Euphytica (2006) 147: 433–440
DOI: 10.1007/s10681-005-9042-y

An SSR-based molecular genetic map of cassava

E. Okogbenin[1,2], J. Marin[1,3] & M. Fregene[1,*]
[1]*International Center for Tropical Agriculture, CIAT, Km 17, Recta Cali-Palmira, AA6713, Cali, Colombia;* [2]*University of Ibadan, Ibadan, Nigeria;* [3]*Universidad del Tolima, Ibague, Tolima, Colombia*
*(*author for correspondence:e-mail: M.Fregene@cgiar.org)*

Received 19 May 2005; accepted 18 October 2005

Key words: cassava, SSR markers, linkage map

Summary

Microsatellites or simple sequence repeats (SSR) are the markers of choice for molecular genetic mapping and marker-assisted selection in many crop species. A microsatellite-based linkage map of cassava was drawn using SSR markers and a F_2 population consisting of 268 individuals. The F_2 population was derived from selfing the genotype K150, an early yielding genotype from an F_1 progeny from a cross between two non-inbred elite cassava varieties, TMS 30572 and CM 2177-2 from IITA and CIAT respectively. A set of 472 SSR markers, previously developed from cassava genomic and cDNA libraries, were screened for polymorphism in K150 and its parents TMS 30572 and CM 2177-2. One hundred and twenty two polymorphic SSR markers were identified and utilized for linkage analysis. The map has 100 markers spanning 1236.7 cM, distributed on 22 linkage groups with an average marker distance of 17.92 cM. Marker density across the genome was uniform. This is the first SSR based linkage map of cassava and represents an important step towards quantitative trait loci mapping and genetic analysis of complex traits in *M. esculenta* species in national research program and other institutes with minimal laboratory facilities. SSR markers reduce the time and cost of mapping quantitative trait loci (QTL) controlling traits of agronomic interest, and are of potential use for marker-assisted selection (MAS).

Introduction

Cassava (*Manihot esculenta* subsp. *esculenta* Crantz) is the principal source of calories for more than 500 million people from mainly poor populations of the tropical regions (Cock, 1985; Best & Henry, 1992). As a staple food, it is the sixth most important crop worldwide (Mann, 1997). Cassava is an alloploid with 36 chromosomes having a DNA content of 1.67 pg per cell nucleus (Awoleye et al., 1994). This value corresponds to 772 mega base pairs in the haploid genome and puts cassava's genome size at the lower end of the range of higher plants (Bennet et al., 1992).

The first genetic linkage map for cassava was constructed with predominantly RFLP markers and a full-sib intra-specific cross (Fregene et al., 1997). The map has so far provided initial tools for genetic analysis of important traits of cassava (Jorge et al., 2000, 2001; Akano et al., 2002; Okogbenin & Fregene, 2002; Okogbenin & Fregene, 2003), as a first step towards such a rational use of molecular markers in cassava breeding.

However an F_1 progeny is not the ideal population for genetic analysis of complex quantitative traits. It cannot be used to detect recessive or epistatic interactions, important gene actions in traits of agronomic interest. The use of full-sib crosses from heterozygous parents alters QTL mapping by redefining mating type at a locus level rather than all loci in parents and also detection of QTL alleles is based on separate maps for each parent. The marker genotype in the F_1 progeny populations results from the independent meioses and crossovers in the maternal and paternal parents thus individual maps are often constructed for each parent (Grattapaglia et al., 1994; Groover et al., 1994; Van Eck et al., 1994).

Furthermore, RFLPs cannot be transferred readily to national programs of the developing world because it is expensive and laborious, furthermore facilities for the radioactive procedures are not available in most laboratories. Microsatellite or simple sequence repeat (SSR) markers are preferable, because they are easy to implement in most laboratories, are reproducible, and amenable for high throughput marker genotyping. Furthermore, PCR-based marker systems such as SSR produce results within a day. SSRs are small tandem repeats of DNA, usually 2–5 bp in length, that occur in most eukaryotic genomes. They are widely applied in plant genome mapping and genetic analysis because of their co-dominant inheritance, high degree of polymorphism and ease of analysis (Akkaya et al., 1992, 1995; Senior & Heun, 1993; Jarret & Bowen, 1994; Plaschke et al., 1995; Roder et al., 1995; Rongwen et al., 1995; Hamwieh et al., 2005).

