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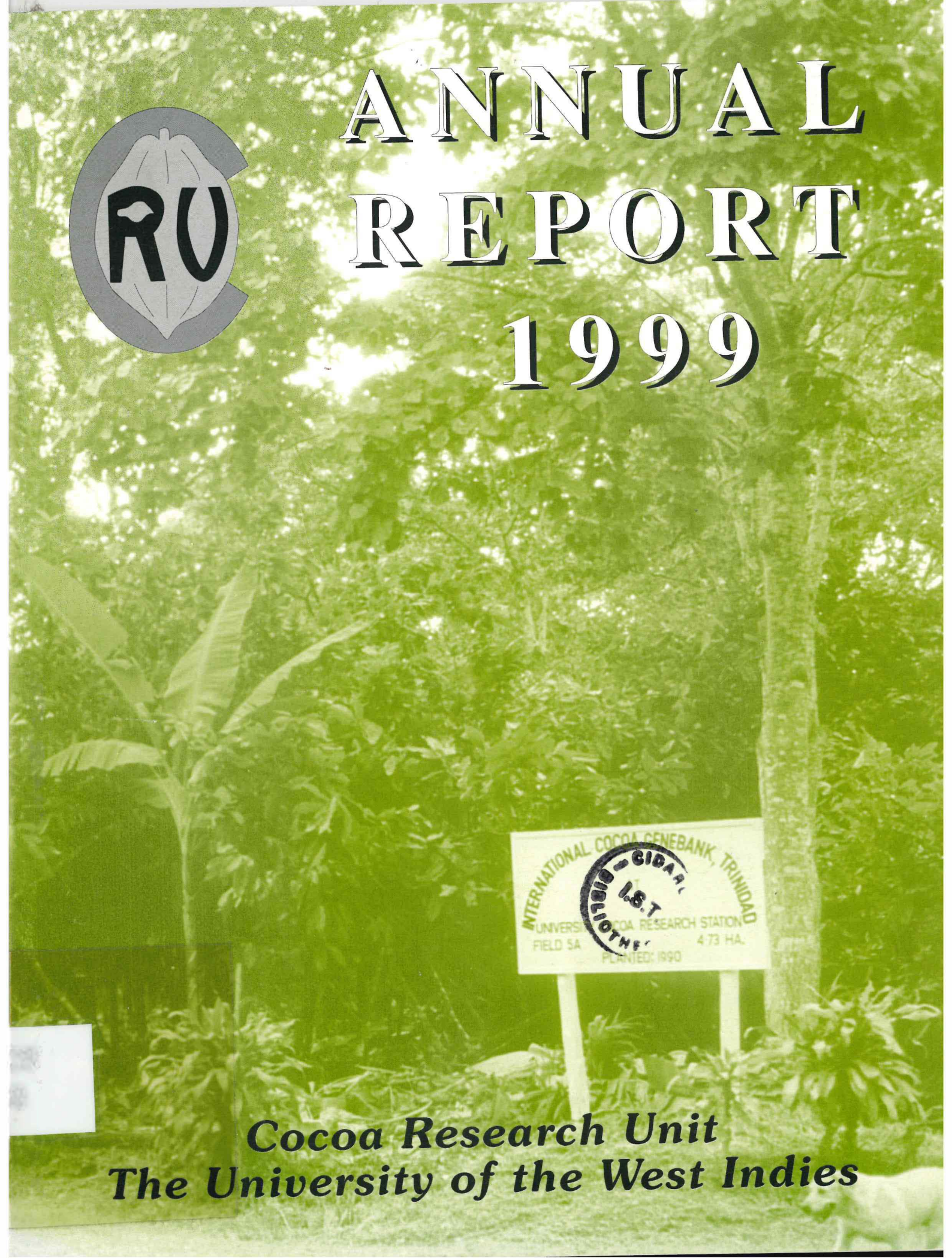

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Theobroma cacao L.: Genome Linkage Map and QTLs for *Phytophthora palmivora* Resistance

