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Pheno-morphological characterization and Harnessing Genetic variability utilizing Diverse Cultivars, Promising Stabilized Cowpea x Rice Bean Inter-specific Derivatives in Fodder Cowpea (*Vigna unguiculata* (L.) Walp sub sp. *unguiculata*)

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ABSTRACT: The present study on morphological characterization of diverse genotypes of fodder-type cowpea (*Vigna unguiculata* (L.) Walp subsp. *unguiculata*) based on DUS guidelines by PPV& FRA was carried out at botany garden of the Department of Genetics and Plant Breeding, College of Agriculture, University of Agricultural Sciences, Dharwad, for 2 seasons *i.e*., *Kharif* 2021 and summer 2022. There is a growing need to develop suitable varieties for a specific region and specific use. Genetic variability is important to select characters, which are heritable unless and until there is a large amount of variability present in the population selection cannot be practiced. All 30 genotypes of fodder-type cowpea were grouped for seven morphological descriptors. However, no variability was observed for terminal leaf width as all the genotypes belonged to the long category except one. Less variability was recorded for the number of branches per plant and terminal leaflet length. The characterization of cowpea genotypes provides valuable information on the diversity of morphological and quantitative characters for strengthening the future breeding plan. The study on genetic variability parameters revealed high heritability coupled with high genetic advance over mean for the traits like stover yield per plant and dry matter content. Genetic correlation studies indicated that traits like dry matter content, leaf to-stem ratio and stover yield per plant showed a significant positive association with green fodder yield per plant and directly influenced the green fodder yield in fodder-type cowpea. Path analysis results suggested that except for days to fifty per cent flowering and days to maturity all traits *viz.,* dry matter content, leaf to-stem ratio and stover yield per plant showed direct positive effects on green fodder yield per plant at genotypic level indicating that direct selection for these traits results in an increase in green fodder yield in cowpea.

Keywords: DUS, Descriptors, Morphological characterization, genotypes.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a self-pollinating, annual herbaceous legume belonging to the family Fabaceae which originated in West Africa (2n=22). It is grown for hay and silage, as well as for vegetables, grains, fresh cut and carry fodder (Roy *et al*., 2016). Cowpea can be grown all year long and is appropriate for inter, mixed, and relay cropping systems. This legume is renowned for its natural capacities, including its capacity to withstand drought and shade, as well as its capacity to grow quickly and cover large areas with ground cover (Fatokun *et al.*, 2009). Farmers find it appealing because of its short growing season and ability to be cut multiple times (KAU, 2015). Due to the ability of the plant's root nodules to fix atmospheric nitrogen, it only needs a small number of inputs. The entire plant is utilised as a valuable, nutrient-rich legume for livestock (Singh and Tarawali 1997). Cowpea leaves and haulms are particularly nutrient-dense. While fiber content is only approximately 6%, the crude protein concentration in grain and leaves ranges from 22 to 30% (Bressani, 1985; Nielsen *et al.*, 1997) and from 13 to 17% in haulms with excellent digestibility (Tarawali *et al.*, 1997).

Globally Cowpea is cultivated in an area of 23.4 million hectares with a production of 18.29 million tonnes and productivity of 637 kg ha^{-1} (Anon., 2019-20). In India Cowpea is grown in an area of 4 million hectares with a production of 2.7 million tonnes and with a productivity of 567 kg ha^{-1} (Anon., 2019-20). Karnataka accounts for 12% of the area under pulses in the country and stands fourth in Cowpea among all pulses. In Karnataka, the total area under Cowpea production is 62009 hectares with a production of 21311 tonnes (Anon., 2019-20).

In the modern era, DUS (distinctness, uniformity and stability) testing is essential for the grant of protection of new plant varieties under the plant varieties and farmers rights. This act has provision to compare the candidate variety with the varieties/ genotypes of common knowledge on a set of relevant characteristics and generally the morphological data is also demanded at the time of filing of the application (Yadav *et al.*, 2013). To categorize and further develop the crop for fodder purposes, the variability existing in the available fodder cowpea germplasm must be evaluated. The goal of this study was to characterize the cowpea genotypes based on guidelines given by PPV & FRA, New Delhi on the qualitative and quantitative morphological characters to establish distinctness among the newly developed genotypes along with old ones.

