Current Journal of Applied Science and Technology

39(48): 262-274, 2020; Article no.CJAST.65093
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Identification of Putative Molecular Markers Associated with Nut Weight and Kernel Weight and Four Growth Traits in Cashew

E. Eradasappa[1*], G. S. Mohana[1], J. D. Adiga[1] and Siddanna Savadi[1]

[1]ICAR-Directorate of Cashew Research, Puttur, Karnataka, India.

Author's contributions

This work was carried out in collaboration among all authors. Author EE performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and author GSM designed the study and corrected the manuscript. Author JDA created the mapping population and author SS corrected the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2020/v39i4831229
Editor(s):
(1) Dr. Rita Andini, Syiah Kuala University, Indonesia.
Reviewers:
(1) Edith Alba Luz Segovia Corrales, National University of Asuncion (Universidad Nacional de Asunción), Paraguay.
(2) Lisandra Abatti, Universidade de Caxias do Sul, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/65093

Original Research Article

Received 25 October 2020
Accepted 30 December 2020
Published 31 December 2020

ABSTRACT

Aim: To identify putative molecular markers linked to some useful traits in cashew using SSR markers in the F_1 population.
Place and Duration of the Study: Study conducted at ICAR-Directorate of Cashew Research, Puttur, Karnataka, India during 2013-17.
Methodology: Population for the study comprised of 83 F_1 plants developed through crossing tall variety Ullal-3 as female parent with dwarf accession NRC-492 as male parent which was planted in the field at 6m x 6m spacing during the year 2009. It was phenotyped along with parents during the year 2017 for the 13 growth and yield related traits and genotyped using 32 polymorphic SSR markers from cashew, almond, pistachio and mango. Single marker analysis was deployed to identify the markers linked to traits by SPSS software.
Results: results revealed three markers such as AL 29, IM 31 and IM 28 to be significantly associated with some traits. Marker AL 29 from Almond species was linked to traits stem girth, nut weight and kernel weight with an explained phenotypic variance of 7.9%, 5.6% and 5.4%,

**Corresponding author: E-mail: era.dasappa@gmail.com;*

respectively. Marker IM 31 from mango species was associated with stem girth, kernel weight, tree height, mean tree spread, nut weight and presented phenotypic variance of 17.6%, 5.7%, 8.2%, 4.5, 4.2% in that order. While the marker IM 28 from mango species showed linked with stem girth and intermodal length with phenotypic variance of 7.6%, 7.5%, 10.4% correspondingly.

Conclusion: The present study has identified three markers AL 29 (from Almond) , IM 28 and IM 31 (from Mango) linked to six traits viz., stem girth, tree height, mean tree spread, intermodal length, nut weight and kernel weight with the limited polymorphic markers using Single Marker Analysis. However, further studies are aimed to validate the linked markers prior to using them in marker assisted selection in cashew.

Keywords: Cashew; F_1 population; single marker analysis; SSR markers.

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is widely grown in east and west coast regions of India and is becoming popular in the plains region. It plays vital role in providing employment to poor people and generating income for the growers and processors. The cashew production in India is 0.786 million tons from an area of 1.00 million ha with an average productivity of 783.10 kg / ha [1]. However, the domestic production is inadequate to meet the demand of cashew industries and hence importing raw nuts from African and east Asian countries.

Since 1950s, Indian cashew breeding programme has developed and released 54 varieties [2] for commercial cultivation. These varieties were developed employing conventional breeding techniques of simple selection or hybridization followed by selection for yield and quality traits. Nevertheless, as cashew is a perennial plant the conventional breeding programmes are in slow pace due to long juvenile period and time required for evaluation, highly heterozygous nature of genotypes, requirement of large experimental fields and environmental effect [3].

Therefore, research efforts on developing DNA based molecular markers are being undertaken to facilitate conventional breeding strategies in cashew in the areas like molecular characterization of germplasm accessions [4,5,6,] diversity analysis [7-10] DNA fingerprinting of varieties [11], development of genetic linkage maps and detection of QTLs [12-15] for horticultural traits. Nowadays, marker assisted selection (MAS) is being used in perennial plant breeding programmes to hasten the process of development of variety [16,17]. Application of MAS in perennial crop breeding programmes has indicated advantages for selection during the juvenile phase [18,19] and for replacing expensive, time-consuming or technically difficult traits [20,21]. The utility of RAPD markers in distinguishing dwarf seedlings in cashew was showed [22]. Bulk Segregant Analysis (BSA) in germplasm bulks at DCR, Puttur, could identify four RAPD markers linked to economic characters like nut weight and plant stature [23].

There have been efforts to map molecular markers linked to economic traits in perennial fruit trees such as mango [24], apple [25], cashew [13-15], pistachio [26] etc. However, major constraint in perennial crops such as cashew is the generation of a suitable mapping population (such as F_2, RIL and NILs) with sufficient number of individuals, which is time and resource intensive. In cashew, cross between two individuals produces heterogeneous F_1 population and may behave akin to F_2 population [27]. Keeping this in view, our study aimed at genetic analysis of existing mapping population (F_1) with the aim of linking molecular markers to some useful traits in cashew using SSR markers.

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was laid out in the year 2006 at Kemminje campus of ICAR-DCR, Puttur situated at a latitude of 12.87 N and longitude of 74.88 E in the Dakshina Kannada District of Karnataka. The soils are lateritic and the annual rainfall is 4000 mm in this region.

2.2 Plant Material

Mapping population for the study comprised of 83 F_1 plants developed through crossing tall variety Ullal-3 as female parent with dwarf accession NRC-492 as male parent.

Characteristic features of parents NRC-492 and Ullal-3 are presented in Table 1.These plants were raised by sowing matured F_1 seed nuts along with parents in the nursery in the polybags and thereafter planting healthy seedlings in the field at 6m x 6m spacing during the year 2009.