L.A. Motilal and O. Sounigo

Introduction

Black pod disease is an important constraint to cocoa production in all producing countries. The disease is caused by several *Phytophthora* species and, until recently, little was known about the genetic basis of resistance to the pathogen. To address this problem, the CAOBISCO project entitled *The use of molecular markers to identify the genetic basis of resistance to Black Pod disease* (Phytophthora*) and identify early screening markers* was developed and implemented in Trinidad, France, Cameroon and Côte d'Ivoire in 1995. In Trinidad, the F1 progeny of the cross IMC 57 x CATONGO was assessed for resistance to *P. palmivora* by means of the leaf disc method of Nyassé *et al.* (1995). Disease resistance in cacao is reportedly polygenic (Warren, 1994) and is therefore, by definition, a quantitative trait. QTL analysis relies on the principle that genes, which influence phenotypic traits, are transmitted along with associated molecular markers. QTLs are useful because they can serve as markers for selection at the seedling stage of development, can position the loci for genes of interest and identify genes associated with a particular locus (Fritz *et al.*, 1995). The aims of this research were to map the cacao genome and to obtain molecular markers linked to the QTLs for *P. palmivora* resistance. In order to obtain a saturated genomic map, markers were determined from isozyme studies and from techniques that involved the polymerase chain reaction (PCR), *viz.*, randomly amplified polymorphic DNA (RAPD-PCR), simple sequence repeat products using PCR (SSR-PCR or microsatellite-PCR) and amplified fragment length polymorphism (AFLP).