Further to examine the available variability and explore the diversity to pick superior parents for hybridization to further enhance quality and yield. The knowledge about genetic variability, heritability, correlation coefficients and other parameters help in further improving the green fodder yield in cowpea through a directed selection of component traits (Ugale *et al.*, 2020). Correlation studies among yield and yield-related traits is useful to practice indirect selection for important traits with high heritability so that ultimately yield as a complex trait with low heritability can be improved. Correlation coefficients, although, very useful in quantifying the size and direction of trait associations can be well interpreted with path coefficient (Baranda *et al.*, 2017). Path coefficient is an excellent means of studying the direct and indirect effects of interrelated components of a complex trait. By determining the interrelationships among grain yield components, a better understanding of both the direct and indirect effects of the specific components can be attained (Chaudhary and Joshi 2005).

The present study was therefore conducted to estimate genetic variability parameters, correlation and path coefficients in cowpea for utilization in selection programs aimed at productivity increase of future genotypes of fodder-type cowpea.

MATERIAL AND METHOD

The material for the present study includes 30 diverse genotypes of fodder-type cowpea. The details of the material used in the study is represented in Table 1 for fodder-type cowpea. The material was sown in the Botany Garden of Department of Genetics and Plant breeding, College of Agriculture, UAS, Dharwad following randomized complete block design with two replications for two seasons *i.e.*, *kharif* 2021 and summer 2022. Each genotype was raised in 5 rows of four-meter lengths with a spacing of 45 cm between the rows and 10 cm between the plants.

Table 1: List of Fodder Cowpea material used in the present investigation.

Sr. No.	Genotypes	Source
1.	BL-1 (National Check)	IGFRI, Jhansi, UP
2.	BL-2 (National Check)	IGFRI, Jhansi, UP
3.	AV-5	GKVK, Bangalore
4.	MFC-09-01 (State Check)	VC Farm, Mandya, UASB
5.	MFC-08-14 (State Check)	VC Farm, Mandya, UASB
6.	MFC-09-12 (State Check)	VC Farm, Mandya, UASB
7.	EC4216	IARI, New Delhi
8.	UPC 5286	GBPUAT, Pantnagar, Uttarakhand
9.	UPC 8705	GBPUAT, Pantnagar, Uttarakhand
10.	UPC 9202	GBPUAT, Pantnagar, Uttarakhand
11.	UPC 4200	GBPUAT, Pantnagar, Uttarakhand
12.	UPC 621	GBPUAT, Pantnagar, Uttarakhand
13.	UPC 622	GBPUAT, Pantnagar, Uttarakhand
14.	UPC 625	GBPUAT, Pantnagar, Uttarakhand
15.	UPC 287	GBPUAT, Pantnagar, Uttarakhand
16.	SHWETA	MPKV, Rahuri, Maharashtra
17.	PKB-4	GKVK, Bangalore
18.	KBC-6	GKVK, Bangalore
19.	KBC-9	GKVK, Bangalore
20.	DCS 47-1	UAS, Dharwad
Interspecific hybrid derivatives between Cowpea × Rice bean in F_{10} generation identified for fodder type		
1.	F10- 204-1-2	Dept. of GPB, UAS, Dharwad
2.	F10- 209-2-1	Dept. of GPB, UAS, Dharwad
3.	F10- 174-2-2	Dept. of GPB, UAS, Dharwad
4.	F10- 190-1-2	Dept. of GPB, UAS, Dharwad
5.	F10- 204-2-2	Dept. of GPB, UAS, Dharwad
6.	F10- 137-1-1	Dept. of GPB, UAS, Dharwad
7.	F10- 127-1-2	Dept. of GPB, UAS, Dharwad
8.	F10- 190-2-2	Dept. of GPB, UAS, Dharwad
9.	F10- 201-2-2	Dept. of GPB, UAS, Dharwad
10.	F10- 128-1-1	Dept. of GPB, UAS, Dharwad

