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Identification of quantitative trait loci associated with yield-related traits in sweet potato (*Ipomoea batatas*)

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ABSTRACT. Yield-related traits in sweet potato are generally thought to be quantitative inheritance traits significantly affected by variations in environmental factors. The objective of this study was to identify inter simple sequence repeat (ISSR) markers linked to top weight, root weight, root number, root shape, root skin color, and flesh color in the sweet potato (*Ipomoea batatas*). Two mapping populations of nearly 120 F_1 plants were derived from a reciprocal cross between 'Nancy Hall' (NH) and 'Tainung 27' (TN27). In total, 100 ISSR primers were screened, among which 18 were identified with scorable polymorphic bands that were amplified. All of these primers were anchored at the 3′-end with 7 different types of dinucleotide, and 90.7% of them were segregated as simplex markers. Two partial linkage maps in the sweet potato which use simplex and double-simplex ISSR markers were constructed. The length of each linkage group spanned a very wide range of 10.7~149.1 centiMorgans (cM). These maps consist of 37 NH and 47 TN27 markers with map lengths of 479.8 and 853.5 cM, respectively. The ISSR markers were fitted to a 3:1 ratio of simplex and duplex segregation for the parental alleles, as the sweet potato was expected to be autohexaploid. Fourteen and nine quantitative trait loci (QTL) linked to specific ISSR markers were detected for traits in (NH × TN27) and (TN27 × NH), respectively. Twenty-two of these QTL-mapped markers were unique to the specific traits, and two were linked to two traits in each linkage map. These markers appear to be applicable to crop improvement.

Keywords: ISSR; Linkage map; Molecular marker; Polyploidy; QTL; Sweet potato.

INTRODUCTION

The sweet potato (*Ipomoea batatas* (L.) Lam), of the family Convolvulaceae, is grown in many countries around the world and ranks as the fifth food crop in developing countries after rice, wheat, maize, and cassava (Food and Agriculture Organization, 2002). There have been few genetic studies on sweet potato, probably due to its self-incompatibility and high level of cross-incompatibility, polyploidy level (hexaploid), and large chromosome number (2n=6X=90) (Magoon et al., 1970; Ozias-Akins and Jarret, 1994). The sweet potato originated from the diploid *I. leucantha* Jacq., from which was derived the tetraploid *I. littoralis* Blume by polyploidization (Nishiyama et al., 1975). Hybridization between these two species might have generated the triploid *I. trifida* Don., which reached the hexaploid status by a doubling of the triploid chromosome set. Further selection and domestication of these wild plants might have given rise to the hexaploid *I. batatas*. Another hypothesis for the origin of sweet potato suggests hybridization between *I. trifida* and *I. triloba*, resulting in the generation of the wild ancestor of *I. batatas* (Srisuwan et al., 2006).

Most traits of economic importance in crop plants are quantitative in nature, each controlled by many genes. If based only on phenotype analyses, selection is difficult by traditional breeding methods in the case of large genotype-environment interactions. The sweet potato has been shown to be sensitive to environmental changes (Lin et al., 2007). There is no reliable field screening technique that can be used year after year or generation after generation. One approach to facilitating the selection and breeding of complex traits is the identification of genetic markers linked to the traits of interest. Furthermore, because the expression of many of these commercially important genes cannot be scored during the early stages of growth, the early and rapid determination of superior genotypes through marker-assisted selection (MAS) prior to field maturity would be of benefit to breeders. DNA markers are abundant and operate independently from environmental conditions. Many inter simple sequence repeat (ISSR)

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markers have been used to extend existing linkage maps in plants, and have been shown to be useful for developing linkage maps (Sankar and Moore, 2001). Genetic analysis of agronomic traits using DNA markers has been limited so far in sweet potato. The difficulty of quantitative trait locus (QTL) mapping in sweet potato using DNA markers lies in the inability to distinguish between different classes of heterozygous genotypes that may possess one, two, or three doses of a fragment representing an allele. Ukoskit and Thompson (1997) reported a polysomic inheritance in sweet potato based on the segregation ratio and genetic linkage relationship of random amplified polymorphic DNA (RAPD) markers. In addition, Kriegner et al. (2003) demonstrated the genetic inheritance, segregation, and linkage of amplified fragment length polymorphism (AFLP) markers in two hexaploid sweet potato varieties. These are the first reported genetic linkage maps that have substantial genome coverage of sweet potato. However, these maps did not detect QTLs associated with yield and related agronomic traits. The objectives of this research were to determine the number and locations of QTLs for yield traits in sweet potato and develop a database enabling the utilization of ISSR markers as selection tools to improve crop characteristics.

MATERIALS AND METHODS

Plant materials and reciprocal crosses

'Tainung 57' (TN57) is one of the most widely grown sweet potato varieties in Taiwan due to its high yields. The F_1-mapped population of TN57 originated from a reciprocal cross between two sweet potato varieties, 'Nancy Hall' (NH) and 'Tainung 27' (TN27). The parents differed in a number of ways. TN 27 produces an extremely high quantity of pollen. NH, introduced from the USA, has a high yield of storage roots with high levels of β-carotene. Male and female parents belonging to different incompatible groups can make crosses by hand pollinations. TN 27 and NH belong to the fifth and third group of incompatibility, respectively (Lin et al., 2007). In order to produce more flowers on both male and female parents, grafting was applied in May 2001. "American Yellow Skin" which produces more flowers than other varieties, was used as rootstock, and TN27 and NH as the scions. One month after grafting, the seedlings of grafting were transplanted to the field at Chia-Yi Agricultural Experiment Station (CAES) in July. Twenty plants of TN27 and NH were then reciprocally cross-hybridized in the field from October to December, 2001. Fertile inter-specific F_1 hybrid seeds from the controlled crosses were harvested from December 2001 to February 2002. Fifty parents and three hundred F_1 seeds from reciprocal crosses were sown in the nursery at CAES in May 2002. After 45 days, one thousand vine cuttings about 40 cm in length were transplanted 50 cm apart within rows (ridges) spaced 120 cm wide between rows. There were two plots in the field: one for the 119 progeny derived from the cross NH (♀) and TN27 (♂), and the other for the 112 progeny from the reciprocal cross TN27 (♀) × NH (♂). Within these two plots, ten plants of each parent were planted. Six traits were assessed in the parents and F_1 populations at harvest: shape, flesh color, skin color, fresh weight and number of storage roots, and fresh weight of the tops (leaves and stems). The data were recorded from December 2002 to January 2003 on an individual plant per plot basis. The shape of the storage root was scored visually using a 1-to-3 scale: with 1, 2, and 3, respectively, being long, round, and oval. The flesh color and skin color of the storage root were examined according to Kidgway's 'Standard Color Chart' with a 1-to-3 rating for yellow, red, and purple.