2.3 Phenotypic Analysis

The F_1 mapping population was phenotyped along with parents during the year 2017 for the traits such as intermodal length (INL) (cm), tree height(TH) (m), stem girth (SG) (cm), mean tree spread (MTS) [(average of N-S and E-W directions(m)], panicle length (PL) (cm), panicle breadth (PB) (cm), apple weight (AWT) (g), nut weight (NWT)(g), kernel weight (KWT) (g) along with testa, kernel weight (KWT) (g) without testa, shell thickness (ST) (cm), shelling percentage (SP) and apple to nut ratio (ANR) as per the methodology described in the experimental manual on cashew by the National Research Center for Cashew [28].

2.4 Genomic DNA Extraction

Fresh young cashew leaves were collected in the early morning from the field and brought immediately to the lab. The genomic DNA was extracted grinding leaf samples in liquid nitrogen and following cetyl trimethyl ammonium bromide (CTAB) extraction buffer method [29] with slight modification. The extracted DNA was quantified using Hoefer Dyna Quant 200 model of Fluorometer (GE Healthcare, Singapore) and its homogeneity was checked on 0.8% agarose gel electrophoresis.

2.5 Polymerase Chain Reaction (PCR)

During the study time, there were only 21 SSR markers in cashew. In order to increase the number of SSR markers, cross species SSR markers consisting of 28 from pistachio, 24 from almond and 65 from mango were also deployed for arriving polymorphic markers. A total of 138 SSR primers from cashew, pistachio, almond and mango were used to assess polymorphism between two parents. In cashew, 21 SSR primers using the sequences published (4) were synthesized from Operon Technologies, Germany through M/s Genetix New Delhi. SSR Primers of 28 in Pistachio [30], 24 in Almond [31] and 65 in Mango [32,33] were got synthesized from M/s. Sigma Aldrich. Polymerase chain reaction was carried out in 25 μL reaction containing 2.5 μL 1x buffer (2 mM), 2.5 μL dNTPs (0.2mM), 2.5 μL MgCl2 (2 mM), 4 μL(F+R) primer(1 μM), and 1 U Taq DNA polymerase (Bangalore Genei, Bangalore) and 2 μL template DNA (30 ng) and 11.25 μL sterile water. Amplification was carried out with the following thermal cycle protocol: 94°C for 2 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, primer annealing at 50°C to 60°C for 1 min, initial extension at 72°C for 2 min and a final extension at 72°C for 6 min. The PCR was performed using Eppendorf thermocycler (Eppendorf, Hamburg, Germany). The PCR products were separated on a horizontal 3.5% superfine agarose (GE Healthcare) gel electrophoresis using 0.5×Tris-acetic acid-EDTA buffer and stained with ethidium bromide (0.5 µg/ml). The image of bands was acquired through UV light using AlphaImager Gel documentation system (Alpha Innotech Corp. USA). The molecular size of the amplicons was determined with 50 and 100 kb ladder DNA as reference marker.

2.6 Study of Parental Polymorphism and Segregation of Markers in Mapping Population

The contrasting parents (Ullal-3 × NRC-492) for growth and yield traits were screened with 138 SSR markers. Scoring of the bands was done as follows, the segregating band from the female parent was scored as 3, male parent as 1, missing as 9 and F_1 as 2 in the mapping population. The genotypic data was subjected for statistical analysis. As per the segregation of SSR markers in the mapping population, parents were interpreted for putative marker locus and the Chi-square test was performed using statistical software SPSS Ver 16 [34].

2.7 Association of Identified Polymorphic Markers with Traits

In order to detect the association of molecular markers for growth and yield traits, single marker analysis [35] was carried out using SPSS Ver.16 software [34]. Regression (R^2) values were computed by one-way analysis of variance (ANOVA) procedure in order to find out the amount of variability explained by markers. Traits were treated as dependent variables while the molecular markers as independent variables in the analysis. Thirteen different growth and yield traits were used to associate with the 32 polymorphic molecular markers.

3. RESULTS AND DISCUSSION

3.1 Phenotyping of Mapping Population for Vegetative and Reproductive Traits

The variability observed for each trait is presented in the Table 2. Internodal length varied from 2 cm to 13.8 cm with a mean of 6.01 cm whereas stem girth varied from 40 cm to 128 cm with a mean of 72.69 cm. Tree height recorded a minimum of 3.1 m and maximum of 9.8 m with a mean of 5.78 m while tree spread showed minimum of 3.33 m and maximum of 9.7 m with a mean of 6.53 m. The weight of cashew apple ranged from 12.5 g to 85 g with an average of 40.67 g while the nut weight varied from 2.37 g to 8.49 g with an average of 5.14 g. The apple to nut ratio varied between 2.81 to 18.63 with a mean of 7.95. Shell thickness varied from 1.73 cm to 3.77 cm with a mean of 2.83 cm whereas shelling percentage varied from 28.22% to 44.2% with a mean of 35.43%. Kernel weight with testa varied from 0.99 g to 2.78 g with a mean of 1.78 g whereas kernel weight without testa varied from 0.88 g to 2.73 g with a mean of 1.65 g. Transgressive segregants were observed for the F_1s for most of the traits. The continuous variation presented by all the traits indicated the quantitative nature of these traits (Fig.1). Considerable variability was observed for the traits phenotyped in the population confirming the quantitative nature of these traits and as a result signifying the suitability of this population for marker trait association studies. The association of molecular markers with desired phenotype of an agronomic trait is a powerful and effective application of molecular biology to plant breeding [36] and hence the present study is more relevant in this context.

