International Journal of Bio-resource and Stress Management https://pphouse.org/ijbsm.php

Crossref

IJBSM August 2022, 13(8):815-821 Print ISSN 0976-3988 Online ISSN 0976-4038 **Article** AR3130

Research Article *Natural Resource Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2022.3130

Assessment of Genetic Diversity Among Cowpea [*Vigna unguiculata* (L.) Walp] Genotypes using SSR Markers

K. Vinay[1], P. Jagan Mohan Rao[2], N. Sandhya Kishore[3] and Y. Hari[3]

[1]Dept. of Genetics and Plant breeding, College of agriculture Rajendranagar, PJTSAU, Hyderabad (500 030), India
[2]Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad (500030), India
[3]Regional Agriculture Research Station, Warangal, PJTSAU (506 007), India

Open Access

Corresponding K.vinayreddy166@gmail.com

0000-0003-2093-7944

ABSTRACT

In the present study, 32 genotypes of cowpea were evaluated for genetic diversity through simple sequence repeats (SSR) markers at Biotechnology laboratory, Regional Agriculture Research station, Warangal, Telangana state, India during during *rabi* (October–February, 2020–21). 25 pairs of SSR primers were employed to analyze the genetic diversity among the genotypes. The polymorphic bands were scored visually as present (1) or absent (0) on a binary matrix and this information was utilized in the calculation of Jackard's similarity matrix using NTSYS-pc version 2.1. Dendrogram was constructed using the Unweighted pair Group Method with an Arithmetic mean (UPGMA) algorithm. Out of 25 markers, only eight markers have shown scorable polymorphism, while fifteen markers exhibited monomorphic banding pattern and remaining two markers showed no banding pattern. A dendrogram of these genotypes based on SSR polymorphism divided into three major clusters at 23% similarity. Genetic similarity among the genotypes ranged from 13–100% with an average of 57%. High genetic similarity of 100% was recorded between the genotypes namely WCP-4 and WCP-6, GC-1712 and PCP-1124, PGCP-69 and CPD-313. The genotypes TPTC-29 and KBC-9 appeared more divergent than remaining genotypes with 26% similarity which indicates that they are genetically more distant from other genotypes and can be utilized in crossing programmes. The present study revealed that microsatellites can be successfully utilized for assessment of genetic diversity to establish relationships among the germplasm lines of cowpea.

KEYWORDS: Cowpea, dendrogram, genetic diversity, germplasm, microsatellites, polymorphism, SSR

***Citation* (VANCOUVER):** Vinay et al., Assessment of Genetic Diversity Among Cowpea [*Vigna unguiculata* (L.) Walp] Genotypes using SSR Markers. *International Journal of Bio-resource and Stress Management,* 2022; 13(8), 815-821. HTTPS://DOI.ORG/10.23910/1.2022.3130.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Funding: Authors confirm that there was no funding received. However, Shriniketan Puranik received institutional fellowship.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 09th June 2022 RECEIVED in revised form on 04th August 2022 ACCEPTED in final form on 16th August 2022 PUBLISHED on 24th August 2022

1. INTRODUCTION

Cowpea [*Vigna unguiculata* (L.) Walp.] is an important leguminous crop with chromosome number (2n=2x=22) with genome size of about 620 million base pairs (Boukar et al., 2019). It belongs to order Rosales, family Fabaceae, genus Vigna and is native to Central Africa (Darlington and Wylie, 1955). The crop is autogamous, but up to 5% outcrossing has been reported in the cultivated varieties, probably due to insect activities (Badiane et al., 2014). Cowpea is mainly cultivated in tropical and subtropical countries such as Africa, Asia, Central and South America. Because of its high protein content, cowpea is referred to as "vegetable meat" and have biological value on a dry weight basis (Ram, 2014). Cowpea is economically grown throughout Indian subcontinent for variety of purposes such as seeds as pulses, long green pods as vegetables, foliage as fodder for cattle, green manuring and cover crop (Meena et al., 2015). The young leaves are used as spinach in eastern and southern Africa (Boukar et al., 2015).

