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MAPPING OF QUANTITATIVE TRAIT LOCI (QTL) RELATED TO YIELD, NUT QUALITY AND PLANT SIZE OF CASHEW(Anacardium OccidentaleL)

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ABSTRACT

Improvement of yield, nut quality and plant size in cashew is a complex process through conventional technique. It takes at least 9-12 years to develop new varieties, this causes inadequate supply of improved planting materials to farmers. To overcome this limitation, we need to use marker-assisted selection. However, to date, MAS in cashew breeding has been hampered by the absence of markers linked to yield, nut quality and plant size. This study aimed at identifying quantitative trait loci associated with yield, nut quality and plant size. To map QTL a genetic linkage map with 761 single nucleotide polymorphism markers was developed using F2 population of cashew derived from ATA19/250 × Cook05. The linkage map consisted of 21 linkage groups covering 2230cM with an average marker distance of 3.3cM. The F2 progenies were evaluated for yield, number of nuts, nut weight, number of kernels, kernel weight, plant size, canopy diameter and percentage outturn in three seasons namely 2013, 2014 and 2015. A total of 13 QTLs were mapped on 8 linkage groups which explained 25.6 - 56% of the total phenotypic variation. The identified QTLs will speed up the development of high-yielding varieties with good nut quality through marker assisted selection.

Keywords: Conventional breeding, Single Nucleotide Polymorphism Markers, Marker Assisted Selection, Linkage map.

Introduction

Cashew(*Anacardium Occidentale* L) is a perennial tree crop mainly grown in tropical countries such as India, Vietnam, Brazil and Africa including Tanzania. In Tanzania cashew is grown in Mtwara, Lindi, Songea and few parts along the Coast areas [1] – [2]. It plays quite substantial role in economic development of many countries around the world [3] – [4]. In Tanzania cashew nuts play a key role as income generating crop as well as food crop [5] – [6]. As income

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generating crop cashew providing foreign exchange, raw materials for industrial uses and employment to women and youth [7] – [8].

Despite these economic importance, production is still limited, due to several factors, such as low yields of local varieties, diseases, pests and shortage or lack of good quality planting materials [9]. Efforts to develop cashew cultivars with high yield, big nuts and manageable plant size have strongly emphasized on generation and selection of superior hybrids by using conventional techniques. However, selection of improved varieties by using conventional techniques is hampered by a long juvenile phase of the crop and the polygenic nature of the quantitative traits [10] – [11]. This limits the production and availability of improved planting materials [12].

Application of molecular markers technology is vital aspect to improve efficiency and effectiveness of ongoing conventional breeding through marker assisted selection [13]. Molecular markers linked to quantitative trait locican facilitate identification of genotypes possessing favorable alleles [14] – [15] – [16] – [17] – [18], and therefore aiding selection of genotypes with desirable characteristics [19] – [20]. QTL is a position along the genome that contains genes which have statistically significant effects on the quantitative trait expression of particular traits [21]- [22]. QTL are identified via statistical procedures that integrate genotypic and phenotypic data [23] – [24], and are assigned to chromosome locations based on the positions of markers on a linkage map [25]. This study aimed at detecting QTLs related to yield, nut quality parameters and plant size that will be used for marker assisted selection (MAS).

Materials and Methods

Planting materials

The study was carried out at Naliendele Agricultural Research Station, located in Mtwara region of Tanzania. The experiment was planted in 2002 and consisted of 200 F_2 genotypes which were obtained by selfing F_1 population developed through controlled crossing of two distinct cashew clones, namely between a dwarf cashew clone (Cook 05) and a common (giant) cashew clone (ATA19/250). The plants were planted in straight rows spaced at 12 x 12 meters.

