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A genetic linkage map of Guinea yam (*Dioscorea rotundata* Poir.) based on AFLP markers

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Abstract A genetic linkage map of the tetraploid white yam (*Dioscorea rotundata* Poir.) was constructed based on 341 co-dominantly scored amplified fragment length polymorphism (AFLP) markers segregating in an intraspecific F_1 cross. The F_1 mapping population was produced by crossing a landrace cultivar TDr 93-1 as female parent to a breeding line TDr 87/00211 as the male parent. The marker segregation data were split into maternal and paternal data sets, and separate genetic linkage maps were constructed since the mapping population was an F_1 cross between two presumed heterozygous parents. The markers segregated like a diploid cross-pollinator population suggesting that the *D. rotundata* genome is an allo-tetraploid (2n = 4*x* = 40). The maternal map comprised 155 markers mapped on 12 linkage groups with a total map length of 891 cM. Three linkage groups consisted of maternal parent markers only. The paternal map consisted of 157 markers mapped on 13 linkage groups with a total map length of 852 cM. Three and one quantitative trait loci (QTLs) with effects on resistance to *Yam Mosaic Virus* (YMV) were identified on the maternal and paternal linkage maps, respectively. Prospects for detecting more QTLs and using marker-assisted selection in white yam breeding appear good, but this is subject to the identification of additional molecular markers to cover more of the genome.

Keywords Yam · *Dioscorea rotundata* · Genetic mapping · Tetraploid · *Yam Mosaic Virus* · (YMV)

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Introduction

Yams (*Dioscorea* spp.) constitute a staple food crop for over 100 million people in the humid and subhumid tropics. They constitute a multi-species, polyploid crop that is vegetatively propagated. Although 90% of the world production, estimated at about 37.7 million metric tons (FAO 1999), occurs in West and Central Africa, the crop is also important in other regions (Coursey 1967; Degras 1993). *Dioscorea rotundata* and *Dioscorea cayenensis* (both known as Guinea yams) are the most important yams in West and Central Africa where they are indigenous, while *Dioscorea alata* (referred to as the water yam or the greater yam) is the most widely distributed species globally. Yams have suffered from research neglect in spite of their importance. Hence, there are many challenges facing efforts aimed at increased productivity of the crop. In recent years, some progress has been made using isozyme (Hamon and Toure 1990; Dansi et al. 2000) and molecular markers (Terauchi et al. 1992; Ramser et al. 1996, 1997; Mignouna et al. 1998) for yam germplasm characterisation and for phylogenetic studies. The genetics of yams is least understood among the major staple food crops (Martin 1966; Zoundjihékpon et al. 1994). The challenges to genetic analysis include poor to non-flowering, heterozygosity, dioecy, the polyploid status of the crop and the lack of classical markers. Although controlled crosses can be made among selected flowering genotypes, the extended juvenile growth habit results in a period of 2–3 years from seed to seed. In addition, various levels of ploidy and the non-existence of a diploid relative of the cultivated polyploid yams have complicated genetic studies. Before the present study, no

sound data had been published to indicate the type of polyploidy present in yams (allo- or auto-polyploidy). The basic chromosome number reported so far is 10 and reported ploidy levels vary from 2n = 4*x* to 2n = 14*x* (Baquar 1980; Zoundjihékpon et al. 1994).

The apparent complexity of the *D. rotundata* genome calls for the application of modern molecular markers to unravel it and to assist in gaining a better insight into the inheritance of factors controlling important agronomic traits. We recently showed that resistance to the most-damaging foliar diseases of yam (anthracnose and yam mosaic disease) is inherited polygenically (Mignouna et al. 2001a, b). Genes controlling such traits are usually distributed among several quantitative trait loci (QTLs), which may not be linked, thus making these traits difficult to manipulate using conventional breeding methods. The recessive nature of *Yam Mosaic Virus* resistance in some *D. rotundata* genotypes means that such resistance cannot be identified at the phenotypic level, demanding refined diagnostic procedures such as molecular mapping for detailed genetic localization of specific genes (Mignouna et al. 2001b). Screening by molecular markers linked to QTLs has the advantage of selecting pairs of parents with genes at different QTLs for the same trait (Solomon-Blackburn and Barker 2001). The usefulness of a saturated linkage map for analysing the complex nature of the inheritance of quantitative traits has already been demonstrated in cowpea (Ubi et al. 2000) and common bean (Miklas et al. 2001).

Amplified fragment length polymorphism (AFLP) and microsatellite markers have been proposed as a class of PCR-based DNA markers for genetic mapping. AFLP markers do not require sequence information, and a large number of markers can be assayed on a single gel (Vos et al. 1995). The high level of polymorphism makes AFLP markers an attractive choice for genetic mapping (Mackill et al. 1996), and the utility of these markers has been demonstrated in several plant genetic studies (Van Eck et al. 1995; Folkertsma et al. 1996; Keim et al. 1997; Miklas et al. 2001). Within the genus *Dioscorea* genetic linkage mapping using scored AFLP and microsatellite markers was first performed on the wild diploid species *Dioscorea tokoro* Makino (Terauchi and Kahl 1999). This wild species is widely distributed in East Asia and is classified into the botanical section Steno-phora, which is phylogenetically distant from the Section Enantiophyllum to which all the important cultivated yams belong within the genus *Dioscorea*, family Dioscoreaceae. The *D. tokoro* map therefore has little immediate use for genetic improvement in cultivated yams.

