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Molecular Genetic Studies and QTL Mapping for Aflatoxin Resistance in Selected Groundnut (*Arachis hypogaea* L.) Breeding Lines

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Abstract

Molecular genetic studies and QTL mapping for aflatoxin resistance in selected groundnut breeding lines were undertaken. One hundred (100) parental breeding lines and eleven (11) progenies in the F2 generation were screened pathologically. Healthy accessions were subjected to in vitro *Aspergillus* seed invasion test. A total of 500 molecular markers (215 SSRs, 205 SNP/EST and 80 RAPD markers) were screened for polymorphism on the parental lines followed by the use of polymorphic markers on the F2 lines. Phenotypic and genotypic data were loaded into Minitab 16.0 and ICIM QTLIciMapping 4.2 software for cluster analysis and QTL linkage map construction respectively. In total, 23 breeding lines were identified as promising *Aspergillus* resistant genotypes. SAMNUT 22 X ICGV-91324 progeny was unique in genetic make-up using all co-dominant markers. Some parents closely associated with resistant F2 genotypes were: ICGV-94379, ICGV-IS-13878, ICGV-IS-13875 and SAMNUT 25. QTL map constructed was 344.32cM long where the various markers are positioned at intervals. A total of 8 markers and QTLs were identified as the most likely linked to the polygenes controlling aflatoxin resistance. Five linkage groups are located at position 124.45cM on the map and they are: IPAHM39 (position 124.45cM), Y70 (position 124.45cM), GA101 (position 124.45cM), EX24 (position 124.45cM), GM1076 (position 124.45cM), GA5 (position 34.48cM), MS120 (position 34.48 cM) and GM2165 (position 344.32 cM). The highlighted groundnut genotypes and markers in this report are recommended in groundnut breeding for aflatoxin resistance in the quest to achieving safe groundnut consumption globally.

Keywords

Aflatoxin Resistance, Groundnut Breeding, Polymorphic Markers, QTL Mapping

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1. Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is an important leguminous food crop valued as a rich source of energy as contributed by oil (48-50%) and protein (25-28%) in the kernels [1, 2]. The crop is cultivated annually on about 24.63 million hectares worldwide with annual production of 41.27 million tons. Nigeria is the highest producer of groundnut in Africa [3]. Despite the economic, social and cultural importance of groundnuts, its productivity and consumption are severely challenged by several biotic factors such as viruses, bacteria, fungi and nematodes, aphids, thrips, leaf miner, red hairy caterpillar and jassids. Termites, white grubs, and storage pests also cause damage to groundnuts. Groundnut weevil and rust-red flour beetle are the major storage insect pests damaging seed. In most breeding

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programs across the world, breeding for resistance to aforementioned pest and diseases have received attention and successes have been achieved due to the availability of resistant breeding lines for diseases in cultivated and wild *Arachis* species [4].

A trending unresolved enigma in groundnut consumption is the presence of mycotoxin called aflatoxin. Mycotoxins cause immunological effects, specific organ damage, cancer, and in some cases, death [4]. Agricultural workers may also suffer from skin and respiratory exposure during crop harvest and storage. Mycotoxin poisoning is a global problem associated with maize, rice, tree nuts and peanuts [4]. Aflatoxins are highly toxic and carcinogenic compounds majorly produced by the fungus, *Aspergillus flavus* during growth of peanut and other crops such as maize [5]. For a long time, aflatoxin contamination in peanut was spuriously considered as a post-harvest problem. The recommended cultural practices and integrated approaches have failed to solve the problem till date. The uptake of molecular breeding approaches in groundnut improvement programs in many African countries is slow due to inadequate infrastructure, high genotyping costs, and human capacities. ICRISAT (International Crop Research Institute for the Semi-Arid Tropics) had made laudable achievements in establishing some breeding lines of groundnut aimed at improving groundnut for yield and other abiotic stresses in Nigeria. Aflatoxin resistance is controlled by polygenes, hence QTL (Quantitative Trait Loci) mapping has been successfully done on maize and this has provided a breakthrough in maize research all over the world.

The aim of this work was to undertake genetic studies of aflatoxin resistance among groundnut breeding lines using molecular approaches. The objectives were to: screen groundnut breeding lines for resistance and susceptibility to aflatoxigentic fungi (field and laboratory); screen molecular markers (SSR, RAPD, SNP/EST) for genetic polymorphism; construct SSR and SNP/EST-based genetic linkage map; identify possible QTLs for aflatoxin resistance; and recommend groundnut breeding lines that are resistant to *Aspergillus flavus* among the whole germplasm.

2. Methodology

2.1. Study Area

Field experimental site was established within the Federal University of Agriculture Makurdi (FUAM) campus along Gbajimba Road as approved by the University management. The University is located in the Northern part of Makurdi Local Government Area of Benue State (Latitude 07° 45.53'N; Longitude 008° 37.41'E). The University falls within a typical Southern Guinea Savannah agro ecological zone. Benue State is popularly known as "the food basket of the nation". Agricultural activities are high in the study area and farmers rely on the research activities of the University as sources of free seeds for their farm work.

