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Full Length Research Paper

Molecular Diversity and Varietal Differences in Seedling Growth Rate among Cowpea Genotypes using SSR Markers

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The present study aimed to evaluate genetic variability in seedling growth rate among cowpea genotypes using both phenotypic characteristics and Simple Sequence Repeat (SSR) markers. Twenty cowpea genotypes were sourced from the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Seedlings were cultivated in controlled conditions within the plant house of the Department of Biotechnology, Federal University of Technology, Akure. Analysis of variance indicated that most growth traits did not show significant differences, except seedling fresh and dry weights. The phenotypic coefficient of variation (PCV) exceeded the genotypic coefficient of variation (GCV) for all traits, with the highest values observed in seedling dry weight (41.20% and 33.62%, respectively), indicating considerable environmental influence. Heritability was generally low, except for seedling fresh weight (63.71%) and seedling dry weight (66.83%). All eight SSR markers were polymorphic, with CP09781 and CEDG093 yielding the highest allele numbers (17). Cluster analysis separated the genotypes into two groups, indicating limited genetic divergence. Consequently, these genotypes may not be ideal for hybridization in future breeding programs. However, seven genotypes (TVu-2300, TVu-16451, TVu-16117, TVu-16143, TVu-16123, TVu-15203, and TVu-12456) exhibited superior seedling growth traits, making them promising candidates for future cowpea breeding initiatives.

Keywords: Molecular diversity, varietal differences, seedling growth rate, SSR markers, cowpea genotypes

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous crop grown mainly in the savannah regions of the tropics and subtropics which belongs to the family Fabaceae) with 22 chromosomes (2n=22. It is an indigenous African grain legume rated as one of the most economically important crops and a veritable source of plant protein (Owolabi *et al*., 2012). Cowpea serves as an important tropical and sub-tropical seed plant within the African continent and outside with the world annual production estimate of 3.9-4.5 million tonnes grown from 12.5-14 million hectares **(**FAOSTAT, 2017; Abdullahi *et al.,* 2019). Cowpea is adapted to high temperatures in the range, 20℃ - 35℃. It does not withstand flooded conditions. It grows under a wide extreme of moisture, and once established, it is fairly tolerant to drought and can give good yields under marginal rainfall (Lazaridi *et al*., 2017). Amidst the indigence of African states, leguminous plants part especially cowpea consumption as the herb was reported in the treatment of malaria, gastric imbalance, liver diseases, and cardio-metabolic risks due it phytochemicals and metabolites (Alemu 2016). The significant low-fat level in legumes was also stated to be associated with a reduction in cholesterol level of coronary heart diseases patients during some clinical trials (Padhi

and Ramdath, 2017) and made it a potential weight restriction diet based on nutritional guidelines fact till date (Aremu *et al.,* 2015). Legumes contain several bioactive substances that play significant metabolic roles in the body system (Barman et al., 2018). The dietary fibre composition is also beneficial against the treatment of obesity and being a well-balanced diet contributor to prevent chronic diseases (Joye, 2020).

Rapid and uniform crop establishment is a key determinant of crop yields (Finch-Savage and Bassel, 2015), making understanding the variability within seeds a vital component of the global seed industry and ensuring crop security. Good quality and viable seed are required for rapid and synchronous seedling which is a prerequisite for successful crop stand establishment, uniform crop growth, and finally the yield (Mia and Shamsuddin, 2009). Seed vigour which is a basic requirement for seedling growth rate has been known as a comprehensive characteristic affected by many factors, such as the genetic background and environmental factors during seed development and storage stages (Bareke, 2020). The cultivars with strong seed vigour are desirable for farmers to get optimum crop stand establishment under optimal field conditions (Damalas, 2019). Good quality and viable seeds are required for rapid and synchronous seedling which is a prerequisite for successful crop stand establishment, uniform crop growth and finally the yield (Mia and Shamsuddin, 2009). Seeds with strong vigour may significantly improve the speed, uniformity of seed germination, the final percentage of germination, field emergence, good crop performance and even high yield under suboptimal conditions (Foolad *et al.,* 2007; Sallaku *et al.,* 2020).

The study of variability and diversity in accessions of cultivated crops could provide vital information for the establishment of a breeding programme, especially when intraspecific hybridization is necessary for the incorporation of new features or mapping purposes (Sarkar et al., 2017). Assessment of genetic diversity and variability in cowpea would enhance the development of cultivars for adaptation to specific production constrain (Magashi et al., 2019). This enables the breeder to operate selection efficiently and subsequently developed appropriate breeding strategies to solve the problems of poor yield as well as improve the nutritive quality of the crop. Knowledge of the genetic variability available within the germplasm collections can enhance the overall effectiveness of cowpea improvement programs (Nkhoma *et al.,* 2020). Collection of the germplasm and assessment of genetic variability is a basic step in any crop improvement programme (Sahoo *et al.,* 2019). Several methods have been used to assess genetic diversity among different soybean accessions including the use of morphological and agronomic traits, isozymes, pedigree information, and DNA markers (Chakraborty *et al.,* 2018). However, the use of morphological and agronomic traits for assessing genetic diversity are highly affected by environmental factors, making examination of distinctiveness difficult (Gupta and Manjaya, 2017). In addition, the use of pedigree information is also affected by uncertain or incomplete data and possible errors in data capture (Oda et *al.,* 2015). The use of DNA markers has been considered more informative, reliable, and reproducible compared to the commonly used conventional methods like phenotypic descriptors and pedigree analysis (Chakraborty *et al.,* 2018). Among the different DNA, markers are; Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphisms (SNPs), Restriction Fragment Length Polymorphisms (RFLPs), Microsatellites/ Simple Sequence Repeats (SSRs) and Randomly Amplified Polymorphic DNAs (RAPDs) have been widely used in studying genetic diversity in soybeans, each with its own merits and demerits (Khare *et al*., 2013; Chakraborty *et al.,* 2018). The objectives of this study are to :). Investigate the extent of variation in seedling growth rate in the cowpea genotypes. ii). identify the genotypes with a rapid growth rate. iii). estimate the genetic components among the cowpea seedlings. iv). determine the extent of molecular diversity among cowpea genotype using Simple Sequence Repeats (SSR) Marker.