To overcome the problems associated with the use of labor intensive RFLP markers and problems with genetic analysis in an F_1 cross of non-inbred parents, we used simple sequence repeat (SSR) markers to conduct genetic mapping in an F_2 population. A genetic map derived using an F_2 population should be much more informative than an F_1 population. In addition, an SSR-based F_2 – derived map of cassava will allow for a higher level of map saturation unlike parent-specific mapping in the F_1. The F_2 – derived map will be of value to studies designed to identify markers associated with traits of interest and for comparative analysis with other related species. In addition, SSR markers associated with traits of interest will facilitate marker-assisted selection (MAS) in a modest cassava breeding program. We report here the construction of the first SSR marker-based genetic map of cassava.

Materials and methods

Plant material and DNA isolation

The F_1 cassava mapping population described in Fregene et al. (1997) was analyzed for early yield and related traits in 1998 and 1999. Based on results obtained and profuse flowering abilities, three F_1 individuals (K68, K145 and K150) were pre-selected and selfed to produce F_2 populations. These F_1 individuals were derived from the cross between 'TMS30572' (female parent), an elite cassava cultivar from the breeding program at the International Institute of Tropical Agriculture (IITA), Nigeria, and CM2177-2′ (the male parent), a successful cassava cultivar resulting from breeding activities at the Centro Internacional Agricultura Tropical (CIAT) in Colombia. The highest germination rate was recorded in K150, with 372 seedlings as compared with 316 and 245 seedlings for K68 and K145 respectively, K150 also showed the highest heterozygosity with SSR markers. The progeny of K150 were therefore selected for genetic mapping studies.

The progeny used for map construction consisted of 268 individuals produced from selfing K 150. From each F_2 genotype, approximately 3 g of young leaf tissue from greenhouse-grown plants was collected in a mortar and immediately frozen in liquid nitrogen. Genomic DNA was extracted from the frozen leaf samples of each individual of the F_2 population and from the grand parents (TMS 30572 and CM 2177-2) and K150 as described by Dellarporta et al. (1983). DNA concentrations were quantified using a DNA fluorometer. DNA quality and integrity were assessed by electrophoresis on agarose gels.

Molecular marker analysis

One hundred and eighty six SSR markers from a genomic library (Mba et al., 2001), 132 SSR markers from a cassava root and leaf cDNA library (Mba et al., 2001 unpublished data), and 154 SSR markers from a genomic library (Fregene et al., 2002 unpublished data) were used, totaling 472 markers. The SSR primer sequences used in this study are available upon request from the International Center for Tropical Agriculture (CIAT, the Spanish acronym) Colombia.

All of the primer pair combinations were first screened with the grandparents (TMS 30572 and CM 2177-2) and K150 (F_1 parent of the F_2 population) to find the polymorphic SSR marker. The selected polymorphic markers were then used for evaluating the whole population. Some of the polymorphic markers with non-specific amplifications and/or too faint products were discarded from the final population assay.

PCR was performed in 96-well plates in PTC200 thermocyclers (MJ Research, Watertown, Mass). Amplifications were carried out in 12.5-μl reactions containing 25 ng of DNA, 5pmoles of each primer, 10 $\times$ of *Taq* polymerase buffer (500 mM KCl, 100 mM Tris-HCI (pH 8.5), and 1 mg/ml gelatin), 1.0 mM of $MgCl_2$, 0.5 mM of dNTPs and 0.25 U of *Taq* polymerase. The final volume was adjusted with sterile distilled H_2O. The PCR profile was: 94 °C for 10 min, followed 95 °C for 4 min, 25 cycles at 95 °C for 1 min, 55 °C for 2 min

and finally 10 min at 72 °C for the final extension. The PCR products were separated by running on 6% polyacrilamide denaturing gels (PAGE) gels and electrophoresed in 1× TBE at 100 W for 2 h using a Bio-Rad sequencing gel rig (BIORAD, California). The amplified products were visualized by silver staining. Two sequential loadings, after an interval of about 20 min, of PCR amplification product of the progeny were done to increase the efficiency of the mapping process.

Genetic linkage analysis

SSR alleles segregating in the mapping population were scored according to the expected classes for an F_2 population. Alleles derived from female grandparent were scored as "A" alleles whereas alleles from the male grandparent were designated "B" alleles. Individuals homozygous for maternal grandparental alleles were scored "AA", heterozygous "AB" and homozygous for paternal grandparental "BB". Marker classes at each locus were summarized for all individuals into the three different genotypic classes expected for a F_2 population and chi square tests for segregation distortion were carried out to compare the observed with the expected 1:2:1 ratio. Chi square analysis was performed at the threshold of $P = 0.001$ to test for significant deviations from expected ratios (segregation distortion).