2.2. Assemblage of Germplasm

One hundred (100) parental breeding lines of groundnut accessions (germplasm) were sourced from the ICRISAT, Nigeria. The germplasm constituted genotypes that were undergoing genetic improvement for different traits with unknown history of aflatoxin resistance and susceptibility. Eleven (11) progenies in the F2 generation breeding lines of aflatoxin resistance were also used. Total plant materials were 111 and coded G1-G111.

2.3. Field Preparation, Design and Seed Multiplication

Field was cleared and 30 long ridges of 35m each were constructed. Inter ridge distance was 0.75m. Size of experimental site was calculated using the formulae: Farm size = Inter-ridge distance x number long of ridges x length of each ridge (0.75 x 30 x 35) = 787.5m^2. Field experiment was a Randomized Complete Block Design. A total of 111 genotypes were planted, three seeds per genotypes in two blocks following randomized sequence. The genotypes consisted of 100 parental lines and 11 F2 progenies.

2.4. Disease Screening of 100 Parents and 11 F2 Progenies in the Field

Plants were properly taken care of by weeding and application of fertilizers. Plant stands were monitored from juvenile stage for the incidence of Crown rot disease of *Aspergillus* to maturity and for the presence (1) or absence (0) of any other known fungal disease using a standard coloured chart. Genotypes that recorded no visible traces of any fungal infection among the three replicates in the two blocks were selected as being disease free after harvesting.

2.5. Molecular Studies

2.5.1. DNA Extraction and Purification

DNA was collected from 15-day old seedlings using the FTA™ Plant Card (Whatman®). Protocol adopted in [6] was used in DNA extraction and purification through the use of FTA purification reagent. Finally, tubes containing DNA discs were air dried for 2 hours and preserved at -20°C.

2.5.2. SSR, SNP/EST and RAPD Based Polymerase Chain Reaction

215 SSR (Simple Sequence Repeats), 205 SNP/EST (Single Nucleotide Polymorphic/Expressed Sequence Taqs) 80

RAPD (Random Amplified Polymorphic DNA) were used. Amplification was done on a thermal cycler (Applied Biosystem in Life Technology 2720 Model). Groundnut SSR protocol reported by [7] was finally adopted after series of temperature/time optimization. Initial denaturation was programmed at 94°C followed by 35 cycles of 94°C for 30 seconds, annealing temperature at 59°C for 60 seconds, and extension temperature at 72°C for 2 minutes, and final extension at 72°C for 20 minutes. Temperature was held at 4°C. SNP, EST and RAPD PCRs were carried out with slight modifications of SSR temperature gradient using the protocol adopted by [8] and [9] after proper optimization.

2.5.3. Agarose and Polyacrylamide Gel Electrophoresis

PCR products were resolved on both agarose and polyacrylamide gel stained with ethidium bromide using a horizontal gel electrophoresis system. After electrophoresis, banding patterns of DNA were viewed by placing gel on a bench top UV transilluminator with UV blocking protective cover. Image of DNA bands were captured using digital camera for scoring.

2.5.4. Selection of Polymorphic Markers

Percentage polymorphism was calculated as the total number of polymorphic bands divided by the total number of bands generated by each marker multiplied by 100 [10]. Markers that scored >70% polymorphism were considered highly polymorphic and they were selected for further genetic work on *Aspergillus* resistant F2 progenies.

2.6. Testing for *Aspergillus* Resistance on Selected Disease Free Lines

Aflatoxin screening of disease free genotypes was done indirectly by evaluating the natural presence of *Aspergillus* species in seeds of harvested accessions. Twenty (20) healthy seeds were randomly picked from each of the 27 screened accessions and were examined for infection by *Aspergillus* species following ICRISAT procedures as outlined in [11]. The seeds were surface sterilized by soaking in 0.1% aqueous solution of $HgCl_2$ for 3 minutes, rinsed in sterilized water before plating in petri dishes containing Czapek-Dox agar with rose Bengal and streptomycin. Incubation of plates was done at 25°C for 7 days. Fungal colonies observed on the seeds were recorded. Number and % of infected of colonized seeds were recorded.

2.7. Use of the Polymorphic Markers on F2 Progenies

Genotyping of 10 selected F2 progenies was carried out using the resulting polymorphic markers consisting of 13 SSR and 11 SNP/EST markers. Genotyping was also done on all resistant breeds was using 11 polymorphic RAPD primers. PCR protocols were adhered to as outlined above.

2.8. Data Analysis

Dendrograms of SSR and SNP based genetic markers were constructed using the Average Linkage method measured on Euclidean similarity using Minitab 16.0 software. Each of these markers were analysed in the resistant F2 progenies. Dendrograms of RAPD marker was constructed using the Ward method measured on Manhattan distance by considering the relatedness and diversity among all resistant breeds. Construction of genetic map and identification of marker gene were done using the genotypic data obtained from F2 progenies and the polymorphic co-dominant markers (SSR and SNP) [12, 13]. ICIM QTLIciMapping 4.2 Version software was used for Genetic Linkage Map Construction. Chromosome width was set at 10.0 cM. A minimum LOD score of 5.0 was used [13] for the identification of putative QTLs. The length of the Chromosome was determined in centiMorgan(cM). The Grouping function had a Threshold value done by Recombination frequency set at 0.3. The Ordering function was set at k-Optimality followed by Ripping and Outputting.

