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Genetic analysis and QTL mapping of early root bulking in an F_1 population of non-inbred parents in cassava (*Manihot esculenta* Crantz)

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Abstract The genetic basis of early bulking in cassava was studied in a replicated, multi-locational trial using 144 F_1 progeny derived from an intra-specific cross between two non-inbred parents. A second, sequential harvest experiment examined the relative importance of eight yield-related traits on early bulking and their QTLs during the crop growth cycle. Our objectives were to identify traits, and genes controlling them, strongly associated with early yield as a first step to marker-assisted improvement of the trait. Multiple linear regression analysis and stepwise regression of early yield on eight yield-related traits revealed harvest index, dry foliage weight and root diameter as the most important factors associated with early yield. A total of 18 QTLs controlling early yield were identified in the first and second experiments and 27 QTLs, 2 for dry foliage weight, 8 for harvest index and 17 for root diameter, in the second experiment. The individual effects of alleles at these QTLs identified ranged from 7% to 33% of the phenotypic variance explained. Seven of 18 QTLs found for early yield (39%) coincided with QTLs associated with one or more traits with significant influence on early yield. The results show that sink and source capacities are very important in determining early yield. The identification of a number of QTLs with positive effect for increased early yield provides an opportunity for marker-assisted selection and improvement of early bulking potential in cassava.

Keywords Early bulking · QTL · Genetic mapping · Cassava

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Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial vegetatively propagated crop grown throughout the lowland tropics for its starchy, thickened roots. The fresh roots of cassava contain 30 to 40% dry matter made up of 70–85% starch and serve in many tropical countries as a major source of calories for humans and animals, and as raw material for industrial starch production. (Cock 1985). But the greatest advantage of cassava is its ability to grow and produce reliable yields in places where cereals and other crops produce nothing at all. Due to drought problems in sub-Saharan Africa, especially in Southern Africa, cassava has assumed greater importance and is the favored food-security crop. A total of 80 million metric tons of cassava are produced annually in Sub-Sahara Africa where agriculture is based on rain-fed farming systems (Dorosh 1988). Cassava has no definite maturation point and harvests can take place 9 to 24 months after planting. Recent findings have revealed that late root bulking in cassava is the most important single factor responsible for the rejection and abandoning of cassava genotypes in sub-Saharan Africa due to demographic and market pressures (Nweke et al. 1994).

Given the expanding demands for cassava as food, feed and industrial raw material, genotypes with high yield and early bulking attributes are highly desired. The need for food production to keep pace with the rapidly expanding human population, especially in marginal rainfall areas with rising population densities, has made it necessary to develop early bulking, high yielding genotypes for sub-Saharan Africa. In addition, Wholey and Cock (1974) had earlier reported that one way of improving the efficiency of crop production in terms of root yield per unit time is by shortening the growth period through the identification of early yielding cultivars. The nature and sequence of the development and bulking of storage roots in cassava have been described in qualitative terms in various studies (Doku 1969; Indira and Sinha 1970; Wholey and Cock 1974; Cock et al. 1979; Lian and Cock 1979b; Dahniya et al. 1982; Ramanujan

and Ghosh 1990). Wholey and Cock (1974) found earliness in root yield to be related to rapid bulking and the rate of bulking differed between cultivars. Lian and Cock (1979b), stressed further that root yield was not only directly influenced by the rate of bulking, but also by the duration of bulking, with highest yielders having the highest rate of bulking for the longest period of time. Findings from these studies suggest that the improvement of early bulking may have a dual output of early varieties and improved crop yields. There is no morphological or visual system to know when cassava plants starts accumulating starch in the roots, except by harvesting the roots, thus making it difficult for breeding projects to identify early bulkers. The objectives of this study were to: (1) investigate the onset of root bulking and determine the root bulking pattern in cassava, (2) determine agronomic traits influencing early root bulking, and (3) detect QTLs linked to early root bulking/yield. Information obtained could also form the basis of a marker-assisted improvement of early bulking in cassava.

Materials and methods

The development of the mapping population and the molecular genetic map that is the source of markers for this study has been described elsewhere by Fregene et al. (1997) and Mba et al. (2001). The parents are non-inbred lines TMS 30572 (the female parent) and CM 2177-2 (the male parent). The female parent was developed at the International Institute of Tropical Agriculture (IITA) while the male parent resulted from breeding at the International Center for Tropical Agriculture (CIAT, its Spanish acronym). The parents were selected for their possession of traits that are of agronomic importance such as early bulking in TMS30572. A total of 144 progeny of the F_1 population were used for the QTL mapping study.

Experiment 1. Identification of extreme phenotypes for early bulking

An experiment was designed to study QTLs for early yield in the F_1 population and to identify a group of very early and very late yielders for further experiments. The trial was established January 1998 in two locations: at CIAT headquarters in Palmira (Valle del Cauca department), and CIAT station in Santander de Quilichao (Cauca department), both in the mid-altitude agro-ecology of Colombia. The experimental site at Palmira (1,000 m above sea level, latitude 3°31′N and longitude 76°21′W), has a soil texture described as mollisol (Lian and Cock 1979a, b). Two rainfall peaks at this location occur in the intervals March to June and October to December. Long-term total annual rainfall is about 1,000 mm, although yearly variations are considerable. The water-holding capacity of the soil is such that cassava rarely suffers from water stress at this site. Mean temperature is 25 ± 1 °C monthly. Solar radiation is normally between 12,000 and 14,500 g cal cm^{-2} mo^{-1} throughout the year.

