Construction of a Genetic Linkage Map based on SSR Markers and Identification of QTLs Related to β-carotene, Dry Matter and Starch Content in Sweet Potato (*Ipomoea batatas* L. Lam)

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A B S T R A C T

Sweet potato (*Ipomoea batatas* L. Lam) is a dicotyledonous tuber crop belonging to the morning glory family, Convolvulaceae. It is one of the staple foods because it is easy to propagate and maintain, and yield well under a variety of adverse conditions. Constructing genetic maps in polyploids has historically been challenging, and has most successfully been accomplished for allopolyploid species. In this study, the parental analysis with 90 SSR primer pairs identified 43.3 per cent polymorphism. The polymorphic SSR primer pairs were used for genotyping the F_1 mapping population and their segregation among mapping population produced 138 segregating loci using SDRF method. From the segregating loci, 69 SSR loci were found to be linked into six different linkage groups and the rest of 69 SSR loci remained unlinked. These segregating markers were used for linkage map construction and the markers were randomly distributed onto the six linkage groups with a total length of 1957.1 cM and the average marker interval of 133.44 cM. Single marker analysis (SMA) was carried out by one-way ANOVA using the phenotypic and genotypic data segregated in the F_1 progenies. The SMA between marker and phenotype resulted in the identification of sixteen SSR marker alleles having association with the β-carotene. SSR markers associated with QTLs related to β-carotene, dry matter and starch content were identified by single marker analysis (SMA), simple interval mapping (SIM) and composite interval mapping (CIM) using Windows QTL Cartographer.

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is a vegetatively propagated, highly heterozygous and outcrossing dicotyledonous crop (Woolfe, 1992). Over 80% of global sweet potato production occurs in the developing world, particularly in Africa and Asia. The importance of sweet potato as a food, feed, and nutrition and income security crop is widely recognized (FAOSTAT, 2017). Sweet potato breeding has relied on the ability of breeders to identify parental genotypes possessing desirable traits and to combine these parents through hybridization schemes such as the polycross and controlled cross

nurseries (Grüneberg *et al.,* 2009a). It has been hypothesized that, crosses of genetically diverse parents result in high levels of heterosis in sweet potato and this approach can be employed to enhance genetic gains. Studies to evaluate this hypothesis are ongoing at the International Potato Center (CIP), Lima, Peru (Grüneberg *et al.,* 2009a). However, such anticipated genetic gains could be limited by the high levels of self- and cross-incompatibilities in some of the diverse parental genotypes (Martin 1965; Gurmu *et al.,* 2013).

The polyploid nature (2n=6x=90) of sweet potato, outcrossing behaviour, and numerous mating incompatibilities, make sweet potato breeding very difficult. Breeding efforts are complicated by the fact that most traits of economic significance in sweet potato exhibit quantitative inheritance (Collins *et al.,* 1999; Jones, 1986). Currently, the sweet potato breeding community lacks a good genetic linkage map to facilitate the breeding process, especially when dealing with complex traits. To date, two independent genetic map of sweet potato have been developed and were not implemented in the breeding programs because of little information on the inheritance of complex traits in sweet potato (Kriegner *et al.,* 2003; Ukoskit *et al.,* 1997).

Molecular marker based linkage maps have been employed successfully in crops such as wheat for tagging resistance genes (Guo *et al.,* 2003; Robert *et al.,* 1999; Prins *et al.,* 2001) and several genes conferring quality traits (Joppa *et al.,* 1997; Mesfin *et al.,* 1999; Parker *et al.,* 1998) have been identified, leading to the implementation of a marker assisted selection (MAS) breeding strategy in some wheat breeding programs as well as other crops (Hamalainen *et al.,* 1997; Howarth and Jadav, 2002; Mohan *et al.,* 1997). In sweet potato, the construction of a fairly dense molecular genetic map will

provide needed information on the mode of inheritance and serve as a guideline for accelerating the introgression of economically important traits into breeding lines by providing important information on trait inheritance, and where feasible and appropriate through the incorporation of a MAS breeding approach into breeding programs. MAS selection for several traits likely inherited by a single gene or few major genes such as resistance to some pathogens (Barker *et al*., 2005; Dillon *et al.,* 2005; Ukoskit *et al.,* 1997), as well as some morphological traits (Babu *et al.,* 2004; Kimura and Hosaka, 2002) could be easily implemented.

Constructing genetic maps in polyploids has historically been challenging, and has most successfully been accomplished for allopolyploid species. This is due to their similarity to diploids in terms of the segregation patterns and chromosomal pairing (Hermsen, 1984; Sybenga, 1996). Genetic mapping in polyploids is difficult for several reasons. First, a large number of possible genotypes are expected in a segregating population due to the larger number of alleles combining in a particular event given the ploidy level of the genome. This is especially true in autopolyploid species. Second, the genotype of an individual is not always readily inferred through its marker phenotype. Third, the type of ploidy (allopolyploidy or autopolyploidy) of many crops is unclear, making it difficult to determine the patterns of inheritance (Ripol *et al*., 1999; Wu *et al.,* 1992).

One commonly used approach to construct molecular genetic maps in polyploids is based on the use of single-dose restriction fragments (SDRF) (Wu *et al.,* 1992). Wu *et al.,* (1992) illustrated this method using both autopolyploid and allopolyploid species with different ploidy levels. Because single-dose

markers are markers present in one parent in a single copy, during the formation of the gametes only half will carry the marker. Thus, disregarding the type of genetic make-up of the genome, half the progeny will possess this fragment and half will not, because the segregation ratio is not affected by the type of ploidy of the plant species. For that reason, SDRF have being used to construct linkage maps in several polyploidy species, including potato, sugarcane, and eucalyptus (da Silva *et al.,* 1993; Ghislain *et al*., 2004; Grattapaglia and Sederoff, 1994; Hoarau *et al.,* 2001; Ripol *et al.,* 1999), and for sweet potato (Kriegner *et al.,* 2003; Ukoskit and Thompson, 1997).

The identification of the homologous chromosomes is very important in genetic mapping analysis in polyploids, since only homologous chromosomes pair and recombine during meiosis. Thus, the identification of all homologous groups is crucial for understanding the genomic constitution of sweet potato and its inheritance mechanism. Sweet potatoes have been characterized as allopolyploids (Jones, 1965; Magoon *et al.,* 1970; Ting and Kehr, 1953) and as autopolyploids (Nishiyama *et al.,* 1975, Ukoskit and Thompson, 1997). The long term objectives of this work are to use the molecular genetic linkage map to facilitate the introgression of desired traits into breeding lines, and open the doors for a map-based cloning alternative for important genes in sweet potato.

The results of our molecular mapping research focused on identifying QTLs in sweet potato for storage root dry matter, starch and β-carotene content. This research will enhance the understanding of the inheritance of complex traits in sweet potato, and provides the initial step towards the identification and location of genes involved in the expression of economically important storage root traits. Furthermore, our finding could lead to the potential application of marker-assisted breeding strategies in sweet potato.

Materials and Methods

Mapping population

A mapping population of 208 progeny derived from a cross between 'ST-14' (female) and 'S-1' (male) sweet potato was used for this study. The vine cuttings were collected from the nursery maintained in ICAR- Central Tuber Crops Research Institute (ICAR-CTCRI) glass house and planted in the field. The phenotypic evaluation of F_1mapping population was recorded based on the tuber flesh colour of the progenies (Aswathy *et al.,* 2015).