DNA isolation and ISSR- Polymerase Chain Reaction (PCR) assay

Young leaves were detached from each plant and frozen at -70°C. Protocols for DNA extraction are described in Hwang et al. (2002). One hundred ISSR primers (no. 801~900, Primer set 9, NAPS Unit) were obtained from the University of British Columbia Biotechnology Laboratory, Vancouver, Canada. The reaction conditions were optimized to improve the banding pattern of each primer. Each PCR contained 20 ng of the genomic DNA template, 1 unit of *Taq* DNA polymerase (Gibco-Life Technologies), 200 μM each of dATP, dCTP, dGTP, and dTTP, 0.2 μM of each primer, and a PCR buffer with a final concentration of 2 mM $MgCL_2$, in a final volume of 20 μl. The PCR was performed in an Eppendorf Mastercycler Gradient Thermal Cycler set with the following thermal program: initial denaturation at 94°C for 3 min, followed by 42 cycles of 94°C for 1 min, 42~50 °C (depending on the primers used) for 90 s, and 72 °C for 2 min; with a final extension at 72°C for 10 min. Amplified products were separated by electrophoresis on 15% non-denaturing polyacrylamide gels and silver-stained as described by Hwang et al. (2002). λ/Hind*III* or a 100-bp ladder was used as the molecular weight marker to determine the sizes of the amplified fragments. Only the most intense, clear, repetitive bands were used for the ISSR analysis.

Marker scoring, marker segregation type and assessment of polyploidy type

Results of the parental survey were analyzed for polymorphic bands using the 100 ISSR primers. ISSR bands were manually screened twice for the absence (0) or presence (1) of dominant markers. Scored markers that were polymorphic in the F_1 populations were divided into three groups on the basis of their presence in one parent and absence in the other, or presence in both parents. The marker dosage was assessed by the expected segregation ratios (presence vs. absence) of the ISSR markers in the mapped progeny, in accordance with the allele dosage expected by hypotheses of the sweet potato as described by Kriegner et al. (2003). Briefly, markers present in just one parent are expected to segregate in a 1:1 ratio if present in a single dose (simplex) or a 4:1 ratio if present in a double dose (duplex). Markers present in both parents

are expected to segregate in a 3:1 ratio if both parents have a simplex configuration (double-simplex). Markers were tested by a χ^2 goodness-of-fit test at a significance level of 5%. Since autopolyploidy is the most likely genetic configuration in the sweet potato (Nishiyama et al., 1975; Ukoskit and Thompson, 1997), we expected 25% of all segregating markers to be duplex and 75% to be simplex (Kriegner et al., 2003). The observed segregation ratio was tested again using a χ^2 test at a 0.05 significance level.

Markers fitting the expected 3:1 and 1:1 ratios were used for the linkage analysis. Each identified polymorphic ISSR marker was named by a primer number followed by a band number (increasing from top to bottom of a gel) as a suffix.

Map construction and QTL analysis

Sweet potato possesses complex meiotic behavior and numerous copies of the same gene in a given genotype. Female and male meioses leading to gamete formation are independent events because they occur on different sporophytes, and the combination of gametes constituting any individual offspring is a random process. Therefore, data from each individual parent can be regarded as being independent. In this manner, an independent map for each of the two parents was constructed using the segregation analysis from the reciprocal crosses. The linkage relationships of 81 double simplex and 7 simplex markers were determined using MAPMAKER 3.0 (Lander et al., 1987). Three point analyses were performed at a log of odds (LOD) score threshold of 5 and a recombination fraction threshold of 0.35. Haldane's map function was used to convert the map distance to a corresponding recombination fraction. QTL Cartographer software using composite interval mapping (CIM) analyses was then carried out to detect putative QTL locations and distances, maximum values of LOD scores, the additive effect, and

Table 1. Primer types, sequences, amplified and polymorphic bands, as well as number of loci in the linkage group of 18 identified inter simple sequence repeat (ISSR) primers.

Primer type	Primer number	Primer sequence (5′→3′)	Amplified band	Polymorphic band	Number of loci linked to the maps
I	807	$(AG)_8$T	11	8	7
	808	$(AG)_8$C	12	6	1
	809	$(AG)_8$G	20	9	5
II	810	$(GA)_8$T	18	6	3
	811	$(GA)_8$C	22	7	3
	812	$(GA)_8$A	17	5	2
III	813	$(CT)_8$T	10	4	3
IV	816	$(CA)_8$T	12	4	1
	817	$(CA)_8$A	15	2	1
	818	$(CA)_8$G	20	9	5
V	819	$(GT)_8$A	14	3	3
VI	822	$(TC)_8$A	10	3	1
	823	$(TC)_8$T	12	4	4
	824	$(TC)_8$G	9	3	2
VII	825	$(AC)_8$T	20	8	6
	826	$(AC)_8$C	23	9	5
	827	$(AC)_8$G	14	8	5
	857	$(AC)_8$CG	12	4	4
Total			271	102	54
Average			15.1	5.7	3

the percentage of phenotypic variations explained by each QTL (Basten et al., 2002). Zmapqtl, model 6 with a window size of 10 cM around the test interval was used for CIM analyses (Zeng, 1994). Permutation analysis was performed to estimate an appropriate LOD threshold score for a QTL of each trait and population (Doerge and Churchill, 1996). After 1,000 repeats of a permutation test, LOD threshold of 2.50 was chosen for CIM corresponding to a genome-wise significance level of $\alpha = 0.05$. The likelihood ratio (LR) test statistic for each interval was -2 $\ln(Lo/L_1)$, where Lo/L_1 is the ratio of the likelihood under the null hypothesis of no QTL to the likelihood under the null hypothesis that there is a QTL in the interval. The LR statistic at a genomic position was distributed as χ^2 with 2 degrees of freedom (df) under the null hypothesis (Jiang and Zeng, 1995). Log-likelihood peaks for each significant QTL were used to position the QTL on the ISSR linkage map.