Linkage analysis was with MAPMAKER/EXP, version 3.0 (Lander et al., 1987). Linked markers were identified using the group command and a recombination value of 0.30 and LOD of 3.0. For each group of markers, three point analysis was performed. Markers within groups were then ordered using the order command with LOD > 2.0. The resulting marker order was examined using the "ripple" command to ascertain the order was at least 100 times better than the second best order. The marker order was considered as the framework for each linkage group.

Recombination frequencies were converted to map distances (cM) using the Kosambi mapping function (Kosambi, 1944). The remaining markers were then placed with the try command. The "error detection" command was used to check for unexpected mistakes in data entering.

Results

SSR polymorphism and segregation of markers

Of the 472 SSR markers, 163 were found to be heterozygous in K150. Eight markers, which were polymorphic in K150, TMS 30572, and CM 2177-2, did not segregate in the F_2 progeny, revealing that these markers may be duplicated loci. Percentage SSR polymorphism was 60% for genomic DNA and 40% for cDNA library. Seventy three percent (122) of the markers evaluated segregated in 1:2:1 ratio. Thirty three markers (27%) showed distorted segregation ($P \leq 0.001$, chi-square test). Results of linkage analysis revealed that markers with distorted segregation were distributed throughout the genome. Deviation from the expected segregation ratios was observed for markers on thirteen LGs. The number of markers showing segregation distortion varied from 1 to 4 per LG. The most extreme examples of segregation distortions in the F_2 were found with marker SSRY 100 on LG 16 where only 16 of 260 plants were "AA" homozygotes, and with marker NS 33 on the same linkage group where only 17 of 235 plants scored were also "AA" homozygotes.

An SSR linkage map

One hundred and twenty two markers were employed in the linkage analysis and 100 of these markers could be assigned to 22 linkage groups (LG1 – LG22), which had 2–8 markers, and a linkage group length varying from 9.7 cM (LG19) to 129.9 cM (LG3) (Table 1). The linkage map of the F_2 population spanned a total of genetic distance of 1236.7 cM (Kosambi cM), with 22 markers remaining unlinked. Markers were randomly distributed on the 22 linkage groups.

The distance between the markers on the map also varied greatly across the different linkage groups. The average marker distance was 17.92 cM, with intervals between loci ranging from 5.6 to 39.8 cM (Figure 1). The size of the LG did not necessarily reflect the number of linked markers. For instance, LG 2, with a total linkage distance of 84.3 cM had 8 mapped loci, whereas LG 12, with a distance of 105 cM was covered by only 5 markers. However the correlation between linkage distance and number of markers was high ($r = 0.75$, $P = 0.05$) indicating that the SSR markers were fairly distributed randomly across the genome. Table 1 provides a summary of SSR marker distribution on different linkage groups showing the size, number of markers and the average marker interval of each LG. The number of LG in this map (22) exceeded the haploid number of chromosomes for cassava ($n = 18$), indicating that the map is not saturated.

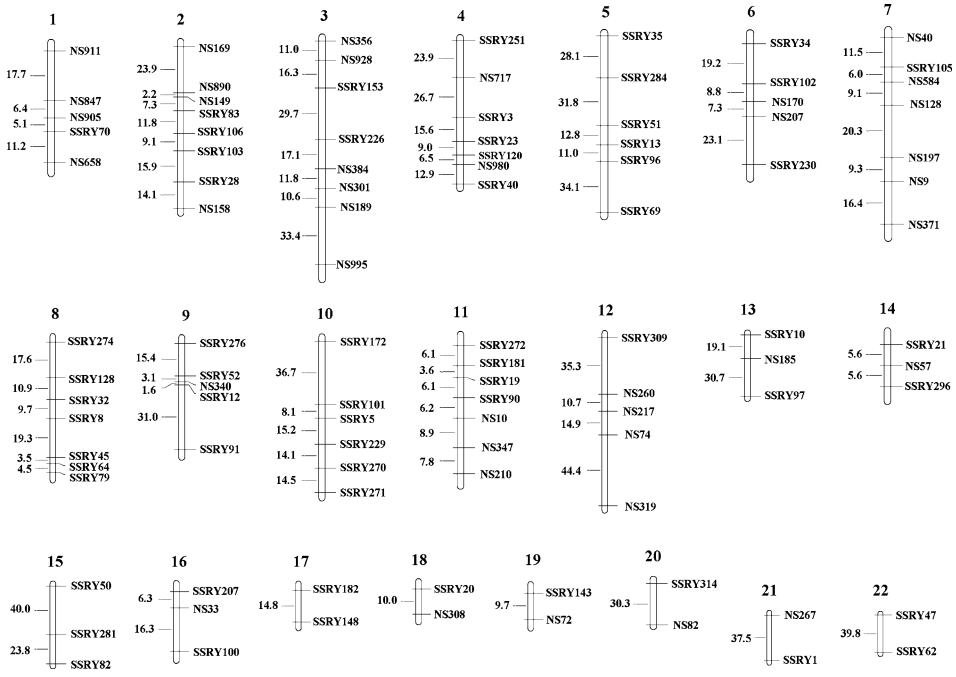

Figure 1. A genetic linkage map of cassava (*Manihot esculenta* Crantz) based upon a F_2 cross and SSR marker.