For the recording of observations, five plants of each genotype were randomly selected from each replication. The observations on various morphological characters related to fodder cowpea as mentioned in the DUS guidelines given by PPV & FRA, New Delhi that were recorded for fodder-type cowpea are listed in Table 2. All recommended crop production and protection practices were followed to raise a good crop. The estimates of PCV and GCV were classified as low, moderate and high according to Sivasubramaniam and Madhavamenon (1973). The estimated heritability was classified as low, moderate, and high according to Robinson *et al.,* (1949). The genetic advance as a percentage of the mean was estimated following the formula of Johnson *et al.* (1955). Data of six yield and yield attributing characters, were subjected to analysis to calculate means, minimum and maximum values, variances and standard errors (SE) and correlation and path coefficient analysis were carried out through R Software.

Table 2: List of observations recorded for morphological characterization based on DUS guidelines by PPV & FRA, New Delhi for fodder-type cowpea.

Sr. No.	Character	Category	Stage of recording observation
1.	Plant height (cm)	Dwarf (<50 cm)	Days to 10% flowering (40-60 DAS
		Tall (50-60 cm)	
		Extra tall (>60 cm)	
2.	Number of primary branches per plant (numbers)	Low (<5)	Days to 10% flowering (50-75 DAS)
		Medium (5-8)	
		High (>8)	
3.	Number of secondary branches per plant (numbers)	Low (<5)	
		Medium (5-8)	
		High (>8)	
4.	Days to 10% flowering	Early (<45 days)	10% of plants with at least one open flower
		Medium (45 – 55 days)	
		Late (>55 days)	
5.	Growth habit	Erect	Days to 10% flowering (50-75 DAS)
		Semi erect	
		Spreading/horizontal	
6.	Terminal leaflet: Length of penultimate leaf(cm)	Short (<5)	Days to 10% flowering (50-75 DAS)
		Medium (5-8)	
		Long (>8)	
7.	Terminal leaflet: Width of penultimate leaf(cm)	Short (<5)	Days to 10% flowering (50-75 DAS)
		Medium (5-8)	
		Long (>8)	

Observations on yield and yield-related traits were recorded for the following traits *i.e*, leaf to-stem ratio, days to 50 percent flowering, days to maturity, green fodder yield per plant (g), dry matter content (%) and stover yield per plant (g).

RESULTS AND DISCUSSION

The identification of promising lines for varietal development programs and the prevention of duplication in the germplasm collection depend on the collection and characterization of existing germplasm. A wide genetic foundation is provided by the diversified germplasm for the creation of various breeding stock. In addition to fulfilling the criteria for new variety registration, the botanical description is also utilized to grant plant variety protection, for which uniqueness is a vital need. Along with example genotypes, the properties of the cowpea genotypes under investigation and the frequency distribution of each descriptor of genotypes were recorded (Table 3). Based on field observations made during different stages of plant growth, cowpea genotypes were classified into various classes.

Morphological characterization of fodder-type cowpea based on DUS guidelines by PPV & FRA, New Delhi. The results revealed that of the 30 genotypes studied in fodder-type cowpea for plant height seven belonged to the dwarf category. It was followed by tall category in 14 genotypes and whereas nine genotypes were grouped in the extra tall category. Further the genotypes were studied for the number of primary branches per plant, it was recorded that 21 genotypes belonged to the low category followed by a medium class with nine genotypes. The germplasm was studied for the number of secondary branches per plant, it was observed that two genotypes belonged to the low category followed by a medium class with 28 genotypes. Out of 30 genotypes, 22 genotypes were grouped into the medium class. It was followed by late category with five genotypes whereas, the early flowering group had three genotypes. Among the total 30 genotypes studied it was observed that semi-erect type formed a major group since it was observed in 22 genotypes. Further, four genotypes showed a spreading type of growth habit. Similarly, erect type growth habit was seen in four genotypes. Based on terminal leaflet length the 30 genotypes were grouped as a medium and long group. Out of the 30, 18 belonged to the medium category and 12 genotypes belonged to long terminal leaflet length.

Table 3: Morphological classification of fodder-type cowpea genotypes based on DUS guidelines by PPV & FRA, New Delhi.