SSR genotyping

Genomic DNA was extracted from young leaves of both the parents and each progenies using a modified cetyltrimethyl ammonium bromide (CTAB) method according to Doyle and Doyle (1990) with minor modifications. The DNAs were quantified by spectrophotometry. The final DNA concentration of each template stock was adjusted to 50 ng/μl. SSR and EST-SSR Primers were selected from the previously published literatures and synthesized by eurofins (High Quality Custom Oligos). Fluorescent SSR Primers were synthesized by Applied Biosystems UK. The forward primer of each of the SSR markers was labeled at the 5′ end of the oligonucleotide using fluorescent dyes (6-FAM, VIC, PET and NED) to enable detection on the ABI 3500 capillary DNA Genetic Analyzer (Applied Biosystem) (Karuri*et al.,* (2010); Buteler *et al*., (1999); Wang *et al.,* (2011)).

DNA samples were quantified and a total of 50 ng of total genomic DNA from each of the samples was used for polymerase chain

reactions. A total of 90 SSR primer pairs were used for the sweet potato DNA amplification reactions. A final volume of reaction mixture was 20 μl containing 10X buffer, 10 mMdNTPs each, 2 μl primer (100 μM), 3 U/μlTaq DNA Polymerase, 50 ng/μl DNA, and ddH$_2$O was used for the PCR. PCR amplifications were performed in a C1000^{TM} Thermal Cycler (BIORAD) under the following cycle profile: 5 min at 94°C, 30 cycles of 1min at 94°C, 2 min at an annealing temperature between 50.0 and 66.0°C (depending on the annealing temperature of the primer) and 2 min at 72°C, a final extension at72°C for 5 min and hold at 4°C for infinite time.

Construction of genetic linkage map and QTL mapping

Parental Screening

The selected primers were first used to screen the parents of a mapping population for polymorphisms, detectable differences in marker patterns. Polymorphisms can take the form of size differences of DNA bands, or presence or absence of a DNA band. Only primers showing a clear polymorphism between the parents will be useful in mapping the population. 90 SSR primer pairs were used for the parental study. The amplified PCR products were run in 6% urea denaturing polyacrylamide gel electrophoresis (PAGE) which was carried out in 1X TBE buffer at 100 W for about 1 hour (Sequi-Gen® GT, BIO-RAD sequencing gel, USA). 3-4μl of PCR product was loaded in the well. For obtaining better resolution of polymorphic bands, the gel is silver stained and after staining the gel shows the banding pattern (Panaud *et al.,* 1996). Band size was estimated using a 100 bp DNA ladder (Fermentas). The bands were scored to see the polymorphism between the two parents and the polymorphic primers were selected for the

progeny screening. Polymorphic bands were used to assign loci for each primer and scored as present (1) or absent (3). Among the amplified primers, only polymorphic SSR primers were used for further screening of each progeny in the mapping population.

PCR amplification for Genetic analyzer

The DNA concentrations were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware).The PCR for DNA amplification was performed in a 10 μl reaction volume. The reaction consisted of 2.0 μl DNA template, 1.0 μl of 10X PCR buffer, 0.1 μl of 40mM dNTPs mix, 1.0 μl fluorescent labeled primer (100 μM), 0.1 μlTaq polymerase (5 U/μl) and 5.8 μl PCR water.

The PCR conditions were as described above and the PCR amplifications were performed in C1000^{TM} Thermal cycler (Bio-Rad, Singapore). Each of the DNA samples were amplified using fluorescent SSR primers independently and the PCR products were pooled for genotyping by capillary electrophoresis using an automated ABI3500 Sequencer (Applied Biosystems).

Capillary electrophoresis

Evaluation of polymorphism and segregation of parental alleles was conducted using the ABI 3500 capillary DNA Genetic Analyzer (Applied Biosystem). The PCR products from each sample were co-loaded in three groups on the basis of dye colour and fragment size to ensure separation of each fragment in comparison with others. For fragment analysis, 1μl of pooled PCR product was added to 9 μl of HiDiformamide/LIZ600 size standard mix (ABI). The run conditions for fragment analysis were; run voltage of 15 KV, sample injection voltage of 2 KV for 10

sec, run temperature of 60°C and laser power of 25 mW.

Data analysis

The amplified fragments were viewed, sized and binned using the Gene mapper v.3.7 software (Applied Biosystems). This software performs allele calls which include peak detection and fragment size matching. The data from the size standard (LIZ) is used to determine a standard curve plotting mobility of the fragments against the known size. Fragments arising from the PCR products were compared with the standard curve and their sizes determined. The allele scores were converted to binary data, that is 1 (allele present) and 3 (allele absent).

Single Marker Analysis (SMA)

The scoring of markers for each of the primer pairs was done using Single Dose Restriction fragment (SDRF) method as described by Wu *et al.,* (1992). SDRF are markers that are present in one parent and absent in the other, and are expected to segregate in a 1:1 ratio (absence or presence) in a heterozygous population. Single marker analysis was performed by one way ANOVA to identify SSR markers associated with β-carotene. A significant F-test (P<0.01) indicated association of marker locus with phenotype.

Linkage analysis

A framework linkage map based on single-dose markers was constructed using a Map Maker 3.0 (Lander *et al.*1987) software. Band presence and absence was scored within the F_1population for marker analysis. Band presence was scored with a '1' while band absence was scored with a '3'; missing data was notated with a hyphen. Each marker was tested for goodness-of-fit to a 3:1 ratio to detect possible segregation distortion. A

linkage map was constructed using MapMaker/EXP 3.0 (Lander *et al.,* 1987).The parameters used included a minimum logarithm of odds (LOD) score of 3 and a maximum map distance of 50.0 centimorgans (cM). Markers within a group were ordered using the order command with LOD of 3.0. The remaining markers were located with the 'Try' command and the map order was re-tested using the 'Ripple' command. The Kosambi mapping function was used to determine map distance between ordered marker loci.

QTL mapping

Single marker analysis was performed using WinQTL Cartographer (Wang *et al.,* 2005) to determine any association between the SSR markers and the trait. Interval mapping (Lander and Botstein1989) and composite interval mapping analysis was also performed using standard algorithms implemented in WinQTL. Significance of the QTLs was established by their respective LOD score or their LR (likelihood ratio). A locus was considered significant if its LOD or LR were higher than their respective threshold value (threshold values were calculated automatically by WinQTL according to the variation of the particular trait data; typical threshold values are LOD 2.5 and LR 11.5).

QTL Cartographer software using composite interval mapping (CIM) analysis was then carried out to detect putative QTL locations and distances, maximum values of LOD scores, the additive effect, and 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 analysis (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 like livelihood ratio (LR) test statistic for each interval was $-2\ln(Lo/L1)$, where $Lo/L1$ 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 chi-square with 2degrees of freedom (df) under the null hypothesis (Jiangand Zeng, 1995). Log-likelihood peaks for each significant QTL were used to position the QTL on the SSR linkage map. Venn diagram was also constructed for the identification of QTLs associated with the β-carotene, dry matter and starch content (Oliveros, 2015).

Results and Discussion

SSR marker analysis of parents and hybrids

Upon screening of the 90 SSR primers for polymorphism on the parents a total of 39 primers were polymorphic, while 47 were monomorphic. The remaining four primers did not amplify. The DNA of the 208 progenies and the parents were amplified using the 39 polymorphic SSR primers. The Chi-square test was used to analyze the goodness-of-fit to the expected segregation ratios for all markers. For the linkage mapping, 39 polymorphic SSR primer pairs were selected (Fig. 1a to 1d).

