A Genetic Linkage Map of Sweetpotato (*Ipomoea batatas* (L.) Lam.) Based on AFLP Markers

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Genetic linkage maps of Tanzania and Bikilamaliya sweetpotatoes (*Ipomoea batatas* (L.) Lam), based on amplified fragment-length polymorphism (AFLP) were developed using a two-way pseudo-testcross. At a LOD score of 5, a total of 656 maternal and 469 paternal markers were ordered in 90 and 80 linkage groups, respectively, covering a total map length of 3655.6 cM for Tanzania and 3011.5 cM for Bikilamaliya. The average distance between markers is 5.9 cM, with only four intervals exceeding 30 cM. Mapping was carried out in two steps. Linkage among simplex markers provided a map framework to which multiple-dose markers were added. The maternal maps contained 91 duplex markers, which allowed identification of 13 homologous co-segregating groups; paternal maps contained 69 duplex markers, allowing identification of 10 homologous co-segregating groups. The exploitation of 215 double-simplex bridging markers detected 15 linkage groups as homologous maternal and paternal groups. Type of polyploidy (autopolyploidy vs. allopolyploidy) was evaluated using the ratio of repulsion to coupling linkages. The absence of linkages in the repulsion phase indicated a high level of polysomic inheritance of homologous chromosomes. The detection of a few repulsion linkages at a lower confidence level, however, demonstrated that there is partial preferential chromosome pairing in sweetpotato.

Maps of highly dense genetic linkages are powerful tools for the localization and map-based cloning of genes, as well as marker-assisted breeding. They also provide information for understanding the biological basis of complex traits (Lee, 1995) and polyploidy (Da Silva, 1993). Different approaches are available for direct mapping of polyploid genomes, most of which have been based on the use of simplex (single-dose) markers (Wu et al., 1992) derived from only one parent and segregating in a 1:1 ratio. The single-dose restriction fragment (SDRF) method has been applied to various polyploid crops such as alfalfa, apple, sugarcane, and potato. Multiplex markers have been exploited to identify homologous co-segregating groups in *Saccharum* (Da Silva, 1993; Ripol et al., 1999) and alfalfa (Yu and Pauls, 1993). Da Silva (1993) was able to include duplex and triplex markers in simplex marker maps and to identify homologous groups.

Sweetpotato is a hexaploid species (2n=6x=90) with a base chromosome number of 15 (Jones, 1965). Cytological studies of sweetpotato are difficult because of the high number of small

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chromosomes and large number of basic groups, so the nature of its polyploidy remains uncertain. Both allopolyploidy (Jones, 1965; Magoon et al., 1970) and autopolyploidy (Nishiyama et al., 1982; Shiotani, 1988) have been proposed. Shiotani and Kawase (1989) postulated the genome constitution of sweetpotato as ($B_1B_1B_2B_2B_2B_2$), but the degree of homology could not be estimated with accuracy. Ukoskit and Thompson (1997) reported a polysomic inheritance in sweetpotato based on the segregation ratio and relationship of the genetic linkages of RAPD markers.

The commonly used mapping population for outbreeding trees and polyploids is the progeny of a cross between two unrelated heterozygous parents (a two-way pseudo-testcross) (Grattapaglia and Sederoff, 1994). With this mapping population, linkage analysis of SDRFs in coupling phase results in two separate linkage maps for each parent based on the male and female sources of markers. The ratio of repulsion linkages versus coupling linkages was used to evaluate type of polyploidy (Da Silva and Sorells, 1996).

In this paper, we report the genetic inheritance, segregation, and linkage of amplified fragment-length polymorphism (AFLP) markers in two hexaploid sweetpotatoes (2n=6x=90). We used an F1 population to construct two separate parental maps, integrating simplex and multiplex markers, and analyzed the genome constitution based on the pattern of inheritance and segregation of the markers. These are the first reported genetic-linkage maps that have substantial coverage of the sweetpotato genome. They provide a framework for tagging the genes and quantitative trait loci (QTL) of the economic traits of the sweetpotato.

