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Identification of quantitative trait loci for dry-matter, starch, and β-carotene content in sweetpotato

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Abstract Development of orange-fleshed sweetpotatoes (OFSP) is desired for the improvement of the food supply and nutritional status of millions of people in developing countries, particularly in sub-Saharan Africa. However, sweetpotato [*Ipomoea batatas* (L.) Lam] breeding is challenging due to its genetic complexity, and marker-assisted breeding tools are needed to facilitate crop improvement. We identified quantitative trait loci (QTL) for dry-matter, starch, and β-carotene content in a hexaploid sweetpotato mapping population derived from a cross between Tanzania, a white-fleshed, high dry-matter African landrace, and Beauregard, an orange-fleshed, low dry-matter sweetpotato cultivar popular in the USA. Two parental maps were constructed using a population of 240 clones. Strong correlations were observed between starch and dry-matter content ($r > 0.8$, $P < 0.0001$) in the storage roots, while moderate correlations ($r = -0.6$, $P < 0.0001$) were observed for β-carotene and starch content. In both parental maps, QTL analysis revealed the presence of 13 QTL for storage root dry-matter content, 12 QTL for starch content, and 8 QTL for β-carotene content. Multiple QTL regression models developed for segregation of alleles in each parent explained 15–24% of the variation in dry-matter content,

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17–30% of the starch content, and 17–35% of β-carotene content. To the best of our knowledge, this research presents the only QTL mapping study published to date for dry-matter, starch, and β-carotene content in sweetpotato. This work improves our understanding of the inheritance of these important traits in sweetpotato, and represents a first step toward the long-term goal of developing marker-assisted breeding tools to facilitate sweetpotato breeding efforts.

Keywords *Ipomoea batatas* · Sweetpotato · Sweetpotato breeding · QTL · Molecular marker · Molecular mapping · Polyploid mapping · Marker-assisted breeding · Vitamin A

Introduction

Sweetpotato [*Ipomoea batatas* (L.) Lam] is the seventh most important crop in the world with an estimated 124 million metric tons produced annually. In the tropics, sweetpotato ranks fifth in terms of caloric contribution after rice, wheat, maize, and cassava (CIP 2010a; FAO 2008; Reddy et al. 2007). In many developing countries, sweetpotato is a staple because they are easy to propagate and maintain, and yield well under a variety of adverse conditions, including drought. The potential of this crop as a food and carbohydrate source is widely recognized (Jarret et al. 1992). Orange-fleshed sweetpotatoes (OFSP), in particular, are a very nutritious food, being an excellent source of β-carotene (a pro-vitamin A precursor) and vitamin C, as well as fiber, iron, potassium, and protein (Woolfe 1992, Low et al. 2007). Sweet, low dry-matter content ($\sim$20%) OFSP genotypes are the predominant types of varieties produced in the United States, but in much of sub-Saharan Africa (SSA) the preferred types are cream- or white-fleshed sweetpotatoes that are higher in dry-matter content (28–30%) and have little to no sweetness (Mwanga et al. 2007). Because of their reduced carotenoid content, these types are not as nutritious as the orange-fleshed types. Therefore, much breeding work in SSA is focused on the development of higher dry-matter, semi-sweet OFSP to address the vitamin A deficiency needs of women and children in order to prevent malnutrition and enhance nutrition and food security (CIP 2010a).

Sweetpotato, a highly heterozygous, generally self-incompatible, outcrossing polyploid with a large number of small chromosomes ($2n = 6x = 90$) poses numerous challenges for plant breeding. Cross incompatibilities are common and each successful cross typically results in the production of less than two botanical seed. Most traits of economical importance in sweetpotatoes, including many resistance traits, are quantitatively inherited or appear to be so due to the polyploid nature of the crop (Cervantes-Flores et al. 2002, 2008b). Marker-assisted breeding (MAB) tools are needed for sweetpotato. Until recently, a complete genetic map of sweetpotato was not even available and only a few studies have explored MAB in sweetpotato. Our group recently developed a medium density sweetpotato linkage map based on AFLPs using a population derived from the cross Tanzania $\times$ Beauregard (Cervantes-Flores et al. 2008a). Here, we report the results of our molecular mapping research focused on identifying quantitative trait loci (QTL) in sweetpotato for storage root dry-matter, starch and β-carotene content. This research improves our understanding of the inheritance of these traits in sweetpotato, and provides the initial step toward the identification and location of genes involved in the expression of these economically important storage root traits. Furthermore, our research is a first step toward implementation of MAB and possible association mapping strategies in this important but little researched food staple.

Materials and methods

Mapping population

The mapping population consisted of 240 progeny derived from a cross between Tanzania (female) and Beauregard (male) sweetpotatoes. The population development and AFLP map construction were described in detail by Cervantes-Flores (2006) and Cervantes-Flores et al. (2008a). The parental clones used in the studies differed significantly for several economically important traits. Tanzania, a landrace from east Africa, develops storage roots with a white- to pale cream-colored flesh (containing only traces of β-carotene) that have dry-matter content of over 30%. Tanzania, which typically requires over 120 days to produce a crop in SSA, yields very poorly in the

USA. In contrast, Beauregard, a major variety produced in the USA that yields very well, produces orange-fleshed storage roots in 100–110 days, and they are high in β-carotene and low in dry-matter content ($\sim$20%).

The original planting materials for the field trials were obtained from virus-indexed, clonal plant materials maintained in greenhouses at North Carolina State University. During 2002, five vegetative cuttings from each clone were planted in the field to increase "seed" of each clone. Storage roots harvested from each clone were stored over the winter and planted in the field in plant propagation beds. After several weeks of growth, 25–30 cm-long stem cuttings were transplanted to the field for evaluation. Replicated tests were conducted in two different locations (the Horticultural Crops Research Station, Clinton, NC, and the Lower Coastal Plains Tobacco Research Station, Kinston, NC) during 2003 and 2004. In each experiment, the mapping clones were planted as 10-plant plots arranged in a randomized complete block design with three replications per experiment. Each experiment was replicated over 2 years per location. Plants were spaced 23 cm within rows on rows spaced 90 cm apart. Approximately 120 days after planting the experiments were harvested. The storage roots were harvested and total yield in kilograms was determined for each plot. After harvest, storage roots of each clone were held in our storage facility as a source of seed for the field experiments to be conducted the following season.

Dry-matter determinations

At harvest, four to five medium-sized storage roots per plot were randomly selected for dry-matter determinations. For each plot or experimental unit, storage roots were peeled and sliced into approximately 0.5 cm chips using a food processor, then a combined sample ($\sim$200 g fresh weight) of slices from each of the storage roots sampled in the plot was obtained and oven-dried at 70°C for 3–5 days. Dry-matter content was determined by determining initial and final weight, and estimating the percentage of dried weight. The same procedures were followed for all replications and locations for a total of 1,440 samples (240 clones $\times$ 3 replications $\times$ 2 sites).

