



International Journal of
**Plant Breeding
and Genetics**

ISSN 1819-3595



Academic
Journals Inc.

www.academicjournals.com

Identification of SNP Markers Associated with Seed Size in Cowpea [*Vigna unguiculata* (L) Walp]

^{1,2}K.F. Egbadzor, ¹M. Yeboah, ¹E.Y. Danquah, ³K. Ofori and ⁴S.K. Offei

¹West Africa Centre for Crop Improvement, University of Ghana, Ghana

²CSIR-Plant Genetic Resources Research Institute, Bunso, Ghana, Ghana

³School of Research and Graduate Studies, University of Ghana, Ghana

⁴College of Agriculture and Consumer Sciences, University of Ghana, Ghana

Corresponding Author: K.F. Egbadzor, West Africa Centre for Crop Improvement, University of Ghana, Ghana

ABSTRACT

Cowpea seed size is an important consumer trait in West Africa where most of the world's production and consumption take place. Association was established between eighteen SNP markers and cowpea seed mass and thickness at $-\log_{10} p \geq 5.00$. Seventy-eight genotypes comprising of gene-bank materials and improved varieties from Ghana and 4 improved genotypes from the University of California Riverside and IITA were used for the study. Genotypes were grown at the University of Ghana and their seeds phenotype after harvest. Leaf tissues were sampled to the KBioscience in the United Kingdom and genotyped with SNP markers. Genome wide Association between the marker scores and phenotypes were analyzed using single trait single environment association mapping algorithm in GenStat. Eighteen of the SNPs were highly associated with seed size. These markers could be exploited for marker assisted breeding of larger seeded cowpea.

Key words: Cowpea, SNP, association mapping, seed size

INTRODUCTION

It is well established that the use of molecular markers together with phenotype is more reliable than phenotype alone in crop improvement. However, lack of access of molecular markers contributes to over reliance on phenotypes despite their limitations especially in the developing world. Among molecular markers, SNP genotyping is a promising platform for providing plant breeders with simplest, useful and most cost-effective genotyping services for marker-assisted selection (Lucas *et al.*, 2012). "An SNP is a single base pair site in the genome that is different from one individual to another" (Acquaah, 2007). These SNPs are suspected to be more abundant in plants than even those in the human genome (Gupta *et al.*, 2001; Zhu *et al.*, 2008).

Cowpea is very important food crop in West Africa where most of the world's production, trade as well as consumption take place. In recent past, cowpea has attracted considerable amount of research attention. Genetic linkage map was developed by Muchero *et al.* (2009). Varshney *et al.* (2007) assessed different types of molecular markers in diversity studies and conservation of cowpea genetic resources. A number of genes have been mapped on cowpea indicating the advance in cowpea research beyond traditional phenotyping. However, the use of SNP in marker trait association in cowpea has not been either reported or well known.

Efforts are being made to address problems of biotic stress which is of very importance in cowpea production. There are pests that feed on practically every part of the cowpea plant resulting in substantial economic loss if left uncontrolled (Jackai and Daoust, 1986; Makoi *et al.*, 2010). Lucas *et al.* (2012) reported markers linked with Thrips resistance in the crop. Different strategies are being employed to reduce insect pests' damage such as seed flavonoids (Lattanzio *et al.*, 2005; Makoi *et al.*, 2010); improved cropping system using integrated management (Fatokun *et al.*, 2000); genetic transformation (Fatokun *et al.*, 2000) among others with the aim of host plant resistance improvement.

One of the important traits desired in cowpea is large seed size in West Africa (Drabo *et al.*, 1984; Langyintuo *et al.*, 2003; Tchiagam *et al.*, 2011; Egbadzor *et al.*, 2013). However, much breeding objectives have not been directly focused on seed size compare with such traits as biotic and abiotic stress tolerance (Hall *et al.*, 1997; Orawu *et al.*, 2013). This is not to say that seed size has never been studied. Conflicting gene actions have been reported to control seed size inheritance in cowpea through classical studies (Drabo *et al.*, 1984). This is indication of complexity of the trait. Association mapping or linkage disequilibrium which is an alternative to traditional QTL mapping is a powerful method in complex traits studies (Abdurakhmonov and Abdulkarimov, 2008) and therefore can be used to better understand inheritance of seed size in cowpea. Association mapping has advantage over QTL mapping in terms of time, allele number and quality of resolution (Zhu *et al.*, 2008).