3.2 Identification of Polymorphic Microsatellite Primers in Parental Lines

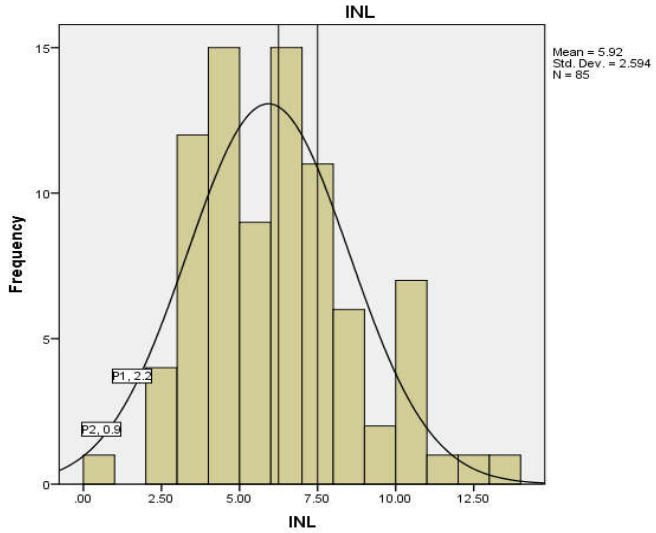
The contrasting parental (Ullal-3 × NRC-492) for growth and yield traits were genotyped with 138 SSR primers from cashew, mango, almond and pistachio, and among them 32 primers (23%) showed polymorphism (Table 3). In those 32 polymorphic markers, 5 are from cashew, 11 from almond and 16 from mango. The

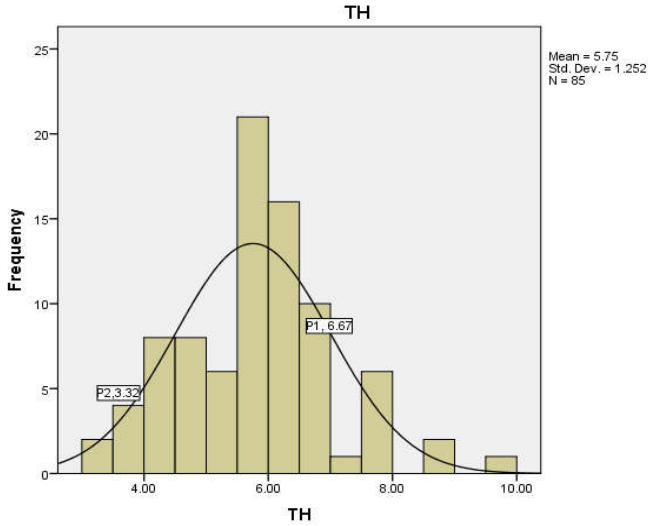
Table 1. Characteristic features of parents NRC-492 and Ullal-3

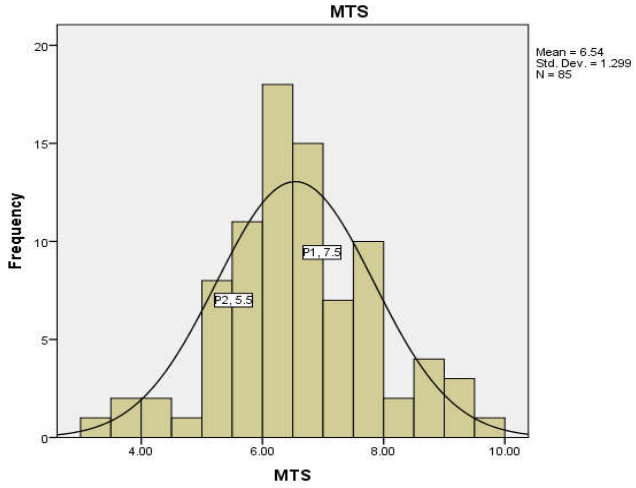
Sl. No.	Trait	NRC- 492	Ullal-3
1	Tree Height(m)	3.32	6.67
2	Mean Tree Spread (m)	5.5	7.5
3	No.of nuts / kg	185-190	140-145
4	Nut weight (g)	5.4	7.3 g
5	Kernel weight (g)	1.8	2.2 g
6	Shelling %	33.3	30.1
7	Export grade	W320	W210
8	Mean Nut yield / tree (kg/tree)	5.0	14.7

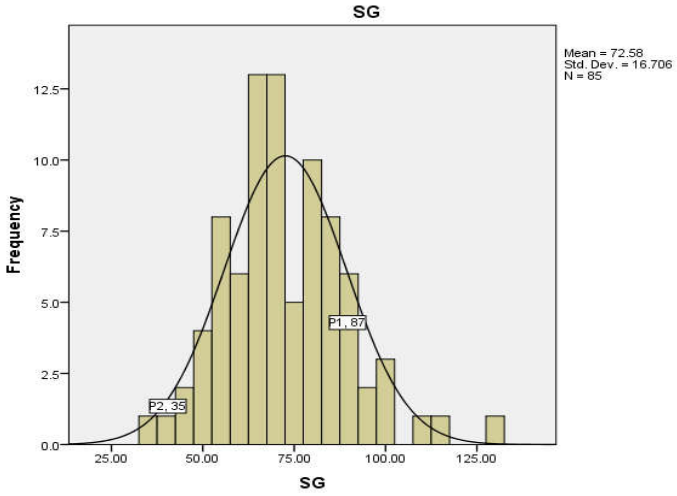
Table 2. Range and mean values of morphological traits in F_1 mapping population (N=83)

Trait	Minimum	Maximum	Mean	SD
Internodal length, (INL) (cm)	2.0	13.80	6.01	2.59
Panicle breadth, (PB) (cm)	17.0	36.0	25.01	4.52
Panicle length, (PL) (cm)	13.70	28.00	19.03	3.00
Tree height, (TH) (m)	3.10	9.80	5.78	1.23
Mean tree spread,(MTS) (m)	3.33	9.70	6.53	1.30
Stem girth,(SG) (cm)	40.00	128.00	72.69	16.28
Weight of apple,(AWT) (g)	12.50	85.00	40.67	16.81
Nut weight, (NWT) (g)	2.37	8.49	5.14	1.59
Shell thickness, (ST) (mm)	1.73	3.77	2.83	0.48
Shelling, (SP) (%)	28.22	44.20	35.43	4.08
Kernel weight (g) [along with testa]	0.99	2.78	1.78	0.43
Kernel weight, (KWT) (g) [without testa]	0.88	2.73	1.65	0.43
Apple to nut ratio, (ANR)	2.81	18.63	7.95	2.53