MATERIALS AND METHODS

The experimental materials comprising twenty cowpea genotypes utilized for this study were obtained from the cowpea germplasm collection of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo – State, Nigeria.

Experimental Design and Layout

The experiment was carried out in two phases comprising fieldwork and laboratory (molecular) analysis.

For the fieldwork, the cowpea genotypes seeds were planted in nursery pots filled with 1kg topsoil in the plant house of the Department of Biotechnology, Federal University of Technology Akure. The experiment was Randomized Complete Block Design (RCBD) with three (3) replications. Twenty (20) genotypes of cowpea were evaluated and 3 pots were allocated to each of the genotypes.

Data were collected on each of the varieties based on the following characters / traits: Days to germination (day), number of leaves per plant, shoot length (cm), root length (cm), seedling length (cm), seedling fresh weight (g), and seedling dry weight (g), at 2WAP and 4WAP respectively.

For the laboratory (molecular) analysis, twenty genotypes of two weeks old cowpea seedlings were used for the DNA extraction following CTAB protocol.

The analysis of variance for different characters was carried out using the mean data to partition variability due to different sources by following Panse and Sukhatme (1961). All statistical analysis was performed using SAS version 9.2 (SAS, 2008) to examine the presence of

statistically significant differences among the genotypes for characters studied.

The components of the genetic parameters were also calculated. This includes phenotypic and genotypic variance (Wricke and Weber 1986), Genotypic coefficient of variation (GCV), and phenotypic coefficient of variation (PCV) were classified as low (0– 10%), moderate (10–20%) and high (> 20%) Johnson et al., (1955) heritability (Hb) percentage was categorized as low (0–30%), moderate (31–60%), and high (> 60) and genetic advance as percentage of mean was classified as low (0–10%), moderate (11–20%) and high (> 20%) Johnson et al., (1955).

MOLECULAR ANALYSIS

DNA Extraction

Two (2) grams of leaves from 2 weeks old of potted cowpea was ground after being surface sterilized with ethanol and 1000µl of freshly prepared modified CTAB extraction buffer (100mMTris-HCl, pH 8.0; 20mM EDTA, pH 8.0; 1.4M NaCl; 2% CTAB; (just before use))was added in mortar and pestle. The resultant mixture was homogenized and incubated in a 60°C water bath for 30min. Following the incubation period, the tube was transferred into a centrifuge at 12,000RPM for 10minutes. The supernatant was transferred into a new sterilized Eppendorf tube, then 10µL of RNases H solution was added and incubated at 37℃ for 5minutes. It was allowed to cool for 7 min and 1000µL chloroform: isoamyl alcohol (24:1) was added in the tubes and centrifuged at 12,000 rpm for 5minutes. The supernatant recovered after centrifugation was transferred into new tubes and up to 500µL Isopropanol was added and kept in -10°Cfreezer for 30minutes for DNA precipitation. The pellet was collected by centrifugation at 12,000 rpm for 2 min and washed with 500µL of chilled 70% ethanol. Pellet was air-dried until no further trace of ethanol. An average of 40 µL nuclease-free water T.E. buffer was added to elute the DNA and stored at -10℃.

Polymerase Chain Reaction (PCR)

SSR primers developed by Schafleitner *et al*., (2013) were used in this study and the primer sequences are shown in Table 2. PCR reaction for SSR was performed in 10 µl volume containing 20 ng template DNA and PCR master mix (NEB). The PCR reaction conditions were an initial denaturation at 95 ^{o}C for 5 min, followed by 35 cycles of 94 ^{o}C for 30 s, 55^{o}C for 30s, 72 ^{o}C for 30 s, and a final extension at 72 ^{o}C for 10 min. The amplified SSR products were separated on 6 % agarose gel electrophoresis and were visualized by silver staining with EZ-Vision. The gels were visualized under blue light using Bluebox^{TM} (USA).

Molecular Data Analysis

Statistical analysis was conducted by scoring SSR bands as co-dominant. These bands were considered polymorphic when they were present in some samples. Percent polymorphism for each marker was generated by the formula: (Number of polymorphic bands/Total number of scored bands) X 100. The polymorphic information content (PIC), a degree of polymorphism, was obtained with the PIC calculator (http://www.liv.ac.uk/*kempsj/pic.html). To analyse the variability, the amplicons were scored based on band weight and the pair-wise genetic similarity between genotypes was generated through Jaccard's co-efficient. Past 3 (PALEONTOLOGICAL STATISTICS VERSION 3) was subjected to generate a dendrogram using the unweighted pair group method average (UPGMA) clustering. The computer program BOOTSTRAP was used to examine the robustness of the dendrogram nodes with 100 bootstraps.

RESULTS AND DISCUSSION

The names and source of the cowpea genotypes are presented in Table 1. The analysis of variance estimates for 20 cowpea genotypes seedling growth rate is presented in Table 3. The cowpea genotypes showed non-significant differences in almost all the characters considered except seedling fresh weight and seedling dry weight.

The mean performance of the 7 characters studied among the cowpea genotypes is presented in Table 4. Genotypes; G_{11}, G_{12} and G_{18} recorded the highest value (6.07 days), and G_{10} the lowest value (2.07 days) for days to germination. Genotype; G_{12} recorded the highest value (36.17 cm) and G_3 the lowest value (15.67cm) for shoot height. For the root length, genotype; G_{18} recorded the highest value (21.97cm) and G_I, V_6 recorded the lowest value (9.50cm). Genotype; G_{I8} and G_3 recorded the highest value (57.63cm) and lowest value (25.63) for seedling length respectively. For a number of leaves per plant, seedling fresh weight and seedling dry weight G_{I3} recorded the highest value (18.33, 20.47g and15.73g) respectively whereas G_I recorded the lowest value (5.67, 4.73g and 3.33g) respectively.

