Applied Tropical Agriculture
Volume 22, No. 2, 101-110, 2017.

Association Studies of Agronomic Traits, Genetic Structure and Phylogeny of Some Selected Nigeria Cowpea Cultivars

Adetumbi, J.A.[1]*, Akinyosoye, S.T.[1] and Amusa, O.D.[2]

[1]Institute of Agricultural Research and Training, PMB 5029, Moor Plantation, Ibadan. Nigeria
[2]Department of Cell Biology and Genetics, University of Lagos, Akoka Lagos, Nigeria
*Corresponding author: jaadetumbi@yahoo.com

ABSTRACT

The genetic component of nine elite cowpea cultivars in Nigeria were analysed using single nucleotide polymorphism (SNP) technology along with their agronomic trait with the aim of understanding their genetic structure, diversity, phylogenic relationship and marker-trait association among the varieties. Twenty-five morpho-agronomic and disease reaction traits of the cultivars were studied. Genotyping was done with 255 SNPs markers, while Association analysis was done using the general and mixed linear models. 160 SNPs (71.0%) were highly polymorphic. There was narrow genetic diversity with three structured populations and three evolutionary paths identified among the cultivars. 93 SNPs (26 SNPs associated with more than one trait) and 84 SNPs (7 SNPs associated with more than one trait) were identified using the General and Mixed Linear Models respectively. Strong associated SNP markers which can be used to improve cowpea production were identified. The study also provides information that can be utilised in cowpea yield and disease resistance improvement programme.

Key words: Cowpea, Association mapping, Genetic diversity, Genetic Structure, Phylogeny

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), is an important multipurpose crop plant in many developing countries in Africa and Asia. It is mostly used for consumption and income earning at both local and international level. Apart from being the cheapest source of protein when compared to fish, meat and egg, this crop is also used as fodder for animal feed (Xiong *et al*., 2016). Despite the usefulness of this grain crop, its production is constantly faced with a lot of biotic and abiotic factors which lowers production. Collaborative efforts of institutions are continuously being made to improve this crop through conventional breeding, marker assisted selection (MAB) and marker assisted breeding (MAB).

The utilization of molecular markers in conjunction with phenotype has been reported to be more reliable when compared to the use of only phenotype in crop improvement. However, non-accessibility to molecular markers in developing countries has contributed to over reliance on the use of phenotypes despite their limitations (Egbadzor *et al*., 2013). Single nucleotide polymorphism (SNP) has become a powerful tool in genome mapping, association studies, diversity analysis and tagging of important genes in plant genomics due to its relative abundance, cost efficiency and high-throughput scoring (Collard and Mackill 2008; Xu and Crouch 2008; Varshney *et al*., 2009). Shi *et al*. (2016) stated that the identification of SNP markers associated with important agronomic traits will not only provide breeders with a powerful resource to assist in selecting for pest and disease resistance but also speed up the development of elite lines in cowpea breeding programs.

Recently, genome wide association studies have received increased attention due to its potential to identify single polymorphism within the gene that is responsible for phenotypic differences. Also, association mapping (AM) uses natural occurring population to identify markers that are associated with the trait of interest and it involves searching for genotype-phenotype correlations among unrelated individuals. It has also been used to validate markers previously identified to be linked to various traits, which suggest the practicability of this approach to use MAS/MAB in breeding programmes (Rathi *et al*., 2014). Another advantage of Association mapping is that no mapping population needs to be developed, as the sampling of non-related individuals represent a series of advantage towards developing and validating MAS in breeding programmes (Jannink *et al.,* 2001). Hence the objective of the study was to analyse the genetic component of some elite cowpea varieties in Nigeria with a view to providing information on their relationship, genetic structure, evolutionary development and identify SNP markers

associated with some important agronomic traits of the cowpea cultivars.

MATERIALS AND METHODS

Experimental location and Plant materials

The experiment was carried out at the screen house of the Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Nigeria. Seeds of nine (9) cowpea genotypes (ERUSU, OLOYIN, IFE BROWN, MODUPE, IFE 98-12, IFE 98-14, IT-84S-2246-4, IT-95-193-12, SOKOTO) obtained from germplasm collections of the Institute of Agricultural Research and Training, Ibadan, Nigeria were used for the study.

