

# Analysis of QTL for yield-related traits in cassava using an F<sub>1</sub> population from non-inbred parents

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**Abstract** A cassava F<sub>1</sub> population raised from the cross SC6 × Mianbao was used to construct a genetic linkage map. The map incorporated 200 polymorphic amplified fragment length polymorphism, sequence-related amplified polymorphism, simple sequence repeat (SSR), and expressed sequence tag (EST)–SSR markers which fit a 1:1 segregation ratio. It comprised 20 linkage groups (LGs) and spanned a genetic distance of 1645.1 cM with an average marker interval of 8.2 cM. Fifty-seven repeatedly detected QTLs (rd-QTLs) for three phenotypic traits (fresh root yield, root dry matter content, and root starch content) were identified in the F<sub>1</sub> population in four trials of year 2003, 2004, 2005, and 2008 by inclusive composite interval mapping. Among the 57 rd-QTLs, 25

rd-QTLs were linked to SSR/EST–SSR markers, which will help to facilitate marker-assisted selective breeding in cassava, and 15 marker intervals on ten LGs showed pleiotropic effects.

**Keywords** Cassava · Genetic map · Breeding

## Introduction

Cassava (*Manihot esculenta* Crantz) is an important tuberous root crop in Africa, South America, and Asia. It is the sixth most important staple crop worldwide and is consumed by 600 million people. Cassava offers the advantages of high photosynthetic efficiency, high root/starch yield, and tolerance of drought and poor soils. Furthermore, cassava is cultivated to be a cash crop for the bioenergy and starch industries in China and other countries. The annual production of cassava in 2010 was 229 million tons and production increased by approximately 5 million tons/year from 2000 to 2010 (FAOSTAT). However, current production is insufficient to meet the rapidly increasing market demand.

The application of molecular marker techniques for quantitative trait locus (QTL) mapping is an effective approach to dissect complex quantitative traits. Cassava is monoecious and protogynous, and thus predominantly outcrossing, which leads to a high degree of heterozygosity that considerably complicates breeding (Hershey and Jennings 1992). QTL

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analysis of complex and quantitative traits in cassava is helpful to identify favorable alleles and to obtain recombinants with desirable traits through marker-assisted selective breeding.

The QTL analyzes so far has focused on disease resistance and root yield/quality. Diseases such as cassava bacterial blight (CBB), cassava mosaic disease (CMD), and cassava in cassava, anthracnose disease (CAD) are important biotic constraints that greatly decrease cassava yield worldwide, particularly in Africa and South America. Jorge et al. (2000, 2001) detected 14 and eight QTLs for tolerance to CBB by a single-marker regression method in the progeny of the cross TMS30572 × CM2177-2 in successive years. Wydra et al. (2004) and Lopez et al. (2007) identified additional QTL associated with resistance to CBB. Several QTLs associated with resistance to CMD and CAD have been identified (Akano et al. 2002; Akinbo et al. 2007; Lokko et al. 2005). These studies have provided marker resources that are useful for breeding disease resistance in cassava, and the identified QTLs/markers could be utilized and further validated by molecular marker-assisted selection (MAS). In addition, Cortés et al. (2002) published ten QTLs associated with resistance to post-harvest physiological deterioration (PPD), and these QTLs could explain 5–13 % of phenotypic variation in the same mapping population by Jorge et al. (2000, 2001) formerly.

Increased root yield and improvement of eating quality are also important objectives in cassava breeding. Okogbenin and Fregene (2002) and Okogbenin et al. (2008) successively identified a series of QTLs that control traits related to cassava root yield in the progeny of the cross TMS30572 × CM2177-2, and a portion of these QTLs were detected repeatedly. Furthermore, Balyejusa Kizito et al. (2007) identified two and six QTLs that control cyanogenic glucoside potential and root dry matter content (DMC), respectively.