The estimates of mean, range and variance components of cowpea seedling growth rate at 4WAP are presented in Table 5. The highest mean value (43.03) was recorded in seedling length while the lowest mean value (4.54) was recorded in days to germination. The genotypic variance varied between 0.35 and 23.61 being maximum in seedling length (23.61) whereas the phenotypic variance between 1.45 and 131.66 being maximum in seedling length (131.66). Generally, the phenotypic variances were higher than the genotypic variance.

The estimates of the genetic parameters for the characters studied among the cowpea genotypes are presented in Table 6. The phenotypic and genotypic standard deviations were recorded for the parameters. Seedling length was found to have the highest phenotypic germination recoded the lowest value for phenotypic

Table 1. Names of cowpea genotypes and source

S/N	Cowpea Sample ID	Source
1	TVu-8825	International Institute of Tropical Agriculture(IITA)
2	TVu-7440	International Institute of Tropical Agriculture(IITA)
3	TVu-297	International Institute of Tropical Agriculture(IITA)
4	TVu-16762	International Institute of Tropical Agriculture(IITA)
5	TVu-2300	International Institute of Tropical Agriculture(IITA)
6	TVu-16072	International Institute of Tropical Agriculture(IITA)
7	TVu-14544	International Institute of Tropical Agriculture(IITA)
8	TVu-16451	International Institute of Tropical Agriculture(IITA)
9	TVu-2269	International Institute of Tropical Agriculture(IITA)
10	TVu-4348	International Institute of Tropical Agriculture(IITA)
11	TVu-16117	International Institute of Tropical Agriculture(IITA)
12	TVu-16143	International Institute of Tropical Agriculture(IITA)
13	TVu-16123	International Institute of Tropical Agriculture(IITA)
14	TVu-9924	International Institute of Tropical Agriculture(IITA)
15	TVu-12390	International Institute of Tropical Agriculture(IITA)
16	TVu-16723	International Institute of Tropical Agriculture(IITA)
17	TVu-15203	International Institute of Tropical Agriculture(IITA)
18	TVu-12456	International Institute of Tropical Agriculture(IITA)
19	TVu-10407	International Institute of Tropical Agriculture(IITA)
20	TVu-12474	International Institute of Tropical Agriculture(IITA)

Table 2. Names and primers sequence of the SSR markers used for the analysis of 20 cowpea genotypes

Marker	Forward	Tm	Reverse	Tm
CEDG156	5´CGCGTATTGGTGACTAGGTATG 3´	54.84	5´CTTAGTGTTGGGTTGGTCGTAAGG 3´	57.38
CP09781	5´CTGACGCATTCAGCATTTTACAGC 3´	55.68	5´ATACGGTTGCGTCCATGTAT 3´	55.68
CEDG127	5´ GGTTAGCATCTGAGCTTCTTCGTC 3´	57.38	5´CTCCTCACTTGGTCTGAAACTC 3´	54.84
CEDG305	5´ GCAGCTTCACATGCATAGTAC 3´	52.40	5´GAACTTAACTTGGGTTGTCTGC 3´	52.97
CEDG020	5´ TATCCATACCCAGCTCAAGG 3´	51.78	5´ GCCATACCAAGAAAGAGG 3´	48.04
CEDG245	5´ GATAGAGCTTAAACCCTC 3´	45.77	5´ CTTTTGATGACAAATGCC 3´	43.49
CEDG132	5´ GGGTGTAATCCGTCAGAGC 3´	55.88	5´ CTTCCCCCTCTTCCGTTCTC 3´	55.88
CEDG093	5´ AAAACCCATGTAAAGTTCA 3´	43.58	5´ CAATCCATTCCCTTCTTAAT 3´	45.63

Table 3. Analysis of variance for seven characters in cowpea genotypes seedling growth rate

Source of Variation	Df	DTG(cm)	SHT(cm)	RL(cm)	SL(cm)	NLPP	SFWT(g)	SDWT(g)
Replication	2	8.24	208.47	126.18	603.72	42.07	126.76 *	95.61 **
Genotypes	19	4.35	163.70	52.48	393.88	40.99	69.61 *	42.11 **
Error	38	3.31	142.36	41.34	323.04	31.31	25.26	13.97

*, ** indicate Significance at 5% and 1% level of probability respectively.
DTG = Days to germination, SHT = Shoot height, RL = Root length, SL = Seedling length, NLPP = Number of leaves per plant, SFWT = Seedling fresh weight, and SDWT = Seedling dry weight.

standard deviation (1.20) and genotypic standard deviation (0.58) respectively.

The PCV of cowpea genotypes for various seedling characters ranged from 26.43% to 41.20%. All the characters showed high PCV; Days to germination (26.43%), Shoot height (26.89%), Root length (26.88%), Seedling length (26.63%), Number of leaves per plant (31.44%), Seedling fresh weight (40.71%) and seedling dry weight (41.20%).

The GCV of cowpea genotypes varied between 9.72% and 33.62%. Two characters showed high GCV; Seedling

Table 4. Mean performance of the cowpea genotypes seedling growth rate at 4wap

Cowpea genotypes	DTG(days)	SHT(cm)	RL(cm)	SL(cm)	NLPP	SFWT(g)	SDWT(g)
G_1	2.93	16.33	9.50	25.83	5.67	4.73	3.33
G_2	4.40	23.00	12.23	35.23	16.33	8.60	6.13
G_3	2.93	15.67	9.97	25.63	7.67	8.03	5.93
G_4	4.25	27.00	15.13	42.13	12.00	12.00	9.07
G_5	5.73	36.00	21.50	57.50	12.67	14.97	11.57
G_6	3.47	16.33	9.50	25.83	8.00	6.77	5.43
G_7	4.53	30.67	16.80	47.47	13.67	12.83	10.00
G_8	6.07	34.33	17.73	52.07	13.00	10.97	8.03
G_9	4.00	20.00	12.40	32.40	9.33	7.57	6.13
G_{10}	2.07	21.33	11.40	32.73	7.33	6.43	5.33
G_{11}	6.07	35.00	18.17	53.17	17.00	14.73	10.07
G_{12}	6.07	36.17	20.43	56.60	15.00	15.80	12.20
G_{13}	4.87	33.90	20.53	54.43	18.33	20.47	15.73
G_{14}	5.60	31.33	18.10	49.43	11.33	19.53	15.47
G_{15}	3.67	20.17	12.37	32.53	6.33	6.97	5.63
G_{16}	3.40	21.33	11.77	33.10	9.67	6.43	4.87
G_{17}	5.27	32.67	20.03	52.70	15.67	16.27	12.87
G_{18}	5.33	35.67	21.97	57.63	14.33	18.13	14.13
G_{19}	4.27	32.33	15.97	48.30	11.67	14.90	11.43
G_{20}	5.87	30.33	15.50	45.83	10.33	10.67	8.70
Grand mean	4.54	27.48	15.55	43.03	11.77	11.84	9.10