The traits recorded

The data were recorded for three consecutive seasons namely, 2013, 2014 and 2015 for the following traits: plant height (PH), canopy diameter (CD), yield per plant (YLD), number of nuts per plant (NN), nut weight (NW), number of kernels per plant (NK), kernel weight (KW) and percentage out turn (%OT). Recording of individual tree yield was done daily from September to December for all three years. Weighing of fresh nuts was carried out by using a mechanical weighing balance Salter Brecknell 235-6S-110. A one kg sample of randomly collected nuts per

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tree was used for nut analysis following protocols described by [26]. The mean nut weight was calculated by dividing sample weight by number of nuts in the sample. Kernel weight was calculated using the formula KW = (WK)/ (NK), where KW = kernel weight, WK = weight of sampled kernels and NK = number of kernels. Equally, percentage kernel out turn was calculated using formula %OT= (KW/NW) × 100; whereby OT% = percentage kernel out turn, KW = kernel weight and NW = nut weight. Total number of nuts per tree was obtained by calculating total weight per tree divided by mean nut weight. Canopy diameter was estimated by computing the mean of the two diameters taken from North-South and East-West of the tree canopy using a measuring tape and plant height was measured using a measuring tape raised parallel to the trunk from the ground level to the top of the tree canopy using a long wooden pole.

DNA extraction

DNA was extracted based on the cetyltrimethyl ammonium bromide (CTAB) method of Doyle and Doyle [27].Approximately 0.25g leaf samples were grounded into fine powder by using mortar and pestle. The powdered tissue was transferred to 1.5 ml eppendorf tube and mixed with 700µl of preheated (65^{o}C) 2% CTAB extraction buffer. The extraction buffer consisted of 0.2M Tris (hydroxyl methyl) amino methane hydrochloride (Tris-HCl), 1.4M sodium chloride (NaCl) at pH of 8.0, 2% (w/v) polyvinyl pyrrolidone (PVP), 0.05M ethylene-diaminetetraacetate (EDTA) and 0.2% (v/v) β-mecarptoethanol. The mixture was shaken vigorously for 10min until all tissue dispersed in the buffer, then transferred to 65^{o}C heating block for 15min and allowed to cool at room temperature for 2min. Then 250µl of ice-cold 5M potassium acetate was added and mixed gently by inverting the tubes 5-6 times and incubated on ice for 20min.

The mixture was then centrifuged at 14 000 rpm for 10min to separate supernatant. The supernatant was transferred to a new 1.5ml eppendorf tube and 700 µl of ice-cold isopropanol was added and mixed gently by inverting the tube 8-10 times. The sample was incubated overnight and centrifuged at 14 000 rpm for 10min. The supernatant was poured off and the last drops of isopropanol were removed by placing the eppendorf tube face down on paper towels. The pellet was allowed to air dry by leaving it on the paper towel for 1h. Then 100µl of 10mM Tris-HCl, 1mM EDTA containing 10mg/ml RNase was added and stored overnight at 4^{o}C to dissolve the pellet. The dissolved DNA was sent to DArTs Canberra University Laboratory in Australia for sequencing and SNP discovery.

Determination of DNA quantity and quality

The DNA quality was determined by using 0.5 µl of DNA mocked with 7 µl of loading dye and incubated at 37^{o}C for 2h. Thereafter the DNA was loaded on 0.8% (w/v) agarose gel in 1x Tris-acetate EDTA buffer and run at 120V for 20min. The run gels were photographed by using Trans

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illuminator spectrophotometer (Super imagine UK) and the picture image interpreted for DNA quality and quantity.

Preparation of Genomic Library Representation (Targets)

Genomic DNA was reduced into short fragments of specific sequence using Pst1 digestion method. It involves digestion of genomic DNA with Pst1 and Mse1 restriction enzymes. The restriction enzymes were selected to digest genomic DNA in fragments of 100-500bp size. The digested DNA was ligated by using barcode adapter and common adapter in a process called digestion/ligation reaction. The dig/lig reaction was carried out in a total volume of 50µl containing 5µl of 10x PCR buffer, 1µl of 10mM dNTPs, 0.1 µl of 100µm PCR1 primer, 0.1µl of 100µm Mse1 primer, 2µl Red Rag (1u/ul), 39.8 MG water, 2µl template of PCR1 primer + Msel primer. PCR mix reagent was run to PCR cycling reactions of 30 cycles as follows: 1min at 94 °C, 1min at 50-60°C, 1min at 72°C, and a final extension step for 5min at 72°C. The PCR products were run on 1.2% agarose gel and their quality was assessed based on the intensity of the bands. Targets with clear bands were selected and pooled together (the pooled targets are called reduced genomic libraries) by using TECAN liquid handling robot while the failed targets were rejected. The pooled amplified targets were purified and their quality checked through a process called Post PCR target quality control.