Materials and Methods

Plant materials and DNA extraction

Seeds of an F1 mapping population originated from a two-way pseudo-testcross between two African sweetpotato landraces (Tanzania and Bikilamaliya). Tanzania is the most widely grown sweetpotato cultivar in sub-Saharan Africa and is resistant to the sweetpotato virus disease (SPVD) complex in East Africa. Bikilamaliya is susceptible to SPVD under the same environmental conditions. Linkage mapping was carried out on a subset of 94 randomly selected plants. Genomic DNA was extracted from young leaves of greenhouse-grown sweetpotatoes using the CTAB method (Doyle and Doyle, 1990).

AFLP assays and marker scoring

A procedure adapted from Zabeau and Vos (1993) was followed for the AFLP analysis. For the selection of primer combinations (PCs), the two parental varieties (Tanzania and Bikilamaliya) were screened with 240 EcoRI/MseI PCs. The PCs yielding a high number of polymorphic fragments for each parental line and with a total number of fragments ranging between 50–100 were selected for generating AFLP markers.

Autoradiographs were manually scored for the absence (0) or presence (1) of AFLP marker bands. Markers were chosen on the basis of their presence in one parent and absence in the other, or presence in both parents. Scored markers were divided into three groups depending on the presence or absence in each parent.

Segregation ratio

The assessment of marker dosage was done by the expected segregation ratios (presence:absence) of F1s for AFLP markers present in one parent in accordance with the allele dosage for three cytological theories of sweetpotato (Table 1). To classify fragments into dosage groups, acceptance regions for simplex, duplex, triplex, and double-simplex markers were constructed.

Simplex markers. All markers present in one parent and absent in the other were tested for goodness of fit to the 1:1 segregation ratio in the progeny by a χ^2 test at

Table 1. Expected segregation ratios (presence:absence) for the inheritance of a single gene in a hexaploid plant, according to three cytological theories based on allele dosage.

Marker dose	Autohexaploid (hexasomic)	Tetradiploid (tetradisomic)	Allohexaploid (disomic)
Simplex	Aaaaaa 1:1	Aaaa aa 1:1	Aa aa aa 1:1
		aaaa Aa 1:1	
Duplex	AAaaaa 4:1	AAaa aa 5:1	Aa Aa aa 3:1
		Aaaa Aa 3:1	AA aa aa -
		aaaa AA -	
Triplex	AAAaaa 19:1	AAAa aa -	Aa Aa Aa 7:1
		AAaa Aa 11:1	AA Aa aa -
		Aaaa AA -	
Quadruplex	AAAAaa -	AAAA aa -	AA Aa Aa -

the 99% confidence level, allowing type I error = type II error = 0.5%. The null hypothesis (1:1 segregation) was tested against the alternative hypothesis (3:1 segregation) since all non-single dose ratios would be 3:1 or greater, regardless of whether sweetpotato is an auto-, autoallo-, or allopolyploid (Wu et al., 1992).

Duplex markers. Among the three cytological theories listed in Table 1, allohexaploidy is the least likely genetic configuration in sweetpotato (Nishiyama et al., 1982; Shiotani, 1988; Ukoskit and Thompson, 1997). Under the assumption of either hexasomic or tetradisomic inheritance, duplex markers are expected to segregate 4:1 (hexasomic) and 3:1, 5:1 (tetradisomic) (Table 1). A 4:1, 5:1 segregation ratio corresponds to a duplex marker of fragments located on homologous chromosomes, compared to a 3:1 segregation ratio for a duplex marker where the individual fragments are located on nonhomologous chromosomes. The latter are not useful for the estimation of linkages among simplex/duplex and duplex/duplex marker pairs. As the population size used in this experiment was not large enough to distinguish between hexasomic and tetradisomic situations, we looked for a common subset of 4:1, 5:1 segregating markers as tested by a χ^2 test for goodness of fit at a 10% confidence level. This set of markers is sufficient to distinguish duplex markers from the expected segregation ratios for triplex markers (11:1, 19:1) and simplex markers (1:1). To separate the 3:1 from 4:1 or 5:1 segregation ratios, however, would require an extremely large population size. Therefore, in our study, calculation of 4:1 and 5:1 segregation ratios was not totally exclusive of 3:1 markers.