Character	Descriptor	Frequency	Name of genotypes
1. Plant height	Dwarf (<50 cm)	7	127-1-2, 201-2-2, 204-2-2, 190-1-2, 190-2-2, UPC 625, UPC 621
	Tall(50-60 cm)	14	UPC 9202, UPC 8705, 137-1-1, UPC 4200, 128-1-1, MFC-08-14, PKB-6, BL-2, PKB-4, 174-2-2, SHWETA, DCS 47-1, 204-1-2, 209-2-1
	Extra tall (>60cm)	9	KBC-6, UPC 287, KBC-9, UPC 622, EC4216, UPC 5286, BL-1, MFC-09-01, MFC-09-12
2. No. of primary branches per plant	Low (<5)	21	127-1-2, 190-1-2, 209-2-1, 204-2-2, UPC 5286, 204-1-2, UPC 9202, 174-2-2, UPC 8705, DCS 47-1, UPC 625, MFC-09-12, BL-2, EC4216, UPC 287, SHWETA, UPC 621, UPC 622, 137-1-1, 128-1-1, PKB-6
	Medium (5-8)	9	UPC 4200, PKB-4, KBC-9, MFC-09-01, KBC-6, 190-2-2, MFC-08-14, BL-1, 201-2-2
3. No. of secondary branches per plant	Low (<5)	2	127-1-2, 174-2-2
	Medium (5-8)	28	PKB-4, 128-1-1, 209-2-1, 190-2-2, SHWETA, 201-2-2, 190-1-2, PKB-6, 204-1-2 KBC-9, 137-1-1, UPC 4200, UPC 9202, KBC-6, MFC-09-12, EC4216, UPC 5286, 204-2-2, UPC 8705, MFC-08-14, BL-1, UPC 287, DCS 47-1, UPC 625, UPC 621, BL-2, UPC 622, MFC-09-01
4.Plant growth habit	Erect	4	BL-2, EC4216, 190-1-2, 190-2-2
	Semi erect	22	MFC-09-01, MFC-09-12, BL-1, UPC 5286, MFC-08-14, UPC 9202, UPC 287, UPC 622, UPC 625, DCS 47-1, SHWETA, PKB-4, PKB-6, KBC-9, 204-1-2, 209-2-1, 174-2-2, 204-2-2, 137-1-1, 127-1-2, 201-2-2, 128-1-1
	Spreading	4	UPC 8705, UPC 4200, UPC 621, KBC-6
5.Days to 10% flowering	Early (<45 days	3	201-2-2, 209-2-1, 174-2-2
	Medium (45-55 days)	22	UPC 9202, MFC-09-12, MFC-08-14, UPC 5286, UPC 8705, SHWETA, BL-2, 204-1-2, MFC-09-01, PKB-6, 127-1-2, UPC 622, KBC-6, UPC 4200, 128-1-1, 190-2-2, 137-1-1, 190-1-2, PKB-4, KBC-9, EC4216, DCS 47-1
	Late (>55 days)	5	UPC 625, UPC 287, UPC 621, BL-1, 204-2-2
6. Terminal leaf length	Medium	29	128-1-1
	Long	1	UPC 2873, 174-2-2, PKB-6, BL-2, UPC 4200, 127-1-2, 204-1-2, 204-2-2, 190-1-2, 209-2-1, 201-2-2, MFC-09-01, MFC-08-14, PKB-4, 190-2-2, UPC 622, MFC-09-12, DCS 47-1, BL-1, KBC-9, SHWETA, 137-1-1, EC4216, KBC-6, UPC 8705, UPC 625, UPC 5286, UPC 621, UPC 9202
7. Terminal leaf width	Medium(5-8 cm)	18	MFC-09-01, MFC-09-12, SHWETA, 127-1-2, 204-1-2, UPC 287, PKB-4, 128-1-1, BL-2, PKB-6, 204-2-2, 190-2-2, 190-1-2, 201-2-2, 209-2-1, UPC 4200, 174-2-2, BL-1
	Long >8 cm)	12	137-1-1, MFC-08-14, DCS 47-1, UPC 5286, UPC 8705, UPC 621, UPC 625, UPC 9202, KBC-9, UPC 622, KBC-6, EC4216

Further based on the terminal leaflet width of the 30 genotypes, 29 belonged to the long category and one genotype belonged to the medium category (Table 3). Similar findings were also observed earlier by Arya *et al.* (2021); Jain *et al.* (2006) in cowpea, Yadav *et al.* (2013) in Indian mustard, Kumar and Sarlach (2015) ; Nguyen *et al.* (2016) in cowpea.

