Revealing QTLs Associated with Fatty Acid Composition and Compactness on an Integrated Linkage Map Of Oil Palm Interspecific Backcross 2 (BC_2)

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Abstract

Background Molecular breeding has opened new avenues for crop improvement with the potential for faster progress. As oil palm is the major producer of vegetable oil in the world, its improvement, such as developing compact planting materials and improving its oil's fatty acid composition, is important. Results This study sought to identify the QTLs associated with fatty acid composition and vegetative traits for compactness in the crop. It integrated two separate interspecific backcross two (BC2) mapping populations to improve the genetic resolution and evaluate the consistency of the QTLs identified. A total of 1,963 markers (1,814 SNPs and 149 SSRs) spanning a total map length of 1793 cM was integrated into a consensus map. The QTL analysis observed 19 and 10 significant genomic loci associated with FAC and compactness, respectively. Conclusions A few genomic regions were revealed to be major loci influencing FAC and compactness. It is hoped that the QTLs identified will be useful tools for marker-assisted selection, accelerate the process of identifying desirable genotypes for breeding.

Background

Global palm oil production now stands at over 65 million tonnes/year, or 34% of the world's vegetable oil production [1,2]. The oil palm commonly planted commercially is the African origin (*Elaeis guineensis)*.. It is the most productive vegetable oil crop, with commercial oil yields of ~4 tonnes/ha/yr [3], but up to 13 tonnes/ha/yr have already been achieved in some breeding trials [4]. Although yield is the primary target, there is also need for disease resistance and tailored fatty acid composition (FAC) for the multivaried uses of the oil.

The oil palm, unfortunately has only a single growing point, and continually grows taller making it more and more difficult to harvest, until well-nigh impossible. Having shorter (dwarf) palms will extend its economic life, with ensuing lower (labour) cost of harvesting. The height increment of current commercial *Dura* x *Pisifera* palms is 40—75 cm/yr [5], and palms can reach 15–18 meters in height, up to 30 meters in a dense forest [6]. A breeding programme for dwarf palms was initiated by Elmina Estate in Malaysia with selfing of the short and famous Malayan Dumpy dura palm E206 [7]. More recently, MPOB, from its Nigerian prospection, identified Population 12 not only for its dwarfness, but also for its high bunch number, good yield, and desirable fruit characteristics [8]. These palms, when crossed with elite materials, are 5–10% shorter than the standard crosses [9]. In improving the palm, progress can be speeded up by the use of biotechnology, such as using molecular markers to screen for desired traits. Recently, quantitative trait loci (QTL) associated with trunk height and bunch weight identified using a linkage map containing 1,085 single nucleotide polymorphism (SNPs) [10]. A study [11], also constructed a consensus linkage map for a population of oil palm using simple sequence repeats (SSRs) and SNPs, and identified a major QTL for stem height on LG 5. In other study using association mapping, a SNP marker SNPG00006 *Fatl* observed to be significantly associated with height increment ($P < 0.05$) [12].

All the above work was carried out on *E. guineensis,* the African oil palm. There is, however, a second oil palm, *Elaeis oleifera*the American oil palm. Although not much commercially planted and produces very low yields, it has several interesting characteristics, such as shortness, less saturated oil and disease

resistance, which may be introgressed to improve *E. guineensis.* Interspecific hybrids of *E. guineensis* and *E. oleifera* have already been made (F1)—they are shorter, but the yield is still very low [13]. There is also a lack of pollen production (as demonstrated by pure *E. oleifera),,* and assisted pollination is even required to produce the low yield. Backcrossing to *E. guineensis* will quickly improve the yield, but just as quickly lose the desirable *E. oleifera* characteristics. In other words, the desire to improve *E. guineensis* by introgressing *E. oleifera* traits has largely come to nought in a painstakingly slow and costly process.