INL
Mean = 5.92
Std. Dev. = 2.594
N = 85
Frequency
P1, 2.2
P2, 0.9
INL


TH
Mean = 5.75
Std. Dev. = 1.252
N = 85
Frequency
P1, 6.67
P2,3.32
TH


MTS
Mean = 6.54
Std. Dev. = 1.299
N = 85
Frequency
P1, 7.5
P2, 5.5
MTS


SG
Mean = 72.58
Std. Dev. = 16.706
N = 85
Frequency
P1, 87
P2, 35
SG

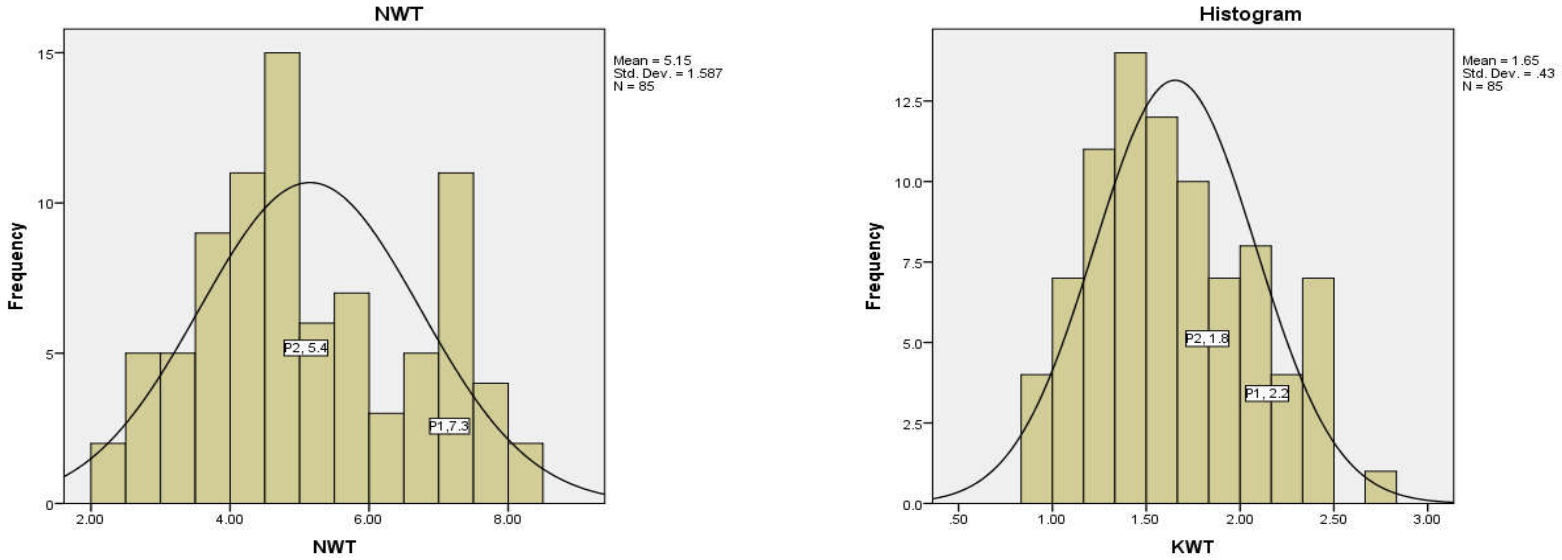
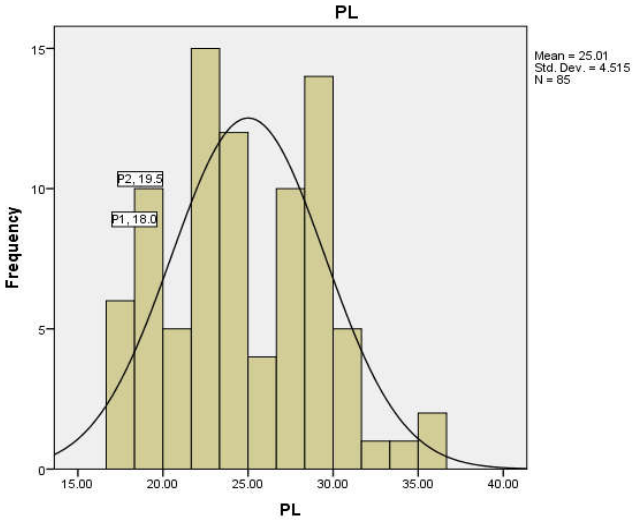
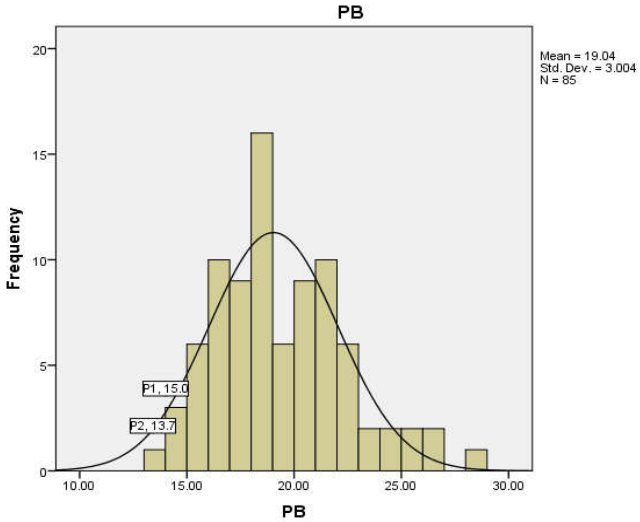
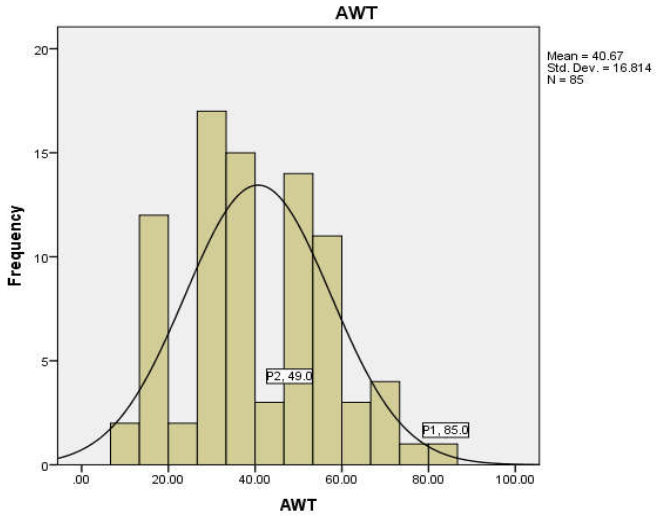
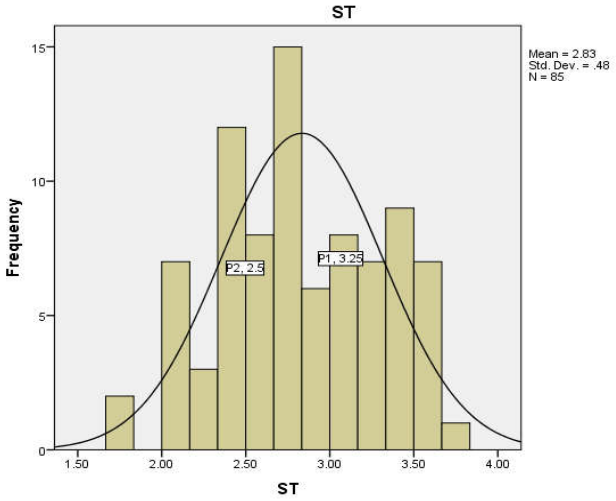


