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Linkage mapping and QTL analysis in coconut (*Cocos nucifera* L.)

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Abstract Different DNA marker types were used to construct linkage maps in coconut (*Cocos nucifera* L.; 2n = 32) for the two parents of the cross Malayan Yellow Dwarf (MYD) × Laguna Tall (LAGT). A total of 382 markers was sufficient to generate 16 linkage groups for each parent. The total genome length corresponded to 2226 cM for the LAGT map and 1266 cM for the MYD map with 4–32 markers per linkage group. Common markers allowed the association of 9 linkage groups for the two parents MYD and LAGT. QTL analysis for the trait early germination identified six loci. These QTLs correlate with early flowering and yield, representing characters which are important in coconut breeding. The co-segregation of markers with these QTLs provides the first opportunity for marker-assisted selection in coconut breeding programmes.

Key words AFLP · DNA markers · Early germination · ISSR · ISTR · RAPD

Introduction

Coconut (*Cocos nucifera* L.) is a perennial oil plant cultivated in coastal regions of the tropics some 20 degrees north and south of the equator. Owing to its importance to the rural communities, it has been termed "tree of life", since from the root to the frond all parts of the coconut palm are utilized for either nutritional or non-food purposes (Persley 1992). Among the oil crops, the coconut palm plays a special role due to the high lauric acid content of its oil (Jones 1991). However, coconut oil as the economically most important product of coconut processing is facing heavy competition in the world market. Furthermore, the increasing age of many coconut plantations, especially in the Philippines, which is still the major export country of coconut oil, is an increasingly important factor that contributes to the decline in coconut production.

A further area of concern for coconut production is the sensitivity of the coconut palm to biotic and abiotic stress conditions. While fungal, bacterial, viral and viroid pathogens contribute substantially to losses in coconut, phytoplasm causing "lethal disease" or "lethal yellowing" represents the most devastating pathogen in coconut (in-depth information can be found at the websites http://www.cicy.mx/dir_acad/cicly/main and http://www.ftld.ufl.edu/lyfacts.htm). With reference to abiotic stress, sensitivity to drought is a highly important factor, since it limits coconut propagation to the coastal areas where rainfalls prevail. Thus, breeding in coconut has to aim for high-yielding hybrids with tolerance or resistance to biotic and abiotic stress factors.

In view of the time-consuming process in coconut breeding, modern molecular technologies like DNA markers have a great potential to assist in and accelerate breeding programmes. Various DNA marker technologies have been applied to coconut biodiversity analysis through DNA fingerprinting [inverse sequence-tagged repeat (ISTR): Rohde et al. 1995; Duran et al. 1997; randomly amplified polymorphic DNA (RAPD): Ashburner et al. 1997; Duran et al. 1997; restriction fragment length polymorphism (RFLP): Lebrun et al. 1998; amplified fragment length polymorphism (AFLP): Perera et al. 1998; simple sequence repeat (SSR): Perera et al. 1999]. Basically, two main groups (Indian Ocean and Pacific Ocean germplasm) of coconut have been identified by the various techniques. Analysis of DNA polymorphisms has also revealed that the two main coconut types "Tall" and "Dwarf" show different degrees of polymorphisms

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with more polymorphism being present in Tall coconut types than in the Dwarf types.

With the availability of F_1 mapping populations from controlled crosses involving (partially) heterozygous parents from both coconut types, linkage mapping of identified polymorphic markers has become possible, and an initial linkage analysis based entirely on ISTR markers has recently been described (Rohde et al. 1999). This work was extended using different DNA marker types including AFLP (Vos et al. 1995), ISTR (Rohde 1996), RAPD (Williams et al. 1990) and ISSR (Zietkiewicz et al. 1994). In this paper we present linkage maps of 16 linkage groups each for the two coconut types Laguna Tall (LAGT), an ecotype indigenous to the Philippines, and Malayan Yellow Dwarf (MYD), which is generally used in coconut breeding programmes around the world.

Materials and Methods

Plant material

An F_1 population of 52 genotypes descending from a cross between a Malayan Yellow Dwarf genotype (MYD20) and a Laguna Tall genotype (LAGT07) was used for linkage mapping and quantitative trait locus (QTL) analyses. Establishment of the MYD × LAGT mapping population has been described in detail elsewhere (Rohde et al. 1999). Genomic DNA was extracted from young spear leaf material as described by Rohde et al. (1995).

Molecular methods

RAPD markers were produced as described by Duran et al. (1997) with modifications by Rodriguez et al. (1997) using a Biometra UNO (Version 2.72bb) thermal cycler and *Taq* polymerase from Gibco/BRL for polymerase chain reaction (PCR) amplification. Random 10-mer primers supplied by Operon Technologies (Alameda, Calif.) were firstly analysed with parental DNAs for the presence of polymorphic fragments and, if appropriate, applied to the mapping population. A total of 28 primers listed in Table 1 were used for linkage mapping. The amplification products were separated by agarose gel electrophoresis in TBE buffer and visualized by ethidium bromide staining applying standard methodology (Sambrook et al. 1989).