Detection of associated SSR markers linked with β-carotene

Single marker analysis (SMA) was carried out using the phenotypic scores and the marker segregation patterns of 208 progenies to identify the SSR markers linked to β-carotene. The SMA between marker and phenotype

resulted in the identification of sixteen SSR markers having association with the β-carotene. The associated markers are IB242[g], IBSSR04[c], IBSSR04[d], GDS0215[d], IB318[f], IBR13[a], IBR13[d], IBR13[e], GDS0252[b], IB286[d], IB297[h], IB297[j], GDS0615[d], GDS0997[b], IBR03[a] and IB3-28[e] respectively. The markers IBSSR04[c], IB318[f], GDS0252[b], IB297[h], GDS0615[d], GDS0997[b], IBR03[a] and IB3-28[e] were linked on the linkage group 1. The associated markers IBSSR04[d] and IBR13[a] are linked on the linkage group 2 and 4. The markers such as IB242[g], GDS0215[d], IBR13[d], IBR13[e], IB286[d] and IB297[j] are unlinked markers but show association with the β-carotene. The one-way ANOVA between markers and phenotype are given in Table 1.

Construction of linkage map

The above mentioned 39 primer pairs produced different patterns of segregation. These markers were established based on the method described by Wu *et al.,* (1992) for SDRF markers. From a total of 138 segregating loci, 69 SSR loci were found to be linked into six different linkage groups and the rest of 69 SSR loci remained unlinked. These segregating markers were used for linkage map construction and the markers were randomly distributed onto the six linkage groups. It was also found that thirty five alleles followed 1:1 Mendelian segregation ratios using Chi-square test and remaining loci showed segregation distortion *ie.,* deviation from normal segregation.

The total map length was 1957.1 cM and the average marker interval was 133.44 cM. The distance between the markers on the map also varied across the different linkage groups. The size of the linkage group did not necessarily reflect the number of linked markers indicating non-uniform distribution of markers. For example, chrom2, with a total

map distance of 132.6 cM had five mapped loci, whereas inchrom3, chrom4, chrom5 and chrom6 was showing a distance of 49.0 cM, 19.1 cM, 44.2cM and 41.9 cM respectively was covered by only two SSR markers. The map lengths of individual linkage groups ranged from a minimum of 19.1 cM (chrom4) to a maximum of 1670.3cM (chrom1), as shown in Table 2 and Fig. 2. On average, a genetic map consists of two markers per linkage group. The lower number of linkages in these maps is due to the low number of markers generated.

Chrom1was the largest linkage group for this mapping population with a kosambi map length of 1670.3 cM comparatively higher negative LOD value (-5978.03) than other linkage groups. This linkage group consists of 56 SSR markers. The average marker interval map distance is 29.82 cM. The "order" command of Map Maker predicted the best marker order of each group. The total length of chrom2 is 132.6 cM, which is third longest among the six linkage groups. It consists of five SSR markers. The best order predicted by "compare" command of MapMaker is IBR12[b], IBR12[a], IBR12[f], IBSSR04[e] and IBSSR04[d] and this gave the most highly negative LOD value (-504.25). The average marker interval map distance is 26.52 cM. The inter-marker distance ranged from 41.9 cM between IBR12[b] and IBR12[a] to 11.3 cM between IBR12[a] and IBR12[f], 40.8 cM between IBR12[f] and IBSSR04[e] and 38.7 cM between IBSSR04[e] and IBSSR04[d] respectively.Chrom3 is one of the shortest linkage group among the four in this linkage map. It has a total length of 49.0 cM and consists of only two markers placed in the order of IBSSR11[b] and IBS10[d] with a negative log likelihood value of -237.44. The average marker interval map distance is 24.50 cM. Only two markers were placed in chrom4 with an order of IBR13[a] and IBR13[b]. This linkage group is the smallest group with a

total map length of 19.1 cM with a log likelihood value of -204.26. The average marker interval map distance is 9.55 cM.Chrom5 consisted of two SSR markers which was the second longest among the six linkage groups. A total kosambi map length of 44.2 cM was observed with the marker data IB297[a] and IB297[b]. This marker order gives a negative LOD value of -234.54. The average marker interval map distance is 22.10 cM.Chrom6was second smallest linkage group for the mapping population with two markers including GDS0615[e] and GDS0615[f] with a total map distance of 41.9 cM was obtained with a negative LOD score of -232.97. The average marker interval map distance is 20.95 cM.

Single marker analysis using QTL cartographer

β-carotene

The SMA between marker and phenotype resulted in the identification of six SSR markers having association with the β-carotene. The associated markers are IB3-28[e], GDS0252[b], GDS0615[d], IBSSR04[d], IBR13[a]and IBR03[a]respectively. The IB3-28[e], GDS0252[b], GDS0615[d] and IBR03[a] respectively were linked on the linkage group 1. The associated markers IBSSR04[d] and IBR13[a] are linked on the linkage group 2 and 4 (Table 3a).

Dry matter

The SMA between marker and phenotype resulted in the identification of six SSR markers having association with the dry matter. The associated markers are GDS0215[a], IB3-28[e], IB324[a], IBSSR04[d], IB297[a] and GDS0615[e] respectively. The associated markers GDS0215[a], IB3-28[e] and IB324[a] respectively were linked on the linkage group 1. The associated marker

IBSSR04[d] is linked on the linkage group 2 (Table 3b). The markers IB297[a] and GDS0615[e] were linked on the linkage group 5 and 6 respectively.

Starch content

The SMA between marker and phenotype resulted in the identification of six SSR markers having association with the starch content. The associated markers are GDS0215[a], IB3-28[e], IB324[a], IBSSR04[d], IB297[a] and GDS0615[e] respectively. The associated markers GDS0215[a], IB3-28[e] and IB324[a] respectively were linked on the linkage group 1. The associated marker IBSSR04[d] is linked on the linkage group 2 (Table 3c). The markers IB297[a] and GDS0615[e] were linked on the linkage group 5 and 6 respectively.

QTL analysis

The QTL analysis provides important information in support of different breeding programs in plants and animals. This study represents a QTL analysis for economically important traits related to sweet potato QTLs associated with β-carotene, dry matter and starch content.

Identification of QTL positions by SIM for β-carotene

The test statistic of simple interval mapping (SIM) analysis for β-carotene trait in the mapping population showed many peaks across the genome, especially on the chrom1.The horizontal line in the graph is the threshold line that was set earlier by either manually or permutation test. The vertical lines in the graphs are lines separating LR values of each chromosome. The LR line above horizontal line shows identification of QTLs at that marker position on that chromosome. The Fig. 3a suggests that a QTL

is present at about 56.0cM from the left end of the chromosome contributed by parent two alleles with the negative additive effect of -0.322, and that this QTL accounts for about 0.07% of the variance in the trait.

The Table 4a shows information on chromosome number, QTL, QTL position, LOD score, additive variance, phenotypic variance for all significant QTLs identified for the trait under study. In the present study, six significant QTLs were identified, five QTLs on chrom1 and one on chrom2. TheQTL1 was flanked by IB1809[c] and IB242[b], QTL2 was flanked by GDS0215[a] andGDS1059[a], QTL3 was flanked by IBSSR04[c] and GDS1059[a], QTL4 was flanked by IB318[b]and IB318[a], QTL5 was flanked by IBSSR04[b] and GDS0997[d] in chrom1 and QTL6 was flanked by IBSSR04[e] and IBSSR04[d] in chrom2. The QTL6 on chrom2, was identified with a LOD value of 3.42 is explaining 0.44% phenotypic variation (Fig. 3b).

Identification of QTL positions by SIM for dry matter content

Two significant QTLs were identified, one each QTL on chrom1 (Fig. 4a) and chrom2 (Fig. 4b). The QTL1 is flanked by J116[c]and J116[d] and QTL2 was flanked by IBSSR04[e] and IBSSR04[d]. The QTL2 on chrom2 is present at about 109.0 cM was identified with a LOD value of 4.88 with the positive additive effect of 1.35 is explaining with 3.39% phenotypic variance in the trait (Table 4b).