Phenotypic evaluation

Four seeds of each genotype were planted and arranged in a Completely Randomised Design (CRD) with four (4) replicates. Germinating plants were thinned to two at 2 weeks after planting. Twenty two (22) morpho-agronomic characters: days to first flowering, days to 50% flowering, terminal leaf length (cm), terminal leaf width (cm), petiole length (cm), number of seeds per pod, number of pod per peduncle, number of pod per plant, total pod weight per plant (g), pod length (cm), total seed weight per plant (g), 100 seed weight per plant (g), days to physiological maturity, seed length (cm), seed width (cm), seed thickness (cm) were obtained based on the descriptors for cowpea (IBPGR, 1983) at various stages of growth of the crop. Also, resistance to viral infection, Cercospora leaf spot and anthracnose were scored at six weeks after planting according to Kumar (2009), Akande *et al*. (2012) and Shamsi and Najimun (2015) respectively.

DNA isolation and genotyping

Samples of the cowpea cultivars were planted in the screen house of IAR&T for fresh leaf tissues. Two weeks after planting, ten leaf discs were cut from the young leaf tissues of each plant and placed into the wells of a storage plate. The plate was sealed with a perforated (gas-permeable) heat seal and placed in a heavy-duty, sealed bag with desiccant to dehydrate and preserve the leaf tissue during transit to LGC genomics in United Kingdom for genotyping. Total DNA was extracted using the oKtopure™ protocol and checked using UV spectrophotometry for quality and quantity of the DNA, while preliminary PCR at a serial dilution was carried out and the results produced were duplicated to identify the best dilution to run the samples. SNP genotyping was done using KASP genotyping reactions (http://www.lgcgroup.com/ourscience/genomics-solutions).

Genetic Diversity and Phylogenic Analysis

Result obtained from SNPs genotyping was used to estimate the genetic diversity and the phylogenic analysis. The major allele frequency, heterozygosity, gene diversity and polymorphic information content (PIC) were calculated for each SNP using PowerMarker 3.2.5 (Liu and Muse, 2005). A frequency based genetic distance matrix calculated using shared allele option was used to construct a phylogenic tree relationship between the sampled cowpea cultivars. Unweighted pair-group method with arithmetic average (UPGMA) method was used to estimate the genetic relationship from the genetic distance between the cowpea germplasm. PowerMarker software was used to perform these analyses and they were displayed in phylogram and dendrogram using the MEGA6 software (Tamura *et al*., 2013).

Population Structure

The population structure (Q) of the samples evaluated was carried out in STRUCTURE v2.3.4 software. The optimum number of populations was selected with a burn-in period of 100,000 steps followed by 100,000 MCMC (Monte Carlo Markov chain replicates). The range of genetic clusters was set from K = 1 to K = 7 with 10 iterations. To determine the true value for K, *ad hoc* statistic ΔK was calculated (Evanno *et al*., 2005).

Association analysis

Marker-trait association analysis was evaluated using TASSEL v5.3.2 (Trait Analysis by Association Evolution and Linkage). SNP genotype data generated was first filtered to remove monomorphic SNP sites. The kinship matrix (K) was estimated from the genotype data in TASSEL program. Both general linear model (GLM) and mixed linear model (MLM) were used accordingly. Significantly associated SNP markers with traits were identified on the basis of their $P < 0.05$ (Bradbury *et al*., 2007)

RESULTS AND DISCUSSION

Genetic diversity and Evolutionary trend among the cowpea cultivars

There was a high similarity among the cultivars. MODUPE and IFE BROWN were the most similar to each other (similarity index = 0.92; distance index = 0.08) while the least genetic similarity relationship was observed between IFE 98-12 and Sokoto white (similarity index = 0.49; distance index = 0.51). There was a moderate to low diversity among the genetic relationship between the nine cowpea cultivars (Table 1). A similar report of low genetic diversity in 19 cowpea accessions from Kenyan national gene bank has been reported by Wamalma *et al*. (2016). However, a study conducted by Patil *et al*. (2013) revealed a high variability (diversity) among 30 cowpea lines using 20 random amplified polymorphic DNA markers (RAPD). The high variability might be due to low heterozygosity resolution power of RAPD marker apart from the

Table 1: Genetic relationship analysis between the nine cowpea samples evaluated

Cowpea genotypes	1	2	3	4	5	6	7	8	9
1	-	0.33	0.41	0.33	0.38	0.08	0.35	0.4	0.16
2	0.67	-	0.21	0.41	0.46	0.3	0.37	0.39	0.16
3	0.59	0.79	-	0.5	0.53	0.38	0.42	0.44	0.19
4	0.67	0.59	0.5	-	0.43	0.29	0.34	0.36	0.46
5	0.62	0.54	0.47	0.57	-	0.36	0.21	0.38	0.51
6	0.92	0.7	0.62	0.71	0.64	-	0.34	0.41	0.31
7	0.65	0.63	0.58	0.66	0.79	0.66	-	0.27	0.43
8	0.6	0.61	0.56	0.64	0.62	0.59	0.73	-	0.43
9	0.65	0.84	0.81	0.54	0.49	0.69	0.57	0.57	-

inbreeding nature which increases homozygosity in the crop.