Cowpea grains have a nutritional profile of 23.4% protein, 60.3% carbohydrates and 1.8% fat, as well as a good source of vitamins (folic acid and vitamin B) and phosphorus necessary for prevention of congenital malformations (Venkatesan et al., 2003, Srivastava et al., 2016). The crop is well known in India and Southeast Asia for its immature tender pods and dry seeds, which serve as a cost-effective source of protein (Khandait et al., 2016).

Cowpea is also known for producing high-quality forage. It produces dense vegetative growth and effectively covers the land preventing soil erosion. It fixes nitrogen as it is a leguminous crop and improves fertility of the soil (Hall et al., 2003, Kumar et al., 2015). Because of its, drought tolerance, soil-restoring properties, smothering nature and multi-purpose uses, it is considered as a versatile legume crop and fits well into most cropping systems as a pulse crop (Das et al., 2020).

The worldwide annual average cowpea production from 2014–2018, was 6.57 mt on a harvested area of 12.4 mha with an average yield of 0.53 t ha^{-1} (Anonymous, 2020). Worldwide, Nigeria is the largest producer of cowpea followed by Niger, Burkina Faso, Cameroon, and Mali.

Crop genetic diversity is crucial for both food security and sustainable development, as it serves as a source of genes needed in the development of better performing and well adapted varieties (Dossa et al., 2016). Food production and security depend on the conservation and wise use of agricultural biodiversity.

Several approaches have been utilized to enhance our knowledge of the nature and extent of variability among cowpea accessions stored in different genetic resource centers. To evaluate the genetic diversity of a given crop, morphological (phenotype) and molecular (genotype) markers have been used. However, most cowpea accessions are characterised primarily based on morphological data, which are fraught with environmental fluctuations (Nkongolo, 2003). Molecular markers, particularly Simple Sequence Repeats (SSR), are playing an increasingly important role in assessment, characterization and conservation of plant genetic resources (Kanavi et al., 2019). The sequences are abundant and randomly distributed throughout the genome, highly polymorphic, inherited co-dominantly and are not influenced by environmental variations and have shown great potential for various genetic studies (Tautz, 1989, Pradeepkumar et al., 2017). They have been used to assess the diversity of various cowpea germplasm from different countries (Li et al., 2001, Uma et al., 2009, Asare et al., 2010, Gupta and Gopalakrishna, 2010, Adesoye et al., 2016, Desalegne et al., 2016, Lal et al., 2016). Therefore, an attempt was made to investigate molecular diversity in the germplasm considered for present study.

2. MATERIALS AND METHODS

2.1. Plant materials and DNA Isolation

In the present study, 32 cowpea genotypes were used for molecular diversity analysis (Table 1). The experimental material was grown during *rabi* (October–February, 2020–21) at C-block of Regional Agricultural Research Station (RARS), Warangal, Telengana state, India. It falls on 17.58° N latitude and 79.40° E longitudes. Molecular analysis was performed at Biotechnology Laboratory at RARS, Warangal. For isolation of genomic DNA, young leaves of 21–28 days were selected. The standard protocol as described by Doyle and Doyle (1987) was followed with few modifications and was then quantified spectrophotometrically on a nano spectrophotometer (Implen, Germany).

2.2. SSR-PCR amplification

25 SSR primers were used to screen cowpea germplasm lines presented in Table 2. The concentration of 30 ng μl^{-1} of genomic DNA was used for SSR-PCR (Eppendorf) amplification. PCR amplification was performed with reaction conditions programmed as Initial denaturation at 94°C for 4 m, followed by 35 cycles of denaturation for 1 m at 94°C. Annealing temperature 55°–60° c for 1 m and extension at 72°C for 1 m. A final extension was performed at 72°C for 7 m and storage at 4°C. The PCR amplified products obtained were loaded on 4% agarose gel which was prepared with 1X TAE buffer as well as ethidium bromide (10 mg ml^{-1}). SYNGENE system was used for documentation of the gel.