RESULTS

Identified ISSR primers

After screening 100 ISSR-PCR products, 18 primers were found to amplify reproducible banding profiles (Table 1). Based on the repeat motif length, these 18 primers were divided into seven dinucleotide groups, AG, GA, CT, CA, GT, TC, and AC, with different anchors at the 3′-end. There were 43, 57, 10, 47, 14, 31, and 69 bands, respectively, amplified from type I to VII primers. These primers amplified 271 discrete bands (15 bands on average per primer) including 102 clear polymorphic bands (6 bands on average per primer). The highest (23) and lowest (9) numbers of bands were generated by primers 826 and 824, respectively. Two to nine polymorphic bands were detected in the 18 identified primers. Number of loci linked in the maps ranged from one to seven per optimized primer, with an average of three polymorphic bands in the linkage groups of the mapped populations. For example, of the eleven amplified bands generated by ISSR 807, eight revealed polymorphisms. Among the eight bands, seven were linked in the NH map (groups 8 and 9, Figure 1) and/or TN27 map (groups 11 and 12, Figure 2).

ISSR marker segregation pattern

In the segregation ratio analysis of the 18 ISSR primers, seven simplex, 81 double-simplex, and nine duplex markers are shown in Table 2. Double simplex alleles were abundant (68.1%) in these plants. For each primer, individual fragments were scored as dominant markers. Fifty-four and 65 amplified PCR products were present in the (♀NH × ♂TN 27) and (♀TN27 × ♂NH) populations, respectively (Table 2). A total of 22 markers (18.5%) did not fit the expected segregation ratios subjecting to a χ^2 test to identify alleles that showed a significant ($p \leq 0.05$) segregation distortion, with deviation toward the presence or absence of a fragment. Since segregation distortion was caused by the reciprocal crosses, the independent map was generated for each population. The 88 simplex and duplex markers were used for the linkage analysis in the parents.

Polyploidy type of sweet potato

A χ^2 analysis was carried out to test polyploidy type for inheritance, as the sweet potatoes are thought to be autopolyploidy. Table 3 shows the 97 DNA bands scored which contained a 3:1 segregation ratio (Kriegner et al., 2003) of simplex (including double-simplex) and duplex markers for parental alleles. Thus, NH and TN27 are autohexaploid.

ISSR linkage analysis

The PCR products, ranging from 150 to 3,000 bp, generated from the ISSR analysis were used to determine the genetic linkage map in sweet potato. Two separate sets of linkage data were obtained, and a specific genetic map was constructed for each parent. A quantitative trait dissection analysis was independently carried out

Table 2. Segregation analysis of 18 inter simple sequence repeat (ISSR) primers based on random chromosome segregation in the (♀NH × ♂TN 27) and (♀TN27 × ♂NH) populations.

Marker type*	ISSR marker			
	NH	TN27	Total	
	Number	Number	Number	Percent (%)
Simplex	0	7	7	5.9
Double simplex	38	43	81	68.1
Duplex	3	6	9	7.5
Distorted	13	9	22	18.5
Total	54	65	119	100

* ISSR markers that fit to the 1:1 (simplex pattern), 3:1 (double simplex pattern), and 4:1 (duplex pattern) ratios were tested by a χ^2 test ($p \leq 0.05$).

Table 3. Chi-square analysis for polyploidy type in sweet potato based on the percentages of simplex vs. duplex markers supporting the autopolyploid nature of sweet potato.

Marker type	Observed number of ISSR markers	Expected number of ISSR markers	
		Autohexaploid	
	Number	Number	%
Simplex (including double-simplex)	88	72.75	75.0
Duplex	9	24.25	25.0
Total	97	97	100.0
χ^2	-	-	12.79***