Our objective was to produce the first comprehensive molecular linkage map for tetraploid *D. rotundata* and to identify QTLs controlling resistance to *Yam Mosaic Virus* (YMV), genus *Potyvirus*, a major constraint to white yam production (Thouvenel and Dumont 1990).

Materials and methods

Mapping population and YMV resistance screening

The ploidy levels of various flowering genotypes of *D. rotundata* were determined using flow cytometry. Three levels of ploidy (tetraploid, hexaploid and octoploid) were found in the cultivars analysed based on previously reported chromosome numbers in yams (Zoundjihékpon et al. 1994). Two tetraploid genotypes with contrasting reactions to *Yam Mosaic Virus*, genus *Potyvirus*, were chosen and crossed for the development of a mapping population. The resistant female parent (TDr 93-1) and susceptible male parent (TDr 87/00211) were planted and cross-pollinated in the field. The susceptible parent TDr 87/00211 was a breeding line, while the resistant parent TDr 93-1 was a popular landrace cultivar that has consistently shown field resistance across locations. Both parents have previously been used to generate F_1 individuals segregating for resistance to a Nigerian isolate of YMV (Mignouna et al. 2001b). The F_1 population used in the present study consisted of 180 individuals. This population served as the source of individuals for YMV resistance screening, and for marker segregation analyses. The virus isolate used, virus inoculation and symptom evaluation were as reported previously (Mignouna et al. 2001b).

DNA extraction

Total genomic DNA was extracted from lyophilised or freshly harvested leaves of the 180 F_1 individuals and the two parental lines according to a modified CTAB procedure (Mignouna et al. 1998). DNA quality was assessed visually after electrophoresis in 1% agarose gels and the concentration was determined from the UV absorbance at 260 nm using a Beckman-spectrophotometer model DU 520.

AFLP markers

AFLP analysis was carried out as described by Vos et al. (1995) using the enzyme combinations *Eco*RI/*Mse*I and *Pst*I/*Mse*I. A total of ten *Eco*RI/*Mse*I and 11 *Pst*I/*Mse*I primer combinations were selected (Table 1) based on a pre-screening of 64 *Eco*RI/*Mse*I and 48 *Pst*I/*Mse*I primer combinations, respectively. The density, complexity and number of polymorphic AFLP fragments were used as selection criteria. The adaptor and primer sequences used were based on the core primer design as described by Vos et al. (1995). AFLP markers were co-dominantly scored using Keygene proprietary scoring software (Vuylsteke et al. 1999). Each polymorphic AFLP fragment was identified by the code referring to the primer combination *Eco*RI/*Mse*I (E/M) or *Pst*I/*Mse*I (P/M), followed by the estimated size of the DNA fragment in nucleotides. A 10-bp DNA ladder from SequaMark (Research Genetics, Huntsville, Ala., USA) was used as standard to estimate the size of the fragments. The primer nomenclature of Keygene was used throughout and the primer sequences can be deduced from the marker designations (Table 1).

Mapping analysis

Linkage analyses were performed with the computer software package JoinMap 2.0 (Stam 1993; Stam and Van Ooijen 1996), and the segregating population was treated as resulting from cross-pollination. Mapping analysis involved the assignment of markers to linkage groups based on LOD scores (minimum LOD score of 3.0), and calculation of pairwise recombination frequencies and corresponding LOD scores for all pairs of markers that belong to a certain linkage group (Stam 1993). Measures of the goodness-of-fit were expressed as "chi-square values" (χ^2). A chi-square value < 2.0 was considered to be reliable for this analysis. It is noted that marker segregation data were obtained by analysing 180 indi-

Table 1 The primer combinations (PCs) used to screen the mapping population, PC nomenclature and the number of scored markers per primer combination

Primer combination	Extension	Number of scored markers
P12/M15	AC/CA	16
P12/M19	AC/GA	13
P13/M16	AG/CC	13
P14/M15	AT/CA	14
P14/M20	AT/GC	10
P14/M22	AT/GT	8
P15/M20	CA/GC	7
P16/M15	CC/CA	10
P16/M16	CC/CC	9
P17/M15	CG/CA	10
P17/M22	CG/GT	10
E11/M55	AA/CGA	24
E11/M57	AA/CGG	23
E11/M58	AA/CGT	21
E12/M50	AC/CAT	21
E12/M59	AC/CTA	23
E12/M60	AC/CTC	26
E13/M51	AG/CCA	23
E13/M60	AG/CTC	21
E14/M49	AT/CAG	16
E14/M61	AT/CTG	23
Total		341

viduals with the primer combination E/M, whereas 90 individuals were analysed with the combination P/M.

QTL mapping

A search for QTLs with effects on resistance to YMV was carried out on the entire mapping population using all the available segregation data. The interval mapping procedure of the software package MapQTL version 4.0 (Van Ooijen and Maliepaard 1996) was used for QTL analysis. The symptom scores of all individuals were used as a phenotypic trait score (quantitative trait), and significant associations were searched for in both the maternal and the paternal linkage maps. An appropriate threshold value for declaring a significant QTL effect was sought through the permutation analysis of the MapQTL software. This was done for all mapped markers. The frequency distribution of the maximum LOD score was then determined. The LOD values at $P = 0.05$ and $P = 0.01$ were taken as the estimated critical values at which to declare the presence of a QTL.