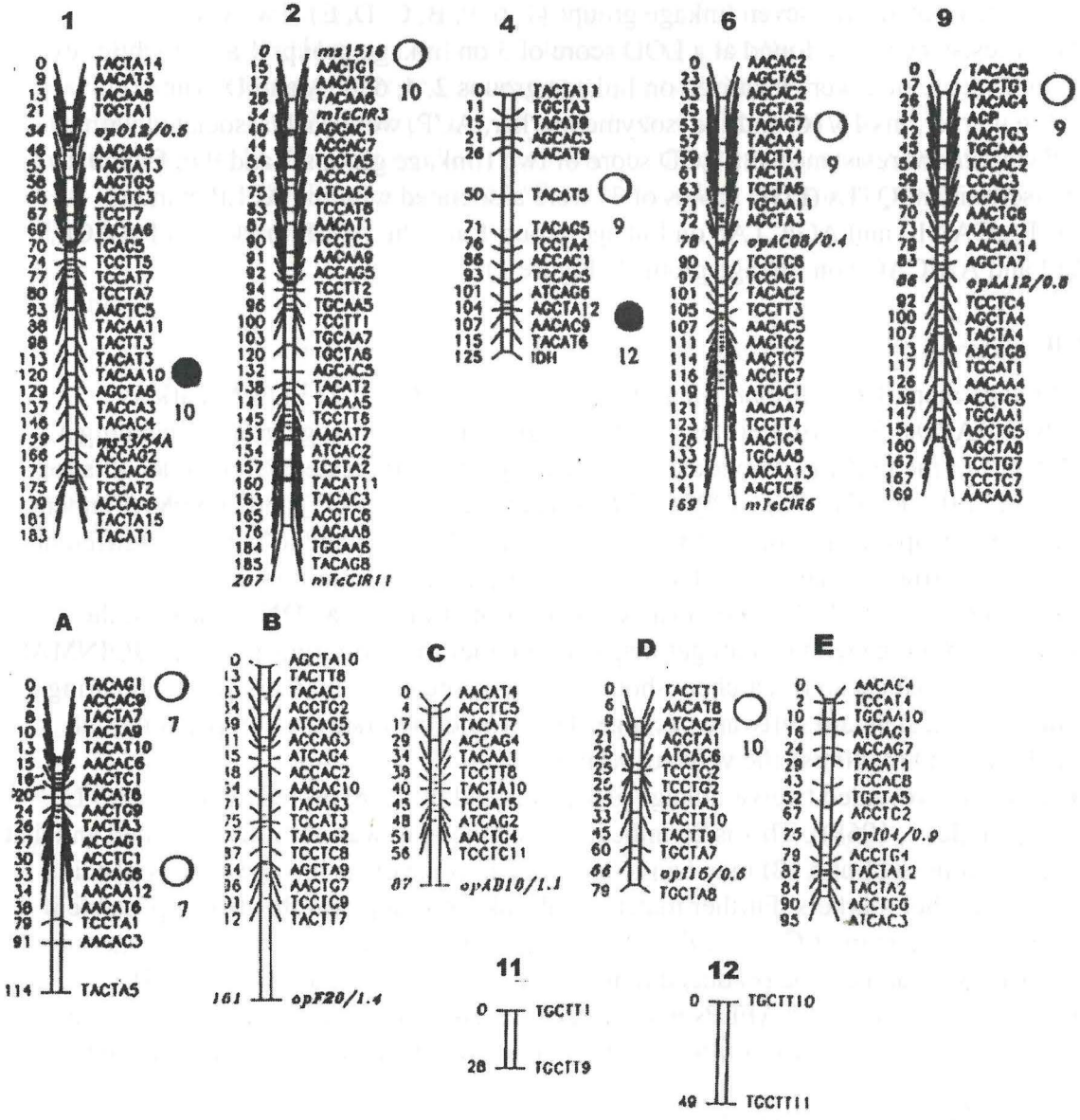
Methods

Individuals in the progeny were scored for *P. palmivora* resistance as determined from the leaf disc inoculation method of Nyassé *et al.* (1995) and for segregating markers from AFLP, microsatellite, RAPD-PCR and isozyme banding patterns. The data were processed using the MAPMAKER program (available on the World Wide Web at mapmaker@genome.wi.mit.edu). QTLs at Log-Likelihood (LOD) scores of 2 and 3 were detected by interval mapping.

Results

The removal of markers at extreme distances at the ends of linkage groups culminated in a total of 213 markers: 199 AFLPs; 7 RAPDs (Operon primers: F20, I15, I04, O12, AA12, AB18, AC08); 5 microsatellites (ms 53/54A, ms 15/16, mTcCIR3, mTcCIR6, mTcCIR11) and two isozymes (acid phosphatase, ACP; icocitrate dehydrogenase, IDH). The resultant genome map is presented in Figure 1.

Figure 1. Genetic linkage map of *Theobroma cacao* L. (A combination of 2 isozymes (IDH and ACP), 5 microsatellites (ms and mTcCIR), 7 RAPDs (op) and 199 AFLPs achieved a total of 213 markers over a total map distance of 1466 cM. Inoculation of leaf discs with *Phytophthora palmivora* (Butl.) Butler enabled the positioning of QTLs at LOD scores of >3 (•) and >2 (○)).



These 213 loci were distributed over 12 linkage groups of which two groups (11 and 12) were each comprised of only two AFLP markers. Five of the remaining 10 linkage groups were matched to the map of Cilas *et al.* (1998) and the remainder were coded alphabetically (Figure 1). These 10 linkage groups had a combined map length of 1389 cM, ranging from 79-207 cM. The average distance between loci was 6.6 cM with a range of 2-39 cM. Isozyme markers were localised in linkage groups 4 (IDH) and 9 (ACP).

Microsatellite markers were present acrocentrically or telocentrically in linkage groups 1, 2 and 6, three microsatellites being localised on the second linkage group. The seven RAPD markers were distributed over seven linkage groups (1, 6, 9, B, C, D, E). Two QTLs for *Phytophthora* resistance were found at a LOD score of 3 on linkage groups 1 and 4 while seven QTLs at a LOD score of 2 were localised on linkage groups 2, 4, 6, 9, A and D. The microsatellite marker, ms 15/16, and the isozyme marker, ACP, were each associated with a QTL for *Phytophthora* resistance at a LOD score of two (linkage groups 2 and 9 in Figure 1). The more discriminant QTLs (LOD scores of 3) were associated with the AFLP markers TA/CAT3, TA/CAA10 and AG/CTA6 on linkage group 1 and the AFLP markers AT/CAG6, AG/CTA12 and AA/CAC9 on linkage group 4 (Figure 1).

Discussion

A genetic linkage map of *Theobroma cacao* L. was constructed with 213 DNA markers (199 AFLPs, 7 RAPDs, 5 microsatellites and 2 isozymes) over 12 linkage groups spanning a total of 1466 cM of the genome. Previously reported maps have indicated genomic lengths for cacao of 1057 cM (Crouzillat *et al.*, 1996), 759 cM (Lanaud *et al.*, 1995) and 793 cM (Flament, 1998). The greater map distance obtained in this study may be attributed to the use of different parents, the use of different markers and the imprecise positioning of terminal markers. Furthermore, Lanaud *et al.* (1995) demonstrated that for the same cacao DNA markers, the MAPMAKER program generated a longer map than another more rigorous program, JOINMAP. It appears therefore that cacao researchers should utilise a core set of markers when preparing genomic maps with a standard software package. This would facilitate the comparison of all maps in the future and maximise the value of results.

The present study revealed twelve linkage groups instead of the expected ten (*T. cacao* L.: 2n=2x=20 (Purseglove, 1968)). This is an indication that the map was not fully saturated and that the two "extra" groups (11 and 12) may be incorporated into the other linkage groups pending the availability of other markers. Further matching of linkage groups would also be possible if some markers from the map of Cilas *et al.* (1998) are used in subsequent work. AFLP markers are reportedly reliable and can be produced rapidly in large numbers (Vos *et al.*, 1995). Furthermore, previously mapped AFLPs may be regarded as potential chromosome-specific markers. This strengthens the case for the selection of a compulsory set of AFLPs for use by cacao geneticists.