Where: DTG = Days to germination; SHT = Shoot height; RL = Root length; SL = Seedling length; NLPP = Number of leaves per plant; SFWT = Seedling fresh weight; SDWT = Seedling dry weight.
G_1 = TVu-8825; G_2 = TVu-7440; G_3 = TVu-297; G_4 = TVu-16762; G_5 = TVu-2300; G_6 = TVu-16072; G_7 = TVu-14544; G_8 = TVu-16451; G_9 = TVu-2269; G_{10} = TVu-4348; G_{11} = TVu-16117; G_{12} = TVu-16143; G_{13} = TVu-16123; G_{14} = TVu-9924; G_{15} = TVu-12390; G_{16} = TVu-16723; G_{17} = TVu-15203; G_{18} = TVu-12456; G_{19} = TVu-10407; G_{20} = TVu-12474

Table 5. Mean, range and variance components of cowpea seedling growth rate at 4wap

Parameters	Mean	Range	Genotypic variance	Phenotypic variance
Days germination(days)	4.54	2.07 - 6.07	0.35	1.45
Shoot height(cm)	27.48	15.13 - 36.17	7.11	54.57
Root length(cm)	15.55	9.50 - 21.97	3.71	17.49
Seedling length(cm)	43.03	25.63 - 57.63	23.61	131.66
No of leaves per plant	11.77	5.67 -18.33	3.22	13.66
Seedling fresh weight(g)	11.84	4.73 - 20.47	14.78	23.20
Seedling dry weight(g)	9.10	3.33 - 15.73	9.38	14.07

Table 6. Estimate of the genetic parameters of cowpea genotypes seedling growth rate

Parameters	σph	σg	PCV	GCV	HB (%)	GA	GAM
DTG(day)	1.20	0.58	26.43	12.78	23.84	0.60	12.98
SHT(cm)	7.39	2.67	26.89	9.72	13.03	1.98	7.22
RL(cm)	4.18	1.93	26.88	12.41	21.23	1.83	11.75
SL(cm)	11.46	4.86	26.63	11.30	17.98	4.25	9.87
NLPP	3.70	1.79	31.44	15.21	23.60	1.80	15.29
SFWT(g)	4.82	3.84	40.71	32.43	63.71	6.33	53.43
SDWT(g)	3.75	3.06	41.20	33.62	66.83	5.16	56.72

Where- σph= Phenotypic standard deviation, σg= Genotypic standard deviation, PCV= Phenotypic coefficient of variability, GCV= Genotypic coefficient of variability, HB%= percentage heritability, GA= Genetic advance, GAM= Genetic advance as percentage of mean. DTG = Days to germination, SHT = Shoot height, RL = Root length, SL = Seedling length, NLPP = Number of leaves per plant, SFWT = Seedling fresh weight, and SDWT = Seedling dry weight.

fresh weight (32.43%), Seedling dry weight (33.62%), moderately high GCV values were recorded in four characters Days to germination (12.78%), Root length (12.41%), Seedling length (11.30%), Number of leaves per

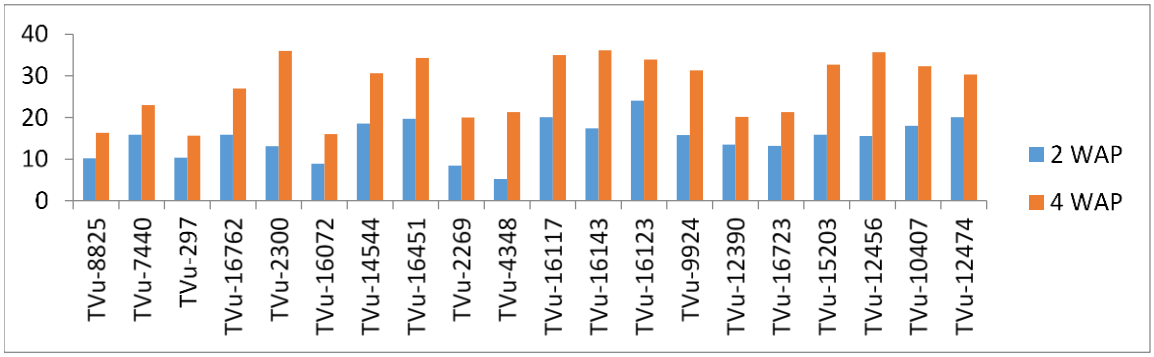

Figure 1. Shoot Height at 2WAP and 4WAP among the Cowpea genotypes

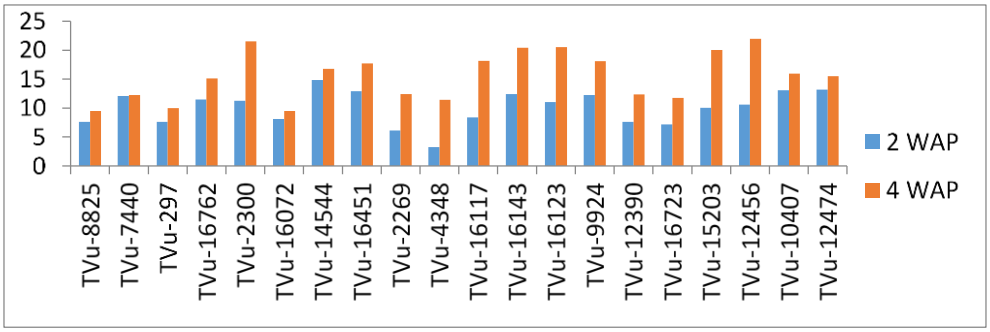

Figure 2. Root Length at 2WAP and 4WAP among the Cowpea genotype

plant (15.21%), and one character showed low GCV; Shoot height (9.71%).

