J. Amer. Soc. Hort. Sci. 122(6):822–828. 1997.

Autopolyploidy versus Allopolyploidy and Low-density Randomly Amplified Polymorphic DNA Linkage Maps of Sweetpotato

Kittipat Ukoskit
Department of Plant and Soil Sciences, Mississippi State University, MS 39762

Paul G. Thompson
Pontotoc Research and Extension Center, 8320 Highway 15 South, Pontotoc, MS 38863

Additional index words. *Ipomoea batatas,* polyploid, simplex markers, nonsimplex markers, homologous linkage group

Abstract. **Low-density randomly amplified polymorphic DNA (RAPD) markers of sweetpotato [*Ipomoea batatus* (L.) Lam.; 2n = 6x = 90] were constructed from 76 pseudotestcross progenies obtained from 'Vardaman' x 'Regal'. Of 460 primers, 84 generating 196 well-resolved repeatable markers were selected for genetic analysis. 'Vardaman' and 'Regal' testcross progenies were analyzed for segregation and linkages of RAPD markers. Type of polyploidy, autopolyploidy, or allopolyploidy is uncertain in sweetpotato and was examined in this study using the ratio of nonsimplex to simplex RAPD markers and the ratio of simplex RAPD marker pairs linked in repulsion to coupling. Both measures indicated autopolyploidy. Low-density RAPD linkage maps of 'Vardaman' and 'Regal' were constructed from simplex RAPD marker linkage analysis. Duplex and triplex markers were then mapped manually into the simplex marker map. Homologous linkage groups were identified using nonsimplex RAPD markers and three homologous groups were found in each of the parent maps. Use of nonsimplex markers increased mapping efficiency. The 'Vardaman' map had a predicted coverage of 10.5% at a 25-cM interval of the genome size of 5024 cM. In 'Regal', genome coverage was estimated to be 5.6% at a 25-cM interval of the genome size of 6560 cM. Therefore, average chromosome length was ≈56 to 73 cM.**

Sweetpotato is the seventh most important food crop worldwide (Jansson and Raman, 1991). It ranks third in value of production and fifth in calorie contribution to human diets in developing countries (Plucknett, 1991). Despite its global importance, genetic information needed for cultivar development is limited. Reasons for this information shortage include the fact that sweetpotato is a hexaploid with reported 2n chromosome numbers of 90 (Jones, 1965; Magoon et al., 1970; Ting and Kehr, 1955) and 88 to 94 (Shiotani, 1988). Chromosomes are difficult to distinguish due to the large number of extremely small chromosomes. Because of this, determining type of polyploidy is difficult and remains uncertain. Autopolyploidy (Nishiyama, 1982; Shiotani, 1988) and allopolyploidy (Jones, 1965; Magoon et al., 1970; Ting et al., 1957) were hypothesized.

Due to genomic complexity, morphological markers and protein markers (isozymes) provide little information for genome analysis in sweetpotato because of the limited number of markers available (Kennedy and Thompson, 1991). In contrast, DNA-based markers offer an unlimited number of genetic markers. Since the markers are directly determined at the DNA level, the expression of a gene is not needed. Epistatic or pleiotropic effects, environmental effects, and developmental stage or tissue-specific gene expression are not limiting factors for DNA-based markers (Tanksley, 1983).

DNA-based linkage maps exist for several crops, such as *Arabidopsis thaliana* (L.) Heynh. (Chang et al., 1988; Nam et al., 1989), rice (*Oryza sativa* L.) (McCouch et al., 1988), maize (*Zea mays* L.) (Helentjaris et al., 1986), and tomato (*Lycopersicum esculentum* Mill.) (Bernatzky and Tanksley, 1986); however, mapping polyploid species has lagged behind mapping diploid species because of the difficulty in determining genotypes from a large number of possible segregation ratios. One approach proposed by Wu et al. (1992) for determining type of polyploidy and for mapping autopolyploids is the single-dose restriction fragment (SDRF) method, i.e., mapping based on a simplex segregation pattern. With this method, the markers segregating in a simplex pattern are mapped. A 1:1 ratio of coupling to repulsion simplex–simplex linkages indicates alloploidy. This method has been used in conjunction with restriction fragment length polymorphism (RFLP) (da Silva et al., 1993) and randomly amplified polymorphic DNA (RAPD) markers (Al-Janabi et al., 1993) in *Saccharum spontaneum* L., a polyploid species, for markers that segregated in a 1:1 ratio; however, other polysomic segregations cannot be mapped with the SDRF method.