But that was in the past using conventional breeding. Now, with DNA-based markers there is promise of more efficient crop improvement by introgressing only the specific genes wanted, rather than half the whole genome of donor palms for some required genes in conventional hybridization. The availability of dense genetic maps for both *E. guineensis* and interspecific hybrid populations [14,15,16,17], as well as markers linked to important quantitative traits such as yield, vegetative measurement and FAC [10,15,18,19,20], have already provided the groundwork for this work. However, and interestingly, no QTL associated with height increment has yet been reported for the interspecific hybrid.

Compact palms with shorter trunks and fronds can be planted at a higher density than the current 148/ha. If the individual palm yields can be maintained, then the yield per unit area will increase [21,22]. In South America, *E. guineensis*-based compact palms [23] have already been developed by multiple rounds of backcrossing OxG hybrids to *E. guineensis [*24], and the outstanding palms cloned for planting [25]. In 2012, it has been reported that the development of an OxG hybrid, known as COMPACT palms, with low height increment (<40 cm/year) and shorter fronds (~6.5 meter), allowing high density planting (180–200 palms/ha) [26]. Backcrossing the COMPACT palms with Deli, Ghana, and Nigeria *E. guineensis* produced fronds of 6.6–6.9 meters which reduced the density to 170 palms/ha.

Interspecific hybrids and their backcrosses also have desirable FACs in their oils. The genomic regions associated with various FACs in an OxG interspecific hybrid [14,20] and interspecific backcross one (BC_1) [16] mapping populations were identified via conventional QTL analysis. A number of these QTLs were validated across interspecific backcross two (BC_2) mapping populations [20]. One of the major restrictions in associating markers to traits in oil palm is the size of the mapping populations employed in such studies. Oil palm breeding trials generally consist of 64 palms per progeny, which is small for effective genetic mapping and QTL analysis. However, a study had shown it possible to develop high quality integrated maps of multi parental populations, which can enhance QTL discovery [15]. This study searched for the QTLs associated with vegetative traits and FAC in two interspecific BC_2 mapping populations - characters important for developing compact palms with higher unsaturated mesocarp oil. It integrated two separates interspecific BC_2 mapping populations to enhance the genetic resolution and observe the consistency of the QTLs detected.

Results

Traits of Interest

The vegetative parameters, carotene content and FACs, observed in the 2.6–1 and 2.6–5 mapping families are summarized in Table 1. All the traits showed wide segregation and followed normal distribution ($P<0.05$, Shapiro-Wilk test) in the 2.6–5 population. However, for population 2.6–1, rachis length (RL), height increment (HI), iodine value (IV) and oleic acid (C18:1) were not normally distributed. The means for RL, petiole cross section (PCS) and IV were slightly higher in 2.6–1, but HI and carotene content were higher in 2.6–5. However, both RL and HI were considerably lower than in the commercial DxP*,* whereby, they are generally >5 and >0.45 meters, respectively (Noh et al., 2012). For FAC, population 2.6–1 had higher stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids, whereas palmitic (C16:0) was slightly higher (35.33%) in the 2.6–5 population.

The relationships between the individual fatty acids were evaluated using Pearson's correlation analysis and consistent results were obtained for both mapping families (Tables 2 and 3). The most abundant saturated fatty acid, C16:0, was negatively correlated with the unsaturated fatty acids (C18:1 and C18:2). A negative correlation was also observed between C18:0 and C16:0. As expected, IV, as an indicator for unsaturation, was positively correlated with C18:1 and C18:2, and negatively with C16:0 and C18:0. In addition, correlations were positive between C18:0 and C18:2 and negative between C18:1 and C18:2. The results corroborated with those from other studies [14,16,20]. Correlation trends for vegetative parameters were similar in both populations. PCS was positively correlated with HI and RL, while RL and HI were negatively correlated.