Comparison with F_1 map

The length of the cassava genome based on genetic mapping in an F_1 cross is estimated to be about 1610 cM (Fregene et al., 1997). The F_1 map (Fregene et al., 1997) of this species differed from the F_2 map with respect to marker type and number, genome coverage (span) and marker density. The F_1 female parent-derived map spans 931.6 cM with 168 markers compared to the F_2 map with 1236.7 cM and 100 markers.

Mapping of SSR markers in the F_1 map have also been conducted (Zarate et al., 2002 unpublished results; Libreros et al., 2002 unpublished results), so far, about 200 SSR markers have been placed on the F_1 map and sixty-seven of the SSR markers are common to both the F_2 population and the F_1 map. Zarate et al. (2002 unpublished results) reported that in the F_1 genetic map, segregation distortion among the 200 SSR markers mapped was about 20%, which was fairly close to that obtained in the F_2 (27%).

A good proportion of the markers mapped in the F_1 were not polymorphic illustrating the need for a larger screening of SSR marker when dealing F_2 as compared with F_1 populations. Forty seven SSR markers were found common to the F_1 and F_2 maps. With the exception of markers NS40, NS9, and NS74, 22 intervals bearing the other 44 markers are shown in Table 2. A majority (43) of the common SSRs showed colinearity between F_1 and F_2 maps (Zarate et al., unpublished data) indicating the reliability of both maps. However some differences were detected for some markers. A few differences in order were evident for some markers in LG5 (SSRY 35, SSRY 13), LG 7 (NS40, NS 9) and LG 12 (NS 74) in the F_2 map. These differences were probably due to statistical inaccuracy associated to the limited number of individuals studied in the F_1 (150 individuals). Other possible reasons include missing data for some markers, very short intervals between markers (which could complicate the exact ordering) or the presence of additional markers between two of the common markers in one of the maps. The mean and variation in the lengths of the linkage groups in the F_2 is also similar with that found in the F_1.

Some marker intervals were found to be consistent between the F_1 and F_2 maps (for example, in LG12 the interval NS 260-NS 217 was 10.5 cM in the F_1 and 10.7 cM in the F_2; in LG2 for SSRY 83 –NS 890 the interval was 9.9 cM in the F_1 and 9.5 cM in the F_2) (Table 2). However, there were also recombination differences between the two maps in some genomic regions (for example in LG10, the interval between SSRY172-SSRY101 was 75 cM in the F_1 and 36.7 in

Table 1. Linkage group size, number of markers, and the average marker interval per linkage group of the F_2 linkage map

Linkage group	Size (cM)	No. of markers	Average marker interval (cM)
1	40.4	5	10.1
2	84.3	8	12
3	129.9	8	16.2
4	94.6	7	15.7
5	117.8	6	23.5
6	58.4	5	14.6
7	72.6	7	12.1
8	65.5	7	10.9
9	51.1	5	12.8
10	88.6	6	17.7
11	38.7	7	6.45
12	105.3	5	26.3
13	49.8	3	24.9
14	11.2	3	5.6
15	63.8	3	31.9
16	22.6	3	11.3
17	14.8	2	14.8
18	10	2	10
19	9.7	2	9.7
20	30.3	2	30.3
21	37.5	2	37.5
22	39.8	2	39.8
$\sum$/mean	1236.7	100	17.9