***Significant at $p \leq 0.01$.
ISSR, inter simple sequence repeat.

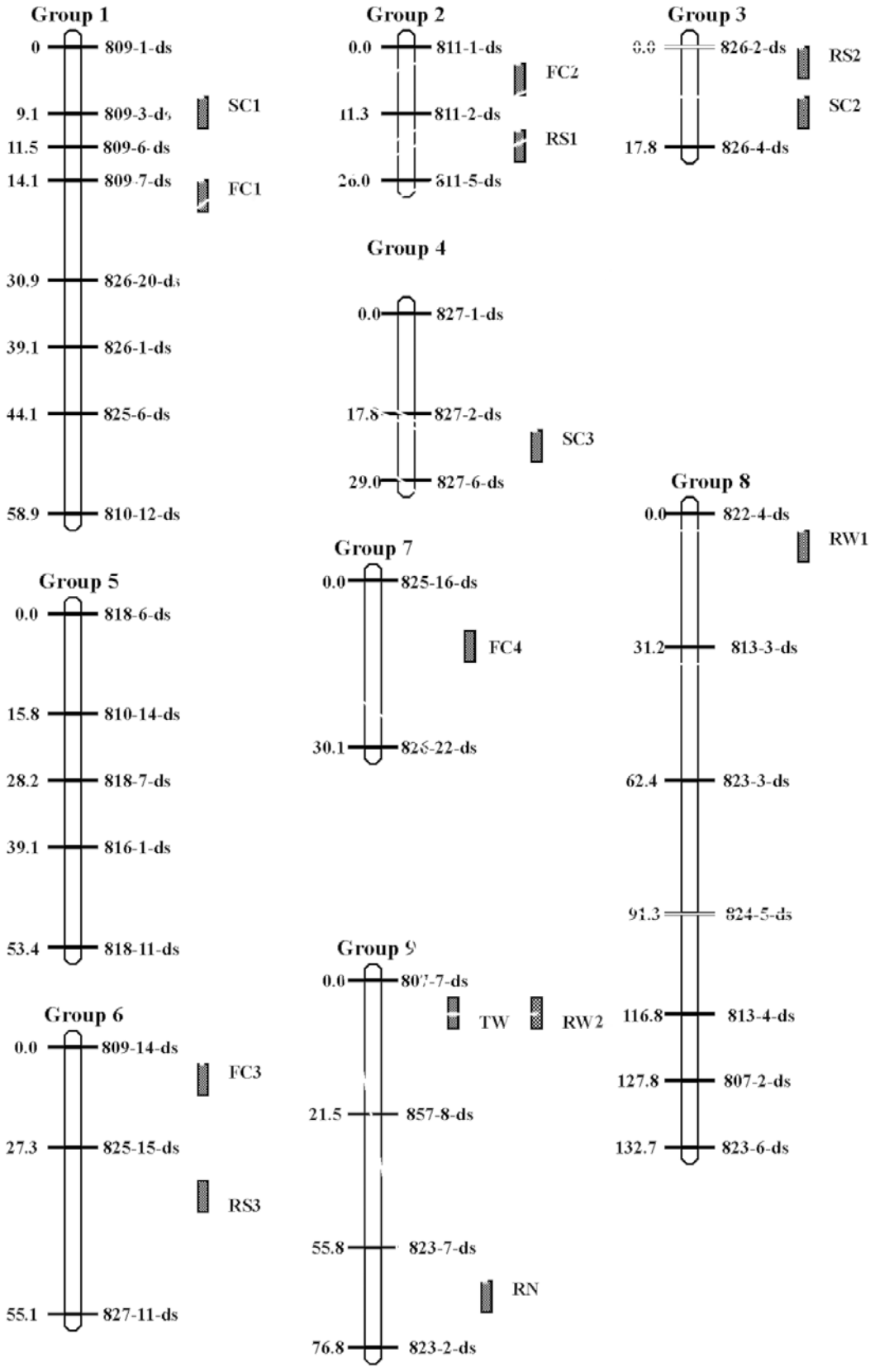

Figure 1. Genetic linkage map of NH. This map shows 37ds ISSR markers that were used to locate quantitative trait loci (QTLs). The marker is presented as the identified inter simple sequence repeat (ISSR) number followed by the band number detected by this primer and marker's segregation type (ds, double simplex). The vertical black bar indicates individual QTLs identified in this study. Markers denoted by SC, FC, RW, RN, and TW were found to be linked to QTLs for skin color, flesh color, and weight of storage roots, as well as top weight. The markers on the left are cumulative map distances in centiMorgans (cM).