In this reaction, 100µl of a pooled sample were drawn and placed into a new tube and 500µl of binding buffer were added to it and spin shortly. The mix was transferred into a column tube and 750µl of wash buffer were added into the column and spin for 1min. The filter of the column tube was then transferred into 1.5ml tube and 50µl of Elution buffer (EB Buffer) were added and left for 1min. The mixture was spin for 1min and then run on 1.2% agarose gel. The amplified pooled targets were subjected to post quantification using 5µl of ethidium buffer, 11µl of diluted DNA and 3µl of NaOH, which were mixed by using multichannel pipets and aliquoted in 96 well plate. The mix was denatured to single strand by using 46µl of ethidium bromide buffer, and left at room temperature for 5min and run on 1.2% agarose gel. The well amplified targets were selected and subjected to hybridization by using cBot to form clusters. Hybridization reaction involved a mix of 994µl of hybrid buffer and 6µl of denatured DNA. Then 75µl from the mix were placed into the template strips and run in the cBot to generate clusters which were then sent for sequencing by using Hiseq2500 sequencer. Hiseq2500 sequencing machine operates by immobilizing the DNA template onto a flow-cell and then each single DNA molecule is amplified using a ‘bridging PCR’ amplification reaction. The DNA sequence is then recorded by utilizing different fluorescent labels that are captured by an optical camera.

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SNP call

The data from Illumina Hiseq2500 were converted into "0" (absent) or "1" (present) using DArTsoft v.7.4.7 (DArT P/L, Canberra, Australia). The markers were scored as binary data (1/0), indicating presence or absence of a marker in genomic representation of each sample. FASTQ software extrapolated the data into three different files namely, silico file which presents the markers, SNPs file row 1 and SNPs file row 2. The data were trimmed based on compound filter criteria, whereby the genotypes with a call rate of 97% and allele-calling of 98% were selected as markers and analyzed for polymorphism information content (PIC) using KD compute software [28]. The informativeness of markers was determined by calculating the PIC of the identified markers, and only markers with PIC equal or higher than 0.1 were scored and used for the analysis. Furthermore, the markers were used to determine QTLs related to yield, nut quality and plant size.

Linkage analysis

Linkage analysis was performed by using JoinMap v 3.0 software package [29].

QTL analysis

The Map QTL version v 4.0 software [30] was used to detect QTLs based on interval mapping method. A LOD score of 3.0 was chosen as minimum to declare the presence of QTLs. The confidence intervals for each QTLs was set at one-LOD support interval ($P < 0.05$) as described by Lander and Botstein [31]. Estimates of QTL positions were obtained at the point where the LOD score assumes its maximum value. Genetic effects of QTLs - additive (a) and dominance (d) effect were calculated simultaneously during the genome scan for QTLs. The QTLs were determined to be largely additive, dominant or recessive. The additive model was tested by forcing the dominance term $d = 0$, a dominant model by forcing $d = a$, and a recessive model by forcing $d = -a$. This model was used to test whether the QTLs were largely additive (a), dominant (d) or recessive (r). The non-parametric, single marker-based Kruskal-Wallis analysis was done to test for significance of the detected QTLs at $P = 0.05$.