Candidate genes identified within the QTL intervals

Candidate genes residing within the QTL interval were identified using the existing oil palm genome assembly [27]. Blast results to the genome build identified 22 candidate genes within the QTL confidence intervals affecting the vegetative traits and FAC. For HI, QTL region LG7 revealed an interesting gene with high similarity to the auxin transport protein *BIG [*GenBank: XM_010943964.2]in oil palm. In addition, we identified *BAM1 [*GenBank: XM_010914345.2]which co-localized with markers in the QTL region associated with RL on LG11. The *ERECTA* gene [GenBank: XM_010910431.1] was found in the QTL interval linked to PCS on LG4. For FAC, two 3-ketoacyl-CoA synthase genes in Arabidopsis, *CUT1* [GenBank: XM_010917870.2] and *KCS11* [GenBank: XM_010916640.2] flanked the QTLs for IV, C16:0 and C18:2 on LG1. For C18:1, the QTL regions on LG1 and LG6 contained an interesting gene with high similarity to *PAH2 [*GenBank: XM_010918326.2; XM_010918327.2; XM_010918329.2; XM_010928080.2; XM_010928081.2; XM_010928083.2; XM_010928084.2; XM_019852014.1; XM_019852015.1]. Details of all 22 genes identified are provided in [see Additional file 2].

Discussion

The traits analyzed in this study - HI, RL, PCS, C16:0, C18:0, C18:1, C18:2, IV and carotene content - are generally highly heritable in oil palm [14,28,29], indicating that they are amenable to selection either via conventional or molecular breeding. This makes the traits attractive for QTL analysis. Four of them (RL, HI, IV and C18:1) in the 2.6–1 mapping family did not follow a normal distribution, but, as reported by [30], deviation from normal distribution did not appear to greatly affect QTL detection. As expected, all the trait

values were intermediate between the means for *E. oleifera* and *E. guineensis,* similar to the observations by other studies [16,31]. The wide distribution for all traits measured, suggests that both BC2 populations are ideal for QTL mapping and so for selection and improvement in oil palm. RL and HI in both populations are considerably lower than in commercial DxP as reported by [32], suggesting that these populations can be used for the development of compact palms.

The genetic linkage maps for the two BC2 populations was successfully integrated, improving the resolution of the combined map. The number of palms in the individual populations used in this study was relatively small compared to in other crops. However, such small family sizes are common in oil palm trials with >64 palms being rare. Although the study focused on highly heritable traits, the small population size could have led to an underestimation of the QTL numbers and restricted the QTL analysis to only the most prominent effects. [33] found that the number of QTLs detected increased with population size. To obviate this limitation, the map resolution and hence QTL detection power, were improved by integrating the two BC2 maps. Other factors, such as the phenotypic measurement accuracy and marker density, also contributed to the QTL detection and localization [34].

Development of an ordered set of markers along the oil palm chromosome allows the genome to be screened systematically for linkages to complex traits. SNP markers employed in this study resulted in better genome coverage, increasing the potential for realization of effective marker-assisted selection (MAS). A total of 1,963 polymorphic loci (1,814 SNPs and 149 SSRs) generated 16 LGs, which is consistent with the 16 chromosome-pairs in oil palm [35]. The genome length observed (1,793 cM) was close to that reported by [14,17] of 1,815—1,867 cM for *E. guineensis.* The average length of the LGs is 112 cM, which is in the range of most agricultural crops [36]. Resolution of the two individual genetic maps was good with an average gap of 0.86 cM (2.6–1) and 1.32 cM (2.6–5). An average interval of 0.91 cM was observed on the BC2 integrated map. This gap was much smaller than those previously reported on oil palm interspecific hybrids of 1.2 - 7.2 cM [14,16,17]. The density of both BC2 genetic maps allowed the genomic segments associated with compactness and FAC traits to be identified. Consistency of the QTLs detected in the independent and integrated maps [see Additional file 3] adds confidence to their detection.