The other location, Santander de Quilichao (altitude 990 m, latitude 3°30′N, longitude 76°31′W), is distinct from the Palmira site more in its edaphic properties, with a less-fertile, sandy loam, acidic-soil type. This location has a similar rainfall pattern with Palmira, but precipitation is much higher and the dry season is less distinct. Long-term annual rainfall is about 1,770 mm, and the mean temperature is 25 ± 1 °C. The experimental design in both locations was a partially balanced triple lattice design with three replicates. Carefully selected stem cuttings of 144 genotypes were planted vertically on ridges at a spacing of 1 × 1 m giving a plant density of 10,000 plants ha^{-1}. The plot size was 20 m^2 planted as five rows of four plants each, to give 20 plants per plot. The experiment was weeded regularly, and plants were protected against insect pests by spraying pesticides.

Because the experiment combined the phenotypic evaluation of early bulking with other traits, including yield, that are traditionally evaluated at 11 months, only three border plants were randomly selected per plot for early yield evaluation. Dry root yield per plot basis was determined from dry matter percentage calculated by measuring specific gravity. Measurements of specific gravity were obtained by weighing roots in air and then in water (weight in air/weight in water – weight in water). This method is based on the correlation which exists between root specific gravity, dry matter and starch content (CIAT 1976). The dry matter percentage was determined using the formula: DM% = [158.3 × (weight in air/weight in water – weight in water)]. Fresh root yield is multiplied by DM% and then divided by 100 to obtain the dry matter yield.

Experiment I. Data analyses

Means of the dry root yield of plants harvested at 7 months after planting (MAP) in the F_1 progeny were calculated using Microsoft Excel. The F_1 progeny was considered as a random sample of all possible progenies derived from the cross of the parental lines. When early yield data was analyzed as a lattice design (SAS LATTICE procedure, SAS institute 1996) no significant increase in efficiency was observed compared to the RCB analysis of variance (SAS ANOVA GLM procedure), therefore only RCB results are presented here.

Broad-sense heritability (H^2) was calculated based on the genotype means using the variance components of the expected mean squares from ANOVA as described by Fehr (1987, p 256). Variance components were determined using the type III sums of squares of the ANOVA. Spearman's rank correlation coefficients of yield were calculated within and across locations to determine the extent or magnitude to which early yield is controlled by the same genes in different environments. For early yield, standard errors were determined for parental and progeny data. The distributions of mean values of the progeny data were examined with the SAS UNIVARIATE procedure.

QTL mapping was based on 143 and 135 markers from the female- and male-derived genetic maps of cassava, respectively. Adjusted means of yield at 7 MAP were employed in single-marker analysis for QTL mapping of the F_1 segregating population using the software package QGENE (Nelson 1997) which is based on a single-factor model. In this program, a representative F statistic, measuring the fit of a linear regression of phenotype on marker genotype is calculated for early yield, and a QTL was declared significant at $P < 0.005$. The resulting R^2 represents the proportion of the phenotypic variance explained. The QTL analysis was also done using the PGRI/QTL conditioning analysis package based on a conditional *t*-test statistic (Liu and Lu 1995). Where three or more linked markers were significantly associated to a putative QTL, these markers were further subjected to a multiple regression analysis. The model was:

$$Y_{ij} = \mu + A_i + B_i + C_i + \cdots + Z_i + \varepsilon_{ij},$$

where Y_{ij} is the trait value of phenotype j with marker score I, μ is the overall mean for the trait, $A_i + B_i + C_i +$ and Z_i represent the effects of linked markers associated with the trait loci and ε_{ij} is the random error. Use of this multilocus model can reduce spurious association of markers with trait loci due to collinearity among markers. We report only QTLs that accounted for 6% and above of the observed phenotypic variance in early yield.

Experiment II: evaluation of extreme genotypes for early bulking by sequential harvest

A second experiment was designed to examine traits associated with early bulking and to understand the source – sink relation-

ships underlying early bulking patterns in cassava. A good knowledge of these factors is required for improvement of early bulking in cassava. The trial was conducted at one location (Palmira) using extreme phenotypes for earliness from the mapping population. The experiment was initiated at the end of 1998 (December), and 80 genotypes selected for this trial were based on the results obtained from our preliminary assessment of earliness for root yield in experiment I in the early part of 1998. The best 40 early bulking progeny and the worst 40 at 7 months after planting (7 MAP), representing the extreme phenotypes of the F_1 population, were used as the population for this study. These individuals are expected to be more informative since they are expected to contain a higher frequency of "+ve" and "–ve" alleles for earliness at QTLs affecting the target trait. Specific expectations for information gain as a function of population size and selection intensity has been discussed elsewhere (Lander and Botstein 1989; Paterson 1998). In addition to the selected genotypes of the F_1 population, a popular land race, *Mandica tres meses*, known for its earliness, was included in the trial as a control for our root bulking studies.