The phylogenic tree drawn using neighbour joining method divided the nine cultivars into three clades (Figure 1). The cultivars in the same clades displayed closer genetic evolutionary background. Although, IT-84S-2246-4 occupies a single evolutionary path away from the other cultivars, it cannot be regarded as an outgroup because, the phylogram showed three simultaneous divergences from a common ancestry leading to three evolutionary paths of which IT-84S-2246-4 occupies a solitary evolutionary path (Figure 1). IFE 98-12, IFE 98-14 and IT-95K-193-12 shared a common ancestry. It will be noted that Ife 98-12 and Ife 98-14 are sister taxa forming the second cladistics group. The third clade featured MODUPE and Ife brown sharing a common ancestry with OLOYIN, ERUSU and SOKOTO. ERUSU appeared to be the most recently diverged cowpea cultivar among the cultivars evaluated considering the evolutionary event as measured by phylogram's branch length (Figure 1). The phylogenetic tree suggested that the cowpea cultivars were distributed to three well-differentiated genetic populations and admixtures similar to the report of Xiong *et al*. (2016).

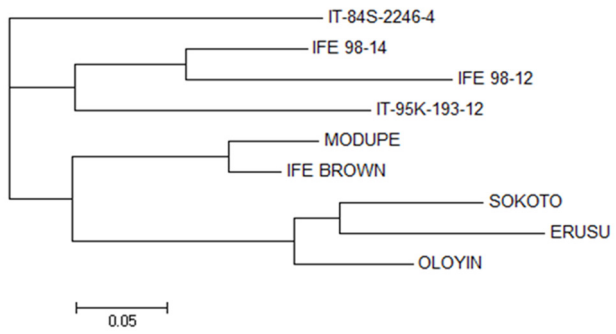

Figure 1: Phylogeny tree constructed for nine cowpea samples evaluated

Shi *et al*. (2016) also reported three phylogenic clades from 249 USDA cowpea accessions collected from 43 countries evaluated

Population structure of Nigerian elite cowpea cultivars

The structure analysis showed that the nine cultivars had a peak at ΔK of 63.51 with K = 3 (Figure 2). Three genetic subpopulation structures with admixtures were identified. IFE 98-12, IFE 98-14 and IT-95-193-12 occupied the first subpopulation structure (Cluster Q1), IFE BROWN, and MODUPE occupied the second subpopulation structure (Cluster Q2) but IT-84S-2246-4 was observed to be an admixture sample for Q1 and Q2 with inclination towards Q2. ERUSU, OLOYIN and SOKOTO occupied the third subpopulation structure (Cluster Q3) (Figure 3).

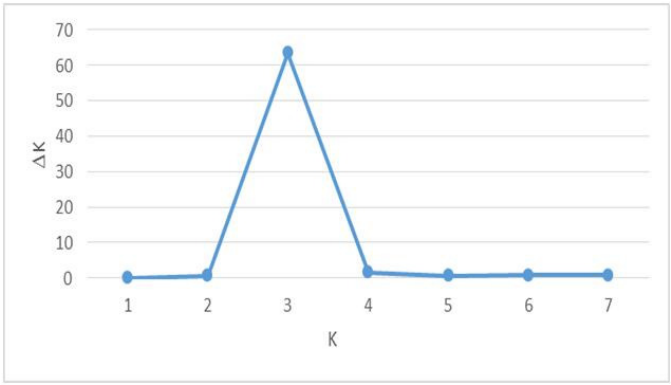

Figure 2: Optimum number of subpopulations among the nine cowpea cultivars (ΔK values for the different numbers of populations assumed (K) in the STRUCTURE analysis)

This report is similar to the work of Xiong *et al*. (2016). Three gene pools were reported from their study of cowpea varieties from 56 countries of 11 geographical regions of the world. Shi *et al*. (2016) also reported three well differentiated genetic subpopulations with some admixtures from 249 cowpea accessions. However, Huynh

et al. (2013) reported two major subpopulations in the world landrace population of cowpeas analysed from 56 countries but stated the existence of a narrow gene pool in African cowpea landraces.