Heritability estimates of cowpea genotypes of the characters studied expressed moderately high and low heritability. The estimates varied between 13.03 and 66.83. Two characters showed high heritability; Seedling fresh weight (63.71%), Seedling dry weight (66.83%) whereas in the other characters, heritability estimates were low. For the expected Genetic Advance (GA) and Genetic Advance as percentage of mean (GAM) of cowpea genotypes characters studied, all the characters recorded low GA which varied between (0.60 and 6.3%).

For GAM, seedling fresh weight and seedling dry weight recorded high GAM values (53.43% and 56.72%) respectively. Moderately high GAM estimates were recorded in days to germination (12.98), root length (11.75) and number of leaves per plant (15.29) while low GAM values were recorded in shoot height (7.22%) and seedling length (9.87%).

Figure 1 showed the shoot height at 2WAP and 4WAP respectively. At 2WAP, genotype; TVu-16143 recorded the highest (24.03cm) shoot height while TVu-4348 recorded the lowest (5.20cm) shoot height. At 4WAP genotype; TVu-16143 recorded highest (36.17cm) shoot height while TVu-297 lowest (15.67cm) height.

Figure 2 showed the root length at 2WAP and 4WAP respectively. At 2WAP, genotype TVu-14544 recorded the highest (14.83cm) root length while TVu-4348 recorded the lowest (3.27cm) length. At 4WAP, genotype TVu-12456 recorded the highest (21.97cm) root length while TVu-16072 the lowest (9.50cm).

Figure 3 below showed the Seedling length at 2WAP and 4WAP respectively. Genotype TVu-16123 recorded the highest (35.07cm) seedling length while TVu-4348 recorded the lowest (8.47) length at 2WAP. Genotype TVu-12456 recorded the highest (57.63cm) seedling length while TVu-297 recorded the lowest (25.63cm) length at 4WAP.

Figure 4 showed the Seedling fresh weight at 2WAP and 4WAP respectively. At 2WAP, genotype TVu-10407 recorded the highest (5.42g) seedling fresh weight while TVu-4348 lowest (1.47) weight. Genotype TVu-16123 recorded the highest (20.47g) while TVu-8825 recorded the lowest (4.73) weight at 4WAP.

Figure 5 showed the Seedling dry weight at 2WAP and 4WAP respectively. At 2WAP, genotype TVu-16117 recorded the highest (3.46g) seedling dry weight while TVu-8825 recorded lowest (0.77g) weight. Genotype TVu-16123 recorded the highest (15.73g) while TVu-8825 recorded the lowest (3.33g) weight at 4WAP.

The allele number and the polymorphic information content of the SSR primers were presented in Table 7. The