The experiment was a randomized complete block design with two replications at CIAT headquarters in Palmira. Selected mature stem cuttings 20-cm long were planted vertically on ridges spaced 1.0-m apart. Plant spacing within the row was also 1.0 m. Each plot of 60 m^2 contained 60 plants of each genotype in a 6×10 (column by row) arrangement. Four plants from the central 32 plants, organized as eight rows of four plants, were sequentially harvested every 3 weeks. The progress of storage root thickening in the F_1 population was assessed at 3-weekly intervals over a 7-month duration beginning at 6 weeks after planting (WAP) through to 30 WAP covering a total of eight central plants, and one border plant, harvests.

At each harvest, data were collected from four plants in a row within a plot for dry root yield and other traits related to bulking. The other traits evaluated were: plant height (cm), plant vigor, fresh foliage (g), root size differentiation, number of roots per plant, root diameter of the biggest five storage roots and harvest index (measured as the ratio of root weight to the total plant weight) with a plant vigor visual rating scale of 1–5, (1- poor; 5- best). Dry matter determination for root and foliage was done by taking 200-g samples of the fresh weight of storage roots and fresh foliage (stems and leaves mixture), and oven-dried to a constant weight to estimate dry matter content. Starch initiation (or commencement of bulking) was also evaluated between 6 and 12 WAP. Samples of roots were randomly picked, sectioned and then stained with iodine for a blue-black coloration test for starch granule presence. This was repeated until starch was detected in the root or all roots had been examined. Similarly, differentiation in root size was checked until the first storage root with a diameter greater than 0.35 cm was observed. Special care was taken at harvest to minimize damage to the roots early in the experiment.

Experiment II. Data analyses

Dry matter yield at every harvest date was correlated with all other traits evaluated using the SAS CORR procedure (SAS Institute 1996). Phenotypic correlation analysis was used to assess the association of these traits with dry matter yield. Regression analyses were performed to determine the linear relationships between dry matter root yield (dependent variable) and other evaluated traits (independent variables) for each harvest time using the SAS REG procedure (SAS Institute 1996). The regression coefficient of each variable trait was accepted as significantly different from zero and declared associated to early bulking at $P < 0.05$. The calculated R^2 or multiple coefficient of determination indicates the proportion of the total variation in yield explained by the model. We also re-evaluated our data in a stepwise regression model to confirm results from multiple linear regression analysis. Traits identified to be strongly associated with early bulking were subjected to QTL analysis as described in experiment I, to identify regions of the genome influencing these traits and by extension of early bulking in cassava.

Because of the curvilinear relationship observed between yield and time, simple non-linear regression of dry matter yield over time (i.e. progressive increase in assimilate accumulation over nine harvest periods) per genotype was done. Bulking rate expressed as the percentage increase in yield per unit time was given by the regression coefficients obtained from the analysis. We also examined bulking rate by determining the average amount of yield-increase per unit time, calculated as the yield at the final harvest divided by the duration of plant growth.

Results

Variation of early bulking in the F_1 mapping population

Phenotypic evaluation of the yield at 7 MAP in the F_1 population at Palmira and Quilichao revealed variation typical of quantitative traits. The normality test showed that phenotypic values of yield were normally distributed at both sites, indicating the suitability of this population for QTL mapping of early yield (data not shown). The broad-sense heritability estimate, on an entry mean basis, was fairly high (0.64), suggesting a strong underlying genetic basis for the observed variation in yield. Phenotypic values of dry root yield at 7 MAP were generally higher at Quilichao with a mean of 640 g/plant (6.4 tons ha^{-1}) as compared with a mean of 539 g/plant (5.4 tons ha^{-1}) obtained at Palmira. The highly fertile conditions associated with mollisols in the Palmira site, supported vigorous top growth especially at the early stage of plant development. The significant correlation ($r = 0.35$, $P < 0.01$) in early yield between the two locations used in this study, suggest some degree of similarity in yield responses at both sites.

In experiment II, the sequential harvest, starch initiation which signals the commencement of assimilate translocation to the roots, and thus storage root thickening, was found to have started in most (79%) of the genotypes as early as 6 WAP. Over 95% of the genotypes showed the presence of starch 3 weeks later. Rapid differentiation in root size was observed, as thickened roots had become very evident (in 75% of the population) at 9 WAP. This is in agreement with the findings of Wholey and Cock (1974), that bulking commences in cassava after 2 months of growth. Root bulking increased with time, but the rate of bulking was different between F_1 individuals. Our results showed that starch initiation time, root size differentiation time, root diameter, plant height, harvest index, dry foliage weight, number of roots and plant vigor were all significantly correlated with dry root yield (data not shown), suggesting these factors as component parts of the root bulking process that underlie yield as a complex trait. Phenotypic correlation coefficients between yield and other evaluated traits within the growth period varied from as low as 0.28 to 0.91. There was variation between the F_1 genotypes for plant height, root diameter, number of roots, plant vigor and dry foliage weight. These results suggest that these variables were involved in the root bulking processes. The total production of dry root yield per plant in Experiment II showed wide variation between genotypes as expected for extreme phenotypes selected from

Table 1 Multiple and stepwise regression results showing *P* levels of traits evaluated at each harvesting stage of early bulking assessment at Palmira over a 30-week period. Significant variables at each evaluation stage