Examples of polyploid genetic mapping include a report by de Winton and Haldane (1931), who showed linkages of seven possible gametic series in the tetraploid *Primula sinensis*. Yu and Pauls (1993) reported cosegregations among 19 RAPD markers in three different gametic series (simplex coupling, simplex repulsion, and asymmetrical repulsion) of tetraploid alfalfa (*Medicago sativa* L.). da Silva (1993) extended the linkage analysis to an autooctaploid and was able to include duplex and triplex markers in simplex marker maps. Polyploidy type was indicated by the proportion of nonsimplex to simplex markers, since autopolyploidy should result in a lower percentage of nonsimplex markers relative to simplex markers than allopolyploidy. He was also able to use this method to identify homologous linkage groups, and most of the available data were included in the map.

Mapping populations obtained from crosses between inbred lines are useful for genetic mapping; however, in sweetpotato, it is difficult to produce inbred lines because of self-incompatibility. One alternative mapping population is a cross between two unrelated heterozygous parents known as a two-way pseudotestcross (Grattapaglia and Sederoff, 1994; Hemmat et al., 1994). With this mapping population, a linkage map can be produced for each parent. This mapping population is suitable for species with self-

Received for publication 26 Sept. 1996. Accepted for publication 26 June 1997. Article J-8990 of the Mississippi Agricultural and Forestry Experiment Station. We gratefully acknowledge support of 92-37311-81354 NRI Competitive Grants Program/USDA. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

incompatibility and a high level of heterozygosity. Since sweetpotato has both of these characteristics, a pseudotestcross population should be suitable for mapping.

In the present study, we used a pseudotestcross mapping population to indicate type of polyploidy and mapped RAPD markers that segregated in a 1:1 ratio. In addition, duplex and triplex markers were inserted in the map.

Materials and Methods

Plant materials. Controlled crosses between 'Vardaman' and 'Regal' sweetpotatoes were made by hand. 'Vardaman' is from the controlled cross M97-4 x HM145. It has high early root yield, drought tolerance, and is susceptible to root-knot nematode. 'Regal' is a polycross seedling out of breeding line W-99, which is a polycross seedling out of breeding line South Carolina-166. 'Regal' has high levels of disease, root-knot nematode, and insect resistance. Seventy-six F_1 pseudo-testcross progenies were obtained and used as a mapping population. DNA isolated from both parents and progenies were used for RAPD analysis.

DNA isolation. A hexadecyltrimethyl ammonium bromine (CTAB) DNA extraction procedure (Gawel and Jarret, 1991) was used to isolate the genomic DNA from 500 mg of young leaves. Leaf samples were ground with a mortar and pestle in 2 to 3 g of polyvinyl-polypyrolidone (pvpp) and 10 mL of liquid nitrogen. CTAB extraction buffer (0.2 M EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, and 2% CTAB plus 0.4% 2-mercaptoethanol added just before use) was added to make a homogenate, which was then poured into Falcon tubes. The mixture was incubated at 65 °C for 1 h. After incubation, an equal volume of 24 chloroform : 1 octanal (v/v) was added to the sample. Proteins were separated by centrifugation at 2500 g_n for 15 min at room temperature. The aqueous phase was drawn off and the DNA was precipitated by adding twice the amount of the aqueous phase of cold ethanol and mixing by quick inversion several times. The precipitated DNA was hooked with a glass rod and washed in 0.2 M sodium acetate for 20 min and 0.01 M ammonium acetate for 10 s. About 1 $\mu g \cdot \mu L^{-1}$ of DNA yield was dissolved in 400 µL of TE buffer (10 mM Tris-HCl pH 8.3, 1 mM EDTA).

RAPD analysis. Polymerase chain reactions (PCRs) were carried out in a 12.5-µL reaction volume containing 50 ng DNA, 0.2 µM primer, 100 µM each of 4 dNTPs, 1.4 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100, and 1 unit Taq DNA polymerase (Promega Inc., Madison, Wis.). The reaction mixtures were overlaid with 15 µL of mineral oil and PCRs were performed in a thermal cycler (GTC-2; Precision Scientific Inc., Chicago). The thermal cycler was programmed for 40 cycles at 94 °C for 1 min, 40 °C for 1 min, 72 °C for 2 min, and 1 cycle of 72 °C for 5 min. DNA products were analyzed by electrophoresis on 1.5% agarose gels at 100 V, stained with 1.5 $\mu g \cdot mL^{-1}$ of ethidium bromide, and photographed under UV light.

