



LINKAGE GROUP ASSEMBLY AND ORDERING AS A SOURCE OF SPURIOUS QTL ESTIMATES IN MANGO

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ABSTRACT

Objective: This study aimed to align genetic linkage groups (LGs) with the mango physical map by comparing single nucleotide polymorphism (SNP) sequences in the 'Alphonso' mango genome.

Theoretical Framework: Few studies have evaluated the impact of linkage group (LG) formation and marker ordering on quantitative trait loci (QTL) estimates for marker-assisted selection (MAS).

Method: SNPs from the mango linkage groups (n=20) were aligned on the chromosomes of the genome sequence of the 'Alphonso' mango (n=20), evaluating the correspondence between the genetic linkage map and the physical map.

Results and Discussion: Four major aberrations were identified: (a) chromosome 1 was divided into LGs 9 and 14; (b) chromosome 4 was split between LGs 1 and 3; (c) LG 8 was formed by combining SNPs from chromosomes 9 and 17 of 'Alphonso'; and (d) chromosome 18 was not represented in any of the 20 LGs. All remaining LGs, except LGs 11, 14 and 20, contained at least one SNP from non-corresponding reference chromosomes. Significant discrepancies in SNP ordering were observed across the 20 LGs, particularly in 12 LGs.

Research Implications: For species with available genome sequences, physical maps, created through SNP sequence Blast alignment, yield better results for quantitative trait mapping aimed at marker-assisted selection.

Originality/Value: QTLs utilization in MAS remains limited. Insufficient attention has been given to limitations in linkage group formation and marker ordering compared to physical mapping as sources of spurious QTL estimates.

Keywords: *Mangifera Indica*, QTL, SNPs.

FORMAÇÃO E ORDENAÇÃO DO GRUPO DE LIGAÇÃO COMO FONTE DE ESTIMATIVAS ESPÚRIAS DE QTL EM MANGA

RESUMO

Objetivo: Este estudo teve como objetivo alinhar grupos de ligação genética (LGs) com mapa físico da manga comparando sequências de polimorfismos de nucleotídeo único (SNP) no genoma da manga 'Alphonso'.

Referencial Teórico: Poucos estudos avaliaram o impacto da formação do grupo de ligação (LG) e ordenação dos marcadores nas estimativas de 'loci' de características quantitativas (QTL) para seleção assistida por marcadores (MAS).

Método: SNPs dos grupos de ligação da manga (n=20) foram alinhados nos cromossomos do sequenciamento genômico da manga 'Alphonso' (n=20), avaliando a correspondência entre mapa de ligação genética e mapa físico.

Resultados e Discussão: Quatro aberrações foram identificadas: (a) cromossomo 1 foi dividido nos LGs 9 e 14; (b) cromossomo 4 foi dividido entre os LGs 1 e 3; (c) LG 8 foi formado pela combinação de SNPs dos

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cromossomos 9 e 17 de "Alphonso"; e (d) cromossomo 18 não estava representado em nenhum dos 20 LGs. Todos os LGs restantes, exceto os LGs 11, 14 e 20, continham pelo menos um SNP de cromossomos de referência não correspondentes. Discrepâncias significativas na ordenação dos SNPs foram observadas nos 20 LGs, particularmente em 12 LGs.

Implicações da Pesquisa: Para espécies com sequências genômicas disponíveis, mapas físicos, criados por meio do alinhamento de sequência de SNP, produzem melhores resultados para mapeamento de QTLs.

Originalidade/Valor: A utilização de QTLs em MAS permanece limitada. Esse trabalho enfatiza as limitações de LG e ordenação de marcadores, em comparação ao mapeamento físico, como fontes de estimativas espúrias de QTLs.

Palavras-chave: *Mangifera Indica*, QTL, SNPs.

ENSAMBLAJE Y ORDENAMIENTO DE GRUPOS DE ENLACE COMO FUENTE DE ESTIMACIONES DE QTL ESPURIAS EN MANGO

RESUMEN

Objetivo: Este estudio buscó alinear los grupos de ligamiento genético (GL) con el mapa físico del mango mediante la comparación de secuencias de polimorfismos de un solo nucleótido (SNP) en el genoma del mango 'Alphonso'.

Marco teórico: Pocos estudios han evaluado el impacto de la formación de grupos de ligamiento (GL) y el ordenamiento de marcadores en las estimaciones de loci de caracteres cuantitativos (QTL) para la selección asistida por marcadores (SAM).

Método: Los SNP de los grupos de ligamiento del mango (n=20) se alinearon en los cromosomas de la secuencia genómica del mango 'Alphonso' (n=20), evaluando la correspondencia entre el mapa de ligamiento genético y el mapa físico.

Resultados y discusión: Se identificaron cuatro aberraciones principales: (a) el cromosoma 1 se dividió en los GL 9 y 14; (b) el cromosoma 4 se dividió entre los GL 1 y 3; (c) el GL 8 se formó mediante la combinación de SNP de los cromosomas 9 y 17 de 'Alphonso'. y (d) el cromosoma 18 no estaba representado en ninguno de los 20 LG. Todos los LG restantes, excepto los LG 11, 14 y 20, contenían al menos un SNP de cromosomas de referencia no correspondientes. Se observaron discrepancias significativas en el ordenamiento de los SNP en los 20 LG, particularmente en 12 LG.

Implicaciones de la investigación: Para las especies con secuencias genómicas disponibles, los mapas físicos, creados mediante el alineamiento Blast de secuencias de SNP, producen mejores resultados para el mapeo cuantitativo de rasgos dirigido a la selección asistida por marcadores.

Originalidad/Valor: El uso de QTL en la selección asistida por marcadores (MAS) sigue siendo limitado. Se ha prestado poca atención a las limitaciones en la formación de grupos de ligamiento y el ordenamiento de marcadores en comparación con el mapeo físico como fuentes de estimaciones falsas de QTL.

Palabras clave: *Mangifera Indica*, QTL, SNP.

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1 INTRODUCTION

Most economically important traits in crops, livestock, and humans are quantitative (i.e., polygenic), including height, weight, disease resistance, and yield (Kaur et al. 2023). Such traits pose significant challenges for marker-assisted selection (MAS) implementation due to their genetic complexity. In contrast, traits governed by single genes or simple inheritance patterns (e.g., the male sterility 'S' locus in onion) allow more straightforward and reliable MAS applications.

According to Kaur et al. (2023), although numerous quantitative trait loci (QTL) associated with key agronomic traits have been mapped across various crops, their direct utilization in MAS remains limited. According to Xu and Crouch (2008), the annual number of publications containing the term "marker-assisted selection" has consistently been threefold lower than those referencing "quantitative trait locus/loci," with the disparity continuing to widen.

The limitations in applying QTLs to MAS stem from small population sample sizes, genotyping and phenotyping errors, and insufficient marker coverage (resulting in linkage maps with large gaps) (Miles and Wayne 2008, Xu and Crouch 2008, Kaur et al. 2023). Insufficient attention has been given to proper marker assembly and ordering in pseudo-chromosomes or linkage maps, which are crucial for robust QTL estimation. Medeiros et al. (2022) identified linkage groups containing segments from multiple chromosomes when comparing genetic and physical maps of the guava genome, demonstrating how improper linkage group assembly and marker ordering can lead to spurious QTL estimates.

Kuhn et al. (2017) noted that genetic and genomic resources for mango (*Mangifera indica*) remain limited and have not significantly advanced global mango breeding efforts. Luo et al. (2016) reviewed the four mango genetic maps available by 2016: 1) the first map constructed using 197 RFLP and 650 AFLP markers; 2) the second using 34 AFLP markers; 3) the third using 81 AFLP markers; and 4) a partial linkage map using 9 microsatellite and 67 RFLP markers. Subsequently, Luo et al. (2016) developed a high-density genetic map using 6,594 SLAF-seq markers, while Kuhn et al. (2017) published a linkage map with 888 SNPs across seven mapping populations, describing it as "a tool for mango breeding".

From this perspective, the present study aimed to evaluate the reliability of the Kuhn linkage map for QTL estimation by comparing SNPs from the mango linkage groups ($n = 20$) developed by Kuhn et al. (2017) with the same SNP set aligned against the chromosome-scale assembly of mango 'Alphonso' ($n = 20$) published by Wang et al. (2020). This comparison was



performed using BLAST analysis to assess the correspondence between genetic and physical maps.

2 THEORETICAL FRAMEWORK

Quantitative trait locus (QTL) analysis is a statistical method that integrates phenotypic data (trait measurements) with genotypic data (typically molecular markers) to elucidate the genetic basis of variation in complex traits (Miles and Wayne 2008). QTL analysis is expected to facilitate: (1) precise map-based cloning of alleles/genes, (2) comparative studies of trait evolution across species, and (3) marker-assisted selection (MAS) (Semagn et al. 2006), the latter being the primary focus of this study. The QTL mapping process involves developing a segregating mapping population for the target trait, constructing a linkage map, and performing QTL analysis - often using interval mapping methods - to identify genomic regions associated with the trait of interest (Semagn et al. 2006).