Table 2. Marker pairs intervals in the F_1 and F_2 Maps

Linkage Group	Marker (SSR)	F_1 (cM)	F_2 (cM)
20	NS82-SSRY314	26.1	30.3
14	SSRY296-SSRY21	20.7	11.2
4	NS980-SSRY40	7.8	12.9
	SSRY3-SSRY23	8.2	15.6
	SSRY251-NS717	19.8	23.9
12	NS260-NS217	10.5	10.7
2	SSRY83-NS890	9.9	9.5
	NS189-NS995	1.2	33.4
3	NS928-SSRY226	44.3	46
9	SSRY12-NS340	7.9	1.6
	SSRY52-NS340	7.3	3.1
5	SSRY35-SSRY284	14.6	28.1
	SSRY13-SSRY284	48.7	44.6
10	SSRY172-SSRY101	75	36.7
	SSRY101-SSRY229	11.4	23.3
16	NS33-SSRY100	71.5	16.3
18	NS308-SSRY20	14	10
13	SSRY10-NS185	10.9	19.1
11	NS210-NS347	5.8	7.8
	NS347-NS10	4.6	8.9
	NS10-SSRY90	5.2	6.2
	SSRY90-SSRY19	5.2	6.1
Average		19.57	18.42

the F_2; for SSRY101-SSRY229 it was 11.4 cM in the F_1 and 23.3 cM in the F_2). The average marker intervals based on 22 marker pairs (Table 2) were 19.57 cM in the F_1 and 18.42 in the F_2 (Table 2), indicating that the average recombination frequencies between both maps were similar.

Discussion

SSR markers are advantageous to applied plant breeding because they are co-dominant, easily assayed and detect high levels of polymorphism (Morgante and Olivieri, 1993) and for these reasons SSR markers have become highly valuable markers to breeders for the purposes of genome and QTL mapping. SSR markers have, thus, become the marker class of choice for the molecular mapping of many crop species (Roa et al., 2000).

The high degree of microsatellite polymorphism, 50%, that we observed in cassava is not surprising and is comparable to the results of other crop species (Udupa et al., 1999; Winter et al., 1999). The polymorphism detected with RFLPs in cassava is lower, an average of 40%. A few markers revealed monomorphic double bands indicating the possibility of duplicated loci for such genomic regions.

Segregation distortions have been reported in several studies and a high frequency of markers showing distorted segregation is common in outcrossing species (Kubisiak et al., 1995; Hanley et al., 2002; Dettori et al., 2001; Liebhard et al., 2002). The level of segregation distortion observed in this study (27%) is within the range reported in plant molecular studies (Schon et al., 1993; Lin et al., 1996; Wang et al., 1998). Deviations of Mendelian segregation ratios may be due to various processes amongst which may be the presence of gametophytic selection for sub lethal genes i.e. genes controlling the viability of pollen, zygote or seedlings, putatively located on one or more of the these linkage groups (Yan et al., 2005). Cassava is an outcrossing species with high genetic load and suffers from severe

inbreeding depression. Segregation distortion in cassava may therefore not be unrelated to the association between heterozygosity and plant vigor found in cassava.

We have constructed the first PCR marker-based genetic linkage map of cassava that contains only SSR loci but the map requires further saturation. The expected number of eighteen linkage groups for a comprehensive linkage map of cassava ($2n = 36$) was exceeded by seven linkage groups, out of which five linkage groups had only two markers, and four linkage groups had only three markers. None of the 47 common SSR markers identified in the F_1 and F_2 populations allowed us to merge some of the small linkage groups. Since most linkage groups are small, it is safe to conclude that the apparent excess of linkage groups might be due to incomplete coverage of the genome with the marker loci. Furthermore, the high percentage of polymorphic SSR markers that remained unmapped indicates that the F_2 map is not yet saturated. We anticipate that the smaller groups will be brought together as new markers are identified.

Marker distribution along the linkage groups (LG) was not uniform, as evident by the mixture of tightly linked loci and regions with low density as observed in the constructed map. This suggests that either recombination events or mapped loci were not evenly distributed throughout the genome. The low density of markers in some of the linkage groups might also correspond to regions highly homozygous and subject to higher recombination frequencies events (Castiglioni et al., 1999).

Differences in map length between the F_1 and F_2 can result from a variation in the number of recombination events in the two maps as well as variations in the numbers and locations of mapped loci. For most of the linkage groups, the order of the markers in both maps is consistent apart from minor differences on some linkage groups. The presence of common markers in both maps favors, not only the identification of homologous linkage groups but also the integration of the F_1 and F_2 maps. Multi-parental genetic mapping recommended by Murranty (1996) is a potential field of application. Through such common markers, QTLs identified using the F_1 map for important agronomic traits, can be revalidated in the F_2. This is useful from the point of view of breeding and stability in different genetic backgrounds, prerequisites for using molecular markers for marker-assisted selection.

