, Uguru MI, Chimdi GO. Estimates of genetic parameters in bambara groundnut {*Vigna subterranea* (L.) VERDC.}. Plant Breeding and Biotechnology. 2019; 7(4): 295-301.
28. Aman AKS, Kumar S, Sharma M, Gupta, N. Genetic variability and character association studies in urd bean (*Vigna mungo* L.) under irrigated and rainfed conditions of Jammu region. The Indian Society of Agricultural Science. 2022; 43(2): 179-188.
29. Venkatesan NM, Thangavel P, Ganesan J. Genetic variability, heritability and genetic advance analysis in segregating generation of black gram [*Vigna mungo* (L.) hepper]. Legume Research-An International Journal. 2005; 28(1): 49-51.
30. Praveena VS, Abraham MA, Kumar VI. Genetic divergence studies in fodder cowpea (*Vigna unguiculata*) using D^2 statistics. Forage Res. 2019; 44(4): 230-6.
31. Varanya A, Gayathri G, Arya K, Thomas UC, Pratheesh P. Genetic variability and genetic parameters analysis of 143 fodder cowpea [*Vigna unguiculata* (L.) Walp] germplasm accessions for yield and yield attributing traits. The Pharma Innovation Journal, 2022; 11(2): 2595-2600.
32. Phogat DS, Panchta RA, Kumari PU, Niwas R, Arya SA. Variability, correlation and path analysis studies in fodder cowpea [*Vigna unguiculata* (L.) Walp]. Trends in Biosciences. 2017;10(3): 1130-2.
33. Singh A, Shweta SV. Estimate of genetic variability, heritability, and genetic advance for yield and its components traits in Indian cowpea (Vigna unguiculata (L.) Walp.). Int. J Pure App. Biosci. 2018; 6(1): 1142-7.
34. Malarvizhi D, Swaminathan C, Robin S, Kannan K. Genetic variability studies in fodder cowpea (*Vigna unguiculata* L. Walp). Legume Research-An International Journal. 2005; 28(1): 52-4.
35. Singh SB, Singh AK, Singh AP. Genetic variability, trait relationship and path analysis for green fodder yield and its components in cowpea (*Vigna anguiculata*) under rainfed environment. Progressive Agriculture. 2010; 10(1): 42-6.

Comment [B25]: Mahalanobis, PC?. Please give full meaning of PC

Comment [B26]: New York: Johan Wiley and Sons; 1952

Comment [B27]: Singh RK, Chaudhary BD.

Comment [B28]: First give edition number of the book. Then- New Delhi: Kalyani publishers; 2012.

Comment [B29]: Gore, PG?. Please give full name

Comment [B30]: Ph.D (Ag.) Thesis: Indian Agricultural Research Institute, New Delhi; 2019.

Comment [B31]: Venkateswarlu O? Full name

Comment [B32]: 282-87?

Comment [B33]: Gupta N

Comment [B34]: 1142-47?

Comment [B35]: 42-46?

36. Thorat A, Gadewar, RD. Variability and correlation studies in cowpea (*Vigna unguiculata*). Int. J. Env. Rehab. Conser. 2013; 4(1):.44-49.
37. Bandi HRK, Rao KN, Krishna KV, Srinivasulu K. Variability, heritability and genetic advance for quantitative characters in rice fallow black gram [*Vigna mungo* (L.) Hepper]. Int. j. curr. microbiol. app. Sci. 2018; 7(2):171-176.
38. Kumar GV, Vanaja M, Lakshmi NJ, Maheswari M. Studies on variability, heritability and genetic advance for quantitative traits in black gram [*Vigna mungo* (L.) Hepper]. Agricultural Research Journal. 2015; 52(4):28-31.
39. Janjal AP, Mehta AK. Studies on genetic divergence in rice bean by using D^2 statistics. Journal of Pharmacognosy and Phytochemistry. 2019; 8(5): 499-502.
40. Sahoo S, Sanadya SK, Sharma AK, Shekhawat SS. Assessment of genetic diversity of mothbean (*Vigna aconitifolia*(Jacq.) Marechal) germplasm. Journal of Food Legumes. 2022; 35(2): 95-9.

Comment [B36]: Gadewar RD

Comment [B37]: 4(1):44-49.
Omit inside full-stop.

Comment [B38]: 95-99?