ISTR analyses were performed as described by Duran et al. (1997) using a total of 24 primer pairs, *Taq* polymerase from GIBCO/BRL and a Biometra UNO thermal cycler for the PCR reactions. Amplified ISTR fragments generated in the presence of [^{33}P]-labelled ISTR primers were separated on sequencing gels and detected by autoradiography (Rohde 1996). Unlabelled DNA fragments (Table 1) were detected by silver staining following the protocol of Bassam et al. (1991).

AFLP fragments were produced with 44 primer combinations according to the protocol of Vos et al. (1995). Different techniques were used for the visualization of amplification products as indicated in Table 1. AFLP fragments were detected by autoradiography, by silver staining according to the protocol of Bassam et al. (1991) or on a LI-COR 4200-S1 DNA sequencer. For this, primers were labelled with the fluorescent infrared dye IRD800 (LI-COR, Lincoln, Neb.) and fragment analysis recorded by a laser system as described by the manufacturer (LI-COR, 1997).

ISSR fragments were generated as described by Fang et al. (1997) with some modifications. PCR products were separated on denaturing 6% polyacrylamide gels in the presence of 7.5 *M* urea and detected by silver staining.

Data analysis and linkage mapping

Polymorphic DNA fragments were scored for presence or absence in parents and F_1 progenies. Linkage analysis between marker fragments, estimation of recombination frequencies and determination of linear orders between linked loci including multipoint linkage analysis and the EM algorithm for handling missing data were performed as described by Ritter et al. (1990) and Ritter and Salamini (1996). The MAPRF programme was applied for the computational methods. First linkage groups were constructed based on fragments specific to either parent. Subsequently, fragments common to both parents were integrated into linkage groups as anchor points as described by Ritter et al. (1990).

QTL analysis

The mapping population was examined for early germination. Following coconut breeders' standardized research techniques (Santos et al. 1995), early germination was determined by the number of days from the date of nut harvest until germination in the nursery. A controlled environment was provided in that nuts were grown in identical soil in polyethylene bags and by shading of the nursery to prevent extreme temperature differences.

QTLs were mapped using least square interval mapping methods developed for backcross progeny according to Knapp et al. (1990) and Knapp and Bridges (1990) and applied to intervals composed of individual markers for both parents. SAS software (SAS Institute Inc. 1989) and, in particular, the procedure PROC NLIN were used for computational analysis. In addition, QTL analyses were performed at single marker loci according to Soller et al. (1976). Progeny genotypes were divided into two subgroups based on the presence or absence of a fragment. The trait means of these two marker classes were tested for significant differences with the two-sample *t*-test using SAS software.

Results

Generation of polymorphic DNA markers

A total of 24 ISTR, five ISSR and 44 AFLP primer combinations were used for the molecular analyses and generated 87, 6 and 235 segregating fragments, respectively. In addition 54 polymorphic fragments descending from 28 RAPD primers were obtained, so that a total of 382 segregating polymorphic DNA markers was available for map construction.

In addition, a high number of non-segregating DNA fragments was obtained with each marker type. On average, only 9.6% of the AFLP, 11.1% of the ISTR, 6.2% of the ISSR and 18.1% of the RAPD DNA fragments segregated between progeny genotypes. The number of polymorphic markers varied considerably between primer combinations with 5, 8 and 14 segregating fragments representing the highest number for individual RAPD, ISTR and AFLP primer, respectively. ISSR primers produced only one or two polymorphic markers. A total of 211 segregating fragments were specific for the LAGT parent, 149 fragments descended from the MYD parent and 22 fragments were present in both parents (common fragments, Table 2). Thirty-six fragments showed significant deviations from the expected segregation ratios (Table 1).

Table 1 Polymorphisms and distorted markers obtained with different molecular marker types in the coconut progeny (MYD × LAGT)