Fig. 1. Genetic variability for intermodal length (INL), tree height (TH), mean tree spread (MTS), stem girth (SG), nut weight (NWT) and kernel weight (KWT) without testa in mapping population of Ullal-3 × NRC-492. Values of parents (P1, P2) are given in text boxes


PL
Mean = 25.01
Std. Dev. = 4.515
N = 85
P2, 19.5
P1, 18.0
Frequency
PL


PB
Mean = 19.04
Std. Dev. = 3.004
N = 85
P1, 15.0
P2, 13.7
Frequency
PB


AWT
Mean = 40.67
Std. Dev. = 16.814
N = 85
P2, 49.0
P1, 85.0
Frequency
AWT


ST
Mean = 2.83
Std. Dev. = .48
N = 85
P2, 2.5
P1, 3.25
Frequency
ST

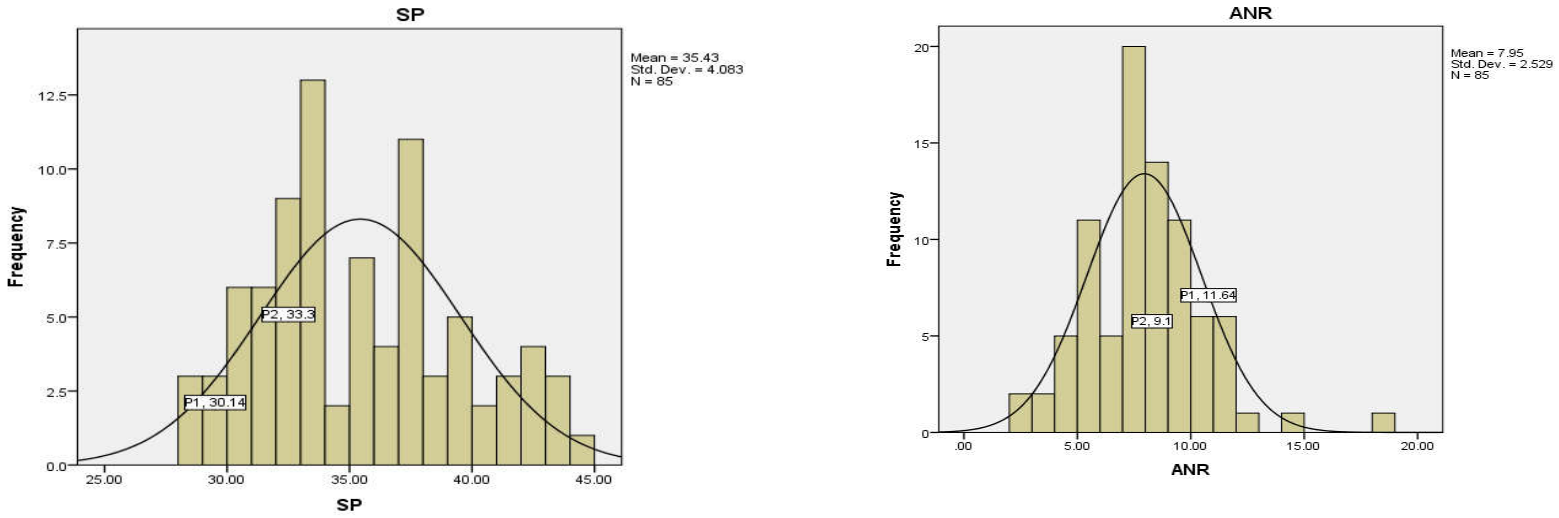


Fig. 2. Continued. Genetic variability for intermodal length (INL), tree height (TH), mean tree spread (MTS), stem girth (SG), nut weight (NWT) and kernel weight (KWT) in mapping population of Ullal-3 × NRC-492. Values of parents (P1, P2) are given in text boxes

polymorphism obtained in the present study was only 23% hence more SSR markers need to be developed for use in molecular breeding of cashew. In the earlier studies in cashew with RAPD marker analysis 71.8% polymorphism was obtained using 10 selected primers which generated 75 loci and among them 52 were polymorphic and in ISSR analysis, with 10 selected primers, a total of 88 bands were generated, of which 77 bands (87.5%) were polymorphic and in SSR analysis, a set of 15 primers was used to generate a total of 33 bands, of which majority i.e. 31 bands (93.3%) were polymorphic [37].