The present map contains 9 QTLs for resistance to *P. palmivora* confirming a polygenic mode of inheritance. The results support the hypothesis of Warren (1994) who postulated a minimum of five unlinked loci. Similar results were obtained for *P. megakarya* on ICS 84, IMC 67, UPA 134 and *P. palmivora* on T 60/887 with five QTLs each (Flament, 1998). Resistance to

Phytophthora in cacao may therefore be under the influence of 5-9 genes and is probably additive in effect as suggested by Fritz *et al.* (1995).

Markers linked with genes controlling economically important traits may be used for marker assisted selection (MAS) in breeding programmes (Byrne *et al.*, 1995). This is especially useful when evaluation using molecular markers is faster than phenotypic evaluation in each cycle of selection (Groh *et al.*, 1998). MAS, therefore, has an important role to play in cocoa breeding programmes. The presence of polygenes for resistance allows for a diverse assemblage of markers to be chosen for the detection of resistant progeny. Thus, while MAS programs give priority to markers associated with QTLs that are highly correlated, several markers associated with less discriminant QTLs over different linkage groups should also be utilised. This increases the likelihood of detecting improved cacao progeny. Tightly associated AFLP markers on linkage groups 1,2, 4, 6, 9, A, and D can be used. It should be noted that ACP was also tightly associated with a QTL for *Phytophthora* resistance in the progeny of completely different parents (Flament, 1998). It appears that MAS cacao breeding programmes may therefore utilise ACP as a common marker.

References

Byrne, M., Murrell, J.C., Allen, B., Moran, G.F. (1995) An integrated genetic linkage map for eucalyptus using RFLP, RAPD and isozyme markers. *Theoetical and Applied Genetics* **91**: 869-875.

Cilas, C., Despréaux, D., Flament, M.H., Kebe, I., Lanaud, C., N'Goran, J., Nyassé, S., Paulin, D., Pieretti, I. and Risterucci, A.M. (1998) Mapping of quantitative trait loci (QTLs) for resistance to *Phytophthora.* Pages 27-61 *in:* Proceedings of the Workshop on the use *of molecular markers to identify the genetic basis of resistance to black pod disease (Phytophthora) and identify early screening markers.* Montpellier, France, 25-26 June 1998, Montpellier, France : CIRAD-CP.

Crouzillat, D., Lerceteau, E., Pettiard, V., Morera, J., Rodriguez, H., Walker, D., Phillips, W., Ronning, C., Schnell, R., Osei, J. and Fritz, P. (1996) *Theobroma cacao* L.: a genetic map and quantitative loci analysis. *Theoretical and Applied Genetics* **93**: 205-214.

Flament, M.H. (1998) Cartographie genetique de facteurs impliques dans la resistance du cacaoyer (*Theobroma cacao* L.) a *Phytophthora megakarya* et a *Phytophthora palmivora. Thèse de doctorat*, l'Ecole Nationale Supérieure Agronomique de Montpellier, France.

Fritz, P.J., Phillips-Mora, W. and Rodriguez, H. (1995*) New tools for 21st century plant breeding. DNA markers: theory and applications.* Turrialba, Costa Rica : Tropical Agriculture Research and Higher Education Centre, Centro Agronómico Tropical de Investigación y Enseñanza.

Groh, S., Khairallah, M.M., González-de-Leon, D., Willcox, M., Jiang, C., Hoisington, D.A. and Melchinger, A.E. (1998) Comparison of QTLs mapped in RILs and their test-cross progenies of tropical maize for insect resistance and agronomic traits. *Plant Breeding* **117**: 193-202.

Lanaud, C., Risterucci, A.M., N'Goran, A.K.J., Clement, D., Flament, M.H., Laurent, V., Falque, M. (1995) A genetic linkage map of *Theobroma cacao* L. *Theoretical and Applied Genetics* **91** (6-7): 987-993.

Nyassé, S., Cilas, C., Herail, C., Blaha, G. (1995) Leaf inoculation as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytophthora* black pod disease. *Crop Protection* **14**(8): 657-663.

Purseglove, J.W. (1968) *Tropical Crops. Dicotyledons*. London, UK : The Longman Group Ltd.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T.V.D., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23** (21): 4407-4414.

Warren, J.M. (1994) Estimation of the number of loci involved in the inheritance of resistance to *Phytophthora palmivora* in the leaves of *Theobroma cacao*. *Plant Pathology* **43**: 73-79