Table 1: List of 32 cowpea varieties used for molecular diversity analysis

Sl. No.	Entry	Source	Sl. No.	Entry	Source
1.	TPTC-29	RARS, Tirupathi	17.	WCP-4	Local germplasm
2.	KBC-9	UAS, Bangalore, Karnataka	18.	WCP-6	Local germplasm
3.	DC-15	UAS, Dharwad, Karnataka	19.	WCP-9	Local germplasm
4.	PCP-0306	MPKV, Rahuri, Maharashtra	20.	WCP-10	Local germplasm
5.	KBC-12	UAS, Bangalore, Karnataka	21.	WCP-12	Local germplasm
6.	CPD-311	RARI, Durgapura, Rajasthan	22.	WCP-15	Local germplasm
7.	KBC-13	UAS, Bangalore, Karnataka	23.	WCP-16	Local germplasm
8.	CPD-313	RARI, Durgapura, Rajasthan	24.	WCP-17	Local germplasm
9.	PGCP-69	GBPUA&T, Pantnagar	25.	WCP-18	Local germplasm
10.	PCP-1124	MPKV, Rahuri, Maharashtra	26.	WCP-21	Local germplasm
11.	SKUA-WCP-149	SKUAST, Srinagar, J&K	27.	WCP-23	Local germplasm
12.	CPD-331	RARI, Durgapura, Rajasthan	28.	PMCP-1131	Palampur, Himachal Pradesh
13.	TC-1901	IARI, New Delhi	29.	WCP-26	Local germplasm
14.	GC-1712	SDAU, S.K,Nagar, Gujarat	30.	WCP-28	Local germplasm
15.	PGCP-70	GBPUA&T, Pantnagar	31.	WCP-29	Local germplasm
16.	WCP-1	Local germplasm	32.	WCP-34	Local germplasm

Table 2: List of primers used in the present study

Sl. No	Markers	Markers Sequence	S l . No	Markers	Markers Sequence
1.	VM4 Forward	AGTAAATCACCCGCAC-GATCG	13.	VM20 Reverse	ATCCAAGATTCGGACAC-TATTCAA
	VM4 Reverse	AGGGGAAATGGAGAG-GAGGAT			
2.	VM5 Forward	AGCGACGGCAACAACGAT	14.	VM21 Forward	TAGCAACTGTCTAAGCCTCA
	VM5 Reverse	TTCCCTGCAACAAAAATA-CA		VM21 Reverse	CCAACTTAACCATCACTCAC
3.	VM8 Forward	TGGGATGCTGCAAAGA-CAC	15.	VM24 Forward	TCAACAACACCTAGGAGC-CAA
	VM8 Reverse	G A A A A C C G A T G C - CAAATAG		VM24 Reverse	ATCGTGACCTAGTGCCCACC
4.	VM9 Forward	A C C G C A C C C G A T T - TATTTCAT	16.	VM25 Forward	CCACAATCACCGATGTCCAA
	VM9 Reverse	A T C A G C A G A C A G - GCAAGACCA		VM25 Reverse	C A A T T C C A C T G C G G G A - CATAA
5.	VM10 Forward	TCCCACTCACTAAAATA-ACCAACC	17.	VM26 Forward	G G C A T C A G A C A C A T A T - CACTG
	VM10 Reverse	G G A T G C T G G C G G C G - GAAGG		VM26 Reverse	TGTGGCATTGAGGGTAGC
6.	VM11 Forward	CGGGAATTA ACGGAGT-CACC	18.	VM27 Forward	GTCCAAAGCAAATGAGTCAA
	VM11 Reverse	CCCAGAGGCCGCTATTA-CAC		VM27 Reverse	TGAATGACAATGAGGGTGC

Table 2: Continue...