Identification of QTL positions by SIM for starch content

Two significant QTLs were identified, one each QTL on chrom1 and chrom2.The QTL1 is flanked by J116[c] and J116[d] and QTL2 was flanked by IBSSR04[e] and IBSSR04[d].The

QTL2 on chrom2 is present at about 109.0 cM was identified with a LOD value of 4.88 with the additive effect of 1.18 is explaining with 3.43% phenotypic variance in the trait (Table 4c; Fig. 5a and 5b).

Identification of QTL positions by CIM for β-carotene

The Table 5a generates information on the chromosome number, QTL, QTL position, LOD score, additive variance, phenotypic variance for all significant QTLs identified for the trait. Seven significant QTLs were identified, six QTLs on chrom1 and one on chrom2. The QTL1 was flanked by IB1809[c] and IB1809[b], QTL2 was flanked by IB1809[b] and IB242[b], QTL3 was flanked by GDS1059[a] and IB1809[a], QTL4 was flanked by GDS0215[b] and GDS0134[a], QTL5 was flanked by IB318[b] and IB318[a], QTL6 was flanked by IBSSR04[b] and GDS0997[d] in chrom1 and QTL7 were flanked by IBSSR04[e] and IBSSR04[d] in chrom2. An additional QTL in the chrom1 was identified in the CIM of the

trait than the SIM. The QTL3 on chrom1 was present at about 220.9 cM identified with a LOD value of 8.12 explaining with 87.56% of phenotypic variance (Fig.6).

Identification of QTL positions by CIM for dry matter content

The Table 5b generates information on the chromosome number, QTL, QTL position, LOD score, additive variance, phenotypic variance for all significant QTLs identified for the trait. Three significant QTLs were identified, one QTL on chrom1 and two QTLs on chrom2. The QTL1 was flanked by J116[c] and J116[d] on chrom1. QTL2 and QTL3 were flanked by IBSSR04[e] and IBSSR04[d] in chrom2. An additional QTL in the chrom2 was identified in the CIM of the trait than the SIM. The QTL3 on chrom2 was present at about 130.0c Midentified with a LOD value of 4.88with the additive effect of 1.83was explaining with 9.16% phenotypic variance in the trait (Fig. 7a, 7b).

Table.1 One way ANOVA for SSR markers associated with β-carotene gene

Sl No.	Linked markers	F (calculated)[f]	P-value	F (critical)
1	IB242[g]	4.749	0.030	3.886
2	IBSSR04[c]	6.484	0.011	3.886
3	IBSSR04[d]	8.795	0.003	3.886
4	GDS0215[d]	6.037	0.014	3.886
5	IB318[f]	6.843	0.009	3.886
6	IBR13[a]	4.110	0.043	3.886
7	IBR13[d]	4.341	0.038	3.886
8	IBR13[e]	7.614	0.006	3.886
9	GDS0252[b]	8.011	0.005	3.886
10	IB286[d]	5.972	0.015	3.886
11	IB297[h]	3.904	0.049	3.886
12	IB297[j]	3.980	0.047	3.886
13	GDS0615[d]	5.735	0.017	3.886
14	GDS0997[b]	4.763	0.030	3.886
15	IBR03[a]	4.788	0.029	3.886
16	IB3/28[e]	3.956	0.048	3.886

[f]- calculated using single factor ANOVA

Table.2 The marker information in the linkage group

Linkage group	Size (cM)	No. of markers	Average marker interval
chrom1	1670.3	56	29.82
chrom2	132.6	5	26.52
chrom3	49.0	2	24.50
chrom4	19.1	2	9.55
chrom5	44.2	2	22.10
chrom6	41.9	2	20.95
Total	**1957.1**	**69**	**133.44**

Table.3a Summary of markers linked to β-Carotene

Chromosome/Linkage group	Marker	b0	b1	-2ln(L0/L1)	F(1,n-2)	pr(F)
01	IB3-28[e]	3.250	0.814	9.653	9.786	0.002 **
01	GDS0252[b]	3.342	0.297	7.935	8.011	0.005 **
01	GDS0615[d]	3.311	0.272	5.712	5.736	0.017 *
01	IBR03[a]	3.074	0.431	4.779	4.788	0.029 *
02	IBSSR04[d]	3.419	-0.291	8.697	8.796	0.003 **
04	IBR13[a]	3.436	0.201	4.109	4.110	0.043 *

Significance at the 5% and 1% levels are indicated by * and ** respectively

Table.3b Summary of markers linked to dry matter content

Chromosome/Linkage group	Marker	b0	b1	-2ln(L0/L1)	F(1,n-2)	pr(F)
01	GDS0215[a]	31.573	-1.044	6.669	6.712	0.010 *
01	IB3-28[e]	31.492	-1.908	4.271	4.274	0.039 *
01	IB324[a]	32.580	-1.714	4.484	4.489	0.035 *
02	IBSSR04[d]	31.170	1.441	17.717	18.316	0.000 ****
05	IB297[a]	30.797	1.059	8.997	9.106	0.002 **
06	GDS0615[e]	30.921	0.883	6.425	6.463	0.011 *

Significance at the 5%, 1% and 0.01% levels are indicated by *, ** and **** respectively

Table.3c Summary of marker linked to starch content

Chromosome/Linkage group	Marker	b0	b1	-2ln(L0/L1)	F(1,n-2)	pr(F)
01	GDS0215[a]	21.099	-0.898	6.526	6.566	0.011 *
01	IB3-28[e]	21.031	-1.651	4.227	4.229	0.040 *
01	IB324[a]	21.969	-1.479	4.416	4.420	0.036 *
02	IBSSR04[d]	20.753	1.254	17.726	18.326	0.000 ****
05	IB297[a]	20.429	0.922	9.014	9.124	0.002 **
06	GDS0615[e]	20.536	0.773	6.516	6.556	0.011 *

Significance at the 5%, 1% and 0.01% levels are indicated by *, ** and **** respectively

Table.4a QTLs identified for β-carotene by simple interval mapping

Sl No.	Chromosome	QTLs	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	56.0	5.13	-0.32	-2.47	0.000
2	chrom1	QTL2	197.3	10.00	0.28	2.64	0.107
3	chrom1	QTL3	216.3	11.84	1.67	-0.55	0.000
4	chrom1	QTL4	728.1	7.99	0.05	-2.50	0.008
5	chrom1	QTL5	777.1	10.95	0.98	-1.81	0.000
6	chrom2	QTL6	115.0	3.42	-0.11	-1.61	0.004

Table.4b QTLs identified for dry matter content by simple interval mapping

Sl No.	Chromosome	QTLs	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	920.5	3.07	-3.95	-9.48	0.000
2	chrom2	QTL2	109.0	4.88	1.35	5.98	0.033

Table.4c QTLs identified for starch content by simple interval mapping

Sl No.	Chromosome	QTL	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	920.5	3.07	-3.43	-8.25	0.000
2	chrom2	QTL2	109.0	4.88	1.18	5.19	0.034

Table.5a QTLs identified for β-carotene by composite interval mapping

| Sl No.	Chromosome	QTL	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	27.0	5.63	0.07	2.43	0.008
2	chrom1	QTL2	58.0	3.53	-0.24	-2.33	0.001
3	chrom1	QTL3	220.9	8.12	0.98	-1.48	0.875
4	chrom1	QTL4	540.8	4.20	-0.18	2.32	0.003
5	chrom1	QTL5	726.1	5.37	0.02	-2.33	0.030
6	chrom1	QTL6	778.1	8.95	0.94	-1.74	0.000
7	chrom2	QTL7	116.0	2.57	-0.09	-1.47	0.002

Table.5b QTLs identified for dry matter content by composite interval mapping

Sl No.	Chromosome	QTL	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	920.5	3.24	-3.22	-9.14	0.006
2	chrom2	QTL2	113.0	4.81	1.43	5.51	0.054
3	chrom2	QTL3	130.0	4.88	1.83	-6.38	0.091