SNPs present in the coding sequences, may determine the mutant phenotype and will show 100% association with the trait and could therefore, be useful, for MAS and gene isolation (Gupta *et al.*, 2001). This research, therefore, aimed at mapping seed size in the cowpea genome using association population of mainly Ghanaian genotypes with SNP markers. Identification of significant association between SNP markers and seed size could help in marker assisted selection of the trait.

MATERIALS AND METHODS

A total of 78 cowpea genotypes were used in the association mapping. Sixty-nine of the genotypes were random samples of gene-bank materials from the Plant Genetic Resources Research Institute of the Council for Scientific and Industrial Research (CSIR-PGRRI), Bunso, collected from across Ghana. There were 5 improved varieties from Ghana and 4 from outside Ghana. Four of the five improved varieties were Tona, Nhyira, Asontem and Zaayura, all in cultivation in Ghana. The fifth variety, labeled "Market" was taken from the market. The four genotypes obtained from outside Ghana were UCR779, CB27, IT82E-18 and IT97K-556-6.

Genotypes were grown in single rows without replication at WACCI farms, University of Ghana in April 2011 under rainfall condition. Dry pods from five plants out of ten per row were bulk harvested and their seeds removed for data collection. Leaf tissues of one week old plants from each genotype were sampled to the KBioscience Laboratory in the United Kingdom for genotyping with SNP markers. Genotype and phenotype data were used for association mapping.

Data collection and analysis: Seeds were dried to approximately 12% moisture content in the sun after harvest and measurement taken on their sizes (mass, length, width and thickness). Seed length, width and thickness were measured with electronic digital calipers and mass of 100 seeds with scale. The facet of the seed representing length, width and thickness is clarified in Fig 1.

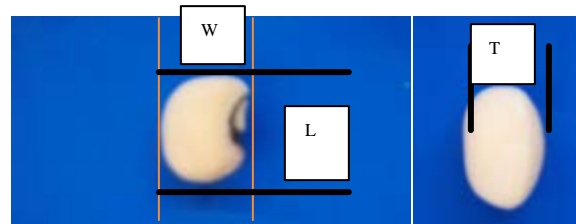


Fig 1: Cowpea seed (W) Width, (L) Length and (T) Thickness

Phenotypic and genotypic data were recorded on excel sheet, the former was imported directly into GenStat for analysis, however, the latter was transported into notepad before importation into GenStat. Preliminary single environment analysis was done on the phenotypic data: vital information including F Probability and heritability estimates reported.

The procedure single trait single environment association mapping (VSN International, 2012) was followed to identify SNP markers linked with seed mass and thickness.

RESULTS

Preliminary single environment analysis was run twice: first with genotypes random in order to estimate heritability and variance parameters, the second time with Genotypes fixed to get unshrunk means for QTL analysis. Vital information from this analysis is presented in Table 1.

Correlations between the various attributes of seed size namely seed mass, seed length, seed width and seed thickness were estimated (Fig. 2).

The markers used for the mapping were fairly distributed across the cowpea genome as shown in Fig. 3 and 4 show strength of association between various markers and seed mass while Fig. 5 shows their positions in the cowpea genome. Figure 6 and 7 are similar to Fig. 4 and 5 but for seed thickness. Some of the markers having significant association with the traits are shown in Table 2.

DISCUSSION

The insignificance of variability in seed length and width in the collection of cowpea studied might mean that these traits might not be important in seed size as compared with thickness and mass. Seed length and width have more to do with seed shape than size. The length and width of cowpea seed are related to shape such as globose, rhomboid and kidney shapes. These shapes are recognized no matter the size of the seed and among other things, it is the length and width of the seed that determine the shape. In this case, there could be small or large kidney shaped cowpea seed. Disproportionate change in length and width may alter the shape of the seed. For instance, increasing the width of a globose seed without a change in the length may change the seed shape to crowder. Increasing the length of globose seed may lead to ovoid if the width remains the same.

Variability in 100 seed mass and seed thickness of the genotypes studied were significantly different from each other (0.006 and 0.016 F. probability, respectively) as shown in Table 1. Although, differences between genotypes with respect to seed width and length were not significant, their F probabilities were quite small (0.08 and 0.06, respectively). Because the differences in the seed width and length were not significant, only 100 seed mass and seed thickness were used for association mapping with the SNP markers. Notwithstanding, heritability

Table 1: Results From preliminary single environment analysis on seed size

Seed trait	Minimum	Maximum	Mean	F. Probability	Heritability
100 seed Mass (g)	6.58	20.27	10.48	0.006	0.96
Width (mm)	4.94	7.53	5.89	0.081	0.82
Length (mm)	5.65	9.9	6.94	0.06	0.85
Thickness (mm)	2.3	5.82	3.56	0.016	0.94

No. of observations (n) is 78 for all traits

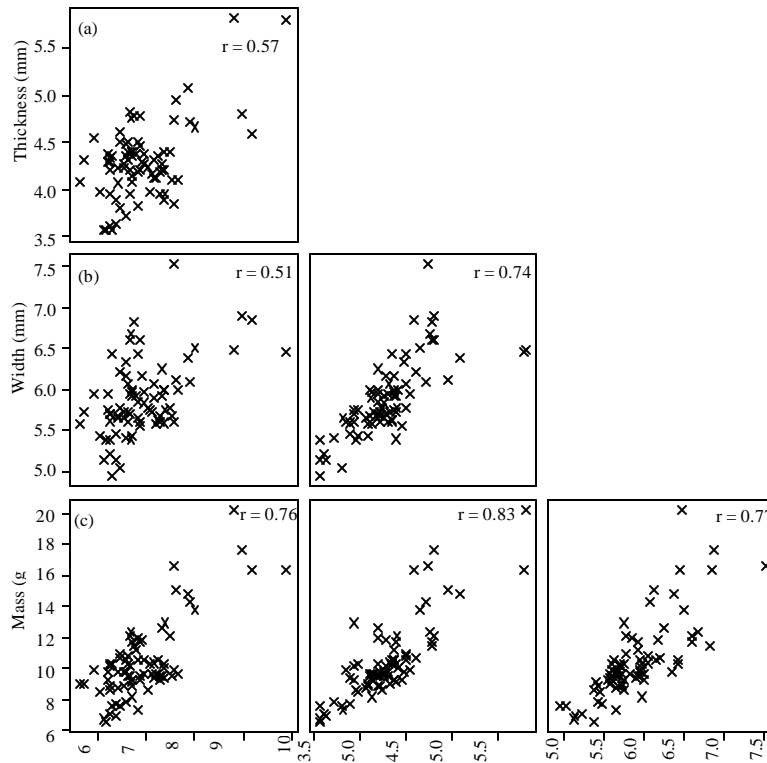


Fig. 2(a-c): Correlation between seed mass, length, thickness and width

estimate for all four seed size traits were very high: between 82 and 96%. It is however, important to note that the heritability estimate for seed width and length (82 and 85%), respectively were lower than that of 100 seed mass (96%) and seed thickness (94%). High heritability for seed size is known in cowpea (Hall *et al.*, 1997).

Significant variability was observed in the collection based on the measured traits (Table 1). With regard to 100 seed mass, the largest seed (20.27 g) was more than three times heavier than the smallest. The difference between the widest (7.53 mm) and the narrowest (4.94 mm) seeds, however was quite small. The longest seed on the other hand was about two times the length of the shortest seed. The seed with the highest value for thickness (5.82 mm) was two and a half times more than the least (2.3 mm).

There was positive correlation between all the traits studied. The highest correlation (0.83) was between 100 seed mass and seed thickness: the traits with significant variability. The smallest

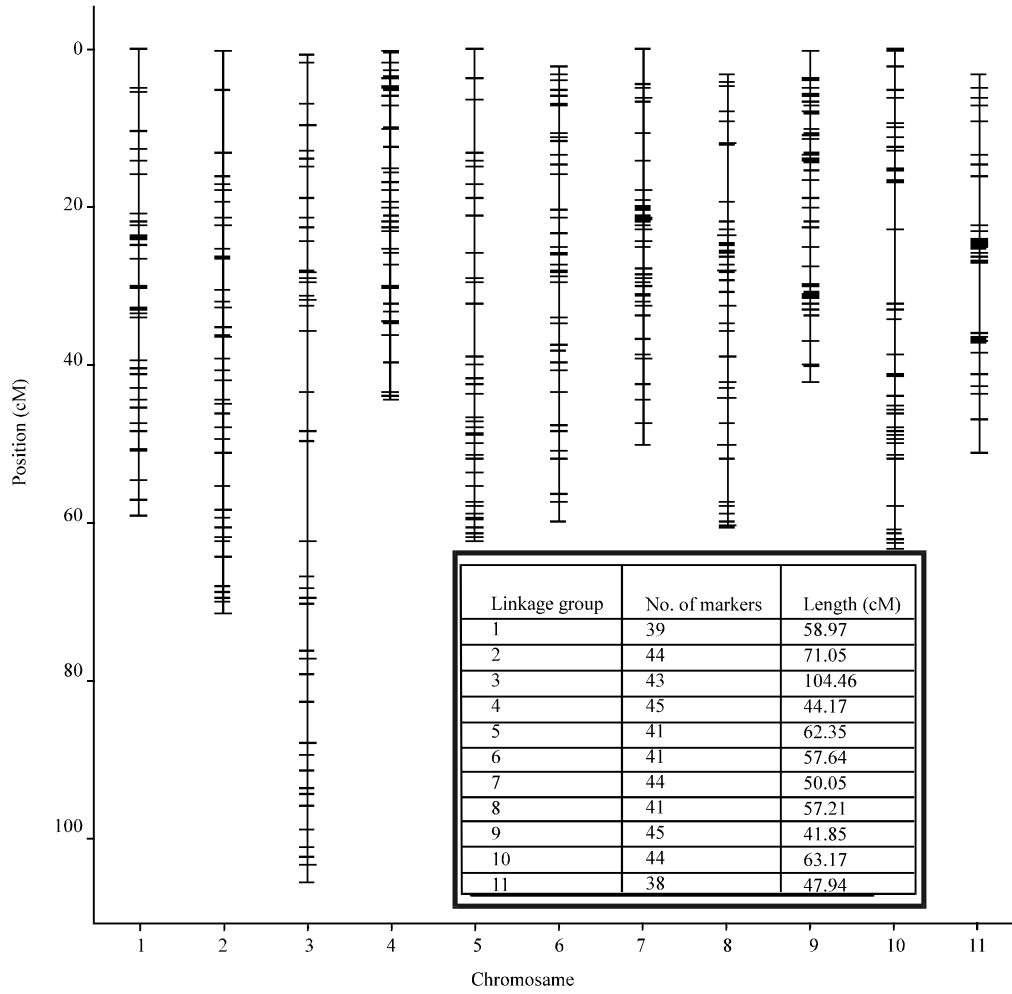


Fig. 3: Markers mapped onto chromosomes

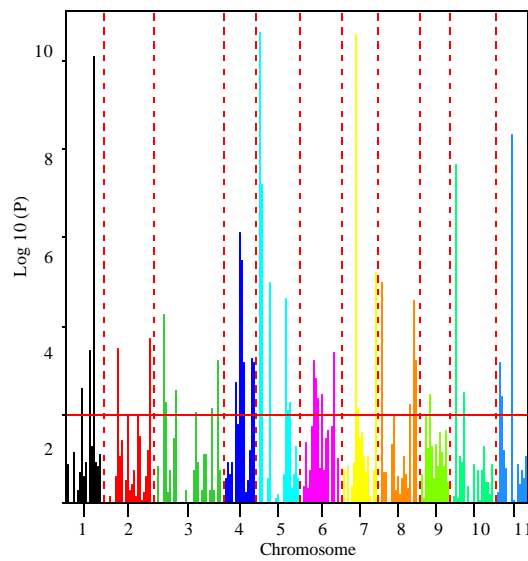


Fig. 4: Markers' association with seed mass at different p levels

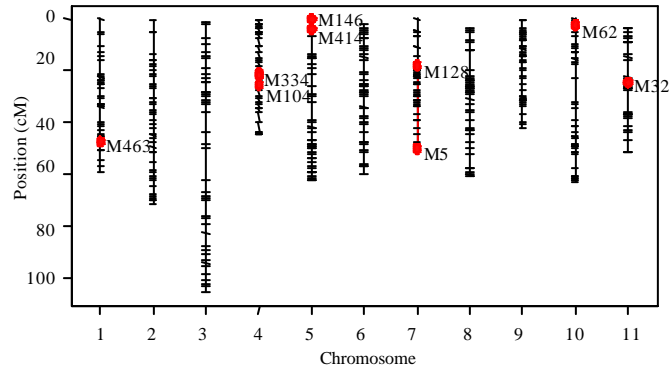


Fig. 5: Positions of markers with significant association with seed mass [$-\log_{10} p \geq 5.0$]

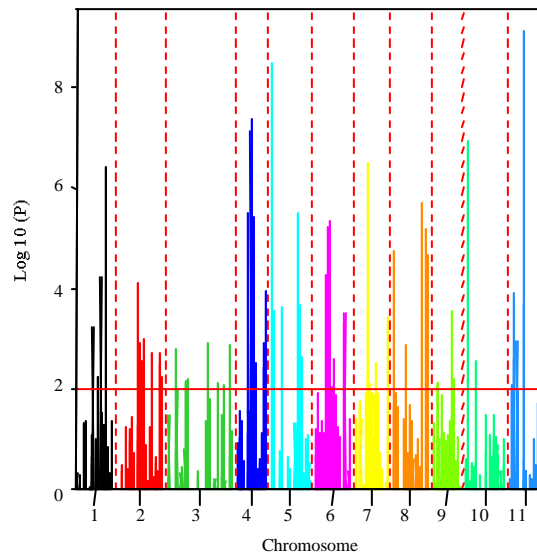


Fig. 6: Markers' association with seed thickness at different p levels

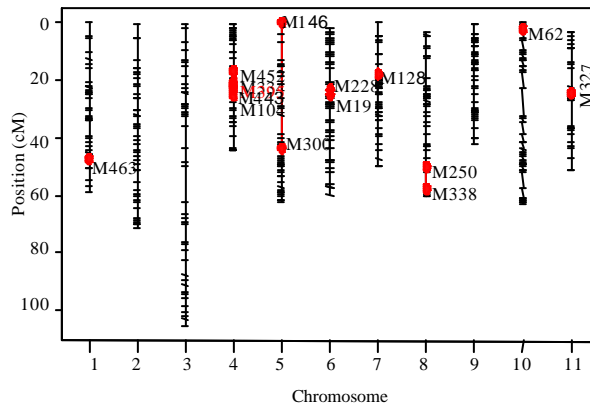


Fig. 7: Positions of markers with significant association with seed thickness [$-\log_{10} (p) \geq 5.0$]

Table 2: Markers with significant association with traits [-log₁₀ (p)≥5.0]

Marker	Linkage group	Position	Trait
9175_1561	1	47.4	Mass and Thickness
8899_1022	4	16.8	Thickness
5061_428	4	21.1	Mass and Thickness
7102_965	4	21.9	Mass and Thickness
8625_1231	4	23.2	Thickness
13269_270	4	25.4	Mass and Thickness
1441_128	5	0.0	Mass and Thickness
7967_1210	5	3.9	Mass
4098_1148	5	43.7	Thickness
17588_963	6	23.3	Thickness
10279_316	6	25.0	Thickness
13872_1420	7	18.0	Mass and Thickness
16822_160	7	50.0	Mass
2395_247	8	50.1	Thickness
5135_477	8	57.7	Thickness
11851_914	10	2.3	Mass and Thickness
12183_298	11	24.4	Mass
4882_531	11	24.6	Mass and Thickness

correlation value of 0.51 was between seed length and width. The strong positive correlation between seed mass and the other three traits suggests that seed mass can be used to represent seed size. The thicker the seed, the heavier it is expected, hence the high correlation between thickness and mass.

In addition to wide variability observed in seed mass between the genotypes, seed mass was easier to measure compared with the three. This also suggests that there could be much more error in measuring seed width, length and thickness than weighing to obtain the 100 seed mass.

Significant associated markers were observed for seed mass on all the eleven chromosomes at the default level of p (2). There was just one associated marker on chromosome 9. Chromosomes 2 and 7 had two associated markers each with seed mass. There were 3 significant markers each for chromosomes 1 and 11. Marker trait significance level was raised from the default of 2 to 5. The number of significant SNPs associated with 100 seed mass also reduced drastically. No marker on chromosomes 2, 3, 6, 8 and 9 were significantly associated with seed mass. Chromosomes 1, 10 and 11 had one significant associated marker each. Chromosome 4 had 3 significant markers while 5 and 7 had two markers. This result does not agree with Fatokun *et al.* (1992) where cowpea seed weight genes were mapped on chromosomes 2 and 6. However, Fatokun *et al.* (1992) used RFLP and not SNP. Kelly *et al.* (2003) also mapped seed weight genes of cowpea on chromosomes 1, 3, 4, 6 and 7 showing some agreement with the current studies.

Trend in significance association between markers and trait for seed thickness was similar to that of 100 seed mass. The two traits shared a number of significant markers. This could be indication that seed thickness is responsible for seed mass and consequently seed size in cowpea. At [-log₁₀ (p)≥5.0], there were 18 significant SNPs for the two traits. Seed mass had only 3 significant markers that were not significant with seed thickness. Seed thickness on the other hand had 7 significant markers that were not significant with seed mass. The significant SNPs shared with seed mass and seed thickness could be studied further as they might be the most important seed size markers in cowpea. In this regard, the single markers with significant association on chromosome 1, 10 and 11 are strongly suggested.

A key issue in the study of heredity and variations at the molecular level is the detection of associations between DNA sequence variation and the heritable phenotypes (Gupta *et al.*, 2001). “SNPs are often linked to genes” (Acquaah, 2007), suggesting the association of these ones with seed size genes. The association between SNP markers discovered in this experiment could be used in marker assisted breeding of large seeded cowpeas which is of economic value in West Africa.

CONCLUSION

Results of the experiment showed seed mass and seed thickness as the major determinants of seed size in cowpea. Seed mass and thickness were significantly different within the population studied. It was also observed that these two traits were highly correlated (0.83). Seed width and length could be more of shape determinants than size. There were significant marker-trait association for both seed mass and thickness for 8 SNPs used in the experiment at $[-\log_{10} p \geq 5.0]$. The markers with significant association with seed size are distributed throughout the cowpea genome from chromosomes 1 to 11 in the exception of 2, 3 and 9. It would be important to estimate the contribution of each of these loci to seed size differences. The distribution of the significant loci among other things confirmed quantitative nature of seed size. Developing on this knowledge can lead to marker assisted breeding of larger seeded cowpea varieties.

ACKNOWLEDGMENTS

We appreciate the benevolence of CSIR-PGRRI for supplying us with the chunk of genotypes used for the studies. We are indebted to the quick response of Mr. I.F. Asare of the seed store section in supplying the germplasm and Mr. D. Ofori a National Service Person at CSIR-PGRRI for his assistance in phenotyping. We thank GCP for providing the financial support and KBioscience for the genotyping service: without them it might not be possible to do this work. We also wish to express our appreciation for support given by the following: Mr. E. Addo of the Biotech Centre, CACS and all staff of WACCI University of Ghana.