Triplex markers. Triplex markers are expected to segregate 11:1 under hexasomic inheritance and 19:1 under tetradisomic. As these segregation ratios cannot be distinguished properly, and estimates of recombination fractions among simplex and triplex markers are associated with large standard errors, triplex markers were not analyzed.

Quadruplex markers. Quadruplex or higher-dose markers were not expected to result in observable segregation in the offspring, except in the case of a quadruplex marker with random chromatid segregation, which would result in a genotype not showing the marker.

Double-simplex markers. AFLP fragments present in a single-dose condition in both parents are expected to segregate in a 3:1 ratio in the population, as tested for H_0 at a 10% significance level to avoid overlapping with the 11:1 segregation class for duplex-simplex markers.

Estimation of recombination fraction (r) and linkage mapping

Linkage analysis and map construction was carried out in two steps.

In the first step, two parental framework maps (maternal and paternal) were constructed using simplex markers. Simplex markers in coupling behave the same in diploids as in polyploids and can be mapped with standard methods. Since detection of repulsion linkages in polysomic polyploids requires extremely large populations, they were not included. Recombination fractions and marker order were obtained under the backcross model using JoinMap 2.0 software (Stam and Van Ooijen, 1995).

In the second step, duplex and double-simplex markers were inserted in the fixed marker order of the parental simplex framework maps. Recombination fractions were estimated by numerically maximizing the log-likelihood (Stam and van Ooijen, 1995), assuming the random pairing of homologous chromosomes and the absence of double reduction.

Duplex markers. As the true underlying mode of chromosome inheritance (hexasomic vs. tetradisomic) in sweetpotato remains uncertain, linkage of simplex/duplex and duplex/duplex markers in coupling were calculated for both hexasomic and tetrasomic inheritance, applying phenotypic frequencies given by Ripol et al. (1999). The sets of pairwise r and LOD estimates were alternatively mapped to the simplex framework map using standard procedures (JoinMap 2.0). All duplex markers placed at different map sites for the hexasomic and tetrasomic inheritance types were removed, resulting in identical maps with an equal number of markers and marker order, with slightly different map distances. The map with the smallest map size was selected for adding double-simplex markers.

Double-simplex markers. Recombination fractions for simplex/double-simplex and double-simplex/double-simplex configurations of marker pairs were estimated according to Meyer et al. (1998).

The final maps comprised simplex, duplex, and double-simplex markers, with each linkage group corresponding to an individual chromosome. Two linkage groups containing the same duplex marker are likely to be homologous. A duplex marker should not align to more than two linkage groups, unless a third group is an unconnected part of one of them. Two linkage groups were declared homologous female and male linkage groups if they possessed the same double-simplex (bridging) marker. Final maps were drawn using DrawMap 1.1 software (Van Ooijen 1994). The expected genome coverage was estimated according to Bishop et al. (1983).

Estimation of polyploidy

For species with levels of high polyploidy, the ratio between the number of detected repulsion versus coupling linkages may provide a crude measurement of preferential chromosome pairing (Sorrels, 1992; Wu et al., 1992). Using Mapmaker's two-point linkage analysis (LOD = 4, $r < 0.3$), the repulsion linkage was analyzed between an original marker and inverted markers.

Results and Discussion

The high multiplex ratio of the AFLP method was used to produce a large number of highly reliable genetic markers for linkage analysis. A total of 112 out of the 240 primer combinations screened were selected and used to generate AFLP markers for 94 individuals of the mapping population. Three genotypes were removed from the data set because they contained many missing data and could have affected the estimation of the genetic distances among the markers.

Segregation ratio

Clearly scorable markers were separated according to their presence in the male and female parents and in both. The observed segregation ratio for each of the 808 female and 641 male markers are shown in Figures 1 and 2. The distributions appear with a large group centered around