Results

Generation of marker data sets

Prior to the current study it was not known whether the yam parental lines were auto- or allo-tetraploid. To investigate the inheritance of the markers the band intensities of the AFLP markers were analysed by Keygene proprietary software. In case of an auto-tetraploid different segregation types are expected which are visualized by the different intensity scores of the markers (Bradshaw et al. 1998). Two classes of markers showing single- and double-dose band intensities were found in this study, suggesting an allo-tetraploid status. In consequence, segregation data were treated like a diploid cross-pollinator population.

Table 2 Mapping data for the yam population (TDr 93-1 × TDr 87/00211) showing the number of markers mapped after 1, 2 or 3 JoinMap-rounds including the mean chi-square value and the distance (cM) per linkage group. Only Parent 1 [(TDr 93-1, <AB × AA>) and (<AB × AB>) markers] were used

Linkage group	JM-round	Mean χ^2	No. of markers	Length (cM)
LG 1	Round 1	0.961	14/16	52.7
	Round 2	0.961	14/16	52.7
	Round 3	1.078	16/16	53.5
LG 2	Round 1	0.435	8/9	65.8
	Round 2	0.435	8/9	65.8
	Round 3	1.292	9/9	61.5
LG 3	Round 1	0.568	9/11	49.9
	Round 2	0.955	11/11	50.0
LG 4	Round 1	0.665	10/10	74.7
LG 5	Round 1	1.526	12/12	71.7
LG 6	Round 1	1.223	16/20	97.3
	Round 2	1.343	19/20	98.8
	Round 3	1.337	20/20	98.7
LG 7	Round 1	1.283	17/19	94.4
	Round 2	1.283	17/19	94.4
	Round 3	1.832	19/19	93.0
LG 8	Round 1	0.635	14/19	149.8
	Round 2	0.809	18/19	172.0
	Round 3	1.162	19/19	172.6
LG 9	Round 1	0.744	13/13	95.0
LG 10	Round 1	0.740	8/9	48.5
	Round 2	0.740	8/9	48.5
	Round 3	1.868	9/9	48.9
LG 11	Round 1	0.854	11/11	39.9
LG 12	Round 1	0.646	6/6	31.4
Total across linkage groups	Round 2	–	147 (95%)	894.9
	Round 3	–	155 (100%)	890.9

Due to parental heterozygosity segregating marker loci could be followed from both parents. In total, three marker segregation types were found: 81 <AB × AB> markers, 118 <AB × AA> markers and 142 <AA × AB> markers, the first giving information about the segregation of both parents, while the latter two types provide information about the maternal and paternal parent, respectively. The final data set consisted of 341 markers. The number of markers scored per primer combination is presented in Table 1.

Mapping the TDr 93-1 × TDr 87/00211 population

The data set of 341 markers was split into two, containing 199 (81 + 118) maternal and 223 (81 + 142) paternal markers, respectively. A separate genetic map was generated for each parent.

The maternal and paternal maps

The 199 markers were allocated to 16 distinct linkage groups (LOD 3.5). After a first mapping analysis it was

Table 3 Mapping data for yam population (TDr 93-1 × TDr 87/00211) showing the number of markers mapped after 1, 2 or 3 JoinMap-rounds including the mean chi-square value and the cM-distance per linkage group. Parent 2 [(TDr 87/00211, <AA × AB>) and (<AB × AB>) markers] were used

Linkage group	JM-round	Mean χ^2	No. of markers	length (cM)
LG 1	Round 1	0.518	15/16	96.5
	Round 2	0.518	15/16	96.5
	Round 3	0.932	15/16	89.9
LG 2a	Round 1	1.103	15/19	85.9
	Round 2	1.103	15/19	85.9
	Round 3	1.554	19/19	117.8
LG 2b	Round 1	1.526	9/12	51.9
	Round 2	1.526	9/12	51.9
	Round 3	2.593	12/12	56.1
LG 3	Round 1	0.481	14/15	48.1
	Round 2	0.481	14/15	48.1
	Round 3	0.808	15/15	51.8
LG 4	Round 1	1.643	8/8	37.4
LG 5	Round 1	1.390	8/8	28.2
LG 6a	Round 1	1.345	19/25	79.7
	Round 2	1.490	21/25	78.1
	Round 3	1.818	24/25	79.2
LG 6b	Round 1	0.915	8/9	27.5
	Round 2	0.915	8/9	27.5
	Round 3	1.953	9/9	24.4
LG 7	Round 1	1.306	13/15	85.7
	Round 2	1.372	14/15	85.9
	Round 3	1.819	15/15	85.9
LG 8	Round 1	0.952	10/11	42.2
	Round 2	0.952	10/11	42.2
	Round 3	0.817	11/11	121.7
LG 9	Round 1	0.431	5/5	22.2
LG 10	Round 1	0.100	4/4	63.7
LG 11	Round 1	1.385	9/11	58.5
	Round 2	1.385	9/11	58.5
	Round 3	2.325	11/11	73.6
Totalising across linkage groups	Round 2		140 (89%)	726.1
	Round 3		157 (99%)	851.9

found that some markers were responsible for a large increase of the chi-square value. Such markers were removed from the data set. In addition, some linkage groups that contained three or fewer markers were discarded. Finally, 155 markers were mapped in 12 linkage groups with a total map length of 891 cM. The size of the linkage groups ranged from 31 cM to 172 cM. The average distance between two adjacent markers was 5.7 cM. In the second round of JoinMap, 95% of all markers were mapped, indicating a high reliability of the data. Furthermore, when all markers were mapped, the chi-square values remained low (Table 2). The genetic linkage map is shown in Fig. 1a.