In the present study, a cassava F<sub>1</sub> population that comprised 208 progeny was raised from the cross SC6 × Mianbao. Amplified fragment length polymorphism (AFLP; Vos et al. 1995), sequence-related amplified polymorphism (SRAP; Li and Quiros 2001), simple sequence repeat (SSR), and expressed sequence tag (EST)–SSR markers were applied to generate a genetic linkage map, which was used to identify QTL for DMC, fresh root yield (FRY), and root starch content (SC) with the F<sub>1</sub> population.

## Materials and methods

### Plant material and field experiments

A cassava F<sub>1</sub> population that comprised 208 progeny was raised from a cross between two non-inbred lines, SC6 (the female parent) and Mianbao (the male parent), at the Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. SC6 is a predominant cultivar that is grown in south China and Mianbao is a landrace grown on Hainan Province that shows tolerance to drought and barren stresses. The F<sub>1</sub> population was designated the population SM. Stem cuttings from the 208 F<sub>1</sub> progeny and the parents were planted in the field, with a randomized block design and one replication in 2003, 2004, 2005, and 2008 in Wenchang, Hainan Province, and each genotype had six individuals. The field management essentially followed normal agricultural practices. About 10 months after planting, three uniform individuals were harvested from each line for the collection of phenotypic data.

### Trait evaluation

Fresh root yield was determined as the average weight of the roots of the three selected individuals. Dry matter root content was determined by drying 200 g slices of storage root (the mixed sample of three individuals) at 60 °C in a forced air dryer to a constant weight. The dried root slices were ground in a household grinder, filtered through a 0.5 mm mesh sieve, and placed in a desiccator until subsequent analysis. Root SC was measured by a polarimetric method (Mitchell 1990).

### AFLP analysis

Total DNA (200 ng) of each line was digested with two restriction enzymes, *EcoRI* and *MseI*. Adapter ligation and two successive PCR amplifications for AFLP analysis were performed in accordance with the method described by Vos et al. (1995) with slight modification. The 5' end of the *MseI* primer was labeled with the fluorescent FAM marker. The PCR products were mixed with loading buffer and denatured. Then, a 1.0 µl aliquot was electrophoresed on an ABI PRISM<sup>TM</sup> 377 DNA sequence analyzer for 2 h,

and the amplified fragments were visualized by FAM fluorescence. The AFLP primers used in the study are listed in Supplementary Table 1.

### SRAP analysis

Sequence-related amplified polymorphism analysis is a PCR-based marker system that uses two primers, a forward primer of 17 bases and a reverse primer of 18 bases. The forward primer consists of a core sequence of 14 bases. The first ten bases at the 5' end comprise a 'filler' sequence of no specific constitution, which is followed by the sequence CCGG and then by three selective nucleotides at the 3' end. The reverse primer of 18 bases is similar to the forward primer, but its 'filler' sequence is followed by AATT instead of CCGG and there are four selective nucleotides. The first five cycles of the PCR amplification comprised denaturation at 94 °C for 60 s, annealing at 35 °C for 60 s, and extension at 72 °C for 60 s, and then the annealing temperature was raised to 50 °C for an additional 35 cycles (Li and Quiros 2001). The PCR products were electrophoresed on a 2 % agarose gel for 30 min at 90 V, and visualized under ultraviolet light. The SRAP primers used in the study are listed in Supplementary Table 1.

### SSR and EST–SSR analysis

Twenty-four SSR primers and 30 EST–SSR primers were first screened against the parents and ten gene pools. Each gene pool consisted of ten individual progeny's DNA. The SSR markers that were polymorphic were used to evaluate the entire population. The PCR products were separated on 6 % denaturing polyacrylamide gels for 1.5 h using a BIO-RAD Sequi-Gen sequencing system (Bio-Rad, USA). The separated products were visualized by silver staining.