Regression	Variables[a]	6 WAP	9 WAP	12 WAP	15 WAP	18 WAP	21 WAP	24 WAP	27 WAP	30 WAP
Multiple regression	SI	0.0040	0.9275	0.5489	0.5575	0.0463	0.7878	0.1399	0.7663	0.3926
	SD	0.0857	0.0192	0.1056	0.5250	0.4214	0.5839	0.8279	0.0995	0.2152
	RD	0.0001	0.0001	0.0002	0.2195	0.0788	0.3129	0.2702	05153	0.3672
	FLG	–	–	0.0254	0.0701	0.0001	0.0001	0.0001	0.0001	0.0001
	HI	–	–	0.0018	0.1418	0.0001	0.0001	0.0001	0.0096	0.0001
	NR	–	–	0.0638	0.0254	0.4147	0.9160	0.0100	0.4294	0.1197
	PH	–	–	0.4876	0.7258	0.2932	0.5566	0.1149	0.2296	0.0980
	PV	–	–	0.1393	0.9957	0.4120	0.3618	0.7240	0.2124	0.1332
Adj. R^2		0.51	0.83	0.82	0.72	0.84	0.82	0.86	0.67	0.82
Stepwise regression	SI	0.0009	ns	ns	ns	ns	ns	ns	ns	ns
	SD	ns	0.0465	ns	ns	ns	ns	ns	0.0122	0.0038
	RD	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	ns	ns	ns
	FLG	–	–	ns	ns	0.0001	0.0001	0.0001	0.0001	0.0001
	HI	–	–	0.0043	ns	0.0044	0.0012	0.0001	0.0001	0.0001
	NR	–	–	ns	0.0011	ns	ns	ns	ns	0.0001
	PH	–	–	ns	ns	ns	ns	ns	ns	ns
	PV	–	–	ns	ns	ns	ns	ns	ns	ns
Adj. R^2		0.52	0.83	0.83	0.73	0.81	0.83	0.85	0.68	0.77

[a] SI, starch initiation; SD, root size differentiation; RD, root diameter; FLG, dry foliage; HI, harvest index; NR, number of storage roots; PH, plant height; PV, plant vigor

the F_1 population. Dry root yield was as low as 154 g (1.54 ton ha^{-1}), and as high as 1,450 g per plant (14.5 tons ha^{-1}) at 30 weeks after planting (WAP). Dry root yield results indicate that some potentially early yielding genotypes exist in the population. Some genotypes out-performed *Mandioca tres meses*, a Brazilian land race, which was introduced into the trial as a check for its early yielding quality, emerging among the top 10% of early yielding individuals in this study. The multiple linear regression model used, with yield as the dependent variable and all other traits as independent variables, adequately explained the variation observed for early bulking. The proportion of variation accounted for by multiple linear regression, given by the multiple coefficient of determination (R^2), was high and the adjusted R^2 varied from 0.67 to 0.86 between 12 WAP and 30 WAP (Table 1). Multiple linear regression revealed that, dry foliage weight and harvest index were the two most important factors influencing early bulking. These traits were evaluated between 12 WAP and 30 WAP, and were found to be significantly associated with early bulking for five of the six evaluations, representing over 83% of the period (Table 1). Stepwise regression (Table 1) again confirmed foliage and harvest index as traits highly influencing early yield. Stepwise regression also revealed root diameter as a very important factor at the initial stages of root bulking, between 6 WAP and 21 WAP (Table 1). Root diameter, which is a measure of the expansion of storage cells in the roots, however, became less important at the later stages of root bulking. Genotypic correlations of foliage, harvest index and root diameter with yield were highly significant at all evaluation stages (Table 2) between 6 WAP and 30 WAP, suggesting a high genetic basis for the observed strong association between these characters and yield. All other traits were most of the times non-significant in their partial regression coefficients and thus weakly related to early bulking. QTL analysis for dry foliage and harvest index, the two most important traits influencing early yield, were conducted to identify genome regions influencing root bulking between 12 WAP and 30 WAP.

Table 2 Genotypic correlation of traits strongly associated with yield at different evaluation stages. ***Statistically significant at the 0.0001 level

Harvest stage	Genotypic correlation coefficients between		
	Foliage and dry root yield	Harvest index and dry root yield	Root diameter and dry root yield
6 WAP	–	–	0.92***
9 WAP	–	–	0.57***
12 WAP	0.80***	0.98***	0.96***
15 WAP	0.50***	0.95***	0.96***
18 WAP	0.79***	0.89***	1.00***
21 WAP	0.75***	0.66***	0.91***
24 WAP	0.46***	0.60***	0.79***
27 WAP	0.62***	0.65***	0.91***
30 WAP	0.70***	0.49***	0.77***

QTL mapping

Early root yield taken at 7 MAP in the F_1 population in 1998, described in Experiment I, was subjected to QTL analysis. Because genotype × environment interaction was significant for early yield, QTL analysis was done separately for each location. For the early root yield for each harvest date in the sequential harvest experiment, Experiment II was also subjected to QTL analysis. The result of QTL analysis for early yield is presented in Table 3. Eighteen QTLs were detected for early yield and the percentages of the observed phenotypic variance

Table 3 QTLs detected for early yield (7 MAP) and associated traits based on single-marker regression analysis in a TMS 30572 × CM 2177-2 cross (F_1 mapping population)