Number	Marker-type	Primers	Number of polymorphic bands	Detection method[a]	Number	Marker type	Primers	Number of polymorphic bands	Detection method[a]
1	ISTR	B5/F7c	5	R	23	AFLP	E35/M33	4	R
2	ISTR	B5/F1a	1	R	24	AFLP	E35/M36	5	R
3	ISTR	B5a/F7	5	R	25	AFLP	E35/M41	5	R
4	ISTR	B5a/F3	5	R	26	AFLP	E37/M34	6	R
5	ISTR	B5a/F1	5	R	27	AFLP	E37/M36	4	R
6	ISTR	B3/F7a	3	R	28	AFLP	E43/M33	9	R
7	ISTR	B2b/F4	4	R	29	AFLP	E43/M36	5	R
8	ISTR	B2a/F4	3	R	30	AFLP	E39/M33	3	R
9	ISTR	B2a/F1	5	R	31	AFLP	E39/M36	4	R
10	ISTR	B1a/F4	8	R	32	AFLP	E39/M41	4	R
11	ISTR	B1a/F3	5	R	33	AFLP	E33/M49	6	R
12	ISTR	B1a/F1	2	R	34	AFLP	E33/M54	5	R
13	ISTR	B9/F6a	5	R	35	AFLP	E34/M49	8	R
14	ISTR	B7/F7a	4	R	36	AFLP	E79/M81	5	S
15	ISTR	B8/F1	2	R	37	AFLP	E63/M66	5	S
16	ISTR	B9/F7	2	R	38	AFLP	E654/M66	2	S
17	ISTR	B2b/F5	4	R	39	AFLP	E63/M64	1	S
18	ISTR	B2b/F3	4	R	40	AFLP	E65/M64	8	S
19	ISTR	B2a/F5	3	R	41	AFLP	E63/M65	8	S
20	ISTR	B1a/F9a	1	R	60	AFLP	E79/M80	4	S
21	ISTR	B5a/F7c	1	R	61	AFLP	E81/M81	7	S
22	ISTR	B5/F9a	6	R	62	AFLP	E81/M82	5	S
75	ISTR	B2a/F6	3	S	63	AFLP	E81/M80	5	S
76	ISTR	B2b/F6	1	S	64	AFLP	E82/M81	8	S
Subtotal:	**No. 24**	**Fragments: 87**			65	AFLP	E82/M82	3	S
					79	AFLP	E86/M83	2	S
42	RAPD	A12	3	E	80	AFLP	E85/M86	3	S
43	RAPD	A18	1	E	81	AFLP	E85/M85	5	S
44	RAPD	A19	5	E	82	AFLP	E83/M86	2	S
45	RAPD	AQ9	1	E	83	AFLP	E83/M85	8	S
46	RAPD	AS11	1	E	84	AFLP	E83/M84	1	S
47	RAPD	B1	1	E	85	AFLP	E83/M83	3	S
48	RAPD	B19	1	E	86	AFLP	E94/M95	5	F
49	RAPD	B20	2	E	87	AFLP	E94/M92	4	F
50	RAPD	C11	2	E	88	AFLP	E94/M91	8	F
51	RAPD	C12	1	E	89	AFLP	E94/M90	7	F
52	RAPD	C13	3	E	90	AFLP	E94/M89	13	F
53	RAPD	C14	1	E	91	AFLP	E94/M88	8	F
54	RAPD	D20	1	E	92	AFLP	E94/M87	2	F
55	RAPD	D6	3	E	93	AFLP	E94/M84	1	F
56	RAPD	D14	1	E	94	AFLP	E94/M83	10	F
66	RAPD	P10	1	E	95	AFLP	E94/M82	1	F
67	RAPD	W3	2	E	96	AFLP	E94/M81	14	F
68	RAPD	W4	3	E	97	AFLP	E94/M79	9	F
69	RAPD	W5	1	E	**Subtotal:**	**No: 44**	**Fragments:**	**235**	
70	RAPD	W11	1	E					
71	RAPD	W14	2	E	57	ISSR	(GA)8YG	1	S
72	RAPD	W15	2	E	58	ISSR	HVH(CA)7	2	S
73	RAPD	X20	1	E	59	ISSR	HVH(TCC)	1	S
74	RAPD	Al18	3	E	77	ISSR	(TCC)5RY	1	S
98	RAPD	X10	4	E	78	ISSR	BDB(CA)7	1	S
99	RAPD	AX11	3	E	**Subtotal:**	**No: 5**	**Fragments:**	**6**	
100	RAPD	AX13	2	E					
101	RAPD	X5	2	E					
Subtotal:	**No: 28**	**Fragments: 54**							

Total: polymorphic bands: 382 primer/primer combinations: 101

Distorted markers
ISTR: 10 RAPD: 11 AFLP: 15 ISSR: 0 Total: 36 (10.6%)
Descendance of markers: LAGT: 23 MYD: 12 common: 1

[a] R, Radioactivity; S, silver staining; E, ethidium bromide; F, fluorescence

Table 2 Characteristics of the parental linkage maps from the cross MYD × LAGT and marker distribution of total and mapped markers (*LG* linkage group)