3. Results and Discussion

Out of 100 parental lines, 17 (17%) accessions were screened to be healthy as given in table 1. They did not show any physical manifestation of crown rot seedling disease of *Aspergillus* nor any other known disease of groundnut. The 17 parental genotypes were good (5/5) in the assessment of overall plant health and they produced good quality seeds in the pods. There were no traces of seed contamination. However, 83 (83%) lines recorded between poor to intermediate health status and were screened out. Out of 11 F2 progenies, 10 (90.9%) were screened to be healthy as given in table 2. They did not show any physical manifestation of crown rot seedling disease of *Aspergillus* nor any other known disease of groundnut. The ten F2 progenies were good (5/5) in the assessment of overall plant health and they produced good quality seeds in the pods. There were no traces of seed contamination. Only one progeny (SAMNUT 24 X ICGV-91324) had intermediate health status and it was screened out. The observed differences in plant health status could have genetic basis [14, 15]. This affects seedlings vigour and overall plant performances [16].

A. flavus is most commonly associated with aflatoxin contamination of susceptible groundnut crops [17]. To address this problem, the use of *Aspergillus* resistant crops has been suggested where selection is done using traditional

genetic methods with molecular marker-assisted selection or by direct genetic modification [18]. Conventional plant breeding using molecular markers are being used to select for genes conferring resistance to *Aspergillus* infection. Among the 17 healthy parental genotypes earlier selected, only 13 (76.5%) were resistant to *Aspergillus* invasion (table 2). This represents 13% of the given population of 100 parental lines. They are: ICGV-IS 13858, ICGV-IS 13874, ICGV-IS 13875, ICGV-IS 13878, ICGV-IS 13881, ICGV-IS 13896, ICGV-IS 13911, ICGV 94379, ICGV 91317, SAMNUT 24, SAMNUT 23, SAMNUT 25 and ICGV-IS 13828. However, ICGV-IS 13955 was the most susceptible genotype recorded with 25% seed colonization by *Aspergillus*. Among the resistant parents, SAMNUT 23, SAMNUT 24 and SAMNUT 25 are crucial in producing F2 resistant lines. All the 10 healthy F2 genotypes earlier selected in the field were found to be *Aspergillus* resistant, which represent 90.9% of the overall F2 population. They are SAMNUT 23 X ICGV-91324, SAMNUT 25 X ICGV-91317, SAMNUT 26 X ICGV-91324, SAMNUT 26 X ICGV-91328, SAMNUT 22 X ICGV-91324, SAMNUT 22 X ICGV-91328, SAMNUT 26 X ICGV-91319, SAMNUT 24 X ICGV-91317, SAMNUT 25 X ICGV-91328 and SAMNUT 24 X ICGV-91328.

In this work, a total of 35 markers representing 7% of screened markers passed the test and were selected (table 3). Thirteen (13) SSR markers were selected as highly polymorphic and they represent 6.05%. Among them, AH4-101 had the highest % polymorphism (94%) followed by GA5 (91%). Among the SNP/EST markers, 11(5.37%) were selected as highly polymorphic. MS-64 was the most polymorphic marker (94%) followed by CP-201/202 marker (92%). Among the RAPD markers, 11(13.75%) were polymorphic and selected but the highest % polymorphism recorded was 81% as observed in OPU15. This result is consistent with previous reports on the polymorphic nature of some of these markers in other crops [19].

Dendrogram of F2 lines using SSR marker (figure 1) gave a divergent relationship. Genetic similarity ranged between 39.45-51.7 maximum indicating wide gene pools among the offsprings. This probably accounted for their resistance to *Aspergillus* invasion hence aflatoxin resistant nature. The most distant among them were SAMNUT 24 X ICGV-91328 offspring, SAMNUT 22 X ICGV-91328 offspring and SAMNUT 22 X ICGV-91324 offspring. Plate 1 represents the gel images of selected SSR primers that amplified the DNAs of the F2 genotypes.

Dendrogram of F2 lines using SNP/EST markers (figure 2) gave a divergent relationship (but closer to some extent). Genetic similarity ranged between 26.62-41.17 maximum which also indicate wide gene pools among the offsprings. Moreover, genetic distance is longer in SNP/EST (26.62) marker than in the SSR (39.45). About 6 out of 10 lines displayed some levels of close relationships at one point or the other. However, the genetic divergence of SAMNUT 25 X ICGV-91328 was noticeable among the clusters. The most outstanding offspring in the SNP/EST genetic make-up in terms of genetic distance and divergence was the SAMNUT 22 X ICGV-91328 hybrid which was also among the three unique offsprings identified with SSR markers concerning aflatoxin resistance trait. Plate 2 represents selected gel images of the F2 hybrids using SNP/EST markers.