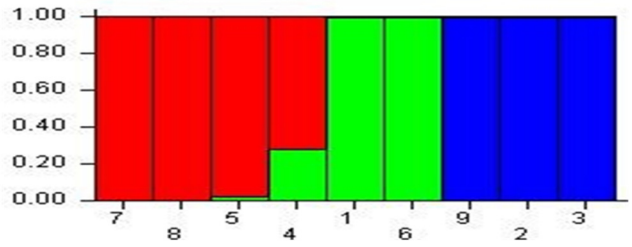

Table 3: Population structure analysis of nine elite cowpea genotypes. Numbers on the x-axis indicates the genotypes (1: Modupe; 2: Oloyin; 3: Erusu; 4: IT-84S-2246-4; 5: IFE 98-12; 6: Ife brown; 7: IFE 98-14; 8: IT-95K-193-12; 9: Sokoto White)

.

Single nucleotide polymorphism diversity within cowpea samples evaluated

One hundred and sixty (160) SNPs markers representing 71% of the 255 SNPs markers were used for analysing the nine cowpea cultivars after removing rear alleles (less than 5%), high-missing ratios (more than 30%) and monomorphic sites (filtration). Six SNP combinations types were observed. These include AG (36.13%), AC (9.68%), AT (10.97%), CT (25.16%), CG (6.45%) and GT (11.61%). Major allele frequency, gene diversity, heterozygosity and Polymorphic Information Content (PIC) of the SNPs ranged between 0.50-0.94, 0.10-0.50, 0.00-0.11, 0.10-0.38 with means of 0.74, 0.36, 0.01 and 0.29 respectively. This result is similar to that of Xiong *et al*. (2016) that suggested existence of SNP variations, mutations and genetic diversity. This study also showed percentage of occurrence of SNPs in the order of AG > CT > GT > AT > AC > CG. Xiong *et al*. (2016) also reported the percentage of these SNP variations in same order for the cowpea genotypes evaluated in their study. The low heterozygosity and PIC values observed in the study indicates a low polymorphism among the cowpea genotypes. This may be due to the small number of sample used for the study or single domestication attributes in this crop. Also, the inherent nature of inbreeding reproduction mechanism of the crop may influence the low heterozygosity observed among the cowpea genotypes as suggested by Badiane *et al*. (2012).

Marker-trait association using combinations of General Linear Model (GLM) and the Mixed Linear Model (MLM)

A total of 93 significant ($P \leq 0.05$) marker-trait associations were identified with GLM models with 26 SNP markers associated with more than one trait (Table 2). However, the MLM model showed a total of 84 significant ($P \leq 0.05$) marker-trait associations with 7 SNP markers associated with more than one trait (Table 3). Small marker effects ($R^2 < 0.05$) for all significant marker-trait associations were observed. This may be due to contributions of multiple QTLs in the natural population and the effect of any individual locus might have been diluted as suggested by some authors (Zhang *et al.,* 2012, Muchero *et al.,* 2013, Zila *et al.,* 2013). Alternatively, the number of SNP markers used in the study might have led to the detection of loci with smaller effects than real, due to weak linkage disequilibrium between marker and causal gene (Massman *et al*., 2011)

Although, Lucas *et al.* (2013) reported 7 SNPs associated with seed size in cowpea using 804 individuals from 8 bi-parents, this study however showed 9 SNPs associated with 100 seed weight with GLM, 2 SNPs with seed length, 3 SNPs with seed thickness, 4 SNPs for seed weight/plant with the MLM. This result might be due to diverse sample used which captures the diverse loci available in the population compared to the use of bi-parents which narrows available loci to the parents. This study however showed no SNP marker associated with seed weight per plant, terminal leaf length, physical maturity and Cercospora leaf spot severity using the GLM model while seed width and vining showed no marker association using MLM model. This study reports multiple SNPs associated to a particular trait and single SNPs significantly associated with multiple traits which indicate either pleiotropy or genetic linkage between the markers associated to traits. These SNPs therefore need to be further studied on other larger similar population or family base population to confirm its marker-trait linkage (Bartholome *et al*. 2016). However, population stratification or relatedness can result in the detection of spurious marker-trait associations (Eu-ahsunthornwattana *et al.* 2014).

Generally, MLM is considered to be more robust as compared to GLM as chances of false positive associations tend to be more with GLM. However, it should be noted that the approach of MLM may not always be rewarding, and the strategy of analysis may differ from trait to trait so that, for a given trait in a given set of genotypes, approach like GLM may be more fruitful (Zhao *et al.*, 2011).