The interspecific hybrid breeding programme in Malaysia aims to develop palms with higher unsaturated oil and compact characteristics, without sacrificing yield. Applying MAS can accelerate the programme as markers can be linked to selected traits to enable early detection. A high logarithm of the odds ratio (LOD) score will provide confidence for integrating the markers in breeding lines, at least in palms of similar genetic backgrounds. In the QTL analysis of vegetative traits, interestingly, the QTLs associated with RL, PCS and HI were located at the same genomic region on LG4, likely representing a major locus influencing compactness in oil palm. Similarly, QTLs for RL and PCS were also located in close proximity on LG8, revealing another major locus for compactness. To date, there are no QTL analysis of vegetative traits in interspecific hybrids. The QTLs detected in this study were compared to those described previously [15], for a segregating *E. guineensis* population. However, most of the QTLs detected in the current BC2 populations were located on different chromosomes compared with those reported by [15], with the exception of those associated with RL which was on LG11. This suggests that separate genomic regions influence compactness in *E. guineensis* and the interspecific hybrids. A previous study [18] reported two QTLs related

to RL and PCS in *E. guineensis.* However, a comparison between similarity of the linkage groups could not be made as the sequence information for restriction fragment length polymorphism (RFLP) markers linked to the traits in the study were not publicly available for further analysis. More specifically on the HI, recent reports revealed QTLs and candidate genes influencing it in *E. guineensis* [10,11]. However, the genomic region linked to HI in this study was different from these two reports.

A total of 13 QTLs were detected for IV, C16:0, C18:0, C18:1 and C18:2 in seven LGs. The results are similar to those of [16,20] on interspecific hybrids, with 19 and 12 QTLs found, respectively. Five of the identified QTLs in this study were similar to those in both reports. The QTL on LG4 for IV on LG15 is in agreement with that by [16]. The major QTLs for IV, C18:1 and C16:0 on LG1 were previously reported for an interspecific hybrid family [20] which shows their potential to be used for making informed decisions in breeding. The results support a previous postulation that the same genomic region has a major influence on the unsaturation (IV) and saturation (C16:0) of palm oil. The QTLs for IV, C18:0 and C18:2, were also located around the same region on LG4, revealing another major locus influencing fatty acid composition. IV was not significantly correlated with C18:0 (at $P<0.05$) in both mapping families, but its QTL overlapped that for C18:0 in LG4. The QTL for C18:0 on LG4 is similar to that reported by [20]. Overlapping of the QTLs map position for unrelated traits could be due to pleiotropic effects [37]. Since similar work for carotene content in oil palm has not been reported, comparison with other research could not be made.

Availability of the oil palm genome sequence [27] has allowed the underlying QTL interval to be positioned on the EG5 physical map to identify potential candidate genes influencing the traits of interest. The auxin transport protein, *BIG,* related to HI, is required for auxin efflux and polar auxin transport (PAT) and could influence auxin-mediated developmental responses (e.g. cell elongation, apical dominance, lateral root production, inflorescence architecture, general growth and development) [38]. *BIG* controls elongation of the pedicel and stem internodes through auxin action. In Arabidopsis, *BIG* also plays a role in the regulation of responses to phytohormones, such as auxin, cytokinins, ethylene and gibberellic acid (GA), particularly during light-mediated stimuli (e.g. shade avoidance and etiolation) [39,40]. *BAM1,* in association with RL, encodes a leucine-rich repeat receptor-like serine/threonine-protein kinase which is involved in cell-cell communication during early anther development, and regulates cell division and differentiation, such as in the formation of shape, size and symmetry of leaves [41]. *ERECTA,* linked to PCS on LG4, regulates aerial architecture (including inflorescence), e.g. shoot apical meristem-originating organ shape, elongation of internodes and pedicels, and adaxial-abaxial polarity, and stomatal patterning, probably by tuning cell division and expansion [42].