Starch and β-carotene analyses

An additional three to five medium-sized roots per plot were selected for starch and β-carotene determinations. To reduce the total number of samples for these determinations the replicates were bulked by clone, thus we only had one sample of each clone per location and year. For each clone, roots were peeled and sliced into approximately 0.5 cm chips using a food processor, from which a bulked sample of approximately 50 g fresh weight was obtained and freeze-dried. After drying, each sample was ground to a fine powder using a coffee grinder under reduced lighting, and sifted using a US Number 35 (500 μm) Standard Sieve (Fisher Scientific International, Hampton, NH, USA). Roughly half of the sifted sample was stored in 100-ml Whirl–Pak® bags (Nasco Whirl–Pak, Fort Atkinson, WI, USA) at 4°C for starch analysis. To prevent oxidative degradation of β-carotene, the remaining half of the ground storage root tissue sample described above was placed in small, approximately 50 ml, bags made of Oxygen Barrier Plastic® film (Cryovac Sealed Air Corporation, Duncan, SC, USA) containing Ageless® Z100 Oxygen Absorber sachets (Ageless Keep Safe, Toronto, Ontario, Canada) and stored at –4°C.

Total starch content was analyzed using a Total Starch Assay® kit (Megazyme International, Wicklow, Ireland), and spectrophotometric readings were conducted using a Spectronic 1201 spectrophotometer (Milton Roy Company, Ivyland, PA, USA) using glucose as sugar control and maize starch as the starch control. The starch content of the storage roots was first calculated as a percentage per dry-weight basis, and later converted to a percentage per fresh-weight basis for analysis.

To quantify β-carotene content, 100–250 mg of the sample was homogenized for 3 min in 10 ml of a hexane:acetone (1:1 v/v) solution, and centrifuged for 10 min. The extraction was repeated twice and the combined supernatant was evaporated in the presence of nitrogen gas. After evaporation, the residue was dissolved in 3 or 5 ml of hexane and the solution was filtered using 25-mm Durapore® (PVDF) membrane filters (Millipore, Billerica, MA, USA) and placed into 5-ml dark glass bottles for analysis. The β-carotene content was analyzed using a ThermoQuest HPLC system (San Jose, CA, USA) as previously described (Teow et al. 2007). Sample vials were

placed in an autosampler cooled to 6°C and covered with aluminum foil to minimize light. Samples (20 µl) were injected into a Sunfire C18 reverse phase column (4.6 × 100 mm, 3.5-µm particle size; Waters Associates, Milford, MA, USA) equipped with an Altech C 18 guard column. Separation was performed at 35°C with a mobile phase of methanol:acetonitrile:chloroform (42.5:42.5:15 v/v) containing triethylamine (0.05% v/v) at a flow rate of 1.2 ml/min. Peaks were monitored at 450 nm with a UV 6000 LP diode array detector. Standard solutions of β-carotene (Sigma–Aldrich, St. Louis, MO, USA) with concentrations of 0.5 µg/ml to 10 µg/ml were used to obtain a standard curve. ThermoQuest Chromatography Data Acquistion Software (version 4.1) was used to collect and process the data. Due to the high cost and labor intensity required to quantify the storage root β-carotene content of each clone in the 240 progenies, these analyses were conducted only in 2003 at both sites.

Genotyping

The development of the Tanzania × Beauregard genetic map was described by Cervantes-Flores et al. (2008a). Briefly, AFLP markers were developed using the Eco/Mse primer combination, according to Vos et al. (1995) with slight modifications for the LI-COR system (Myburg et al. 2001). Markers that were polymorphic between the parents were scored as 1 or 0, depending on their presence or absence in the progeny. A framework linkage map based on single-dose markers was constructed using a combination of JoinMap 3.0 (Van Ooijen and Voorrips 2001) and MapMaker (Lander et al. 1987) software. The parental maps consisted of 726 and 947 single-dose AFLP markers ordered into 90 and 86 linkage groups (genetic chromosomes) for Beauregard and Tanzania, respectively.

Data analysis

Prior to the QTL analyses, analysis of variance (ANOVA) procedures (PROC MIXED, SAS v9.1, SAS Institute, Cary, NC, USA) were used to determine if there were any significant differences between the replications of our tests within each location and by year. In those cases when replications were not significantly different within each location, dry-matter content and yield data values were averaged across replications for each location and year to simplify subsequent QTL analyses. Starch and β-carotene content values were analyzed as collected since only one data point was obtained from each year by location combination. To determine associations between AFLP markers and phenotypic traits, single-point QTL analysis was performed using WinQTL Cartographer (Wang et al. 2005). Interval analysis (Lander and Botstein 1989) and composite interval mapping analysis was also performed using standard algorithms implemented in WinQTL. A locus was considered significant if its calculated LOD or LR (likelihood ratio) value was higher than the respective threshold value. Threshold values were calculated automatically by WinQTL according to the variance characteristics of the trait data. Typical threshold values were LOD 2.5 and LR 11.5. Additionally, to overcome limitations of mapping procedures, we performed single-point analysis on all markers disregarding their segregation ratios and relation to each other using PROC CORR in SAS v9.1. Correlations between phenotypic traits were calculated using PROC CORR.

Markers with significant effects were analyzed for interactions (alpha = 0.05) with other markers in the dataset using PROC GLM (SAS v9.1). Markers demonstrating significant effects (positive or negative) were analyzed in multiple regression models to determine the percent variation explained in a trait by the multiple marker model. To assess if there were any other interactions with other markers from the map that were not initially significant by themselves, an iterative analysis using PROC GLM was conducted. Potential interactions were considered significant and were further analyzed if $P < 0.05$. QTL were graphically displayed on the linkage group by rectangular bars spreading through the markers associated to the variation of the trait (see Figs. 4, 5.)

Results

Dry-matter content

The storage root dry-matter content of the mapping population was distributed normally and ranged from 15 to 35% with a population mean of 25% (Fig. 1). The average dry-matter content was 18 and 30% for storage

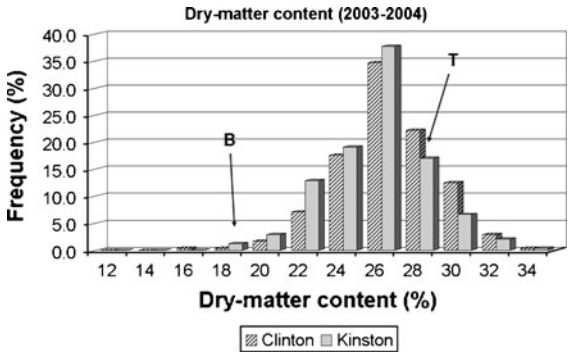


Fig. 1 Frequency histogram of the distribution of dry-matter content in the progeny of the Tanzania (T) × Beauregard (B) mapping population. *Bars* represent the percentage of clones in the population with a dry-matter content observed in given range of dry-matter values (e.g. 20–21.9%) in each site (Clinton and Kinston) averaged over 2 years (2003–2004)

roots of Beauregard and Tanzania, respectively. ANOVA of storage root dry-matter content revealed that location and year had no significant main effects on dry-matter content, but there were significant interactions between the location and year. However, no significant differences were observed between the same progeny genotypes across different locations or years (Table 1). Therefore, data from the three replications of each location and year combination was averaged for the respective QTL analyses. Transgressive segregation was observed in the population, with some progeny exhibiting higher levels and others lower levels of dry-matter content than either parental clone. Dry-matter content was highly correlated with starch content ($r > 0.8$; Fig. 2), and only slightly with β-carotene content ($r = -0.3$; Fig. 3).