CONCLUSION

This study revealed that the nine cowpea cultivars had low genetic diversity and heterozygosity indicating a narrow genetic base. However, regardless of the number of population, cowpea cultivars can be clearly distinguished to three genetic structures and phylogenic path. SNP technology revealed significant markers that can be used for cowpea genetic association studies and breeding programmes. It's ability to detect polymorphism at a single nucleotide makes is a more effective tool than other molecular marker technologies.

Table 2: Association between significant SNP markers loci and agronomic traits determined by the General Linear Model (GLM)

Trait	SNP	sig	R^2	Trait	SNP	Sig	R^2	Trait	SNP	sig	R^2
Pod length	13709_659	***	3.47E-05	100 seed weight	1278_268	*	2.79E-05	Leaf petiole length	13551_722	*	1.52E-05
	13080_585	***	3.47E-05		13551_722	*	2.79E-05		1278_268	*	1.52E-05
	12428_146	***	3.47E-05		10833_1266	*	2.66E-05		12685_433	*	1.52E-05
	11_533	***	3.47E-05		10763_123	*	2.66E-05		3194_319	*	1.38E-05
	10331_516	***	3.47E-05		4146_1588	*	2.65E-05		13005_643	*	1.38E-05
	10172_1642	***	3.47E-05		1912_1062	*	2.65E-05		11118_399	*	1.38E-05
	12119_480	***	3.47E-05		17067_979	*	2.65E-05		10969_452	*	1.38E-05
	10288_378	***	3.47E-05		13342_813	*	2.65E-05		10941_471	*	1.38E-05
	11851_914	**	3.48E-05		12584_1346	*	2.65E-05		10738_1400	*	1.38E-05
	1285_722	**	3.47E-05	Number of pods per peduncle	11597_678	**	3.70E-05		8944_1233	*	1.38E-05
	10905_418	*	2.87E-05		13386_815	*	4.95E-05		894_153	*	1.38E-05
	13017_290	*	2.71E-05		1003_1507	*	4.95E-05		1366_307	*	1.38E-05
Number of days to first flowering	16822_160	***	5.70E-05		4780_374	*	4.89E-05		13586_1058	*	1.38E-05
	13693_225	**	5.26E-05		13764_285	*	4.89E-05		13137_722	*	1.38E-05
	13063_1329	**	5.26E-05		12363_196	*	4.89E-05		11514_619	*	1.38E-05
	12393_305	**	5.26E-05		11783_1366	*	4.89E-05		10115_384	*	1.38E-05
	1339_101	*	5.06E-05		11233_1096	*	4.89E-05		1285_722	*	1.63E-05
	12324_917	*	5.06E-05		10500_1030	*	4.89E-05		10292_823	*	1.63E-05
	1202_1215	*	5.06E-05		11985_1833	*	4.85E-05		13222_1855	*	1.63E-05
	11028_734	*	5.06E-05		13652_572	*	4.85E-05		12991_691	*	1.36E-05
	12905_686	*	5.27E-05		13563_863	*	4.85E-05	Leaf pigmentation	6410_648	**	3.10E-06
Number of days to germination	12119_480	**	7.50E-05		12568_234	*	4.85E-05		13017_290	**	2.95E-06
	10288_378	**	7.50E-05		9673_1553	*	4.70E-05		12308_1447	**	3.09E-06
	13709_659	**	7.50E-05		13707_697	*	4.70E-05	Number of nodes	10269_488	*	2.20E-06
	13080_585	**	7.50E-05		12918_1176	*	4.70E-05	Viral infection severity	7229_909	*	1.87E-05