Table 3. Polymorphic primers and their sequences used to study the segregation of markers in the F_1 mapping population

Sl.No.	Primer name	Primer sequence (5'-3')	Tm(∘C)
1	CS-3	F:CAAAACTAGCCGGAATCTAGC R: CCCCATCAAACCCTTATGAC	58.2
2	CS-5	F:ATCCAACAGCCACAATCCTC R: CTTACAGCCCCAAACTCTCG	60.3
3	CS-7	F:GGAGAAAGCAGTGGAGTTGC R: CAAGTGAGTCCTCTCACTCTCA	60.3
4	CS-8	F:TCCACAAAATCAGCCTCCAC R: GAGCGCTCGTGTCCTGTACT	60.3
5	CS-13	F:ACTGTCACGTCAATGGCATC R: GCGAAGGTCAAAGAGCAGTC	60.3
6	Al 22	F:TGCAAGTTGAATGTGGCAAT R:CTTTGGGTAGTGCAGGGATG	64.1
7	Al 24	F:GCTTGGAAAAGGGTCTCCTA R: CCACCTCAGTTTTGACAAATGAA	62.0
8	Al 27	F:CAGACCGTCGTGTTGAAGTC R: GACCCGAATCGGAGTTGTAA	63.4
9	Al 35	F:TTGAATCGGAGTTGGAAAGAA R: CGGTGCTGGGAGAATCGT	63.3
10	Al 36	F:GATGGTACCTGAAGCGGAGGA R: TGGTCTAAATACCGCGAAGG	67.4
11	Al 17	F:CAGCAATGTTTATGCAGGGTAA R: TGAATATTTGGATTGCGAAGG	63.5
12	AL 30	F:GAAACTCAGTGGCACAATCG R: GCAGGAGTTTCGAAAGGAAG	63.3
13	AL 29	F: TGCAAAGAAAAACGGAGAGG R: GAAACTCAGTGGCACAATCG	64.0
14	AL 38	F:GTGGCAAATGTTGGCAAAG R: AACACAAAGCAGCACCAAGA	64.0
15	AL 40	F:GACCTCATCAGCATCACCAA R: TTCCCTAACGTCCCTGACAC	63.8
16	AL 50	F:TCGAAGGAGGATGAAGTTGC R: ATATCACGAGGGGCAAAATG	64.2
17	IM 7	F: GTGTTCAGAATACCGGCCA R: ACCCCTGCATGATTTTGACT	59.99
18	IM 8	F:TGGGAGAGAGATCAAATCGC R:GGTTCCAAACCTAGCCTTCC	59.94
19	IM 11	F: GTCGATGCCTGGAATGAAGT R: AAGCATCGAACAGCTCCAAT	60.21
20	IM 12	F:TTATTTGCGTTTAATGTGAGAATTA R:AGGGGGAGGGAAGAAGATAA	58.98
21	IM 39	F:GACCTCCTCCTTCAGCTGTTC R:CCTTGCTTATGTTGGGTGAAA	59.98
22	IM 4	F:AAGGAATTTTCCATTTTGGG R:AGCAACAGCAGCCATAGGAT	59.87

Sl.No.	Primer name	Primer sequence (5'-3')	Tm(∘C)
23	IM 9	F: AACTCAGGCCCCTCTTCAA R: TTGGGGTCTAGCAAACAAGA	60.074
24	IM 21	F: ACAAAGTTGGATGCCCAGA R: GGATGGATATCAGAAGGGCA	60.111
25	IM 30	F: CTAGAATTTTTGAAAAGATAATGTGA R:TTTCACATACAAGTTAGTTGGGG	57.10
26	IM 31	F:TTGGGTGAATTTGCTGTCTG R:GCAGGAATTGCAAACCCATA	60.84
27	IM 35	F: TTGGTAAGTTCGAAGTCGG R: GGGCAGAGACTAGTCGAACG	58.56
28	IM 38	F:TCGCACATCATTACGTCAAA R:GCCAGAGCTACTGCTTCGAT	59.75
29	IM 6	F: GATCCGACTTTAAGAGCCCC R: CCTGTCCCCGATTACAGAGA	60.23
30	IM 15	F:CCACAGACTTAAACTCACTCCAT R:TGAAAAGCCTTTCTGGCAGT	59.99
31	IM 27	F:CATTGTTTGTACACATGCGATCT R:TCAAGCCCAAACTCAAGTCA	59.41
32	IM 28	F:TTTTTATCCCTTAATTTTTGTAACCA R:GGGCTTGATCTCAGCCACTA	60.36

Table 4. Single marker analysis and chi-square tests (χ2) for SSR markers in the F_1 mapping population (N=83)

Parentage	Number of polymorphic loci tested for SMA	Number of SSR marker liked to various traits	Number of unlinked SSR markers	Marker loci not following expected ratio (1:1)	Marker loci following expected ratio (1:1)
Ullal-3 x NRC-492	32	20	12	17	3

3.3 Single Marker Analysis and Chi-square Tests (χ2) for SSR Markers in the F_1 Mapping Population

The Chi-square tests were conducted for each marker to find out segregation distortion from the expected allele frequency ratio of 3: 1. Among the 32 polymorphic markers tested, 20 were found linked to various traits. Out of 20 linked markers, 17 maker loci were in concurrence with the expected ratios and 3 marker loci were not following the Mendelian inheritance (Table 4).

3.4 Markers linked to Traits

The single marker analysis revealed three markers such as AL 29, IM 31 and IM 28 to be significantly associated with some traits and their association is presented in Table 5. Marker AL 29 from Almond species was found significantly associated with stem girth, nut weight and kernel weight and explained phenotypic variance of 7.9%, 5.6% and 5.4%, respectively. Marker IM 31 from mango species was found significantly associated with stem girth, kernel weight, tree height, mean tree spread, nut weight and presented phenotypic variance of 17.6%, 5.7%, 8.2%, 4.5, 4.6% in that order. Whereas the marker IM 28 from mango species showed significant association with stem girth and intermodal length with phenotypic variance of 7.5%, 10.4% correspondingly.