Table.5c QTLs identified for starch content by composite interval mapping

Sl No.	Chromosome	QTLs	QTL Position	LOD score	Additive effect	Dominance effect	R^2
1	chrom1	QTL1	488.1	2.57	-1.97	-5.90	0.022
2	chrom1	QTL2	920.5	2.88	-2.74	-7.61	0.006
3	chrom2	QTL3	110.0	3.96	1.11	4.78	0.034
4	chrom2	QTL4	130.0	3.93	1.38	-5.56	0.069

Fig.1a Parental Screening of SSR primers

M P1 P2 M
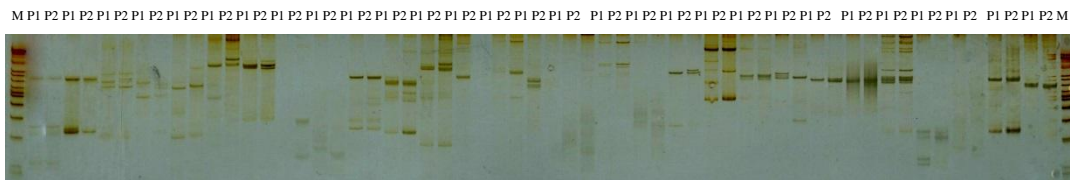
Lane M: Marker; P1: S-1; P2: ST-14

Fig.1b Parental Screening of EST-SSR primers

P1 P2 P1
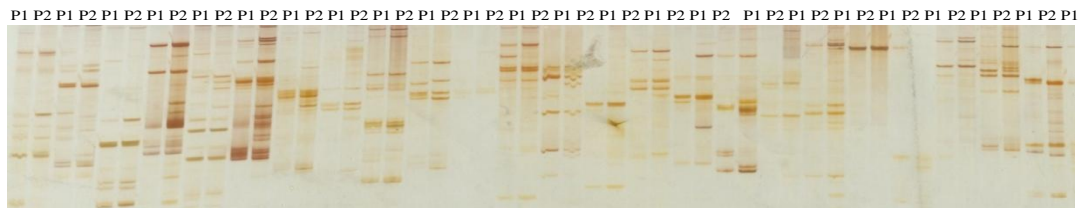
Fig.1c Progeny screening of SSR primer IBS01

F$_1$ progenies →   P1 P2 M →

F$_1$ progenies ←   P1 P2 M
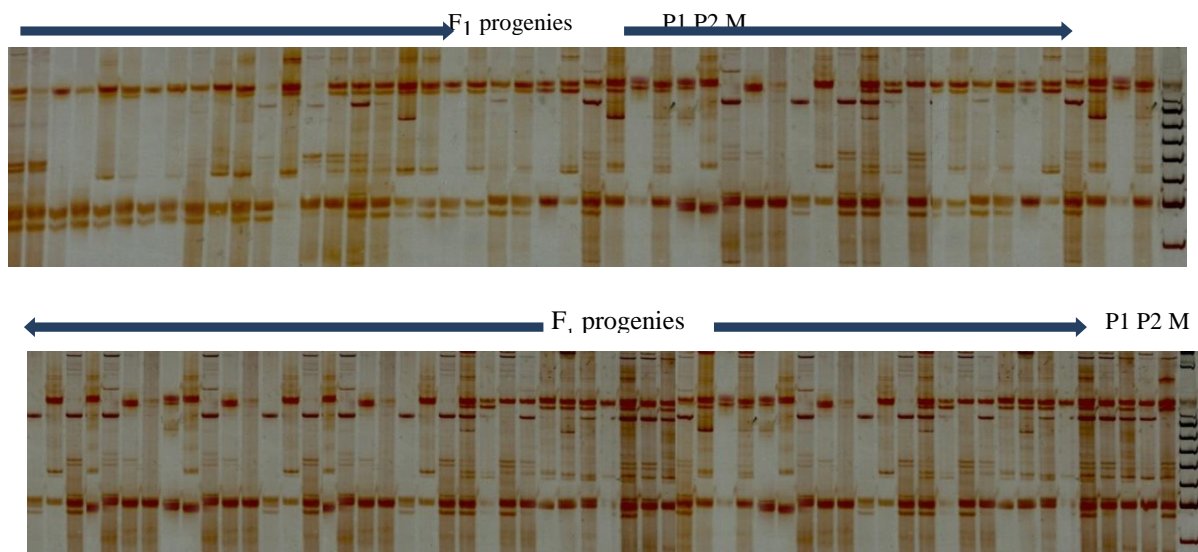
Fig.1d Allelic peak size difference segregated in the progenies for different fluorescent primers
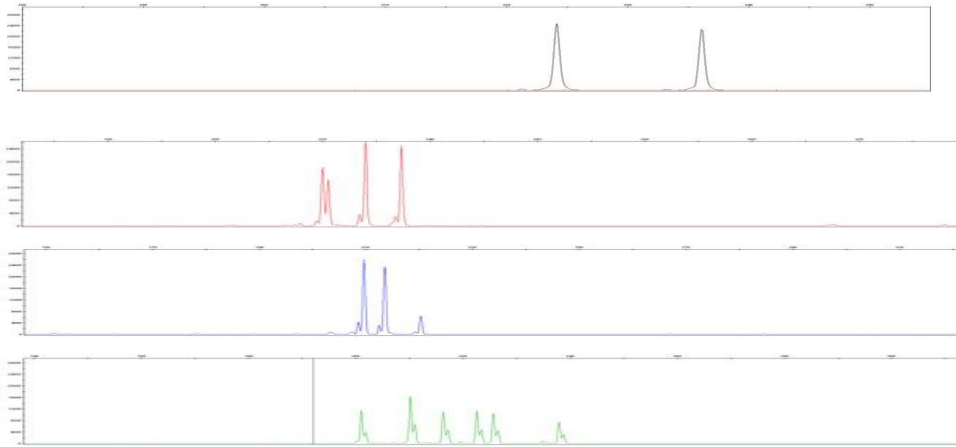
Fig.2 Construction of linkage map of the mapping population S1× ST14
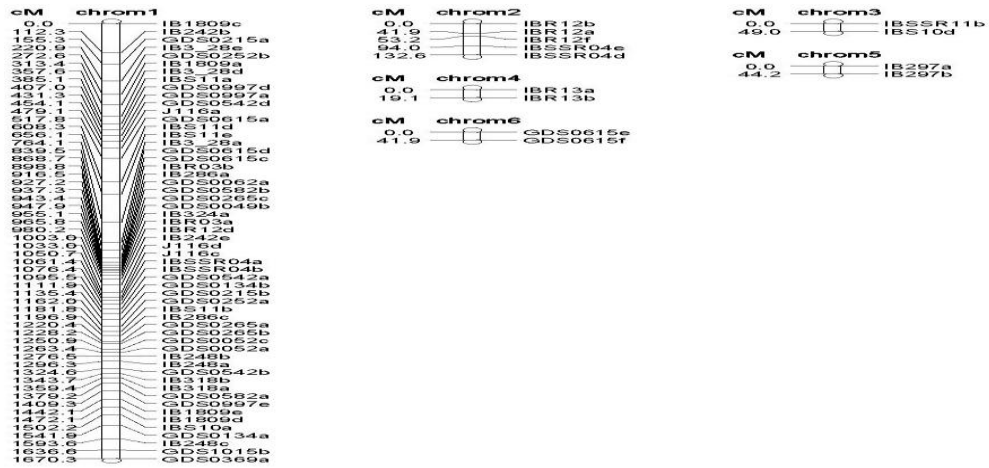
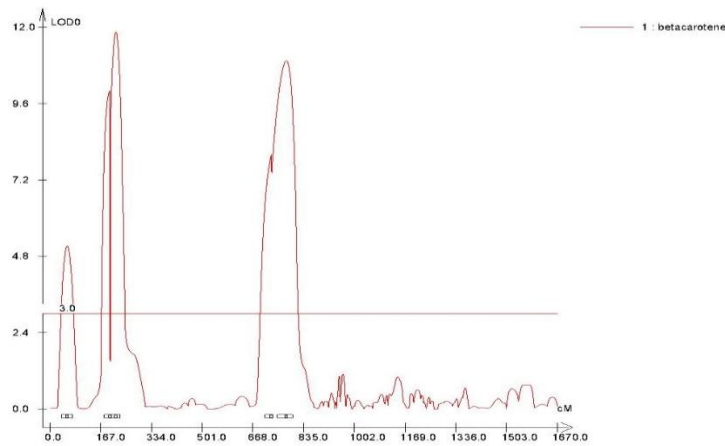**Fig.3a** Graphical representation of QTLs by SIM for β-carotene in Chrom1