A Linkage maps

Parent LAGT						Parent MYD				
LG	Name	Length (cM)	Individual markers	Common markers	Total	Name	Length (cM)	Individual markers	Common markers	Total
1	I	110.2	13	0	13	L1 (VII)	42.1	8	1	9
2	II	123.1	11	0	11	L2 (VIII)	77.4	13	1	14
3	III	106.1	14	1	15	L3 (X)	103.2	16	3	19
4	IV	99.3	11	0	11	L4	111.1	9	0	9
5	V	147.2	14	2	16	L5 (XIII)	148.3	27	5	32
6	VI	166.1	11	0	11	L6	125.4	22	0	22
7	VII	122.3	11	2	13	L7 (XI)	116.9	7	1	8
8	VIII	165.8	20	1	21	L8 (V)	70.6	4	2	6
9	IX	154.7	11	1	12	L9	80.6	4	0	4
10	X	173.2	16	3	19	L10	41.8	4	0	4
11	XI	142.3	14	1	15	L11	59.1	4	0	4
12	XII	162.4	11	0	11	L12 (III)	59.7	3	1	4
13	XIII	135.3	9	5	14	L13	87.9	6	0	6
14	XIV	139.4	8	0	8	L14	35.4	4	0	4
15	XV	142.3	6	0	6	L15 (XVI)	44.9	3	2	5
16	XVI	136.4	8	2	10	L16 (IX)	61.3	3	1	4
	Total:	**2226.1**	**188**	**18**	**206**	**Total:**	**1265.6**	**137**	**17**	**154**
Total markers both maps:									**325**	**18**

B Marker distribution of total and mapped markers

Descendence of markers	ISTR		AFLP		RAPD		ISSR		Total	
	Total	Mapped	Total	Mapped	Total	Mapped	Total	Mapped	Total	Mapped
LAGT	50	45	126	122	32	17	3	3	211	187
MYD	32	31	92	86	22	18	3	3	149	138
Comon markers	5	5	17	13	0	0	0	0	22	18
Total:	**87**	**81**	**235**	**221**	**54**	**35**	**6**	**6**	**382**	**343**

Construction of linkage maps

Linkage maps of 16 linkage groups each were obtained for the two parents of the mapping population (Fig. 1A,B). Their characteristics are summarized in Table 2. Linkage groups of the LAGT map contained 6–21 markers each and were between 99.3 and 173.2 cM in length (Table 2). The total LAGT map length was 2226 cM. The MYD map was 1266 cM in length and was made up of linkage groups with 4–32 markers each. Despite this range, marker distribution was quite variable in the different linkage groups. Many markers clustered in a few linkage groups, while 6 linkage groups were composed of only 4 markers each (Table 2). The sizes of the linkage groups varied between 35.4 and 148.3 cM.

In total, 343 (89.8%) of the 382 markers could be mapped to the 32 linkage groups. Association of the residual 39 markers – although linkage to mapped markers on linkage groups was apparent – was ambiguous, so that they were discarded. Only 6 ISTR and 14 AFLP markers remained unmapped, while 35% of the RAPD markers could not be assigned clearly to any linkage group. Based on the integration of common markers into linkage groups for both parents, it was possible to assign nine homologous chromosomes for LAGT and MYD. This assignment is reflected in the names of the linkage groups of the MYD parent (Roman numbers in brackets). Except for c40/6, all common markers could be integrated into the maps. This marker was not found to be statiscally linked to marker 78/1 on MYD linkage group L1 and was, therefore, omitted in Fig. 1B.

QTL analyses

On the average, progeny genotypes germinated after 58 days (Table 3). This value is in good agreement to that reported by Fremond et al. (1971) in their observation of germination of MYD × WAT progenies (cited by Balingasa and Santos 1977). However, there was considerable variation of trait expression in the individual MYD × LAGT progeny genotypes as reflected by the corresponding minimum and maximum values (52 and 160 days, respectively) and a coefficient of variation of 47.2% (Table 3).

Six QTLs were detected for early germination showing absolute effects between 20.5 and 26 days of reduc-