Two most important mapping programs have been applied for linkage groups building, adopting different strategies and algorithms:

1. OneMap ((Margarido et al. 2007) implements a maximum likelihood-based algorithm that simultaneously estimates linkage and linkage phases for diverse marker types, including fully informative markers (segregating 1:1:1:1) and partially informative markers (segregating 1:2:1, 3:1, and 1:1) in full-sib families from outbred parent plants (Wu et al. 2002). Wu et al. (2002) identified 18 possible cross combinations based on parental genotypes,
2. JoinMap4 (Van Ooijen 2006) implements a Monte Carlo multipoint maximum likelihood algorithm (Van Ooijen, 2011) capable of simultaneously estimating recombination frequencies and determining marker order based on five segregation types. This methodology was implemented in JoinMap4.1, utilizing a simulated annealing ordering algorithm that optimizes marker arrangement by minimizing the sum of recombination frequencies between adjacent segments.



3 METHODOLOGY

3.1 MANGO LINKAGE MAPS AND SNPS SEQUENCES

The linkage groups developed by Kuhn et al. (2017) served as the primary analytical framework. According to their methodology, the assembly and alignment of the mango genome were performed using two mapping programs - JoinMap4 (Van Ooijen 2006) and OneMap (Margarido et al. 2007) - applied to each of the seven mapping populations. The authors employed these complementary programs as follows:

- 1) OneMap was used to establish 20 linkage groups through recursive analysis, progressively increasing the LOD threshold (in increments of 0.1) until achieving 20 linkage groups (LGs) with a minimum of 10 markers per LG. This process was conducted separately for all seven mapping populations (Kuhn et al. 2017);
- 2) The resulting OneMap LGs provided the initial marker groupings for JoinMap4 analysis. JoinMap4 calculations employed default parameters for population grouping and Maximum Likelihood (ML) mapping. The program's Strongest Cross Link (SCL) function incorporated ungrouped markers with SCL values ≥ 5.0 into appropriate groups. The most informative map, derived from the TA \times KP population, served as the template for other population maps in JoinMap4, which were subsequently merged using the software's integration functions to generate the consensus map (Kuhn et al. 2017).

The SNP markers analyzed by Kuhn et al. (2017) originated from three institutions: the Department of Agriculture and Fisheries, Queensland (DAFQ, Australia); the Subtropical Horticulture Research Station (SHRS, USA); and the Agriculture Research Organization (ARO, Israel). Kuhn et al. (2017) provided sequences for 1,409 SNPs in their supplementary materials (Table 2). Among these, 1,054 SNP assays were processed using Fluidigm technology (South San Francisco, CA, USA) and analyzed on a Fluidigm EP-1 platform across all mapping populations.

BLAST analysis of SNP sequences against the 'Alphonso' reference genome

Basic Local Alignment Search Tool (BLAST) analysis was performed by aligning the mango SNPs (query sequences from Kuhn et al. 2017) against the 'Alphonso' reference genome (subject sequence from Wang et al. 2020), available through the National Center for Biotechnology Information (NCBI) under accession number GCA_011075055.1_CATAS_Mindica_2.1_genomic. The analysis procedure was as follows:



1) Blast software was downloaded and installed in a personal computer, 2) the *Mangifera indica* genome was downloaded from NCBI site (https://www.ncbi.nlm.nih.gov/genome/?term=mangifera&utm_source=gquery&utm_medium=search), 3) a 'mango genome' folder was created to unzip the downloaded 'Alphonso' genome, 4) a multifasta file .fna with the SNPs was added to the folder, 5) commands in the DOS prompt: > cd desktop, >cd "genoma mangifera", >blastn -query ARQUIVO.fna -subject GCA_011075055.1_CATAS_Mindica_2.1_genomic.fna -out resultado.txt -outfmt 7.

Each mango SNP sequence had a length of approximately 231 nucleotides. The BLAST output provided for each SNP included: percentage identity, alignment length, genomic start/end positions, E-value, and bit score. SNP-to-chromosome assignments were determined by selecting alignments with the lowest E-values and highest bit scores, even when SNPs showed multiple genomic hits. SNPs were subsequently ordered according to their positions in the 'Alphonso' chromosome assembly. Comparative alignment between the linkage groups (Kuhn et al. 2017) and reference chromosomes (Wang et al. 2020) was visualized using Microsoft Excel and PowerPoint, with corresponding SNPs connected by manually drawn lines.

4 RESULTS AND DISCUSSIONS

The implemented BLAST protocol for aligning SNP sequences against the 'Alphonso' genome successfully identified Kuhn et al. (2017) SNPs with e-values $< 1e-21$ and bit-scores > 191 (data not shown), which mapped to the 20 linkage groups originally published by Kuhn et al. (2017). The total number of blasted SNPs (1,201) exceeded the number of genotyped SNPs (1,054; Table 1 and Kuhn et al. 2017 supplementary data), due to repetitive SNPs showing similar e-values and bit scores that mapped to multiple positions across the 'Alphonso' chromosomes.


Table 1

Number of mango SNPs (#SNPs)/'Alphonso' chromosome and linkage groups (LG) (Kuhn et al., 2017) 'blasted' against each one of the 20 chromosomes (Chr) of the Mangifera indica 'Alphonso' reference genome CATAS_Mindica_2.1 (GCA_011075055.1) (Wang et al. 2020)

Chr	#SNPs/Chr	LG	#SNPs/LG	Chr	#SNPs/Chr	LG	#SNPs/LG
1	91	9	34	11	56	13	1
1		14	26	11		12	35
2	56	4	34	11		5	2
2		8	1	12	73	15	45
3	95	17	52	12		2	1
3		19	1	12		19	1
3		2	1	13	68	13	41
4	89	3	25	13		17	1
4		1	28	13		4	1
5	93	16	67	14	64	15	1
5		10	1	14		20	18
6	62	19	31	15	41	6	25
7	57	11	25	16	48	7	29
7		2	1	16		9	1
7		17	1	17	31	8*(Chr9)	19
8	61	10	35	17		16	1
8		16	2	18	34	missing	missing
9	61	17	1	19	38	5	30
9		8*(Chr17)	23	19		12	1
9		6	2	19		17	1
10	49	2	28	20	34	18	21
Total SNPs				1201		694	

The 91 SNPs from chromosome 1 (Chr) (Wang et al. 2020) were divided into linkage groups (LGs) 9 and 14 from Kuhn et al.'s (2017) mapping, with 34 and 26 SNPs, respectively. This division was also observed for the 89 SNPs from chromosome 4 (Chr), which were split between LGs 1 and 3 with 25 and 28 SNPs respectively (Table 1). LG 8 from Kuhn et al. (2017) was composed of 23 and 19 SNPs from chromosomes 9 and 17, respectively, of the 'Alphonso' mango reference genome (Table 1), indicating significant limitations for mapping and molecular marker-assisted selection applications.

The remaining LGs, except LGs 11, 14 and 20, contained at least one SNP from non-corresponding reference chromosomes: LG 2 contained one SNP each from chromosomes 3, 7 and 12; LG 4 contained one SNP from chromosome 13; LG 5 contained two SNPs from chromosome 11; LG 6 contained two SNPs from chromosome 9; LG 8 contained one SNP from chromosome 2; LG 9 contained one SNP from chromosome 16; LG 10 contained one SNP from chromosome 5; LG 12 contained one SNP from chromosome 19; LG 13 contained one SNP from chromosome 11; LG 15 contained one SNP from chromosome 14; LG 16 contained two SNPs from chromosome 8 and one from chromosome 17; LG 17 contained one SNP each from chromosomes 7, 13 and 19; and LG 19 contained one SNP each from chromosomes 3 and 12



(Table 1). Chromosome 18 of the 'Alphonso' mango reference genome (Wang et al. 2020) was not represented in any of the 20 linkage groups (Table 1), indicating another major limitation of the linkage map presented by Kuhn et al. (2017).

In the formation of genetic linkage groups (Kuhn et al. 2017), four major aberrations were observed when compared to the 'Alphonso' reference genome: a) chromosome 1 was divided into linkage groups 9 and 14; b) chromosome 4 was divided into linkage groups 1 and 3; c) LG 8 was formed by combining SNPs from chromosomes 9 and 17 of 'Alphonso'; and d) chromosome 18 was not represented in any of the 20 linkage groups. The remaining LGs, except LGs 11, 14 and 20, contained at least one SNP from non-corresponding reference chromosomes, indicating limitations in the mango linkage map (Kuhn et al. 2017). Similarly, Medeiros et al. (2022) also reported DNA segments from different chromosomes when comparing genetic linkage maps with the physical map of guava (*Psidium guajava*).

Santos et al. (2023) reported the formation of 23 linkage groups in the Haden × Tommy Atkins cross, one of the seven populations in Kuhn et al.'s (2017) study, indicating that the 20 linkage groups reported by Kuhn et al. (2017) are inconsistent and were somehow, statistically or otherwise, forced to match the $n = 20$ of the mango genome.