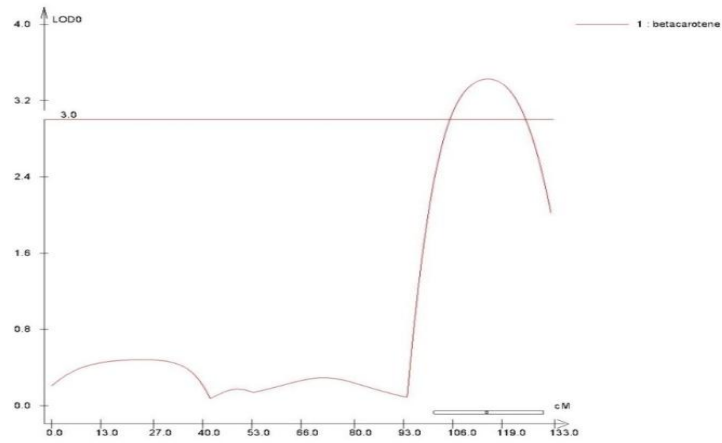**Fig.3b** Graphical representation of QTLs by SIM for β-carotene in chrom2

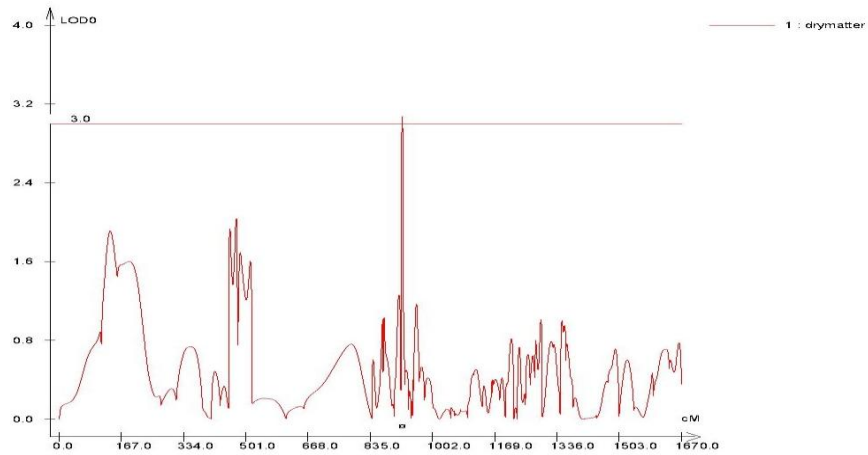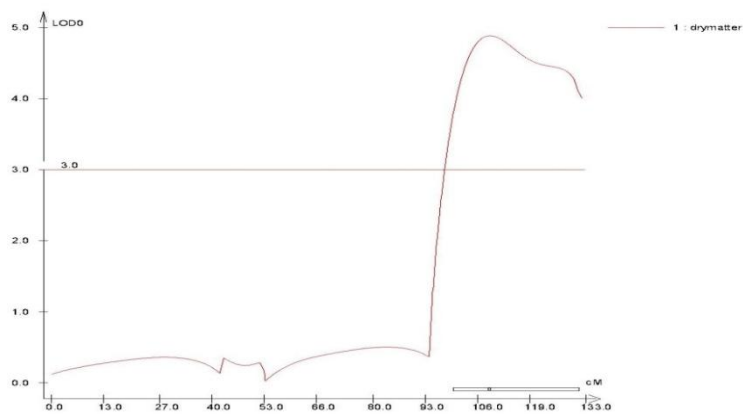**Fig.4a** Graphical representation of QTLs by SIM for dry matter content in chrom1

Fig.4b Graphical representation of QTLs by SIM for dry matter content in chrom2

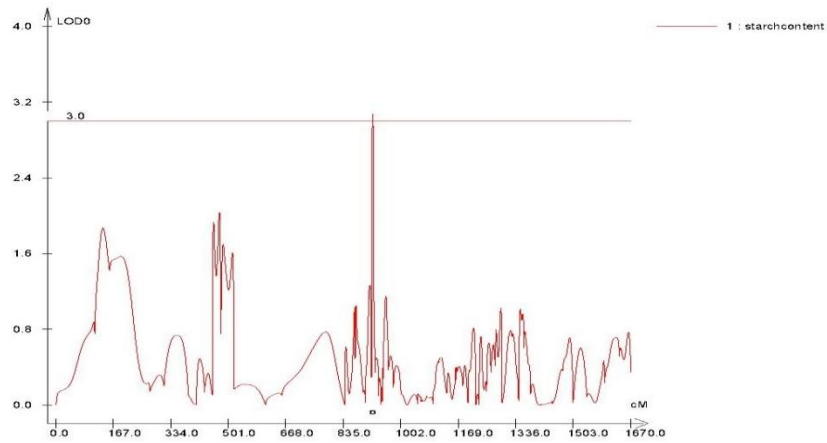**Fig.5a** Graphical representation of QTLs by SIM for starch content in chrom1

Fig.5b Graphical representation of QTLs by SIM of starch content in chrom2
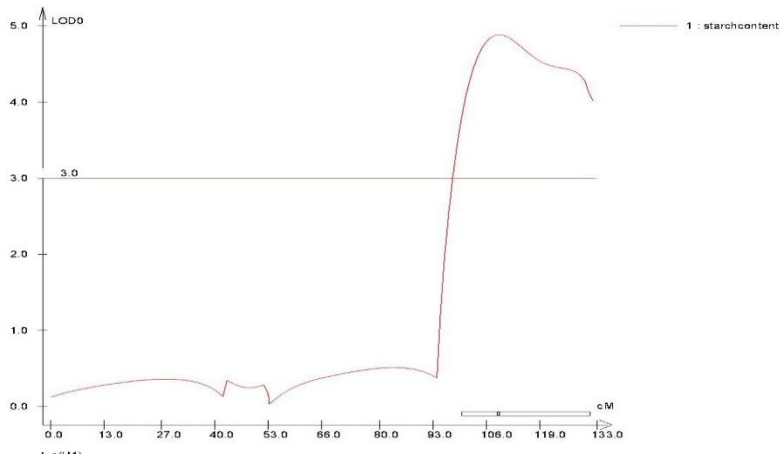