Sl. No.	Markers	Markers Sequence	Sl. No.	Markers	Markers Sequence
7.	VM12 Forward	TTGTCAGCGAAATAAGCAGA-GA	19.	VM28 Forward	GAATGAGAGAAGTTACG-GTG
	VM12 Reverse	CAACAGACGCAGCCCAACT		VM28 Reverse	GAGCACGATAATATTTG-GAG
8.	VM14 Forward	AATTCGTGGCATAGTCA-CAAGAGA	20.	VM30 Forward	CTCTTTCGCGTTCCA-CACTT
	VM14 Reverse	ATAAAGGAGGGCATAGGGAG-GTAT		VM30 Reverse	GCAATGGGTTGTGGTCT-GTG
9.	VM16 Forward	TCCTCGTCCATCTTCACCTCA	21.	VM34 Forward	AGCTCCCCTAACCTGAAT
	VM16 Reverse	CAAGCACCGCATTAAAGT-CAAG		VM34 Reverse	TAACCCAATAATAAGACA-CATA
10.	VM17 Forward	GGCCTATAAATTA-ACCCAGTCT	22.	VM36 Forward	ACTTTCTGTTTTACTCGA-CAACTC
	VM17 Reverse	TGTGTCTTTGAGTTTTT-GTTCTAC		VM36 Reverse	GTCGCTGGGGGTGGCT-TATT
11.	VM18 Forward	AGCCGTGCACGAAATGAT	23.	VM38 Forward	GATGGTTGTAATGGGAGA-GTC
	VM18 Reverse	TGGCCTCTACAACAACACTCT		VM38 Reverse	TCGTGGCATGCAGTGTCAG
12.	VM19 Forward	TATTCATGCGCCGTGACACTA	24.	VM39 Forward	GATGGTTGTAATGGGAGA-GTC
	VM19 Reverse	TCGTGGCACCCCCTATC		VM39 Reverse	AAAAGGATGAAATTAG-GAGAGCA
13.	VM20 Forward	GGGGACCAATCGTTTCGTTC	25.	VM40 Forward	TATTACGAGAGGCTATT-TATTGCA
				VM40 Reverse	CTCTAACACCTCAAGT-TAGTGATC

2.3. Data analysis

DNA bands generated from PCR amplification were subjected to binary system where 25 SSR markers were scored for presence (1) or absence (0) of band. To avoid poor reproducibility, the faint and diffused bands were excluded from scoring. Only distinct and unambiguous bands were utilized. The band sizes were estimated by comparing the amplified products to a 100 bp DNA ladder. Such data was used to calculate Jackard's similarity matrix using NTSYS-pc version 2.2 (Rohlf, 1988). Similarity matrix were organized for all pairs of accessions with the help of Jackard's similarity coefficient and dendrogram for genetic diversity was constructed using UPGMA (unweighted pair-group method with arithmetic mean analysis).

3. RESULTS AND DISCUSSION

3.1. SSR polymorphism

32 genotypes of cowpea were subjected to SSR assay to analyze molecular diversity by using 25 SSR markers. Out of 25 SSR markers, only eight markers showed polymorphic pattern (VM-8, VM-9, VM-10, VM-16, VM-18, VM-28, VM-36, VM-39) and 15 markers exhibited monomorphic banding pattern and remaining 2 markers showed no banding pattern. The observed % polymorphism was 32% for all the markers studied indicating the presence of less variable SSR loci for these markers in the germplasm studied. The level of polymorphism detected in our study was low to moderate which is in agreement with previous series reported by several cowpea researchers which may be due to the hindrance induced by a single domestication event in addition to its inherent mechanism of self-pollination. (Li et al., 2001, Badiane et al., 2004, Diouf and Hilu, 2005, Sarr et al., 2021).

Eight SSRs generated a total of 27 alleles. The number of alleles ranged between 2–5 locus^{-1} with an average of 3.37 alleles. A maximum number of alleles (5) were generated by VM-28 and a minimum of two alleles was produced by marker VM-10. Gel profiling of cowpea genotypes for

marker VM-28 was given in Figure 1. The Polymorphic Information Content (PIC) was determined for each marker to assess the informativeness of the marker to detect polymorphism. PIC value refers to the value of a marker's ability to detect polymorphism within a population, depending upon the number of alleles detected and their distribution frequency. Thus, it gives an assessment of the marker's discriminating power. (Nagy et al., 2012). In the present study, the PIC values (Figure 2) among eight polymorphic SSR markers ranged from a minimum of 0.702 (VM-10) to maximum of 0.945 (VM-28) with an average value of 0.86, indicating that these markers are highly informative in detection of polymorphism. However, there are various findings that had reported more number of alleles in cowpea collected from Senegal in which alleles ranged from 2 to 15 (Sarr et al., 2021). In contrast Devi and Jayamani (2019) reported 1 to 3 alleles in local cowpea