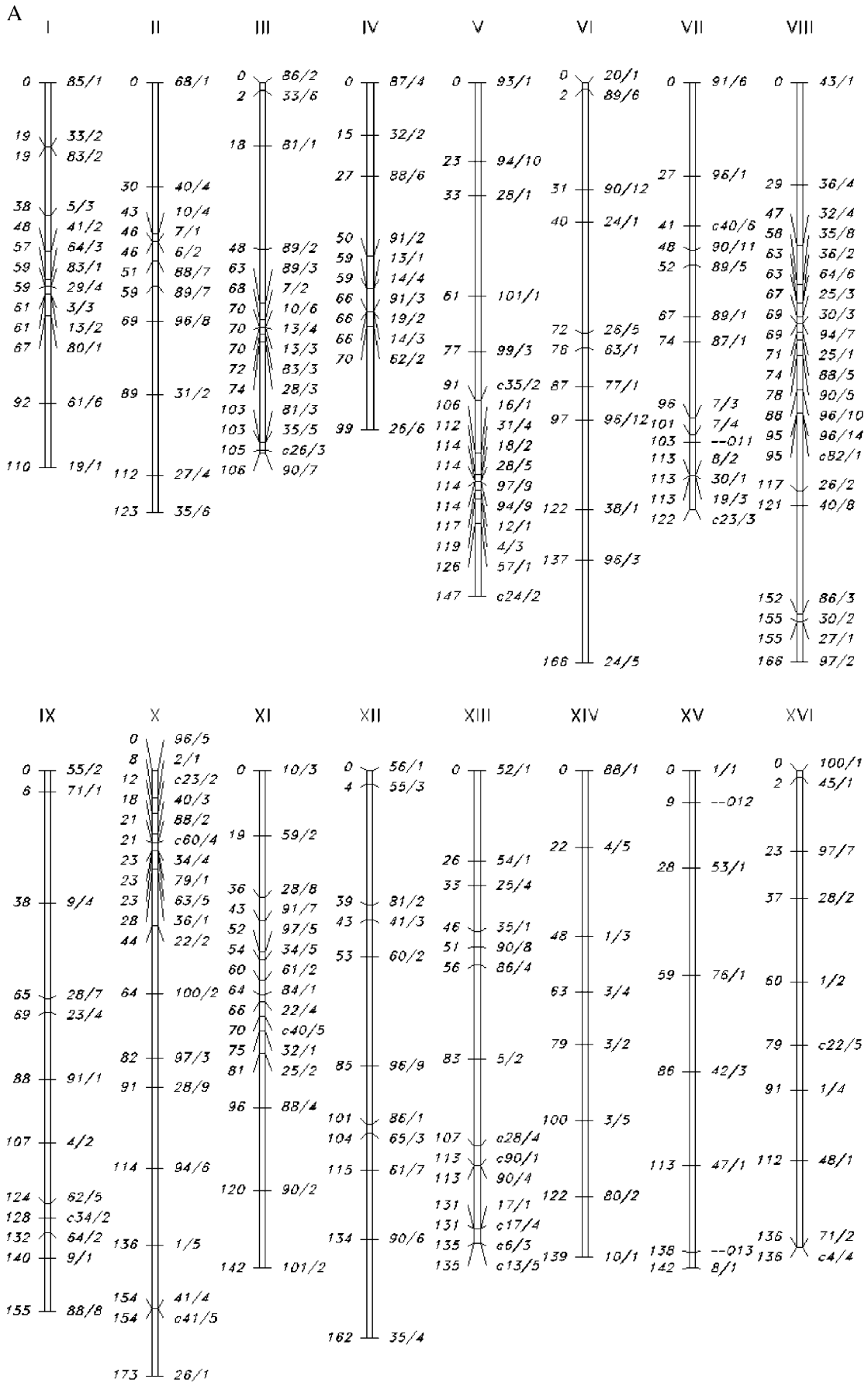
A
I
0 85/1
19 33/2
19 83/2
38 5/3
48 41/2
57 64/3
59 83/1
59 29/4
61 3/3
61 13/2
67 80/1
92 61/6
110 19/1
II
0 68/1
30 40/4
43 10/4
46 7/1
46 6/2
51 88/7
59 89/7
69 96/8
89 31/2
112 27/4
123 35/6
III
0 86/2
2 33/6
18 81/1
48 89/2
63 89/3
68 7/2
70 10/6
70 13/4
70 13/3
72 83/3
74 28/3
103 81/3
103 35/5
105 c26/3
106 90/7
IV
0 87/4
15 32/2
27 88/6
50 91/2
59 13/1
59 14/4
66 91/3
66 19/2
66 14/3
70 62/2
99 26/6
V
0 93/1
23 94/10
33 28/1
61 101/1
77 99/3
91 c35/2
106 16/1
112 31/4
114 18/2
114 28/5
114 97/9
114 94/9
117 12/1
119 4/3
126 57/1
147 c24/2
VI
0 20/1
2 89/6
31 90/12
40 24/1
72 26/5
76 63/1
87 77/1
97 96/12
122 38/1
137 96/3
166 24/5
VII
0 91/6
27 96/1
41 c40/6
48 90/11
52 89/5
67 89/1
74 87/1
96 7/3
101 7/4
103 --Q11
113 8/2
113 30/1
113 19/3
122 c23/3
VIII
0 43/1
29 36/4
47 32/4
58 35/8
63 36/2
63 64/6
67 25/3
69 30/3
69 94/7
71 25/1
74 88/5
78 90/5
88 96/10
95 96/14
95 c82/1
117 26/2
121 40/8
152 86/3
155 30/2
155 27/1
166 97/2
IX
0 55/2
6 71/1
38 9/4
65 28/7
69 23/4
88 91/1
107 4/2
124 62/5
128 c34/2
132 64/2
140 9/1
155 88/8
X
0 96/5
8 2/1
12 c23/2
18 40/3
21 88/2
21 c60/4
23 34/4
23 79/1
23 63/5
28 36/1
44 22/2
64 100/2
82 97/3
91 28/9
114 94/6
136 1/5
154 41/4
154 c41/5
173 26/1
XI
0 10/3
19 59/2
36 28/8
43 91/7
52 97/5
54 34/5
60 61/2
64 84/1
66 22/4
70 c40/5
75 32/1
81 25/2
96 88/4
120 90/2
142 101/2
XII
0 56/1
4 55/3
39 81/2
43 41/3
53 60/2
85 96/9
101 86/1
104 65/3
115 61/7
134 90/6
162 35/4
XIII
0 52/1
26 54/1
33 25/4
46 35/1
51 90/8
56 86/4
83 5/2
107 c28/4
113 c90/1
113 90/4
131 17/1
131 c17/4
135 c6/3
135 c13/5
XIV
0 88/1
22 4/5
48 1/3
63 3/4
79 3/2
100 3/5
122 80/2
139 10/1
XV
0 1/1
9 --Q12
28 53/1
59 76/1
86 42/3
113 47/1
138 --Q13
142 8/1
XVI
0 100/1
2 45/1
23 97/7
37 28/2
60 1/2
79 c22/5
91 1/4
112 48/1
136 71/2
136 c4/4