Results

Phenotypic performance of F_2 mapping population

The F_2 cashew population, obtained by selfing F_1 population developed through controlled crossing of two distinct cashew clones, namely a dwarf cashew clone (Cook 05) and a common (giant) cashew clone (ATA19/250), exhibited considerable variability for the eight morphological traits (Table 1). During the three years of observation, the annual yield ranged from 1.5 to 21.9 kg per tree with an average of 9.0 kg per tree. The traits with the highest variability were number of kernels, number of nuts, yield and canopy diameter, while plant

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height, nut weight, percentage out-turn and kernel weight exhibited lower variability as revealed by the CV% values.

Table 1: Performance of F_2 cashew genotypes with respect to eight morphological traits

Trait	Minimum	Maximum	Mean	Std. Deviation	CV%
Yield per Tree (kg)	1.5	19.9	9.00	3.8	42.7
Nut Weight (g)	4.3	8.9	6.2	0.9	15.1
Number of nuts	255.5	4 020.1	1 470.7	684.2	46.5
Number of Kernels	244.7	3 956.5	1 390.7	653.9	47.0
Kernel Weight (g)	0.9	3.6	1.9	0.4	22.7
% Out-turn	12.4	36.8	27.2	5.2	19.2
Plant Height (m)	3.5	6.4	5.0	0.6	11.6
Canopy Diameter (m^2)	2.3	12.2	6.9	2.2	32.2

The study revealed that yield was positively correlated with the number of nuts (r = 0.908), number of kernels (r = 0.918), canopy diameter (r = 0.506) and plant height (r = 0.372). However, there was insignificance negative correlation between yield and either nut weight, kernel weight or percentage out-turn (Table 2). On the other hand, nut weight was negatively correlated with both number of nuts (r = -0.366) and number of kernels (r = -0.346), but positively correlated with kernel weight (r = 0.535). Both number of nuts and number of kernels showed positive correlation with plant height and canopy diameter, but these two parameters were negatively correlated with kernel weight. The results also revealed that the percentage out-turn was positively correlated with kernel weight (r = 0.561), but had insignificance negative correlation with nut weight (r = -0.082).

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Table 2: Pearson's correlation coefficients between quantitative traits calculated from 200 F_2 cashew genotypes

Variables	YLD	NW	NN	NK	KW	%OT	PH	CD
YLD	**1**							
NW	-0.043	**1**						
NN	**0.908**	**-0.366**	**1**					
NK	**0.918**	**-0.346**	**0.988**	**1**				
KW	-0.060	**0.535**	**-0.237**	**-0.223**	**1**			
%OT	0.071	-0.082	0.058	0.096	**0.561**	**1**		
PH	**0.372**	**-0.160**	**0.400**	**0.397**	-0.092	0.083	**1**	
CD	**0.506**	-0.027	**0.489**	**0.479**	-0.016	-0.014	**0.290**	**1**

Key: KW = Kernel Weight, NN = Number of Nuts, YLD = Yield, NW = Nut Weight, PH = Plant Height, CD = Canopy Diameter, %OT = Percentage Outturn, NK = Number of Kernels

QTL analysis

Of the 200 F_2 genotypes subjected to QTL mapping using 761 SNP markers, 165 genotypes produced useful data. From the 761 SNPs markers used for QTL mapping only eight (8) SNP markers namely; SNP638, SNP761, SNP330, SNP499, SNP625, SNP981, SNP561 and SNP796 detected thirteen (13) QTLs for eight traits, namely yield (YLD), number of nuts (NN), number of kernels (NK), nut weight (NW), kernel weight (KW), % out-turn (%OT), plant height (PH), and canopy diameter (CD) (Table 3). This study identified a total of 13 QTLwith LOD scores that ranged from 3.0 to 4.4 (average of 3.4) (Table 3, Fig. 1)on eight linkage groups.The QTL explained an average phenotypic variance of 41.07, which ranged from 25.6 to 56 (Table 3).

For yield one QTL was detected on LG 14 in Year 1 and 3 at position 10cM that accounted for 31.8% of the phenotypic variation (Table 3).