### Construction of the genetic linkage map

JoinMap3.0 was used to construct a linkage map (Stam 1993) with the population SM. The threshold of fit was set to  $\leq 5.0$  with LOD scores  $> 1.0$  and a recombination frequency  $< 0.4$ . The population SM was considered to be a CP-type (cross pollination between two heterozygosity lines) population because the genetic background of the two parents was heterozygous. Hence, each AFLP, SRAP, and SSR marker was assumed to be located at a single locus. The parent-specific markers that showed 1:1 segregation in the population SM were scored and recorded as  $lm \times ll$  (marker in female parent) and  $nn \times np$  (marker in male parent). Only the markers that showed 1:1 segregation were

**Table 1** Summary of measurements of fresh root yield per individual (FRY), dry matter root content (DMC), and starch content (SC) for SC6, Mianbao, and the population SM in 2003, 2004, 2005, and 2008 in Wenchang, Hainan Province, China

	Trial	FRY (g)	DMC	SC
SC6/Mianbao	2003	1050.0 $\pm$ 85.6/625.0 $\pm$ 45.7 <sup>a</sup>	0.367/0.4610	0.276/0.344
	2004	1813.0 $\pm$ 189.1/837.0 $\pm$ 90.7	0.341/0.4784	0.217/0.354
	2005	2800.0 $\pm$ 321.6/1280.0 $\pm$ 106.4	0.325/0.3611	0.185/0.232
	2008	967.0 $\pm$ 47.3/237.0 $\pm$ 32.1	0.400/0.404	0.298/0.288
Population SM	2003	1039.0 <sup>b</sup>	0.390	0.275
		190.0–2490.0	0.231–0.529	0.133–0.399
	2004	1169.3	0.444	0.317
		233.0–2513.0	0.260–0.568	0.151–0.405
	2005	1641.1	0.390	0.246
	90.0–4900.0	0.202–0.559	0.089–0.368	
	2008	1015.8	0.408	0.304
		30.0–4070.0	0.277–0.570	0.180–0.405

FRY: the average value of three individuals, DMC and SC: the mixed sample of three individuals

<sup>a</sup> The mean  $\pm$  standard deviation

<sup>b</sup> Upper values are the mean of population SM, lower values are the range

used to generate the genetic map; all other markers were excluded.

### Mapping of QTL

Inclusive composite interval mapping (ICIM), as implemented in the IciMapping 3.0 software (Li et al. 2007), was used to detect QTL for FRY, DMC, and SC in four trials. Model 7 of the procedure was employed, and the likelihood of a QTL and its corresponding effect was estimated at every 1 cM. A LOD score of 2.5 was set as the threshold for declaring the presence of a QTL. The dominance ( $d$ ) and additive ( $a$ ) values for each QTL that were given by the program were used to calculate the ratio of  $d/a$ . The QTLs based on the average data of 4 years/single year data were list in Table 3/Supplementary Table 1.

## Results

### Phenotypic evaluation

Measurements for the FRY, DMC, and SC traits in the population SM and the two parents are listed in Table 1. The FRY of SC6 was higher than that of Mianbao in all 4 years ( $t$ -test,  $P = 0.029$ ), whereas the DMC of SC6 was lower than that of Mianbao in all 4 years ( $t$ -test,  $P = 0.107$ ). The SC of SC6 was lower than that of Mianbao in 3 years (2003, 2004, and 2005), but slightly higher in 2008.

### Construction of a genetic linkage map

Out of a total of 367 polymorphic markers, 200 markers showed 1:1 segregation and were assigned to 20 linkage groups (LGs) with JoinMap. The genetic map consisted of 139 AFLP markers, 21 SRAP markers, 11 SSR markers, and 29 EST–SSR markers, and was designated the SM11 genetic map because the mapped markers all conformed to a 1:1 segregation ratio (Table 2; Fig. 1). The markers were not distributed evenly among different LGs; the largest LG contained 33 markers, and the smallest LG only contained three markers. The length of the LGs varied from 43.5 cM (LG19) to 125.1 cM (LG17). The SM11 genetic map spanned a total of 1645.1 cM (Kosambi units). The marker intervals ranged from 0.1 to 39.4 cM and the average marker interval was 8.2 cM.