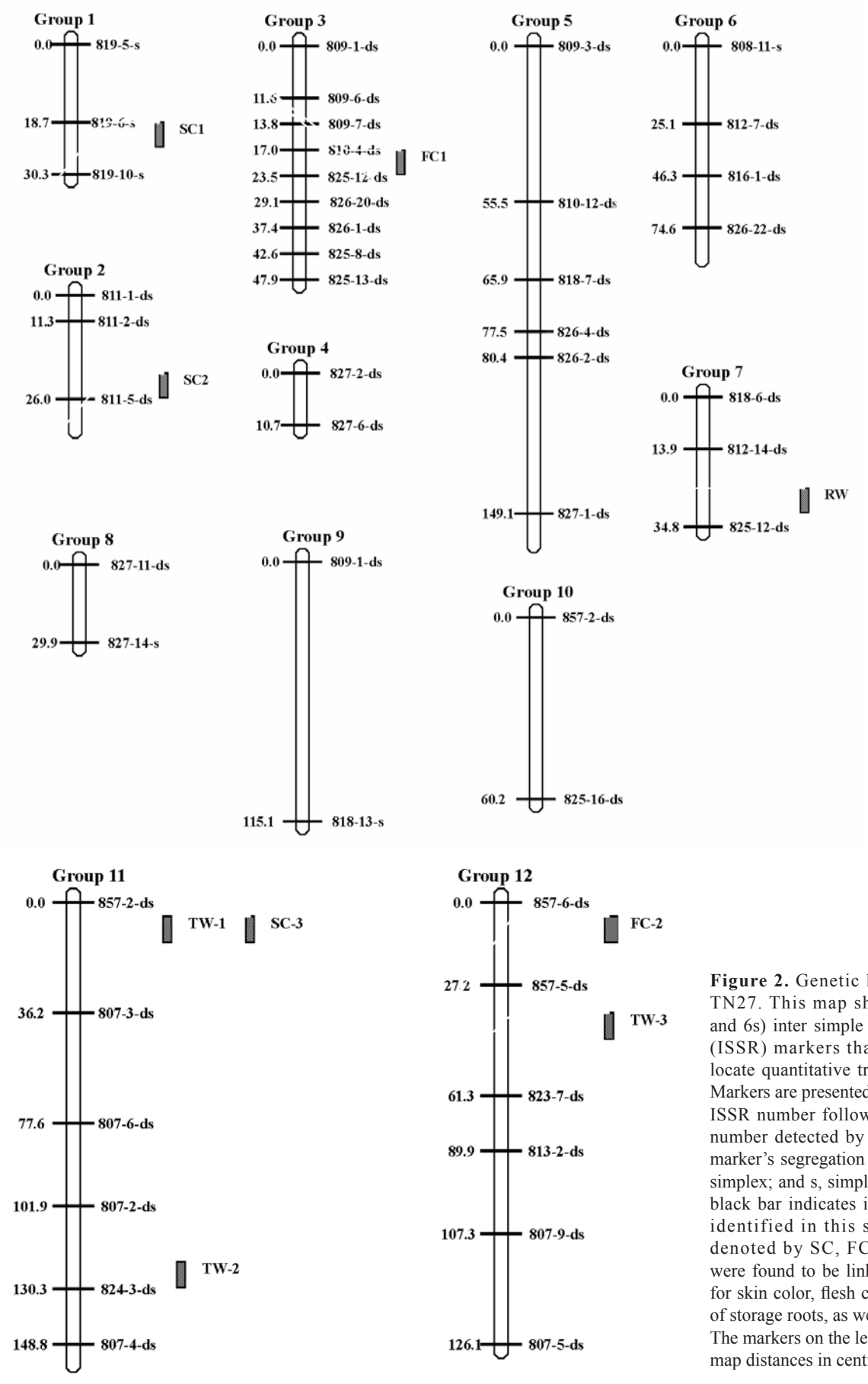

Figure 2. Genetic linkage map of TN27. This map shows 47 (41 ds and 6s) inter simple sequence repeat (ISSR) markers that were used to locate quantitative trait loci (QTLs). Markers are presented as the identified ISSR number followed by the band number detected by this primer and marker's segregation type (ds, double simplex; and s, simplex). The vertical black bar indicates individual QTLs identified in this study. Markers denoted by SC, FC, RW, and TW were found to be linked to the QTLs for skin color, flesh color, and weight of storage roots, as well as top weight. The markers on the left are cumulative map distances in centiMorgans (cM).

for both NH × TN27 and TN27 × NH, and this led to the detection of individual specific QTLs. The NH map includes 37 ISSR markers on 9 linkage groups covering 479.8 centiMorgans (cM), with an average spacing of 12.9 cM between markers (Figure 1). The TN27 map consists of 47 ISSR markers on 12 linkage groups covering 853.5 cM, with an average spacing of 17.7 cM between markers (Figure 2). The linkage groups obtained were numbered at random. The length of each group was < 100 cM, except for group 8 (132.7 cM) in the NH map, and groups 5 (149.1 cM), 9 (15.1 cM), 11 (148.8 cM), and 12 (126.1 cM) in the TN27 map. Distorted loci were clustered on groups 1, 5, and 8 in the NH group (Figure 1), and on groups 3, 5, 11, and 12 in the TN27 map (Figure 2). Simplex (1:1) and double simplex (3:1) segregation patterns of polymorphisms were scored in both the NH and TN27 populations. The majority of markers found in these two linkage maps were double-simplex (3:1), and no simplex (1:1) markers were identified in the NH group (Figure 1). Cumulative Haldane map distances were used in this study, because we assumed that no interference occurred between insufficient markers. Twenty-one markers remained unmapped.

QTL analysis of the traits

QTL mapping detects and identifies regions of a genome associated with variations in a quantitative trait of interest. The CIM analysis increased the control of the genetic background and resolution of QTL mapping (Zeng, 1994). Fifteen and nine significant QTLs (LOD > 2.5) associated with yield traits were identified in NH × TN27 (Table 4) and TN27 × NH (Table 5), respectively. Both tables present linkage locations of putative QTLs, LOD scores, QTL positions (cumulated distances from the upper distal marker), the closest markers (peaks) of QTLs, the additive effect, and the percentage of phenotypic variation explained (PVE) by each QTL (R^2 % or QTL effect). QTLs were not evenly dispersed on the identified groups. The most significant QTL controlling top flesh weight was linked to the 807-7 marker, accounting for 20.51% of the phenotypic variance with an LOD score of 4.9 (Table 4). Storage root weight was affected by two QTLs linked to the 822-4 and 807-7 markers, which individually explained 25.13% and 29.52% of the phenotypic variation, with LOD scores of 3.2 and 3.4, respectively. Three QTLs for skin color of the storage root with LOD scores of 4.1~5.8 explained 14.18%~20.45% of the phenotypic variation. Furthermore, four QTLs were identified and scattered in four different genomic regions for the flesh color of the storage root. Phenotypic variations of single QTLs were in a range of 14.10%~27.80%. The identification of multiple genomic regions for both the skin and flesh color of storage root suggests that these two traits are polygenetically controlled. Three QTLs linked to 811-2, 826-2, and 825-15 were detected for the storage root shape, with PVEs of 20.01%, 26.08%, and 29.76%, respectively. Only one noticeable QTL, linked to 823-7, was identified for the root number, and explained only 14.82% of the phenotypic variation. The additive effects of all QTLs were positive values for all traits in the ♀NH × ♂TN 27 population (Table 4), consistent with the NH seed parent contributing to the phenotypes (Lin et al., 2007). The mapped QTLs for top weight, root weight, skin color, and flesh color were detected in TN27 × NH (Table 5). No marker was detected for either root shape or root number in this population. QTLs linked to 857-2, 824-3, and 857-5 markers respectively explained 16.0%, 20.23%, and 24.66% of the phenotypic variance for top weight. In addition, the genomic region of 825-12 explained 28.44% of the phenotypic variation for root weight. Three QTLs categorized into three linkage groups controlling skin color accounted for 20.41% (819-6 marker)~28.32% (811-5 marker) of the PVE. Moreover, two QTLs with close LOD scores were mapped for flesh color. All of the additive effects in the ♀TN27 × ♂NH population had positive values (Table 5) which were derived from the TN27 seed parent for top weight, root weight, root skin color, and flesh color (Lin et al., 2007).

Of the 23 mapped QTLs, all were liked to double-simplex markers except for one simplex marker 819-6 (group 1, Figure 2).