Fig. 1A

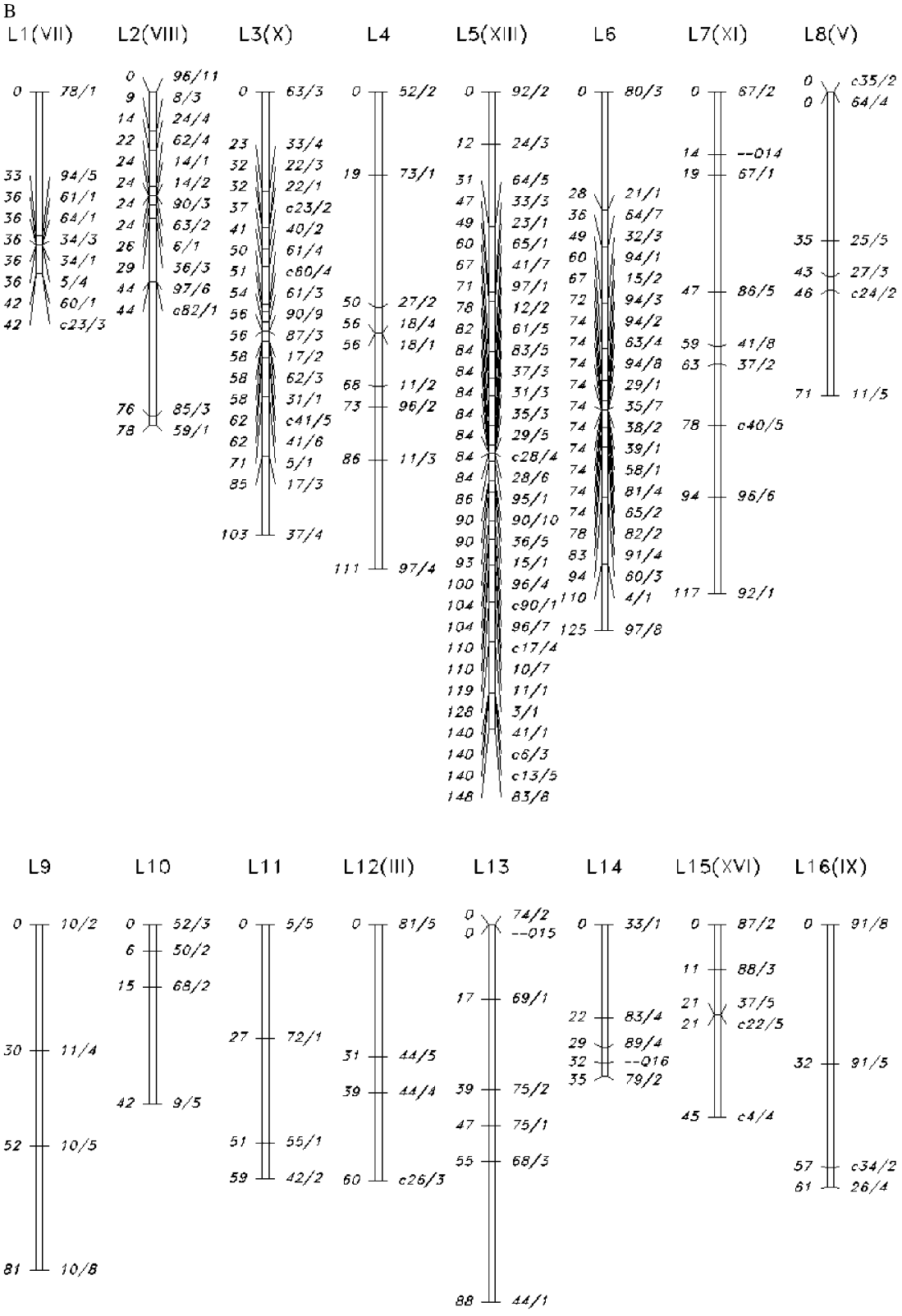

Fig. 1B

Fig. 1A, B Linkage maps of coconut genotypes LAGT07 (**A**) and MYD20 (**B**). "–Qi" represent OTLs for early germination. Common markers carry the prefix "c". Distances are given in centi-Morgans (cM, Kosambi units; Kosambi 1944). See Tables 2 and 3 for details on linkage groups and QTLs