The RAPD marker based genetic diversity among all the 23 resistant lines (parents and offsrings) identified so far is presented in figure 3. There are 5 sub sub-clusters (1-5 from L-R respectively), all branching from two main clusters. Each of the sub clusters has mixes of parent-offspring genotype except sub cluster 2 where all the 5 genotypes are all parents. Sub cluster 1 is made up of 4 F2 lines and 1 parent. These mixes are an indication of closeness in genetic relationship in certain traits as confirmed in the genetic similarity level of 87.5 in some clustered genotypes. However, main cluster 1 clearly circumscribes between some unique parents and hybrid genotypes. The parents include: ICGV-IS 13896 (R), SAMNUT 23 (R), ICGV 91317 (R), SAMNUT 24 (R) and ICGV-IS 13828, all resistant to *Aspergillus* phenotypically. The circumscribed offsprings are: SAMNUT 26 X ICGV-91328, SAMNUT 24 X ICGV-91328, SAMNUT 25 X ICGV-91317 and SAMNUT 25 X ICGV-91328. In sub cluster 3, a close relationship was observed between SAMNUT 22 X ICGV-91328 (the genetically distinct offspring) and SAMNUT 23 X ICGV-91324.

The potential advantages of the SSR/SNP/EST molecular markers over the RAPD markers are the feasibility to target multiple traits for improvement and provide tools to tap new alleles from wild species having understood that aflatoxin resistance is a polygenic trait. These markers are specific, co-dominant (distinguishes heterozygous loci), reproducible and highly sensitive [20, 21]. The integration of molecular breeding approaches offers many advantages. It provides tools to target traits of economic importance that remained poorly or not amenable to conventional breeding approaches in part due to their quantitative nature of inheritance. The identification of genomic regions, popularly called QTLs for quantitative traits is now routine in molecular breeding [15, 22, 23]. QTL provides the most promising long term protection against aflatoxin crop pollution [24].

Table 4 gives the statistics of QTL parameters. Figure 4 displays the actual genetic linkage map of aflatoxin resistance constructed for groundnut based on the SSR, SNP and EST polymorphic markers. The linkage map located on chromosome 1 in the genome is 344.32cM (centiMorgan) long. The position of the markers on the chromosome shows

that TC3E05 occupies the lowest position of 0.00 cM followed by a group of marker genes including MA114, GA5 and MS120 that occupies position 32.48cM on the map. The farthest markers on the map are Y60 and GM2165 that occupy position 344.32 cM. There are 3 clustered groups of markers occupying about the same position on the map. Two groups appear before the centromere including position 32.48 cM harbouring 3 markers at a locus and 124.05 cM harbouring 6 markers at same locus while a clustered group is positioned after the centromere at 344.32cM. The chromosome clearly shows the relative position of markers on the linkage map constructed for aflatoxin resistance in groundnut in ascending order of their positions.

QTL mapping is essentially a set of procedures used to detect and locate quantitative trait loci [13]. It identifies and locates potential genes controlling a quantitative trait using the expected association between putative genes and the known genetic markers. It is a combination of linkage mapping and quantitative genetics approaches to find an association between genetic marker and a phenotype that can be measured [12, 25]. The roles of molecular markers in genetic mapping cannot be overemphasized [13] enabling the construction of genetic Linkage maps of many plant species which were limited in size until the advent of molecular mapping. The lower the recombination frequency, the greater the chances that a given marker will be close to a gene of interest [26]. In this work, a total of 8 markers and QTLs were identified as the most likely linked to the polygenes controlling aflatoxin resistance with a recombination frequency of 0.000. Five linkage groups are located at position 124.45cM on the map and they are: IPAHM39, Y70, GA101, EX24 and GM1076, a combination of simple sequence repeats, single nucleotide polymorphism and expressed sequence tags markers. Others are GA5, MS120 (located at position 34.48 cM) and GM2165 located at the farthest end of the map (344.32 cM). The arrangement of the markers based on their relative recombination frequency is shown in figure 5 where TC3E05 marker had RF of 50% thus very unlikely to be linked to gene controlling aflatoxin.

Table 1. Preliminary Pathological screening of Groundnut Genotypes.

Genotype	Total screened in the field	Number and % infected	Number and % non infected
Parents	100	83 (83%)	17 (17%)
F2 progenies	11	1(8.1%)	10 (90.9%)
Total	111	84 (75.68%)	27(24.32%)

Table 2. In vitro Screening for *Aspergillus* growth on seeds selected disease free genotypes.