The 223 markers in the paternal data set were mapped to 16 distinct linkage groups (LOD 7.5). A procedure similar to that described above was followed, resulting in a total of 157 markers that were mapped to 13 linkage groups with a total length of 852 cM. The 13 linkage groups ranged in size from 24 cM to 121 cM. In the second round of JoinMap 89% of all markers were mapped, which indicates the reliability of the data. Again, chi-square values remained low when all markers were mapped (Table 3). The genetic map is shown in Fig. 1b.

Table 4 Presentation of identical markers in the linkage groups of the parent-1 and parent-2 maps

Linkage groups containing identical markers		No. of identical markers
Parent 1 (93-1)	Parent 2 (87/00211)	
LG 1	LG 1	9
LG 2	LG 2a	4
LG 2	LG 2b	3
LG 3	LG 3	3
LG 4	LG 4	4
LG 5	LG 5	1
LG 6	LG 6a	8
LG 6	LG 6b	3
LG 7	LG 2a	2
LG 7	LG 7	4
LG 8	LG 8	4
LG 8	LG 7	1
LG 8	LG 11	2
LG 9	LG 5	1
LG 9	LG 9	4
Total 155 markers	Total 157 markers	Total 53

Comparison of the parental genetic maps

Comparison of the parental maps showed a great resemblance in grouping and marker order. Nine linkage groups were found in both parental maps and the two maps were connected by a total of 53 <AB × AB> markers. On the basis of this comparison the linkage-group numbers of both parental maps were coordinated. Six of the 53 common markers mapped to different linkage groups (Table 4). All other <AB × AB> markers were placed in similar groups with the corresponding order. Among the remaining 28 AB × AB markers, 17 were mapped in the two parental maps (ten markers were mapped in the P1 map only while seven others were mapped in the P2 map only). The remaining 11 <AB × AB> markers did not map at all. The linkage groups did not contain enough <AB × AB> markers that were segregating in both parents to allow combination of the maternal and paternal maps. Three linkage groups consisted of maternal markers only, whereas two linkage groups had only paternal markers. These five linkage groups probably represent genomic regions unique to the respective parents. The total length of the parental genetic maps is similar (891 and 852 cM for the maternal and paternal maps, respectively). There are still some gaps in the genome to be covered, because 44 markers are still not mapped.

Distortion of marker segregation

The segregation ratios of the <AA × AB> / <AB × AA> and the <AB × AB> types were 1:1 and 1:2:1, respec-