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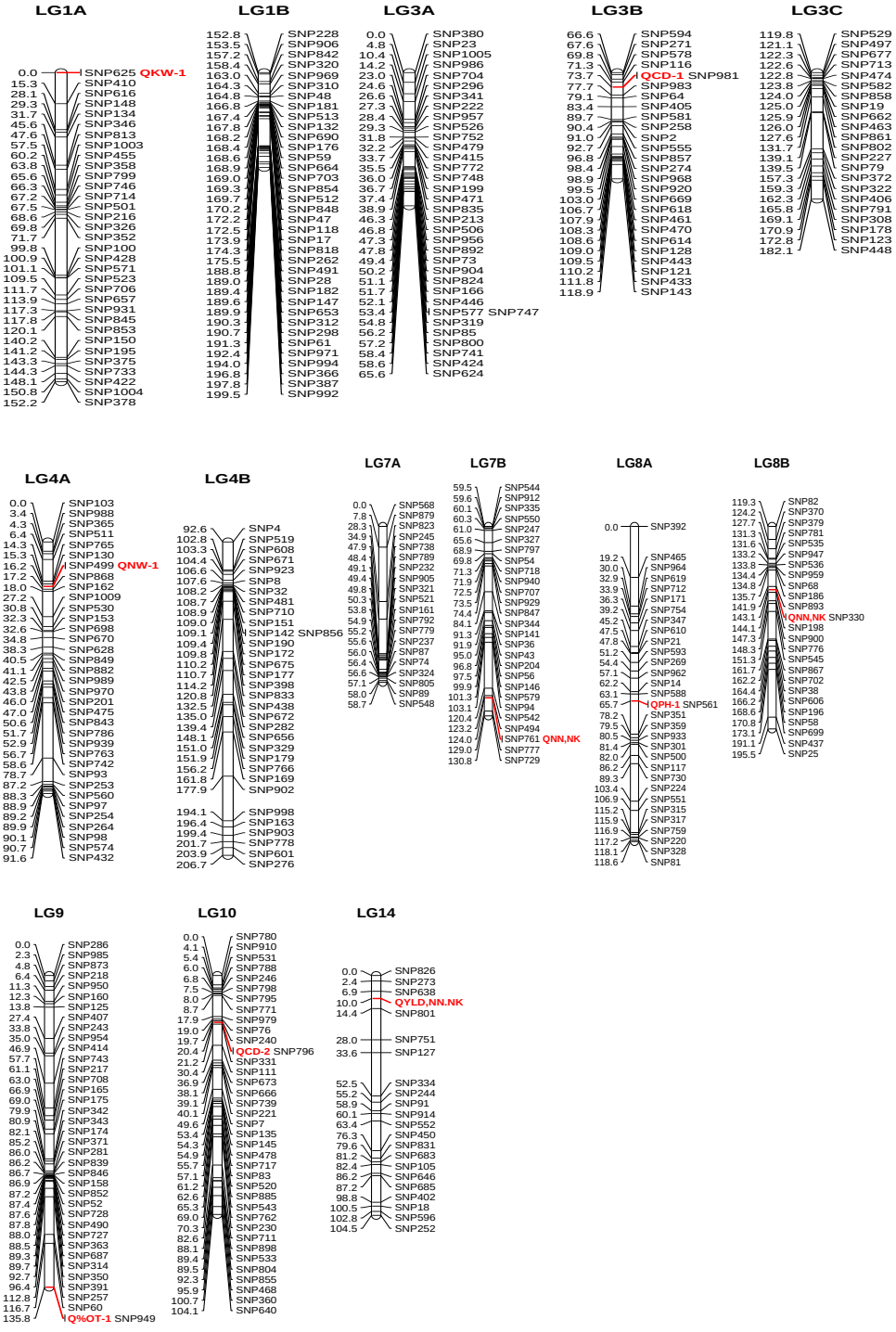

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Figure 1: QTLs mapped into Linkage map of cashew derived from 165 F_2 cashew genotypes obtained from selfing F_1 population developed through controlled crossing of two distinct cashew clones, namely a dwarf clone Cook05 and a common clone ATA19/250