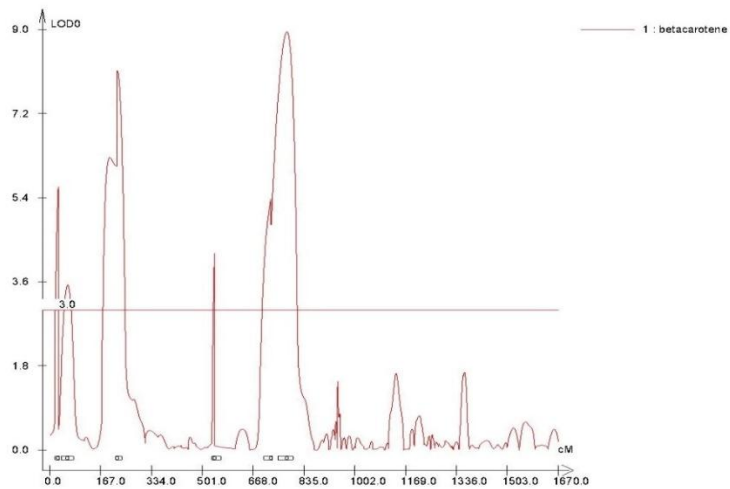**Fig.6** Graphical representation of QTLs by CIM for β-carotene in chrom1

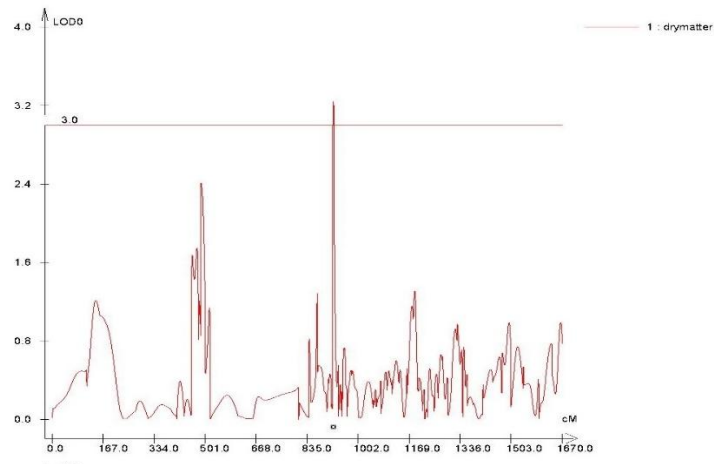**Fig.7a** Graphical representation of QTLs by CIM for dry matter content in chrom1

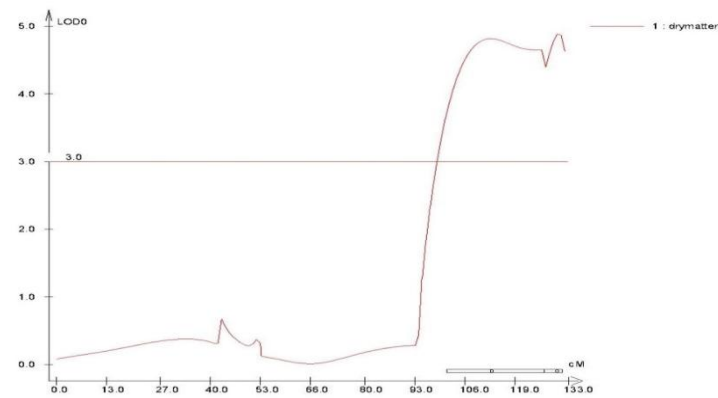**Fig.7b** Graphical representation of QTLs by CIM for dry matter content in chrom2

Fig.8a Graphical representation of QTLs by CIM for starch content in chrom1
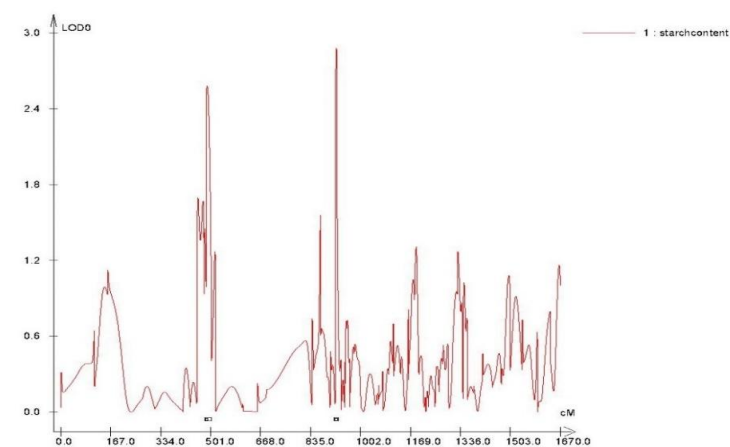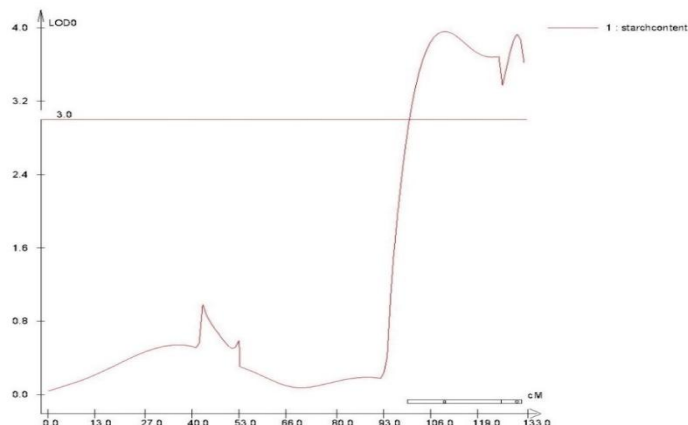
Fig.8b Graphical representation of QTLs by CIM for starch content in chrom2

Identification of QTL positions by CIM for starch content

The Table 5c generates information on the chromosome number, QTL, QTL position, LOD score, additive variance, phenotypic variance for all significant QTLs identified for the trait under study. Four significant QTLs were identified, two QTL on chrom1 and two QTLs on chrom2. The QTL1 was flanked by J116[a] and GDS0542[d] and QTL2 was flanked by J116[c] and J116[d]in chrom1, QTL3 and QTL4 were flanked by IBSSR04[e] and IBSSR04[d] in chrom2. An additional QTL in the chrom2 was identified in the CIM of the trait than the SIM. An additional QTL on chrom1 was present at about 920.5cMand was identified with a LOD value of 2.88. The QTL3 and QTL4 in chrom2is having positive additive effect of 1.11 and 1.38is explaining with 3.43% and 6.9% phenotypic variance in the trait (Fig. 8a and 8b).

The need for more detailed molecular genetic studies of sweet potato is great considering the significant potential that this crop has contributed greatly to the food security and nutritional needs of poverty stricken countries (Hagenimana and Low, 2000; Low *et al.*, 2001), as well as its potential use as an energy crop (Ganguli and Dean, 2003; Hall and Smittle, 1983).

Only five papers to date have reported on the construction of linkage maps in sweet potato, and the maps have been based on analysis of randomly amplified polymorphic DNA (RAPD), simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP) (Cervantes-Flores *et al.*,2008a; Kriegner *et al.*, 2003; Li *et al.*, 2010; Ukoskit and Thompson, 1997; Zhao *et al.*, 2013). Sweet potato has been characterized as an allo hexaploid (Jones, 1965; Magoon, 1970; Ting and Kehr, 1953; Sinha and Sharma, 1992). Shiotani and Kawase (1989) proposed the genome constitution of sweet potato as B1B1B2B2B2B2 based on the occurrence of frequent formation of tetravalents and hexavalents.

However, recent reports have suggested that sweet potato is an autohexaploid based the segregation ratio of molecular markers (RAPD, SSR, and AFLP) from a genetic linkage analysis (Cervantes-Flores *et al.*, 2008a; Kriegner *et al.*, 2003; Ukoskit and Thompson, 1997; Zhao *et al.*, 2013). In addition, these reports indicated that some preferential pairing occurs based on distorted segregation in some markers of different dosages in sweet potato. Thus, recent studies have supported the hypothesis that the genome structure of sweet potato is mainly autohexaploid with some preferential pairing.