Accession code	Groundnut genotype	Generation	Number and % of infected of colonized seeds	Susceptibility profile
G3	ICGV-IS 13856	Parental	2 (10%)	S
G5	ICGV-IS 13858	Parental	0 (0%)	R
G10	ICGV-IS 13874	Parental	0 (0%)	R
G11	ICGV-IS 13875	Parental	0 (0%)	R
G12	ICGV-IS 13877	Parental	4 (20%)	S
G13	ICGV-IS 13878	Parental	0 (0%)	R
G14	ICGV-IS 13881	Parental	0 (0%)	R
G17	ICGV-IS 13896	Parental	0 (0%)	R
G20	ICGV-IS 13911	Parental	0 (0%)	R
G29	ICGV-IS 13955	Parental	5 (25%)	S
G30	ICGV-IS 13971	Parental	1 (5%)	S
G68	ICGV-IS 13967	Parental	6 (30%)	S
G70	ICGV 94379	Parental	0 (0%)	R
G71	ICGV 91317	Parental	0 (0%)	R
G73	SAMNUT 24	Parental	0 (0%)	R
G80	SAMNUT 23	Parental	0 (0%)	R
G81	SAMNUT 25	Parental	0 (0%)	R
G97	ICGV-IS 13828	Parental	0 (0%)	R
G101	SAMNUT 23 X ICGV-91324	F2	0 (0%)	R
G102	SAMNUT 25 X ICGV-91317	F2	0 (0%)	R
G103	SAMNUT 26 X ICGV-91324	F2	0 (0%)	R
G104	SAMNUT 26 X ICGV-91328	F2	0 (0%)	R
G105	SAMNUT 22 X ICGV-91324	F2	0 (0%)	R
G107	SAMNUT 22 X ICGV-91328	F2	0 (0%)	R
G108	SAMNUT 26 X ICGV-91319	F2	0 (0%)	R
G109	SAMNUT 24 X ICGV-91317	F2	0 (0%)	R
G110	SAMNUT 25 X ICGV-91328	F2	0 (0%)	R
G111	SAMNUT 24 X ICGV-91328	F2	0 (0%)	R

S=susceptible to *Aspergillus* R=resistant to *Aspergillus*.

Table 3. Statistics of Polymorphic Markers.

Marker type	Total screened	polymorphic markers	% Polymorphic markers	Highest % Polymorphism
SSR	215	13	6.05	94% AH4-101
RAPD	80	11	13.75	81% OPU15
SNP/EST	205	11	5.37	94% MS-64
Total	500	35	7	

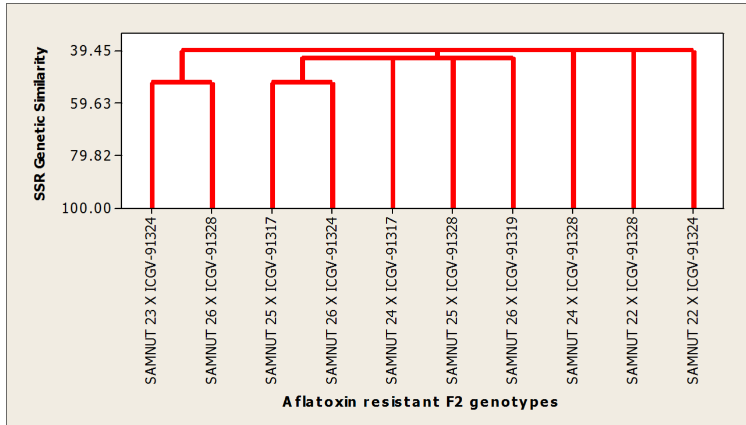

Figure 1. Dendrogram of Aflatoxin resistant resistant F2 genotypes based on polymorphic SSR markers.

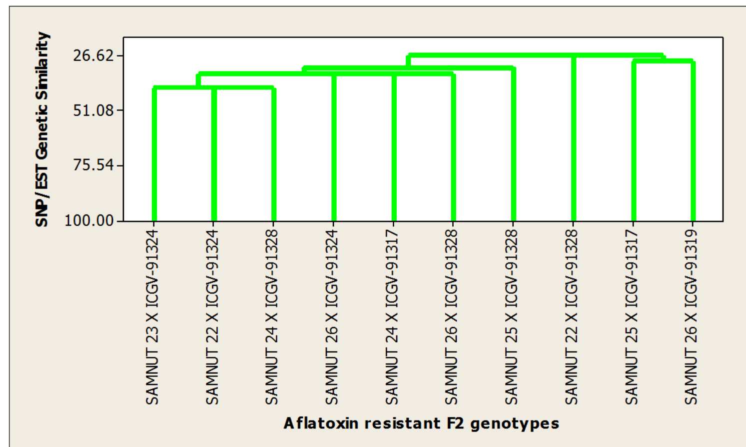

Figure 2. Dendrogram of Aflatoxin resistant resistant F2 genotypes based on polymorphic SNP/EST markers.

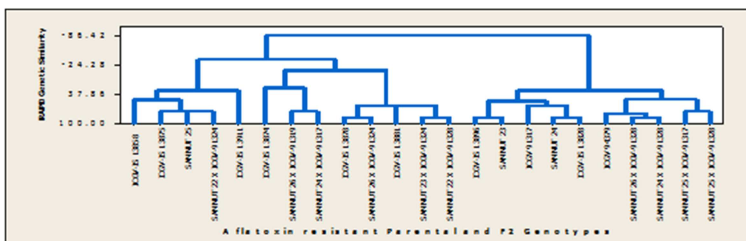

Figure 3. Genetic diversity among all resistant genotypes based on polymorphic RAPD markers.