Primer screening. A total of 460 random decamer primers, set G, I-Y (20 primers/set) from Operon Technologies, Alameda, Calif., and number 501-600 from the Univ. of British Columbia, Vancouver, was screened using the two parents. Based on those results, primers were selected to maximize the number of polymorphic bands per primer. The selected primers were used to amplify the DNA from two independent sets of individuals. In the first replication, DNAs from 37 progenies and 2 parents were amplified with all selected primers. For the second replication, another set of 39 individuals was assayed independently by preparing separate PCR reactions and amplifying with another thermal cycler. With this procedure, the repeatability of the scorable markers could be determined. Only those markers that were scored the same in both replications were used for determining segregation ratios.

Segregation and linkage analysis. The mapping population was based on a pseudotestcross configuration, therefore data were collected and used to construct separate linkage maps for each parent. Expected genetic segregation ratios were based on the assumption of random chromosome segregation. Since nonsimplex markers greater than triple dose in parental lines do not segregate in an F_1 backcross, three different hexasomic ratios were considered: 1:1, 4:1, and 19:1 (presence : absence), representing simplex, duplex, and triplex markers, respectively (single-, double-, and triple-dose fragments, respectively). The segregation ratios were tested for goodness-of-fit by a χ^2 test ($\alpha \leq 0.05$). Polyploidy type was tested by using the combined data from both parents and comparing the proportion of simplex markers to nonsimplex markers (duplex and triplex markers) (da Silva, 1993). Autohexaploidy should result in 25% nonsimplex and 75% simplex markers compared to 37.5% and 62.5%, respectively, for allohexaploidy.

To confirm type of polyploidy further, the proportion of simplex–simplex linkages in coupling phase to repulsion phase was determined. In allopolyploids, the linkages estimated in coupling and repulsion phase are identical to those in diploids and should appear with equal frequency. For autopolyploids, repulsion phase linkage is very difficult to detect because the chance of chromosome pairing between two informative homologous chromosomes carrying contrasting alleles at the two loci is $1/(h-1)$ (h = number of homologous chromosomes). Therefore, a comparison of the number of coupling- and repulsion-phase linkages can indicate the degree of preferential pairing among chromosomes (Sorrells, 1992; Wu et al., 1992). Linkages in repulsion phase were analyzed using the data of both parents. Each score of original data was inverted and MAPMAKER was then used to perform a linkage analysis between the original data and the inverted data using a two-point analysis with a LOD score of 3.0 and $r \leq 0.35$. Nonsignificant and significant χ^2 tests for 1:1 ratios (repulsion : coupling) indicate allopolyploidy and autopolyploidy, respectively (Wu et al., 1992).

Linkage relationships among simplex markers were determined using MAPMAKER version 3.0 (Lander et al., 1987) using two-, three-, and multipoint analysis. The maximum detectable recombination fraction (r) for linkage between simplex markers in the coupling phase for autopolyploids using a family size of 75 is 0.37 (Wu et al., 1992). Therefore, a maximum recombination fraction of 0.25 with a minimum logarithm of the odds (LOD) score of 4 was used to give high confidence for discriminating spurious linkages. The recombination fractions were presented (in cM) as a Kosambi mapping function.

After linkages among simplex markers were determined, two-point linkage analyses in coupling phase were performed between 1) simplex and duplex markers (simplex–duplex asymmetrical coupling), 2) duplex and duplex markers (biduplex coupling), and 3) duplex and triplex markers (duplex–triplex asymmetrical coupling). A χ^2 test for detection of linkage was performed to test the null hypothesis of independent segregation ($r = 0.5$) between two marker types. Recombination fractions were estimated by the maximum likelihood method (Mather, 1964).

Genome size (in cM) was estimated from the partial linkage maps of simplex markers using the method of moments procedure (Hulbert et al., 1988). Assuming a continuous genome and considering only pairwise comparisons between markers, the genome size (in cM) can be expressed as

Table 1. Segregation analysis of 196 randomly amplified polymorphic DNA (RAPD) markers, based on random chromosome segregation, in 'Vardaman' and 'Regal' sweetpotatoes.