Table 3: Thirteen 13 QTL associated with eight traits of cashew and their corresponding SNP markers, LOD score and percentage of phenotypic variance explained (%PVE)

Trait	Year	QTL*	LG	Position (cM)	Marker	LOD	%PVE
Yield	Y1	QYLD-1	14	10	SNPS638	3.43	31.8
	Y3	QYLD-1	14	10	SNPS638	3.43	31.8
NN	Y1	QNN-1	7	124	SNPS761	4.3	30.4
	Y2	QNN-1	7	124	SNPS761	4.3	30,4
	Y1	QNN-2	8	143	SNPS330	3.2	25.6
	Y3	QNN-2	8	143	SNPS330	3.2	25.6
	Y1	QNN-3	14	10	SNPS638	3.3	30
	Y3	QNN-3	14	10	SNPS638	3.3	30
NK	Y1	QNK-1	7	124	SNPS761	4.3	28.6
	Y2	QNK-1	7	124	SNPS761	4.3	28.6
	Y1	QNK-2	8	143	SNPS330	3.2	52.2
	Y3	QNK-2	8	143	SNPS330	3.2	52.2
	Y1	QNK-3	14	10	SNPS638	3.3	53
	Y3	QNK-3	14	10	SNPS638	3.3	53
NW	Y2	QNW-1	4	16.1	SNPS499	3.08	40

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	Y3	QNW-1	4	16.1	SNPS499	3.08	40	
KW	Y2	QKW-1	1	0	SNPS625	3.1	44	
	Y3	QKW-1	1	0	SNPS625	3.1	44	
%OT	Y1	QOT-1	9	136	SNPS949	9	56	
	Y2	QOT-1	9	136	SNPS949	9	56	
PH	Y1	QPH-1	8	65.7	SNPS 561	4.4	51	
	Y2	QPH-1	8	65.7	SNPS561	4.4	51	
CD	Y1	QCD-1	3	73.7	SNPS981	3.2	40	
	Y2	QCD-1	3	73.7	SNPS981	3.2	40	
	Y1	QCD-2	10	20.4	SNPS796	3.4	46	
	Y3	QCD-2	10	20.4	SNPS796	3.4	46	
Avg.						3.4	41.07	

Key: CD = Canopy Diameter, KW = Kernel Weight, NK = Number of Kernels, NN = Number of Nuts, NW = Nut Weight, PH = Plant Height, %OT = Percentage Outturn, YLD = Yield.

***** The name of the QTLs includes Q followed by an abbreviation of the trait name and a serial number.

On the other hand, three QTL were detected for both number of nuts and number of kernels in Year 1 and 2 on LG 7 at position 124, Year 1 and 3 on LG 8 at 143cM and LG 14 at positions 10. The three QTLs accounted for 30.4%, 25.6% and 30.0% % of the phenotypic variation. For nut weight (NW) one QTL was detected on LG 4 at position 16.1cM in Year 2 and 3, and accounted for 40.0% of the variation, while for kernel weight (KW) one QTL was detected on LG 1 in Year 2 and 3 at position 0 cM, and accounted for 44% of the variation. The analysis also revealed one QTL each for %OT and plant height (PH) on LG 3 and LG 8, respectively in Year 1 and 2. Two QTL were also detected for canopy diameter (CD) in Year 1 and 2, one on LG 2 at position 73.7cM accounting for 40% of the variation and the second one in Year 1 and 3 on LG 10 that accounted for 46% of the phenotypic variation. Three QTL, namely QYLD-1 for yield,

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QNN-3 for number of nuts and QNK-3 for number of kernels were detected on the same linkage group 14 and at the same position (10 cM) (Table 4).

Table 4: QTLs for yield, number of kernel and number of nuts found on the same linkage group 14

Trait	QTL	LG	Position (cM)	Marker	LOD	%EXP
Yield	QYLD-1	14	10	SNPS638	3.43	31.8
NN	QNN-3	14	10	SNPS638	3.3	30
NK	QNK-3	14	10	SNPS638	3.3	53

The study revealed two modes of gene action (inheritance) namely, additive, dominance or recessive as revealed by d/a mode (Table 5).