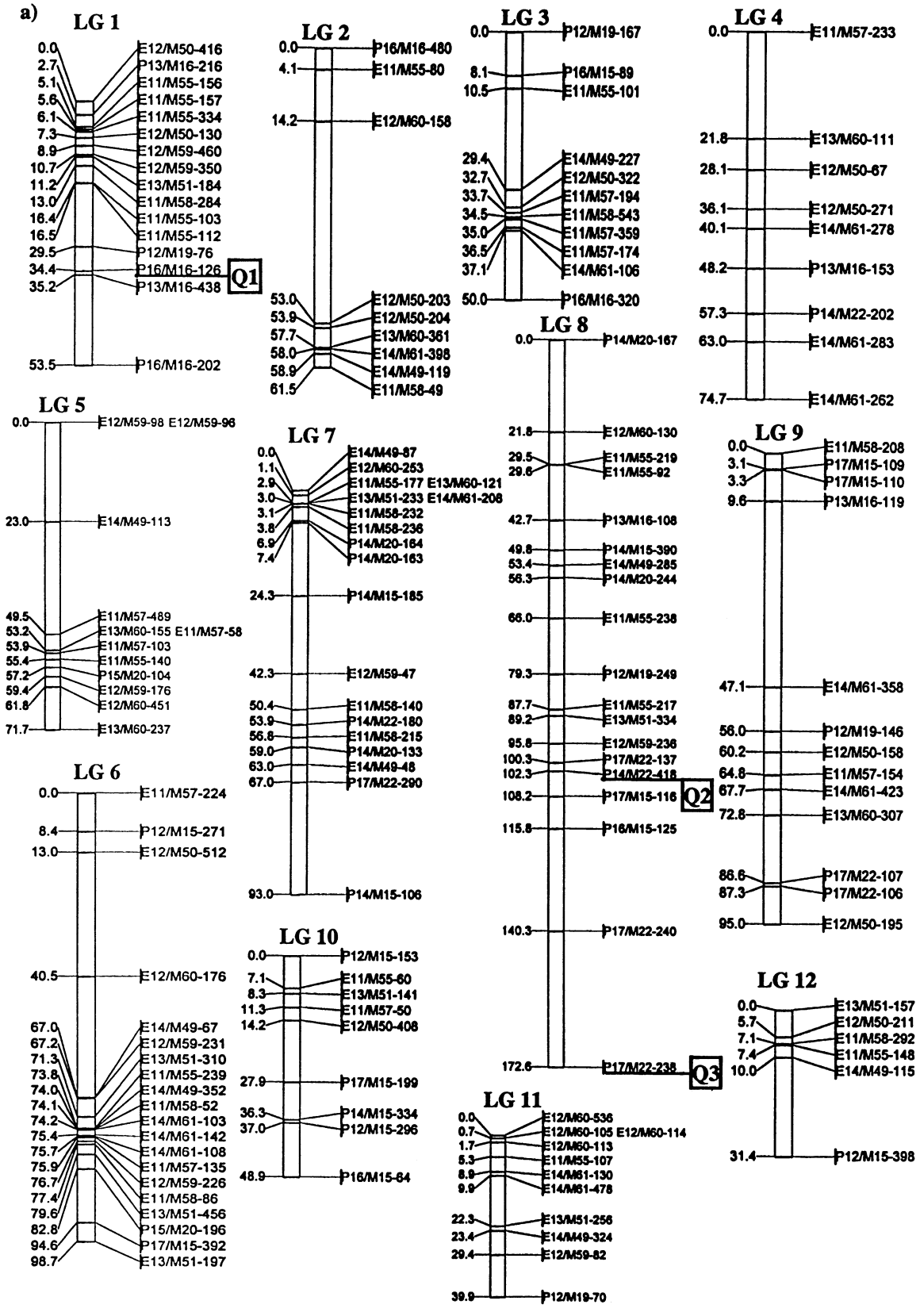
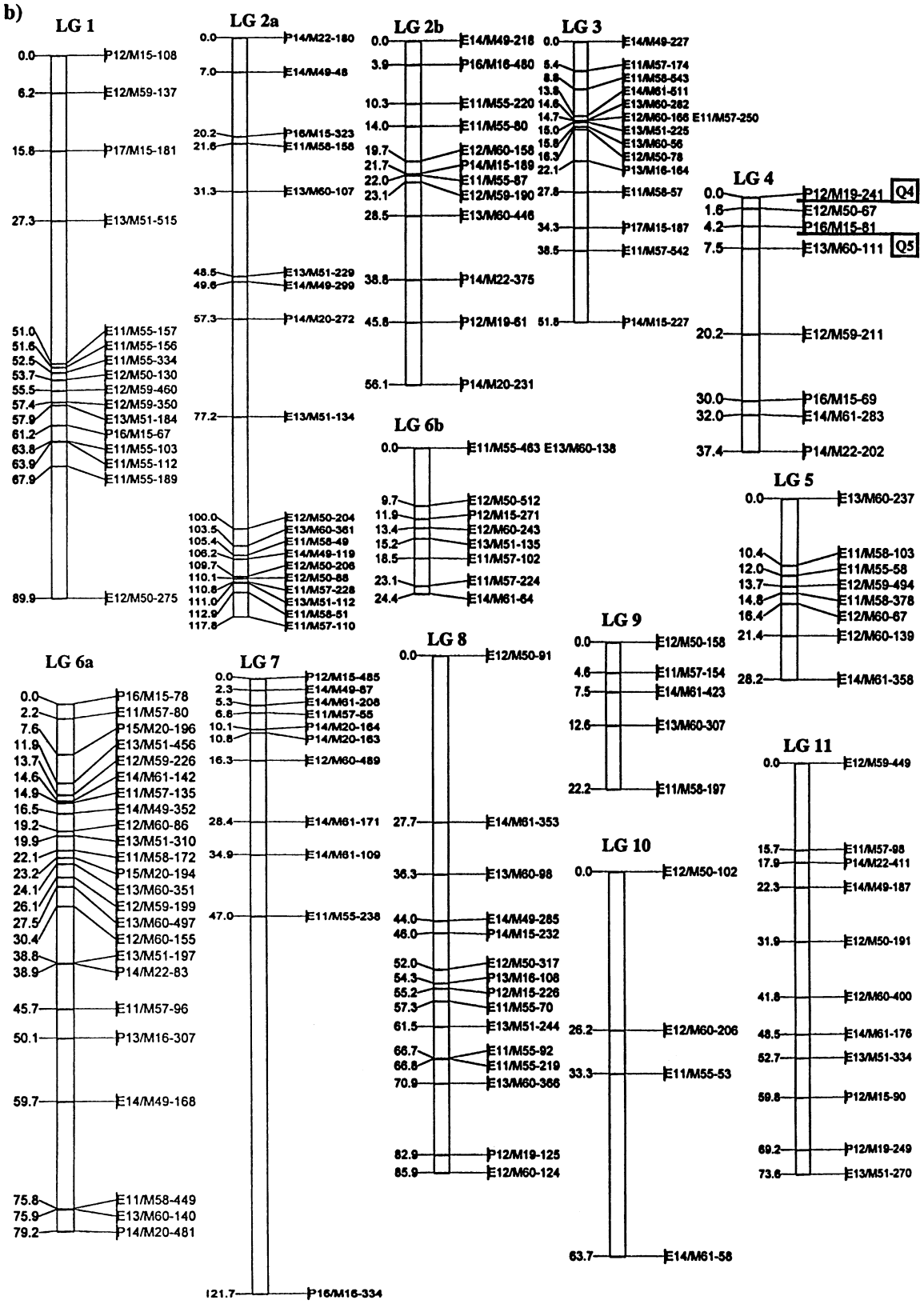