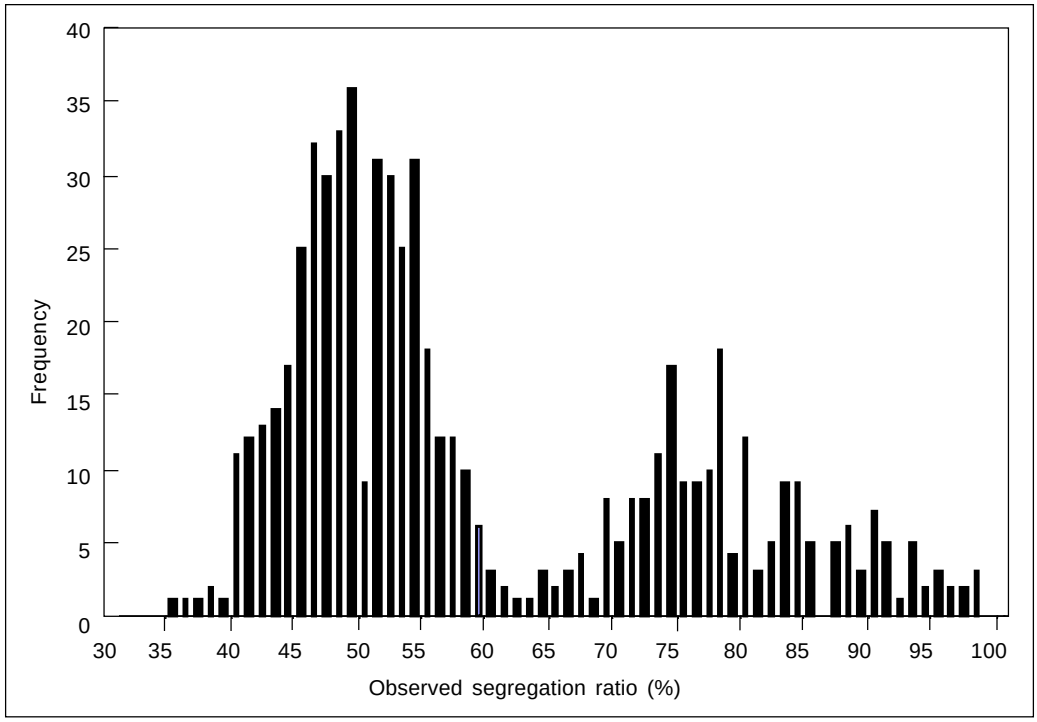

Figure 1. Observed segregation ratios for 808 markers in Tanzania.