Quantitative trait loci analysis revealed the presence of 13 regions having significant effects on the variation of storage root dry-matter content in both parental maps. In Beauregard eight significant regions were identified (Fig. 4). Four of the regions had a positive effect on dry-matter content and were associated with markers E35M4511 (linkage group [LG] B05.26, $P = 0.0247$), E32M3202 (LG B07.40, $P = 0.0098$), E40M4010 (LG B11.61, $P = 0.0138$), and E36M5408 (LG B00.89, $P = 0.049$). An additional four loci, markers E42M3421 (LG B01.03, $P = 0.0056$), E43M5403 (LG B04.23, $P = 0.0007$), E36M5103 (LG B11.62, $P = 0.0055$), and E34M4906 (LG B12.70, $P = 0.0006$) exhibited a negative effect on storage root dry-matter content. Based on the probability levels observed and percent variation explained in storage root dry-matter content, on average, the negative effects were stronger than the positive effects.

Significant interactions between markers E35M4 511 and E32M3202 ($P = 0.0283$), E32M3202 and E42M3421 ($P < 0.0001$), E35M4511 and E43M5403 ($P = 0.045$), and E42M3421 and E43M5403 ($P = 0.045$) in Beauregard were detected by PROC GLM.

Table 1 ANOVA of dry-matter content in progeny of the Tanzania × Beauregard mapping population

Source	*df*	SSQ	MS	*F* value	*P* value
Clone	237	13,429	56.66	8.64	<0.0001
Location[a]	1	0.74	0.74	0.00	0.9999
Year	1	1478.13	1478.13	0.21	0.9999
Location*year	1	5689.76	5689.76	14.80	<0.0001
Rep (location*year)	8	3031.15	378.89	142.93	<0.0001
Clone*environment[b]	608	3993.03	6.56		
Clone*location	235	1510.37	6.43	1.14	0.1884
Clone*year	216	1601.43	7.41	1.32	0.0325
Clone*year*location[c]	157	881.23	5.62	2.12	<0.0001
Residual[d]	1,630	4321.02	2.65		

Replicated trials were conducted in two different locations (Clinton and Kinston, NC, USA) during 2003 and 2004

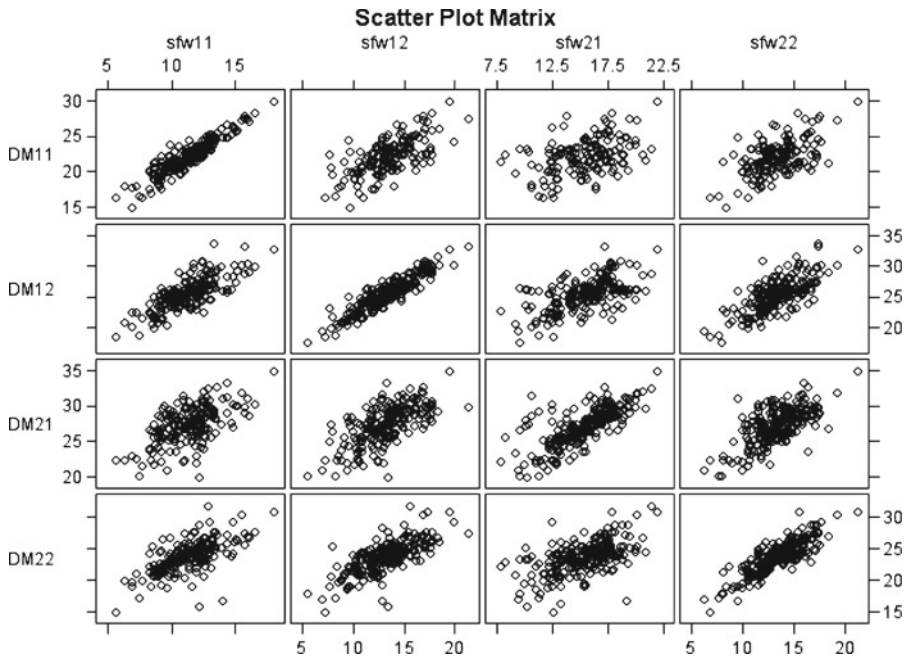
df degrees of freedom, *SSQ* sum of squares, *MS* mean sum of squares (SSQ/*df*)

[a] Mixed MS were used in ANOVA for testing significant effects of location, year, and location*year

[b] Pooled MS used in ANOVA for testing significant effects of clone

[c] MS used in ANOVA for testing significant effects of clone*location, and clone*year

[d] MS used in ANOVA for testing significant effects of clone*year*location, and rep(loc*year)

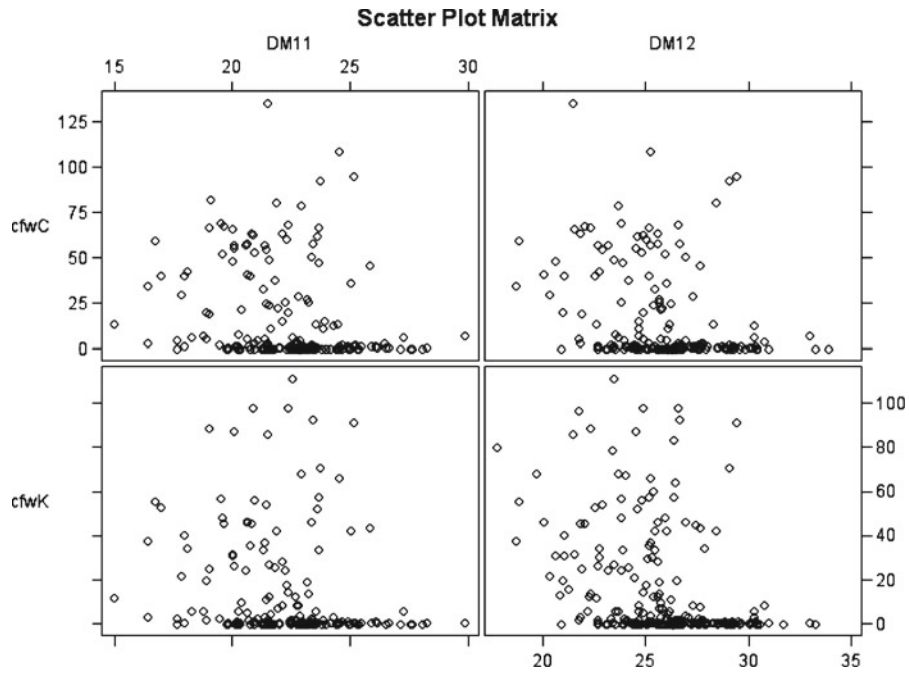


Pearson Correlation Coefficients Prob> |r| under H0: Rho=0				
	sfw11	**sfw12**	**sfw21**	**sfw22**
DM11	0.92125 <.0001	0.66526 <.0001	0.40531 <.0001	0.57319 <.0001
DM12	0.77229 <.0001	0.91931 <.0001	0.58778 <.0001	0.73639 <.0001
DM21	0.58422 <.0001	0.66443 <.0001	0.73321 <.0001	0.66550 <.0001
DM22	0.64489 <.0001	0.72115 <.0001	0.55703 <.0001	0.84335 <.0001

Fig. 2 Pearson correlation coefficients between starch and dry-matter content in the progeny of the Tanzania × Beauregard mapping population (DM = dry-matter content, sfwb = starch content as a percentage of fresh weight basis; sfw11, swf12, swf21, and swf22 = Clinton-2003, Kinston-2003, Clinton-2004, and Kinston-2004, respectively)

Fitting all significant regions and the most significant interactions in an eight-marker model explained roughly 24% of the observed variation in storage root dry-matter content due to the segregation of alleles in Beauregard.