All linkage maps previously constructed in sweet potato have utilized a two-way pseudo-testcross method employing F_1 progeny. With this method, two separate linkage maps for each parent are constructed based on the expected segregation ratios of markers in the mapping progeny. In hexaploid mapping progeny, several variations in segregation ratio have been detected based on allele dosage (simplex, duplex, or triplex) and affected by cytological characteristics (autohexaploid, tetradiploid, or allohexaploid) (Jones, 1967). Among the markers, only simplex markers show a simple segregation ratio (1:1) for any cytological characteristic, indicating their utility as molecular markers for linkage map construction. Previous studies have employed simplex markers to construct a framework map and then inserted duplex, triplex, and double simplex markers into the map (Cervantes-Flores *et al*., 2008a; Kriegner *et al*., 2003; Li *et al.*, 2010; Ukoskit and Thompson, 1997; Zhao *et al*., 2013). Thus, obtaining a number of simplex markers is important for linkage map construction.

In the present study, 69 SSR markers were found to be mapped into six different linkage groups and the rest of 69 SSR markers remained unlinked. The markers were randomly distributed on to the six linkage groups. The distance between the markers on the map also varied across the different linkage groups. The size of the linkage group did not necessarily reflect the number of linked markers indicating non-uniform distribution of markers. The linkage group 1 consists of 56 makers with a total map distance of 1670.3 cM. The average marker interval map distance is 29.82cM.The linkage group 2, with a total map distance of 132.6 cM had five mapped loci, whereas linkage group 3, 4, 5 and 6 was showing a distance of 49.0 cM, 19.1 cM, 44.2 cM and 41.9 cM respectively was covered by only two SSR markers. Kriegner *et al.,* (2003) reported632

'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.

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 to 149.1 centi Morgans (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 (Chang *et al*.,2009).

In sweet potatoes quantitative trait loci (QTL) are identified showing associations with dry matter, starch and β-carotene content. This was developed *via* mapping population generated from crosses between varieties with white-fleshed, high dry matter content and orange fleshed with low dry matter content. In the present study of parental maps constructed using a population of 208 progenies revealed the presence of six marker search associated to β-carotene, dry matter and starch content. Cervantes-Flores *et al.,* (2011) reported the parental maps constructed using a population of 240 clones revealed the presence of 13 QTLs for storage root dry matter content, 12 QTLs for starch content and eight QTLs for β-carotene content. Fourteen molecular

markers associated with root dry matter content of sweet potato were selected from 903 markers generated with four AFLP primers. The application of these markers revealed the phenotypic grouping without error (Labonte *et al.,* 2002). Fifty seven sweet potato genotypes with high dry matter content and resistant to sweet potato virus disease (SPVD) were characterized using four SSR markers. Mcharo and Labonte (2010) developed the molecular markers associated with β-carotene of sweet potato using multivariate selection of AFLP markers in F_1 half-sib population and their parents.

The genetic linkage maps and phenotypic data were imported into QTL Cartographer to identify significant QTL for the traits analyzed (Wang *et al.,* 2005). Each trait was run independently. The genetic association of the phenotypic data was analyzed using three different methods: single marker analysis (SMA), interval mapping (SIM) analysis, and composite interval mapping (CIM) analysis. Simulations have shown that comparing different analysis can be useful for determining robustness of the data. To establish a significance threshold for each QTL, the data was permuted 1000 times at a confidence level of alpha 0.05, and a walk speed of 1 cM. Only QTLs at or above the LOD threshold for a given trait are reported. The size of a given QTL was determined as the width at two LOD units below the QTL peak and neighboring QTL were required to be a minimum of 5 cM apart at their peaks. Single point, IM and CIM analysis yielded somewhat similar results, suggesting that the QTL observed are significant and real. The correspondence of the results obtained with single point ANOVA and QTL mapping methods may be related to the density of our maps. Low distance between the markers placed into the maps increases the power of single point analysis to locate significant QTLs with similar confidence as the more powerful QTL mapping methods (Doerge *et*

al., 1997). As a matter of fact, the higher power of interval analysis or more complex QTL analysis methods is better realized when the distance between markers is higher than 10 cM and this distance is uniform across the map.

For β-carotene trait, six and seven QTLs were identified in the simple interval mapping (SIM) and composite interval mapping (CIM) respectively. An additional QTL was identified in the CIM. For the traits dry matter and starch content a new QTL was identified in the chrom2 for CIM. From these results, concluded that CIM has identified QTLs with sharp peaks and some of the new QTLs were identified in CIM which were not identified in SIM.

The present study, revealed six QTLs having significant effects on the variation of trait, β-carotene content by simple interval mapping. Out of six QTLs, five QTLs were linked to chrom1 and one QTL to chrom2. Four QTLs showed positive effects on phenotypic variation and two QTLs had a negative effect on variation. In CIM, seven QTLs showed significant effect on the variation of trait, β-carotene content. Among them QTL3 observed a positive additive effect of 0.98 with a range of 87.5% of phenotypic variation. Cervantes-Flores *et al.,* (2011) reported QTL analysis of β-carotene content revealed the presence of eight QTLs having significant effects on the variation of the 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 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 coloured with only traces of β-carotene. 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 (Ducreux *et al.,* 2005; Ye *et al.,* 2000), has confirmed the presence of many of the genes needed for the production of β-carotene in genotypes showing no traceable carotenoids.

Two and three significant QTLs with flanking regions were identified in the interval and composite interval mapping for the trait dry matter content in the given mapping population.

The QTLs has positive and negative additive effects on the variation. In CIM, two QTLs (QTL2 and QTL3) were localized in a single flanking region having a positive additive effect with a 5.4 to 9.1% of phenotypic variation. Dry matter content was highly correlated with starch content and only slightly with β-carotene content. Similar results were also observed in Cervantes-Flores *et al.,* (2011). QTL analysis revealed the presence of 13 regions having significant effects on the variation of storage root drymatter content in both parental maps. In 'Beauregard' eight significant regions were identified. Four of the regions had a positive effect on dry matter content and were associated with markers E35M4511 (LG B05.26, P=0.0247), E32M3202 (LG B07.40, P=0.0098), E40M4010 (LG B11.61, P=0.0138) and E36M5408 (LG B89, 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 drymatter content. Based on the P levels observed and percent variation explained in storage root dry matter content, on average, the negative effects were stronger than the positive effects.

According to Chang *et al.,* (2013) interval mapping and multiple quantitative trait locus (QTL) model (MQM) analysis were used to identify QTLs for starch content with a mapping population consisting of 202 F_1 individuals of a cross between Xushu 18, a cultivar susceptible to stem nematodes, with high yield and moderate starch, and Xu 781, which is a resistant to stem nematodes, low yield and high starch content. Six QTLs for starch content were mapped on six linkage groups of the Xu 781 map, explaining 9.1-38.8% of the variation. All of the six QTLs had a positive effect on the variation of a starch content which indicated the inheritance derived from the parent Xu 781. Two QTLs for starch content were detected on two linkage groups of the Xushu 18 map, explaining 14.3 and 16.1% of the variation, respectively. They had a negative effect on the variation, indicating the inheritance derived from the parent Xu 781. Seven of eight QTLs were colocalized with a single marker which is the first report on the development of QTLs co localized with a single marker in sweet potato. In the present study, two QTLs for starch content were mapped in two linkage groups in interval mapping one with a negative effect on chrom1 and another with a positive effect on chrom2, explaining 0-3.43% of variation. In CIM, four QTLs were detected on two linkage groups with a negative effect of 0.65-2.28% of variation and a positive effect of 3.43-6.93% of variation, respectively. Three of four QTLs were colocalized with a single marker which is the second report on the development of QTLs colocalized with a single marker in sweet potato. These QTLs and their colocalized markers can be used in marker-assisted breeding for the starch content of the sweet potato.

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How to cite this article: