

Identification of Quantitative Trait Loci (QTLs) Conferring Dry Matter Content and Starch Content in Cassava (*Manihot esculenta* Crantz)

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Abstract: Cassava tubers are an excellent source of carbohydrate and a competitive source of starch most traded internationally. It is a highly desirable raw material for food and industrial purpose due to its high dietary carbohydrate content. The economic value of cassava products lies in the DMC (dry matter content). Cassava roots contain up to 80-90 per cent of carbohydrate by dry weight and 80 per cent of carbohydrate is starch. Increasing world population, limited land area, changing climatic condition and food scarcity demanded the need for improved cassava starch. Yield of cassava tubers is related to both tuber volume and DMC and thus DMC can be improved by cassava breeding. Thus QTL mapping of DMC is very much relevant to understand the genetic effects controlling the traits. The current study focused on QTL mapping for DMC and SC (starch content) to identify and study the favourite alleles using Windows cartographer version 2.5. Single marker analysis (SMA) identified seven marker alleles associated with DMC and eight marker alleles associated with SC. Using interval mapping, a single QTL for DMC was identified in chrom21 flanked by SSRY110^b and SSRY182^b. On the other hand, five QTLs for SC were identified by simple interval mapping (SIM) and a single QTL in chrom17 with R² value of 12% and at a LOD value 5 using composite interval mapping (CIM). The exact position of the QTLs and its interactions were studied using MIM and the genetic effect of QTLs controlling DMC was found to be over-dominance. But in the case of SC, the QTL interaction was identified and found to be additive x additive epistatic interaction.

Keywords: Cassava, Dry Matter Content, Linkage Map, Quantitative Trait Loci, Starch Content

1. Introduction

Cassava is an important tuber crop and a highly desirable raw material for food and industrial purpose due to its high dietary carbohydrate content feeding more than 800 million people [8]. According to Food and Agriculture Organization Corporate Statistical Database FAOSTAT [21], the global cassava production was estimated to be 277.8 MT and the Indian production was accounted to be 4651000 ton from an area of 228000ha. Cassava usage varied significantly by region and about 60 to 70% of cassava produced globally is used for food and 40% of the total cassava production is used for starch extraction [23]. Apart from food, cassava starch and its derivatives are used in the making of confectionery, sweeteners,

glues, plywood, textiles, paper, biodegradable products, monosodium glutamate and drugs. Cassava chips and pellets are used in making animal feed and alcohol production.

Cassava is a starchy root crop grown in small-scale by farmers and large-scale by industries due to the high demand for starch. Starch content (SC) and dry matter (DM) were two important parameters that affect the use of cassava as food and an industrial raw material [24]. On an average, Cassava tuber contains about 70% moisture, 20-30% carbohydrate, 1.0-1.8% crude protein, 1.5-3.5% crude fiber, 0.35-0.45% fat and 8-28 mg HCN/kg of dry mass [18]. Kawuki et al. [31] reported a wide genetic variability for DMC in Africa. DMC was affected by a number of genetic and environmental factors include age of the plant, crop

season, location and efficiency of canopy to trap sunlight [36]. It was usually highest before the onset of rain and dropped after the rain. This was due to the mobilization of starch from root to leaves in order to establish re-growth [9]. Allocation of DM to the storage root varied with the growth stages and DM was almost zero during the early stages to 80 per cent during the late growth stage [1]. Micronutrients in cassava is very low and several researches were carried in the cassava tubers to improve the micronutrient content such as provitamin A [7]. Biofortification of cassava largely depends on various agronomic parameters such as starch content, DMC, resistance to diseases and pests etc. DMC is one such parameters influences the cassava tuber texture after boiling and it is also studied that there is no negative relationship between carotenoids and DMC [15].

The storage root is a key organ for the direct production of cassava which contains high amount calories, but low protein, fat, mineral and vitamin content. Eighty per cent of carbohydrate in cassava is starch and 80-90 per cent carbohydrate is contributed by dry weight Wanatsanam *et al.*, [57]. Starch is the main storage carbohydrate synthesized from sucrose, inside amyloplast. This starchy root crops have becomes a significant feed stock for both food and commodity industries besides rice and cereals. With the increasing world population, extremely unpredictable environmental condition, limiting land area for cultivation and food scarcity strengthened the need for the development of new cassava cultivars with improved starch through cassava breeding program. Two main factors that determine the yield of cassava tubers are tuber volume and dry matter content. Yield therefore can be improved by increasing the dry matter content which itself reflects the economic value of cassava products Teye *et al.*, [53]. Significant correlation exists between starch and dry matter content in storage roots were reported by Cervantes-Flores *et al.*, [16]. Transcriptomic data integration of starch biosynthesis indicated that the genes involved in starch biosynthesis are more up-regulated and the carbon source for biosynthesis is from D-glucose-1-phosphate (G1P) Wanatsanam *et al.*, [57]. QTL mapping of such complex traits is very much relevant to identify the favorable alleles and to understand the genetic effects controlling the traits. With this in mind, the present study focused to identify QTLs controlling DMC and SC in cassava using F₁ mapping population derived from a cross between MNga-1 and CI-732.

2. Materials and Methods

In this study, two cassava cultivars *viz.*, CI-732 (local variety with high DMC and SC) and MNga-1 (CMD resistant variety with low DMC and SC) were selected as female and male parents based on the field trials conducted in ICAR-central Tuber Crops Research Institute (ICAR-CTCRI) during 2011-2015. The two cultivars were contrasted in DMC, SC, tuber skin colour, tuber rind colour and tuber flesh colour. CI-732 produced tubers with brown coloured skin, pink coloured rind and cream coloured flesh. On the other

hand, MNga-1 produced tubers with ivory coloured skin, white coloured rind and white coloured flesh. The parental lines were crossed and produced 114 F₁ progenies named as KM-1 to KM-114. The DMC and SC in the parental lines and F₁ progenies were determined by both oven dry method and by using electronic balance.

2.1. Phenotypic Evaluation

2.1.1. Determination of Dry Matter Content Using Dry Oven Method

The dry matter content in newly harvested cassava tubers of first clonal trials was analyzed by oven dry methods. In this method, a total of 50g of fresh peeled cassava tubers obtained from three different sections (head, middle and tail) were chopped into small pieces was dried at 60°C for 72h in an oven. Each F₁ genotype was replicated three times and weight of dried sample was expressed as percentage of fresh weight [4].

$$\text{Dry matter (\%)} = \frac{\text{weight of dried sample (g)}}{\text{weight of fresh sample (g)}} \times 100$$

2.1.2. Determination by Starch Content Using Electronic Balance

The starch content in newly harvested cassava tubers were performed by electronic balance. Firstly, the tubers were properly cleaned in water to remove the soil particles. With clean water, lift the bucket to the empty basket until the basket is completely immersed in water. Weighed the empty basket immersed in water and recorded the weight of basket indicated by the device. Lift the bucket back to the lower position. Weighed 3-6 kg clean cassava tubers into the basket in air. Lift the bucket to the basket containing cassava tubers until the basket was completely immersed in water and weighed again. Starch content of the sampling was calculated by a microprocessor in the indicating device. The SC was calculated automatically by microprocessor attached with the indicating device using the formula

$$\text{Starch content (SC)} = \frac{(SG - 1.00906)}{0.004845} \%$$

Where, SG is the specific gravity

Analysis of variance (ANOVA) was performed for DMC and SC and the means were compared with DUNCAN's multiple range test. The correlation study between DMC and SC was also performed.

2.2. QTL Mapping

The QTL mapping of DMC and SC of F₁ progenies was done separately to find out the markers associated with the particular traits using the genetic linkage map already constructed [56]. The QTL mappings were carried out using three different methods *viz.*, single marker analysis (SMA), simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM) in Windows QTL Cartographer version 2.5. A significant LOD value for a particular trait in interval mapping was decided by the genome-

wide significant threshold value which itself was calculated by means of permutation test carried out at 1000 replicates, 5% level of significance and at a walk speed of 1cm [35]. CIM was performed using the parameters viz., forward and backward regression, Model-6 standard model, 10 control markers and a walk speed of 1cm [26, 27, 61, 62] The Broad-sense heritability (H^2) of the trait was calculated using the formula

$$H^2 = \frac{V_G}{V_P} \times 100$$

V_G is the total genetic variance (additive, dominant and epistatic) and V_P is the total phenotypic variance

The narrow sense heritability (h^2) of the QTLs for a particular trait was calculated using the formula

$$h^2 = \frac{V_A}{V_P} \times 100$$

V_A is the total additive variance and V_P is the total phenotypic variance

The expected advance or gain from the current breeding population were calculated using the formula

$$G_c = (k)(\sqrt{V_P})h^2$$

G_c = genetic advance or gain, $\sqrt{V_P}$ = square root of phenotypic variance, k = Selection intensity (1.76), h^2 = narrow sense heritability