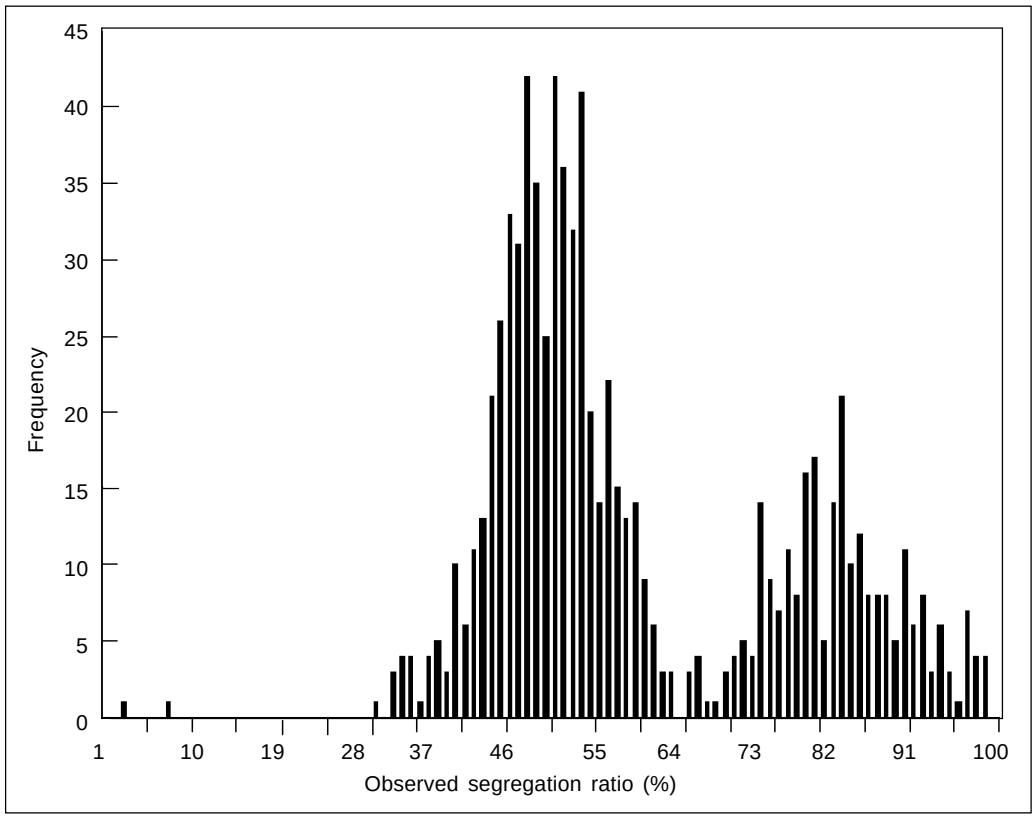

Figure 2. Observed segregation ratios for 641 markers in Bikilamaliya.

5% of the expected segregation ratio for simplex markers and a smaller group around 8% where ratios of 3:1, 4:1, or 5:1 duplex markers are expected. At higher frequencies, triplex markers or quadruplex markers were observed, as expected under random chromatide segregation. Segregation analysis resulted in 951 simplex markers. A total of 298 markers fitted into a 4:1 or 5:1 acceptance region as expected for duplex markers under hexasomic and tetrasomic inheritance, respectively. Eighteen markers showed a segregation ratio significantly lower than 1:1, which was the lowest expected segregation ratio; these were removed as skewed markers. Of 540 segregating markers present for both parents, 215 markers fitted into the 3:1 segregation ratio for double-simplex markers.

Estimation of polyploidy based on repulsion linkage

Type of polypoidy (autopolyploidy vs. allopolyploidy) was investigated using the ratio of coupling to repulsion linkages. For diploids and disomic polyploids (allopolyploids), linkages in the repulsion phase should be equal to those in the coupling phase, compared to autopolyploids, where an extremely large sample size is required for detecting repulsion linkages (Wu et al., 1992). Simplex marker scores were inverted and added to the original marker scores. In diploids—as in allopolyploids—a homologous recessive allele of dominant single-dose fragments may be simulated by its 'marker image' and this can be used to detect linkages in the repulsion phase. In autopolyploids, a total of m-1 alleles (where m = ploid number) hide behind a dominant simplex marker, so the image of a simplex marker score only approximates a recessive allele. Calculation of repulsion linkages in polysomic genomes, therefore, is limited to codominant markers.

Pairwise linkages among original and inverted markers were estimated using Mapmaker 3.0 (Lander et al., 1987). We did not find a single linkage in repulsion phase (at LOD = 4 and a maximum recombination fraction of 0.4), which would be highly unlikely if sweetpotato displayed strict disomic segregation. As suggested by Wu et al. (1992), this excludes allopolyploidy, indicating a high polysomy of sweetpotato chromosomes within a base set.

A few repulsion linkages detected at LOD = 3, r = 0.5 gave some evidence for partially preferential pairing, which supports a genome constitution ($B_1B_1B_2B_2B_2B_2$) with a certain degree of homology among the two genomes, as proposed by Shiotani and Kawase (1989). Chromosome association in many autopolyploid species is likely to be a combination of random and preferential chromosome association (Wu et al., 1992).

These results suggest that polysomic inheritance plays a major role in sweetpotato, with the occurrence of partially preferential pairing among certain chromosomes.

Framework map constructed with simplex markers

Two linkage maps were constructed, based on 539 simplex markers from maternal sources and 421 from paternal sources, using standard JoinMap 2.0 procedures (Stam and van Ooijen, 1995). To avoid false linkage detection, a LOD score of 5 was set as the linkage threshold for grouping markers. This reduces the rate of false positives to 10^{-5}, reducing the number of false linkages to less than 1 in 100,000. Markers that could not be put in the map during 'round one' or 'round two' of the JoinMap mapping procedure were omitted. A total of 44 markers for the female parent and 50 for the male parent remained unlinked. There were 90 linkage groups for Tanzania, with 48 major and 42 minor groups of three or two markers; for Bikilamaliya, this was 80 linkage groups, with 42 major and 38 minor groups. The resulting linkage groups provided a framework into which higher-dose markers

were mapped (for example, see Figure 3). Grouping was consistent through a range of LOD scores of from 4 to 6, suggesting that the groups formed were highly reliable. Linkage groups have been named randomly until they can be aligned to chromosome karyotypes.