The single marker analysis indicated significant linkage of six traits with three markers. The extent phenotypic variation explained (R^2) by the markers linked to traits was not more than 20% and it ranges from 4.6% to 17.6%. This implies that for success of marker-trait association studies in cashew, it necessitates good number of polymorphic markers. Considering the limited number of polymorphic markers, linkage map was not constructed for the present population and only marker-trait association analysis was carried out. It has come to our notice that, successful mapping studies were conducted even in the absence of a linkage map in chickpea [38]. It is worth mentioning here that the markers

Table 5. List of markers linked to traits and their Chi-square values in the F_1 mapping population

Sl.No.	Marker	Trait	Cal t value	R^2 (%)	P value	χ2 (Cal)	P value
1	AL 29	SG	4.54*	7.9	0.014	0.93	0.34
		NWT	3.45*	5.6	0.037		
		KWT	3.35*	5.4	0.040		
2	IM 31	SG	18.56**	17.6	0.000	0.59	0.44
		KWT	6*	5.7	0.016		
		TH	8.28**	8.2	0.005		
		MTS	4.91*	4.5	0.030		
		NWT	4.95*	4.6	0.029		
	IM 28	SG	4.33	7.5	0.016	1.052	0.31
		INL	5.77**	10.4	0.005		

** Significant at 5 %, ** Significant at 1 %, χ2 (Tab) = 3.841 at 5% significance*

Table 6. List of three highly significant associated markers overlapped with one or more than one trait/s and following mendelian segregation (1:1) in the mapping population

Sl.No	Marker	Trait	R^2 (%)	$P < 0.05$
1.	AL 29	SG	7.9	0.014
	IM 31		17.6	0.000
	IM 28		7.5	0.016
2.	AL 29	NWT	5.6	0.037
	IM 31		4.6	0.029
3.	AL 29	KWT	5.4	0.040
	IM 31		5.7	0.016
4.	IM 31	THMTS	8.2	0.005
			4.5	0.030

linked to traits such as tree height, tree spread, stem girth have significant positive correlation with nut yield [39-42] and therefore linked markers will be helpful in marker assisted selection of desirable genotype in the seedling stage and thus reduce the time and space for required for cashew variety development.

3.5 Overlapped Association of Markers with Traits

Besides, the above three linked markers showed overlapped association with the traits (Table 6). All the three markers AL 29, IM 31 and IM 28 were significantly co-associated with stem girth. Similarly, two linked markers viz., AL 29, IM 31 were co-associated with nut weight and kernel weight whereas IM 31 alone was linked with tree height as well as mean tree spread.

4. CONCLUSION

The present study has identified three markers AL 29 (from Almond) , IM 28 and IM 31 (from Mango) linked to six traits viz., stem girth, tree height, mean tree spread, intermodal length, nut weight and kernel weight with the limited polymorphic markers using Single Marker Analysis. These will be deployed for marker assisted selection in cashew to hasten the process of variety development. It is known that some QTL mapping studies have been carried out in cashew by researchers especially from EMBRAPA, Brazil. Thus, further studies are aimed to develop more SSR primers in cashew to facilitate linkage map development and to identify QTLs and validate linked markers in the present study prior to using them in marker assisted breeding in cashew.

ACKNOWLEDGEMENTS

Authors thank Director, ICAR-DCR, Puttur for financial assistance and other facilities provided for the conduct of experiment. Authors also thank technical assistant Ms. Harshita for assistance in collecting field data.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAOSTAT. Food and agriculture organization, Rome, Italy; 2018. Accessed: 01.09.2020. Available: http://www.faostat.org
2. Eradasappa E. Improved Cashew varieties and their recommendation. In: Eradasappa E, Mog B, Savadi S, Mangalassery S. EdITORS.). Training manual on cashew production and Post Harvest Technologies: ICAR-directorate of cashew research, Puttur, Karnataka, India. 2020;3:10-31.
3. Barros LM, Cavalcanti JJ, Paiva JR. Crisóstomo JR. Hibridação de caju. In Borém A (ed.) Hibridação artificial de plantas. Editora UFV, Viçosa. 2009:214-50.
4. Croxford AE, Robson M, Wilkinson MJ. Characterization and PCR multiplexing of polymorphic microsatellite loci in cashew (*Anacardium occidentale* L.) and their cross-species utilization. Mol. Ecol. Notes. 2006;6:249-51.
5. Thimmappaiah, Santhosh WG, Shobha D, Melwyn GS. Assessment of genetic diversity in cashew germplasm using RAPD and ISSR markers. Sci. Hort. 2009;120:411-17.
6. Thimmappaiah, Melwyn GS, Shobha D, Shirly RA. Assessment of cashew species for molecular diversity. Journal of Plantation Crops. 2009;37:146-51.
7. Mneney EE, Mantell SH, Bennett M. Use of random amplified polymorphic DNA (RAPD) markers to reveal genetic diversity within and between populations of cashew (*Anacardium occidentale* L.). J. Hort. Sci. Biotech. 2001;76:375-83.
8. Dhanaraj AL, Rao EVVB, Swamy KRM, Bhat MG, Prasad DT, Sondur SN. Using RAPDs to assess the diversity in Indian Cashew (*Anacardium occidentale* L.) germplasm. Journal of horticulture science and biotechnology. 2002;77:41-7.
9. Dasmohapatra R, Rath S, Pradhan B, Rout GR. Molecular and agromorphological assessment of cashew (*Anacardium occidentale* L.) genotypes of India. Journal of Applied Horticulture. 2014;16(3):215-21.
10. Bhadra T, Obaidullah A, Sultana M, Ahmed M, Islam M. Genetic diversity analysis in cashew (*Anacardium occidentale* L.) germplasm using RAPD marker. Journal of the Bangladesh Agricultural University. 2019;17(4):461-65.
11. Archak S, Gaikwad AB, Gautam D, Rao EVVB, Swamy KRM, Karihaloo JL. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR and AFLP) for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome. 2002;46:362-69.
12. Cavalcanti JJV. Genetic mapping and qtl identification in cashew (*Anacardium occidentale* L.). Reading: University of Reading. (Ph.D Thesis). 2004;178.
13. Cavalcanti JJV, Wilkinson MJ. The first genetic maps of cashew (*Anacardium occidentale* L.). Euphytica; 2007;157:131-43.
14. Cavalcanti JJV, Santos FHC, Silva FP, Pinheiro CR. QTL detection of yield-related traits of cashew. Crop Breeding and Applied Biotechnology. 2012;12:60-66.
15. Mzena GP, Kusolwa PM, Rwegasira GM, Yao NK. Mapping of quantitative trait loci (qtl) related to yield, nut quality and plant size of cashew (*Anacardium Occidentale*L). International Journal of Agriculture, Environment and Bioresearch. 2017;2(06):307-23, Available:http://www.researchgate.net/publication/322519839.
16. Myles S. Improving fruit and wine: what does genomics have to offer? Trends Genetic. 2013;29(4):190 – 6.
17. Ru S, Main D, Evans K, Peace C. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet Genomes; 2015;11:1–12.
18. Bus V M, Esmenjaud D, Buck E, Laurens F. Application of genetic markers in rosaceous crops. Genetics and genomics of rosaceae. 2009;6:563–99.
19. Karaagac E, Vargas A, De Andrés M, Carreño I, Ibáñez J Carreño J. Marker assisted selection for seedlessness in table grape breeding. 2012;8:1003–15.
20. Collard BCY, Mackill DJ. Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. Biological Sciences. 2008;363(1491):557–72.
21. Varshney RK, Beier U, Khlestkina E, Kota R, Korzun V, Röder M. Single nucleotide polymorphisms in rye: discovery, frequency and applications for genome mapping and diversity studies. Theor. Appl genet. 2007;114:1105 – 16.
22. Neto PS, Maruta I, Takaiwa F, Oono K, Matsumoto K, Gomes JA. 1 Identification