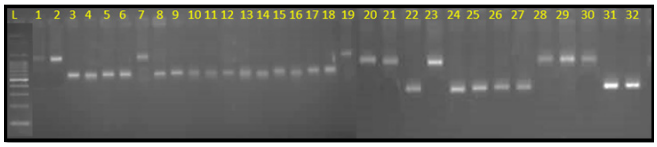

Figure 1: PCR amplification of 32 cowpea genotypes with SSR marker VM-28. (The lane numbers written on the gel corresponds to the list of cowpea genotypes as given in Table 1)

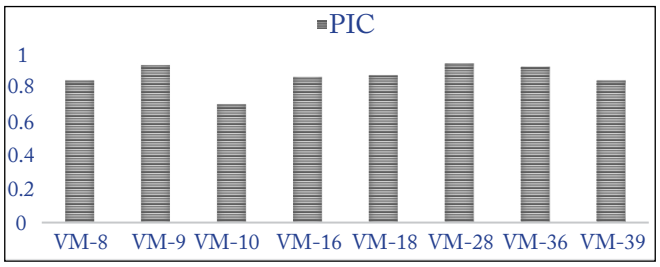

Figure 2: Graphical representation of PIC values of markers

accessions, while Sonker et al. (2019) reported 1 to 10 alleles in cowpea accessions obtained from Scientific and Applied Research Centre (SARC), Meerut (UP). These findings were in agreement with recent reports on the number of alleles detected using SSR makers in other legumes, such as, 4 to 12 in mungbean (Suman et al., 2019), 2 to 5 in yardlong bean (Saha et al., 2020), 1 to 7 in chickpea (Vashist et al., 2019) and 2 to 6 in pea (Ram et al., 2021).

3.2. Dendrogram analysis

A dendrogram (Figure 3) was constructed based on Jaccard's similarity coefficients using UPGMA (Unweighted Pair Group Method with Arithmetic mean) and SAHN (Sequential, Agglomerative, Hierarchical and Nested) clustering algorithm of NTSYS-pc version 2.1 software. 32 genotypes of cowpea were grouped into three major clusters (Table 3) i.e., cluster I, II and III at a cut-off Jaccard's similarity coefficient of 0.23. Cluster I was formed at a