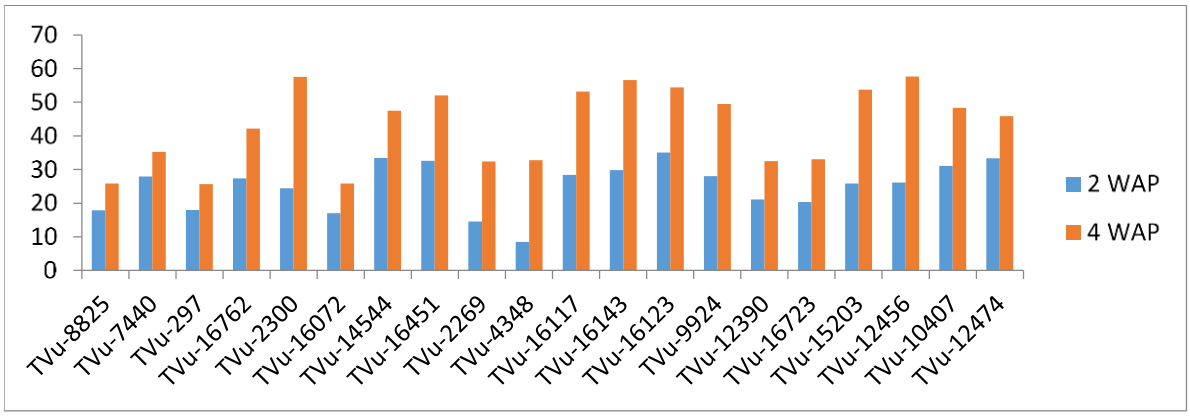

Figure 3. Seedling Length at 2WAP and 4WAP among the Cowpea genotypes

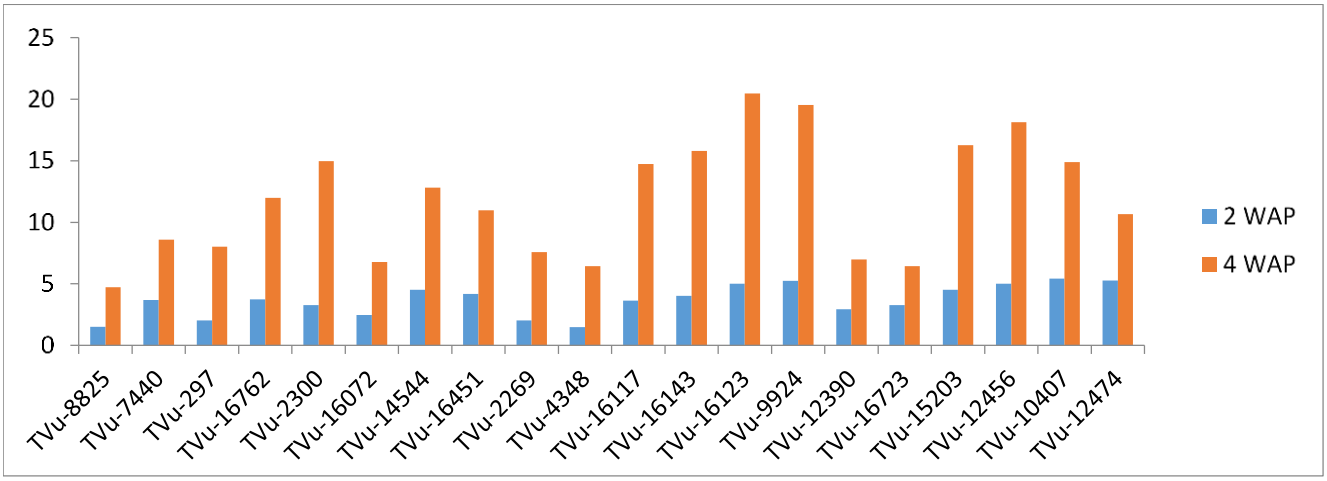

Figure 4. Seedling fresh weight at 2WAP and 4WAP among the Cowpea genotypes

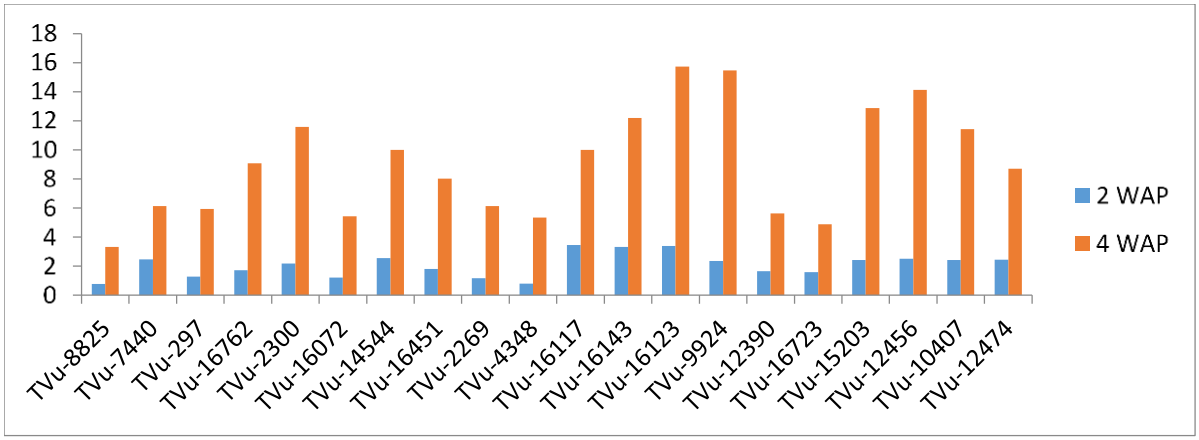

Figure 5. Seedling Dry Weight at 2WAP and 4WAP among the Cowpea genotypes

total number of alleles identified at 8 primer loci across the 20 genotypes of cowpea is 123. These alleles ranged between 13 and 17 per locus. The average number of alleles per locus is 15.375. The loci with the highest number of alleles are found in CEDG093 and CP09781 (17). The PIC value of each marker is estimated base on

Table 7. Alleles number and polymorphic Information Content (PIC) for the Primers used

Marker	Allele No	Percentage allele No (%)	PIC	Percentage PIC
CEDG156	16.0000	13.008	0.9202	12.549
CP09781	17.0000	13.821	0.9258	12.625
CEDG127	15.0000	12.195	0.9038	12.325
CEDG305	13.0000	10.569	0.9030	12.315
CEDG020	14.0000	11.382	0.9087	12.392
CEDG245	16.0000	13.008	0.9256	12.623
CEDG132	15.0000	12.195	0.9199	12.545
CEDG093	17.0000	13.821	0.9258	12.625
Mean	15.3750	12.500	0.9166	12.500

Table 8. Primers used among cowpea genotypes showing its major allele frequency and gene diversity

Marker	Allele No	Major Allele Frequency	Gene Diversity
CEDG156	16.0000	0.1500	0.9250
CP09781	17.0000	0.1500	0.9300
CEDG127	15.0000	0.2000	0.9100
CEDG305	13.0000	0.1500	0.9100
CEDG020	14.0000	0.1500	0.9150
CEDG245	16.0000	0.1000	0.9300
CEDG132	15.0000	0.1000	0.9250
CEDG093	17.0000	0.1500	0.9300
Mean	15.3750	0.1438	0.9219

its number of alleles and the distribution of alleles. There is significant variation in the PIC for all the studied SSR loci. The PIC values ranged from 0.9030 (CEDG093) to 0.9258 (CEDG132 and CP09781) with an average of 0.9144 per locus. On average, 62.5% of the SSR markers (CEDG156, CP09781, CEDG245, CEDG132, and CEDG093) recorded PIC estimates above 0.9166, while 37.5% of the markers (CEDG127, CEDG305 and CEDG020) recorded PIC estimates of below 0.9166.

The estimates of the major allele frequency and gene diversity of the primers are presented in Table 8. The major allele frequency ranged between 10% and 20% being maximum in CEDG305 (0.20) and minimum in (CEDG245 and CEDG132 respectively). The average major allele frequency across the loci was 0.14 (14%). On average, 75% of the markers utilized for this study recorded major allele frequency above 0.14. As regards to gene diversity of the primers, it ranged from 0.91 to 0.93 with an average of 0.92. The highest gene diversity was observed in CEDG127 and CEDG132. On average, 67.5% of the primers recorded gene diversity above 0.92.

Plate 3a to 3h depicted the DNA bands amplified by the 8 SSR markers across the 20 Cowpea genotypes respectively.