3. Result and Discussion

Cassava tubers are an excellent source of carbohydrate and a competitive source of starch most traded internationally. The

increasing demand for starch established the large-scale commercial planting of cassava. The economic value for cassava products lies in the DMC and is regarded as the true biological yield. The yield of cassava tubers can be improved by cassava breeding programmes. In the current study, DMC in parental lines and F_1 mapping population were analyzed by oven dry method. DMC of male and female parent was found to be 38% and 54% respectively. The DMC of F_1 progenies were also taken and found that out of the 114 individuals, 21 individuals showed DMC below 40, 28 individuals with a DMC between 40 and 45, 45 individuals between 46 and 50 and 20 progenies showed DMC above 50. The high DMC ranging from 58-55 was observed in four progenies named KM-2B (57.41), KM-83 (56.32), KM-103 (56.15) and KM-32 (55.78). These genotypes were found statistically significant from other genotypes. The mean trait value was found to be 45.07 with a variance of 35.75%. The distribution of DMC among F_1 progenies were skewed towards high DMC (above 45%) indicating the influence of female parent.

The SC of parental lines viz., MNga-1 and CI-732 were analyzed in electronic balance and was found to be 21 and 27 respectively. The mean trait value of the SC was found to be 24.43 with a variance of 19.7%. In this study, 53 individuals showed SC falls between 26 and 30, 38 individuals with SC between 21 and 25, five individuals showed SC above 30 and 20 individuals with SC below 20. The SC was found to be highest in genotype KM-32 (33) and was on par with KM-23 (30.5), KM-24 (30.5) and KM-25 (31). The positive correlation between DMC and SC was found to be $r = 0.735$ (Table 1).

Table 1. DMC and SC of F_1 progenies derived from MNga-1/CI-732.

Sl. No.	DMC	SC	Sl. No.	DMC	SC	Sl. No.	DMC	SC
1	44.19	25.0	39	50.20	26.0	77	31.09	18.0
2	50.43	27.4	40	43.48	26.5	78	45.59	30.0
3	57.41	28.0	41	49.27	25.5	79	35.20	12.0
4	41.32	22.0	42	52.15	28.5	80	41.80	19.0
5	52.56	27.0	43	41.34	23.5	81	56.11	29.5
6	25.60	12.0	44	45.14	26.0	82	49.23	23.5
7	36.72	17.0	45	34.50	15.0	83	41.12	22.0
8	50.43	28.0	46	40.83	21.0	84	49.18	29.0
9	45.16	25.5	47	45.70	26.5	85	40.36	25.0
10	50.69	27.5	48	47.05	26.0	86	33.18	18.0
11	48.28	28.5	49	44.36	25.0	87	38.00	19.5
12	43.11	22.5	50	33.80	13.5	88	48.81	26.5
13	48.15	29.0	51	47.93	24.0	89	38.30	15.0
14	45.00	22.0	52	42.07	25.0	90	51.71	29.0
15	50.22	26.5	53	50.02	23.0	91	37.43	18.0
16	38.81	26.0	54	48.68	23.0	92	48.27	27.5
17	45.14	21.0	55	37.01	28.5	93	47.06	20.0
18	48.48	30.5	56	49.13	26.0	94	40.78	26.5
19	49.69	30.5	57	47.56	23.5	95	39.52	26.5
20	49.14	31.0	58	50.31	26.0	96	43.14	24.0
21	54.55	30.0	59	49.79	26.5	97	43.60	22.0
22	55.74	33.0	60	52.60	28.5	98	35.59	23.0
23	38.23	22.0	61	41.47	24.0	99	30.47	17.0
24	47.56	27.5	62	49.34	27.5	100	48.58	28.0
25	50.90	27.5	63	44.83	22.5	101	39.32	15.0
26	48.57	25.5	64	48.07	27.0	102	42.63	21.5
27	49.56	26.5	65	45.82	20.0	103	55.10	28.5

Sl. No.	DMC	SC	Sl. No.	DMC	SC	Sl. No.	DMC	SC
28	48.82	29.5	66	56.31	20.0	104	48.48	24.0
29	44.49	24.0	67	52.82	30.5	105	42.71	22.5
30	49.00	28.0	68	42.39	22.5	106	46.10	23.5
31	44.87	25.0	69	42.64	24.0	107	46.57	24.0
32	45.58	22.5	70	39.40	12.5	108	29.50	11.0
33	46.99	26.5	71	41.57	29.0	109	47.65	28.5
34	41.12	17.5	72	46.46	27.0	110	44.43	22.5
35	35.81	23.0	73	39.70	22.5	111	44.69	23.0
36	49.16	28.5	74	44.13	27.0	112	49.58	23.0
37	51.06	29.5	75	45.92	26.0	113	48.26	29.0
38	45.75	28.5	76	41.38	27.0	114	46.32	24.0