Fig. 1 Genetic linkage map of Guinea yam (*D. rotundata*) based on F_1 progeny from maternal line (TDr 93-1) × paternal line (TDr 87/00211). (**a**) Maternal (**b**) Paternal parent map. Map distances (shown on the left of the linkage groups) represent genetic distances in Kosambi centiMorgans (cM). Locus names are on the right side of the linkage groups. The loci are named with (1) the code referring to the corresponding primer combination (Table 1), followed by (2) the estimated size of the DNA fragment in nucleotides. Markers associated with QTL for YMV resistance are marked Q1 to Q5 and explained 24, 22, 35, 13 and 16% of the phenotypic variance, respectively. Note that the association with markers Q2 and Q3, and Q4 and Q5 may not be independent. LG = linkage group


b)
LG 1
0.0 P12/M15-108
6.2 E12/M59-137
15.8 P17/M15-181
27.3 E13/M51-515
51.0 E11/M55-157
51.6 E11/M55-156
52.5 E11/M55-334
53.7 E12/M50-130
55.5 E12/M59-460
57.4 E12/M59-350
57.9 E13/M51-184
61.2 P16/M15-67
63.8 E11/M55-103
63.9 E11/M55-112
67.9 E11/M55-189
89.9 E12/M50-275
LG 2a
0.0 P14/M22-180
7.0 E14/M49-48
20.2 P16/M15-323
21.6 E11/M58-158
31.3 E13/M60-107
48.5 E13/M51-229
49.6 E14/M49-299
57.3 P14/M20-272
77.2 E13/M51-134
100.0 E12/M50-204
103.5 E13/M60-361
105.4 E11/M58-49
106.2 E14/M49-119
109.7 E12/M50-206
110.1 E12/M50-88
110.8 E11/M57-228
111.0 E13/M51-112
112.9 E11/M58-51
117.8 E11/M57-110
LG 2b
0.0 E14/M49-218
3.9 P16/M16-480
10.3 E11/M55-220
14.0 E11/M55-80
19.7 E12/M60-158
21.7 P14/M15-189
22.0 E11/M55-87
23.1 E12/M59-190
28.5 E13/M60-446
38.8 P14/M22-375
45.8 P12/M19-61
56.1 P14/M20-231
LG 3
0.0 E14/M49-227
5.4 E11/M57-174
8.8 E11/M58-543
13.8 E14/M61-511
14.6 E13/M60-282
14.7 E12/M60-166 E11/M57-250
15.0 E13/M51-225
15.8 E13/M60-56
16.3 E12/M50-78
22.1 P13/M16-164
27.8 E11/M58-57
34.3 P17/M15-187
38.5 E11/M57-542
51.8 P14/M15-227
LG 4
0.0 P12/M19-241 Q4
1.6 E12/M50-67
4.2 P16/M15-61
7.5 E13/M60-111 Q5
20.2 E12/M59-211
30.0 P16/M15-69
32.0 E14/M61-283
37.4 P14/M22-202
LG 5
0.0 E13/M60-237
10.4 E11/M58-103
12.0 E11/M55-58
13.7 E12/M59-494
14.8 E11/M58-378
16.4 E12/M60-67
21.4 E12/M60-139
28.2 E14/M61-358
LG 6b
0.0 E11/M55-463 E13/M60-138
9.7 E12/M50-512
11.9 P12/M15-271
13.4 E12/M60-243
15.2 E13/M51-135
18.5 E11/M57-102
23.1 E11/M57-224
24.4 E14/M61-64
LG 6a
0.0 P16/M15-78
2.2 E11/M57-80
7.6 P15/M20-196
11.9 E13/M51-456
13.7 E12/M59-226
14.6 E14/M61-142
14.9 E11/M57-135
16.5 E14/M49-352
19.2 E12/M60-86
19.9 E13/M51-310
22.1 E11/M58-172
23.2 P15/M20-194
24.1 E13/M60-351
26.1 E12/M59-199
27.5 E13/M60-497
30.4 E12/M60-155
38.8 E13/M51-197
38.9 P14/M22-83
45.7 E11/M57-96
50.1 P13/M16-307
59.7 E14/M49-168
75.8 E11/M58-449
75.9 E13/M60-140
79.2 P14/M20-481
LG 7
0.0 P12/M15-485
2.3 E14/M49-87
5.3 E14/M61-208
6.8 E11/M57-55
10.1 P14/M20-164
10.8 P14/M20-163
16.3 E12/M60-489
28.4 E14/M61-171
34.9 E14/M61-109
47.0 E11/M55-238
121.7 P16/M16-334
LG 8
0.0 E12/M50-91
27.7 E14/M61-353
36.3 E13/M60-98
44.0 E14/M49-285
46.0 P14/M15-232
52.0 E12/M50-317
54.3 P13/M16-108
55.2 P12/M15-226
57.3 E11/M55-70
61.5 E13/M51-244
66.7 E11/M55-92
66.8 E11/M55-219
70.9 E13/M60-366
82.9 P12/M19-125
85.9 E12/M60-124
LG 9
0.0 E12/M50-158
4.6 E11/M57-154
7.5 E14/M61-423
12.6 E13/M60-307
22.2 E11/M58-197
LG 10
0.0 E12/M50-102
26.2 E12/M60-206
33.3 E11/M55-53
63.7 E14/M61-58
LG 11
0.0 E12/M59-449
15.7 E11/M57-98
17.9 P14/M22-411
22.3 E14/M49-187
31.9 E12/M50-191
41.8 E12/M60-400
48.5 E14/M61-176
52.7 E13/M51-334
59.8 P12/M15-90
69.2 P12/M19-249
73.6 E13/M51-270

tively. In this study, 28 of the 341 scored markers (8%) showed some distorted segregation. Of those 28 markers ten were found to be on linkage groups 1 and 2a of the paternal parent. Four markers in linkage group 1 and six in linkage group 2a showed distorted segregation ratios. Twelve markers with distorted segregation ratios could not be mapped. The low level of distortion in segregation that occurs in this population may be ascribed to selection during the generation of the mapping population.

