

Genetic variability studies in seven accessions of cowpea (*Vigna unguiculata* L. Moench.) using nine quantitative traits and chlorophyll content of leaves

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Abstract

Seven accessions of *Vigna unguiculata* were evaluated for variation in phenotypic traits and chlorophyll content of the cowpea leaves. Each accession was planted in a plastic container in triplicates in a Completely Randomised Block Design and evaluated based on nine quantitative traits. The morphological characters such as plant height, number of leaves per plant, leaf area, peduncle length, number of pods per plant, terminal leaf length, terminal leaf width, number of main branches and number of nodes were determined at maturity. The leaf chlorophyll content was determined using spectrophotometer set at a wavelength of 634nm, 648nm and 470nm. Accession UAMO910556 has the highest plant height of 91.58cm, followed by accession IT97K-499-35 with plant height of 50.33cm, while accession NGB0050 has the lowest plant height of 22.43cm. Accession T107K-297-13 has the highest peduncle length, number of main branches, number of nodes on main stem, number of leaves and highest number of pod which are significantly different from other accessions for the trait studied. Accession T107K-297-13 also has the highest content of chlorophylls a and b and highest total chlorophyll content. This shows that accession T107K-297-13 is the most diverse genotype with high quality of trait and so it could be selected for further breeding work.

Introduction

Vigna unguiculata also known as cowpea, has different names such as horse gram in English.¹ It belongs to the family of fabaceae, genus *Vigna* and species *unguiculata*. It is an annual herb, in which savannah zone of West Africa has the largest population of about 85.00% of cowpea. Cowpea is an important legume in the tropics and subtropics including Africa, Asia, Central South America, parts of Southern Europe and United States.^{2,3} The seed protein contents is between 23 and 32% of seed weight rich in lysine and tryptophan, and a substantial amount of mineral and vitamins.⁴ Cowpea fixes 80% of its nitrogen requirement for growth from the atmosphere,⁵ thereby reducing nitrogen fertilizer demand and costs of crop production. Many parts of cowpea are used in human and animal feeding.⁶

Cowpea seeds have been found to prevent birth defects in the brain and spineduring pregnancy due to the presence of the highest contents of folic acid and B vitamin.⁷ It is also known to have a low amount of fat and high level of fiber which can prevent heart diseases by reducing the low-density lipoprotein.⁸

The genetic advancement of the crop is limited due to the knowledge of the genetic diversity that is inadequate of the existing germplasm. Analysis of the extent of genetic diversity in each population of genotype and genetic similarity between populations of each taxon, is important to relate the genetic diversity to environmental factors, to identify populations with unique genetic constitution and to suggest the best genotype for breeding work.⁹ According to Udensi and Edu,¹⁰ the future of plant breeding depends on the utilization and conservation of plant breeding resources for the improvement of mankind's conditions. Ghalmi *et al.*¹¹ observed that *ex-situ* conservation of potentially useful populations requires a clear understanding of the genetic variation and distinctiveness of the populations. This characterization of germplasm of crops is feasible by studying the genetic diversity between and within the collection. According to Trethowan and Mujeeb-Kazi,¹² genetic variability study was necessary for the rapid genetic improvement of crop species. Li *et al.*¹³ opined that the utilization of introduced germplasm in breeding programmes is much more important than the direct release of introduced material for cultivation, which means that the future of plant breeding depends on the proper conservation of plant genetic resources.

Before undertaken crop improvement process in any species, a thorough knowledge of the degree of genetic variability existing in the crop for various characters is significant. The information on the diversity of nature and magnitude in the plant material and the character of various associations is necessary for yield improvement. This characterization of germplasm of crops is feasible by studying the genetic diversity between and within the collection. Genetic diversity is the sum of genetic characteristics

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within any species or genus¹⁴ while genetic variability is the variation within the genetic characteristics.¹⁵ Thus analysis of genetic relationships in crop species is an important component of crop improvement.¹⁰ Obviously, various researchers have adopted different approaches in order to estimate genetic diversity in a given germplasm, which includes principal component analysis, divergence analysis, coefficient of parentage, utilization of morphological, agronomical as well as biochemical data.¹⁶⁻¹⁹

In the current study, the morphological traits which are based on the physical attributes of the plant have been used to form the phylogenetic relationships within and between the species and variability study of the compared cowpea character. Morphological traits could be influenced by *nurture* (environmental factor) and may therefore result in changing relationship patterns.²⁰ Influence of the environmental factor on the performance of crops can be reduced or controlled by conducting research in the green house. Protected crops are less apt to be damaged by wind, rain, and hail so the percentage of marketable products is higher. Yield is often higher as well, if optimum growing conditions for each crop is provided. Greenhouses protect crops from many diseases, particularly those that are soil borne and splash onto plants in the rain. And greenhouse crops may be protected from common field pests. The main goal of this paper is to assess the genetic variation among seven accessions of *V. unguiculata* using some quantitative traits, to identify accession with superior characteristics and to determine variation in the chlorophyll content of the leaves of *V. unguiculata*.

Materials and Methods

The *V. unguiculata* seeds used for this research were obtained from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria. The experiment was carried out in the screen house of the Department of Plant Science and Biotechnology, Federal University Oye-Ekiti, Ekiti State, Nigeria. The seven accessions of *V. unguiculata* seeds used in this study were those available at the time of collection and are shown in Table 1.

Soil preparation

The soil used for this experiment was collected from the Federal University Oye-Ekiti, Ekiti State. The soil was mixed and sieved to remove any pebble and stone from it. A total number of twenty-one plastic containers perforated underneath were filled with equal amount of soil.

Table 1. *Vigna unguiculata* accessions.

S/N	Accession number
1	NGB/06/0050
2	NG/SA/07/063
3	IT07K-318-33
4	T107K-297-13
5	UAM091055-6
6	IT97K-499-35
7	IT89KD-288

Experimental design

Twenty-one plastic containers were perforated underneath and labelled according to the accessions used. 3kg of soil sample was weighed and put inside the plastic buckets. Two seeds were planted per bucket for each accession and this was done in triplicates. The soil in the plastic buckets was regularly watered to ensure that the soil was moist enough for plant germination.

Morphological analysis

The experiment was carried out in the screen house following Completely Randomised Block Design in August 2018. The following quantitative traits were determined for each cowpea accession at the tenth week of planting. Terminal Leaf Length (cm) was measured with the aid ruler, Terminal Leaf Width (cm) was measured with ruler, Number of Main Branches was done by counting, Number of Nodes on Main Stem was done by counting, Peduncle Length (cm) measured with ruler, Plant Height (cm) was determined with the aid of ruler or measuring tape, Number of Pods and Number of Leaves were determined by counting.

Chlorophyll content

After 10 weeks of planting, fresh leaves were collected. 0.25g of the leaves sample was macerated *i.e.* crushing in 5mL of 80% acetone using mortar and pestle. The extract was placed in water bath at 70 degrees Celsius for few minutes in order to decolorize. The supernatant was read at absorbance 663nm, 645nm and 652nm using spectrophotometer and expressed in mg g⁻¹ fresh weight basis.

$$\text{Chla } (\mu\text{g/L}) = 0.0127 \text{ A}_{663} - 0.00269 \text{ A}_{645}$$

$$\text{Chlb } (\mu\text{g/L}) = 0.0029 \text{ A}_{663} - 0.00468 \text{ A}_{645}$$

Total Chl ($\mu\text{g/L}$) = 0.0202 A₆₆₃+0.00802 A₆₄₅, according to Arnon's (1949) equation.

Statistical analysis

The data obtained from the study were analysed using Statistical Package for the Social Sciences (SPSS) (Version 22). The significant difference was calculated at 1% confidence limit using Analysis of Variance (ANOVA), a statistical method for testing differences between two or more means. The means were separated using Duncan Multiple Range Technique (DMRT).

Results

Phenotypic variability of nine quantitative and qualitative traits among the seven accessions of *V. unguiculata* at maturity is shown in Table 2 and Figure 1, respectively. The accessions exhibited significant variation with respect to eight quantitative characters. Taking plant height for instance, UAM0910556 recorded the highest length of 91.58cm while NGB0050 recorded the least of 22.43cm. The accessions IT97K-499-35, T107K-297-13, NGS63, IT07K-318-33 and IT89KD288 were significantly different at P<0.05.

The result of peduncle length as shown in Table 2 shows that T107K-297-13 has the highest length of 18.29cm which is not significantly different from UAM0910556 of length 17.15cm, while IT97K-499-35 has the lowest length of 4.00cm.

Accession UAM0910556 has the highest terminal leaf length of 10.72cm, followed by T107K-297-13 of 7.24cm while IT89KD288 has the lowest length of 1.93cm, which are significantly different from NGB0050, NGS63 and IT07K-318-33.

UAM0910556 has the highest terminal leaf width of 4.85cm

which is significantly different from TI07K-297-13 of width 3.46, while IT89KD-288 has the lowest width of 0.72cm and TI07-499-35, NGS63, NGB0050 were not significantly different from IT07-318-33 (Table 2).

Based on the result reported in Table 2, Accession IT07-318-33 has the highest number of main branches of 24.67 which is not significantly different from NGS63 of 24.33 while TI07-499-35 has the lowest number of main branches of 4.33. Accession NGB0050 of 18.33 was significantly different from UAM0910556 of 17.67, IT89KD-288 of 15.33 and IT07-318-33.

In Table 2 below, the result of the leaf area shows that UAM0910556 has the highest leaf area of 42.74, followed by TI07K-297-13 of 16.64 while IT89D-288 has the lowest leaf area of 2.74 and IT07K-318-33 was significantly different from NGB0050 and NGS63.

IT07-318-33 has the highest number of main branches of 24.67 which is not significantly different from NGS63 of 24.33 while TI07-499-35 has the lowest number of main branches of 4.33. Accession NGB0050 of 18.33 was significantly different at $P < 0.05$ for number of nodes on main stem (Table 2).

The result of number of leaf as shown in Table 2 shows that TI07K-297-13 has the highest number of leaf of 57.00 which is significantly different from NGS63 of 54.66 and TI07-499-35 has the lowest number of leaf of 17.00.

Accession TI07-297-13 has the highest number of pods of 11.00, followed by UAM0910556 of 8.00 which is not significantly different from NGS63 of 7.67. Accession IT89KD-288 of 3.33 is significantly different from IT07-318-33 and NGB0050 and TI07-499-35 has the lowest number of pod of 1.66 (Table 2).

Accession IT07K-297-13 has the highest chlorophyll content of 8.83 while IT89D-288 has the lowest chlorophyll content of 3.93 and IT07K-99-35, NGB0050 and UAM0910556 were not significantly different from each other while NGS63 of 7.09 is significantly different from TI07K-318-33 of 6.87 (Figures 2 and 3).

Discussion

The analysis of variance revealed significant differences for quantitative traits among the seven accessions of *V. unguiculata* (Table 2). Significant differences observed for some of the characters showed presence of great amount of variability in all the characters studied.

The significant differences observed among the quantitative traits may be due to the differences in the genotypes and as a result of the differences in the genetic potential of different cowpea geno-

types. This in agreement with the findings of Smith and Smith²¹ and Aryio,²² who stated the role differences in genetic makeup of different varieties in yield determination of okra. The characters showing high range of variation have more possibility for improvement by using them to develop better varieties of cowpea. All the eight characters under study exhibited variability as shown in Table 2. Characters such as plant height, peduncle length, number of pods which have wide range of variations in their mean values indicates high range of genetic variability of these characters and thus providing greater scope for selecting desirable genotypes. This is in accordance with the findings of Singh *et al.*²³ and Reddy *et al.*²⁴ who worked on okra.

Also, plant height in this study varied significantly among the cowpea accessions studied (Table 2). UAM0910556 recorded the highest length of 91.58cm, followed by IT97K-499-35, NGS63, TI07K-297-13, IT07K-318-33, IT89KD288 and NGB0050 recording mean plant height of 50.33cm, 37.05cm, 35.19cm, 29.23cm, 25.73cm and 22.43cm respectively. Final plant height is of particular interest for breeding programme because tall and thin stems increase rate of lodging near harvesting time²⁵ and this can lead to loss of dry matter and subsequent decrease in fruit yield.²⁶ This is also in accordance with the findings of several authors²⁵⁻²⁸ who worked on rice and okra respectively. According to Zhang *et al.*,²⁹ plant height is an important agronomic trait of rice that directly affects the yield of this crop. The dwarf phenotype is beneficial for rice lodging, but if the plants are too short, it will lead to insufficient growth and ultimately affect the yield potential of rice.

The accessions used in this study showed highly significant differences in total chlorophyll content, accession TI07K-297-13



Figure 1. Vegetative stages of cowpea plant in the screen house.

Table 2. Quantitative traits of seven accessions of *Vigna unguiculata*: mean values.

Accession	PH(cm)	PDL(cm)	TLL(cm)	TLW(cm)	NMB	LA	NMS	NL	NP
NGB0050	22.43 ^a	5.98 ^a	4.51 ^a	1.92	18.33 ^a	8.86	18.33 ^a	50.33	4.67 ^a
NGS63	37.05 ^b	8.20 ^b	4.01 ^b	1.84	24.33 ^b	7.52	24.33 ^b	54.66	7.67 ^b
IT07K-318-33	29.23 ^c	7.83 ^c	4.27 ^c	2.14	13.00 ^c	11.61	13.00 ^c	40.33	5.00 ^c
TI07K-297-13	35.19 ^d	18.29 ^d	7.24 ^d	3.46	24.67 ^b	16.64	24.67 ^a	57.00	11.00 ^d
UAM0910556	91.58 ^e	17.15 ^d	10.72 ^e	4.85 ^a	17.67 ^d	42.74	17.67 ^d	45.67	8.00 ^b
IT97K-499-35	50.33 ^f	4.00 ^f	2.17	1.03	4.33 ^e	4.43	4.33 ^e	17.00	1.67 ^e
IT89KD288	25.73 ^g	4.15 ^g	1.93	0.72	15.33 ^f	2.74	15.33 ^f	28.33	3.33 ^f

PH, plant height; PDL, peduncle length; TLL, terminal leaf length; TLW, terminal leaflet width; NMB, number of main branches; LA, leaf area, NMS, number of nodes on main stem; NL, number of leaves; NP, number of pod. The mean value with different superscript was significantly different at $P < 0.05$ while the probability mean value without superscript was not significant.

had higher total chlorophyll content than all other accession with 22.20 $\mu\text{g/L}$. Total chlorophyll content increase for all the accession and this increase can be associated with plant growth stage. Leaf chlorophyll is a key indicator of leaf greenness, and it is often used to investigate leaf nutrient deficiencies and changes in chlorophyll.³⁰ Canopy chlorophyll content is also an indicator of seasonal carbon uptake in forest ecosystems.³¹ Significant correlations between chlorophyll content and leaf nitrogen have been reported in many agricultural crops.³² Miri³³ reported that chlorophyll content index was significantly and positively correlated with grain yield and a harvest index in Iran.

Conclusions

The genetic variability study of cowpea using some morphological traits shows accession T107K-297-13 as the most diverse genotype with high quality trait because plant height is an important trait that affects plant yield. Accession T107K-297-13 also had high total chlorophyll content which indicates the photosynthetic capacity of the plant per unit area of leaf and this determines the rate of photosynthesis of the plant and consequently high productivity of this plant, This makes this accession T107K-297-13 to be

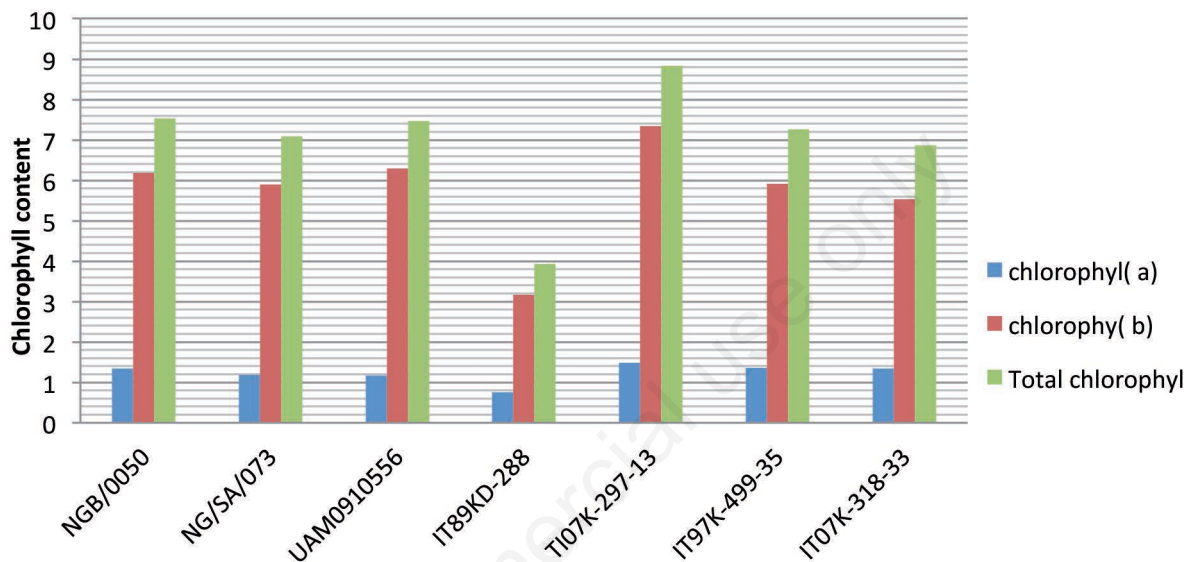


Figure 2. Chart representing the chlorophyll content among the accessions.

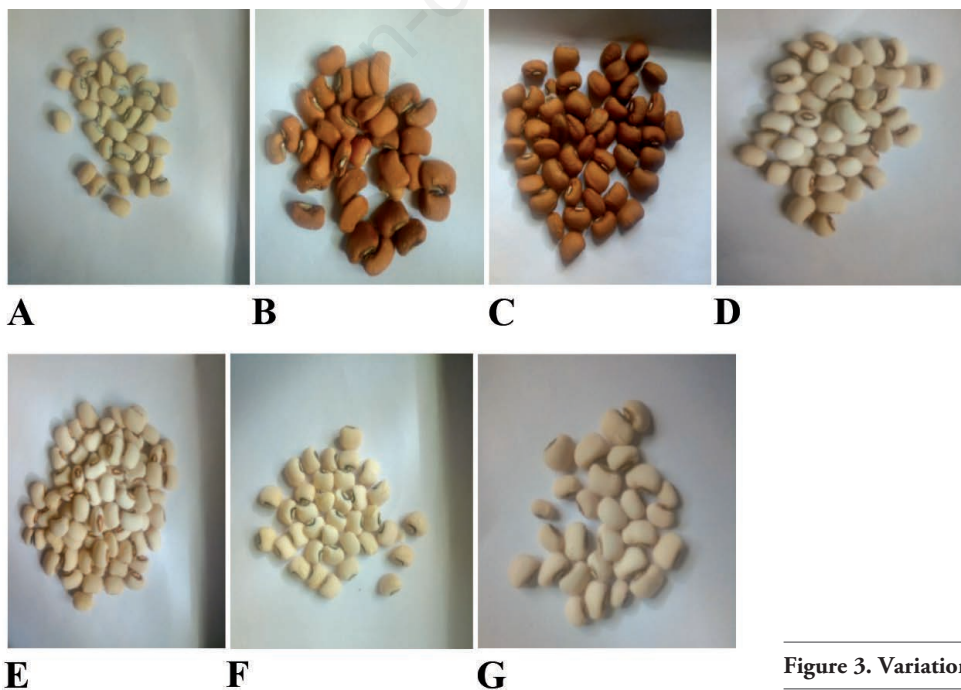


Figure 3. Variation in the colour of cowpea seeds.

diverse from others in terms of high metabolic rate, glucose production, yield and fruit quality. Our findings may be useful in further studies to investigate the breeding potential of accession T107K-297-13 in terms of fruit quality and yield of cowpea.