The alignment of LG 9 showed discrepant SNP order relative to chromosome 1, particularly in the first 10 cM and between 76-81 cM, while LG 14 showed minor discrepancies in SNP order relative to chromosome 1 (Figure 1-A), noting that this chromosome was divided into two LGs (Kuhn et al. 2017) (Table 1). LG 4 showed non-conformity in SNP order, particularly between 30-53 cM, while LG 17 showed major discrepancies in SNP order relative to chromosome 3, covering nearly 100 cM (Figure 1-A). Minor SNP order discrepancies were observed in LG 1 when compared to chromosome 4, while LG 3 showed discrepancies between 39-41 cM, 63-65 cM and 65-67 cM when compared to chromosome 4 (Figure 1-A), noting that this chromosome was divided into LGs 3 and 1 by Kuhn et al. (2017).

The alignment of SNPs from LG 16 (Kuhn et al. 2017) showed major non-conformity with the SNP order of chromosome 5 from the 'Alphonso' reference genome, particularly between 181-228 cM (Figure 1-B). LGs 19, 11, 10, 8 and 2 showed minor discrepancies in SNP order when blasted against chromosomes 6, 7, 8, 9 and 10 respectively of the 'Alphonso' reference genome (Figure 1-B).

Minor discrepancies in SNP order were observed in LGs 12, 6 and 7 (Kuhn et al. 2017) when blasted against chromosomes 11, 15 and 16 (Figure 1-C), respectively, of the 'Alphonso' reference genome (Wang et al. 2020). Significant inconsistencies were observed when blasting



LGs 15, 13 and 20 against chromosomes 12, 13 and 14, respectively, of the 'Alphonso' reference genome (Figure 1-C).

Minor discrepancies in SNP order were observed in LGs 8 and 5 (Kuhn et al. 2017) when blasted against chromosomes 17 and 19 (Figure 1-D), respectively, of the 'Alphonso' reference genome (Wang et al. 2020). Significant inconsistencies were observed when blasting LG18 against chromosome 20 of the 'Alphonso' reference genome (Figure 1-D).

Major discrepancies were observed in the SNP ordering within the 20 linkage groups of Kuhn et al. (2017), particularly in LGs 9, 4, 17, 3, 16, 19, 10, 12, 15, 20, 5 and 18 (Figure 1). Simulations of some software for genetic linkage map construction indicated that JoinMap4.1, the software used by Kuhn et al. (2017), showed ordering that was far from the predefined order of 20 markers (Zhang et al. 2015). In this scenario, QTL mapping becomes extremely limited and compromised, resulting in false linkages between markers and regions controlling agronomically important traits.

Figure 1

A. Alignment of the mango linkage groups (Kuhn et al. 2017) (left) and mango physical chromosomes (right) based on the same SNPs and mapped to the 1, 2, 3 and 4 chromosomes of the Mangifera indica 'Alphonso' reference genome CATAS Mindica_2.1 (GCA_011075055.1)

SNP - LG 9	cM	To Chr	SNP - Chr 1	To LG	bp
mango_rep_c4696	0	1	SSKP008C1_A700T	9	41176
MI_0466	0.548	1	MI_0561	9	385815
MI_0563	1.047	1	Mango_rep_c4696	9	593357
mango_rep_c3663	3.305	1	MI_0466	9	784244
MI_0554	4.824	1	Mango_rep_c3663	9	903234
SSKP008C1_A700T	8.698	1	MI_0563	9	936920
MI_0561	14.196	1	MI_0554	9	1145722
Contig942	18.067	1	Contig942	9	1618793
mango_rep_c9029	43.776	1	MI_0350	9	2478660
MI_0350	44.378	1	MI_0110	9	2534890
MI_0110	45.592	1	MI_0268	9	2696402
MI_0268	48.946	1	Mango_rep_c9029	9	2897344
mango_rep_c11906	62.803	1	Mango_rep_c11906	9	3531176
mango_rep_c758	66.98	1	Mango_rep_c758	9	4082294
Contig5786	67.754	1	Contig5786	9	4188937
MI_0340	76.257	1	MI_0074	9	4403075
MI_0114	76.612	1	SSKP136C1_A519G	9	4742800
MI_0494	77.148	1	MI_0491	9	4835273
Contig756	77.818	16	MI_0494	9	4896922
MI_0491	77.99	1	MI_0114	9	4969855
SSKP136C1_A519G	79.445	1	MI_0340	9	5130965
MI_0074	80.626	1	MI_0148	9	5618618
MI_0148	88.849	1	SSKP041C1_A653T	9	5785581
MI_0318	94.321	1	MI_0318	9	6011854
SSKP041C1_A653T	97.305	1	Contig1087	9	6542986
Contig1087	102.962	1	MI_0035	9	6745914
MI_0035	106.725	1	MI_0417	9	6952016
MI_0417	109.215	1	MI_0402	9	7911973
MI_0402	122.355	1	Mango_rep_c9549	9	8449500
mango_rep_c9549	124.504	1	MI_0497	9	9364446
MI_0142	128.837	1	MI_0142	9	9700652
MI_0497	129.648	1	MI_0008	9	15157352
MI_0008	142.072	1	MI_0133	9	15613230
MI_0133	143.132	1			

SNP - LG 14	cM	To Chr	SNP - Chr1	To LG	bp
Contig1373	0	1	Contig1373	14	20512298
SSKP047C1_C678T	9.508	1	Contig2887	14	21295021
Contig2887	13.297	1	SSKP047C1_C678T	14	21811563
MI_0030	25.776	1	Mango_rep_c11313	14	22755102
SSKP006C1_G845T	31.325	1	SSKP006C1_G845T	14	22841672
mango_rep_c11313	32.415	1	MI_0030	14	22890486
mango_rep_c2650	34.257	1	Mango_rep_c2650	14	23081724
MI_0025	36.869	1	MI_0025	14	23347132
MI_0253	44.133	1	MI_0253	14	23533600
SSKP046C2_A827G	53305	1	SSKP046C2_A827G	14	24446611
mango_rep_c1316	57.35	1	Mango_rep_c1316	14	24576970
MI_0138	59.468	1	MI_0138	14	24818479
mango_rep_c4311	60.99	1	Mango_rep_c4311	14	24853057
mango_rep_c3432	60.4	1	MI_0158	14	24853157
MI_0158	60.42	1	Mango_rep_c4311	14	24932013
Contig1365	68.215	1	Contig1365	14	25458299
mango_rep_c12371	72.084	1	Mango_rep_c12371	14	25513357
MI_0451	75.587	1	Mango_rep_c6097	14	26005290
mango_rep_c6097	76.191	1	MI_0526	14	26371352
MI_0526	77.125	1	MI_0451	14	26389664
SSKP141C2_C347T	83.093	1	Mango_rep_c2016	14	26879986
mango_rep_c2016	85.28	1	SSKP141C2_C347T	14	27044842
mango_rep_c16226	85.25	1	SSKP141C2_C347T	14	27045041
mango_rep_c2828	85.895	1	Mango_rep_c2828	14	27127265
SSKP058C2_A431G	108.488	1	Mango_rep_c16226	14	27372769
MI_0202	114.59	1	SSKP058C2_A431G	14	29093394
			MI_0202	14	29297975



SNP LG-4	cM	To Chr	SNP-Chr2	To LG	bp
SSKP021C1_C1039T	223,198	2	SSKP021C1_C1039T	4	2769441
SSKP109C1_C764T	220,276	2	SSKP109C1_C764T	4	3296127
Contig398	198,474	2	Contig398	4	4428543
SSKP116C1_A524G	156,84	2	SSKP116C1_A524G	4	12845786
Mi_0024	149,379	2	SSKP050C1_C518T	4	13468796
SSKP050C1_C518T	148,744	2	Mi_0024	4	13581644
Mi_0300	146,369	2	Mi_0300	4	13716950
Mi_0467	146,033	2	Mi_0467	4	13731944
Mi_0290	125,903	2	Mi_0533	8	13924205
Mi_0150	122,375	2	Mi_0290	4	15123776
mango_rep_c5750	109,471	2	Mi_0150	4	15253317
Contig6253	107,126	2	Contig6253	4	16544620
Mi_0426	105,574	2	Mango_rep_c5750	4	16797477
Mi_0334	104,965	2	Mi_0426	4	16851936
Mi_0548	100,469	2	Mi_0334	4	16887589
Mi_0302	100,203	2	Mi_0548	4	17170226
Contig929	98,816	2	Mi_0302	4	17196131
Mi_0284	86,434	2	Contig929	4	17351125
Mi_0239	83,224	2	Mi_0284	4	18225412
Mi_0230	56,822	2	Mi_0239	4	18380580
mango_rep_c9407	53,502	2	Mi_0230	4	19900168
Contig2241	53,266	2	Mango_rep_c9478	4	20151263
Mi_0045	41,097	2	Mango_rep_c9407	4	20496307
Mi_0066	40,346	2	SSKP055C3_A517G	4	21042590
Mi_0124	40,062	2	Mi_0088	4	21167636
mango_rep_c8049	38,933	2	Mi_0066	4	21215818
Mi_0088	38,293	2	Mi_0045	4	21220927
SSKP055C3_A517G	38,22	2	Mi_0124	4	21419936
Mi_0335	34,772	2	Mango_rep_c8049	4	21554003
Mi_0487	33,893	2	Mi_0487	4	22087839
mango_rep_c9478	30,377	2	Mi_0335	4	22111413
Mi_0443	22,413	2	Mi_0443	4	22782169
Mi_0221	21,15	2	Mi_0221	4	22848116
Mi_0549	17,974	2	Mi_0549	4	22925039
mango_rep_c8614	0	2	Mango_rep_c8614	4	24017087

