

RESEARCH ARTICLE

Genetic Variability of Exotic Cowpea Genotypes for Agro-Morphological Traits in Mid-Western Region of Nepal

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ABSTRACT

To assess the genetic variability of the cowpea genotypes for yield, a field experiment was conducted at research field of National Grain Legumes Research Program (NGLRP), Khajura, Banke, Nepal during summer season of 2019. Twelve cowpea genotypes were evaluated in Randomized Complete Block Design (RCBD) with three replications. Analysis of variance indicated that all the genotypes were significantly different for an early stand, 50% flowering days, 90% maturity days, pods per plant, seeds per plant, final stand, 100 seed weight, grain yield except for plant height. Genotypes namely IT10K-815-5, IT10K-973-1, IT82D-889 were top performer cowpea genotypes. Traits namely seeds per pods, pods per plants, plant height, hundred grain weight and grain yield had moderate to high genotypic and phenotypic coefficient of variation (GCV and PCV), heritability and genetic advance as a percentage of mean (GAM). All these traits had > 17% GCV and PCV, > 60% heritability and > 30% GAM. Multivariate analysis showed that twelve genotypes were grouped under three clusters and three principle components with eigenvalue more than one having 83.6% total variability. Genotypes belong to three different cluster and principle component showed considerable genetic diversity and can be used in further breeding programs. Genotypes such as IT08K-150-12 and IT82-1337 belong to the second cluster had the highest grain yield of 2.22 ton ha⁻¹ and 2.20 ton ha⁻¹ respectively.

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Introduction

Cowpea (*Vigna unguiculata* L. Walp.) (2n=2x=22) is a member of the Phaseoleae tribe of the Leguminosae family. Cowpea seed grain is used for human consumption as an affordable source of protein and constitutes a supplement green fodder along with cereal's straw for livestock. In Nepal, grain legumes rank fourth in terms of acreage (around 11% of total cultivated land) and 5th in terms of production after rice, maize and wheat (MoAD, 2018/19). Actual data on area, production was not recorded in case of cowpea but Kandel et al. (2019) reported estimated area, production and productivity of green cowpea are 4620.7 ha, 56790.10 mt and 12.3 ton ha⁻¹ respectively.

There were few researches carried out earlier to evaluated or characterized cowpea accession in different parts of countries. Pandey et al. (2006) evaluate five vegetable type cowpea varieties during 2003-2004 at Tanahun. Similarly, Bhattarai et al. (2012) evaluate four cowpea genotypes with two check varieties during 2016 at Lamjung. Kandel et al. (2019) also evaluate seven cowpea genotypes during 2018 at Dang.

For efficient crop breeding program for yield improvements requires information on the nature and magnitude of variation in the available germplasm, the associations between yield and other agronomic characters, and the degree of environmental influence on the expression of these traits (Edukondalu et al., 2017). Binodh et al. (2008) reported that information on character association in

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crops is an important for effective and rapid selection in crop improvement. Collection, characterization and evaluation of available cowpea germplasm, quantification of the magnitude of diversity and classification into groups facilitate identification of genetic variability that enables breeders to select traits of interest for an improvement program. Information on the nature and degree of genetic diversity would assist plant breeders in choosing the best genotypes as parents for hybridization (Souza and Sorrells, 1991).

Cluster analysis is frequently used to classify crops accessions and can be used by breeders and geneticists to identify subsets of accessions that have potential utility for specific breeding or genetic purposes. Many researchers such as Musvosv (2009) and Gerrano et al. (2015) used to classified cowpea accession by using cluster analysis. Multivariate analysis based on principal component analysis (PCA) is mostly used to evaluate the magnitude of genetic diversity among the germplasm (Brown-Guedira, 2000). The

PCA divides the total variance into different factors. The present study was carried out to analyze cowpea genotypes in term of agro-morphological and yield related traits and assess genetic variability present within the population and can be used for the further yield improvement programs.

Materials and Methods

Experimental Site

This experiment was carried out at research field of NGLRP, Khajura, Banke, Nepal. The experiment is situated at 181 masl, 28 °06'N latitude and 81 °37'E longitude. Study area lies in the tropical belt of Nepal. The area has humid type weather with cold winter, very hot summer. Climatological data was shown in figure 1. Soil of experimental area was found to be sandy loam to clayey loam having pH ranges from 6-7.5.