Marker	RAPD marker					
	Vardaman[z]		Regal[y]		Total	
type[x]	No.	%	No.	%	No.	%
Simplex	76	74.5	67	71.3	143	72.9
Duplex	17	16.7	21	22.3	38	19.4
Triplex	4	3.9	3	3.2	7	3.6
Distorted	5	4.9	3	3.2	8	4.1
Total	102	100	94	100	196	100

[z]Presence of amplified DNA product in 'Vardaman' but absent in 'Regal'.
[y]Presence of amplified DNA product in 'Regal' but absent in 'Vardaman'.
[x]Simplex, duplex, and triplex markers are RAPD markers that segregated 1:1, 4:1, and 19:1 (band present : band absent), respectively. Distorted markers did not fit a ratio for any known inheritance pattern. Ratios were tested by χ^2 analysis ($\alpha \leq 0.05$).

$$G_Z = M(M-1)/X_Z/K_Z$$

where, for a given LOD score Z, G_Z is the genome size (in cM), M is the number of markers analyzed, X_Z is the maximum distance (in cM) between linked markers, and K_Z is the number of two-point linkages. Two-point linkages at LOD values of 2.0, 3.0, and 4.0 between the markers analyzed were determined and used to provide three estimates. The estimated genome size was obtained by taking the average of those three estimates.

The expected proportion of a genome covered by a linkage map with n markers randomly placed, $E(C_n)$, was estimated according to Bishop et al. (1983) as

$$E(C_n) = 1\text{-}p_{1,n}$$

where $p_{1,n}$ is the probability that a genome is not covered by a linkage map and is given by

$$p_{1,n} = 2r/n+1 \{(1 - X/2G)^{n+1} - (1 - X/G)^{n+1}\} + (1 - rX/G)(1 - X/G)^n$$

where r is the number of chromosomes, X is the distance between pairs of markers (in cM), and G is the genome size (in cM).

Results and Discussion

Primer screening. The average number of amplified products was 9.61 bands per primer tested. Of 460 primers, 13 primers (2.8%) failed to produce amplified products, 5 primers (1.1%) yielded unclear bands, 247 primers (53.7%) did not produce scorable polymorphisms between two parents, and 195 primers (42.4%) yielded at least one polymorphic band. Of the 195 polymorphic primers, the average number of polymorphic bands per primer was 2.02. The 84 primers that generated 196 well-resolved repeatable markers (2.36 markers per primer) were selected for genetic analysis. Those markers were analyzed for segregation ratios and used to construct linkage maps.

Segregation analysis. A separate segregation analysis was completed for each parent. Only those markers that segregated in simplex, duplex, or triplex inheritance patterns were considered. The number of markers segregating in each parent was similar for all plexes (simplex, duplex, and triplex) of markers (Table 1). About 75% and 71% of markers were simplex in 'Vardaman' and 'Regal', respectively. About 17% of 'Vardaman's' and 22% of 'Regal's' markers were duplex. Only 4% and 3% triplex markers were obtained in 'Vardaman' and 'Regal', respectively. Markers that did not fit a ratio for any known inheritance pattern (distorted) accounted for 5% and 3% of the markers in 'Vardaman' and 'Regal', respectively.

The proportion of nonsimplex to simplex markers was used as an indication of the type of polyploidy, i.e., autoploidy vs. alloploidy (da Silva, 1993). In an autohexaploid, doses of nonsimplex markers greater than a triple dose in parental lines are not expected to segregate in the F_1 population; therefore, nonsimplex markers included duplex and triplex markers. With the assumption of nonpreferential chromosome pairing in an autohexaploid, 25% and 75% nonsimplex and simplex markers, respectively, are expected. Under an allohexaploid hypothesis, bivalent preferential chromosome pairing and disomic segregation are assumed. The expected percentage of nonsimplex markers and simplex markers is 37.5% (25% of duplex markers + 12.5% of triplex markers) and 62.5%, respectively. The observed percentages of nonsimplex and simplex markers were 23.9% and 76.1%, respectively (Table 2). The nonsignificant χ^2 for the autohexaploid hypothesis presented in Table 2 indicated that the hexaploid sweetpotato is autopolyploid.