DISCUSSION

ISSR in the sweet potato genome

Differences in number of anchors contributed to differences in amplification. In this study, only 3′-end-anchored primers that generated seven different amplification patterns were found (Table 1). Among these primer types, the AG, GA, CA, TC, and AC motifs amplified more bands than did the CT and GT motifs, indicating that SSRs with the AG, GA, CA, TC, and AC repeats are abundant in the sweet potato genome. There are more AG, GA, CA, TC, and AC binding sites in the region that can be amplified. This finding is in agreement with the results of Hu et al. (2003), reporting that GA repeats were abundantly distributed and highly polymorphic in the sweet potato. Meanwhile, AT, TA, GC, and CG motifs did not amplify stable, clear bands in this study probably because (A, T) and (C, G) dinucleotides are self-complementary within the primer. Furthermore, trinucleotide and tetranucleotide motifs with or without an anchor were not successfully amplified, suggesting that SSRs with long repeats are less intriguing in the genome. SSRs might not be evenly distributed in the sweet potato genome. ISSR information regarding the motif type, and frequency and polymorphism of different SSR motifs would be useful for developing new microsatellite markers for sweet potato, which would be suitable for constructing a linkage map.

Inheritance, segregation, and linkage of ISSR markers

Previously, the reciprocal crosses between NH and TN27 were made to determine the pattern of inheritance

Table 4. Quantitative trait locus (QTL) analysis by linkage analysis in the sweet potato derived from a ♀NH × ♂TN 27 population. Cartographer was used to determine genomic locations of the log of odds (LOD) scores, QTL positions (cM), closest markers, additive effect, and phenotypic variation explained (PVE) by each QTL (%).

Trait	Name of QTL	Linkage group	LOD	QTL position	Nearest ISSR marker	Additive effect	PVE (r^2 %)
Top weight	TW	9	4.9	10.01	807-7	198.72	20.51
Root weight	RW1	8	3.2	15.42	822-4	121.24	25.13
	RW2	9	3.4	11.30	807-7	109.38	29.52
Root skin color	SC1	1	5.8	10.14	809-3	2.80	20.45
	SC2	3	4.1	16.01	826-4	2.41	14.18
	SC3	4	4.3	27.81	827-6	1.83	15.90
Root flesh color	FC1	1	2.7	16.28	809-7	1.54	10.67
	FC2	2	5.9	5.90	811-1	1.98	27.80
	FC3	6	2.5	4.02	809-14	2.54	14.10
	FC4	7	2.5	12.00	825-16	2.09	20.42
Root shape	RS1	2	9.1	17.31	811-2	1.51	20.01
	RS2	3	9.4	8.01	826-2	1.80	26.08
	RS3	6	8.9	39.31	825-15	2.31	29.76
Root number	RN	9	2.7	57.27	823-7	10.62	14.82

ISSR, inter simple sequence repeat.

Table 5. Qualitative trait locus (QTL) analysis by linkage analysis in sweet potato derived from a ♀TN27 × ♂NH population. Cartographer was used to determine genomic locations of the log of odds (LOD) scores, QTL positions (cM), closest markers, additive effect, and phenotypic variation explained (PVE) by each QTL (%).

Trait	Name of QTL	Linkage group	LOD	QTL position	Closest ISSR marker	Additive effect	PVE (R^2 %)
Top weight	TW1	11	5.2	13.92	857-2	114.27	16.0
	TW2	11	5.8	128.55	824-3	140.44	20.23
	TW3	12	6.0	34.23	857-5	127.68	24.66
Root weight	RW	7	2.5	32.91	825-12	328.73	28.24
Root skin color	SC1	1	2.9	20.38	819-6	1.92	20.41
	SC2	2	5.0	24.30	811-5	1.18	28.32
	SC3	11	4.2	15.16	857-2	1.71	22.84
Root flesh color	FC1	3	3.9	18.12	810-4	1.62	29.94
	FC2	12	3.8	10.61	857-6	1.01	22.84

ISSR, inter simple sequence repeat.

and improve the breeding efficiency for the quality and yield traits of sweet potato. The results have shown that substantial differences of mean and coefficient of variance in the measured traits existed in the F_1 populations of the reciprocal crosses. Maternal effect on top weight between progeny groups have been observed using NH as the seed parent (Lin et al., 2007). In this study, two mapping populations were derived from the same reciprocal crosses for the segregation analysis. Different segregation types are recognized for polyploids showing multivalent pairing at meiosis. According to the theories of sweet potato inheritance (Kumagai et al., 1990; Kriegner et al., 2003), different segregation ratios of DNA markers can be observed and scored in the F_1 population due to the allele dosage. In our study, after the reciprocal crosses, two F_1 heterozygous populations were used to construct two separate parental maps using simplex markers and analyze the genome constitution of sweet potato. Much of the polymorphism between parental bands was masked by the marker dosage, which might have reduced the number of individual ISSR alleles that could be scored in the population. When mapping simplex markers, the segregation ratios and linkage equations are equivalent to diploids for coupling phase linkages, which allows the use of mapping software designed for diploids. Table 2 shows that up to 74.0% (5.9% + 68.1%) of all polymorphic loci behaved as simplex markers, and provided an adequate number of markers for the construction of a genetic linkage map (Figures 1, 2). The differences in the two maps are from segregation distortion (Table 2). Thus, the use of simplex allelic ISSR marker types should be significantly more efficient in the study. Segregation distortion may be caused various processes, including gametic selection of specific chromosome pairing factors, unequal crossover between chromosomes, or associations between heterozygosity and plant vigor (Kriegner et al., 2003). Hexaploids may have a decreased percentage of a distorted segregation ratio (Table 2). It is also interesting to note that a deviation toward the presence or absence of a fragment depended on which seed parent donated the simplex alleles. For instance, simplex alleles with segregation distortion from the NH × TN27 exhibited a deviation toward an absence of the fragment (Table 2, Figure 1). However, simplex alleles with segregation distortion from the TN27 × NH exhibited deviation toward the presence of the fragment, and occurred on linkage groups 1, 6, 8, and 9 (Table 2, Figure 2). The ability to observe level of marker segregation on homologs is an advantage of the allele-dose mapping method. These results are in agreement with Stein et al. (2007) where they reported that 91% of the AFLP markers showed 1:1 segregation ratio indicating its heterozygous simplex state, either in Q4188 or Q417 seed parents. This condition facilitated the identification of simplex markers for mapping at the teraploid *Paspalum notatum* (Bahia grass). About 9% of the markers corresponded to 3:1 pattern, which were informative for identifying homologous groups between maternal and paternal maps.