Table 5: Gene action of individual QTLs affecting yield (YLD), number of nuts (NN), number of kernels (NK), nut weight (NW), kernel weight (KW), % Out-turn (%OT), plant height (PH) and canopy diameter (CD) in F_2 cashew population

Trait	QTL	LG	Position (cM)	Marker	LOD	%EXP	a	d	GIC	d/a	Mode
Yield	QYLD-1	14	10	SNPS638	3.43	31.8	-1	1	1	-1	d,r
NN	QNN-1	7	124	SNPS761	4.3	30.4	1.5	-2.9	1	1.9	d
	QNN-2	8	143	SNPS330	3.2	25.6	1.4	-2.5	1	-1.8	d,r
	QNN-3	14	10	SNPS638	3.3	30	-1	2	1	-1.1	d,r
NK	QKN-1	7	124	SNPS761	4.3	28.9	0.3	0.4	1	1.5	d
	QKN-2	8	143	SNPS330	3.2	52	0.1	0.5	1	3.5	d
	QKN-3	14	10	SNPS638	3.3	53	0.2	0.5	1	2.9	d
NW	QNW-1	4	16.1	SNPS499	3.08	40	0.1	-2.8	1	-15.9	d
KW	QKW-1	1	0	SNPS625	3.1	44	0.4	-2.9	1	8.3	d
%OT	QOT-1	9	136	SNPS949	3	56	0.3	-2.6	0.9	-16.3	d
PH	QPH-1	8	65.7	SNPS561	4.4	51	0.3	-0.2	0.9	0.6	a
CD	QCD-1	3	73.7	SNPS981	3.2	40	0.2	0.2	1	0.8	a

QCD-2	10	20.4	SNPS796	3.4	46	-0.2	-0.2	1	0.8	a

Legend: a = additive, d = dominance, GIC = genetic information content

Non-parametric, single marker-based Kruskal-Wallis analysis was conducted to identify significant marker-trait association. The analysis revealed highly significant marker-trait association for twelve out of the thirteen identified QTLs, while two QTL, one QTL for QCD-2, located at position 20.4cM, and the second Q%OT – 1, located at position 136cM did not show a significant association with its corresponding markers (SNPS796) and (SNPs 949) respectively (Table 6).

Table 6: Significant quantitative trait loci (QTLs) associated with yield, nut quality parameters and plant size of F_2 cashew population

Trait	**QTL**	**LG**	**Position (cM)**	**Marker**	**LOD**	**% EXP**	Kruskal-Wallis P-Value	Significance level
Yield	QYLD-1	14	10	SNPS638	3.43	31.8	**0.01**	**
NN	QNN-1	7	124	SNPS761	4.3	30.4	**0.05**	*
	QNN-2	8	143	SNPS330	3.2	25.6	**0.001**	***
	QNN-3	14	10	SNPS638	3.3	30	**0.01**	**
NK	QKN-1	7	124	SNPS761	4.3	28.9	**0,05**	*
	QKN-2	8	143	SNPS330	3.2	52	**0.05**	*
	QKN-3	14	10	SNPS638	3.3	53	**0.01**	**
NW	QNW-1	4	16.1	SNPS499	3.08	40	**0.05**	*
KW	QKW-1	1	0	SNPS625	3.1	44	**0.05**	*
%OT	QOT-1	9	136	SNPS949	3.0	56	**NS**	
PH	QPH-1	8	65.7	SNPS561	4.4	51	**0.01**	**
CD	QCD-1	3	73.7	SNPS981	3.2	40	**0.05**	*
	QCD-2	10	20.4	SNPS796	3.4	46	**NS**	

NS = Not significant

Discussion

The study aimed at detecting QTLassociated with yield, number of nuts, nut weight, kernel weight, canopy diameter, percentage out turn (%OT), and plant height. The QTL analysis was done using an F_2 cashew mapping population comprising 165 genotypes. The F_2 population exhibited high variation in all traits as revealed by CV% (Table 1). The high degree of variability within the segregating F_2 population indicates that there was high likelihood of identifying quantitative traits loci associated with yield, nut quality and plant size. In this study correlation

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coefficients were calculated to determine the degree of association between different parameters of the population. The highly significant correlation coefficients between traits revealed in this study indicated the possibility of having genetic correlations between those traits. These results are similar to those obtained by Paterson *et al*[32] who reported that the correlated traits are most likely to have genetic correlation.