In terms of FAC, the *CUT1*and *KCS11* genesare associated with the QTLs for IV, C16:0 and C18:2 on LG1. *CUT1* is required for elongation of C24 fatty acid, an essential step in cuticular wax production [43]. This wax is composed of long-chain, aliphatic hydrocarbons derived from very-long-chain fatty acids (VLCFAs). For *KCS11,* this gene is active on both saturated and mono-unsaturated acyl chains C16 to C20 [44]. It is involved in the pathway of fatty acid biosynthesis, which is part of lipid metabolism. The magnesium-dependent phosphatidate phosphatase gene, *PAH2,*related to C18:1, is involved in catalyzing the dephosphorylation of phosphatidate to yield diacylglycerol and may function indirectly as a repressor of multiple enzymes involved in phospholipid biosynthesis. However, at this stage, their involvement and

influences in controlling compactness and FAC of oil palm are still speculative. Further studies are necessary to characterize these genes to determine their functions in regulating the traits in oil palm.

Conclusions

CONCLUSION

The oil palm planted area in Malaysia has expanded rapidly in the last few decades largely by assuming the land for other crops, such as rubber and coconut, and secondary forest [45]. However, there is now limited land for further expansion. Thus, improving productivity is the only way. Developing compact palms is a step in that direction, as it can prolong the economic lifespan of the palms, and allow higher planting density to increase the yield per area. Lower HI, shorter RL and smaller PCS are preferred since more nutrients can be channeled into FFB production instead of vegetative growth [32]. In fact, compact palms at a density of 180/ha are being touted [46] for a possible 20% increase in yield. Breeding for compact palms can be accelerated using MAS, as QTLs can allow quick identification of the traits of interest and early selection of the desired genotypes.

In addition, reducing saturated and increasing unsaturated FAs will open up the prospects for oil palm to compete more effectively with other oil crops, such as soybean, rapeseed and sunflower, in the liquid oil sector [47]. There are already efforts to alter the FAC of palm oil both through traditional breeding [48] and genetic engineering [49], but these approaches are still very much in their infancy. Identifying the QTLs associated with FAC and the resulting candidate genes, can prove useful for selection or genetic manipulation in the quest.

Methods

Mapping Populations

The two independent mapping families used in this study were interspecific hybrid backcross two (BC_2) populations referred to as '2.6–1' and '2.6–5', consisting of 74 and 80 palms, respectively. The two populations were derived by crossing a common BC_1 interspecific hybrid as male parent (335/5.2–5/23.96) with two different *E. guineensis* palms. For Population 2.6–1, the maternal palm was a Nigerian tenera (T128) crossed with a Serdang pisifera. For Population 2.6–5, the female parent was a self of T128. The genesis of the two mapping populations was illustrated by [20]. Both BC_2 mapping populations and their parental are in-situ planted in United Plantations Berhad (UPB), Teluk Intan. Spear leaves were harvested from each palm including the parental palms for DNA extraction. UPB maintains the palms at their facilities in Teluk Intan, Perak and collected the matured oil palm bunches for fatty acid analysis.

Vegetative Measurements and Fatty Acid Composition

The vegetative traits were measured non-destructively [50]. Among the measurements were rachis length (RL) and petiole cross-section (PCS) area, both on Frond 17 following the standard procedure. Height increment (HI) was measured from ground to the base of Frond 41. The difference in height between two

years of measurement is the increment, to be divided by the number of years to obtain the annual increment. FAC of the mesocarp oil was determined using PORIM Test Method [51].

Development of linkage map and QTL analysis

SNP and SSR data were analysed as described previously [20,52,53]. A total of 4,451 SNPs and 40 candidate SNPs flanking various fatty acid and oil biosynthesis related genes were genotyped using the Illumina Infinium assay and iPLEX, respectively. A genetic linkage map was first constructed separately for each population using JoinMap 4.1 [54]. Subsequently, the two maps were integrated, and QTL analysis carried out using Genstat 18th edition (VSN International).

Phenotyping and Marker Sequence Similarity Search

Using SAS version 9.3, the t-test and Duncan analyses were carried out to compare the phenotypic values of the different genotypes. For comparison, palms were grouped according to their genotypes and phenotypic values averaged for each genotype. The sequences of markers flanking all QTLs identified were extracted from the oil palm genome build (EG5) [27] and searched for sequence similarity (BLASTN and BLASTX) against the NCBI databases. Sequences with significant similarity (BLASTN e-value of < 1e–25 and 90 % identity over total sequence length) to genes of interest were shortlisted for further analysis. Putative functions of the selected genes were derived from *UniProt,* a freely accessible database of protein sequence and functional information, and literature.