The pairing of chromosomes can range from preferential (paired with same chromosome in the set) to completely random (equally segregated). Autopolyploidy is determined by the random pairing of homologous chromosomes during meiosis (Doerge and Craig, 2000; Ming et al., 2007). In the case of autohexaploids, 12 different alleles can theoretically be independently segregated in a population. The implications of this for the study of quantitative traits are particularly significant, as these different allelic combinations could result in the phenotypic transformation of an essentially Mendelian character into a trait which exhibits continuous variation. With a dominant marker system, each locus is treated as biallelic to deal with the multiple copies of one locus for autopolyploids. In this case, Wu et al. (1992) and da Silva and Sorrells (1996) first estimated a genetic map of autopolyploids with simplex markers, which represented only one homolog and segregated 1:1 in the progeny. Later, Ripol et al. (1999) demonstrated that any dominant markers could be used at an unobservable dosage level. Luo et al. (2001) and Hackett et al. (2001) then reported that with co-dominant markers, each locus can be treated as being multiallelic. Recently, Cao et al. (2005) showed that the availability of co-dominant markers for autopolyploids are relatively rare, and dominant markers (i.e., simplex markers) have the advantage of being rich in plants and easily scored. In sugarcane, different QTL markers associated with simplex markers have been mapped (Sills et al., 1995; Guimaraes et al., 1997; Ming et al., 2002; Aitken et al., 2006). In our study, the segregation results (Tables 2, 3) showed that the ISSR marker system plays an important role in the genetic analysis of the sweet potato.

QTLs linked to ISSR markers

To our knowledge, this is the first report of the detection of QTLs relating to yield and related traits in the sweet potato. ISSR 807-7 and 857-2 were found to be linked to QTLs for top weight and root weight (group 9, Figure 1) as well as for top weight and skin color (group 11, Figure 2), respectively, but other markers for each trait differed. Thus, largely different sets of genes for different traits might be expressed in different populations. There were 14 QTLs detected in NH × TN27 for six traits (Table 4), while only nine QTLs were identified in the four traits of TN27 × NH (Table 5). Different QTLs generally controlled the same traits in populations from reciprocal crosses. Some genes that are important in one parent might not be expressed in the other parent, or vice versa, suggesting the cross direction has an effect on the expression. These QTLs are directional cross dependent. Barrett et al. (2002) demonstrated that a reciprocal difference in recombination frequencies highlights the importance of considering which parent to use as the female when generating mapping populations. Differences in maternal and paternal recombination frequencies have been reported in wheat (Wang et al., 1995). It is also possible that genetic epistasis occurred in the F_1 populations from the reciprocal cross

leading to linkage disequilibrium of the quantitative genes and resulting in different QTLs associated with different traits in these two populations. Populations of the reciprocal cross were effective in identifying QTLs controlling morphological traits in sweet potatoes.

An additive effect was detected at marker loci for all quantitative traits. The markers are present in both parents reflect a higher mean performance of those individuals with the band compared to without the band. The QTL data (Tables 4, 5) generally supported directional selection for increased top weight, root weight, and root number. The presence of favorable QTL alleles in both parents represents a strong likelihood for recovering transgressive segregants and provides a source of new alleles for plant breeding. Our results obtained using ISSR on the sweet potato show it is possible to resolve multiple alleles following a polysomic segregation pattern. These ISSR markers were unique to specific bands and traits under the designed cross. The association of specific marker alleles with different QTL alleles will allow a breeder to select combinations of desirable alleles from both parents in the progeny. However, as the number of genome regions to be transferred increases, the utility of MAS becomes less clear, as larger and larger populations would be necessary to identify favorable QTL combinations. Thus, in selecting sweet potato lines for a desired trait, the individual marker may be directly and immediately applied, depending on the goals of the breeding program.

Possible pleiotropic effects

Several regions of the genome affect more than one trait. This may be due to closely linked multiple genes or the pleiotropic effects of a single gene. QTL mapping studies of crop plants have found that domestication often involves major alleles at genes with pleiotropic effects and epistatic interactions (Tanksley, 1993). The ISSR 807-7 marker, located on linkage group 9, was associated with top weight and root weight in the NH × TN27 population (Figure 1). The coincidence of the QTL location in the 807-7 marker for the two traits was in agreement with the significantly high correlation coefficients, $r = 0.68$, $p \leq 0.001$ (Lin et al., 2007). Multiple alleles might exist for both loci, some of which affected top weight and others which did not. Thus, the 807-7 marker appears to be linked to independent QTLs that may have pleiotropic effects on top weight and root weight. MAS for favorable alleles at QTLs controlling traits with low-to-moderate heritability can result in appreciable selection gains. MAS is superior for phenotypic selection of low-heritability traits, particular when traits are difficult or costly to phenotype (Yousef and Juvik, 2002). From the MAS using the ISSR 807-7 marker, both top weight and root weight can simultaneously be increased. Improvement in top weight can be accomplished by indirect selection for fresh weight of the storage roots, suggesting that top weight may be used as an indicator for root weight. Papers from Ming et al. (2002) and Ram and Hemaprabha (1998) showed that selection based on a correlated trait, while is more easily selected, is frequently practiced in conventional breeding programs. Likewise, the ISSR 857-2 marker on linkage group 11 showed linkage to a QTL mapped for top weight and skin color in the TN27 × NH population (Figure 2). One or both of these QTLs might correspond to two or more tightly linked genes with different effects on top weight and skin color. Whether a pleiotropic effect is truly involved here is uncertain, since top weight and skin color are not independent traits. The r value between these two traits was non-significant (Lin et al., 2007).