Quantitative trait locus (QTL) analysis

The phenotypic response to YMV was characterized by continuous variation. Only mild symptoms (disease severity score of 2) were recorded for plants of the resistant landrace TDr 93-1, while plants of the susceptible breeding line TDr 87/00211 showed typical mosaic symptoms (disease severity score of 4). Progeny of the cross TDr 93-1 × TDr 87/00211 segregated 124 resistant: 56 susceptible. The observed segregation fitted a genetic ratio of 2.48:1 ($\chi^2 = 0.51$; $P = 0.47$), which can be expected when two simplex heterozygotes are crossed, indicating the possible modifying effect of the susceptible parent. A 3:1 ratio was also possible by the χ^2 test, suggesting the action of two dominant genes in simplex status, but there was only a weak fit to this ratio ($\chi^2 = 3.58$, $P = 0.06$).

Since the population was considered to behave like a diploid cross-pollinator, the enhanced interval mapping method was applied. Three QTLs with effects on the resistance to YMV were identified on linkage groups 1 (1) and 8 (2) of the maternal parent TDr 93-1 at a LOD score mininum of 4.6 ($P = 0.05$). However, just one QTL (P17/M22-238; 35% of phenotypic variance) on linkage group 8 remained significant when the more stringent LOD value of 5.8 ($P = 0.01$) was applied. These three QTLs were associated with the marker P16/M16-126 on linkage group 1, which explained 24% of the phenotypic variance, and P14/M22-418 and P17/M22-238 on linkage group 8, that explained 22% and 35% of the phenotypic variance, respectively. Two QTLs for YMV were detected on the paternal linkage group 4 at a LOD score value of 4.5 ($P = 0.05$). At a higher LOD value of 5.4 ($P = 0.01$) only one of these two was retained. These QTLs were associated with the markers P12/M19-241 and P16/M15-81 that explained 13% and 16% of the phenotypic variation, respectively. This analysis showed that both parents contributed to the phenotypic resistance of the progeny.

Discussion

Density and distribution of *Pst*I versus *Eco*RI markers

The *Pst*I/*Mse*I data-set contained fewer markers per primer combination compared to that of *Eco*RI/*Mse*I. Nevertheless, the band density of the *Pst*I/*Mse*I gels was comparable with that of *Eco*RI/*Mse*I. Although yam is predominantly a dioecious species, the level of heterozygosity has been shown to be rather low, ranging from 12.5 to 50% for *D. rotundata* (Zoundjihékpon et al. 1994). This could explain the relatively low level of polymorphic markers detected by *Pst*I. Markers on the maternal linkage map were randomly distributed over the 12 linkage groups except for three (LG1, LG8 and LG11; $P < 0.05$), whereas in the paternal linkage map, five linkage groups (LG 5, 6, 7, 10 and 12) were found to have markers that were not randomly distributed ($P < 0.05$). In general the distribution of the AFLP markers was satisfactory and the fact that some *Eco*RI/*Mse*I markers were clustered in specific regions is not peculiar to the yam genome. The clustering of *Eco*RI/*Mse*I markers in specific chromosomal regions has been reported in AFLP-based linkage maps of plants such as potato (Van Eck et al. 1995), barley (Qi et al. 1998), soybean (Keim et al. 1997), *Arabidopsis* (Alonso-Blanco et al. 1998) and maize (Vuylsteke et al. 1999).

Genome structure of *D. rotundata*

The observed segregation of AFLP markers reflected a disomic inheritance that revealed an allo-tetraploid structure for *D. rotundata*. Using isozyme markers in genetic studies of selected tetraploid varieties of yams and their F_1 progeny showed a segregation pattern as in a diploid species (Zoundjihépkon et al. 1994). These results confirm the allo-tetraploid origin of yam, although there are no known diploid wild ancestors of Guinea yams.

This raises the question whether the yam genome is polyploid; could the basic chromosome number be 20? Evidence of the polyploid nature of yams (*Dioscorea* spp.) relies on the basic chromosome number of other species such as *Dioscorea tokoro* and *Dioscorea gracillima* which have $2n = 2x = 20$ (diploid) chromosomes. Baquar (1980) reported no species in Africa with 20 chromosomes and no case of natural polyploidy above 40 chromosomes has been recorded in any of the wild species he investigated (cited by Hahn 1995). Because yam chromosomes are small in size, cytogenetics has been difficult. Nevertheless, chromosome pairing in intra-specific crosses in *D. rotundata* shows normal meiosis, with 20 bivalents (IITA 1993). The number of linkage groups identified for each of the parental genomes (12 and 13) is lower than the expected number of 20 (assuming disomic inheritance) which would correspond to the 20 gametic chromosomes of yam. The results support earlier empirical evidence of disomic inheritance, based on the presence of 20 bivalents found at the pachytene stage of meiosis in yam (IITA 1993), and disomic inheritance of isozyme markers in yam (Zoundjihékpon et al. 1994). Since the diploid ancestors are not known, and following the hypothesis of Hahn (1995), *D. rotundata* should have a genome constitution of RRBB. This may have happened as a result of hybridization between a putative wild diploid with a genome constitution RR

(*D. rotundata*) and another one with a genome constitution BB (*Dioscorea burkilliana*), which gave rise to a diploid hybrid RB. Subsequently, the hybrid chromosome number was doubled spontaneously to produce the present *D. rotundata* (RRBB) an allotetrapoid, an event thought to have happened thousands of years ago (Hahn 1995). Since this is an ancient event, it is not surprising that both molecular (AFLP) and isozyme markers showed diploid segregation patterns.