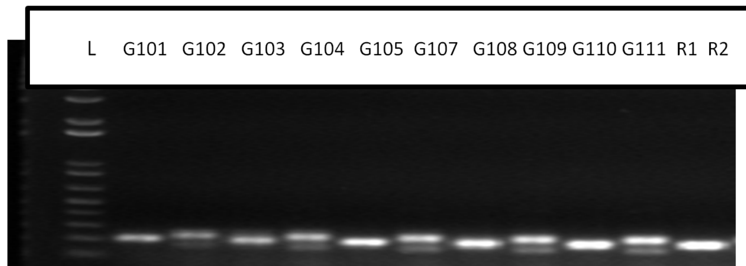

Plate 1. Amplification of F2 resistant genotypes using SSR primer GA101.

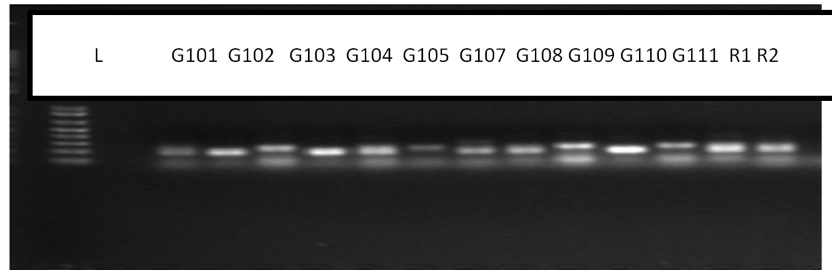

Plate 2. Amplification of F2 resistant genotypes using SNP primer CP-201/202.

L= 100bp ladder
G101= SAMNUT 23 X ICGV-91324
G102= SAMNUT 25 X ICGV-91317
G103= SAMNUT 26 X ICGV-91324
G104= SAMNUT 26 X ICGV-91328
G105= SAMNUT 22 X ICGV-91324
G107= SAMNUT 22 X ICGV-91328
G108= SAMNUT 26 X ICGV-91319
G109= SAMNUT 24 X ICGV-91317
G110= SAMNUT 25 X ICGV-91328
R1, R2= Reference

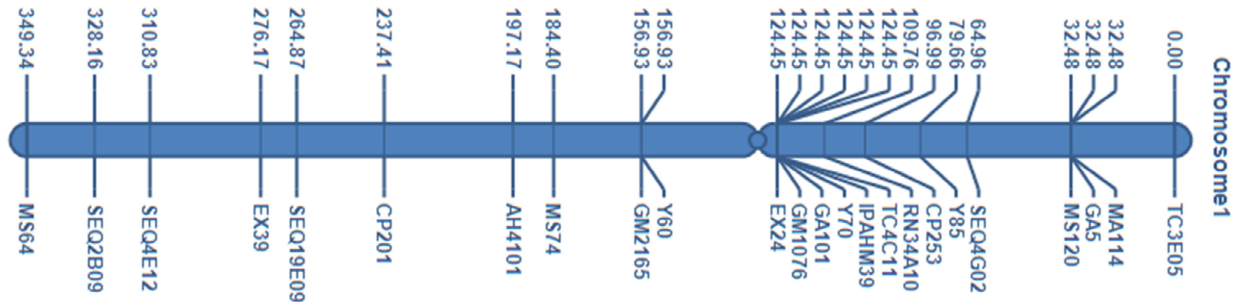

Figure 4. Genetic Linkage Map of Aflatoxin Resistance Constructed for Groundnut.

Table 4. QTL Map Parameters and Statistics.

Chromosome Name	Marker Name	Interval (cM)	Position (cM)	Recombination Frequency	Interference	Chromosome Length (cM)
Chromosome1	TC3E05	0.0000	0.0000	0.5000	0.0000	344.3234
	MA114	32.4821	32.4821	0.2857	0.0000	
	GA5	0.0000	32.4821	0.0000	0.0000	
	MS120	0.0000	32.4821	0.0000	0.0000	
	SEQ4G02	32.4821	64.9641	0.2857	-2.2083	
	Y85	14.6947	79.6588	0.1429	3.5000	
	CP253	17.3287	96.9875	0.1667	4.2857	
	RN34A10	12.7706	109.7581	0.1250	1.5000	
	TC4C11	14.6947	124.4528	0.1429	0.0000	
	IPAHM39	0.0000	124.452	0.0000	0.0000	
	Y70	0.0000	124.4528	0.0000	0.0000	
	GA101	0.0000	124.4528	0.0000	0.0000	
	EX24	0.0000	124.4528	0.0000	0.0000	
	GM1076	0.0000	124.4528	0.0000	0.0000	
	MS64	27.4653	151.9181	0.2500	1.5000	
	SEQ2B09	21.1824	173.1006	0.2000	3.8333	
	SEQ4E12	17.3287	190.4292	0.1667	1.3333	
	EX39	34.6574	225.0866	0.3000	-1.8333	
	SEQ19E09	11.2996	236.3862	0.1111	2.5000	
	CP201	27.4653	263.8515	0.25000	1.2500	
	AH4101	40.2360	304.0875	0.3333	-1.5000	
	MS74	12.7706	316.8581	0.1250	1.0000	
	Y60	27.4653	344.3234	0.2500	0.0000	
	GM2165	0.0000	344.3234	0.0000	0.0000	