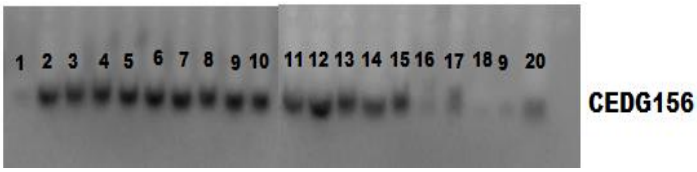

Plate 1a. Gel electrophoresis. DNA bands amplified by CEDG156 Marker across 20 Cowpea genotypes

Plate 3b. Gel electrophoresis. DNA bands amplified by CP09781 Marker across 20 Cowpea genotypes

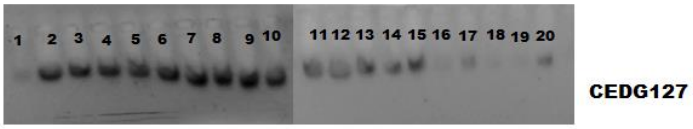

Plate 3c. Gel electrophoresis. DNA bands amplified by CEDG127 Marker across 20 cowpea genotypes

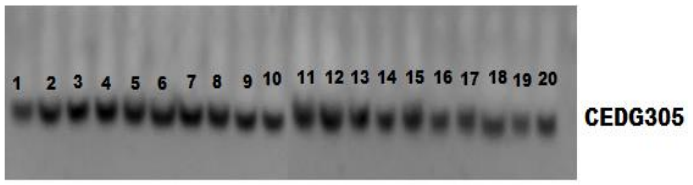

Plate 3d. Gel electrophoresis. DNA bands amplified by CEDG305 Marker across 20 Cowpea genotypes

Plate 3e. Gel electrophoresis. DNA bands amplified by CEDG020 Marker across 20 Cowpea genotypes

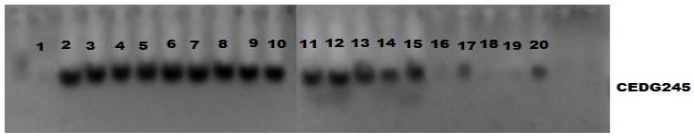

Plate 3f. Gel electrophoresis. DNA bands amplified by CEDG245 Marker across 20 Cowpea genotypes

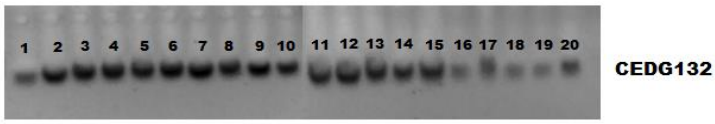

Plate 3g. Gel electrophoresis. DNA bands amplified by CEDG132 Marker across 20 Cowpea genotypes

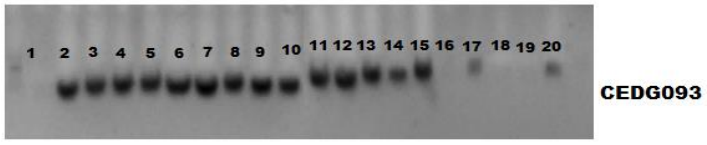

Plate 3h. Gel electrophoresis. DNA bands amplified by CEDG093 Marker across 20 cowpea genotypes

A dendrogram based on UPGMA cluster analysis of the SSR data is shown in Figure 6. The dendrogram showed 2 clusters that are not so distantly separated from one another.

The distribution of the cowpea genotypes is presented in Table 9. The 20 cowpea genotypes were distributed into 2 different clusters (cluster A and cluster B respectively). Cluster A comprises 17 cowpea genotypes generated from the constructed dendrogram. The cowpea genotypes (TVu-8825, TVu-7440, TVu-297, TVu-16762, TVu-2300, TVu-16072, TVu-14544, TVu-16451, TVu-2269, TVu-4348, TVu-16117, TVu-16143, TVu-9924, TVu-12390, TVu-16723, TVu-15203, TVu-12474) cluster B comprises of 3 cowpea genotypes (TDVu-12456, TVu-10407, TVu-16123). Cluster A and cluster B showed Jaccard similarity index or genetic distance of 1 and 0.75 respectively.

DISCUSSION

The non-significant differences observed in almost all the characters studied indicate that the genetic components of the parental materials are intact. The phenotypic variances were generally higher than their respective genotypic variances revealing the role of environmental factors in the expression of these characters in the genotypes (Kumar *et al.,* 2019). Most of the characters recorded low heritability and low genetic advance estimates which imply that these characters are being governed or controlled by non-additive gene action and the interaction between genotypes and the environment (Sahoo *et al*., 2019). This report is contrary to that of Olajide and Ilori (2018) who reported the gene interaction in seedling characters for drought tolerance as both dominance and additive gene action. Hence, these characters may be improved upon their hybridization.

From the mean performance estimates, genotypes; TVu-2300, TVu-16451, TVu-16117, TVu-16143, TVu-16123, TVu-15203, and TVu-12456 exhibited high