Mapping of multiplex markers

The recombination fraction (r) and LOD scores for simplex/duplex and duplex/duplex marker configurations were calculated assuming (a) hexasomic and (b) tetrasomic chromosome inheritance. The absolute differences of pairwise estimates (of r and LOD scores) under the two inheritance types were low, with no more than seven duplex markers placed on different sites on the framework map. These were therefore removed. Duplex markers were assigned to one or two chromosomes, establishing a base-group connection within chromosomes.

The final maps comprised 678 markers for Tanzania, with a total map length of 3655.6 cM, and 481 markers for Bikilamaliya, covering a total map length of 3011.5 cM. The maternal map consisted of 90 linkage groups (71 groups with four or more markers), compared to the paternal map of 80 linkage groups (56 with four or more markers). Of 160 mapped male and female duplex markers, a total of 49 were aligned to two linkage groups, which allowed us to order 60 chromosomes to 13 female and 10 male homologous co-segregating groups. The maternal and paternal maps contained 70 and 31 double-simplex (bridging) markers, respectively, which allowed identification of 17 linkage groups as maternal and paternal homologous groups.

The largest female linkage group was 129 cM and the largest male group was 99.5 cM, with average spacing between marker loci of 5.9 cM with only four intervals exceeding 30 cM. This should provide reasonable genome coverage for the detection of quantitative trait loci (QTL). The percentages of mapped simplex, duplex, and triplex markers were 90%, 54%, and 30%, respectively.

Genome coverage

To avoid overestimating genome coverage, only framework markers were used in this procedure. The expected proportion of genome coverage was estimated using 656 markers for the female map and 469 for the male. Under the assumption of random marker distribution, the probability of being within 20 cM of any existing marker will be 91% for any new female marker and 88% for any new male marker (Bishop et al., 1983).

The derived linkage maps have not yet been resolved into the expected 90 linkage and 15 homologous groups. This is because the total length of the sweetpotato genome was not covered, and the small linkage groups, in particular, might represent unconnected parts of other linkage groups. Therefore, additional markers would be required to cover the entire genome and facilitate the merging of all homologous groups. Codominant markers are also needed to confirm the homology relations of chromosomes.