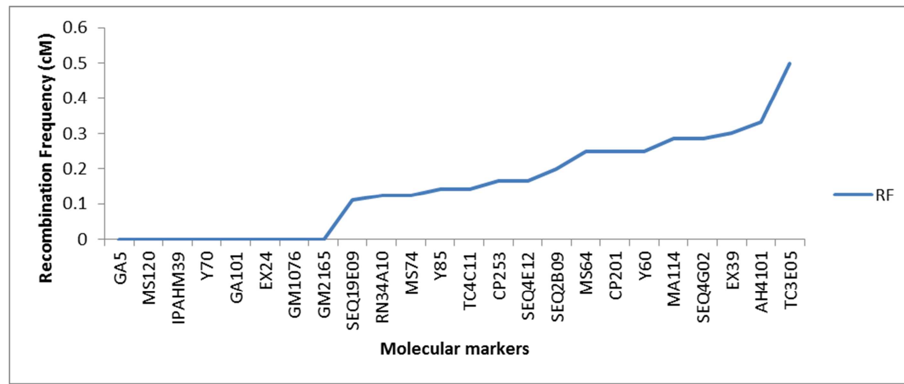

Figure 5. Recombination Frequency of markers on the linkage map

4. Conclusion

In total, 23 breeding lines have been identified as promising genotypes for afalatoxin resistance. SAMNUT 22 X ICGV-91324 progeny was unique in genetic make-up using all co-dominant markers. Some parents closely associated with resistant F2 genotypes were: ICGV-94379, ICGV-IS-13878, ICGV-IS-13875 and SAMNUT 25. QTL map constructed is 344.32cM long where the various markers are positioned at intervals. A total of 8 markers and QTLs are identified as most likely linked to the polygenes controlling aflatoxin resistance. Five linkage groups are located at position 124.45cM on the map and they are: IPAHM39 (position 124.45cM), Y70 (position 124.45cM), GA101 (position 124.45cM), EX24 (position 124.45cM), GM1076 (position 124.45cM), GA5 (position 34.48cM), MS120 (position 34.48 cM) and GM2165 (position 344.32 cM). The above highlighted groundnut genotypes and markers are recommended in groundnut breeding for aflatoxin resistance in the quest to achieving safe groundnut consumption globally.

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References

[1] Jambunathan, R. (1991). Groundnut quality characteristics. In: Uses of Tropical Grain Legumes. *Proceedings of a Consultants Meeting, ICRISAT*, 267-275.

[2] Atasie, V. N., Akinhanni, T. F., Ojiodu, C. C. (2009). Proximate Analysis and Physico-chemical properties of groundnut (*Arachis hypogaea* L.). *Pakistan Journal of Nutrition*, 8: 194-197.

[3] Food and Agricultural Organization (FAO) (2015). Available at: http://faostat.org/peanut. Retrieved on 12/12/2015, 15:10 GMT.

[4] Janila, P., Nigam, S. M., Manish, K. P., Nagesh, P., Rajeev, K. V. (2013). Groundnut improvement: use of genetic and genomic tools. *Frontiers in Plant Science*, 4: 23-28.

[5] Aziz, A. F., Rahmatollah, F., Ali, E. (2016). Seasonal Study of Aflatoxin M1 Contamination in Milk of Four Dairy Species in Iran. *Food Control*, 68: 77-82.

[6] Aguoru, C. U., Omoigui, L. O., Olasan, J. O. (2015a). Comparative Optimized Protocols of DNA Extraction and Purification using FTA PlantSaver Card and DNAzol Methods for Eggplant Studies in North Central Nigeria. *Open Access Library Journal,* 2:1-5.

[7] Kanyika, B. T. N., Lungu, D., Mweetwa, A. M., Kaimoyo, E., Njug'e, V. M., Monyo, E.S., Siambi, M., He, G., Prackash, C. S. (2015). Identification of groundnut (*Arachis hypogaea* L) SSR markers suitable for multiple resistance traits for QTL mapping in African germplasm. *Electronic Journal of Biotechnology*, 18:61-67.

[8] Egbadzor, K. F., Ofori, K, Yaboah, L. M., Opoku-Agyeman, M. O., Danquah, E. Y., Offei, S. K. (2014). Diversity in 113 cowpea [*Vigna unguiculata* (L) Walp] with 458 SNP markers. *Springerplus*, 3: 541-547.