SNP LG-17	cM	To Chr	SNP-Chr3	To LG	bp
Mi_0281	156,703	3	Mi_0281	17	2125508
mango_rep_c9129	148,908	3	Contig1872	17	261959
Contig4872	146,49	3	Mango_rep_c9129	17	629462
Mi_0167	145,906	3	Mi_0167	17	751317
Mi_0328	141,794	3	Mi_0328	17	1122891
mango_rep_c5571	139,554	3	Mango_rep_c5571	17	1238093
Contig3337	129,61	3	Contig3337	17	1553774
Contig4330	127,052	3	Contig4330	17	1681799
Mi_0550	116,059	3	Mi_0129	17	2354488
Mi_0129	115,184	3	Mi_0550	17	2407777
mango_rep_c2747	106,321	3	Mango_rep_c19967	17	2861171
mango_rep_c19967	105,194	3	Mango_rep_c2747	17	2873581
Mi_0368	104,689	3	Mi_0368	17	2927603
Mi_0166	99,444	3	Mi_0166	17	3552969
mango_rep_c7019	98,619	3	Mango_rep_c7019	17	3611964
Mi_0063	97,241	3	Mi_0063	17	3751767
mango_rep_c1966	96,287	3	Mango_rep_c1966	17	3779559
Mi_0071	92,919	3	Mi_0071	17	4259587
Contig1878	90,765	3	Mi_0132	17	4601960
Mi_0132	89,408	3	Mi_0382	17	4746609
Contig1073	88,592	3	Mango_rep_c7055	17	5026408
Mi_0382	88,287	3	Mango_rep_c3609	17	5038666
Mi_0321	86,684	3	Contig3004	17	5045899
Contig2524	79,132	13	Contig1914	17	5208121
mango_rep_c3609	78,464	3	mango_rep_c8265	17	5354551
Contig3004	78,464	3	Mi_0121	17	5413355
mango_rep_c7055	77,676	3	Mango_rep_c15212	17	6419737
Mi_0121	77,307	3	Mango_rep_c10961	17	6736900
Contig1914	75,712	3	Mi_0492	17	6974904
mango_rep_c8265	74,484	3	Mi_0116	17	6994633
Mi_0002	72,674	3	Mi_0235	17	7082271
Mi_0397	70,859	3	Mango_rep_c34886	17	7295708
mango_rep_c10593	70,341	3	Mango_rep_c2961	17	7295969
mango_rep_c15212	62,471	3	Mi_0473	17	7523819
mango_rep_c10961	53,906	3	Mango_rep_c9532	17	7550141
Mi_0168	53,566	3	Mango_rep_c8252	17	7701810
Contig2668	52,648	9	Mi_0505	17	7793828
Mi_0116	50,895	3	Mango_rep_c5787	17	8682235
Mi_0492	50,687	3	Mango_rep_c8171	17	8880513
mango_rep_c3766	49,088	3	Mango_rep_c417	17	8932779
Mi_0235	48,084	3	Mi_0135	17	10526519
Mi_0535	47,558	3	SSKP009C1_A627T	17	10671379
mango_rep_c34886	45,337	3	Mi_0535	17	14919699
mango_rep_c2961	45,149	3	Mango_rep_c3766	17	15966441
Mi_0473	44,519	3	Mi_0168	17	18214363
mango_rep_c9532	41,829	3	Contig2715	2	18245541
mango_rep_c8252	37,626	3	Mi_0397	17	20422455
Mi_0505	36,553	3	Mango_rep_c10593	17	20976026
Contig2556	30,53	19	Mi_0002	17	21172833
mango_rep_c5787	28,211	3	Contig1052	19	21632739
mango_rep_c8171	26,9	3	Contig1071*	17	21632739
mango_rep_c417	26,828	3	Mi_0321	17	22098449
SSKP009C1_A627T	0.14	3	Contig1878	17	22774816
Mi_0135	0	3	Contig1073	17	22876520

SNP - LG 1	cM	To Chr	SNP - Chr 4	To LG	bp
Mi_0520	111.24	4	Contig871	1	13993390
Contig871	105.849	4	Mango_rep_c24937	1	13993415
mango_rep_c24937	105.604	4	Mi_0520	1	14298008
Mi_0186	95.072	4	Mi_0186	1	14886799
Mi_0242	91.707	4	Mi_0242	1	15127120
Mi_0204	89.59	4	Mi_0204	1	15208153
Contig2867	83.745	4	Contig2867	1	15515955
Mi_0445	82.659	4	Mi_0445	1	15905673
Mi_0400	78.35	4	Mi_0486	1	16417706
Mi_0486	77.819	4	Mi_0400	1	16496660
Mi_0018	72.999	4	Mango_rep_c10257	1	16868436
mango_rep_c10257	72.515	4	Mi_0018	1	16966816
mango_rep_c3169	70.451	4	Mango_rep_c3169	1	17199368
Mi_0140	69.331	4	Mi_0140	1	17199651
Mi_0405	68.302	4	Mi_0405	1	17245187
mango_c33092	53.673	4	Mango_c33092	1	18064217
Contig4503	46.313	4	Contig4503	1	18341926
Mi_0456	44.466	4	Mi_0456	1	18351526
Mi_0323	36.315	4	Mango_c41026	1	19081726
mango_c41026	34.127	4	Mi_0323	1	19256516
Mi_0016	32.737	4	Mi_0016	1	19349568
Mi_0423	29.575	4	Mi_0423	1	19649982
Mi_0276	23.612	4	Mi_0276	1	19961442
mango_rep_c4916	18.261	4	Mi_0304	1	20147371
Mi_0304	18.203	4	Mango_rep_c4916	1	20155570
Mi_0414	7.743	4	Mi_0414	1	20606530
Contig1746	3.237	4	Contig1746	1	20841390
Contig167	0	4	Contig167	1	21200634