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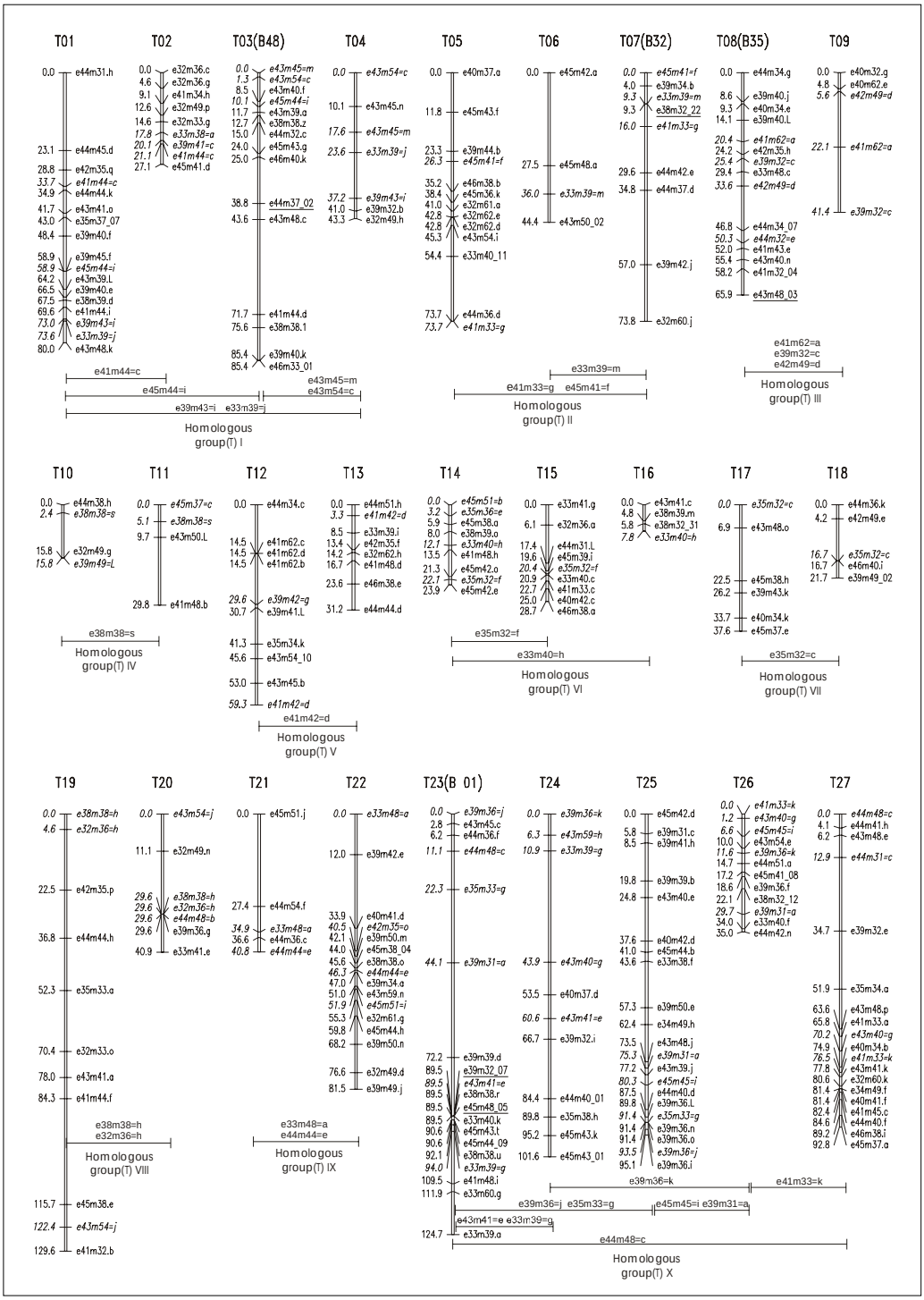
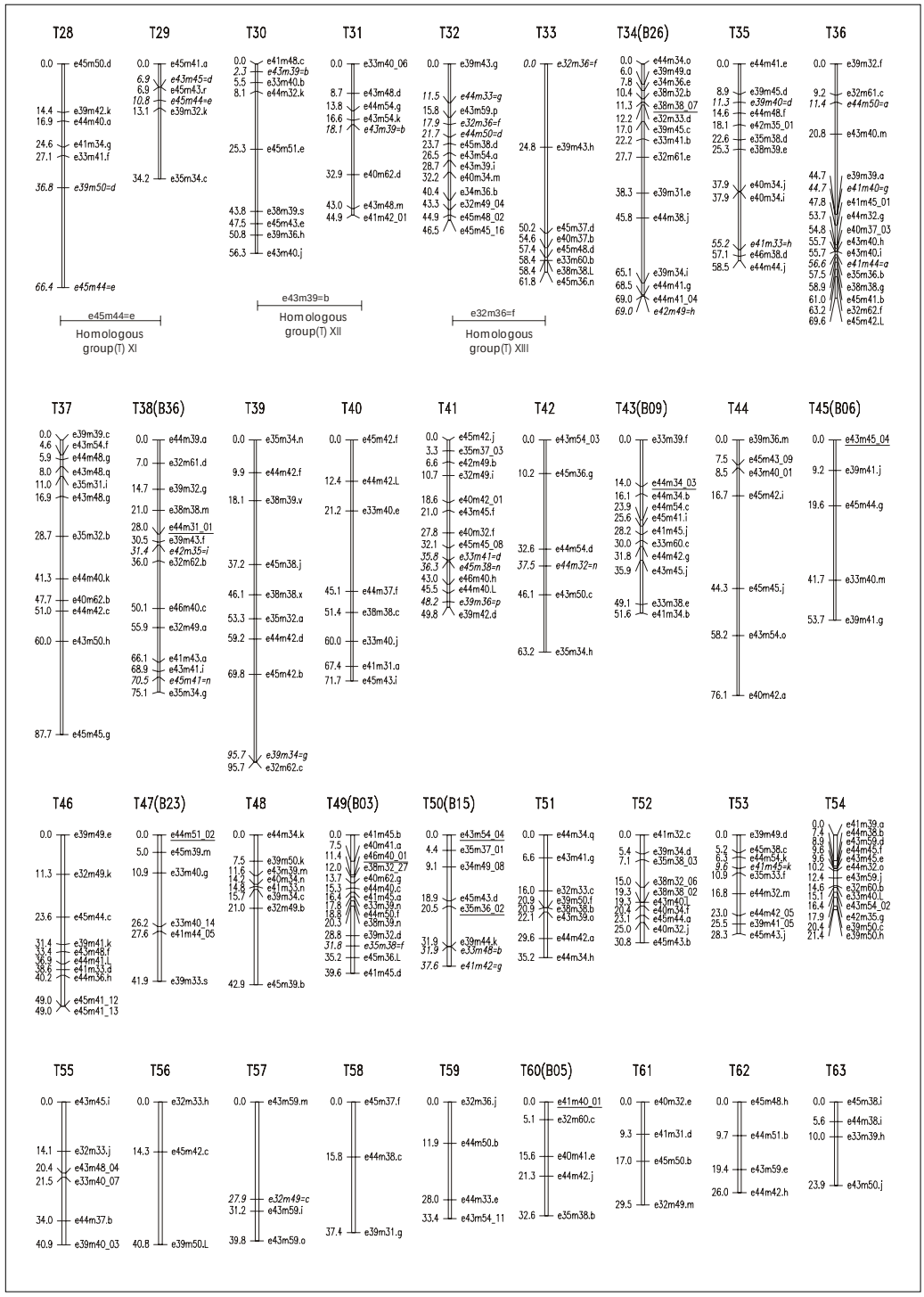