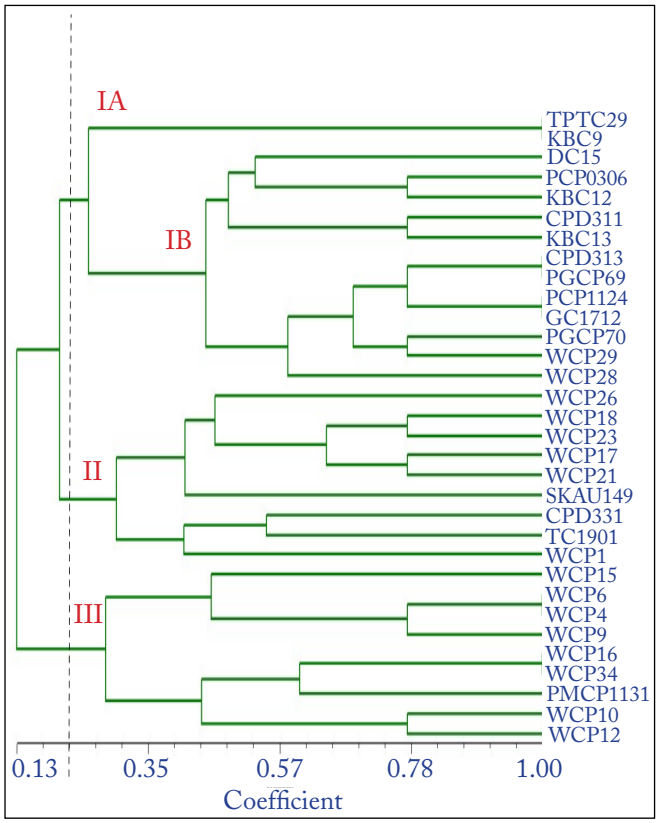

Figure 3: Dendrogram of 32 cowpea genotypes using SSR markers

Table 3: Grouping of 32 cowpea genotypes based on dendrogram analysis

Cluster No.	No. of genotypes	Genotypes
IA	2	TPTC-29, KBC-9
IB	12	DC-15, PCP-0306, KBC-12, CPD-311, KBC-13, CPD-313, PGCP-69, PCP-1124, GC-1712, PGCP-70, WCP-29, WCP-28
II	9	WCP-26, WCP-18, WCP-23, WCP-17, WCP-21, SKAU-WCP-149, CPD-331, TC-1901, WCP-1
III	9	WCP-15, WCP-6, WCP-4, WCP-9, WCP-16, WCP-34, PMCP-1131, WCP-10, WCP-12

similarity coefficient of 0.26 with 14 genotypes which was further grouped into two minor sub-clusters IA and IB at similarity coefficients 0.26 and 0.45 respectively. Cluster II appeared at similarity coefficient of 0.30 while cluster III was formed at 0.28 similarity coefficient. The genotypes in each cluster were clearly distinguished from one other. Cluster analysis revealed presence of low magnitude of diversity among the genotypes studied. The values for

similarity coefficient ranged from 0.13–1.00. The genotypes grouped close to 0.13 showed more dissimilarity whereas the genotypes grouped close to 1.00 showed high similarity. The average of similarity coefficient of all 32 genotypes was found to be 0.57. High similarity of 100% was observed between the genotypes WCP-4 and WCP-6, GC-1712 and PCP-1124, PGCP-69 and CPD-313, WCP-16 and WCP-34. The genotypes namely TPTC-29 and KBC-9 were found to be more diverse with 26% similarity. The maximum number of genotypes i.e., 12 were formed under cluster IB (DC-15, PCP-0306, KBC-12, CPD-311, KBC-13, CPD-313, PGCP-69, PCP-1124, GC-1712, PGCP-70, WCP-29, WCP-28), followed by cluster II (WCP-26, WCP-18, WCP-23, WCP-17, WCP-21, SKAU-WCP-149, CPD-331, TC-1901, WCP-1) and cluster III (WCP-26, WCP-18, WCP-23, WCP-17, WCP-21, SKAU-WCP-149, CPD-331, TC-1901, WCP-1) with 9 genotypes while a minimum of two genotypes accumulated under cluster IA (TPTC-29, KBC-9). The genetic background of 32 cowpea accessions was moderate according to the molecular dendogram and this information will reduce the overall time required in screening large populations of potential parents in identifying breeding stock. The similar results were obtained by Li et al. (2001), Devi and Jayamani (2019), though, contradictory results were observed in Malawian landraces by Nkongolo (2003). In general, the dendogram of accessions and level of polymorphism detected in this study supported the established view that genetic diversity in cowpea is mode rate (Zannouou et al., 2008 and Sarr et al., 2021).

4. CONCLUSION

The existence of moderate to low genetic similarity between genotypes indicated the presence of less variable SSR loci for these markers among the genotypes studied. Highest similarity was observed between WCP-4 and WCP-6, GC-1712 and PCP-1124, PGCP-69 and CPD-313. The genotypes namely TPTC-29 and KBC-9 appeared more divergent than remaining genotypes. The marker data generated in present study is of great significance and can be utilized in marker assisted breeding programmes.

5. REFERENCES

Adesoye, A.I., Folayan, D.V., Ladejobi, O.O., 2016. Genetic relationships among bacterial blight resistant cowpea (*Vigna unguiculata*) lines as revealed by simple sequence repeat markers. Nigerian Journal of Genetics 31, 90–101.

Anonymous, 2020. FAOSTAT Crop Statistics 2019. Food and Agriculture Organization of the United Nations, Rome, Italy. Available at http://www.fao.org/faostat/en/#data. Accessed on 7th September, 2020.