QTL analyses in the F_1 population were done using the RFLP based genetic map constructed by Fregene et al. (1997). The mapping of 200 SSR markers in the F_1 and the development of an SSR based F_2 genetic map is expected to improve QTL mapping efficiency for complex traits in cassava. This F_2 population holds great potential for the detection of QTL of agronomic interest in view of marker-assisted selection. This SSR map will complement genetic analysis in cassava and should provide us the additional opportunity to estimate genetic effects of QTLs. Development of an F_2 map provides a different generation to study the QTLs and their genetic effects. In an F_2 population, one can determine the effect of different gene action on phenotype because all three possible gene dosages at a locus are represented. This cannot be done exhaustively in an F_1 population. Thus an F_2 population can be used to map recessive and epistatic genes from either parent (Patterson et al., 1991) unlike the F_1.

Dominance effects can be determined and fully exploited in generations of F_1 populations. Dominance effects are likely to play an important role in the performance of the crop since cassava is highly heterozygous. Once an elite clone is identified and selected in a breeding scheme, it can be propagated vegetatively, therefore carrying along the dominance effects. However, because inbreeding is not often carried out in cassava improvement programs, a sizeable genetic load (undesirable or deleterious genes) may prevent large and sustained genetic gains (Ceballos et al., 2004). Therefore, the genetic improvement of cassava could benefit from the use of F_2 populations in genetic mapping studies, because F_2 populations (or inbreeding) can improve the probabilities of detecting useful recessive traits. However, because inbreeding depression can be a problem in cassava, tolerance to inbreeding in cassava needs to be improved. Development of S_1 or S_2 inbred families with tolerance to inbreeding is currently an on-going activity at CIAT (Ceballos et al., 2004).

In marker assisted breeding, co-dominant markers such as SSRs are effective in identifying desirable genotypes at early stages of selection. Therefore an F_2 SSR based map is an important pre-requisite for molecular marker-assisted selection (MAS) to increase the efficiency of cassava breeding. Efforts are on-going to develop more SSR markers and to construct a more saturated F_2 map for QTL detection studies.

Acknowledgments

We are grateful to Roosevelt Escobar and Edgar Barrera for laboratory assistance and to Jose Antonio Lopez,

and Jairo Valencia for their help during plantings and evaluations. This research was funded by grants from CIAT and Rockefeller Foundation (RF). EO was supported by doctoral fellowship awarded by the RF.

References

Akkaya, M.S., A.A. Bhagwat & P.B. Cregan, 1992. Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132: 1131–1139.

Akkaya, M.S., R.C. Shoemaker, J.E. Specht, A.A. Bhagwat & P.B. Cregan, 1995. Integration of simple sequence repeat DNA markers into a soybean linkage map. Crop Sci 35: 1439–1445.

Allard, R.W., 1988: Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. J Hered 79: 225–238.

Awoleye, F., M. Van Duren, J. Dolezel & F.J. Novak, 1994. Nuclear DNA content and in vitro induced somatic polyploidization (*Manihot esculenta* Crantz) cassava breeding. Euphytica 76: 195–202.

Bennet, M.D., J.B. Smith & J.S. Heslop-Harrison, 1992. Nuclear DNA amounts in Angiosperms. Proc R Soc London Ser B 216: 179—199.

Berloo, R. & P. Stam, 1998. Marker assisted selection in autogamous RIL populations: a simulation study. Theor Appl Genet 96: 147–154.

Best, R. & G. Henry, 1992. Cassava: towards the year 2000. *In* International network for cassava genetic resources. Report of the first meeting of the International Network for Cassava Genetics International Crop Network Series number 10. International plant Genetic resources institute (IPGRI). 10: 3–11.

Bryne, M., J.C. Murrell, J.V. Owen, A. Kriedemann, E.R. Williams & G.F. Moran, 1997. Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in Eucalyptus nitens. Theor Appl Genet 94: 674–681.

Castiglioni, P., P. Ajmone-Marsan, R. Van Wijk & M. Motto, 1999. AFLP markers in amolecualr linkage map of maize: codominant scoring and linkage group distribution. Theor Appl Genet 99: 425–431.

Ceballos, H., C.A. Iglesias, J.C. Perez & A.G.O. Dixon, 2004. Cassava breeding: opportunities and challenges. Plant Molecular Biology 56: 503–516.

Churchill, G.A. & R.W. Doerge, 1994. Empirical threshold values for quantitative trait mapping. Genetics 138: 963–971.

Cock, J.H., 1985. Cassava:new potential for a neglected crop. Westview press, Boulder Colorado USA.

Dellarporta, S.L., J. Wood & J.R. Hicks, 1983. A plant DNA minipreparation: version II. Plant Mol Biol Rep 1: 19–21.

Dettori, M.T., R. Quarta & I. Verde, 2001. A peach linkage map integrating RFLPs, SSRs, RAPDs, and morphological markers. Genome 44: 783—790.