Figures 3. Maternal ('Tanzania') linkage maps Linkage groups are named at their top; a second name in parenthesis indicates the corresponding linkage group on the paternal map. Each marker locus is identified by



the corresponding combination of primers used for its generation. A letter or a number at the end of each marker identifies the individual fragments amplified by the same primer combination.

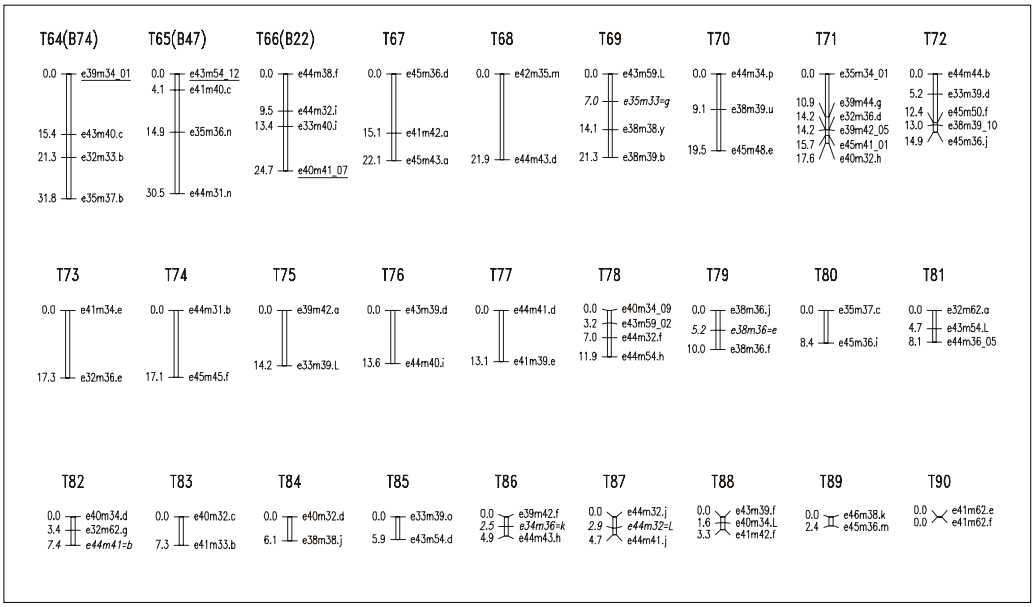


Figure 3. (continued)

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