**Table 2** Linkage group size, number of markers, and average marker interval per linkage group in the cassava genetic linkage map

Linkage group	Size (cM)	No. of markers	Marker type	Average marker interval (cM)
LG1	105.7	21	AFLP	5.0
LG2	50.0	10	AFLP	5.0
LG3	114.7	14	AFLP	8.2
LG4	47.7	5	AFLP	9.5
LG5	83.8	15	AFLP	5.6
LG6	55.3	8	AFLP	6.9
LG7	98.0	11	AFLP	8.9
LG8	80.7	5	AFLP	16.1
LG9	88.7	4	AFLP	22.2
LG10	56.3	7	SRAP	8.0
LG11	95.0	5	AFLP	19.0
LG12	80.9	7	SSR, EST	11.6
LG13	97.6	5	SSR, EST	19.5
LG14	50.2	4	SSR, EST, AFLP	12.5
LG15	118.9	33	AFLP	3.6
LG16	70.5	6	AFLP	11.7
LG17	125.1	27	SSR, EST, SRAP	4.6
LG18	115.6	6	SSR, AFLP	19.3
LG19	43.5	4	SSR, EST	10.9
LG20	67.1	3	EST	22.4
Mean	82.3	10	–	8.2

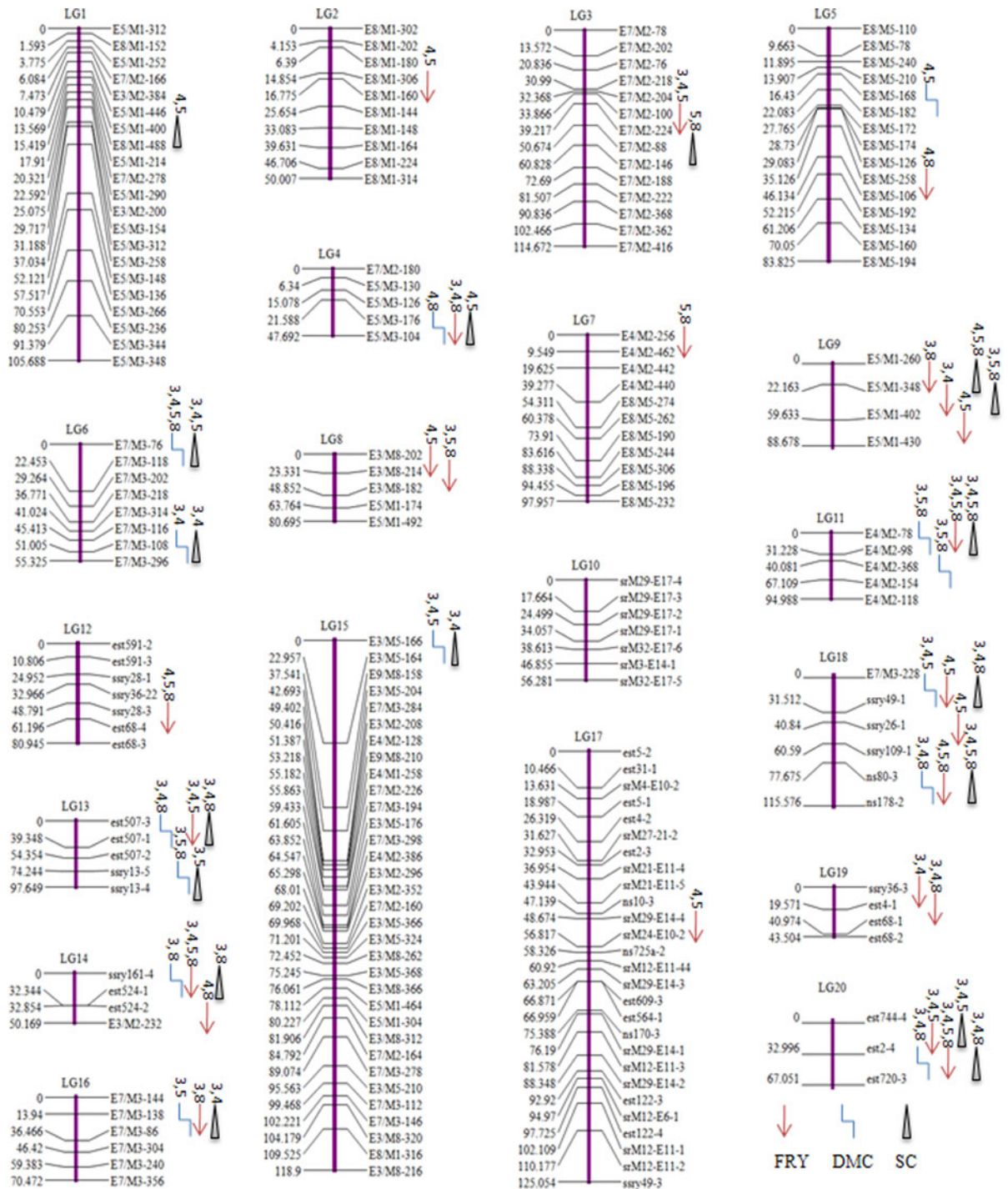
AFLP amplified fragment length polymorphism, SRAP sequence-related amplified polymorphism, SSR simple sequence repeat, EST–SSR expressed sequence tag–SSR

### QTL mapping

The results of QTL mapping for the three traits that were analyzed in the four trials are listed in Supplementary Table 1. For each trait, the analysis was carried out for the phenotypic data obtained in each individual trial as well as the average phenotypic data from all four trials. Although the interaction effects for QTL  $\times$  environment were significant for some QTL, but most of the QTL that were detected repeatedly across environments were also detected when the average values of the four trials (see Table 3; Fig. 1).

For FRY, 60 QTLs were detected, for which the phenotypic variation explained (PVE) ranged from 1.60 to 48.38 % with an average value of 26.75 %. Among these 60 QTLs, 13 QTLs were detected





**Fig. 1** SM11 genetic map and the positions of repeatedly detected QTL with the phenotypic data of four trials in the population SM. E5M1, the AFLP primer combination E5 and M1; srM4-E10, the SRAP primer combination me4 and em10

repeatedly in two trials, eight QTLs were detected repeatedly in three trials, and four QTLs (*qfry3b*, *qfry11a*, *qfry14a*, and *qfry20b*) were detected

repeatedly in all four trials. Eleven repeatedly detected QTLs (rd-QTLs) were linked to SSR markers and their average PVE was 17.42 %.

**Table 3** Identified QTL for three phenotypic traits detected in the population SM using the average phenotypic data of the four trials