Estimation of mean, PCV, GCV and heritability for yield and yield attributing traits in diverse genotypes of fodder-type cowpea. Results presented in Table 4 showed that the phenotypic coefficient of variation was higher than the genotypic coefficient of variation. It indicates that apparent variation is due to genotypes and the influence of the environment also. The high phenotypic coefficient of variation and genotypic coefficient of variation was recorded for dry matter content (%) followed by stover yield per plant (g) and green fodder yield per plant. The GCV and PCV values indicated that a lot of variabilities exist among the genotypic and phenotypic level and better chances of improvement are possible by selection (Vir and Singh 2014). These findings are in agreement with the findings of previous workers (Nwosu *et al.*, 2013; Kharde *et al.*, 2014).

Heritability in a broad sense indicates the percentage of transfers of traits from one generation to the next. Depending upon the heritability of the traits characters were categorized into heritable, medium heritable, and low heritable traits. In the present investigation, an attempt was made to estimate the broad sense of heritability for different characters. High heritability (>70%) estimates were recorded for days to 50% flowering followed by stover yield per plant (g) and days to maturity. Dry matter content (%), leaf to-stem ratio, and green fodder yield per plant showed medium heritability. High heritability indicated that these characters were highly heritable and governed by additive gene effects (Nwosu *et al.,* 2013). The phenotypic values could give a fairly good idea about their genetic material. The findings conform to the findings of Bhadru and Navale (2012); Kharde *et al.* (2014). High genetic advance as a percentage of the mean was recorded for dry matter content (%) followed by stover yield per plant (g), days to 50% flowering and leaf stem ratio. These finding indicated that leaf to-stem ratio and hence green fodder yield per plant can be improved much better way by selection.

Correlation coefficient and path coefficient analysis in diverse genotypes of fodder-type cowpea. The phenotypic and genotypic coefficients of correlation were determined among yield components in all possible character combinations and are presented in Table 5. For most of the characters, a genotypic correlation was higher in magnitude than a phenotypic correlation. The number of days to 50 % flowering had a positive association with days to maturity. In contrast, a significant negative association was shown with leaf to-stem ratio and green fodder yield per plant. The trait showed a non-significant negative correlation with dry matter content and stover yield per plant per plant. Days to maturity exhibited a non-significant positive correlation with days to fifty percent flowering, leaf to-stem ratio, and stover yield per plant.

Further, this trait exhibited a non-significant negative correlation with the dry matter content and green fodder yield per plant. Dry matter content exhibited a significant positive correlation with leaf to-stem ratio, stover yield per plant and green fodder yield per plant. Further, this trait exhibited a non-significant negative correlation with days to fifty percent flowering, and days to maturity. Leaf to-stem ratio exhibited a significant positive correlation with dry matter content, and green fodder yield per plant. However, this trait exhibited a non-significant positive correlation with stover yield per plant. A negative correlation was observed between leaf to-stem ratio and days to fifty percent flowering in a significant manner while it recorded a negative non-significant association with days to maturity. Green fodder yield per plant exhibited a positive significant correlation with leaf to-stem ratio, followed by dry matter content and stover yield per plant whereas negative significant correlation with days to 50 % flowering at the genotypic and phenotypic level, respectively.

Table 4: Analysis of variance for yield and yield attributing traits in diverse genotypes of fodder-type Cowpea.

Sources of variation	Degrees of freedom	MSS					
		Days to fifty per cent flowering	Days to maturity	Dry matter content	Stover yield per plant	Leaf to-stem ratio	Green fodder yield per plant
Replications	1.00	0.02	0.02	101.40	96.27	0.03	817.70
Genotypes	29.00	40.36**	47.89**	188.53**	253.08**	0.05**	1213.27**
Error	29.00	3.76	7.77	25.46	81.67	0.01	622.46

* Significant at 5% level of probability; ** significant at 1% level of probability

Table 5: Estimation of components of genetic variability for yield and yield attributing traits in diverse genotypes of fodder Cowpea.