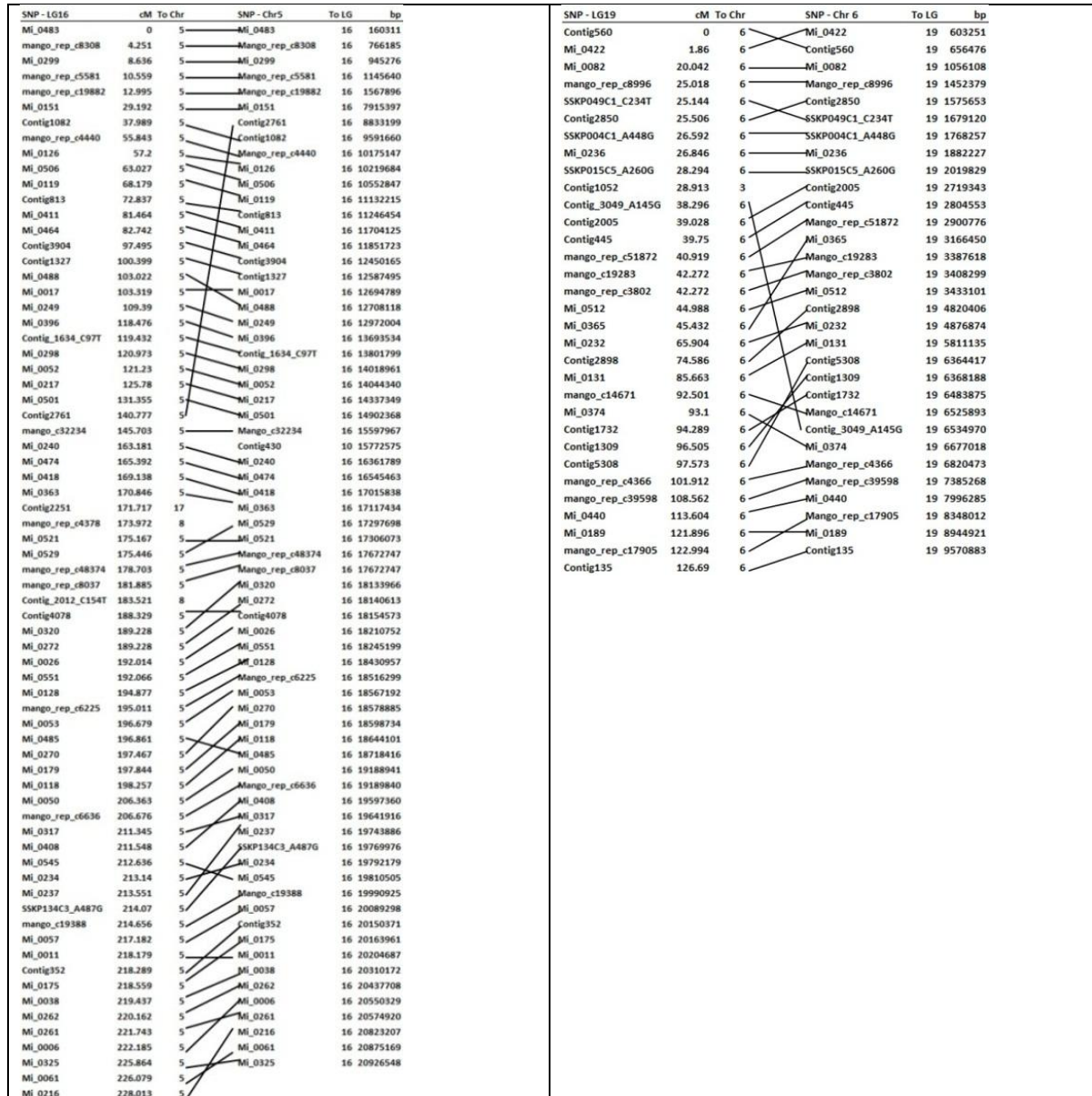
SNP - LG3	cM	To Chr	SNP - Chr 4	To LG	bp
mango_c15408	0	4	Mango_c15408	3	1442004
SSKP131C1_A837G	6.743	4	SSKP131C1_A837G	3	2561552
mango_rep_c5624	17.139	4	Mango_rep_c5624	3	3112434
Mi_0115	28.765	4	Mi_0115	3	4180909
Mi_0556	30.119	4	Mi_0556	3	4363733
Mi_0540	33.941	4	Mi_0540	3	5043205
Mi_0215	34.633	4	Mi_0215	3	5100849
Mi_0152	39.23	4	Mi_0399	3	8658388
Mi_0333	40.03	4	Mi_0152	3	9102077
Mi_0254	40.2	4	Mi_0333	3	9195871
Mi_0399	40.782	4	Mi_0254	3	9321822
Mi_0388	42.022	4	Mi_0388	3	9921130
mango_rep_c6478	61.782	4	Mango_rep_c6478	3	11368088
Mi_0044	62.822	4	Mi_0044	3	11368262
SSKP080C1_0462T	63.175	4	Mi_0248	3	11463396
SSKP113C1_A491T	63.391	4	SSKP111C1_A758C	3	11503428
SSKP081C1_C270T	63.823	4	SSKP081C1_C270T	3	11503921
SSKP082C3_A172C	63.904	4	SSKP080C1_0462T	3	11512273
Mi_0248	64.33	4	SSKP082C3_A172C	3	11518815
SSKP111C1_A758C	64.644	4	Mi_0393	3	11949997
mango_rep_c3739	65.536	4	Mi_0371	3	11973768
Mi_0371	66.966	4	Mango_rep_c3739	3	12283207
Mi_0393	67.393	4	Mi_0067	3	12486668
Mi_0067	75.281	4	Mi_0471	3	12556442
Mi_0471	75.749	4	Mi_0428	3	12816625
Mi_0428	79.433	4			

Source: Wang et al. 2020



Figure 1

B. Alignment of the mango linkage groups (Kuhn *et al.*, 2017) (left) and mango physical chromosomes (right) based on the same SNPs and mapped to the 5, 6, 7, 8, 9 and 10 chromosomes of the *Mangifera indica* 'Alphonso' reference genome CATAS_Mindica_2.1 (GCA_011075055.1)





SNP - LG11	cM	To Chr	SNP - Chr7	To LG	bp	SNP - LG10	cM	To Chr	SNP - Chr8	To LG	bp
SSKP054C1_A8610	0	7	MI_0181	2	3623247	MI_0258	0	8	MI_0258	10	673543
mango_rep_c50209	6.714	7	SSKP054C1_A8610	11	15081654	MI_0477	0.713	8	MI_0477	10	693546
mango_rep_c28055	8.041	7	Mango_rep_c6932	11	15419796	MI_0468	0.714	8	MI_0532	10	730643
mango_rep_c6932	8.642	7	Mango_rep_c50209	11	15510606	MI_0532	0.733	8	MI_0468	10	764067
mango_rep_c14171	12.777	7	Mango_rep_c28055	11	15530801	Contig662	5.625	8	Contig662	10	995410
SSKP027C2_C495T	14.13	7	MI_0159	11	15907254	mango_rep_c4658	11.35	8	Mango_rep_c4658	10	1398097
MI_0159	14.132	7	Mango_rep_c14171	11	15997661	MI_0311	40.166	8	Contig_2012_c154T	16	2536758
SSKP026C1_A1002T	14.817	7	SSKP026C1_A1002T	11	16296361	MI_0390	44.794	8	MI_0311	10	2697000
Contig1567	15.615	7	SSKP027C2_C495T	11	16299029	Contig430	67.439	8	Mango_rep_c4378	16	2700124
mango_rep_c48384	17.685	7	Contig1567	11	16317010	SSKP107C1_A1014G	72.669	8	MI_0390	10	2947119
mango_rep_c52196	17.758	7	Mango_rep_c48384	11	16473751	MI_0478	74.803	8	Contig430	10	4292080
MI_0100	18.373	7	Mango_rep_c52196	11	16497955	mango_rep_c53253	75.738	8	SSKP107C1_A1014G	10	4681912
MI_0229	28.535	7	MI_0100	11	16852025	Contig889	78.918	8	MI_0478	10	4961363
MI_0048	30.817	7	MI_0229	11	17331773	MI_0153	91.619	8	Mango_rep_c53253	10	5045608
MI_0386	34.545	7	MI_0048	11	17609700	MI_0097	92.11	8	Contig889	10	5090438
MI_0448	35.024	7	MI_0386	11	17806765	mango_rep_c14290	92.375	8	Mango_rep_c10854	10	6176131
Contig343	37.477	7	MI_0448	11	17825307	mango_rep_c10854	92.432	8	Mango_rep_c14290	10	6188988
SSKP025C1_A784G	42.873	7	Contig343	11	18059257	mango_rep_c7681	94.229	8	MI_0097	10	6212490
MI_0307	43.05	7	Contig1071	17	18097698	Contig4240	94.5	8	MI_0153	10	6241408
SSKP045C3_C621T	57.464	7	SSKP045C3_C621T	11	18277010	mango_rep_c4763	98.897	8	Mango_rep_c7681	10	6433075
mango_rep_c8480	62.363	7	MI_0307	11	18357541	mango_rep_c2106	99.57	8	Contig4240	10	6435901
mango_rep_c4422	64.899	7	SSKP045C3_C621T	11	18756840	MI_0105	101.44	8	Mango_rep_c4763	10	6669722
MI_0136	68.44	7	Mango_rep_c8480	11	19032564	Contig3651	103.006	8	Mango_rep_c2106	10	6708326
MI_0051	75.161	7	Mango_rep_c4422	11	19371963	MI_0345	104.187	8	MI_0105	10	6788959
MI_0005	77.238	7	MI_0136	11	19618856	MI_0177	104.692	8	Contig3651	10	6951371
			MI_0051	11	20101471	MI_0476	109.715	8	MI_0476	10	7118871
			MI_0005	11	20393512	Contig1199	112.813	8	MI_0345	10	7180827
						MI_0103	113.089	8	MI_0177	10	7196284
						SSKP122C1_C873T	118.233	8	Contig1199	10	7587219
						MI_0231	118.387	8	MI_0103	10	7711452
						MI_0373	120.042	8	Mango_rep_c10386	10	7974419
						mango_rep_c10386	120.517	8	SSKP122C1_C873T	10	8008462
						MI_0438	131.666	8	MI_0231	10	8065502
						MI_0278	136.577	8	MI_0373	10	8263035
						Contig1344	137.977	8	MI_0438	10	9057728
						Contig866	142.09	8	Contig1344	10	9173333
						MI_0442	145.071	8	MI_0278	10	9349041
						MI_0518	162.389	8	Contig866	10	9576784
						mango_rep_c9158	171.735	8	MI_0442	10	9682631
						Contig1686	174.79	8	MI_0518	10	10857894
						MI_0329	186.529	8	Mango_rep_c9158	10	11355056
									Contig1686	10	11785404
									MI_0329	10	12676773