Abbreviations

1. Interspecific backcross two (BC2)
2. Fatty acid composition (FAC)
3. Quantitative trait loci (QTL)
4. Interspecific backcross one (BC1)
5. Rachis length (RL)
6. Height increment (HI)
7. Iodine value (IV)
8. Petiole cross section (PCS)
9. Oleic acid (C18:1)
10. Stearic acid (C18:0)
11. Linoleic acid (C18:2)
12. Palmitic acid (C16:0)
13. Linkage group (LG)
14. Single nucleotide polymorphism (SNP)
15. Simple sequence repeat (SSR)

16. Marker-assisted selection (MAS)
17. Logarithm of the odds ratio (LOD)
18. Oil palm genome build (EG5)
19. Restriction fragment length polymorphism (RFLP)
20. Polar auxin transport (PAT)
21. Gibberellic acid (GA)
22. Very-long-chain fatty acids (VLCFAs).

Tables

Table 1: Summary of vegetative traits and fatty acid composition (FAC) in the 2.6-1 and 2.6-5 BC_2 mapping populations

Population		2.6-1					2.6-5				
Category	**Variable**	**Mean**	**SD**	**CV**	**N**	**Range**	**Mean**	**SD**	**CV**	**N**	**Range**
Vegetative	RL (m)	5.28	0.67	12.78	72	2.45 – 6.80	5.17	0.61	11.75	69	3.53 – 6.56
	HI (m)	0.34	0.07	19.82	72	0.21 – 0.60	0.38	0.06	16.18	69	0.27 – 0.53
	PCS (cm^2)	26.62	5.58	20.95	72	10.80 – 40.85	26.49	5.20	19.61	69	15.68 – 40.50
FAC	Carotene (ppm)	1031	343	33.27	54	221-1965	1162	468	40.27	58	441 – 2738
	IV	65.13	2.55	3.92	54	60.19 – 69.86	63.40	3.22	5.07	58	57.12 – 71.60
	C16:0 (%)	31.06	2.47	7.94	54	24.73 – 36.68	35.33	2.85	8.06	58	26.85 – 41.69
	C18:0 (%)	6.15	1.31	21.38	54	3.29 – 9.43	3.79	0.92	24.30	58	2.11 – 6.48
	C18:1 (%)	48.61	2.87	5.91	54	40.92 – 57.18	47.02	3.67	7.80	58	37.58 – 54.48
	C18:2 (%)	12.86	1.41	10.95	54	9.60 – 16.29	12.67	2.05	16.16	58	8.15 – 17.65

Table 2: Pearson’s correlations between individual fatty acids in the 2.6-1 and 2.6-5 mapping populations

Population	2.6-1				2.6-5			
	C18:0	C18:1	C18:2	IV	C18:0	C18:1	C18:2	IV
C16:0	-0.26	-0.74*	-0.03	-0.75*	-0.32*	-0.71*	-0.04	-0.71*
C18:0		-0.24	0.03	-0.22		-0.16	0.26*	-0.18
C18:1			-0.48*	0.51*			-0.62*	0.20
C18:2				0.50*				0.55*

*significant at $p \leq 0.05$

Table 3: Pearson's correlations between vegetative parameters in the 2.6-1 and 2.6-5 mapping populations

Population	2.6-1		2.6-5	
	RL	HI	RL	HI
HI	-0.21		-0.08	
PCS	0.45*	0.10	0.28*	0.48*

*significant at $p \leq 0.05$

Table 4: Distribution of markers on the 16 linkage groups (LG) of the BC_2 genetic map