Asare, A.T., Gowda, B.S., Galyuon, I.K.A., Aboagye, L.L., 2010. Assessment of the genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp] germplasm from Ghana using simple sequence repeat markers. Plant Genetic Resources: Characterization and Utilization 8(2), 142–150.

Badiane, F.A., Diouf, M., Diouf, D., 2014. Cowpea. In: Singh, M., Bisht, I.S., Dutta, M. (Eds.). Broadening the Genetic Base of Grain Legumes. Springer, New Delhi, 95–114.

Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E., Haruna, M., Diallo, S., Umar, M.L., Olufajo, O., Fatokun, C., 2019. Cowpea (*Vigna unguiculata*): Genetics, genomics, and breeding. Plant Breeding 138(4), 415–424.

Boukar, O., Fatokun, C.A., Huynh, B.L., Roberts, P.A., Close, T.J., 2016. Genomic tools in cowpea breeding programs: status and perspectives. Frontiers in Plant Science 7, 757.

Darlington, C.D., Wylie, A.P., 1955. Chromosome Atlas of Flowering Plants (2nd Edn.). George Allen and Unwin Ltd, London, 56–62.

Das, S., Karak, C., Roy, S., 2020. Genetic variability, correlation and path analysis studies in cowpea [*Vigna unguiculata* (L.) Walp.]. International Journal of Economic Plants 7(1), 123–128.

Desalegne, B.A., Mohammed, S., Dagne, K., Timko, M.P., 2016. Assessment of genetic diversity in Ethiopian cowpea [*Vigna unguiculata* (L.) Walp.] germplasm using simple sequence repeat markers. Plant Molecular Biology Reporter 34(5), 978–992.

Devi, S.M., Jayamani, P., 2020. Molecular genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] genotypes. Journal of Food Legumes 33(1), 6–9.

Diouf, D., Hilu, K.W., 2005. Microsatellites and RAPD markers to study genetic relationship among cowpea breeding lines and local varieties in Senegal. Genetic resources and crop evolution 52(8), 1057–1067.

Dossa, K., Wei, X., Zhang, Y., Fonceka, D., Yang, W., Diouf, D., Liao, B., Cisse, N., Zhang, X., 2016. Analysis of genetic diversity and population structure of sesame accessions from Africa and Asia as major centers of its cultivation. Genes 7(4), 14.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochemical Bulletin 19(1), 11–15.

Gupta, S.K., Gopalakrishna, T., 2010. Development of unigene-derived SSR markers in cowpea (*Vigna unguiculata*) and their transferability to other Vigna species. Genome 53(7), 508–523.

Hall, A.E., Cisse, N., Thiaw, S., Elawad, H.O., Ehlers,

J.D., Ismail, A.M., Fery, R.L., Roberts, P.A., Kitch, L.W., Murdock, L.L., Boukar, O., 2003. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crops Research 82(2–3), 103–134.

Kanavi, P.M.S., Rangaiah, S., Krishnaprasad, T.B., 2019. Molecular characterisation and genetic diversity analysis through SSR markers in germplasm lines of green gram [*Vigna radiata* (L.) Wilczek]. International Journal of Bio-resource and Stress Management 10(1), 298–305.

Khandait, R., Jain, P.K., Prajapati, S., Dangi, A.S., 2016. Correlation and path coefficient analysis for yield and its attributing traits in diverse genotypes of cowpea [*Vigna unguiculata* (L.) Walp.]. International Journal of Bio-resource and Stress Management 7(1), 8–17.

Kumar, S., Phogat, D., Bhusal, N., 2015. Characterization of elite forage cowpea genotypes for various DUS traits. Forage Research 40(4), 232–236.

Lal, H., Rai, N., Rai, K.K., Tiwari, S.K., 2016. Microsatellites markers to study genetic relationships among cowpea (*Vigna unguiculata*) genotypes. Indian Journal of Agricultural Sciences 86(5), 654–660.

Li, C.D., Fatokun, C.A., Ubi, B., Singh, B.B., Scoles, G.J., 2001. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Science 41(1), 189–197.

Meena, H.K., Krishna, K.R., Singh, B., 2015. Character associations between seed yield and its components traits in cowpea [*Vigna unguiculata* (L.) Walp]. Indian Journal of Agriculture Research 49(6), 567–570.