Table 3 Results of QTL analyses for the trait early germination

QTL	Descendance of QTL	Effect (d)	Interval	Chrom[a]	Interval length (cM)	Position[b]	Probability[c] (%)	R2[d] (%)
Q11	LAGT	22.7	7/4—8/2	VII	9.6	9.6	3.4	12.4
Q12	LAGT	24.7	1/1—53/1	XV	27.9	9.2	4.0	14.3
Q13	LAGT	26.4	47/1—8/1	XV	29.2	24.7	3.6	11.0
Q14	MYD	24.8	67/2—67/1	L7 (XI)	19.2	14.4	1.7	14.3
Q15	MYD	20.5	74/2—69/1	L13	17.3	0	1.1	12.7
Q16	MYD	22.7	89/4—79/2	L14	6.8	3.6	4.7	13.0
Mean:	58.2	Min:	52	Max:	160	CV:	47.2	

[a] Location of interval on linkage group (Table 2)
[b] Position of QTL with respect to the upper flanking marker of the interval (Fig. 1A,B)
[c] Probability for the null hypothesis of no QTL
[d] Portion of total variance explained by the QTL

tion in germination time. Half of them descended from the Laguna Tall parent and were located on marker intervals of two different chromosomes (Table 3). The proportion of the total variance explained by a single QTL varied between 11% and 14.3%.

Discussion

The generation of segregating polymorphic DNA markers

Various PCR-based DNA marker techniques were used to generate polymorphic DNA fragments for the parental coconut genotypes LAGT and MYD as well as the 52 F_1 progenies of the MYD × LAGT mapping population. Based on a comparison of the different marker types, we consider AFLP and ISTR markers to be more efficient than RAPD or ISSR markers, since the average number of fragments per primer combination for the former was found to be considerably higher than that for RAPD or ISSR markers. With the exception of ISSR, a large number of amplification products was obtained by AFLP, ISTR and RAPD, but only a relatively low percentage segregated in the MYD × LAGT mapping population. Both LAGT and MYD belong to the Pacific Ocean germplasm group, but similar results have been obtained in a mapping population with parents of different origins [East African Tall (EAT) × Pemba Red Dwarf (PRD); Rohde et al., unpublished results], where EAT is Indian Ocean germplasm, while PRD is a representative of the Pacific Ocean germplasm group (Rohde et al. 1995).

This low percentage of segregating DNA markers in coconut contrasts with results obtained from other crops like potato where some 38 and 49 segregating polymorphisms per AFLP primer combination were observed in a non-inbred offspring (van Eck et al. 1995). Also, with a woody species like *Larix*, 12–41 segregating AFLP fragments per primer combination were detected with a corresponding mapping population (Arcade et al. 1999). On the other hand, polymorphisms are quite frequently found within genebank coconut accessions that represent a broad range of germplasm (Rohde et al. 1995; Duran et al. 1997; Rodriguez et al. 1997). For EAT genotypes, for example, an average of 34% dissimilarities were found (Duran et al. 1997). As for AFLP and ISTR markers in the MYD × LAGT mapping population, 83.5% of the generated DNA fragments were not polymorphic, but common to both parents, indicating that the two parents – despite their morphological differences – share a large number of identical loci and a high degree of homozygosity in either one or both parents.

As mentioned above, the number of segregating fragments was considerably higher in the LAGT parent. However, this parent also revealed a higher number of non-segregating, parent-specific fragments (272 fragments in LAGT, 206 in MYD), while the percentage of segregating fragments within the total of parent-specific fragments was similar for both parents (59.2% in MYD and 58.1% in LAGT). Lebrun et al. (1998) indicated that dwarf genotypes are generally considered to be autogamous, while tall genotypes are more allogamous. However, taking into account that autogamy will lead to an increased level of homozygosity, our findings suggest that there is a similar degree of homozygosity in the dwarf and tall genotypes used to establish the mapping population. This high level of homozygosity in the tall genotype might result from the reduced number of genotypes which enter into the actual breeding programme. Further studies are necessary to investigate whether the reduced number of amplification products for the MYD parent genotype has resulted, for example, from an increased level of conserved repetitive regions or from deletions.