In Tanzania, five regions were observed to have significant effects on the variation of storage root dry-matter content. Of these, four exhibited a positive effect and were associated with markers E35M3603 (LG T01.05, $P = 0.0064$), E36M3808 (LG T05.25, $P = 0.0224$), E31M3208 (LG T06.32, $P = 0.0021$), and E46M6011 (LG T07.37, $P = 0.0486$; Fig. 5). The fifth region exhibited a negative effect on dry-matter content and was associated with marker E43M3524 (LG T02.07, $P = 0.025$). No significant interactions ($P < 0.05$) were observed between these significant regions, or between any of these regions and other non-significant regions (data not shown). The complete model with all significant regions explained approximately 15% of the total variation of dry-matter content in the progeny.



Pearson Correlation Coefficients Prob> |r| under H0: Rho=0		
	DM11	**DM12**
cfwC	-0.20938 0.0057	-0.32434 <.0001
cfwK	-0.19241 0.0139	-0.34535 <.0001

Fig. 3 Pearson correlation coefficients between β-carotene content and dry-matter content in the progeny of the Tanzania × Beauregard mapping population (DM = dry-matter content, cfw = β-carotene content as a percentage of fresh weight basis; C = Clinton, K = Kinston; DM11 = Clinton-2003; DM12 = Kinston 2003, respectively)

Starch content

The total starch content in the mapping population ranged from 5.4 to 21.9% on a fresh-weight basis, with a population mean of 15%. Starch content was distributed normally (Fig. 6). ANOVA of starch content showed no significant effects for locations and years (Table 2). However, there was a significant interaction between progeny phenotype and year. This difference was observed for data from Clinton in the second year, and therefore a separate analysis was performed for the data of the second year from that location. Correlation analyses (PROC CORR, SAS v9.1) demonstrated a strong, positive correlation between starch and dry-matter content ($r > 0.8$, $P < 0.0001$), and a moderately negative correlation with β-carotene ($r > -0.6$, $P < 0.0001$; Fig. 7). Transgressive segregation was observed in the progeny with lower or higher percentages of starch than either parent present in a small number of clones.

Quantitative trait loci analyses revealed a total of 12 regions with significant effects on the variation of starch content due to segregation in both parents (Figs. 4, 5). In Beauregard, three regions located near markers E42M3525 (LG B07.39, $P = 0.0262$), E32M3202 (LG B07.40, $P = 0.0063$) and E40M 4010 (LG B11.61, $P = 0.0442$) were observed to have a significant and positive effect on starch content. Four other regions E42M3421 (LG B01.03, $P = 0.025$), E43M5403 (LG B04.23, $P < 0.0001$),

E36M5103 (LG B11.62, $P = 0.0009$) and E34M4 906 (LG B12.70, $P = 0.04$) exhibited significant negative effects on the variation of starch content. The only significant interactions detected were between regions associated with markers E42M3421 and E32M3202 ($P = 0.0034$). After fitting all significant markers and their significant interactions into a multiple regression model, approximately 30% of the total variation in starch content in the storage root was explained.

In Tanzania, five regions were observed to have significant effects on the variation of storage root starch content. Three loci, E37M4203 (LG T01.04, $P = 0.0412$), E36M3808 (LG T05.25, $P = 0.0165$), and E31M3208 (LG T06.32, $P = 0.0017$) exhibited significant positive effects. Two loci, located near markers E43M3524 (LG T02.07, $P = 0.05$) and E32M4908 (LG T71, $P = 0.0285$), had a significant negative effect on the variation of starch. No significant interactions were observed between these regions, or between these regions and other non-significant regions. A multiple regression model including all five significant regions explained approximately 17% of the total variation of starch content in the storage roots.

Analysis of the starch content data for 2004 from the Clinton test, which differed significantly from the other three experiments, revealed four more chromosomal regions having a significant effect on starch content. These regions were associated with markers E44M3502, E32M3508, E44M5414, and E39M5920, and were located on LGs T01.04, T02.10, T12.70, and T77, respectively. Only the fourth region had a negative effect on starch content.

Most of the regions with significant effects on the variation of starch content in the progeny were also detected as having significant effects on the variation of dry-matter content, and therefore were also associated to the same markers (Figs. 4, 5). This was not surprising, given the high correlation of dry-matter and starch content ($r > 0.8$),

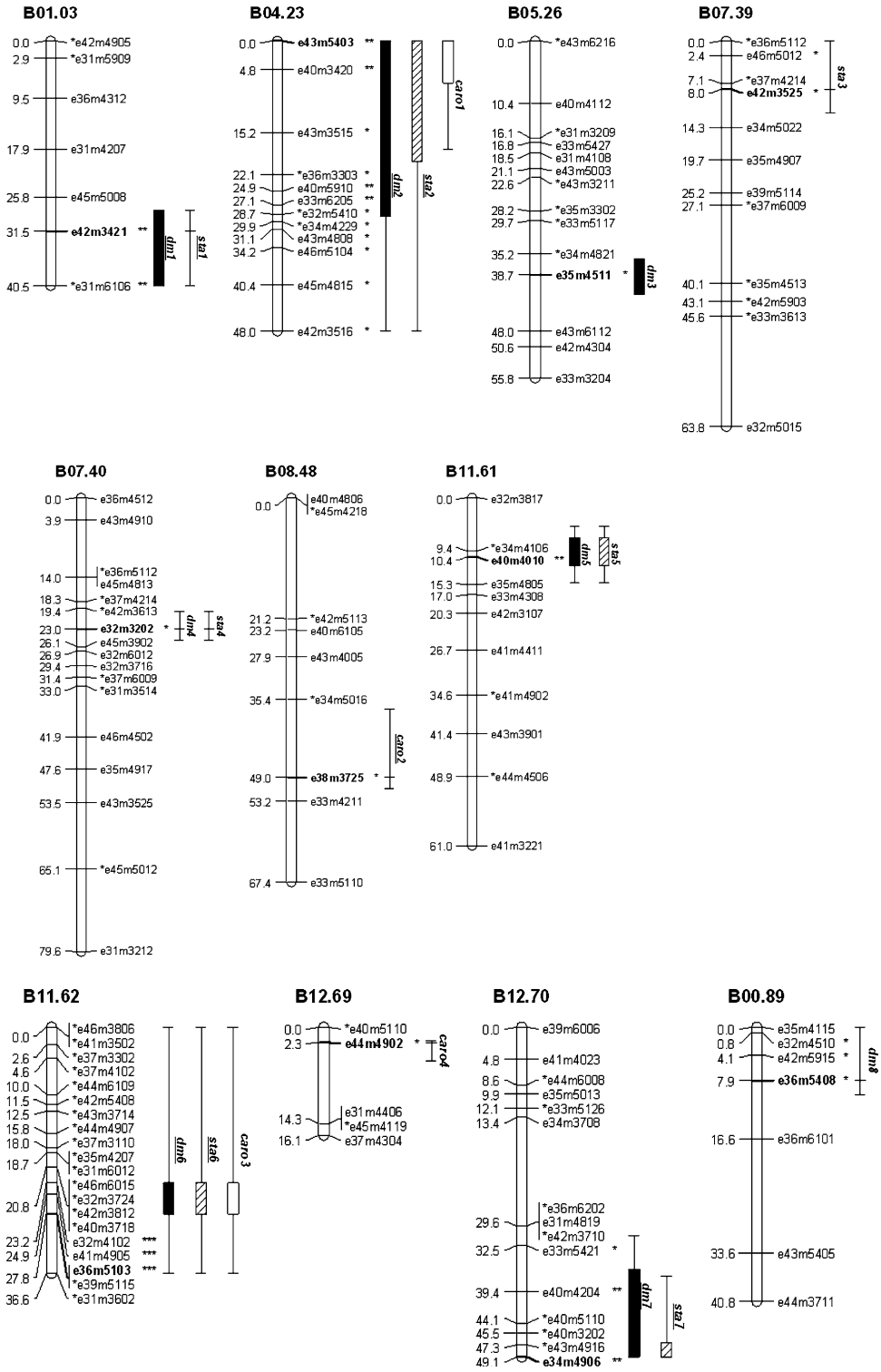
β-Carotene content

The distribution of β-carotene content in the storage roots in this population was highly skewed (Fig. 8), with most clones (70%) showing low to undetectable levels (<5 μg/g of fresh sweetpotato sample) of carotenoids. ANOVA of the β-carotene levels of the pooled storage root samples from both locations were only slightly different (alpha = 0.0307), showing primarily a clone effect (Table 3). Due to this difference, both data sets were used to search for QTL, and a significant QTL was declared when it was significant for both locations. Transgressive segregation was observed, with approximately 15% of the clones containing more β-carotene than the orange-fleshed Beauregard. Correlation analyses indicated that β-carotene content was moderately and negatively correlated with starch content ($r = -0.6$, $P < 0.0001$; Fig. 7), and weakly correlated with dry-matter content ($r = -0.3$, $P < 0.01$; Fig. 3).

Quantitative trait loci analyses on both parental maps revealed the presence of eight QTL with significant effects on the variation of β-carotene content. In Beauregard, four loci linked to markers E43M5403 (LG B04.23), E38M3725 (LG B08.48), E36M5103 (LG B11.62), and E44M4902 (LG B12.69) were significant for β-carotene content (Fig. 4). The first three QTL were highly significant and had a positive effect on the variation, that is, the presence of the marker was correlated with higher levels of β-carotene, while the fourth (E38M3725, LG B08.48) had a negative effect and its effect was primarily due to its interaction with another region. We detected significant interactions between markers E43M5403 and E36M5103 ($P = 0.0008$) on LGs B04.23 and B11.62, and between markers E36M5103 and E38M3725 ($P = 0.0029$) on LGs B11.62 and B08.48. When fitting all significant QTL and their significant interactions, the multiple-marker model explained approximately 35% of the observed variation in the β-carotene content (Table 3).

In Tanzania, four loci located near markers E45M3611 (LG T13.74), E40M3105 (LG T13.76), E46M3901 (LG T78), and E36M4015 (LG T82) had significant effects on the variation of β-carotene content (Fig. 5). The first three regions had a positive

Fig. 4 Beauregard linkage groups showing significant QTL ▶ for β-carotene content, starch content, and dry-matter content. Significance at 5, 1 and 0.1% is indicated by *, ** and ***, respectively. The *markers* most significantly associated with the trait, according to ANOVA analysis, are shown in *bold*. QTL are shown as *vertical bars* on the *right side* of the respective linkage group. *Shaded boxes* QTL are named and according to the trait they affect (dm = dry-matter content; sta = starch content; and caro = carotene content). QTL with positive effect are indicated by *normal text*, and those with negative effect are indicated by *underlined text*


B01.03
0.0 *e42m4905
2.9 *e31m5909
9.5 e36m4312
17.9 e31m4207
25.8 e45m5008
31.5 e42m3421 **
40.5 *e31m6106 **
dm1
sta1
B04.23
0.0 e43m5403 **
4.8 e40m3420 **
15.2 e43m3515 *
22.1 *e36m3303 *
24.9 e40m5910 **
27.1 e33m6205 **
28.7 *e32m5410 *
29.9 *e34m4229 *
31.1 e43m4808 *
34.2 *e46m5104 *
40.4 e45m4815 *
48.0 e42m3516 *
dm2
sta2
caro1
B05.26
0.0 *e43m6216
10.4 e40m4112
16.1 *e31m3209
16.8 e33m5427
18.5 e31m4108
21.1 e43m5003
22.6 *e43m3211
28.2 *e35m3302
29.7 *e33m5117
35.2 *e34m4821
38.7 e35m4511 *
48.0 e43m6112
50.6 e42m4304
55.8 e33m3204
dm3
B07.39
0.0 *e36m5112
2.4 e46m5012 *
7.1 *e37m4214
8.0 e42m3525 *
14.3 e34m5022
19.7 e35m4907
25.2 e39m5114
27.1 *e37m6009
40.1 *e35m4513
43.1 *e42m5903
45.6 *e33m3613
63.8 e32m5015
sta3
B07.40
0.0 e36m4512
3.9 e43m4910
14.0 *e36m5112
e45m4813
18.3 *e37m4214
19.4 *e42m3613
23.0 e32m3202 *
26.1 e45m3902
26.9 e32m6012
29.4 e32m3716
31.4 *e37m6009
33.0 *e31m3514
41.9 e46m4502
47.6 e35m4917
53.5 e43m3525
65.1 *e45m5012
79.6 e31m3212
dm4
sta4
B08.48
0.0 e40m4806
*e45m4218
21.2 *e42m5113
23.2 e40m6105
27.9 e43m4005
35.4 *e34m5016
49.0 e38m3725 *
53.2 e33m4211
67.4 e33m5110
caro2
B11.61
0.0 e32m3817
9.4 *e34m4106
10.4 e40m4010 **
15.3 e35m4805
17.0 e33m4308
20.3 e42m3107
26.7 e41m4411
34.6 *e41m4902
41.4 e43m3901
48.9 *e44m4506
61.0 e41m3221
dm5
sta5
B11.62
0.0 *e46m3806
*e41m3502
2.6 *e37m3302
4.6 *e37m4102
10.0 *e44m6109
11.5 *e42m5408
12.5 *e43m3714
15.8 *e44m4907
18.0 *e37m3110
18.7 *e35m4207
*e31m6012
*e46m6015
20.8 *e32m3724
*e42m3812
*e40m3718
23.2 e32m4102 ***
24.9 e41m4905 ***
27.8 e36m5103 ***
*e39m5115
36.6 *e31m3602
dm6
sta6
caro3
B12.69
0.0 *e40m5110
2.3 e44m4902 *
14.3 e31m4406
*e45m4119
16.1 e37m4304
caro4
B12.70
0.0 e39m6006
4.8 e41m4023
8.6 *e44m6008
9.9 e35m5013
12.1 *e33m5126
13.4 e34m3708
29.6 *e36m6202
e31m4819
*e42m3710
32.5 e33m5421 *
39.4 e40m4204 **
44.1 *e40m5110
45.5 *e40m3202
47.3 *e43m4916
49.1 e34m4906 **
dm7
sta7
B00.89
0.0 e35m4115
0.8 e32m4510 *
4.1 e42m5915 *
7.9 e36m5408 *
16.6 e36m6101
33.6 e43m5405
40.8 e44m3711
dm8

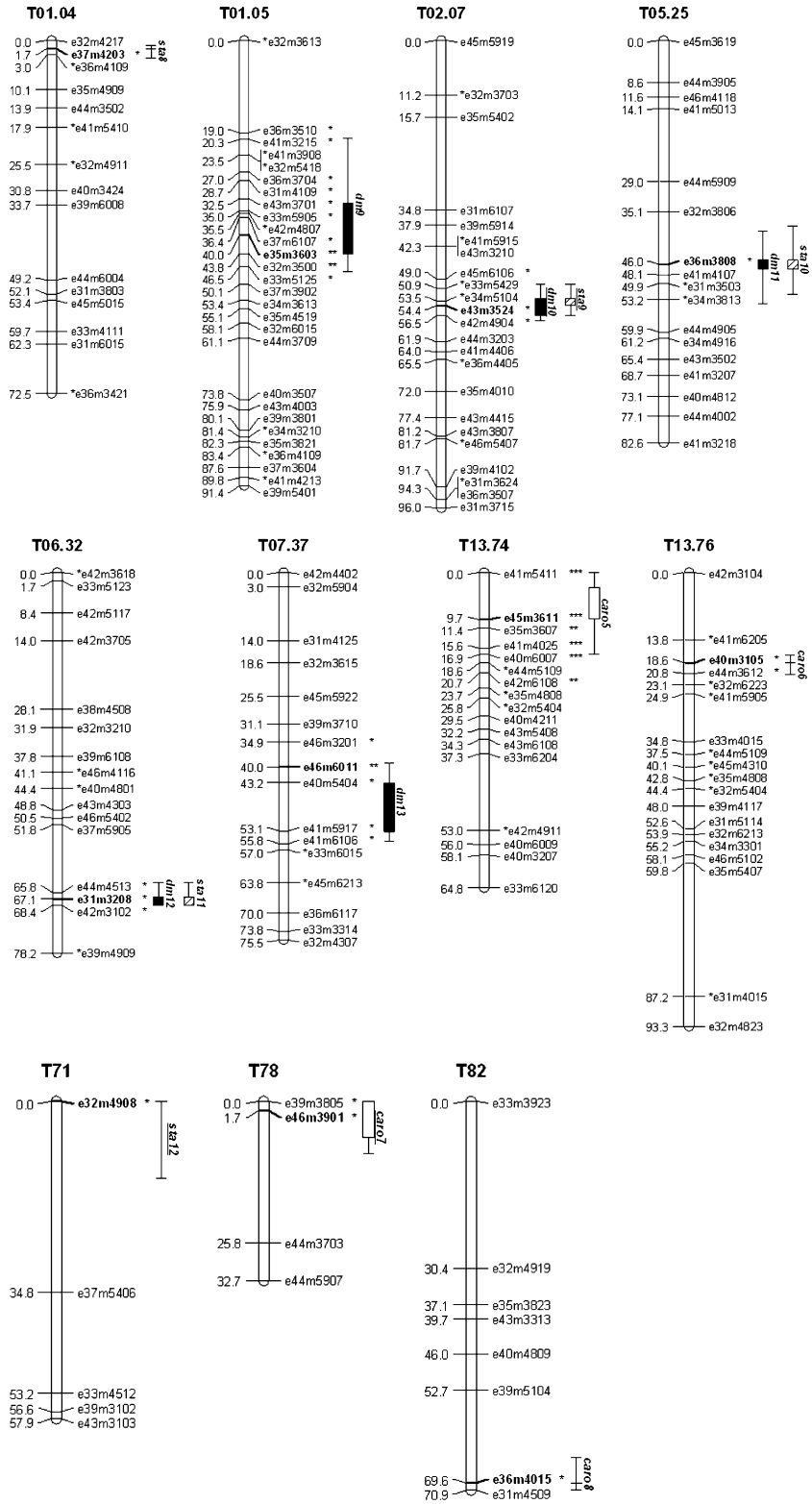


Fig. 5 Tanzania linkage groups showing significant QTL for β-carotene content, starch content, and dry-matter content. Significance at 5, 1 and 0.1% is indicated by *, ** and ***, respectively. The *markers* most significantly associated with the trait, according to ANOVA analysis, are shown in *bold*. QTL are shown as *vertical bars* on the *right side* of the respective linkage group. *Shaded boxes* QTL are named and according to the trait they affect (dm = dry-matter content; sta = starch content; and caro = carotene content). QTL with positive effect are indicated by *normal text*, and those with negative effect are indicated by *underlined text*

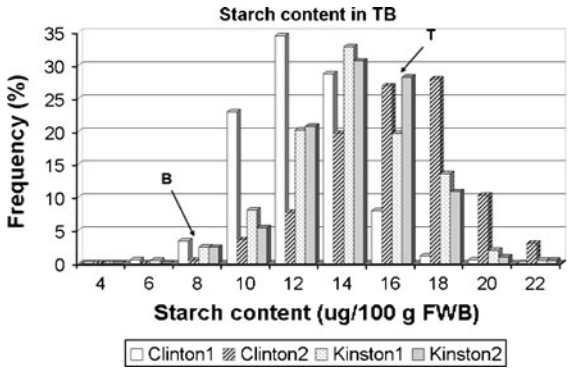


Fig. 6 Histogram of the distribution of starch content expressed as a percentage on a fresh weight basis in the progeny of the Tanzania (T) × Beauregard (B) mapping population. *Bars* represent the percentage of clones in the population with a starch content observed in a given range of starch content (e.g. 10.1–11.9 μg/100 g) in each site (Clinton and Kinston) during 2003 and 2004)

effect and the fourth a negative effect on the total variation of β-carotene content. No significant interactions were observed. The total variation explained by these four regions after fitting them in the model was 17% (data not shown).

Discussion

Over the last four decades, sweetpotato usage has diversified from that of a subsistence, food security or famine relief crop in the developing world to an important food and cash crop with superior nutritional properties (Padmaja 2009; Scott and Maldonado 1999). The development of value-added products from sweetpotato has flourished particularly well in Asia. For example, in Japan the nutrient-rich alcoholic beverage "Pa-Puru" has been developed and commercialized using purple-fleshed sweetpotatoes, and new cultivars for a variety of dried sweetpotato products have been developed (Saigusa and Ohba 2006). Likewise, in China, starch extracted from sweetpotato is being used to make noodles, vermicelli, and sheet jelly; refined starch; starch derivatives (amylophosphate, amylum acetate, and soluble starch); and starch residues (fodder, maltose and sugar residues, brewing products; CIP 2010a). More recently, sweetpotatoes have also been considered to be a potential energy crop due to their ability to produce tremendous amount of starch-based biomass which can be converted into ethanol and various carbohydrate-based polymers via fermentation (Hall and Smittle 1983; Matsuda and Kubota 1984). These new uses of sweetpotato have stimulated breeders to develop better adapted, disease- and insect-resistant, high-starch, high-yielding sweetpotatoes, as well as high β-carotene, high dry-matter and high-yielding OFSP varieties to address nutritional and industrial needs globally.

The need for improved breeding tools to facilitate sweetpotato improvement is great when one considers the significant potential that this crop has for contributing to the food security and nutritional needs of poverty-stricken countries (Hagenimana and Low 2000; Low et al. 2001, 2007), as well as its potential use as an energy crop (Ganguli and Dean 2003; Hall and Smittle 1983). To the best of our knowledge, no previous QTL mapping studies of dry-matter, starch, and β-carotene content have been published for sweetpotato. Extensive germplasm evaluations of

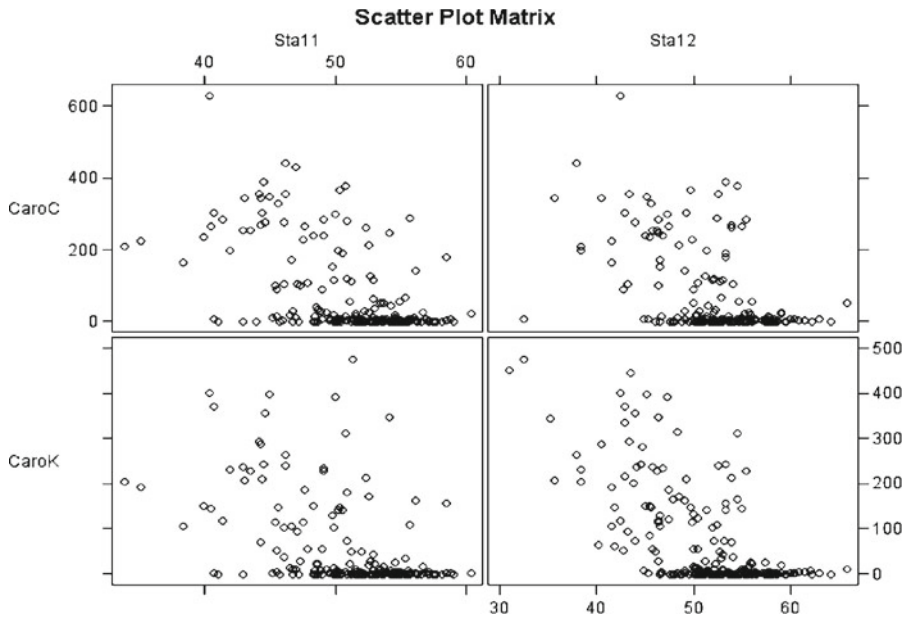
Table 2 ANOVA of starch content in the progeny of the Tanzania × Beauregard mapping population

Source	*df*	SSQ	MS	*F* value	*P* value
Year	1	2.11	2.11	0.003	0.9652
Location	1	580.11	580.11	0.85	0.5258
Year*location[a]	1	682.23	682.23	181.85	<0.0001
Clone	237	3133.56	13.22	6.24	<0.0001
Clone*year	223	475.95	2.21	1.04	<0.0001
Clone*location	200	582.50	2.91	1.37	0.4064
Error	103	218.26	2.12		

Replicated trials were conducted in two different locations (Clinton and Kinston, NC, USA) during 2003 and 2004

df degrees of freedom, *SSQ* sum of squares, *MSE* mean sum of squares (SSQ/*df*)

[a] MSE used in the ANOVA to test the significant effects of year and location



Pearson Correlation Coefficients Prob> |r| under H0: Rho=0		
	Sta11	**Sta12**
CaroC	-0.52501 <.0001	-0.51055 <.0001
CaroK	-0.47487 <.0001	-0.64256 <.0001

Fig. 7 Correlation between starch and β-carotene content in the progeny of the mapping population (CaroC = β-carotene content on a dry-weight basis—Clinton, CaroK = β-carotene content on a dry-weight basis—Kinston, sta11 amd sta12 = starch content as a percentage of dry-weight basis in Clinton-2003 and Kinston-2003, respectively)

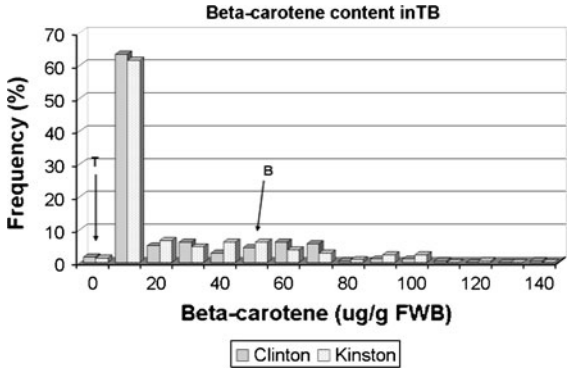


Fig. 8 Histogram of the distribution of β-carotene content expressed on a fresh-weight basis (μg/g FWB) in the progeny of the Tanzania (T) × Beauregard (B) mapping population. *Bars* represent the percentage of clones in the population with a β-carotene content observed in a given range in Clinton and Kinston during 2003

Table 3 ANOVA of β-carotene content in the progeny of the Tanzania × Beauregard mapping population

Source	*df*	SSQ	MS	*F* value	*P* value
Clone	220[a]	250114.54	1136.88	11.20	<0.0001
Location	1	482.75	482.75	4.76	0.0307
Error	161	16345.34	101.52		

Replicated trials were conducted in two different locations (Clinton and Kinston, NC, USA) during 2003

df degrees of freedom, *SSQ* sum of squares, *MSE* mean sum of squares (SSQ/*df*)

[a] Samples for year 1 were pooled for each location and used for the analysis. Genotypes with missing data for both locations were omitted from the analysis

the dry-matter and starch content have been conducted by several researchers (Brabet et al. 1998; Chen et al. 2003; Hagenimana et al. 1999; Okuno

et al. 1998). Several breeding efforts focused on improving these traits in a variety development program have been implemented (K'osambo et al. 1998; Komaki et al. 1998; Kumagai et al. 1990; La Bonte et al. 2000). The availability of dense linkage maps for sweetpotato has enabled us to conduct extensive QTL mapping studies to identify putative regions of the sweetpotato genome affecting the expression of these important traits. These resources provide important information on the inheritance and linkage of these traits, and provide opportunities for future studies focused on MAB in sweetpotato.

As expected, dry-matter, starch, and β-carotene content exhibited quantitative inheritance in our population. Their variation was attributed to multiple loci derived from both parents, ranging from 7 to 13 significant regions dependent on the trait. Single point, interval mapping and composite interval mapping analyses yielded similar results, suggesting that the QTL observed are significant and real. The correspondence of the results obtained with single point ANOVA and the multiple marker QTL mapping methods may be related to the density of our maps. Small distances between markers increase the power of single point analysis to locate significant QTL with similar confidence to the more powerful interval analysis QTL mapping methods (Doerge et al. 1997).

Our QTL analyses of β-carotene content revealed the presence of eight QTL having significant effects on the variation of this trait. Four loci in Beauregard and an additional four from Tanzania explained roughly 35% and 17% of the observable variation in β-carotene in the progenies, respectively. This is interesting because β-carotene is only present in measurable amounts in Beauregard where it is readily observable as dark orange flesh. In contrast, the storage roots of Tanzania are white- to pale cream-colored with only traces of β-carotene (approximately 1 μg/g of the dried sweetpotato root sample). As might be expected, most of the loci segregating in the orange-fleshed Beauregard had a positive effect. That is, the presence of the marker was correlated with higher levels of β-carotene. It is not too surprising that the white-fleshed Tanzania also contributed to β-carotene production as previous studies in other plant species, such as rice and *Arabidopsis* (Beyer et al. 2000; Ducreux et al. 2005; Ye et al. 2000), have confirmed the presence of many of the genes needed for the production of β-carotene in genotypes showing no traceable carotenoids. This was accomplished by inserting only the missing genes necessary to complete or complement the biosynthetic pathway. This was also confirmed by the observation of mRNA of genes in the carotenoid pathway in both orange mutant tissues and the unpigmented wild-type tissues in a study conducted in *Brassica olearacea* (Li et al. 2001).

β-Carotene was negatively correlated with starch ($r = -0.6$) and dry-matter content ($r = -0.3$) in sweetpotato storage roots, suggesting that genotypes containing higher levels of β-carotene are more likely to have lower levels of starch and therefore lower dry-matter content. This observation is consistent with what we observed in the mapping population, and it confirms our applied breeding program experiences, in that cream- or pale-fleshed sweetpotatoes usually have higher dry matter content compared to the dark orange-fleshed clones. Further, it is very difficult to obtain dark orange (>50 μg/g fresh-weight basis) high dry-matter (>30%) sweetpotatoes from breeding populations. However, several clones (e.g., clones TB52, TB97, TB152, and TB258) were observed with high levels of β-carotene (60–100 μg/g fresh-weight basis) and dry-matter (25–30%), as well as cream-fleshed genotypes with low levels of dry matter (e.g., TB24, TB56, TB184, etc.). The negative correlation may be associated with the fact that both starch and β-carotene are synthesized inside plastids (amyloplast and chromoplast, respectively), and thus it is possible that the two substrates may be competing for the same organelles. During development of tobacco floral nectarines, Horner et al. (2007) observed that levels of starch and β-carotene changed inversely and according to the conversion of amyloplast to chromoplast, with an intermediate form "amylochromoplast", containing both β-carotene and starch molecules.

Due to the need for improved and more nutritious sweetpotato clones that provide increased vitamins and minerals, several international projects funded by nonprofit international donors have been initiated in SSA (CIP 2010b, McKnight Foundation 2009). These programs are focused on developing "biofortified" sweetpotato cultivars with high contents of β-carotene and dry-matter. To date, these projects have produced several sweetpotato clones with higher contents of β-carotene and dry-matter content

compared to those clones popular in Africa (Mwanga et al. 2007). The increase of both β-carotene and starch content were necessary for the acceptance of these new clones by the SSA population, because of their preference for sweetpotatoes with high dry-matter content. However, breeding sweetpotatoes of higher quality for SSA is very challenging, since the increase in quality has to be accompanied by an increase in insect and disease resistance and yield, aspects of extreme importance due to high pathogen and insect pressure in the region. Significant progress has been made in this area and as a result several improved clones with higher content of β-carotene have been released (CIP 2010b). However, the levels of β-carotene and dry-matter content in these new cultivars are still below the desired levels to supply the proper amount of vitamins. Consequently, much work is needed to obtain good quality sweetpotatoes with both traits.

Storage root starch and dry-matter content were highly correlated ($r > 0.8$) in this study, which was expected because the total starch content in sweetpotato storage roots ranges from 30 to 65% of the dry-matter content. Many loci affect the inheritance of starch content. In our mapping population, at least 12 different loci exhibited significant additive effects, both positive and negative. The existence of several regions with significant effects agrees with the fact that there are several key enzymes involved in the biosynthetic pathway of starch (Buléona et al. 1998; Kreuze et al. 2009). Increasing storage root starch levels is fairly easy to accomplish by combining clones with different high starch backgrounds, causing as a consequence the increase in the total dry-matter content in the root. However, combining high starch into a finished cultivar is more problematic when one considers all of the other disease resistance, yield, storage and quality traits needed to produce a commercial cultivar. Komaki et al. (1998) used clones from diverse germplasm to increase the total dry-matter and starch content of sweetpotatoes in Japan, obtaining starch levels of up to 40% (fresh weight basis) in their new clones.

The strong correlation of starch content and dry-matter content ($r > 0.8$; Fig. 2) in sweetpotato storage roots has been confirmed by our observation that 9 out of the 12 putative QTL for starch are also significant for dry-matter content. These putative QTL mapped to the same chromosomal region, which could imply that they refer to the same QTL given that the two traits are strongly correlated and could be under the control of common genes. However, because starch only accounts for approximately 60% of the dry-matter content (Woolfe 1992; Brabet et al. 1998), there are also other chromosomal regions that have significant effect on the variation of dry-matter that are not necessarily significant for starch content. These regions may be involved with the synthesis of the other plant products that account for the rest of the dry-matter content, which are not clearly defined, but are composed of fibers, proteins, minerals, etc. (Woolfe 1992). Nevertheless, the most important component of dry-matter for industrial purposes is starch, and breeding for higher starch content is a major aspect of many sweetpotato breeding programs.

Transgressive segregation, in which clones exhibit either higher or lower values than either parent for the particular trait, was observed for all traits studied in the progeny, including yield, which is not presented in this work. Transgressive segregation can be explained by the accumulation or loss of certain favorable alleles in the progeny due to the high levels of heterozygosity of the parents, rather than by complementation with other favorable alleles which can produce this type of segregation (Tanksley 1993; Young 1996). The presence of transgressive segregation in sweetpotato is not unusual as we have seen it on many occasions in our applied breeding program, especially when crosses are made between distinct genetic materials such as Beauregard and Tanzania.

To the best of our knowledge, this work presents the only QTL mapping study conducted to date for β-carotene, starch, and dry-matter content, all of which are important traits in sweetpotato. However, since this is the first attempt to locate QTL associated with these traits in sweetpotato, further analyses are needed to confirm and validate these regions. This research improves our understanding of the inheritance of these complex traits in sweetpotato, and represents a first step toward our long-term goal of developing improved tools to facilitate sweetpotato breeding efforts worldwide.

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