Population	2.6-1		2.6-5		Integrated	
LG	Map Length (cM)	No. Markers	Map Length (cM)	No. Markers	Map Length (cM)	No. Markers
1	115	121	88+23*	95+9*	138	146
2	101	87	97	62	113	107
3	55	48	58	42	57	54
4	174	178	212	117	195	202
5	25.2 + 14.8*	21+17*	53	22	100	44
6	106	129	126	82	122	135
7	78	173	85	117	113	189
8	122	132	175	97	152	154
9	82	56	37	15	83	157
10	88	129	93	93	116	138
11	128	166	133	125	145	179
12	108	130	124	91	124	144
13	77	127	57	96	80	135
14	98	77	88	69	99	94
15	69	119	52 + 46*	79+10*	98	142
16	50	34	63	33	58	43
Total	1505	1744	1564	1254	1793	1963

* Sub-groups

Table 5: QTLs associated with compactness traits and FAC identified on the interspecific BC_2 integrated map	BC_2 Integrated Map			
HI	Closest Marker	LG	Position (cM)	LOD
	SNPM00563	4	4.27	7.77
	SNPM04201	7	79.90	3.03
	SNPM04928	7	110.78	3.83
6.6RL	SNPM03201	4	11.30	3.79
	SNPM03772	8	92.56	3.17
	SNPM03676	11	38.69	4.17
PCS	SNPM00563	4	4.27	7.70
	sMg00027	4	52.30	3.15
	SNPM03375	4	192.47	3.25
	sEg00213	8	139.86	3.81
C16:0	SNPM00796	1	133.4	3.59
C18:0	SNPM00563	4	4.27	3.18
C18:1	SNPM03299	1	74.43	4.08
	sMo00040	6	0.00	3.85
	SNPM00274	12	31.38	5.39
C18:2	SNPM01602	1	124.87	4.62
	SNPM00249	4	3.43	8.19
	SNPM01190	15	70.32	5.14
IV	SNPM01452	1	132.53	13.02
	SNPM04197	3	15.78	3.72
	SNPM01114	4	0.0	3.12
	SNPM03285	15	98.11	5.98
	SNPM02704	16	0.0	5.03
Carotene	SNPM03595	2	102.45	3.20
	SNPM02349	3	4.35	5.12
	SNPM00729	4	181.83	3.32
	SNPM03960	7	108.53	3.73
	SNPM01230	10	1.26	3.09

SNPM03921	10	77.39	3.63	

Table 6: Correlation between segregation of markers linked to QTLs (highest LOD) and specific traits

No.	Trait	Marker	LG	Genotype	N	Mean ± SE
1	C16:0	SNPM00796	1	aa	89	33.81c ±0.37
				ab	23	31.20d ±0.37
2	C18:0	SNPM00563	4	aa	96	4.64d ±0.15
				ab	16	6.67c ±0.36
3	C18:1	SNPM00274	12	aa	34	47.24c±0.61
				ab	64	48.10c ±0.43
				bb	11	47.19c ±0.87
4	C18:2	SNPM00249	4	aa	43	13.17c ±0.27
				ab	69	12.51d ±0.21
5	IV	SNPM01452	1	aa	62	62.94d ±0.31
				ab	44	65.64c ±0.45
				bb	6	67.33c ±0.81
6	Carotene	SNPM02349	3	aa	38	1321.57c ±78.76
				ab	50	1009.57d ±46.83
				bb	23	924.46d ±61.44
7	RL	SNPM00323	11	aa	29	4.90c ±0.78
				ab	78	5.45d ±0.49
				bb	33	4.99d ±0.65
8	HI	SNPM00971	4	aa	47	0.33d ± 0.06
				ab	82	0.36d ± 0.06
				bb	12	0.45c ± 0.07
9	PCS	SNPM00563	4	aa	109	25.67d ±0.50
				ab	32	29.57c ±0.89

Means of the phenotypes were compared using the independent ttest for a marker with two genotypes, and Duncan's test for markers with three genotypes (SAS 9.3 statistical package). Means of the different genotypes (markers) associated with each trait were significantly different (indicated by alphabets c and d) at $P<0.05$.