SNP - LG8	cM	To Chr	SNP - Chr9	To LG	bp	SNP - LG 2	cM	To Chr	SNP - Chr10	To LG	bp
MI_0493	247.848	9	Contig2668	17	260149	MI_0543	135.589	10	Contig3006	2	262169
MI_0349	246.721	9	MI_0493	8	1214497	Contig3006	133.16	10	MI_0543	2	457830
MI_0460	244.508	9	MI_0349	8	1265734	Contig1627	110.064	10	Contig1627	2	2166335
MI_0542	242.811	9	MI_0460	8	1422430	MI_0312	100.517	10	Contig3071	2	2846875
Contig_6532_A130T	209.899	9	MI_0542	8	1529158	Contig3071	100.156	10	MI_0312	2	2919807
SSKP115C1_A717G	198.315	9	Contig_6532_A130T	8	2986773	mango_rep_c21634	99.824	10	Mango_rep_c21634	2	2985745
Contig2997	198.314	9	SSKP115C1_A717G	8	6256081	mango_rep_c5764	91.83	10	Mango_rep_c5764	2	3144816
MI_0187	195.856	9	Contig2997	8	6502356	MI_0246	89.951	10	MI_0246	2	3290207
mango_rep_c5137	195.33	9	MI_0187	8	8122507	MI_0266	85.667	10	MI_0266	2	3488245
MI_0279	194.885	9	SSKP038C1_A444G	8	8368945	MI_0172	85.435	10	MI_0172	2	3510272
SSKP038C1_A444G	194.846	9	MI_0279	8	8448777	MI_0181	84.19	7	Contig1205*	2	4207417
MI_0314	192.55	9	Mango_rep_c5137	8	8992256	Contig1205	70.337	10	Contig1205	2	4207816
SSKP085C1_C285G	190.122	9	MI_0314	8	9360817	Contig2715	66.315	3	MI_0188	2	4511311
mango_rep_c3996	187.743	9	SSKP085C1_C285G	8	9629880	MI_0188	63.782	10	SSKP011C2_A415G	2	5104070
SSKP037C2_A644G	182.744	9	SSKP037C2_A644G	8	10368022	SSKP011C2_A415G	51.27	10	MI_0274	2	6173949
MI_0533	171.349	2	Mango_rep_c3996	8	10444144	MI_0274	34.815	10	Mango_rep_c4965	2	6190890
MI_0068	169.277	9	Mango_rep_c13088	8	11518552	mango_rep_c4965	33.92	10	Mango_rep_c3010	2	6374875
mango_rep_c13088	168.57	9	MI_0068	8	11521351	mango_rep_c3010	32.955	10	Mango_rep_c2935	2	6456471
MI_0117	163.122	9	MI_0117	8	12211279	mango_rep_c2935	31.248	10	Mango_rep_c13463	2	6457920
MI_0339	154.529	9	MI_0339	8	12357509	Contig1500	31.195	10	Contig1500	2	6537560
MI_0122	150.189	9	MI_0122	8	12712330	mango_rep_c13463	31.009	10	MI_0264	2	6600778
MI_0481	145.674	9	MI_0481	8	12913022	MI_0264	30.563	10	MI_0040	2	7127512
SSKP012C3_C375T	144.307	9	SSKP012C3_C375T	8	13262772	MI_0040	24.366	10	Mango_rep_c11503	2	7724098
			Mango_rep_c8984	8	15016915	mango_rep_c11503	14.322	10	Contig636	2	9425930
			SSKP087C1_A198G	6	15269338	MI_0058	8.052	10	MI_0003	2	9962895
			MI_0482	6	18066779	SSKP052C3_C791G	3.376	10	MI_0058	2	12755701
						SSKP051C1_C695T	3.207	10	SSKP052C3_C791G	2	12902682
						Contig636	2.598	10	SSKP051C1_C695T	2	12904549
						MI_0003	0	10			

Source: Wang et al. 2020

**Figure 1**

C. Alignment of the mango linkage groups (Kuhn et al. 2017) (left) and mango physical chromosomes (right) based on the same SNPs and mapped to the 11, 12, 13, 14, 15 and 16 chromosomes of the Mangifera indica 'Alphonso' reference genome CATAS_Mindica_2.1 (GCA_011075055.1)

SNP - LG12	cM	To Chr	SNP - Chr11	To LG	bp	SNP - LG15	cM	To Chr	SNP - Chr12	To LG	bp
Mi_0244	0	11	Contig2232	13	205180	SSKP039C1_C414T	166.222	12	Mango_rep_c25371	15	46445
Mi_0294	0.345	11	Mango_rep_c2497	12	689197	Mi_0379	160.318	12	Mi_0289	15	4951117
mango_rep_c2497	1.225	11	Mi_0244	12	869763	Mi_0289	153.197	12	Mi_0379	15	5995215
SSKP104C1_A463G	3.245	11	Mi_0294	12	896127	mango_rep_c25371	146.138	12	SSKP039C1_C414T	15	6460781
SSKP105C1_A936G	3.469	11	SSKP105C1_A936G	12	1070562	Contig6170	133.211	12	Mango_rep_c7997	15	7344495
Mi_0391	4.144	11	SSKP104C1_A463G	12	1072845	Contig878	132.942	12	Contig6170	15	7742165
SSKP013C1_A574G	12.868	11	Mi_0391	12	1175242	mango_rep_c7997	130.169	12	Contig878	15	7742165
Contig_3454_A139G	14.483	11	SSKP013C1_A574G	12	1598153	Contig2622	129.147	14	Mango_rep_c9915	15	8720027
SSKP029C1_C509T	18.993	11	Contig_3454_A139G	12	1696302	mango_rep_c9915	111.156	12	Mi_0562	15	9148757
SSKP031C1_A370G	18.993	11	Mi_0503	5	1802828	Mi_0562	100.215	12	Mi_0028	15	9194860
Mi_0504	19.019	11	SSKP031C1_A370G	12	1987029	Mi_0028	98.91	12	Mi_0233	15	9287203
Contig2019	19.499	11	SSKP029C1_C509T	12	1987793	Mi_0233	97.352	12	Mi_0195	15	9407851
Mi_0109	20.238	11	Mi_0504	12	1989806	Mi_0195	94.284	12	Mi_0251	15	9584464
Mi_0433	21.65	11	Contig2019	12	2008411	Mi_0251	89.985	12	Contig1205*	2	9730687
Mi_0156	22.456	11	Mi_0109	12	2050617	Mi_0441	89.142	12	Mango_rep_c39	15	9790005
Mi_0313	27.541	11	Mi_0433	12	2102539	Contig2048	89.142	12	Contig2048	15	9790151
Mi_0193	34.295	11	Mi_0156	12	2128916	mango_rep_c39	89.142	12	Mi_0441	15	9790451
SSKP030C1_G486T	35.899	11	Mi_0313	12	2375509	Mi_0377	85.504	12	Mi_0377	15	10000575
Mi_0530	53.364	11	Mi_0444*	5	2582411	SSKP023C1_G385T	80.948	12	SSKP010C1_C421T	15	10010433
Mi_0352	53.607	11	Mi_0193	12	2634978	SSKP010C1_C421T	80.772	12	SSKP023C1_G385T	15	10108927
Mi_0378	59.218	11	SSKP030C1_G486T	12	2808983	Contig_1650_A91G	80.456	12	Contig_1650_A91G	15	10260868
Mi_0212	59.507	11	Mi_0530	12	3482962	Mi_0096	70.884	12	Mi_0096	15	10604128
mango_rep_c16724	59.618	11	Mi_0352	12	3494051	Mi_0059	69.379	12	Mi_0059	15	10685936
Contig640	60.323	11	Mango_rep_c16724	12	3744072	mango_rep_c1665	68.166	12	Mango_rep_c1665	15	10803258
mango_rep_c7501	67.721	11	Contig640	12	3905310	SSKP007C1_C621T	64.13	12	SSKP007C1_C621T	15	11051704
Mi_0472	69.746	11	Mi_0212	12	3906004	Mi_0211	62.374	12	Mi_0211	15	11408669
Contig_1658_A98G	72.772	11	Mi_0378	12	3921867	Mi_0256	62.051	12	SSKP034C1_A537G	15	11450004
mango_rep_c21404	76.486	11	Mango_rep_c7501	12	4497177	Mi_0296	60.898	12	Mi_0296	15	11548896
Mi_0208	80.915	11	Mi_0472	12	4785187	mango_rep_c24145	60.435	12	Mango_rep_c24145	15	11631385
SSKP129C1_C847T	96.309	11	Contig_1658_A98G	12	5024780	SSKP034C1_A537G	58.138	12	Mi_0256	15	11675623
Mi_0054	96.948	11	Mango_rep_c21404	12	5411114	SSKP098C2_A234G	45.534	12	SSKP098C2_A234G	15	12900445
Mi_0326	96.956	11	Mi_0208	12	5575463	Mi_0252	32.62	12	Mi_0037	15	13515783
SSKP077C2_A650G	123.026	11	Mi_0054	12	6270434	Mi_0205	28.661	12	Mi_0252	15	13519205
mango_rep_c6972	139.219	11	SSKP129C1_C847T	12	6343755	Mi_0243	22.531	12	Mi_0205	15	13627810
SSKP065C1_C260G	148.84	11	Mi_0326	12	6359161	Mi_0037	20.036	12	Mi_0243	15	13870577
			SSKP077C2_A650G	12	7808670	mango_rep_c7620	15.545	12	Mi_0499	15	14162900
			Mango_rep_c6972	12	9842366	Mi_0499	14.836	12	SSKP015C5_A260G*	19	14226379
			SSKP065C1_C260G	12	11703474	Mi_0287	12.13	12	Mango_rep_c7620	15	14293252
					SSKP138C2_C598G	7.297	12	Mi_0287	15	14543232	
					Mi_0070	6.069	12	SSKP138C2_C598G	15	14879928	
					SSKP014C1_A851G	5.299	12	SSKP014C1_A851G	15	15119181	
					mango_rep_c58273	4.604	12	Mi_0070	15	15285720	
					Mi_0043	1.445	12	Mi_0043	15	15685136	
					Mi_0409	0	12	Mi_0409	15	15805674	
								Mango_rep_c58273	15	15823469	