Wide genetic variability for DMC has been reported in Africa [30] and the total phenotypic variation in DMC was due to genetic differences in an experiment with inbred and local cassava clones [5]. Thus the present study focused on QTL mapping to identify favourable alleles controlling the complex traits such as DMC and SC, and is very much helpful in marker assisted selection. In this study, a genetic linkage map was constructed using 70 polymorphic SSR primer pairs. Each primer pairs were scored using single dose restriction fragment (SDRF) and the segregation pattern were studied using χ^2 ($p \leq 0.05$). Ninety three (82.3%) primers showed 1:1 disomic segregation pattern and twenty marker alleles (17.69%) showed deviations from Mendelian segregation pattern. A genetic linkage map was constructed which comprises 24 linkage groups spanned a genetic distance of 1165.9cM and the random distribution of SSR markers throughout the genome were revealed through the correlation analysis ($r = 0.67$) between linkage distance and numbers of markers in each linkage group [56].

QTLs controlling these complex quantitative traits were

studied using different QTL mapping studies. SMA using simple linear regression identified seven marker alleles, which were found to be strongly associated with DMC *viz.*, SSRY21^c, SSRY59^c, SSRY59^d, NS185^c, NS185^d, SSRY49^c and SSRY182^b at 5% level of significance. On the other hand, eight marker alleles were found to be associated with SC *viz.*, SSRY314^a, NS308^b, SSRY59^c, SSRY59^d, SSRY182^b, NS185^c, NS185^d and SSRY38^d which was distributed in five linkage groups (Table 2). The distribution of markers controlling the DMC in different linkage group also explained the polygenic control of DMC. This is consistent with the studies of Kizito *et al.* [4] identified six QTLs controlling the DMC in four different linkage groups in cassava and also in favour of previous reports [25, 29]. QTLs controlling the root yield traits were studied by Okogbenin and Fregene [42] and Okogbenin *et al.* [44], root productivity and plant architecture [43] plant and first branch height, which were associated with root yield was reported by Boonchanawiwat *et al.*, [6] and root yield and starch content [51].

Table 2. SSR markers associated with DMC and SC identified by SMA.

Sl. No.	Linkage group	Markers	F-value of DMC	F-value of SC
1	chrom6	SSRY21 ^c	6.051*	-
2	chrom14	SSRY314 ^a	-	7.41**
3	chrom14	NS308 ^b	-	7.41**
4	chrom17	SSRY59 ^c	4.57*	7.58**
5	chrom17	NS185 ^c	4.57*	7.58**
6	chrom18	SSRY59 ^d	4.57*	7.58**
7	chrom18	NS185 ^d	4.57*	7.58**
8	chrom20	SSRY49 ^c	3.96*	-
9	Chrom21	SSRY182 ^b	6.196*	4.45*
10	chrom24	SSRY38 ^d	-	8.93**

*,** - Probability at 5% and 1% level of significance respectively

In SMA, the association of single marker with that particular trait was revealed but the exact QTL positions, its effects and gene interaction were undetermined. This was made through interval mappings which scan the whole genome. Through SIM and CIM, a single QTL for DMC was identified in chrom21 flanked by SSRY110^b and SSRY182^b (Table 3 and Figure 1). The exact position of the QTL was determined by MIM using MIM forward scan and was found at a position of 28cM in chrom21. On the other hand, five QTLs for SC were identified by SIM were distributed in five different linkage groups *viz.*, chrom17, 18, 19, 21 and 24 at a

LOD value of 5. The maximum R^2 was 11% was explained by QTLs at chrom17, 18 and 24. The QTLs present in chrom19 and 21 showed R^2 values of 1% and 4% respectively (Table 4 and Figures 2, 3). Then CIM was performed to narrow down the QTL positions and identified a single QTL in chrom17 with R^2 value of 12% and at a LOD value 5 (Table 5 and Figure 4). The exact position, gene effects and gene interactions of the QTLs were analyzed by MIM and identified two main QTLs in chrom17 and chrom18 present at 5cM and 22cM respectively (Table 6).

Table 3. QTLs for DMC identified by CIM in Windows Cartographer 2.5.