Linkage map of the sweet potato

Only nine and twelve linkage groups were constructed with 37 and 47 linked ISSR markers found in the NH and TN27 maps, respectively. On average, a genetic map consists of four markers per linkage group on either parent. The low number of linkages in these maps is due to the low number of markers generated. A large number of chromosomes decreases the probability of linkages among markers compared to other species with fewer chromosomes. Sweet potato chromosomes are extremely small and differ considerably in size, which may be partially responsible for the differential distribution of markers to the different linkage groups observed in our study. The sweet potato species used here is an autohexaploid (Table 3), so a complete genetic map will have 90 linkage groups. The size of linkage groups is related to recombination, which is due to population size and polymorphism levels. The majority of markers detected in this study were double simplex, suggesting that the parents were related to each other and shared the same markers. This reduced polymorphism levels and thus number of markers detected. Due to insufficient markers and mapping populations, a partial linkage map was constructed in our study. Kriegner et al. (2003) reported 632 ('Tanzania') and 435 ('Bikilamaliya') AFLPs ordered in 90 and 80 linkage groups, respectively. Total map lengths were 3655.6 and 3011.5 cM, respectively, with an average distance of 5.8 cM between adjacent markers. Our ISSR maps cover 537.9 ('NH') and 885.8 cM ('TN27'), which correspond to 14.7% ~ 29.4% of the genome, when compared to the AFLP maps. A complete map of the sweet potato can be achieved by mapping additional markers in the current populations and by combining the results of this study with those of independent studies on the same or related varieties of sweet potato. Further aligning genes mapped in different mapping populations is also possible using common markers as anchors. The efficiency of detecting co-segregation groups differed between NH and TN27 maps (9 vs. 12). One of the explanations for this outcome could be the origin of both parental genotypes. This induced polyploidy constitution could have given rise to many loci at a multiplex condition (i.e. no segregation), reducing the efficiency for detecting linkages in some genomic regions.

The QTLs that we detected had a broad range of effects on individual traits, ranging from 29.76% (QTL-RS3) to 14.10% (QTL-FC3, Table 4). Tanksley (1993)

characterized QTLs as potentially major if they explained > 10% of the phenotypic variance in the mapping population, and such QTLs were found to be quite common in crop plants. In addition, no common markers for root number or root shape were found in the TN27 × NH population, suggesting that different sets of major genes govern root number and root shape. Targeting QTLs for these two traits would be ineffective for improving yields. Only one QTL was mapped for top weight (r^2 = 20.51%) and root number (r^2 = 14.82%) in the NH × TN27 population, and root weight (r^2 = 28.24%) in the TN27 × NH population, suggesting that additional undetected QTLs, as well as environmental factors, contribute to these traits in the sweet potato. Lande and Thompson (1990) proposed that quantitative traits are often controlled by the effects of a few major QTLs which act in concert with a number of smaller-effect QTLs. The QTLs identified for each trait in our study should be considered a low estimate because the population sizes used for QTL mapping were probably not sufficient to detect QTLs with small effects, i.e., no QTL with a phenotypic variation smaller than 14.10% was detected. Furthemore, insufficient population size may also cause overestimate the PVE. Thus, QTLs located in those regions of the genome may have gone undetected. Nevertheless, identification of multiple QTLs associated with a particular trait or suite of traits may provide a reference point for further investigations of important morphological traits. Subsequent studies are needed to reduce recombination frequencies between marker loci and target genes. It should be noted that our results are based on the phenotypic data of a single year at one location. Mapped QTLs for the yield-related traits we reported here could well be unique to these two particular populations and/or this particular environment, and results here should be considered preliminary.

CONCLUSIONS

This study is the first to address the primary set of yield-related qualitative trait loci (QTLs) that have given rise to the various phenotypes seen in the sweet potato from reciprocal crosses. QTL mapping in the sweet potato is complicated by the possibility of segregation for three or more alleles at a locus and by a lack of preferential pairing; however, the subset of polymorphic alleles that shows simplex segregation ratios can be used to locate QTLs. A number of putative QTLs for the measured traits that can aid studies of fundamental physiology and the genetics of traits identified. Most linked markers were unique to a specific parent and trait, and may be useful in crop improvement programs employing MAS. MAS for yield-related traits can be efficiently conducted by selecting individual that contain QTL-linked markers, which would thus facilitate conventional breeding using either NH or TN27 as a donor parent. The ISSR system developed in this study not only provides insights into the genomic organization of this typical representative of present-day sweet potato cultivars in Taiwan, but also provides an important resource for both comparative mapping and evolutional genetic studies among and between related crops.

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辨識與甘藷產量有關之數量性狀基因座

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與甘藷產量有關的性狀通常被認為是數量遺傳，顯著受環境因子變異之影響。本研究目的為辨識與甘藷地上部鮮重、根鮮重、根數、根形狀、根皮色與肉色相關之簡單序列重複 (ISSR) 標誌因子的數量性狀基因座。甘藷‘Nancy Hall’(NH) 與’臺農 27 號’(TN27) 正、反交所產生 120 株 F_1 的兩個族群，共篩選 100 個 ISSR 引子，根據擴大的、可評分的多型性條帶，辨識出其中 18 個條帶。所有引子皆於 3′ 端錨接七種雙核苷酸，其中 90.7% 以簡單 simplex 標識因子分離 ； 使用 simplex 與 double-simplex 之 ISSR 標識因子建立兩個甘藷的部份連鎖圖譜，每一連鎖群的長度是 10.7~149.1 centiMorgans (cM)，這些圖譜由 NH 的 37 個與 TN27 的 47 個標識因子組成，圖譜長度分別為 479.8 與 853.5 cM。ISSR 標識因子符合六倍體親本對偶基因 simplex 與 duplex 的 3:1 分離比。14 與 9 個與專一性 ISSR 標識因子連鎖之數量性狀基因座，分別檢測 NH × TN27 與 TN27 × NH 之性狀，其中 22 個數量性狀基因座圖繪之標誌因子，對於特殊性狀而言具有特殊性 ； 每個連鎖圖譜中，有兩個 QTL 圖繪之標誌因子與兩個性狀連鎖，這些標誌因子應可應用於作物改良。

關鍵詞 ： 簡單序列重複 ； 分子標誌因子 ； 甘藷 ； 多倍體 ； 連鎖圖譜 ； 數量性狀基因座。