[9] Sai, D. K., Rani, K. R., Usha, R. (2016). Assessment of genetic diversity in groundnut (*Arachis hypogaea* L.) genotypes using PCR based RAPD markers. *Biotechnological Research*, 24(4): 142-147.

[10] Dughdugh P., Adedzwa K. D., Omoigui O. L., Olasan, J. O., Ugbaa S. M. (2017). Identification of genetic polymorphism among groundnut genotypes using simple sequence repeats (SSR) markers. *Proceedings of the Genetic Society of Nigeria 41st Annual Conference, University of Agriculture Makurdi*, Pp253-257.

[11] Mehan, V. K. (1989). Screening groundnuts for resistance to seed invasion by *Aspergillus flavus* and to aflatoxin production [a review]. ICRISAT Center, India, International Crops Research Inst. for the Semi-Arid Tropics, Patancheru, A. P. (India).- Patancheru, A. P. (India): ICRISAT, 1989. p. 323-334"

[12] Andargie, M., Rem, S. P., Geoffrey, M. M., Michael, P. T. (2013). Quantitative Trait Loci of flowering time related traits identified in recombinant inbred lines of cowpea (*Vigna unguiculata*). *Genome*, 56: 289-294.

[13] Cosson, P., Decrooq, V., Revers, F. (2014). Development and characterization of 96 microsatellite markers suitable for QTL mapping and accession control in an *Arabidopsis* core collection. *Plant Methods*, 10: 2-6.

[14] Chen, Z. Y., Brown, R. L., Damann, K. E., Cleveland, T. E. (2010). PR10 expression in maize and its effect on host resistance against Aspergillus flavus infection and aflatoxin production. *Molecular Plant Pathology*, 11:69-81.

[15] Nigam S. N. (2000). Some strategic issues in breeding for high and stable yield in groundnut in India. *Journal of Oilseeds Research,* 17: 1-10

[16] Olasan, J. O., Aguoru, C. U., Ugbaa, M. S., Ekefan, E. J. (2017). Physiological Characterization of Groundnut (*Arachis hypogaea* L.) Seedlings using Vigour and Performance Indices. *Proceedings of the Genetic Society of Nigeria 41st Annual Conference, University of Agriculture Makurdi*, Pp84-91.

[17] Jake, C. F., Pawan, K., Liming, Y., Spurthi, N., Nayak, B. T. and Scully, R. D. (2015). Resistance to *Aspergillus flavus* in maize and peanut: Molecular biology, breeding, environmental stress, and future perspectives. *The Crop Journal*, 3: 229-337.

[18] Upadhyaya, H. D., Dronavalli, N., Singh, S., Dwivedi, S. L. (2012). Variability and stability for kernel iron and zinc contents in ICRISAT mini core collection of Peanut. *Crop Science,* 52: 2628-2637.

[19] Aguoru, C. U., Omoigui, L. O., Olasan, J. O. (2015b). Molecular Characterization of *Solanum* Species (*Solanum aethiopicum* complex; *Solanum macrocarpon* and *Solanum anguivi*) using Multiplex RAPD primers. *Journal of Plant Studies*, 4(1): 27-34.

[20] Broomfield, A. and Bourna, D. (2007). Basic techniques in molecular genetics. *Journal of Laryngology and Otology,* 112(3): 230-234.

[21] Demir, K., Bakır, M., Sarıkamış, G. and Acunalp, S. (2010). Genetic diversity of eggplant (*Solanum melongena*) germplasm from Turkey assessed by SSR and RAPD markers. *Genetics and Molecular Research,* 9 (3): 1568-1576.

[22] Baozhu, G., Natalie, D. F., Xiaoping, C., Chun-Hua, W., Jiujiang, Y. (2011). Gene Expression Profiling and Identification of Resistance Genes to *Aspergillus flavus* Infection in Peanut through EST and Microarray Strategies, *Toxins*, 3: 737-753.

[23] Pandey, M. K., Monyo, E., Ozias-Akins, P., Liang, X., Guimaraes, P., Nigam, S. N. (2012). Advances in *Arachis* genomics for peanut improvement. *Biotechnology Advances,* 30: 639-651.

[24] ICRISAT (2015). International Crops Research Institute for the Semi-Arid Tropics Annual Report of 2015. Retrieved from: www.icrisat.org. On 15/12/2015, 11:30 GMT.

[25] Ma, J., Yao, M., Ma, C., Wang, X., Jin, J., Wang, X., Chen, L. (2014). Construction of a SSR Based Genetic Map and Identification of QTLs for Catechins Content in Tea Plant (*Camellia sinensis*). *PLoS ONE*, 9(3): e93131.

[26] Gautami, B., Pandey, M. K., Vadez, V., Nigam, S. N., Ratnakumar P., Krishnamurthy, L. (2012). QTL analysis and consensus genetic map for drought tolerance traits based on three RIL populations of cultivated groundnut (*Arachis hypogaea* L.). *Molecular Breeding,* 32: 757-772.