QTLs	LG	Intervals	Additive effects (a)	Dominance Effects (d)	d/a	Gene Action (GA)	LOD	R ² value
QTL1	chrom21	SSRY110 ^b - SSRY182 ^b	-1.04	-13.22	12.7	OD	2.5	32%

Table 4. QTLs for SC identified by SIM in Windows Cartographer 2.5.

QTLs	LG	Intervals	Additive effects (a)	Dominance Effects (d)	d/a	Gene Action (GA)	LOD	R ² value
QTL1	chrom17	SSRY59 ^c - NS185 ^c	1.07	-12.18	-11.38	OD	6.5	11%
QTL2	chrom18	SSRY59 ^d - NS185 ^d	-1.06	-12.18	11.38	OD	6.5	11%
QTL3	chrom19	SSRY35 ^d - SSRY284 ^c	0.27	-12.4	-45.92	OD	5.6	1%
QTL4	chrom21	SSRY110 ^b - SSRY182 ^b	-0.35	-12.4	35.43	OD	5.5	4%
QTL5	chrom24	NS158 ^d - SSRY38 ^d	-0.15	-12.4	82.66	OD	5.10	11%

Table 5. QTLs for SC identified by CIM in Windows Cartographer 2.5.

QTLs	LG	Intervals	Additive effects (a)	Dominance Effects (d)	d/a	Gene Action (GA)	LOD	R ² value
QTL1	chrom17	SSRY59 ^c - NS185 ^c	1.11	-11.43	-10.29	OD	5.0	12%

Table 6. QTLs for SC identified by MIM in Windows Cartographer 2.5.

QTLs	LG	Intervals	Additive variance (A)	Dominance Variance (D)	QTL Pos cM	Interaction (AA)	R ² value
QTL1	chrom17	SSRY59 ^c - NS185 ^c	1.92	-9.56	5	-3.58	79%
QTL2	Chrom18	SSRY59 ^d - NS185 ^d	0.79	-2.03	22		

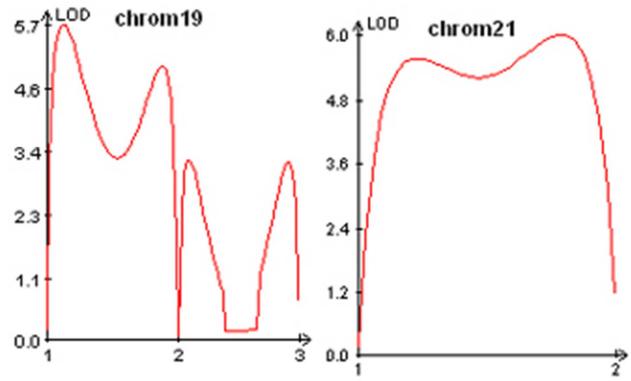
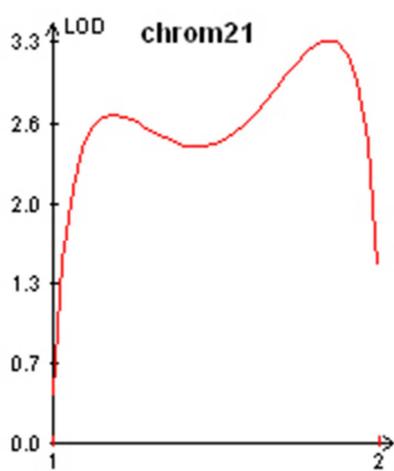


Figure 1. QTL for DM identified in chrom 21 using SIM and CIM.

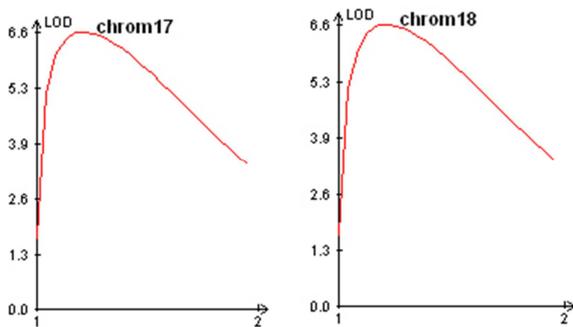


Figure 2. QTL associated with SC in chrom17 and chrom18 using SIM.

Figure 3. QTL associated with SC in chrom19, chrom21 and chrom24 using SIM.