Linkages. No linkage in repulsion phase was detected among simplex markers in either map. The ratio of simplex marker pairs

Table 2. Chi-square analysis for polyploidy type in sweetpotato based on the percentages of nonsimplex compared to simplex randomly amplified polymorphic DNA (RAPD) markers using combined data of 188 markers.

Marker	Observed no. of RAPD markers		Expected no. of RAPD markers			
			Autohexaploid		Allohexaploid	
type	No.	%	No.	%	No.	%
Nonsimplex[z]	45	23.9	47.0	25.0	70.5	37.5
Simplex	143	76.1	141.0	75.0	117.5	62.5
Total	188	100	188.0	100	188.0	100
χ^2			0.11^{NS}		22.87^{**}	

[z]Nonsimplex markers include duplex and triplex markers.
[NS,**]Nonsignificant or significant at $\alpha \leq 0.001$.

linked in repulsion vs. coupling phase, 0:1, indicated nonpreferential pairing of homologous chromosomes or a high degree of autopolyploidy, further substantiating segregation analysis data. Both methods to determine polyploidy type indicated autopolyploidy and were in contrast to microscopic evidence for allopolyploidy presented by Jones (1965), Magoon et al. (1970), and Ting and Kerr (1955). Although the progeny numbers used in this study were adequate to distinguish ratios of simplex from nonsimplex markers and repulsion from coupling linkages, progeny from a cross between two parents were used and represented a restricted sample of the species. Also, RAPD markers identified covered a relatively small part of the genome. Therefore, a conclusion as to polyploidy type in sweetpotatoes should not be made until additional genotypes are evaluated. To establish linkage relationships, 76 and 67 simplex RAPD markers were analyzed in 'Vardaman' and 'Regal', respectively, and 25 and 20 RAPD markers were linked and mapped in 'Vardaman' and 'Regal', respectively (Figs. 1 and 2). For 'Vardaman' and 'Regal', 51 and 46 simplex RAPD markers remained unlinked, respectively.

In 'Vardaman', 25 simplex RAPD markers covered 265.4 cM. The minimum and maximum distances between two marker loci were 4.0 cM and 27.5 cM, respectively. The estimated genome size based on the method of Hulbert et al. (1988) was 5024 cM. The genome coverage based on the equation of Bishop et al. (1983) was 10.5%. In 'Regal', 20 simplex RAPD markers covered 173.1 cM. The minimum and maximum distances were 0 and 25.3 cM, respectively, and the estimated genome size was 6560 cM. The genome coverage was 5.6%. Therefore, each chromosome is ≈56 to 73 cM in length. Genome size estimates with low marker numbers tend to overestimate actual sizes (Chakravarti et al., 1991). Therefore, these estimates are probably higher than actual values. Greater numbers of markers should provide a more precise estimate; however, the genome statistics presented above provide preliminary information.

The next step in the linkage mapping was to include nonsimplex markers. Sixteen possible gametic series based on nonpreferential chromosome pairing for an autohexaploid involving two linked loci segregating for dominant markers are presented in Table 3. Of those sixteen series, only three were included in the map because the power of χ^2 detection was too low for other series. The three gametic series included were simplex–duplex asymmetrical coupling $[(AB)_1(aB)_1(ab)_4]$, biduplex coupling $[(AB)_2(ab)_4]$, and duplex–triplex asymmetrical coupling $[(AB)_2(aB)_1(ab)_3]$. The powers of χ^2 detection at $\alpha = 0.05$ and a mapping population size of 76 were 99%, 100%, and 94%, respectively, for those three gametic series (Agresti, 1990). For comparison, the power of linkage detection for simplex–triplex asymmetrical coupling is 52%, which is too low for linkage determinations.

In 'Regal', 1407, 441, and 63 two-point tests were completed for simplex–duplex asymmetrical coupling, biduplex coupling, and duplex–triplex asymmetrical coupling, respectively, whereas 1292, 289, and 68 two-point tests were completed for simplex–duplex asymmetrical coupling, biduplex coupling, and duplex–triplex asymmetrical coupling, respectively, in 'Vardaman'. The recombination fraction of a marker pair significant for independent segregation was estimated using the general gametic proportions presented in Table 4.