Trait[a]	QTL[b]	Map	Linkage group	Marker[c]	Trial[d]	%PVE[e]	Effects	*P*-value[e]	Harvest period
EYLD	dryD.1	Female	D	GY42	Q-98	16	–166.94	0.0004	7 MAP
	dryD.2	Female	D	GY181	Q-98	16	–162.87	0.0004	7 MAP
	dryUD.1	Male	UD	GY24	Q-98	14	152.18	0.0009	7 MAP
	dryF.1	Female	F	GY194	SHT-99	10	–156.68	0.0045	6, 21, 30 WAP
	dryF.2	Female	F	GY122	SHT-99	10	–154.97	0.0047	6, 18, 27, 30 WAP
	dryF.3	Male	F	GY55	SHT-99	19	138.45	0.0039	21 WAP
	dryG.1	Male	G	GY6	SHT-99	26	–0.13	0.0013	6 WAP
	dryJ.1	Male	J	K10	Q-98	16	–167.35	0.0003	7 MAP
	dryL.1	Female	L	CDY131	P-98	12	144.01	0.0033	7 MAP
	dryL.2	Female	L	CBB1	SHT-99	19	65.61	0.0045	18 WAP
	dryQ.1	Female	Q	rP3	SHT-99	28	212.12	0.0005	24, WAP
	dryR.1	Male	R	GY48	SHT-99	24	211.33	0.0009	27 WAP
	dryUM.1	Female	D	Ai18b	Q-98	17	–167.25	0.0003	7 MAP
	dryUM.2	Female	L	GY31	SHT-99	11	132.93	0.0033	30 WAP
	dryUM.3	Female	L	GY120	SHT-99	10	124.69	0.0048	30 WAP
	dryUM.4	Female	L	GY202	SHT-99	16	154.52	0.0003	18, 27, 30 WAP
	dryUM.5	Female	S	GY153	SHT-99	10	–185.52	0.0047	24 WAP
	dryUM.6	Female	S	GY212	SHT-99	11	–46.72	0.0036	18 WAP
FL	fswJ.1	Female	J	CDY76	SHT-99	33	–178.85	0.0002	24 WAP
	fswL.1	Female	L	CDY131	SHT-99	25	78.03	0.0018	24 WAP
HI	hiA.1	Male	A	rBEST	SHT-99	32	0.12	0.0001	18 WAP
	hiE.1	Male	E	nGY162	SHT-99	19	–0.080	0.0047	18 WAP
	hiF.2	Male	F	rGY55	SHT-99	19	–0.55	0.0044	18 WAP
	hiJ.1	Female	J	GY34	SHT-99	16	–0.09	0.0011	27 WAP
	hiK.1	Female	K	rCDY106	SHT-99	23	–0.10	0.0026	15, 27 WAP
	hiUM.1	Female	S	GY212	SHT-99	18	–0.037	0.0000	15 WAP
	hiUM.2	Female	S	GY142	SHT-99	29	–0.046	0.0009	15 WAP
	hiUM.3	Female	S	GY153	SHT-99	20	–0.070	0.0001	15 WAP
RD	rdB.1	Female	B	GY197	SHT-99	14	5.24	0.0009	21, 27 WAP
	rdD.1	Male	D	GY25	SHT-99	7	2.97	0.0024	30 WAP
	rdF.1	Male	F	rCDY107-1	SHT-99	22	–0.20	0.0024	6 WAP
	rdF.2	Male	F	rGY55	SHT-99	12	2.867	0.0011	15, 18 WAP
	rdF.3	Male	F	GY21	SHT-99	11	–0.095	0.0023	6 WAP
	rdI.1	Female	I	rGY201-1a	SHT-99	11	–4.15	0.0042	24 WAP
	rdI.2	Female	I	rB3a	SHT-99	13	–2.64	0.0007	30 WAP
	rdM.1	Female	M	GY192	SHT-99	10	0.14	0.0006	6 WAP
	rdM.2	Male	M	nrGY67	SHT-99	9	–1.12	0.0049	9 WAP
	rdQ.1	Female	Q	rP3	SHT-99	29	7.03	0.0004	15, 18, 21, 27 WAP
	rdQ.2	Female	Q	rGY74	SHT-99	20	6.95	0.0031	18, 21 WAP
	rdR.1	Male	R	rGY48	SHT-99	20	6.13	0.0037	12, 15, 18, 27 WAP
	rdUM.1	Female	M	GY215	SHT-99	14	0.18	0.0008	6 WAP
	rdUM.2	Female	S	GY212	SHT-99	17	–5.22	0.0002	6, 9, 12, 15, 18, 21, 24, 27 WAP
	rdUM.3	Female	S	GY142	SHT-99	29	–7.44	0.0040	12, 15, 18, 21, 24 WAP
	rdUM.4	Female	S	GY153	SHT-99	16	–5.10	0.0003	6, 9, 12, 15, 18, 21, 24, 27 WAP
	rdUM.5	Male	UF	GY53	SHT-99	20	–0.19	0.0022	6 WAP
BR	bF.1	Female	F	GY211	SHT-99	16	–6.84	0.0004	
	bF.2	Female	F	GY37	SHT-99	12	–5.77	0.0021	
	brH.1	Female	H	rGY211	SHT-99	21	–0.31	0.0036	