Characters	Mean	Range		Vg	Vp	GCV (%)	PCV (%)	h^2_{bs}	GAM(%)
		Minimum	Maximum						
Days to fifty percent flowering	53.10	36.75	59.00	18.30	22.06	8.06	8.84	82.96	15.12
Days to maturity	80.20	72.00	92.25	20.06	27.83	5.58	6.58	72.09	9.77
Dry matter content (%)	10.71	5	20	8.25	12.36	26.82	32.83	66.72	45.12
Leaf to-stem ratio	102.72	87.75	127.75	85.71	167.38	9.01	12.60	51.21	13.29
Stover yield per plant (g)	1.24	0.99	1.48	0.02	0.03	11.46	13.35	73.64	20.25
Green fodder yield per plant (g)	249.08	203.00	303.50	295.40	917.87	6.90	12.16	32.18	8.06

Table 6: Genotypic correlation coefficients of yield and yield attributing traits among 30 diverse genotypes of fodder Cowpea.

Characters	Days to fifty per cent flowering	Days to maturity	Dry matter content	Leaf to-stem ratio	Stover yield per plant	Green fodder yield per plant
Days to fifty per cent flowering	1 .000	0.013	-0.235	-0.393 *	-0.121	-0.598 **
Days to maturity		1 .000	-0.096	0.105	0.092	-0.246
Dry matter content			1 .000	0.478 **	0.421 *	0.783 **
Leaf to-stem ratio				1 .000	0.137	0.803 **
Stover yield per plant (g)					1 .000	0.517 **
Green fodder yield per plant						1 .000

* Significant at 5% level of probability; ** significant at 1% level of probability

Table 7: Phenotypic correlation coefficients of yield and yield attributing traits among 30 diverse genotypes of fodder Cowpea.

Characters	Days to fifty per cent flowering	Days to maturity	Dry matter content	Leaf to-stem ratio	Stover yield per plant	Green fodder yield per plant
Days to fifty per cent flowering	1 .000	0.038	-0.178	-0.281 *	-0.185	-0.355 **
Days to maturity		1 .000	-0.065	0.126	0.044	-0.049
Dry matter content			1 .000	0.282*	0.320 *	0.528**
Leaf to-stem ratio				1 .000	0.069	0.327 *
Stover yield per plant (g)					1 .000	0.166
Green fodder yield per plant						1 .000

* Significant at 5% level of probability ; ** significant at 1% level of probability

Yield is a complex character, influenced by environmental fluctuations. Therefore, direct selection for yield as such will not be reliable and fruitful. Hence, selection criteria based on yield components would be helpful to select suitable plant types. The knowledge of the inter-relationship between yield components and the relative weightage should be given to different yield components to obtain maximum gain. Thus, constructions of selection indices will be helpful to discriminate desirable genotypes based on their phenotypic performance (Vir and Singh 2014). This provides experimental frame work for doing a straight forward analysis of variances, other necessary calculations and identifications of crosses likely to produce viable hybrids and crosses for pure line breeding.

UPC 621
Spreading Growth Habit

MFC 09- 01
Semi Erect Growth Habit

Plate variation in growth habit in fodder cowpea

CONCLUSIONS

The current investigation found that there is significant genetic variability among the fodder cowpea genotypes that were studied. The 30 genotypes of fodder-type cowpea varieties were all categorized into distinct groups based on the current characterization of each trait in line with the DUS guidelines by PPV & FRA, New Delhi. These genotypes and varieties can be utilized as a reference and as parents in breeding initiatives. The results of the study on genetic variability indicated that the traits leaf to-stem ratio, dry matter content, stover yield per plant and green fodder yield per plant were least influenced by environment and governed by additive gene action, thereby offering increased scope for improvement and developing high yielding fodder cowpea varieties.

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Conflict of Interest. None.

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