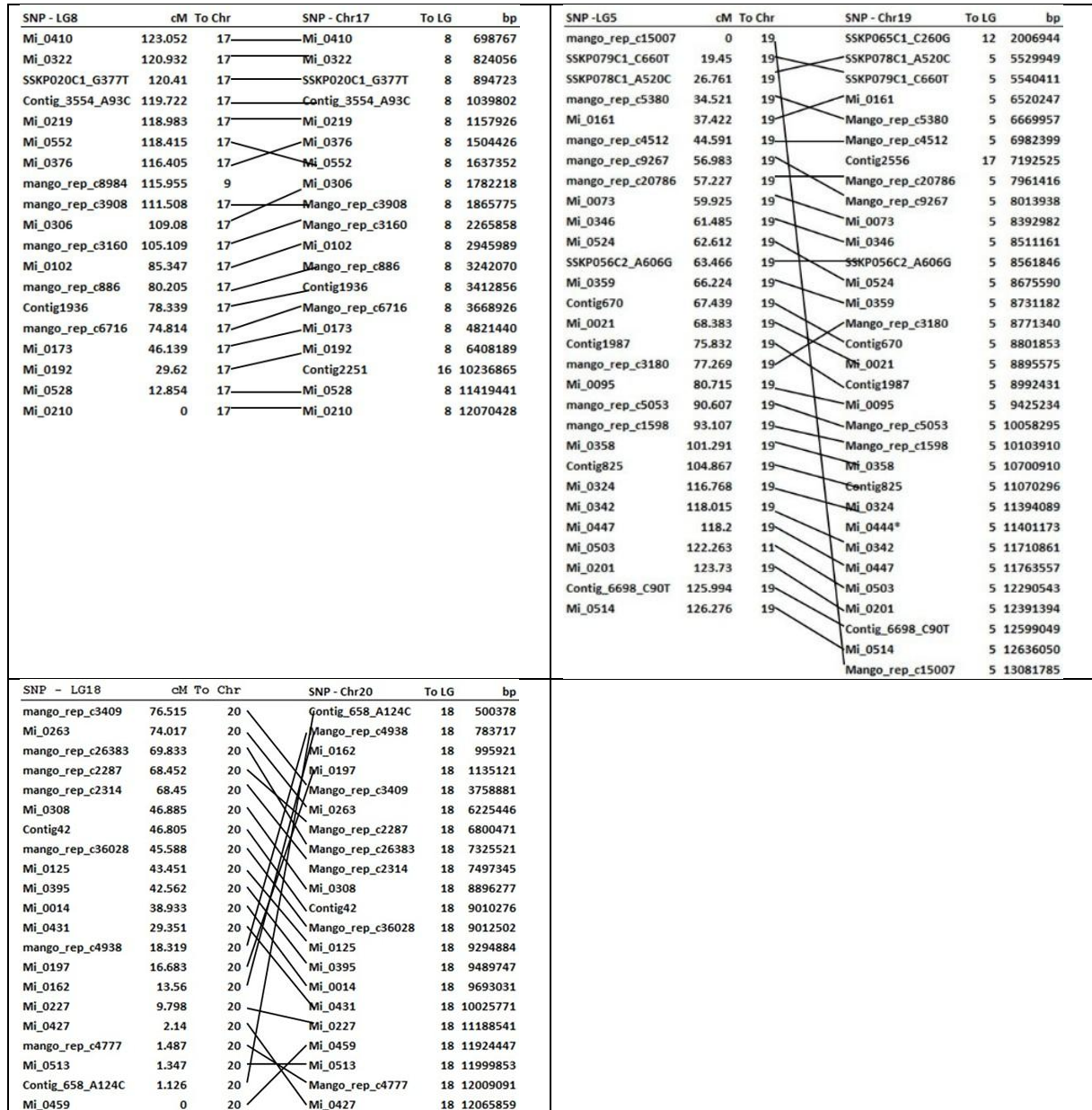
SNP - LG13	cM To Chr	SNP - Chr13	To LG	bp	SNP - LG20	cM To Chr	SNP - Chr14	To LG	bp
Mi_0546	154.948	13	Mi_0546	13 681465	Mi_0214	156.067	14	Mi_0214	20 390403
Contig763	110.173	13	Contig763	13 5251164	Mi_0218	155.214	14	Mi_0218	20 449978
Mi_0065	100.182	13	Mi_0065	13 6453565	Mi_0213	153.201	14	Mi_0517	20 678335
mango_rep_c3242	99.776	13	Mango_rep_c3242	13 6671576	Mi_0403	152.078	14	Mi_0213	20 688814
Mi_0013	99.259	13	Mi_0013	13 6671676	Mi_0517	151.993	14	Mi_0403	20 811205
Mi_0394	97.721	13	Mi_0449	13 7476312	Mi_0250	131.869	14	Mi_0297	20 1466064
Mi_0449	96.781	13	Mi_0500	13 7727701	Mi_0297	131.107	14	Mi_0250	20 1513292
Mi_0500	96.469	13	Mi_0394	13 7768251	Mi_0360	121.434	14	Mi_0360	20 2136242
mango_rep_c7670	94.257	13	Mango_rep_c7670	13 8271093	Mi_0042	116.996	14	Mi_0042	20 2547564
Contig2080	91.496	13	Contig_6876_G118T	13 8943986	Contig255	115.822	13	Mango_rep_c4540	20 2549886
Contig_6876_G118T	90.816	13	Contig2080	13 8987070	Mi_0209	114.142	14	Contig117	20 2670863
Contig2232	76.441	11	Contig2524	17 9385227	mango_rep_c4540	113.765	14	Mi_0209	20 2841922
mango_rep_c4227	74.057	13	Contig2241	4 9722046	Mi_0453	113.267	14	Mi_0453	20 2902036
Contig_2032_A105C	67.926	13	Mango_rep_c4227	13 9867940	mango_c16893	112.809	14	Mango_c16893	20 3034667
SSKP120C1_A561C	58.339	13	Contig_2032_A105C	13 10368690	Contig117	108.923	14	Mi_0315	20 3980736
SSKP119C1_C705T	57.533	13	SSKP120C1_A561C	13 10788444	Mi_0315	97.411	14	Mi_0086	20 4123782
SSKP117C1_A422C	57.131	13	SSKP117C1_A422C	13 10789100	Mi_0086	96.316	14	Mi_0062	20 4565757
SSKP118C1_C423G	57.13	13	SSKP118C1_C423G	13 10790615	Mi_0062	89.366	14	Contig735	20 4727666
Contig3148	55.223	13	SSKP119C1_C705T	13 10795683	Contig735	86.225	14	Mango_rep_c4496	20 4843746
mango_rep_c6017	55.134	13	Mango_rep_c6017	13 10822810	mango_rep_c4496	80.525	14	Contig2622	15 5275826
Contig1447	54.683	13	Contig1447	13 10894868	Contig2601	74.009	3	Mi_0330	20 5388878
mango_rep_c5036	54.414	13	Mango_rep_c5036	13 10899209	Mi_0046	73.076	14	Mi_0046	20 5429185
mango_rep_c8159	33.206	13	Contig3148	13 10950351	Mi_0330	72.411	14	Mi_0357	20 5456184
Mi_0498	33.131	13	Mi_0498	13 12096109	Mi_0357	71.12	14	Mi_0343	20 5472557
mango_rep_c8423	31.473	13	Mango_rep_c8159	13 12132783	mango_rep_c8905	70.406	14	Mango_rep_c8905	20 5533725
Mi_0009	28.383	13	Mango_rep_c8423	13 12216908	mango_rep_c15051	69.614	14	Mi_0277	20 5577893
Mi_0194	28.056	13	Mi_0009	13 12441619	Mi_0277	68.592	14	Mango_rep_c15051	20 5608632
Mi_0084	27.711	13	Mi_0194	13 12504138	Mi_0343	67.468	14	SSKP003C1_C682T	20 6024683
Mi_0207	26.271	13	Mi_0084	13 12570705	SSKP003C1_C682T	57.583	14	Mi_0341	20 6430078
Mi_0566	21.002	13	Mi_0207	13 12646978	Mi_0341	45.595	14	Mi_0145	20 7271359
Mi_0547	18.767	13	Mi_0566	13 13101212	mango_rep_c4542	33.939	14	Mango_rep_c4542	20 7448438
Mi_0012	16.737	13	Mi_0547	13 13354718	Mi_0145	30.756	14	Mi_0450	20 8211230
Contig6127	16.661	13	Contig6127	13 13416403	Mi_0450	19.22	14	Mi_0160	20 8769515
Mi_0310	16.006	13	Mi_0012	13 13479624	Mi_0160	13.255	14	Mi_0519	20 11826283
Contig1989	11.407	13	Mi_0310	13 13618550	Mi_0519	10.2	14	Mango_rep_c12866	20 13138054
mango_rep_c10207	9.754	13	Contig1989	13 13924226	mango_rep_c12866	5.659	14	SSKP062C1_C365T	20 13559075
Mi_0081	6.673	13	Mango_rep_c10207	13 13931822	SSKP062C1_C365T	3.944	14	SSKP062C1_C365T	20 13602717
Mi_0029	5.61	13	Mi_0081	13 14129331	SSKP064C1_C176G	3.469	14	SSKP064C1_C176G	20 13640161
Mi_0139	4.473	13	Mi_0139	13 14161077	Mi_0265	1.76	14	Mi_0265	20 13951692
mango_rep_c4635	0.791	13	Mi_0029	13 14226689	Contig548	0.891	14	Contig548	20 14349110
Contig1142	0.383	13	Contig1142	13 14666630	Mi_0260	0.389	14	Mi_0076	20 14360021
mango_rep_c23136	0	13	Mango_rep_c23136	13 14687019	SSKP018C1_G427T	0.023	14	Mi_0260	20 14517734
			Mango_rep_c4635	13 15278428	Mi_0076	0	14	SSKP018C1_G427T	20 14701152