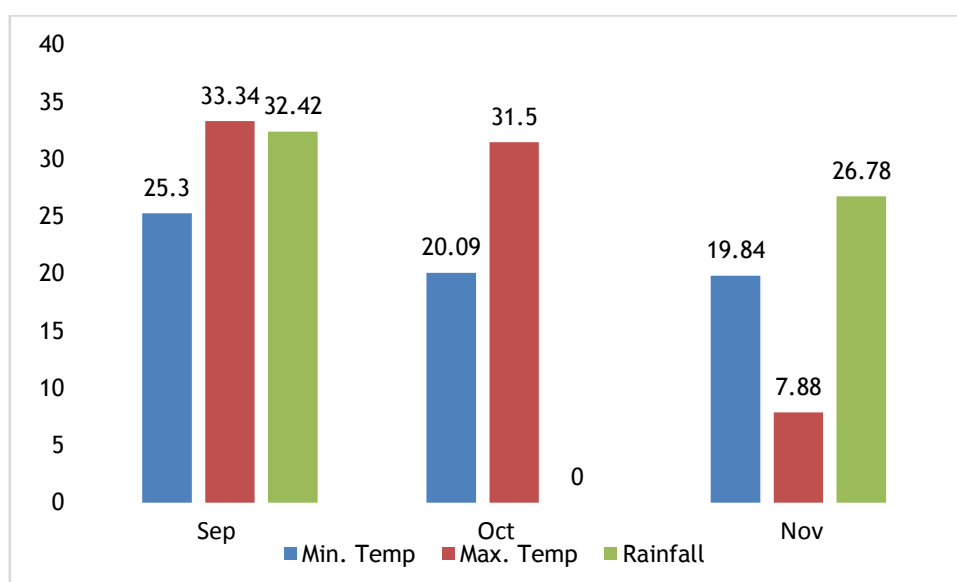


Figure 1. Climatological data of research station during research period

Experiments Design and Treatment Details

Twelve cowpea genotypes were laid out in RCBD with three replications during September to November of 2019. Two seeds were planted per hole, and were later thinned to one plant per hill after germination. Individuals plot size was 2× 4 =8 m². Plants were spaced at 75 cm between rows and 20 cm within rows. Data was collected based on agro-morphological traits as plant height, early stand, final stand, number of pod per plant, number of seed per pod, weight of 100 seed from five randomly selected plants from field whereas phenological traits such as days to 50% flowering and days to 90% maturity.

Statistical Analysis

All collected data were entered in Microsoft Excel version 19 and analysis of data were carried out by statistical package R (version 3.6.0). Mean and LSD at 5% level of significant

were computed. Multivariate analysis such as clustering and principle component analysis were carried out by Minitab version 14.

The genotypic and phenotypic coefficients of variation were calculated by the methods suggested by Lush (1940) and Chaudhary and Prasad (1968).

$$GCV = (\sigma_g / \bar{x}) \times 100 \quad (1)$$

$$PCV = (\sigma_p / \bar{x}) \times 100 \quad (2)$$

Where,

σ_g = Genotypic standard deviation

σ_p = Phenotypic standard deviation

\bar{x} = General mean of the trait

Categorized the value of GCV and PCV as, 0-10%: low, 10-20%: moderate and >20%: high (Sivasubramanian and Menon, 1973)

Falconer (1996) suggested the formula for heritability as:

$$H = \frac{V_g}{V_p} \times 100 \quad (3)$$

Where:

H = heritability in broad sense

Vp = phenotypic variance

Vg = genotypic variance

Categorization of percentage heritability as suggested by Robinson et al. (1949) as follows:

0 - 30% : low

30 - 60% : moderate

> 60% : high

Falconer (1996) suggested the formula for genetic advance as percentage of mean (GAM) estimation as:

$$GAM = GA / \bar{x} \times 100 \quad (4)$$

Where:

GAM=genetic advances as percent of mean

GA=genetic advances under selection

\bar{x} =population mean.

GAM was categorized based on categorize suggested by Johnson et al. (1955) as, 0-10%: low, 10-20%: moderate, >20%: high.

Genotypic and phenotypic coefficient of correlation between two characters was determined by using variance and covariance components (Weber and Moorthy 1952) whereas path analysis was computed using formula given by Dewey and Lu (1959).

Planting Materials

All studied genotypes were obtained from the International Institute for Tropical Agriculture (IITA) provided all studied genotypes to NGLRP except for Surya which was released variety by NARC and used as a standard check (Table 1).

Table 1. Genotypes used in research

S.N.	Genotypes	Year of introduction
1	IT10K-815-5	2013
2	IT08K-150-12	2013
3	IT010K-827-11	2013
4	IT11K-61-82	2013
5	IT07K-299-6	2013
6	IT07K-243	2013
7	IT10K-973-1	2013
8	IT10K-836-2	2013
9	IT82D-889	2013
10	IT82-1337	2013
11	IT07K-291-92	2013
12	Surya (std.check)	2004

Results and Discussion

All the genotypes were significantly variations with all the studies traits. Musvosvi (2009) reported highly significant differences were obtained among the genotypes for number of days to 50% flowering, number of days to first 50% mature

Pods, pod length and weight of 100 seeds, which means that these characteristics can be improved by selection in breeding program, which is accordance to our findings (Table 2).

Table 2. Mean square analysis of cowpea genotypes

Character	Genotype (MSS)Df=11	Replication (MSS)Df=2	Error (MSS)Df=22
Early stand	226.30**	1718.50	64.60
Flowering (50%)	22.21**	2.52	2.40
Maturity (90%)	38.00**	34.75	8.48
Plant height(cm)	149.40 ns	375.30	79.40
Pods/plant	7.35**	3.52	2.19
Seeds/pod	9.54*	9.19	3.71
Final stand	270.40*	2161.80	108.10
Grain yield (ton ha ⁻¹)	0.30**	0.21	0.05
Hundred grain weight (g)	13.14**	1.44	2.20

Df= Degree of freedom. MSS= mean sum of square, ** significant difference at 1% level, * significant difference at 5% level, ns= non-significant.

Early Stand and Final Stand

All the genotypes were significantly varied for both traits. For early stand all genotypes were significantly at par with standard check (Surya). All genotypes were statistically at par with standard check (Surya) except IT08K-150-12 for final stand (Table 3).

Flowering and Maturity Days

Genotypes such as IT07K-243, IT07K-299-6, were earlier flowering genotypes whereas genotypes such as IT07K-291-92, IT10K-973-1 delayed flowering. Flowering days varies from 38-48 which was higher than earlier researcher findings. Ishiyaku and Singh (2003) reported a range of 36-42 days to 90% flowering for two cowpea cultivars in Nigeria. Cobbinah et al. (2011) reported 31-38 days to 50% flowering in some cultivars. Significant different for maturity days among characters. Maturity days varied from 63-72 days. IT10K-815-5, IT010K-827-11, IT07K-299-6, IT07K-243, IT82D-889, IT82-1337 (Table 3).

Table 3. Mean performance of cowpea genotypes

Genotypes	Early stand	Flowering (50%)	Maturity (90%)	Plant height (cm)	Pods per plant	Seeds per pod	Final stand	Grain yield (ton ha ⁻¹)	Hundred grain weight (g)
IT10K-815-5	185 ^{ab}	46 ^{ab}	72 ^a	54.0	13.66 ^a	11.33 ^{abcd}	154 ^{ab}	2.44 ^a	21.00 ^{abc}
IT08K-150-12	174 ^b	44 ^{bc}	66 ^{bcd}	53.2	13.33 ^{ab}	9.00 ^d	137 ^b	2.22 ^{ab}	23.33 ^a
IT010K-827-11	182 ^{ab}	44 ^{bc}	68 ^{abc}	43.2	11.66 ^{abcd}	9.66 ^{bcd}	159 ^{ab}	1.46 ^e	17.33 ^d
IT11K-61-82	198 ^a	46 ^{bc}	63 ^d	41.6	9.00 ^e	11.00 ^{abcd}	168 ^a	1.84 ^{bcde}	17.33 ^d
IT07K-299-6	187 ^{ab}	48 ^a	73 ^a	34.4	9.33 ^{de}	11.00 ^{abcd}	160 ^{ab}	1.77 ^{cde}	17.00 ^d
IT07K-243	172 ^b	48 ^a	72 ^a	35.8	12.00 ^{abc}	11.00 ^{abcd}	153 ^{ab}	1.78 ^{bcde}	19.00 ^{cd}
IT10K-973-1	182 ^{ab}	38 ^e	64 ^{cd}	48.8	11.00 ^{bcde}	12.66 ^{abc}	155 ^{ab}	2.40 ^a	22.67 ^a
IT10K-836-2	186 ^{ab}	45 ^{bc}	72 ^a	50.2	12.00 ^{abc}	13.00 ^{ab}	167 ^a	2.05 ^{abcd}	20.00 ^{bc}
IT82D-889	201 ^a	45 ^{bc}	72 ^a	53.5	13.00 ^{ab}	14.33 ^a	161 ^{ab}	2.38 ^a	21.00 ^{abc}
IT82-1337	178 ^{ab}	43 ^{cd}	69 ^{abc}	44.8	13.00 ^{ab}	11.00 ^{abcd}	144 ^{ab}	2.20 ^{abc}	21.66 ^{ab}
IT07K-291-92	182 ^{ab}	41 ^{de}	65 ^{cd}	41.6	10.33 ^{cde}	9.33 ^{cd}	150 ^{ab}	1.65 ^{de}	20.00 ^{bc}
Surya	192 ^{ab}	44 ^b	71 ^{ab}	54.3	13.00 ^{ab}	14.33 ^a	168 ^a	2.11 ^{abc}	19.00 ^{cd}
LSD (0.05)	23.97	2.61	5.50	17.19	2.55	3.439	28.15	0.44	2.46
CV (%)	7.68	3.50	4.73	22.01	12.89	17.79	10.66	12.91	7.33
Mean	185.22	44.36	69	46.33	11.70	11.47	156.61	2.02	19.94
F-test	**	**	**	ns	**	*	*	**	**

*at 0.05 level of significant, **at 0.01 level of significant, ns=non-significant, CV= coefficient of variation, LSD= Least significant difference.

Plant Height (cm)

Analysis of variance showed that there was significant difference among the genotypes for plant height. All genotypes were statistically at par with standard check except IT07K-299-6. El-Nahrawy (2018) also reported significant different for plant height 24 cowpea genotypes, which was accordance to our findings (Table 3).

Pods Per Plant and Seed Per Pods

Both traits were significantly difference among the studied genotypes. All genotypes were statistically at par with standard check (Surya) except IT11K-61-82, IT07K-299-6, IT07K-291-92. Pods per plant ranged from 9-14 with mean value of 11. Gerrano et al. (2015) reported pods per plants range from 10-31. All genotypes were statistically at par with standard check (Surya) except IT08K-150-12, IT07K-291-92 for seed per pods traits (Table 3).

Hundred Grain Weight (g)

Highly significant difference for 100 seed weight among all genotypes. Genotypes like IT08K-150-12, IT10K-973-1 higher than standard check. Mean value of 100 seed weight was 19.66. All the genotypes are significantly variations with all the studies traits. Musvosvi (2009) reported highly significant differences were obtained among the genotypes

for number of days to 50% flowering, number of days to first 50% mature pods, pod length and weight of 100 seeds, which means that these characteristics can be improved by selection in breeding program, which is accordance to our findings, which was higher than reported by Idahosa et al. (2010) found hundred-seed weight ranged from 8.97 to 13.40 g in Nigeria for eight cowpea lines.

Grain Yield (ton ha⁻¹)

Analysis of variance indicated that there was significant variation among all studied genotypes in term of yield parameter Table 3. Yield ranges from 1.46-2.44 ton ha⁻¹ with mean yield of 2.02 ton ha⁻¹. Genotypes IT10K-815-5 was the highest yielder followed by IT10K-973-1 and IT82D-889. Gerrano et al. (2015) reported significant different among genotypes for grain yield (Table 3).

Correlation Analysis

Grain yield was positive and highly significant correlation with plant height, hundred grain weight where as positive and significant association with pods per plant. Early stand, maturity days, seed per pod were positive non-significant association with grain yield whereas traits such as flowering days and final stand were negative non association with grain yield (Table 4). Previous researcher who works on cowpea reported similar findings with us. Negative

association between grain yield and 50% flowering (Manggoel and Uguru, 2011). Positive and significant association with grain yield and pods per plants (Manggoel and Uguru, 2011). Kalambe et al. (2019) reported the characters plant height, no. of pods per plant, no. of seeds per pod and 100 seed weight showed the positive and significant correlation with pod yield per plant. Yadav et al. (2003), revealed that studying 28 F1s and 28 F2s, green pod yield per plant had positive and significant association with plant height, pods per cluster and seed per pod which all are accordance to our findings.

Table 4. Correlation studies of different traits of cowpea genotypes

	ES	FD	MD	PH	PD	SD	HG W	FS	GY
ES	1								
FD	.052	1							
MD	.048	.626*	1						
PH	.264	-.390	.062	1					
PD	-.229	-.051	.424	.720**	1				
SD	.606*	-.020	.411	.454	.240	1			
HG W	.332	.574	.193	.620*	.606*	.048	1		
FS	.747**	.233	.229	-.002	.354	.639*	.636*	1	
GY	.157	-.262	.110	.741**	.588*	.520	.762**	-.158	1

ES= Early stand, FD= 50% Flowering days, MD= 90% Maturity days, PH=Plant height (cm), PD= Pod per plant, SD= Seed per pod, HGW= Hundred grain weight (g), FS= Final stand and GY=Grain yield (ton ha⁻¹). **Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

Path Analysis

Hundred grain weight exhibited highest direct contribution to grain yield followed by seeds per pod, flowering days, plant height and early stand respectively. Highest negative contribution to grain yield by traits such as pods per plants, final stand and maturity days respectively (Table 5). Patil et al. (1989) reported that pods per plant, 100 grain weight and seeds per pod had greatest positive direct effect on yield. In our result pods per plant showed direct negative effect to grain yield but indirect positive contribution to hundred seed weight. Path analysis revealed that dry matter in pod, pods per plant, seeds per pod and plant height was the main components of green pod yield in the early generation of cowpea (Yadav et al., 2003), which all are accordance to our findings.

Table 5. Path coefficient analysis of different traits of cowpea genotypes (Bold vale indicate direct effect)

	ES	FD	MD	PH	PD	SD	HG W	FS
ES	0.132	0.007	0.006	0.035	-0.030	0.080	-0.044	0.099
FD	0.022	0.417	0.261	-0.163	-0.021	-0.008	-0.240	0.097
MD	-0.002	-0.023	0.037	-0.002	-0.016	-0.015	0.007	-0.008
PH	0.090	-0.134	0.021	0.342	0.246	0.155	0.212	-0.001
PD	0.067	0.015	-0.124	-0.210	0.291	-0.070	0.177	0.103
SD	0.285	-0.009	0.193	0.213	0.113	0.470	0.023	0.300
HG W	-0.281	-0.487	-0.163	0.526	0.514	0.041	0.848	-0.539
FS	-0.156	-0.049	-0.048	0.000	0.074	-0.133	0.133	0.209
Total	0.157	-0.262	0.110	0.741	0.588	0.520	0.762	-0.158

ES= Early stand, FD= 50% Flowering days, MD= 90% Maturity days, PH=Plant height (cm), PD= Pod per plant, SD= Seed per pod, HGW= Hundred grain weight (g), FS= Final stand and GY= Grain yield (ton ha⁻¹).

Genotypic and Phenotypic Coefficient of Variation (GCV and PCV) Heritability(H), Genetic Advance (GA) and Genetic Advance as Percentage of Mean (GAM)

GCV range from 7.73% of early stand to 26.53% for grain yield. Seed per pod, pod per plant, plant height and grain yield showed higher GCV value. Early stand, maturity days and final stand exhibit low GCV whereas flowering days and hundred grain weight showed moderate GCV. PCV was higher than corresponding GCV value for all study traits. All the traits showed the highest heritability value (Table 6). The high values for broad-sense heritability will help in transferring the genetic characteristics from the parents to offspring (Rashwan, 2010). High heritability coupled with high genetic advance was observed for plant height, number of pods per plant and number of branches per plant (Thorat and Gadewar, 2013). High genetic advance was recorded for seed yield per plant by Khan et al. (2015). Sabale et al. (2018) reported lower PCV recorded for maturity day and flowering day. Traits namely seed per pods, pod per plant, plant height, hundred grain weight and grain yield showed moderate to high GCV and PCV value along with high heritability and GAM. The character plant height, number of pods per plant and seed yield per plant showed high phenotypic and genotypic variance (Idahosa et al., 2010;

Manggoel et al., 2012; Massey et al., 2017; Nair et al., 2018) in cowpea.

Table 6. GCV, PCV, H, GA and GAM studies on different traits of cowpea genotypes

Traits	GCV	PCV	H	GA	GAM
Early stand	7.73	8.86	0.76	25.70	13.88
50% flowering days	10.43	11.00	0.90	9.04	20.37
90% maturity days	8.60	9.58	0.81	10.97	15.89
Final stand	9.78	11.82	0.68	26.09	16.66
Seed per pod	25.12	30.22	0.69	4.94	43.03
Pod per plant	21.85	25.21	0.75	4.59	39.01
Plant height (cm)	23.93	30.70	0.61	17.80	38.42
Hundred grain weight (g)	17.66	19.16	0.85	6.69	33.53
Grain yield (ton ha ⁻¹)	26.53	28.94	0.84	1.02	50.10

GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, H = Heritability, GA= Genetic advance and GAM= Genetic advance as percentage of mean.

Cluster Analysis

Cluster 1 consisted of six genotypes (IT10K-815-5, IT010K-827-11, IT10K-973-1, IT07K-299-6 and IT07K-243), cluster 2 consists of 2 genotypes (IT08K-150-12, IT82-1337) and cluster 3 consisted of 4 genotypes (IT11K-61-82, IT10K-836-2, Surya, IT82D-889). Genotypes belongs to cluster 1 have highest hundred grain weight and lowest plant height and lowest grain yield. Cluster 2 genotypes have highest pod per plants, lowest final stands and seed per pods along with highest grain yield. Similarly genotypes of 3rd have highest early stands and final stands, seeds per pods and maturity days and plant, medium yielder, late flowering, lowest pods per plants, lowest hundred grain yield (Figure 1, Table 7). Clustering of twenty-five genotypes of cowpeas was reported at the Agricultural Research Council-Rodepla at Vegetable and Ornamental Plant Institute South Africa, in 2011 and 2012 by Gerrano et al. (2015). Likely Musvosv (2009) at Zimbabwe was evaluated of nine cowpea genotypes at Zimbabwe clustering at results from analysis of variance and cluster analysis showed that there was existence of enough variation and suggesting that the Zimbabwe cowpea genetic resource have adequate variation for future use in endeavors to improve of the crop.

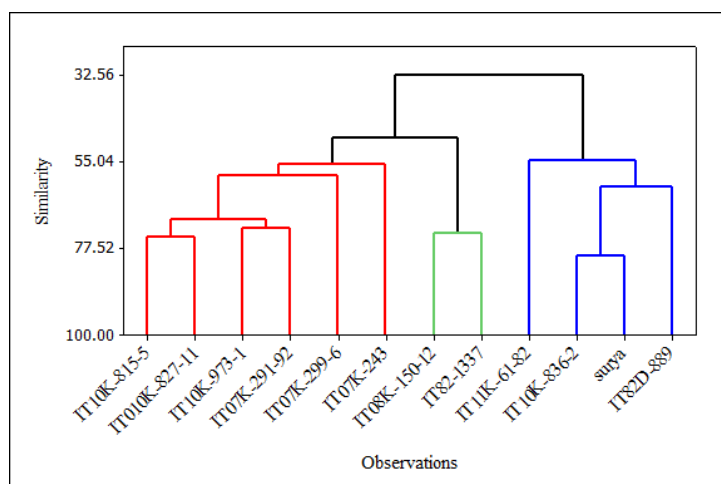


Figure 2. Clustering of genotypes of cowpea with average linkage and Euclidean distance

Table 7. Agro-morphological and yield component traits of cowpea genotypes with in among 3 cluster

Variable	Cluster 1	Cluster2	Cluster3	Grand centroid
Early stand	182.11	176.16	194.41	185.22
50% flowering days	44.38	43.50	44.75	44.36
Plant height (cm)	41.36	47.40	49.93	46.32
Pods per plant	11.94	12.50	10.83	11.66
Seed per pods	11.16	9.33	13.41	11.61
Maturity days	69.11	67.50	69.58	69.00
Final Stand	154.88	140.83	166.25	156.33
Hundred grain weight (g)	21.69	19.73	18.33	19.95
Grain yield (ton ha ⁻¹)	1.88	2.21	2.03	1.99

Principle Component Analysis (PCA)

In principle component analysis (PCA), three principal components with eigenvalues more than 1 explained 83.6% of the total variance. The first component accounted for 41.2% of variability and was positively related with number of hundred grain weight. The second component accounted for 25% variability, was positively related with days to flowering, maturity days. The third component contributed 18% of total variance; it had positive relationship with pods for plants, plant height, maturity days and grain yield (Figure 2, Table 8). Gerrano et al. (2015) evaluated 25 cowpea genotypes in south Africa and occupied five different principle components with 79.30 total variance among the genotypes for agro-morphological and yield related traits.