of cashew (*Anacardium occidentale* L.) seedlings with RAPD markers. Acta hort. 1995;370:21-6.

23. Bhat MG, Nagaraja KV, Rupa TR. Cashew research in India. J. Hortl. Sci. 2010;5(1):1-16.
24. Schnell RJ, Ronning CM, Knight RJ. Identification of cultivars and validation of genetic relationship in (Mangifera indica L) using RAPD markers. Theor Appl Genet. 1995;90(2):269–74.
25. Kenis K, Keulemans J, Davey MW. Identification and stability of QTLs for fruit quality traits in apple. Tree Genet Genomes. 2008;4:647–61.
26. Kafkas S, Ozkan H, Ak BE, Acar I, Atli HS, Koyuncu S. Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR and RAPD Markers. J. Am. Soc. Horticult. Sci. 2006;131:522-29.
27. Ferreira ME, Grattapaglia D. Introducao ao uso de marcadores moleculares em analise genetic. Brasilia, EMBRAPA-CENARGEN; 1996.
28. NRCC. Experimental manual on cashew. National Research Center for Cashew, Puttur, Karnataka, India. 2005;19-26.
29. Mneney EE, Mantell SH, Tsoktonidis,G, Amin S, Bessa AMS. RAPD profiling of tanzanian cashew (*Anacardium occidentale* L.). Proceedings of international cashew & cocoa conference dar es salaam, Tanzania; 1998.
30. Ahmad R, Ferguson L, Southwick SM. Identification of pistachio (*Pistacia vera* L.) nuts with microsatellite markers. The Journal of American Society for Horticultural Science. 2003;128: 898-903. DOI:10.21273/ JASHS.128.6.0898
31. Mnejja M, Garcia-Mas J, Arús P. Development and transportability across Prunus species of 42 polymorphic almond microsatellites. Mol Ecol. Resour. 2005;5(3):531-535.
32. Honsho C, Nishiyama K, Eiadthong W, Yonemori K. Isolation and characterization of new microsatellite markers in mango (Mangifera indica). Mol. Ecol.Notes. 2004;5(1):152-54.
33. Duval MF, Bunel J, Sitbon C, Risterucci AM. Development of microsatellite markers for mango (*Mangifera indica* L.). Mol. Ecol.Notes. 2005;5(4):824-26.
34. SPSS Inc. Released. SPSS for Windows, Version 16.0. Chicago, SPSS Inc; 2007.
35. Halward T, Stalker T, Larue E, Kochert G. Use of single-primer DNA amplifications in genetic studies of peanut (*Arachis hypogaea* L.),.Plant Molecular Biology. 1992;18(2):315–25.
36. Grattapaglia D Bertolucci FLG, Penchel R, Sederoff RR. Genetic mapping of quantitative trait loci controlling growth and wood quality traits in Eucalyptus grandis using a maternal half sib family and RAPD markers. Genetics. 1996;144:1205-14.
37. Thimmappaiah, Shobha D, Mohana GS, Adiga JD, Bhat PG. Fingerprinting of released varieties of cashew based on DNA markers. Vegetos. 2016;29:4.
38. Chandra S, Buhariwalla HK, Kashiwagi J. "Identifying QTL linked markers in marker-29 deficient crops, in Proceedings of the 4th International Crop Science Congress, Brisbane, Australia; 2004.
39. Rao AVDD, Ravisankar C, Reddy MLN. Correlation between nut yield and plant characters in cashewnut (*Anacardium occidentale* L.). J. Res. ANGRAU. 2002;30(1):98-100.
40. Abraham M, Mahapatro GK, Mathew J. Canopy structure in various cashew types and yield performance. J.Plantation Crops. 2007;35(2):116-18.
41. Mohana GS, Nayak MG, Eradasappa E.Genetic architecture of cashew germplasm accessions. Journal of Plantation Crops. 2017;45(1):57-65.
42. Adiga JD, Mohana GS, Eradasappa E, Meena RK, Saroj PL. Development of jumbo nut hybrid and character association studies in cashew (*Anacardium occidentale* L.). Vegetos. 2017;30 (Special). DOI:10.5958/2229-4473.2017.00093.3

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/65093