Declarations

Ethics approval and consent to participate

Not Applicable

Consent for publication

Not Applicable

Availability of data and material

All data generated or analysed during this study are included in this published article [and its supplementary information files]

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

ZY, RS, KS and RN conceived and designed the experiments. ZY, KK, and JJ performed and analyzed the experiments. ZY and RS thoroughly interpreted the data and revised the manuscript for intellectual content. TNC, MM, MDA, LETL, OLCL and MOA coordinated the project and participated in the direction of the study. ZY, KK, SM and RS wrote the manuscript. All authors discussed the results and commented on the manuscript.

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Figures

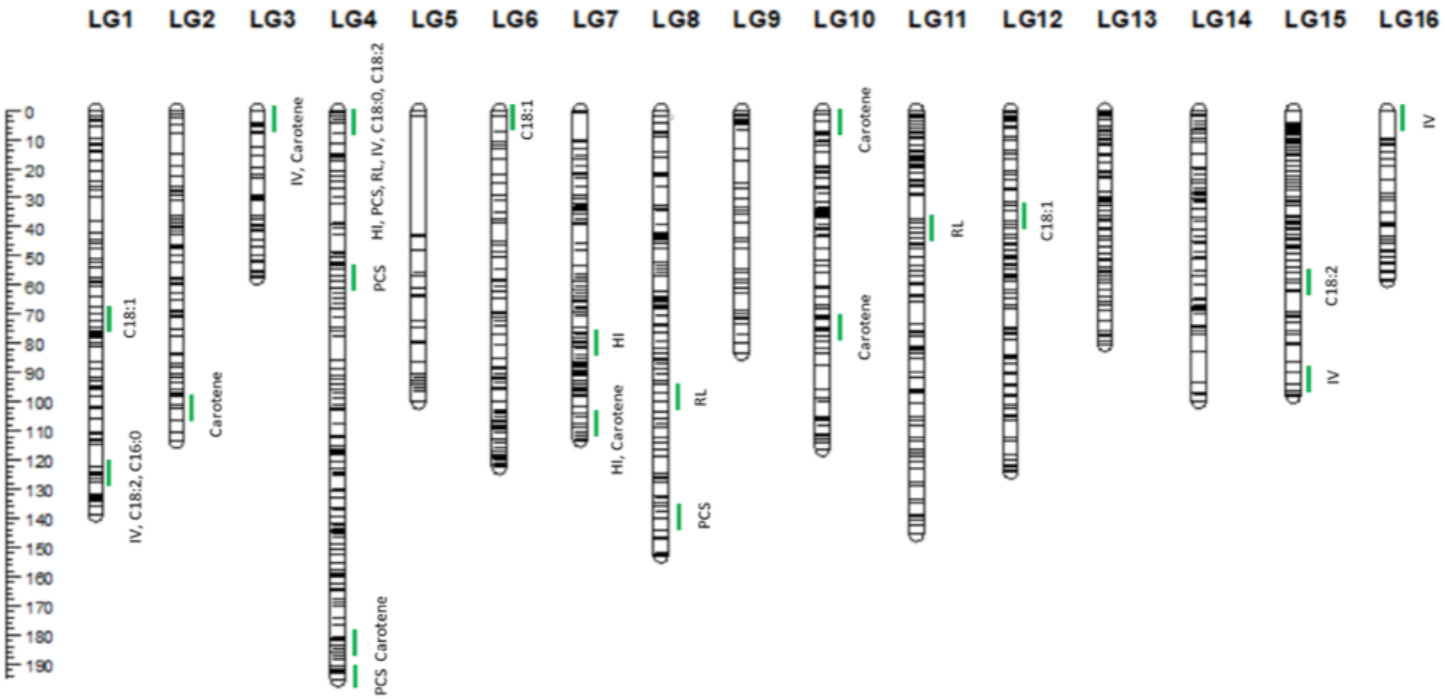

Figure 1

Integrated linkage groups 1-16 of BC2 populations and distribution of QTLs associated with compactness traits and FAC

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