[a] EYLD, early yield; FL, foliage dry weight; HI, harvest index; RD, root diameter; BR, bulking rate

[b] QTLs are named by parameter abbreviations, linkage group designation, and the number of the QTL if there are more than one in a linkage group

[c] Marker significantly associated with trait variation

[d] Q = Quilichao, P = Palmira, 98 = 1998, SHT-99 = sequential harvest trial in 1997

[e] Percent phenotypic variance explained. *P* value refers to the probability of the association between QTL and the marker. Where a QTL is identified more than once in the harvest period, *P*-value and PVE is given for the last harvest date for which the QTL was identified

UM = unassigned markers

explained by individual QTLs ranged from 10 to 28%. Five QTLs in Quilichao and one QTL in Palmira were identified in Experiment I. All of the QTLs were unique to a single site suggesting a strong environmental effect on yield, and this is in agreement with the strong genotype by environment effect observed for yield (data not shown). Twelve QTLs were detected in Experiment II, the sequential harvest trial (SHT-99), in Palmira in 1999. Eight QTLs were found to be associated with an increase in early yield. The QTLs identified in SHT-99 were

detected at different stages of the crop's growth cycle. We identified three QTLs having alleles with phenotypic effects above 20%, and these include *dryG.1* (26%) at 6 WAP, *dryQ.1* (28%) at 24 WAP and *dryR.1* (24%) at 27 WAP. Our results showed that QTLs for early yield also mapped to the same locations controlling other traits influencing yield (see following sections).

Regression analysis of the results from SHT-99 showed the weight of dry foliage as one of the three most important traits influencing early yield (the others being harvest index and root diameter). QTL analysis detected two regions that were significantly associated with foliage (Table 3). The two QTLs were *fswJ.1* and *fswL.1* on linkage groups J and L of the female map; an allele at *fswJ.1* decreased the phenotypic value of dry foliage by 33%, whereas an allele at *fswL.1* increased foliage by 25% of the phenotypic variance, suggesting both as major QTLs. The QTL *fswL.1* mapped to the same location for the QTL (*dryL.1*) identified for early yield. Eight major QTLs on the male- and female-derived maps were detected for harvest index (Table 3). The highest percentage of the observed phenotypic variance was explained by an allele of *hiA.1*, which was the only QTL with a positive effect for increasing harvest index. Three harvest index QTLs, *hiF.2, hiUM.1* and *hiUM.3*, mapped to the same locations identified for early yield QTLs, *dryF.2*, dryUM.6 and dryUM.5 respectively. Alleles for decreased harvest index were associated with QTLs decreasing yield, except in one case, and it confirms the utility of harvest index as a selection parameter for yield.

Root diameter, was associated with 17 QTLs, having effects ranging from 11% to 29% of the phenotypic variance (Table 3). Alleles from the female parent at five marker loci contributed to increased root diameter, whereas only three from the male increased root diameter. The strongest effect was observed for *rdQ.1* on linkage groups Q of the female-derived map, which accounted for 29% of the phenotypic variance. Five of the QTLs were found either associated with yield or harvest index or both. Root diameter QTLs, *rdF.2*, *rdQ.1*, *rdUM.2*, *rdUM.3* and *rdUM.4* coincided with yield QTLs *dryF.2*, *dryQ.1*, *dryUM.2*, *dryUM.3* and *dryUM.4*, respectively. In each case, the direction of the effect of the alleles for each dry matter QTL and the corresponding QTL for yield was the same. Three of the QTLs decreasing root dry matter (*rdUM.2*, *rdUM.3* and *rdUM.4*) also mapped to the location of three QTLs (*hiUM.1*, *hiUM.2* and *hiUM.3*) associated with a decrease in harvest index (Table 3). Two root-diameter QTLs (rdUM.2 and rdUM.4) were detected frequently in different harvests of the sequential harvest experiment. They were identified in eight of the nine harvests, representing 89% of the harvest period suggesting high stability of these QTLs.

Bulking rate

Three QTLs associated with the rate of dry matter accumulation in our study determined both the percentage increases in yield, and the amount of yield per unit time. Simple non-linear regression based on the power-curve function gave the best regression fit for a yield – time relationship for each genotype, and the derived regression coefficients were used for QTL mapping. The percentage increase in dry weight of the tuberous root per unit time per unit area (bulking rate) as given by the regression coefficient, revealed only one QTL (*brH.1*) on the female-derived map. This QTL, *brH.1*, had an allele for bulking rate that explained 21% of the observed phenotypic variance, suggesting it to be a QTL of major effect for this trait (Table 3). This locus however has a decreasing effect on the rate of bulking. Bulking rate, as determined by the average dry matter accumulated per unit time, resulted in two QTLs on chromosome F of the female-derived map (*brF.1* and *brF.2*). Both QTLs have alleles with decreasing effects on the rate of bulking, and the observed phenotypic variance explained was 16% for the *brF.1* allele whereas that for *brF.2* accounted for 12% of the variation. One of the QTLs on chromosome H (*brH.1*) coincided with a QTL (*dryF.2*) having a decreasing effect on dry yield.

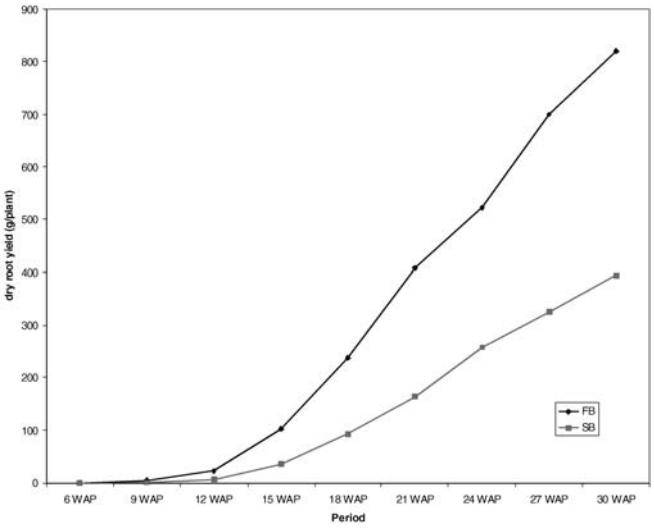

Fig. 1 Increase in dry root weight of two cassava phenotype groups – the fast bulking (*FB*) and slow bulking (*SB*) types – between 6 and 30 WAP. *indicates significant difference between the the two groups by *t*-test analysis

Early and late bulking phenotypes

SB phenotypes showed poor starch in the roots, and total biomass accumulation compared to the FB phenotypes (Figs. 1 and 2). Foliage remained the most active sink throughout the evaluated growth period for SB phenotypes (Fig. 3) while at 24 WAP storage roots became the dominant sink for FB phenotypes (Fig. 4). Starch initiation and root size differentiation commenced 6 and 17 days earlier for the FB group, thus extending the bulking duration for this group of phenotypes. Bulking rate expressed as the average dry root yield accumulated per

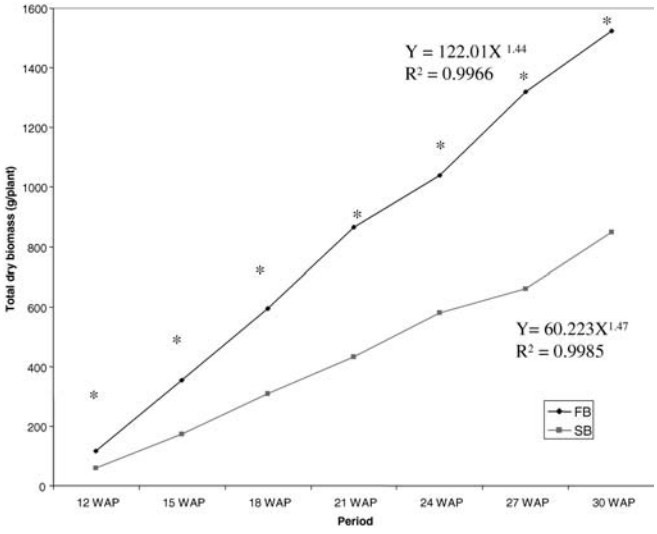

Fig. 2 Total dry biomass increase in fast (*FB*) and slow(*SB*) bulking cassava phenotypes from the F_1 mapping population between 6 and 30 WAP. *indicates significant difference between the two groups for each specific harvest date by *t*-test analysis. ^{ns}non-significant difference

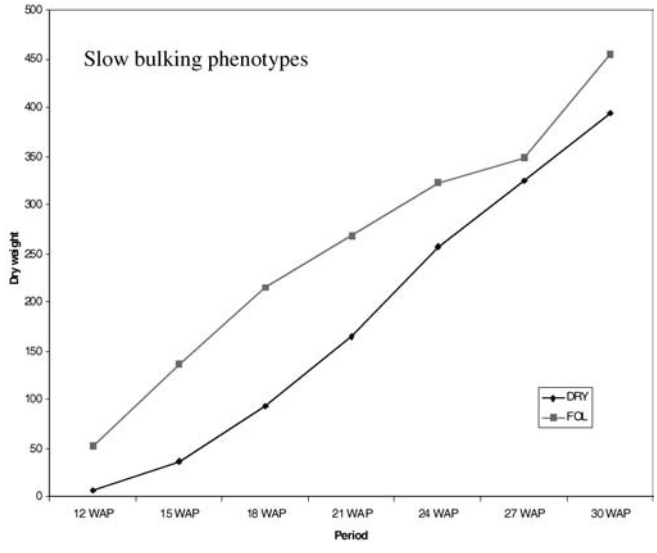

Fig. 3 Trend in dry foliage and dry root yield increase for slow bulking phenotypes in the bulking experiment at Palmira

unit time (28.12 g and 12.76 g for FB and SB respectively) was highly significant ($P < 0.0001$) between the two groups, implying differences in dry matter accumulation in storage roots resulting in a significantly higher yield for the FB phenotypes at 30 WAP. Our observation that dry foliage and harvest index were the two most-important traits influencing bulking, suggests that total biomass is a critical factor for good crop performance and increased early yield. Total biomass was significantly different between the two groups for all harvest periods (Fig. 2).

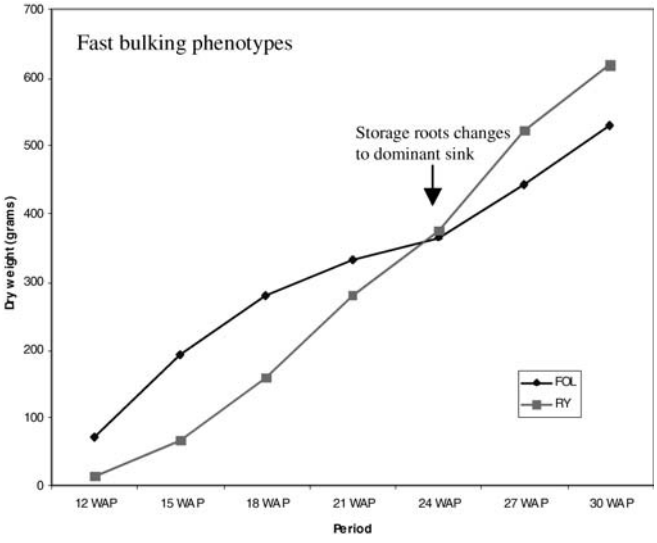

Fig. 4 Trend in dry foliage and dry root yield increase for fast bulking phenotypes in the bulking experiment at Palmira

Discussion

Our studies revealed a high level of genetic variation in the early yield potential of the F_1 genotypes analyzed, and impressive yields above 10 tons in the dry weight of harvested cassava roots per plant at 30 weeks after planting (WAP). The dynamics of storage root development in cassava was analyzed based on its growth and development in sequential harvests (Experiment II). Our observation of rapid enlargement of roots as early as 9 WAP indicates that roots were sufficiently strong sinks to attract carbohydrates for deposition at a very early phase in the growth cycle of cassava. Regression analysis showed root diameter, dry foliage and harvest index were the most important factors for root bulking, suggesting that both source and sink capacities were important for determining early yield.

Root diameter appeared to be the most important factor at the initial phase of root bulking, whereas harvest index and foliage emerged as the most influential factors at the late phase of root development during the 7-month evaluation period. Foliage consists of leaves that represent the site of carbohydrate production, while harvest index is concerned with the distribution of the carbohydrates produced between the top and the roots. Dry matter distribution to the roots increased with age of the plant and this may reflect an increase in source activity as the plant developed and in the sink action as the storage roots enlarged, and the cambium both lengthens and increases in circumference (Hunt et al. 1977). The results show that sink and source capacities are very important in determining early yield.

High source and sink capacity in fast-bulking (FB) genotypes resulted in high total biomass for the FB

group. Past studies in cassava indicate the usefulness of treating yield as a function of biomass and harvest index (Cock et al. 1979; Kawano and Thung 1982; Cock 1983, 1987; Kawano and Jennings 1983; Kawano 1987, 1990; Tan 1987). Williams (1974) pointed out that cultivars, which distributed the greater proportion of dry matter to the roots, also accumulated a greater total amount of dry matter, with roots primarily accounting for the additional accumulation in dry matter. Cassava yield-improvement can therefore be achieved by simultaneously improving both the harvest index and the total biological yield (Kawano 1987; Tan 1987). An enhanced harvest index has been obtained in crops by modification of the sink-source relationship (Veltkamp 1985; Ho 1988). A striking observation was that FB phenotypes bulked at a faster pace, leading to a change from foliage to roots as the dominant sink at 24 weeks after planting (WAP). In the slow-bulking (SB) groups, foliage remained the dominant sink throughout the duration of the early bulking trial, suggesting that this group have an inefficient bulking system.

A total of 45 QTLs were identified in the F_1 population for yield foliage, harvest index, root diameter and bulking rate. Eighteen QTLs were detected for yield, two for dry foliage, eight for harvest index, 17 for root diameter and three for bulking rate. The high number of QTLs identified for yield agrees with its quantitative patterns of inheritance as expected for a trait controlled by many genes. The detection of many QTLs for root diameter and harvest index, which were both found to be highly and significantly correlated with yield, confirms yield as a complex trait, thus making it less amenable for manipulation in breeding programs. Some of the QTLs identified for yield were linked to one or more of the three related traits (dry foliage, harvest index and root dry matter) found in this study to be most influential on early bulking, including bulking rate. The three QTLs detected in Quilichao in 1998, were also found to be involved with QTLs at 11 MAP (data not shown) suggesting that some QTLs controlling early yield are also involved with late yield. QTLs controlling yield, dry foliage, harvest index and root diameter were detected in one or more harvest dates, suggesting that individual QTLs showed different stability. This indicates that some QTLs involved in the control of traits act at different stages, and not all through the period of the crop's growth. Some QTLs were however found to be very stable (e.g. *rdUM.2* and *rdUM.4* for root diameter) leading to their detection most of the times during the harvest schedules, suggesting active roles for these QTLs throughout the growth cycle for the traits they control.

The dissection of a complex trait such as early yield is of great assistance to breeders in selecting parents and designing crosses to enhance the early yield potential of cassava. To further sharpen the power of these experiments to detect QTLs affecting early yield, allelic bridges, markers with unique alleles from both parents, that segregate in a ratio 1:1:1:1, can be used to avoid confounding problems encountered with analyzing segregation from the gametes of only one parent. F_2 populations are also more informative than the F_1 particularly for detecting recessive genes and genes having epistatic interactions. To this end we have generated an F_2 population to further elucidate the genetics of early bulking using molecular markers.

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