Nagy, S., Poczai, P., Cernak, I., Gorji, A.M., Hegedus, G., Taller, J., 2012. PIC calc: an online program to calculate polymorphic information content for molecular genetic studies. Biochemical Genetics 50(9–10), 670–672.

Nkongolo, K.K., 2003. Genetic characterization of Malawian cowpea [*Vigna unguiculata* (L.) Walp] landraces diversity and gene flow among accessions. Euphytica 129, 219–228.

Pradeepkumar, T., Mathew, D., Roch, C.V., Veni, K., Midhila, K.R., 2017. Genetic interrelationship among cowpea varieties elucidated through morphometric, RAPD and SSR analyses. Legume Research 40(3), 409–415.

Ram, H., 2014. Vegetable Breeding Principles and Practices (3rd Edn.). Kalyani publishers, New Delhi, 460–473.

Ram, H., Hedau, N.K., Chaudhari, G.V., Choudhary, M., Kant, L., 2021. Genetic diversity assessment in Pea (*Pisum sativum L.*) using Microsatellite Markers. International Journal of Bio-resource and Stress Management 12(4), 402–408.

Rohlf, F.J., 1988. NTSYS-pc - Numerical Taxonomy and Multivariate Analysis System (Version 2.2). Applied Biostatistics Inc., New York, 16–18.

Saha, N.R., Farabi, S., Azad, A., Hasanuzzaman, M., Haque, M.S., 2020. Microsatellite marker based genetic diversity analysis among the germplasm of an orphan legume yardlong bean (*Vigna unguiculata L. Walp.*) in Bangladesh. Plant cell biotechnology and molecular biology 21(47-48), 43–52.

Sarr, A., Bodian, A., Gbedevi, K.M., Ndir, K.N., Ajewole, O.O., Gueye, B., Foncéka, D., Diop, E.A., Diop, B.M., Cissé, N.,Diouf, D., 2021. Genetic diversity and population structure analyses of wild relatives and cultivated cowpea [*Vigna unguiculata* (L.) Walp.] from Senegal using simple sequence repeat markers. Plant Molecular Biology Reporter 39(1), 112–124.

Sonker, S., Sharma, T., Tandan, N., 2019. SSR based molecular characterization and diversity analysis of cowpea. Plant Archives 19(2), 995–1001.

Srivastava, S., Chopra, A.K., Sharma, P., Kumar, V., 2016. Amendment of sugar mill wastewater irrigation on soil bio hydrological properties and yield of cowpea [*Vigna unguiculata* (L.) Walp] in two seasons. Communication in Soil Science and Plant Analysis 48(5), 511–523.

Suman, S., Rani, B., Sharma, V.K., Kumar, H., Shahi, V.K., 2019. SSR marker based profiling and diversity analysis of mungbean [*Vigna radiata* (L.) Wilczek] genotypes. Legume Research 42(5), 585–59.

Tautz, D., 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17(16), 6463–6471.

Uma, M.S., Hittalamani, S., Keshava Murthy, B.C., Viswanatha, K.P., 2009. Microsatellite DNA marker aided diversity analysis in cowpea [*Vigna unguiculata* (L.) Walp]. Indian Journal of Genetics and Plant Breeding 69(1), 35–43.

Vashist, U., Boora, K.S., Kumar, M., 2019. Evaluation of genetic diversity among chickpea (*Cicer arietinum*) genotypes using PCR based simple sequence repeats markers. The Pharma Innovation Journal 8(2), 182–188.

Venkatesan, M., Prakash, M., Ganesan, J., 2003. Correlation and path analysis in Cowpea [*Vigna unguiculata* (L.) Walp.]. Legume Research 26(2), 105–108.

Zannouou, A., Kossou, D.K., Ahanchédé, A., Zoundjihékpon, J., Agbicodo, E., Struik, P.C., Sanni, A., 2008. Genetic variability of cultivated cowpea in Benin assessed by random amplified polymorphic DNA. African Journal of Biotechnology 7(24), 4407–4414.