This study revealed 13 QTL with minimum LOD score of 3.0 which were consistent for two years (Table 3, Fig 1). OTL is the position along the genome that contains gene responsible for a certain specific traits [33]. The presence of these QTL explained the presence of genes that influence expression of specific traits contributed by the ATA19/250 male parent, a tall (12m) with big nuts (>6g) and Cook 05 female parent a dwarf (5m) with small nuts (<6g). The high percentage of phenotypic variance explained (%PVE) (25.6-56%) by the thirteen identified QTL for eight traits for the yield, nut quality parameters and plant size in two years suggested that these are effective QTLs. Similar results were obtained by Anderson *et al*.[34], who reported that high percentage of phenotypic variance explained (%PVE) is the most important aspect in understanding the effectiveness of identified QTL and proposed that the higher the %PVE the higher the existence of true QTLs.

Three QTLs (QYLD-1, QNN-3, and QNK-3) are mapped on linkage group 14 at position 10cM (Table 4). The presence of three QTL on the same linkage group at the same location indicates that the linkage group 14 has loci with pleiotropy effect. Pleiotropic effect is the situation whereby a single loci has ability to detect more than one trait. The co-localization of these traits is in agreement with the highly significant positive correlation found between yield and number of kernels (0.918); and yield and number of nuts (r = 0.908) (Table 2). However, further genetic studies of the regions containing these QTL would be needed to distinguish between pleiotropy and gene linkage. The high correlation between number of nuts and yield, suggest that either of the QTL detected for yield or QTL detected for number of nuts or number of kernels could be used simultaneously in marker-assisted selection. The study also revealed QTL for plant height, number of nuts and number of kernels found on the same linkage group 8 but at different locations (Fig.1)

In addition the high genetic information coefficient (GIC) of 0.99 – 1 revealed by the 13 QTLs implied that the associated SNP markers were highly informative and should be used in marker assisted selection. Similar results were reported by Reyes- Valdes and Williams [35] who reported that if GIC = 1, it means that there is complete or maximum markers information and the QTLs should be used for marker assisted selection. The interval mapping was analyzed to identify types of gene action affected by the QTLs. The study revealed three modes of gene action (inheritance) i.e. additive, dominance or recessive as revealed by d/a mode (Table 5).

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Gene action refers to the behavior or mode of expression of genes in a genetic population. The analysis indicated that all three dosage of gene action are present at a loci. Thus the corresponding QTL were either additive, dominant or recessive (Table 5). These results provide evidence that F_2 populations can be used to map different gene actions from either parents [36] unlike F_1 populations. Three QTLs (QYLD-1 for yield and QNN-2, QNN-3 for number of nuts) showed partially dominant or recessive gene action. This suggests that cashew yield is controlled by both major and minor genes (D, R). This result is in agreement with theoretical argument that yield is a complex trait which is controlled by major and minor genes [37].

Dominance effects shown by QTLs associated with number of nuts (QNN-1), number of kernels, nut weight, kernel weight and %OT and additive effects detected for plant height and canopy diameter (Table 5) suggest that the mode of gene action ranged from complete additive to over dominance. The non-parametric, single marker-based Kruskal-Wallis analysis was conducted to identify significant marker-trait association (Table 6). The highly significant marker-trait association for twelve out of the thirteen identified QTLs implied that the QTLs identified in this study should be considered for marker assisted selection. Similar results have been reported by Anderson *et al*., [38] who presented significant QTLs and proposed that the highly significant QTLs should be selected as candidate QTLs for marker assisted selection.

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