A preliminary study on the genetic mapping of *D. rotundata* also revealed diploid segregation patterns for alleles at RAPD, isozyme and a few AFLP marker loci (Mignouna and Asiedu 1999). In that study, the detection and estimation of linkage was carried out according to the pseudo test-cross method using single-dose markers (SDM) (Wu et al. 1992) because the genome constitution of yam was not known (allo-versus auto-polyploid). This approach resulted in a rather limited linkage map comprising four linkage groups made of 12 markers each for the male and female parents (Mignouna and Asiedu 1999), mainly because only a small proportion of RAPD and AFLP markers that were polymorphic between the two parents fit the condition of SDM. This situation explains the use of a more-polymorphic source of markers such as AFLPs in the current study. The advantages of AFLP-based markers in generating linkage maps (Becker et al. 1995; Castiglioni et al. 1998), in the identification of markers associated with disease resistance loci (Meksem et al. 1995; Rouppe van der Voort et al. 1998), and in genetic analyses (Vos et al. 1998; Vuylsteke et al. 1999) are well documented in many plant species. The present study has proven that AFLP markers were adequate to generate framework linkage maps of yam and also to identify QTLs for desirable traits, as exemplified by the resistance to YMV. However, there is still a need to generate additional molecular markers in order to combine the two maps and also to identify homoeologous linkage groups.

The total maternal and paternal map lengths of 891 cM and 852 cM, respectively, covered roughly 56% of the yam genome based on a known total diploid *D. tokoro* map length of 800 cM (Terauchi and Kahl 1999). If the yam genome is estimated to be 1,000 – 2,000 cM as inferred from the AFLP markers in this study, then the 155 loci of the female map covered 891/2,000 (about 45% of the yam genome) while the 157 loci of the paternal map covered 852/2,000 (43% of the genome). These estimates are the minimum genome coverage and, as more markers are added to the linkage maps, the unmapped markers from the present study could be fitted in the genetic linkage map. The genome size was estimated by Feulgen-stained root tip nuclei of tetraploid *D. rotundata* to be 0.8 pg per haploid nucleus, and thus is equivalent to that of species such as soybean, rice and spinach (Conlan et al. 1995). Considering the haploid nuclear DNA content of *D. rotundata* to be 800 Mbp/1C the physical distance per map unit could be estimated as 800 Mbp/2,000 cM = 400 kb per cM, making map-based gene cloning feasible in white yam.

Use of the genetic linkage map in marker-assisted selection of *D. rotundata*

The continuous nature of the variation for YMV resistance in the progeny, and the absence of monogenic inheritance, indicated that YMV resistance is inherited quantitatively. Segregation of the progeny gave a good fit to a genetic ratio of 2.48:1 which can be expected when two simplex heterozygotes are crossed, assuming chromatid segregation (Flis 1995; Mignouna et al. 2001b). This indicates the possible modifying effect of the susceptible parent, and suggests that the parents are genotypic mixtures carrying both susceptibility and resistance determinants which interact (Mignouna et al. 2001b). The components of this resistance have been identified in the present study, as three QTLs in the maternal parent and two in the paternal parent. It is not surprising that there were more QTLs in the resistant maternal parent compared to the susceptible paternal one. Since these loci seem to have major effects on YMV resistance, the QTLs identified by AFLP markers could be cloned and sequenced in order to design sequence-tagged-site (STS) specific primers (Solomon-Blackburn and Barker 2001). When this is done, it will be useful to test the candidate markers in other genetic backgrounds and mapping populations as diagnostic markers for YMV and for their potential use in marker-assisted selection. Developing resistant varieties to YMV is primarily based on the identification of useful sources of resistance. However, field screening for YMV resistance is not only laborious, but also unreliable (Mignouna et al. 2001b). Mapping the chromosomal locations of molecular markers linked to genes for resistance to yam viruses or specific virus strains would be useful in identifying and pyramiding such genes in improved varieties.

The present molecular map of *D. rotundata*, although incomplete, opens new avenues for marker-based selection in the breeding of this important tropical staple food crop. Disease resistance gene clusters (covering a diverse range of pathogens) have been found in crops such as potato (De Jong et al. 1997; Hämäläinen et al. 1998; Van der Voort et al. 1999). With additional markers (SSRs, AFLPs and RFLPs) prospects appear good for better coverage of the yam genome in preparation for marker-assisted selection for many morphological and physiological trait loci that are segregating among the progenies of this cross. Traits such as nematode resistance, tuber dormancy and tuber quality, that are currently under field evaluation, could also be mapped in this population and used to assist yam breeding.

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