SNP - LG6	cM To Chr	SNP - Chr15	To LG	bp	SNP - LG7	cM To Chr	SNP - Chr16	To LG	bp
Contig3281	0	15	Contig3281	6 4530356	Mi_0381	151.12	16	SSKP075C1_A358G	7 74185
mango_rep_c53352	3.955	15	Mango_rep_c53352	6 4969658	SSKP075C1_A358G	145.614	16	Mi_0381	7 164024
Mi_0536	7.294	15	Mi_0536	6 5452862	mango_rep_c5276	135.496	16	Mango_rep_c5276	7 889563
Mi_0336	9.01	15	Mi_0336	6 5852040	Mi_0137	134.773	16	Mi_0137	7 901382
Mi_0170	9.446	15	Mi_0170	6 6539793	Mi_0271	133.1	16	Mi_0271	7 1031430
Mi_0380	9.692	15	Mi_0380	6 6588456	Mi_0157	131.194	16	Mi_0157	7 1403888
SSKP087C1_A198G	10.57	9	Mi_0078	6 9389756	Mi_0465	127.738	16	Mi_0465	7 1591565
Mi_0078	12.409	15	Mi_0245	6 10034398	SSKP145C1_C347T	124.003	16	SSKP145C1_C347T	7 1836791
Mi_0245	14.423	15	Mi_0437	6 10452858	Mi_0149	109.914	16	Mi_0149	7 2296849
Mi_0437	18.657	15	Contig_3450_A115T	6 11021009	Mi_0164	102.782	16	SSKP103C1_C430T	7 2677522
Contig_3450_A115T	23.833	15	Mi_0004	6 11221279	Mi_0083	99.198	16	Mi_0164	7 2699731
Mi_0004	25.765	15	Contig2937	6 12104987	SSKP103C1_C430T	98.894	16	Mi_0083	7 2732715
Contig2937	43.205	15	SSKP132C1_C377T	6 12524481	Mi_0123	92.823	16	Mi_0123	7 3281313
SSKP132C1_C377T	49.648	15	Mi_0288	6 12618529	Mi_0238	91.517	16	Contig756	9 3294001
Mi_0288	51.157	15	Contig388	6 12682065	Mi_0092	66.52	16	Mi_0238	7 3326896
SSKP001C2_C1509G	56.87	15	SSKP001C2_C1509G	6 12811702	Mi_0169	66.12	16	Mi_0092	7 4787365
Contig388	57.677	15	Mi_0019	6 13190345	Mi_0080	57.598	16	Mi_0169	7 4816176
Mi_0019	60.47	15	Mi_0174	6 13288552	mango_rep_c5591	50.741	16	Mi_0092	7 4837842
Mi_0174	61.327	15	Mi_0090	6 13313485	Contig952	50.606	16	Mi_0080	7 7998374
Mi_0090	61.904	15	Mi_0455	6 13354135	mango_rep_c11550	48.485	16	Mango_rep_c5591	7 8818242
Mi_0455	62.562	15	Mi_0206	6 14110919	Mi_0147	44.887	16	Contig952	7 8986177
Mi_0206	76.218	15	Mi_0482	6 14575347	Mi_0033	41.989	16	Mango_rep_c11550	7 9335065
Mi_0482	79.711	9	Mango_rep_c13398	6 14698801	Mi_0415	41.446	16	Mi_0147	7 9545143
mango_rep_c13398	80.435	15			Mi_0375	30.212	16	Mi_0033	7 9820254
					Mi_0199	14.183	16	Mi_0415	7 9877854
					mango_rep_c3252	0.449	16	Mi_0375	7 10534563
					Mi_0369	0.002	16	Mi_0199	7 11417562
					mango_c11445	0	16	Mi_0369	7 13664484
								Mango_c11445	7 13664584
								Mango_rep_c3252	7 13754659

Source: Wang et al. 2020

**Figure 1**

*D. Alignment of the mango linkage groups (Kuhn et al. 2017) (left) and mango physical chromosomes (right) based on the same SNPs and mapped to the 17, 19 and 20 chromosomes of the *Mangifera indica* 'Alphonso' reference genome CATAS_Mindica_2.1 (GCA_011075055.1)*



Source: Wang et al. 2020

To overcome the limitations of the mango genetic linkage map, Santos et al. (2023) employed BLAST alignment of SNPs from the Haden × Tommy Atkins population against the 20 chromosomes of the 'Alphonso' mango (physical map) for genome-wide association studies (GWAS) of resistance to dieback diseases caused by *Lasiodiplodia theobromae* and



Neofusicoccum parvum fungi. An identical GWAS procedure, but using different SNPs (133,590), was adopted by Ali et al. (2025) to identify polyembryony loci in mango.

The strategy of BLAST-aligning mango SNPs against the 'Alphonso' reference genome (Wang et al. 2020) recovered 166 SNPs that had been excluded from previous analyses for various reasons, such as aberrant segregation patterns or being unmappable (Kuhn et al. 2017). The physical map approach incorporated all genotyped SNPs, enabling better genome coverage.

The first genetic linkage map, proposed by A. Sturtevant (1913), pioneered gene mapping on chromosomes. Sturtevant used crossover frequency to identify recombinant individuals in *Drosophila*, establishing gene positions, order, and distances (in cM) on chromosomes. A genetic linkage group is defined as a set of markers where each marker is linked (in cM) to at least one other in the same set, while a physical map represents the nucleotide distance (bp) between markers on a sequenced chromosome.

Marker-assisted selection (MAS) uses molecular markers linked to agronomically important traits to accelerate cultivar development: marker presence indicates the trait of interest. Despite this appealing concept, MAS has contributed minimally to releasing improved cultivars with greater abiotic stress tolerance, with few exceptions mainly for quality traits (Henkrar and Udupa 2020).

According to Evans et al. (2021), practical outcomes of QTL mapping for MAS remain limited due to multiple factors including small population sizes, linkage group gaps, and unsaturated maps. Insufficient attention has been given to limitations in linkage group formation and marker ordering compared to physical mapping as sources of spurious QTL estimates. For species with available genome sequences, physical maps created through SNP sequence BLAST alignment yield better results for quantitative trait mapping aimed at MAS applications

5 CONCLUSION

Four major aberrations were identified: (a) chromosome 1 was divided into LGs 9 and 14; (b) chromosome 4 was split between LGs 1 and 3; (c) LG 8 was formed by combining SNPs from chromosomes 9 and 17 of 'Alphonso'; and (d) chromosome 18 was not represented in any of the 20 LGs. All remaining LGs, except LGs 11, 14 and 20, contained at least one SNP from non-corresponding reference chromosomes. Significant discrepancies in SNP ordering were observed across the 20 LGs, particularly in 12 LGs.



Grouping and ordering of linkage genetic maps must be taken into account as a source of spurious QTLs estimates. For species with available genome sequences, physical maps yield better results for quantitative trait mapping aimed at marker-assisted selection.

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