REFERENCES

- Abdurakhmonov, I.Y. and A. Abdukarimov, 2008. Application of association mapping to understanding the genetic diversity of plant germplasm resources. *Int. J. Plant Genomics*, 10.1155/2008/574927
- Acquaah, G., 2007. Principles of Plant Genetics and Breeding. Blackwell Publishing Ltd., Malden, Pages: 569.
- Drabo, I., R. Redden, J.B. Smithson and V.D. Aggarwal, 1984. Inheritance of seed size in cowpea (*Vigna unguiculata* (L.) Walp.). *Euphytica*, 33: 929-934.
- Egbadzor, K.F., M. Yeboah, S.K. Offei, K. Ofori and E.Y. Danquah, 2013. Farmers key production constraints and traits desired in cowpea in Ghana. *J. Agric. Extension Rural Dev.*, 5: 14-20.
- Fatokun, C.A., D.I. Menancio-Hautea, D. Danesh and N.D. Young, 1992. Evidence for orthologous seed weight genes in cowpea and mung bean based on RFLP mapping. *Genetics*, 132: 841-846.
- Fatokun, C.A., S.A. Tarawali, B.B. Sign, P.M. Korawa and M. Tumo, 2000. Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference 3, September 4-8, 2000, International Institute of Tropical Agriculture IITA, Ibadan Nigeria.
- Gupta, P.K., J.K. Roy and M. Prasad, 2001. Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. *Curr. Sci.*, 80: 524-535.

- Hall, A.E., B.B. Singh and J.D. Ehlers, 1997. Cowpea breeding. *Plant Breed. Rev.*, 15: 217-274.
- Jackai, L.E.N. and R.A. Daoust, 1986. Insect pests of cowpea. *Ann. Rev. Entomol.*, 31: 95-119.
- Kelly, J.D., P. Gepts, P.N. Miklas and D.P. Coyne, 2003. Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Res.*, 82: 135-154.
- Langyintuo, A.S., J. Lowenberg-DeBoer, M. Faye, D. Lambert and G. Ibro *et al.*, 2003. Cowpea supply and demand in West and Central Africa. *Field Crops Res.*, 82: 215-231.
- Lattanzio, V., R. Terzano, N. Cicco, A. Cardinali, D.D. Venere and V. Linsalata, 2005. Seed coat tannins and bruchid resistance in stored cowpea seeds. *J. Sci. Food Agric.*, 85: 839-846.
- Lucas, M.R., J.D. Ehlers, P.A. Roberts and T.J. Close, 2012. Markers for quantitative inheritance of resistance to foliar Thrips in cowpea. *Crop Sci.*, 52: 2075-2081.
- Makoi J.H.J.R., A.K. Belane, S.B.M. Chimphango and F.D. Dakora, 2010. Seed flavonoids and anthocyanins as markers of enhanced plant defence in nodulated cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Res.*, 118: 21-27.
- Muchero, W., N.N. Diop and P.R. Bhat, 2009. A consensus genetic map of cowpea [*Vigna unguiculata* (L.) Walp.] and synteny based on EST-derived SNPs. *Proc. Nat. Acad. Sci. USA.*, 106: 18159-18164.
- Orawu, M., R. Melis, M. Laing and J. Derera, 2013. Genetic inheritance of resistance to cowpea aphid-borne mosaic virus in cowpea. *Euphytica*, 189: 191-201.
- Tchiagam, J.B.N., J.M. Bell, D.F. Ngakeu, N.Y. Njintang and E. Youmbi, 2011. Diallel analysis of cowpea (*Vigna unguiculata* (L.) Walp.) for some physical properties of seed under the Sudano-guinean conditions. *Agric. Biol. J. N. Am.*, 2: 698-707.
- VSN International, 2012. GenStat for Windows. 15th Edn. VSN International, Hemel Hempstead, UK.
- Varshney, R.K., K. Chabane, P.S. Hendre, R.K. Aggarwal and A. Graner, 2007. Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Sci.*, 173: 638-649.
- Zhu, C., M. Gore, E.S. Buckler and J. Yu, 2008. Status and prospects of association mapping in plants. *Plant Genome*, 1: 5-20.