Trait	LG	QTL	Position (cM)	Left marker	Right marker	LOD	PVE <sup>a</sup> (%)	EstA	EstD	<i>ld/a</i> <sup>b</sup>	GA <sup>c</sup>	Trial <sup>c</sup>	
FRY	7	<i>fry7</i>	2	E4/M2-256	E4/M2-462	2.51	11.93	9.56	602.50	63.05	OD	5,8	
	8	<i>fry8a</i>	10	E3/M8-202	E3/M8-214	2.74	3.78	-21.28	171.08	-8.04	OD	4,5	
	8	<i>fry8b</i>	36	E3/M8-214	E3/M8-182	5.42	21.07	-9.13	406.31	-44.51	OD	3,5,8	
	9	<i>fry9a</i>	10	E5/M1-260	E5/M1-348	2.85	2.78	57.78	-128.37	-2.22	OD	3,8	
	9	<i>fry9b</i>	41	E5/M1-348	E5/M1-402	4.16	1.60	5.96	-104.31	-17.50	OD	3,4	
	9	<i>fry9c</i>	74	E5/M1-402	E5/M1-430	4.05	5.06	-20.03	-194.78	9.72	OD	4,5	
	11	<i>fry11</i>	15	E4/M2-78	E4/M2-98	4.41	10.94	1.91	289.48	151.50	OD	3,4,5,8	
	12	<i>fry12</i>	58	ssry28-3	est68-4	4.95	23.10	-18.85	478.56	-25.39	OD	4,5,8	
	13	<i>fry13</i>	20	est507-3	est507-1	4.39	3.90	-21.78	-147.62	6.78	OD	3,4,5	
	14	<i>fry14</i>	15	est524-1	est524-2	6.80	18.35	-13.16	534.31	-40.61	OD	3,4,5,8	
	18	<i>fry18a</i>	15	E7/M3-228	ssry49-1	3.83	1.25	35.21	-50.88	-1.45	OD	4,5	
	18	<i>fry18b</i>	95	ns80-3	ns178-2	5.18	4.24	55.45	-118.53	-2.14	OD	4,5,8	
	19	<i>fry19</i>	30	est4-1	est68-1	2.95	3.58	73.74	-145.74	-1.98	OD	3,4,8	
	20	<i>fry20a</i>	17	est744-4	est2-4	4.40	2.39	50.68	-79.14	-1.56	OD	3,4,5	
	20	<i>fry20b</i>	50	est2-4	est720-3	4.99	3.13	64.36	-23.07	-0.36	PD	3,4,5,8	
	DMC	1	<i>dmc1</i>	14	E5/M1-400	E8/M1-488	3.19	10.76	0.01	0.04	4.69	OD	4
		3	<i>dmc3</i>	50	E7/M2-224	E7/M2-88	3.51	13.60	-0.01	-0.06	9.21	OD	8
		4	<i>dmc4</i>	35	E5/M3-176	E5/M3-104	3.20	4.11	0.00	0.02	4.68	OD	4,8
		6	<i>dmc6</i>	11	E7/M3-76	E7/M3-118	3.55	5.61	-0.01	0.01	-2.33	OD	3,4,5,8
		10	<i>dmc10</i>	8	srM29-E17-4	srM29-E17-3	2.63	3.67	0.00	-0.01	2.59	OD	8
11		<i>dmc11a</i>	15	E4/M2-78	E4/M2-98	3.68	1.89	0.00	-0.01	-5.83	OD	5,8	
11		<i>dmc11b</i>	81	E4/M2-154	E4/M2-118	3.18	2.22	0.00	-0.01	14.13	OD	5	
12		<i>dmc12</i>	72	est68-4	est68-3	2.60	7.50	0.00	0.03	-17.07	OD	4	
13		<i>dmc13a</i>	20	est507-3	est507-1	4.21	1.18	0.00	0.00	-0.31	PD	3,4,8	
13		<i>dmc13b</i>	65	est507-2	ssry13-5	2.80	4.76	0.00	-0.02	3.49	OD	4,5	
13		<i>dmc13c</i>	86	ssry13-5	ssry13-4	3.28	2.87	-0.01	0.00	0.71	PD	5,8	
16		<i>dmc16</i>	26	E7/M3-138	E7/M3-86	2.58	2.25	0.00	0.01	3.29	OD	3,5	
20		<i>dmc20a</i>	14	est744-4	est2-4	5.16	4.98	0.01	-0.01	-1.22	OD	5	
20		<i>dmc20b</i>	53	est4	est720-3	5.51	6.32	0.01	-0.01	-1.30	OD	3,4,8	
SC	1	<i>sc1</i>	14	E5/M1-400	E8/M1-488	6.33	15.91	1.27	2.80	2.21	OD	3,4,5	
	3	<i>sc03</i>	50	E7/M2-224	E7/M2-88	3.35	11.72	-0.53	-5.69	10.77	OD	5,8	
	4	<i>sc04</i>	35	E5/M3-176	E5/M3-104	2.95	1.73	-0.01	-1.08	84.70	OD	4,5	
	5	<i>sc05</i>	27	E8/M5-182	E8/M5-172	3.30	7.77	-0.73	1.59	-2.18	OD	4	
	6	<i>sc06</i>	11	E7/M3-76	E7/M3-118	3.15	3.30	-0.45	0.85	-1.91	OD	3,4,5	
	9	<i>sc09</i>	41	E5/M1-348	E5/M1-402	4.13	1.04	0.14	-0.70	-4.84	OD	3,5,8	
	11	<i>sc11a</i>	15	E4/M2-78	E4/M2-98	4.16	2.66	0.47	-0.85	-1.82	OD	3,4,5,8	
	11	<i>sc11b</i>	54	E4/M2-368	E4/M2-154	3.33	1.46	0.20	0.78	3.93	OD	8	
	11	<i>sc11c</i>	81	E4/M2-154	E4/M2-118	3.24	3.02	0.13	-1.35	-10.09	OD	4	
	12	<i>sc12</i>	71	est68-4	est68-3	2.60	4.80	-0.27	1.81	-6.75	OD	4	
	13	<i>sc13</i>	87	ssry13-5	ssry13-4	2.65	1.13	-0.34	0.10	-0.28	PD	3,5	
	16	<i>sc16</i>	25	E7/M3-138	E7/M3-86	2.50	2.12	0.13	1.06	7.92	OD	3,4	
	20	<i>sc20a</i>	14	est744-4	est2-4	5.11	3.83	0.61	0.29	0.46	PD	3,4,5	
	20	<i>sc20b</i>	51	est2-4	est720-3	4.70	2.53	0.48	-0.45	-0.94	D	3,4,8	