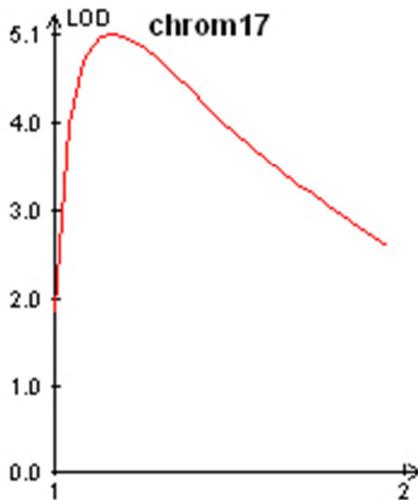


Figure 4. QTL associated with SC in chrom17 using CIM.

Important traits in crops were controlled by quantitative genes which have different actions [39]. Understanding the genetic architecture requires the decomposition of genetic effects into additive, dominance and epistatic components [22, 19, 31]. In the present study, the additive and dominant effects of the QTLs controlling DMC were found to be -1.04 and -13.22 respectively. Also the R^2 value explained by these QTL was found to be 32%. The genetic effect of QTLs controlling DMC was found to be over-dominance which was strongly supported by previous study conducted by Kizito *et al.* [33] and in that study, out of six QTLs identified for DMC, five QTLs showed dominance or over dominance and one QTL showed additive gene action. Amma *et al.* [3] and Amma and Sheela [2] also reported a non-additive gene control for dry matter and starch content using classical studies. On the other hand, Wolfe *et al.* [59], Kawano *et al.* [29] and IITA [25] reported polygenic additive control of root DMC in cassava. Several diallel studies in cassava indicated that non-additive genetic effects in root yield traits were strong [11, 10, 12, 28, 45, 46, 60, 34, 55, 14, 17]. In addition to cassava, non-additive effects were reported and utilized in the breeding of potato [32], Eucalyptus [49] and loblolly pine [40]. A few attempts that have been made to understand the genetics of starch and starch-related traits in cassava that had been reported to be polygenically controlled. There is a positive correlation exist between DMC and SC. The interaction between the QTLs controlling SC was studied and identified a strong additive x additive epistatic (AA) interaction between QTLs. The R^2 value explained by QTL interaction was found to be 79%. The total phenotypic and genotypic variance explained by the trait was found to be 19.59 and 15.64 respectively.

Non-additive variations are common in cassava, especially for low heritability traits. In the present study, the H^2 of DMC was found to be 32.7%. The H^2 and h^2 of this QTL interaction was calculated and found to be 79.8% and 43% respectively. The H^2 and h^2 of this QTL interaction was calculated and found to be 79.8% and 43% respectively. The genetic gain from the selection was found to be 3.35. The non-additive

variation also explained the slow genetic gain in cassava [13] and low accuracies have been reported for genomic prediction of yield compared with CMD resistance and DMC [20, 38]. Inbreeding to convert dominance variance to additive and better control epistatic combinations, as in maize has been suggested as a solution to non-additive genetics [14]. Moreover, the moderate broad sense heritabilities of root yield and starch content inferred that these traits were strongly influenced by the environment [50]. Such traits were expected to be difficult to handle by direct selection [41].

Generally, QTL underlying related traits tend to map in the same genomic regions or adjacent regions in the same linkage group [52]. Therefore, QTL for yield and yield associated traits such as yield components (starch content, % dry matter, seed number and seed weight) and yield-related traits (plant architecture, biomass and harvest index) appeared to be clustered in the genome [48]. In the present study, it was also found that five QTLs identified by SMA and single QTL from interval mapping were common to both DMC and SC. The QTL affecting different traits within the same genomic regions could be explained by pleiotropic effects or the close linkage of multiple genes [43]. The major indicators for pleiotropic QTL were overlapped confidence interval regions of separate QTL, trait correlations and environmental correlations [54]. The consistent QTL and the coincident QTL controlling different traits should be useful for MAS [59, 47]. Thus the genetic variation in root yield trait such as DMC and SC in cassava was greatly explained by the genetic effects (additive and dominance). Performance of best hybrid therefore depends mainly on additive and dominant variance but gets an extra boost from epistasis. In a clonally propagated crop such as cassava, non-additive genetic effects can be effectively exploited by the identification of superior genetic individuals as varieties.

4. Conclusion

This study focused to identify QTLs controlling the traits such as SC and DMC in cassava. The study is conducted using the statistical package Windows cartographer version 2.5. The genetic effect of QTLs controlling DMC was found to be over-dominance and that of SC was found to be additive x additive epistatic interaction.

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