References

- Singh A, Dwivedi B, Raghaw P, et al. Review on standardization and phytochemical of *Vigna unguiculata*. Int J Pharm Res Scholar 2015;4:506-16.
- Singh BB, Chamblis OL, Sharma B. Recent advances in cowpea breeding. In: Singh BB, Mohan Raj DR, Dashiell KE, Jackai LEN, eds. Advances in cowpea research. IITA and Japan International Research Centre for Agricultural Sciences (JIRCAS) copublication. Ibadan, Nigeria: IITA; 1997. pp. 30-49.
- Kaga A, Yoon MS, Tomooka N, Vaughan DA, 2000. Collection of *Vigna* spp. and other legumes from the islands of southern Oki-nawa prefecture, Japan. Report to IPGRI and East Asia Plant Genetic Resources Coordinators. Japan: National Institute of Agrobiological Resources. pp. 2-25.
- Hall AE, Cisse N, Thiaw S, et al. Development of cowpea cultivars and germplasm by the Bean/Cowpea CRSP. Field Crops Res 2003;82:103-34.
- Asiwe JAN, Belane A, Dakora FD. Evaluation of cow-pea breeding lines for nitrogen fixation at ARC-Grain Crops Institute. Potchefstroom, South Africa. Paper presented at the 16th International Congress on Biological Nitrogen Fixation, Montana, USA, 14-19 June, 2009.
- Diouf D. Recent advances in cowpea (*Vigna unguiculata* (L.) Walp) omicsresearchfor genetic improvement. Afr J Biotechnol 2011;10:2803-10.
- Timko MP, Singh BB. Cowpea, a multifunctional legume In: Moore PH, Ming R, eds. Genomics of tropical crop plants. New York, NY: Springer Science and Business Media; 2008. pp. 227-258.
- Phillips RD, Mcwatters KH, Chinnan MS, et al. Utilization of cowpeas for human food. Field Crops Res 2003;82:193-213.
- Geleta M, Bryngelsson T, Bekele E. Genetic diversity of *Guizotia abyssinica* (L.f.) Cass. (Asteraceae) from Ethiopia using amplified polymorphic DNA (RAPD). Genet Resour Crop Evol 2007;54:601-14.
- Udansi OU, Edu NE. Evaluation and identification of genetic variation pattern in cowpea [*Vigna unguiculata* (L.) Walp] accessions using multivariate analyses. J Basic Appl Sci 2015;11:149-58.
- Ghalmi N, Malice M, Jacquemin JM, et al. Morphological and molecular diversity within Algerian cowpea (*Vigna unguiculata* (L.)Walp.) landraces. Genet Resour Crop Evol 2010;57:371-86.
- Trethowan RM, Mujeeb-Kazi A. Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. Crop Sci 2008;48:1255-65.
- Li CD, Fatokun CA, Ubi B, et al. Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers. Crop Sci 2001;41:189-97.
- Rao VR, Hodgkin T. Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell Tissue Org Cult 2002;68:1-19.
- Agbolade JO, Popoola JO, Kioko JI, et al. Comparative variability and heritability of traits in vegetative and floral characters among accessions of two minor legumes. Indian J Agric Res 2019;53:178-83.
- Matus IA, Hayes PM. Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. Genome 2002;45:1095-106.
- Mohammadi SA, Prasanna BM. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci 2003;43:1235-48.
- Jaradat AA, Shahid M, Al-Maskri A. Genetic diversity in the Batini barley landrace from Oman: II. Response to salinity stress. Crop Sci 2004;44:997-1007.
- Ahmad Z, Ajmal SU, Munir M, et al. Genetic diversity for morpho-genetic traits in barley germplasm. Pak J Bot 2008;40:1217-24.
- Smith JSC, Smith OS. The description and assessment of distances between inbred lines of maize. II: The utility of morphological, biochemical and genetic descriptors and scheme for the testing of distinctiveness between inbred lines. Maydica 1989;34:151-61.
- Ariyo OJ. Genetic diversity in West African Okra (*Abelmoschus caillei* (A.Chev.) Stevels) Multivariate analysis of morphological and agronomic characteristics. Gen Resour Crop Evol 1993;40:125-32.
- Adeniji OT. Inheritance studies in West African okra (*Abelmoschus caillei*). Agriculture Thesis, University of Agriculture, Abeokuta, Nigeria; 2003.
- Singh B, Pal AK, Singh S. Genetic variability and correlation analysis in okra [*Abelmoschus esculentus* (L.)Moench.]. J Horticult 2006;63.
- Reddy MT, Haribabu K, Ganesh M, et al. Genetic variability analysis for the selection of elite genotypes based on pod yield and quality from the germplasm of okra (*Abelmoschus esculentus* L. Moench). J Agr Technol 2012;8:639-55.
- Doku AH. Characterisation of 18 African rice (*Oryzagalaberrima*) in Ghana. Thesis diss., Department of Nuclear Agriculture and Radiation Processing, University of Ghana; 2011. pp. 87-102.
- Esthiet JA, Brisibe AE. Morphological characterization and yield traits analysis in some selected varieties of okra (*Abelmoschus Esculentus* L. Moench). Adv Crop Sci Technol 2015;3:1-5.
- Akinyele BO, Oseikita OS. Correlation and path coefficient analyses of seed yield attributes in okra (*Abelmoschus esculentus* (L.) Moench). Afr J Biotechnol 2006;14:1330-6.
- Myanmar A. Evaluation of okra germplasm. ARC-AVRDC Training Report; 1995. pp. 55-76.
- Zhang Y, Chunsheng Y, Jianzhong L, et al. OsMPH1 regulates plant height and improves grain yield in rice. PLoS One 2017;12:e0180825.
- Ali MM, Al-Ani A, Eamus D, Tan DK. Leaf nitrogen determination using non-destructive techniques - a review. J Plant Nutr 2017;40:928-53.
- Croft H, Chen JM, Froelich NJ, et al. Seasonal controls of canopy chlorophyll content on forest carbon uptake: Implications for GPP modelling. J Geophys Res Biogeosci 2015;120:1576-86.
- Wang Y, Wand D, Shi P, Omasa K. Estimating rice chlorophyll content and leaf nitrogen concentration with a digital still colour camera under natural light. Plant Method 2014;10:36.
- Miri HR. Grain yield and morpho-physiological changes from 60 years of genetic improvement of wheat in Iran. Exp Agriculture 2009;45:149-63.