GA gene action, A additive ( $ld/a = 0-0.2$ ), PD partial dominance ( $ld/a = 0.21-0.80$ ), D dominance ( $ld/a = 0.81-1.20$ ), OD overdominance ( $ld/a > 1.2$ )

<sup>a</sup> Proportion of the phenotypic variation explained by the QTL estA, additive effect; positive additive effects indicate that the SC6 allele increases the value of the trait, negative additive effects indicate that the Mianbao allele increases the value of the trait estD, dominance effect

<sup>b</sup> Ratio of dominance effect to additive effect for each QTL

<sup>c</sup> Trial in which QTL was significant: 3, 2003; 4, 2004; 5, 2005; 8, 2008

For DMC, 46 QTLs were detected, for which the PVE ranged from 1.09 to 26.37 % with an average value of 10.37 %. Among these 46 QTLs, six QTLs were detected in two trials, eight QTLs were detected in three trials, and one QTL (*qdmc6a*) was detected in all four trials. Seven rd-QTLs were linked to SSR markers. The average PVE for these rd-QTLs was 6.55 % with a maximum value of 23.92 %, and five of them were detected in three trials.

For SC, 38 QTLs were detected, for which the PVE ranged from 1.11 to 30.04 % with an average value of 12.21 %. Eight QTLs were detected in two trials, seven QTLs were detected in three trials, and two QTLs (*qsc11* and *qsc18b*) were detected in all four trials. Seven rd-QTLs were linked to SSR markers, and had a maximum PVE of 4.06 %. One of the rd-QTL was detected in four trials, whereas the other rd-QTLs were detected in two or three trials.

For the three traits, in addition to the 25 SSR-linked rd-QTLs, 31 AFLP-linked rd-QTLs and one SRAP-linked rd-QTL were detected. Among the latter 32 rd-QTLs, the average PVE was 19.51 % for FRY, 8.09 % for DMC, and 10.57 % SC. Although these 32 QTLs had high PVE values, it is difficult to use AFLP and SRAP markers for MAS; hence, they would need to be converted first into sequence-characterized amplified region (SCAR) or other simple PCR markers.

In total, 57 rd-QTLs were identified for the three traits in the population SM. Of these rd-QTLs, 27 rd-QTLs were detected in two trials, 23 rd-QTLs were detected in three trials, and seven rd-QTLs were detected in all four trials. The rd-QTLs were not distributed evenly among LGs. Ten LGs contained most of the rd-QTLs, and 15 marker intervals contained QTLs that corresponded to two or three traits (Fig. 1; Table 4), which indicated that these genome regions have pleiotropic effects.