Doege, R.W., 1993: Statistical methods for locating quantitative trait loci with molecular markers. PhD dissertation. North Carolina State University.

Eck van, H.J., J.M.E. Jacobs, P. Stam, J. Ton, W.J. Stiekema & E. Jacobsen, 1994. Multiple alleles for tuber shape in diploid potato detection by qualitative and quantitative genetic analysis using RFLPs. Genetics 137: 303–309.

El-Sharkawy, M.A. & J.H. Cock, 1990. Photosynthesis of cassava (*Manihot esculenta*). Expl Agric 26: 325–340.

Fregene, M., E. Okogbenin, C. Mba, F. Angel, M.C. Suarez, J. Guttierez, P. Chavarriaga, W. Roca, M. Bonierbale & J. Tohme, 2001. Genome mapping in cassava improvement: Challenges, achievements and opportunities. Euphytica 120:159–165.

Fregene, M., F. Angel, R. Gomez, F. Rodriguez, P. Chavariaga, W. Roca, J. Tohme & M. Bonierbale, 1997. A molecular genetic map of cassava. Theor Appl Genet 95: 431–441.

Grattapaglia, D., F.L.G. Bertolicci & R. Sederoff, 1995. Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Theor Appl Genet 90: 933–947

Grattapaglia, D. & R. Sederoff, 1994. Genetic linkage maps of *Eucalyptus grandis* and *E. urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137: 1121–1137.

Groover, A., M. Devey, T. Fiddler, J. Lee, R. Megraw, T. Mitchel-Olds, B. Sherman, S. Vujcic, C. Williams & D. Neale, 1994. Identification of quantitative trait loci influencing wood specific gravity in an outbred pedigree of loblolly pine. Genetics 138: 1293–1300.

Gruneberg, J.B., 1938. An analysis of the pleiotropic effects of a new lethal mutation in the rat (*Mus norvegicus*). Proc R Soc Lond B 125: 123–144.

Hamwieh, A., S.M. Udupa, W. Choumane, A. Sarker, F. Dreyer, C. Jung & M. Baum, 2005. A genetic linkage map of *Lens* sp. Based on microsatellite and AFLP markers and the localization of fusarium vascular kilt resistance. Theor Appl Gent 110: 669–677.

Hanley, S., J.H.A. Barrer, J.W. Van Ooijen, C. Aldam, S.L. Harris, I. Ahman, S. Larsson & A. Kart, 2002. A genetic linkage map of willow (*Salix viminalis*) based on two *Lycopersicon esculentum* x *L. pennellii* F_2 populations. Theor Appl Genet 99: 254–271.

Jarret, R.L. & N. Bowen, 1994. Simple sequence repeats (SSRs) for sweet potato germplasm characterization. Plant Genet Res Newslet 100: 9–11.

Jorge, V., M. Fregene, C.M. Velez, M.C. Duque, J. Tohme & V. Verdier, 2001. QTL analysis of field resistance to *Xanthomonas axonopodis* pv *manihotis* in cassava Theor. Appl Genet 102: 564–571.

Jorge, V., M.A. Fregene, M.C. Duque, M.W. Bonierbale, J. Tohme & Verdier, 2000. Genetic mapping of resistance to bacterial blight disease in cassava (*Manihot esculenta* Crantz). Theor Appl Genet 101: 865–872.

Kawano, K., 1990. Harvest index and evolution of major food crop cultivars in the tropics. Euphytica 46: 195–202.

Kosambi, D.D., 1944. The estimation of map distances from recombination values. Ann Eugen 12: 172–175.

Kubisiak, T.L., C.D. Nelson, W.L. Nance & M. Stine (1995). RAPD linkage mapping in a longleaf pine x slash pine F1 family. Theor appl Genet 90:1119–1127.

Lander, E.S. & D. Botstein, 1989. Mapping Mendelian factors underlying quantitative traits by using RFLP linkage maps. Genetics 121: 185–199.

Lander, E.S., P. Green, J. Abraham, A. Barlow, M.J. Daly, S.E. Lincoln & L. Newburg, 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174–181.

Liebhard, R., L. Gianfrancesschi, B. Koller, C.D. Ryder, R. Tarchini, E. Van de Weg & C. Gessler, 2002. Development and characterization of 140 new microsatellites in apple (Malus x domestica Borkh). Mol Breed 10: 217–241.

Lin, S.Y., T. Sasa & M. Yano, 1998. Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa*, using backcross inbred lines. Theor Appl Genet 96: 997–1003.