	12428_146	**	7.50E-05			12731_670	*	4.70E-05		Pod weight	1278_268	*	5.90E-05
	11_533	**	7.50E-05			12505_1312	*	4.70E-05			13551_722	*	5.90E-05
	10331_516	**	7.50E-05			12096_1122	*	4.70E-05		Petiole pigmentation	13017_290	**	1.77E-06
	10172_1642	**	7.50E-05			1208_173	*	4.70E-05			6410_648	**	1.80E-06
	10905_418	*	6.95E-05			10974_245	*	4.70E-05			12308_1447	**	1.79E-06
	1285_722	*	7.50E-05			10530_1801	*	4.70E-05		Seed length	13551_722	*	3.03E-05
	11851_914	*	7.50E-05			10384_1814	*	4.70E-05			1278_268	*	3.03E-05
Number of days to 50% flowering	12393_305	**	6.56E-05			15385_870	*	4.69E-05		Seed thickness	10630_719	**	2.80E-05
	13693_225	*	6.53E-05			1181_710	*	4.68E-05		Seed width	13551_722	**	5.51E-05
	13063_1329	*	6.53E-05			13281_572	*	4.62E-05			1278_268	**	5.51E-05
	1339_101	*	6.15E-05			10661_873	*	4.62E-05		Terminal leaf width	13506_333	*	1.07E-05
	12324_917	*	6.15E-05			10466_465	*	4.62E-05		Vining	12793_473	***	3.55E-06
	1202_1215	*	6.15E-05			13286_530	*	4.61E-05			1285_722	*	6.48E-05
	11028_734	*	6.15E-05			131_524	*	4.61E-05			10292_823	*	6.48E-05
	12905_686	*	6.73E-05			12712_158	*	4.61E-05		Number of pods	1278_268	*	6.83E-05
	16822_160	*	6.61E-05			12553_1026	*	4.61E-05			13551_722	*	6.83E-05
Pod pigmentation	13294_282	***	2.89E-13			12265_692	*	4.61E-05			10905_418	*	5.36E-05
	12183_298	***	2.89E-13			10389_646	*	4.61E-05		Pod curvature	12484_660	*	7.05E-05
	11484_541	***	2.89E-13			10120_329	*	4.61E-05			10951_1216	*	7.05E-05
	12905_686	***	2.89E-13			10000_1243	*	4.61E-05					

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, R^2: Marker fraction of the total phenotypic variation explained

Table 3: Association between significant SNP marker loci and agronomic traits determined by the Mixed Linear Model (MLM)

Trait	Marker	Sig	R^2	Trait	Marker	Sig	R^2	Trait	Marker	Sig	R^2
Number of days to germination	1003_1507	***	0.13	Number of days to germination	13017_290	**	1.82E-10	Number of days to first flowering	1101_164	***	4.41E-06
	13386_815	***	0.13		11764_552	**	8.07E-09		12439_253	***	2.33E-06
	12439_253	***	0.17		2918_753	**	4.75E-10		1003_1507	***	2.55E-06
	1101_164	***	0.03		8273_1205	**	4.75E-10		13386_815	***	2.55E-06
	1181_710	***	0.04		10941_471	**	9.81E-09	Leaf petiole length	1003_1507	***	4.96E-07
	10811_937	***	0.01		10969_452	**	9.81E-09		13386_815	***	4.96E-07
	13693_225	***	1.14E-03		3194_319	**	9.81E-09		1181_710	***	5.34E-08
	13551_722	***	1.43E-04		12261_1773	**	3.21E-10		1101_164	***	5.45E-09
	13747_573	***	2.23E-03		11303_254	**	1.29E-09		12439_253	***	5.39E-08
	10466_465	***	1.75E-08		11557_405	**	1.75E-10	Leaf pigmentation	1181_710	*	1.67E-09
	10661_873	***	1.75E-08		11564_339	**	1.75E-10		1003_1507	*	1.72E-07
	13281_572	***	1.75E-08		12185_1210	**	1.75E-10		13386_815	*	1.72E-07
	12568_234	***	2.79E-06		10630_719	**	5.45E-10	Number of nodes	1003_1507	***	2.82E-06
	13652_572	***	2.79E-06		12324_917	**	9.14E-09		13386_815	***	2.82E-06
	12363_196	***	9.80E-09		1339_101	**	9.14E-09		1181_710	***	1.39E-06
	13764_285	***	9.80E-09		13063_1329	**	1.41E-02	Pod curvature	12439_253	*	2.11E-08
	10780_756	***	9.52E-09		1278_268	*	7.69E-11	Pod length	1003_1507	***	7.63E-03
	11054_889	***	9.52E-09		13665_260	*	2.08E-10		13386_815	***	7.63E-03
	10384_1814	***	1.08E-08		12712_158	*	3.33E-09		13294_282	*	1.27E-05
	10974_245	***	1.08E-08		131_524	*	3.33E-09	Number of pods	1181_710	***	3.47E-08
	12096_1122	***	1.08E-08		10650_1563	*	9.25E-10		11484_541	*	7.07E-08
	12731_670	***	1.08E-08		11371_619	*	3.84E-10		12183_298	*	7.07E-08
	12918_1176	***	1.08E-08		12991_691	*	5.85E-11	Number of pod per peduncle	1101_164	***	1.80E-08
	11138_624	***	2.96E-08		11613_1075	*	4.04E-10		1003_1507	***	3.17E-08
	11770_1166	***	2.96E-08		11622_232	*	4.04E-10		13386_815	***	3.17E-08
	10500_1030	***	1.04E-09		11854_589	*	4.04E-10		1181_710	**	8.72E-09
	11233_1096	***	1.04E-09		13402_898	*	4.04E-10		1101_164	***	2.44E-07