## Discussion

Cassava ranks as the sixth most important food crop in the world, but basic research on this crop has lagged behind that on other crops and relatively few researchers actively study cassava, especially in the field of quantitative genetics research. The first segregated cassava population to be analyzed was the progeny of the cross TMS30572 × CM2177-2, from which two genetic maps were constructed; one map consisted of

**Table 4** QTL regions that associated with two or three traits

Marker interval		LG	FRY <sup>a</sup>	DMC <sup>a</sup>	SC <sup>a</sup>
E5/M3-176	E5/M3-104	LG4	3,4,8	4,8	4 <sup>a</sup> ,5
E7M3-76	E7M3-118	LG6	–	3,4,5,8	3,4,5
E7M3-108	E7M3-296	LG6	–	3,4	3,4
E5M1-260	E5M1-348	LG9	3,8	–	4,5,8
E5M1-348	E5M1-402	LG9	3,4	–	3,5,8
E4M2-78	E4M2-98	LG11	3,4,5,8	3,5,8	3,4,5,8
est507-3	est507-1	LG13	3,4,5	3,4,8	3,4,8
ssry13-5	ssry13-4	LG13	–	3,5,8	3,5
ssry161-4	est524-1	LG14	3,4,5,8	3,8	3,8
E3M5-166	E3M5-164	LG15	–	3,4,5	3,4
E7M3-138	E7M3-86	LG16	3,8	3,5	3,4
E7M3-228	ssry49-1	LG18	4,5	3,4,5	3,4,8
ns80-3	ns178-2	LG18	4,5,8	3,4,8	4,5,8
est744-4	est2-4	LG20	3,4,5	–	3,4,5
est2-4	est720-3	LG20	3,4,5,8	3,4,8	3,4,8

<sup>a</sup> Trial in which QTL was significant: 3, 2003; 4, 2004; 5, 2005; 8, 2008

394 markers and the other consisted of 100 SSR markers (Fregene et al. 1997; Okogbenin et al. 2006). An additional five segregated populations were developed in Colombia (Akano et al. 2002), Nigeria (Lokko et al. 2005; Akinbo et al. 2007), Thailand (Kunkeaw et al. 2010), and China (Chen et al. 2010). These populations were used to construct genetic maps and/or identify QTL for resistance to CMD and CAD, for example. However, most of these QTL were identified in a single-marker analysis and in only one case by the composite interval mapping method. This shortcoming may explain why many false positive QTL were identified and some genuine QTL were missed. Hence, in the present study, the ICIM method (Li et al. 2007) was applied to minimize the false detection rate and increase the detection power.

The 57 rd-QTLs that were recognized for the three traits in the population SM were identified by the ICIM method using the phenotypic data from the four trials. Among these 57 rd-QTLs, 25 rd-QTLs were linked to SSR markers, and 15 marker intervals on ten LGs showed pleiotropic effects. With reference to the genetic map of Balyejusa Kizito et al. (2007) identified a QTL for DMC that was located in the shared marker interval NS80–NS178 on LG18, which contained QTL for all three traits in the present study. Fregene et al. (1997) constructed a genetic map that consisted mainly

of RFLP markers using the F<sub>1</sub> progeny of the cross TMS30572 × CM2177-2, and mapped a series of QTLs on this map, but in practice it is difficult to use RFLP markers in MAS. Subsequently, Okogbenin et al. (2006) constructed a genetic map based on SSR markers using the F<sub>2</sub> progeny (K150) of the cross TMS30572 × CM2177-2, and mapped several QTLs on this map (Okogbenin et al. 2008). None of the published maps contained any shared markers; hence it was impossible to identify common QTLs among different reports, which in turn was an obstacle to the application of published identified QTL in relation to MAS of cassava.

The SM11 genetic map incorporated only 11 SSR and 29 EST–SSR markers., and most of the AFLP or SRAP markers clustered into one LG alone (LG1–LG11, LG15, and LG16). On the basis of these results, the strategy for construction of a cassava genetic map should be modified. SSR and EST–SSR markers should form the major components of a cassava genetic map, and there were 472 SSRs and 431 EST–SSRs that could be used currently. AFLP or SRAP markers should be considered as supplementary components to fill in gaps. In addition, the use of highly inbred lines as parents is needed to develop segregated populations for the construction of genetic maps and QTL analysis in the future.

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