Mann, C., 1997. Reseeding the green revolution. Science 277: 209 –220.

Mba, R.E.C., P. Stephenson, K. Edwards, S. Melzer, J. Mkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme & M. Fregene, 2001. Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome:towards an SSR-based molecular genetic map of cassava. Theor Appl Genet 102: 21–31.

Mogante, M., & A.M. Olivieri, 1993. PCR-amplified microsatellite markers in plant genetics. Plant J 3: 393–427.

Murranty, H., 1996. Power of tests for quantitative trait loci detection using full sib families in different schemes. Heredity 76:156–165.

Nweke, F.I., A.G.O. Dixon, R. Asiedu & S.A. Folayan, 1994. Cassava varietal needs of farmers and potential for production growth in Africa. COSCA working paper 10.

Okogbenin, E. & M. Fregene, 2002. Genetic and QTL mapping of early root bulking in an F_1 mapping population of non-inbred parents in cassava (*Manihot esculenta* Crantz). Theor Appl Genet 106: 58–66.

Okogbenin, E., & M. Fregene, 2003. Genetic mapping of QTLs affecting productivity and plant architecture in a full-sib cross from non-inbred parents in cassava (*Manihot esculenta* Crantz). Theor Appl Genet 107: 1452–1462.

Olsen, K.M., & B.A. Schaal, 2001. Microsatellite variation in cassava (*Manihot esculenta,* Euphorbiacea) and its wild relatives: further evidence for a southern Amazonian origin of domestication. American Journal of Botany 88(1): 131–142.

Patterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln & S.D. Tanksley, 1988. Resolution of quantitative traits into Medelian factors, using a complete linkage map of restriction length polymorphisms. Nature 335: 721–726.

Patterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S. Lander & S.D. Tanksley, 1991. Mendelian factors underlying quantitative traits in tomato:comparison across species, generations and environments. Genetics 127: 181–197.

Plaschke, J., M.W. Ganal, & M.S. Roder, 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers Theor. Appl Genet 91: 1001–1007.

Rector, B.G., J.N. All, W.A. Parott, & H.R. Boerma, 1998. Identification of molecular markers linked to quantitative trait loci for soybean resistance to corn earworm. Theor Appl Genet 96: 786–790.

Roa, A.C., P. Chavarriaga-Aguirre, M.C. Duque, M.M. Maya, M.W. Bonierbale, C. Iglesias & J. Tohme, 2000. Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: allelic polymorphism and degree of relationship. Am J Bot 87: 1647–1655.

Roder, M.S., J. Plaschke, S.U. Konig, A. Borner, M.E. Sorrels, S.D., Tanksley & M.W. Ganal, 1995. Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246: 327–333.

Rongwen, J., M.S. Akkaya, A.A. Bhagwat, U. Lavi, & P.B. Cregan, 1995. The use of microsatellite DNA markers for soybean genotype identification. Theor Appl Genet 90: 43–48.

SAS Institute Inc, 1996. SAS/STAT software:changes and enhancement for release 6.12, Cary, NC: SAS Institute Inc. 158pp.

Senior, M.L., & M. Heun, 1993. Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36: 884–889.

Shapiro, S.S. & M.B. Wilk, 1965. An analysis of variance for normality (complete samples). Biometrika 52: 591–611.

Spickett, S.G. & J.M. Thoday, 1966: Regular responses to selection 3. Interaction between located polygenes. Genet Res 7: 96–121.

Titterington, D.M., A.F.M. Smith & U.E. Makov, 1985. Statitical analysis of finite mixture distributions. John Wiley and sons. N.Y.

Udupa, S.M., L.D. Robertson, F. Weigand, M. Baum & G. Kahl, 1999. Allelic variation at (TAA)n microsatellite loci in a world collection of chickpea (*Cicer arietinum* L.) germplasm. Mol Gen Genet 261: 354–363.

Winter P, T. Pfaff, S.M. Udupa, B. Huttel, P.C. Sharma, S. Sahi, R. Arreguin-Espinoza, F. Weigand, F.J. Muehlbauer & G. Kahl, 1999. Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L) genome. Mol Gen Genet 262: 90–101.

Xu, Y., L. Zhu, J. Xiao, N. Huang, & S.R. McCouch, 1997. Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, double-haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Gent 253: 535–545.

Yan, Z., A. Denneboom Hattendorf, O. Dolstra, T. Debener, P. Stam & P.B. Visse, 2005. Construction of an integrated map of rose with AFLP, SSR, PK, RGA, RFLP, SCAR and morphological markers. Theor Appl Genet 110: 766–777.