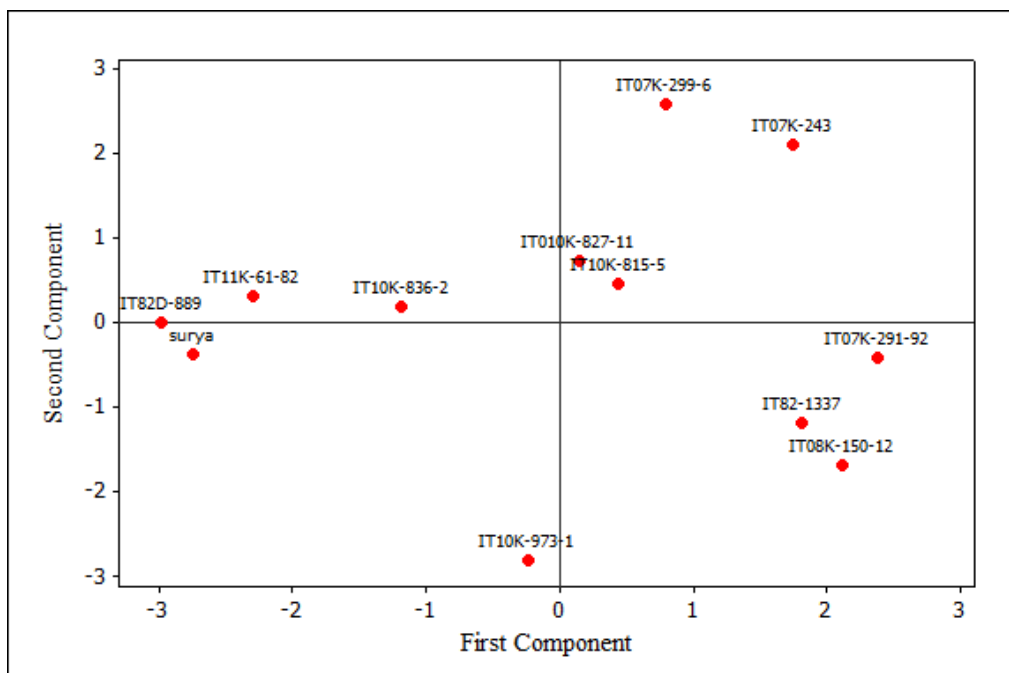


Figure 3. Principle component analysis of cowpea genotypes

Table 8. Principle component for 12 cowpea genotypes for agro-morphological and yield component traits

Variable (average value)	PC1	PC2	PC3
Early stand	-0.457	0.06	-0.07
50% flowering	-0.022	0.59	0.17
Pods per plant	0.259	0.04	0.56
Seed per pods	-0.495	0.03	-0.02
Plant height (cm)	-0.283	-0.44	0.30
90% maturity days	-0.066	0.40	0.54
Hundred grain weight (g)	0.449	-0.02	-0.28
Final stand	-0.426	0.21	-0.22
Grain yield (ton ha ⁻¹)	-0.105	-0.48	0.35
Eigenvalue	3.68	2.21	1.63
Proportion	0.412	0.25	0.18
Cumulative	0.412	0.65	0.83

Conclusion

All the genotypes were significantly different for all studied traits except plant height, so genotypes possess sufficient genetic variability and it can be utilized in crop improvement program. Genotypes such as IT10K-815-5, IT08K-150-12, IT10K-973-1, and IT82-1337 were promising cowpea genotypes having high PCV, heritability along with genetic advance for traits such as seeds per pods, pods per plants, plant height, hundred grain weight and grain yield, considered for selection in further yield improvement program. Further multi location evaluation of these genotypes in mid-western region and conclude based on result of different environments.

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