	11783_1366	***	1.04E-09		15385_870	*	7.37E-11	Pod weight	1181_710	***	2.31E-07
	4780_374	***	1.04E-09		12925_1154	*	9.16E-11		1003_1507	***	1.67E-08
	10378_737	***	5.04E-09		10172_1642	*	1.49E-10		13386_815	***	1.67E-08
	11043_1347	***	5.04E-09		13709_659	*	1.49E-10	Seed length	1101_164	***	1.64E-07
	11118_399	***	2.71E-03		12023_173	*	5.48E-09		1181_710	***	1.36E-07
	10331_516	**	3.79E-09		12029_2782	*	5.48E-09	Seed thickness	1101_164	***	3.93E-08
	11_533	**	3.79E-09		10905_418	*	9.99E-11		12439_253	***	8.02E-09
	12428_146	**	3.79E-09	100 Seed weight	1181_710	**	5.42E-08	Seed weight /plant	1003_1507	*	1.84E-09
	13080_585	**	3.79E-09	No of days to 50% flowering	1181_710	***	1.20E-05		13386_815	*	1.84E-09
	10738_1400	**	7.61E-10		1003_1507	***	1.20E-05		1181_710	*	1.84E-10
	13005_643	**	7.61E-10		13386_815	***	1.20E-05		1101_164	*	3.62E-09
	12393_305	**	1.92E-09	Immature Pod pigmentation	11484_541	*	1.02E-11	Terminal leaf length	1181_710	**	2.88E-09
	10279_316	**	3.56E-09		12183_298	*	1.02E-11		12439_253	**	1.76E-09
	11961_645	**	3.56E-09	Cercoscophora Severity	1101_164	***	1.02E-06		1003_1507	**	1.72E-08
	12037_561	**	3.56E-09		12439_253	***	4.28E-07		13386_815	**	1.72E-08
	12959_58	**	3.56E-09		1181_710	**	1.90E-08	Terminal leaf width	1003_1507	***	8.25E-09
	13187_984	**	3.56E-09	Physiological maturity	12439_253	***	1.67E-08		13386_815	***	8.25E-09
	13553_1085	**	3.56E-09	Petiole pigmentation	1181_710	***	3.58E-09		1101_164	***	3.25E-09
	13563_863	**	5.36E-08		1003_1507	**	2.83E-09		12439_253	**	1.26E-09
	1281_790	**	5.79E-10		13386_815	**	2.83E-09				

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, R^2: Marker fraction of the total phenotypic variation explained

ACKNOWLEDGEMENT

The Authors acknowledged Generation Challenge Programme, Mexico for funding the genotyping of the cowpea lines at LGC genomics. UK

REFERENCES

Akande, S.R., Olakojo, S.A., Owolade, O.F., Adetumbi, J. A., Adeniyan, O. N., and Ogunbodede, B. A. (2012) Planting Date Affects Cowpea Seed Yield and Quality at Southern Guinea Savanna, Nigeria. *Seed Technology,* 34: 79 – 88.

Badiane, F.A., Gowda, B.S., Cisse, N., Diouf, D., Sadio, O. and Timko, M.P. (2012) Genetic relationship of cowpea (*Vigna unguiculata*) varieties from Senegal based on SSR markers. *Genetics and Molecular Research,* 11: 292-304.

Bradbury, P. J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S. (2007) TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics,* 23: 2633-2635.

Bartholome J., Blink M.C, Van Heerwaarden J, Chancerel E, Boury C, Lesur I, Isik F, Bouffier L and Plomion C. (2016) Linkage and association mapping for two major traits used in the maritime pine breeding program: height growth and stem straightness. *PLos ONE* 11: e0165323.doi:10.1371/journal.pone.0165323.

Collard B.C.Y. and Mackill D. J. (2008) Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society* B 12 (363): 557-572.

Egbadzor K. F,, Yeboah M, Danquah E.Y., Ofori K. and Offei S. K., (2013) Identification of SNP Markers associated with seed size in Cowpea (*Vigna unguiculata* (L) Walp). *International Journal of Plant Breeding and Genetics* 7: 115-123.

Eu-ahsunthornwattana J, Miller E. N., Fakiola M., Jeronimo, S.M.B., Blackwell J. M. and Cordell H. J. (2014) Comparison of Methods to Account for Relatedness in Genome-Wide Association Studies with Family Based Data. *PLoS Genetics.* 10:e1004445.doi:10.1371/journal.pgen.1004445

Evanno G, Reganut E and Goudet J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology Notes* 14: 2611-2620.