Arrangement of DNA markers into coconut linkage maps

The analysis of segregating DNA markers established 16 independent linkage groups for the coconut genotypes LAGT and MYD, respectively (i.e.: lateral markers were not statistically linked to any other lateral marker of any other linkage group). These 16 linkage groups may correspond to the 16 chromosomes of the haploid coconut genome (2x = 32). Moreover, the presence of common markers made it possible to identify a total of nine homologous chromosomes in each parent. With several common markers of the same order present in chromosomes of both parents, it is possible to combine the information of markers from different individuals as described by Ritter and Salamini (1996). In this way the number of markers available per chromosome can be increased. However, this situation was only present in two of the nine homologous chromosome pairs. Therefore, more codominant markers are needed to combine marker

information from different parents. The mapping of recently developed coconut microsatellites (SSRs, Rivera et al. 1999; Perera et al. 1999) and of single nucleotide polymorphisms (SNPs) established during a sequencing programme of coconut genes belonging to homeotic gene families (Rohde, unpublished) offers one possibility to achieve this goal by analogy to strategies described, for example, for barley (Castiglioni et al. 1998).

Although most of the segregating polymorphic markers could be assigned to linkage groups, considerable differences were noticed depending on the marker type. This was specifically evident for RAPD markers, one-third of which could not be assigned unambiguously. Also, the degree of distorted segregations was higher for this marker type. Problems related to the reproducibility of RAPD markers and the comigration of equally sized fragments are well-known for this marker type (Black 1993). The results presented here suggest that the application potential of RAPD markers may also be limited for linkage mapping. Since some RAPD markers map to the distal ends of linkage groups or represent the majority of markers for linkage groups such as MYD L10 and L11 (Fig. 1B), future efforts will be directed to covering these regions by other marker types.

The total length of linkage maps differed for LAGT and MYD. Relative to the LAGT map, the MYD map was approximately 50% shorter, which resulted from a reduced number of markers, and a higher number of markers could not be assigned to linkage groups. Therefore, the genome coverage of the MYD map is more reduced than that of the LAGT map. On the other hand, the total map length of the LAGT parent is relatively high, while in other species, like for example, *Prunus*, which has a small genome of 2n = 16 chromosomes, the linkage map comprises only 491 cM covered by 246 markers (Joobeur et al. 1998). Also, in potato, linkage maps comprise 1034 cM for 2n = 24 chromosomes and 304 mapped loci (Gebhardt et al. 1991). In contrast, in conifers which have a relatively large genome, a linkage map of *Picea abies* with 17 linkage groups extends to 3584 cM containing 185 loci (Binelli and Bucci 1994). The nuclear DNA amount of coconut (Röser et al. 1997; estimated genome size of 3.6×10^9 bp) is also very high relative to that of other herbaceous species and could explain the relatively large size of the coconut map. According to Gebhardt et al. (1991) genomic regions with aberrant segregation ratios of markers might be related to the introgression of wild species. The finding that coconut map distortions are low compared to the situation in other crops could be explained by the fact that "normal" coconut genotypes have been used for the cross.

QTL analysis

Molecular methods have only recently been introduced into coconut breeding, and the plant material required for these applications differs from that used in classical breeding. Therefore, unselected progenies of a large size and elevated age are not currently available for this perennial crop. The early germination trait evaluated corresponds to that which is currently at our disposal from the young genotypes of the mapping population. Preliminary data of other characters like girth size and leaf production are also available, but repeated measurements over longer time periods are necessary to draw valid conclusions (Santos et al. 1995).

Despite of the relatively small size of the mapping population (52 F_1 progenies), a number of QTLs were detected for the trait under study, since a considerable amount of variation is present in the data as reflected in the corresponding coefficient of variation. As mentioned above, QTL analysis was performed by single marker *t*-tests and interval regression which involved four marker classes. QTLs indicated in Table 3 refer only to those detected by interval mapping and also found to be highly significant by *t*-tests. On the other hand, several additional significant trait mean differences were detected using *t*-tests. This latter approach, however, is less sensitive, particularly with a small sample size, and does not allow an estimation of the recombination frequency between the marker and the QTL (Lander and Botstein 1989). Consequently, a small putative QTL close to a marker may show the same effect as a large QTL located at a longer distance from a marker (Lander and Botstein 1989; Knapp et al. 1990). Nevertheless, the small progeny size decreases the sensitivity of detecting QTLs, and only main QTLs can be detected, while smaller ones might remain unrevealed. This effect is also visible in the reduced number of significant, adjacent intervals pointing to a QTL in the neighbourhood (results not shown). On the other hand, smaller QTLs might be less stable across different environments and different germplasm and, therefore, their use is also limited.

According to Wuidart (1979) and Santos et al. (1995), early germination is linked to precocity and flowering. This has been earlier established by Liyanage who obtained a highly significant positive correlation of 0.437 between sprouting of seednuts and flowering of palms and a negative correlation of 0.424 between sprouting and yield (cited in Menon and Pandalai 1958). Therefore, seednuts which germinate early eventually flower within a shorter period and are more productive than those which germinate at a much later time. Hence, the QTLs detected in this study for the trait early germination point to genomic locations of genes for important characters. Molecular markers closely linked to this trait will provide the opportunity to select promising genotypes at early seedling stages through marker-assisted selection.

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