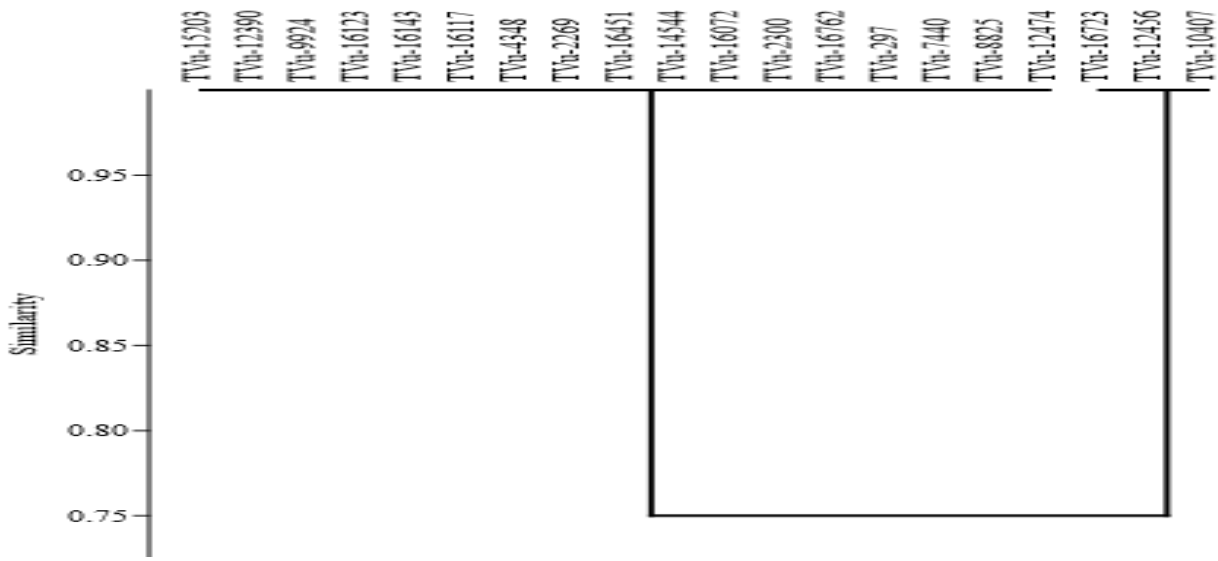

Figure 6. UPGMA based cluster analysis of 20 Cowpea genotypes using SSR Markers

Table 9. Distribution of 20 Cowpea Genotypes into different Clusters

S/no	Cluster number	No of cowpea genotypes	Genotypes
1	A	17	TVu-8825, TVu-7440, TVu-297, TVu-16762, TVu-2300, TVu-16072, TVu-14544, TVu-16451, TVu-2269, TVu-4348, TVu-16117, TVu-16143, TVu-9924, TVu-12390, TVu-16723, TVu-15203, TVu-12474
2	B	3	TVu-12456, TVu-10407, TVu-16123

estimates for the characters studied. This implies that the seedlings possess high seed vigour and viability. Hence, these genotypes will perform optimally and excellently when planted on the field. Although, cowpea takes 3-5 days on average to germinate. From this study, these genotypes required longer days to germinate and they were still able to stand out among the other genotypes for the characters studied.

Assessment of genetic variability within a germplasm is of interest for practical purposes such as breeding to predict the ability to combine the breeding material (Rauf, 2019). Morphological or phenotypic variability has long been performed in many plants either alone or combination with biochemical or molecular assay (Masalla *et al*., 2018). Although for most characters, interactions between genotype and environment complicate the assessment process. Molecular markers have been successfully used in cowpea research to select parents for hybrid production for intraspecific and interspecific classification and analysis of variation (Osei *et al.,* 2018). SSR markers have been commonly used in evaluating genetic diversity and relatedness among organisms because of their abundance in genomes and high allelic polymorphism, codominance and easy manipulation by PCR (Ahmed *et al*., 2017). SSR markers were reported as superior in terms of high information content and discrimination power owing to high allelic variation which allows clear identification of genotypes compared to most DNA as well as biochemical and morphological markers (Mafakheri et al., 2017). Molecular diversity is based on the naturally occurring polymorphism without the influence of environmental factors and gene expression whereas morphological and physiological diversity is largely influenced by environmental conditions and cultural practices (Boopathi, 2020). Hence, molecular data should be given preference over morphological and physiological data in genetic diversity or variability studies (Bhandari *et al*., 2017). The reliability, reproducibility and authentic results obtained from using Simple Sequence Repeat (SSR) markers have made it widely preferred in genetic diversity studies (Mohamed, 2018). The 8 primers utilized for this study amplified the DNA bands and a total of 123 alleles were detected with a range of 13-17. The reason for the wide variation in the number of alleles recorded in this present study may be due to the difference in a number of genotypes, number and distribution of the SSR loci. The number of alleles recorded in this study was found to be very high compared to other studies conducted in cowpea using SSR markers by some other researchers such as Sawadogo *et al.,* 2010; Adetiloye *et al*., 2013;

Carvalho *et al.,* 2017 who recorded allele number ranging from 5 to 12 and 2 to 5 respectively. The variations in the number of alleles detected may be due to the types of primers used and the rate of polymorphism of each primer pairs. The number of alleles observed in this study is comparable to those reported by Ali *et al*., (2015). They detected the highest alleles number of 17 while the lowest alleles number was 2. Based on the Polymorphic Information Content (PIC) values, these primers were highly informative for discriminating and distinguishing the genotypes and were also helpful to study the phylogenetic relationship (Mukuze *et al.,* 2020). In the present study, the polymorphic information content (PIC) ranged from 0.9030 to 0.9258 with a mean of 0.9166. This finding is contrary to that of Badiane *et al.,* 2012 and Asare *et al*., 2010 who reported PIC ranging from 0.08 to 0.33 and 0.07 to 0.66 respectively. The informativeness of PIC value measured by Botstein *et al.,* 1980 scale revealed that the mean PIC value ≥ 0.50 is highly informative, 0.25 -0.50 implies reasonably informative and < 0.25 is slightly informative and loci with many alleles and PIC value near 1; are most desirable (Botstein *et al.,* 1980). The gene diversity observed in this present study ranged between 0.9100 and 0.9300. The results of gene diversity reflect the proportion of polymorphic loci across the genome (Bohra et al., 2017). The gene diversity value recorded in this study was found higher than previous studies reported by Zhao *et al*., (2018) in soybean. The present investigation has provided a useful insight into the informativeness of the SSR markers utilized and can be exploited in future cowpea breeding programs.

CONCLUSION

In the present study, it can be concluded that cowpea genotypes were not significantly different for most of the characters studied. Hence, it shows that the cowpea genotypes were not distantly related. The high phenotypic variance estimates coupled with low heritability estimates and low Genetic advance value implied that the expression of the considered characters was under the control of dominance gene action and also under environmental factor influence other than genetic factors. Hence, these characters cannot be improved upon by direct selection. It can also be concluded that the 8 Simple Sequence Repeat (SSR) markers utilized for this study were highly polymorphic and informative and can be utilized in the future cowpea improvement programme. In addition, it can be concluded from the study that the cowpea genotypes utilized for this research were not distantly related hence, crosses between them might not give a good progeny. It can also be gathered from this study that the 7 genotypes; TVu-2300, TVu-16451, TVu-16117, TVu-16143, TVu-16123, TVu-15203 and TVu-12456 exhibited good seedling vigor based on their mean performance estimates. Hence, they can be selected as promising genotypes to be utilized for future cowpea breeding program.

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