Huynh B-L, Close T.J, Roberts P. A, Hu Z, Wanamaker S, Lucas M. R, Chiulele R, Cisse N, David A, Hearne S, Fatokun C, Diop NN. and Ehlers J. D. (2013) Gene Pools and the Genetic Architecture of Domesticated Cowpea. *The Plant Genome* 6: 1-8.

International Board for Plant Genetic Resources (IBPGR) (1983) Cowpea Descriptors. Rome, Italy. 29p

Jannink, J. L., M.C. Bink and R.C. Jansen. (2001) Using complex plant pedigrees to map valuable genes. *Trends Plant Sci*. 6:337

Kumar L . (2009) Methods for the diagnosis of Plant Virus diseases, Laboratory Manual. IITA. p. 94.

Liu K and Muse SV (2005) PowerMaker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21: 2128-2129.

Lucas MR, Huynh B-L, Vinholes P., Cisse N., Drabo I, Ehlers J.D., Roberts P.A. and Close T.J. (2013) Association studies and legume synteny reveals haplotypes determining seed size in *Vigna unguiculata. Frontiers in Plant Science* 4 (96): 1-9.

Massman J, Cooper B, Horsley R, Neate S, Dill-Macky R, Chao S, Dong Y, Schwarz P, Muehlbauer G. J and Smith K. P (2011) Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. *Molecular Breeding* 27:439–454.

Muchero W, Roberts P.A, Diop N.N, Drabo I, Cisse N, Close T. J, Muranaka S, Boukar O and Ehen J. D. (2013) Genetic architecture of delayed senescence, biomass, and grain yield under drought stress in cowpea. *PLoS ONE* **8**: E70041. doi:10.1371/journal.pone.0070041.

Patil D. M, Sawardekar S. V, Gokhale N. B, Bhave S. G, Sawant S. S, Sawantdesai S. A, Lipne K. A, Sabale S. N and Joshi S. N. (2013) Genetic diversity analysis of cowpea (*Vigna unguiculata* (L.) Walp.) by using RAPD markers. *International Journal of Innovative Biotechnology and Biochemistry* 1: 15-23.

Rathi S, Pathak K., Yadav R.N.S, Kumar B and Sarma R.N. (2014) Association studies of dormancy and cooking quality traits in direct-seeded *indica* rice. *Journal of. Genetics* 93: 1-10.

Shamsi S and Najmun N (2015) Disease severity and mycoflora associated with anthracnose on leaves of five angiosperms. *Bangladesh Journal of Science Research* 28: 103-111.

Shi A, Buckley B, Mou B, Motes D, Morris J. B, Ma J, Xiong H, Qin J, Yang W, Chitwood J, Weng Y, and Lu W (2016) Association analysis of cowpea bacterial blight resistance in USDA cowpea germplasm. *Euphytica* 208: 143-155.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30**:** 2725-2729.

Varshney R. K, Nayak S. N, May G. D and Jackson S. A. (2009) Next generation sequencing technologies

and their implications for crop genetics and breeding. *Trends in Biotechnology* 27: 522-530.

Wamalwa E. N, Muoma J and Wekesa C. (2016) Genetic diversity of cowpea (*Vigna unguiculata* (L.) Walp.) accession in Kenya Gene Bank Based on Simple sequence repeat markers. *International Journal of Genomics.* Article ID 8956412, 5 pages http://dx.doi.org/10.1155/2016/8956412.

Xiong H, Shi A, Mou B, Qin J, Motes D, Lu W, Ma J, Weng Y, Yang W and Wu D. (2016) Genetic diversity and population structure of cowpea (*Vigna unguiculata* L. Walp). *PLoS ONE.* 11: e0160941.doi:10.1371/journal.pone.0160941

Xu Y and Crouch J. H. 2008. Marker-assisted selection in plant breeding: from publications to practice. *Crop Science* 48:391-407

Zhang D. L, Hao C. Y, Wang L. F and Zhang X. Y. (2012) Identifying loci influencing grain number by microsatellite screening in bread wheat (*Triticum aestivum* L.). *Planta* 236:1507-1517

Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GL, Islam MR, Reynold A, Mezey J, McClung A. M, Bustamante C. D and McCouch S.R. (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. *Nature Communications* 2:467

Zila C. T, Samayoa L. F, Santiago R, Butrón A and Holland J. B. (2013) A genome-wide association study reveals genes associated with Fusarium ear rot resistance in a maize core diversity panel. *G3* 3 :2095-2104.

*****