Only marker pairs involving nonsimplex markers with

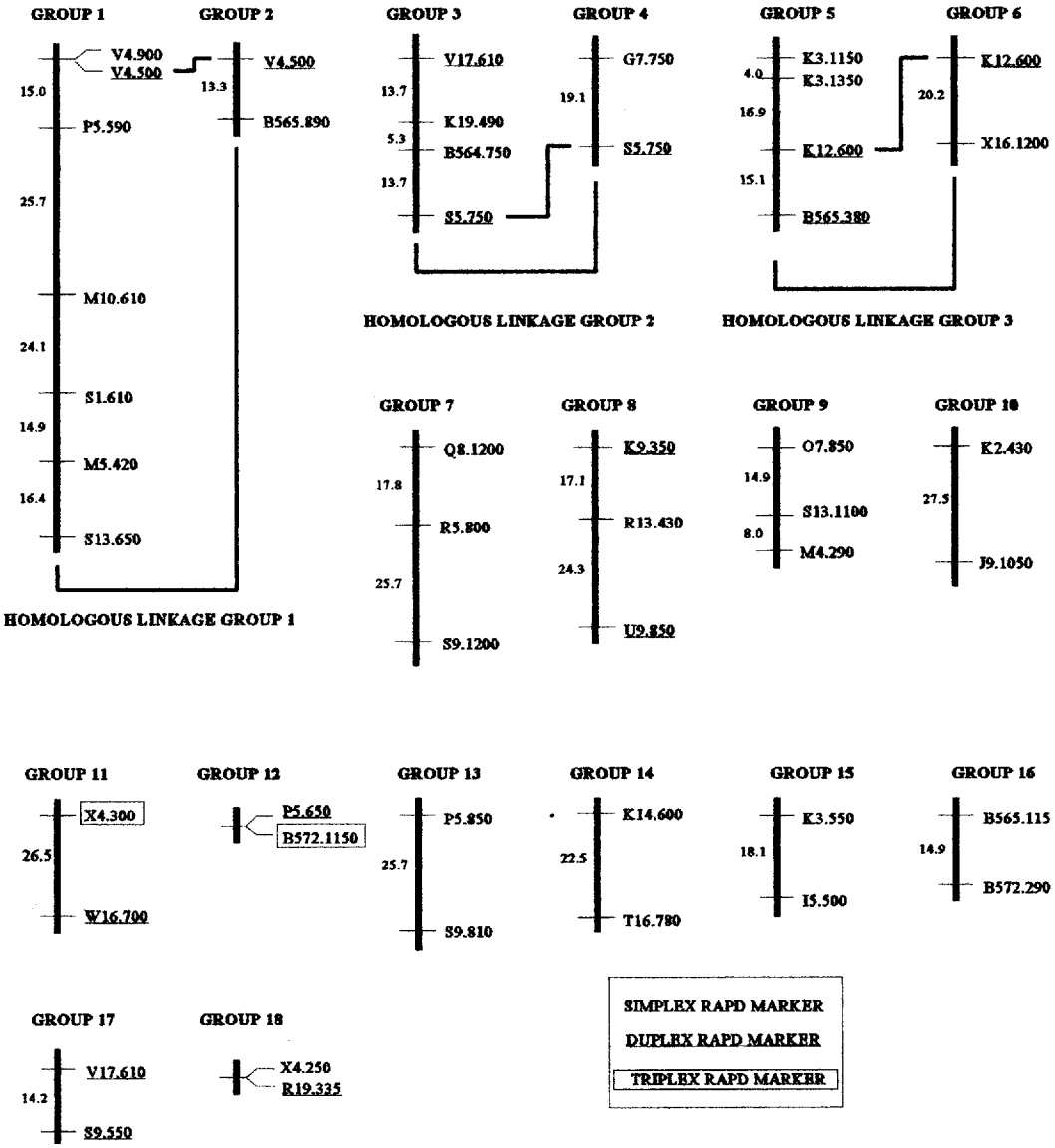

Fig. 1. Partial genetic linkage map of the hexaploid sweetpotato 'Vardaman'. Estimated map distances are shown on the left (in cM) using a Kosambi mapping function. Letters and numbers on the right indicate the randomly amplified polymorphic DNA (RAPD) marker locus designated by primer number and approximate molecular weight in base pairs. Putative homologous groups are indicated by the lines between two doses of a duplex marker located in two linkage groups. Simplex markers are located in the map by MAPMAKER V.3 with a minimum LOD of 4.0 and a maximum r of 0.25. Nonsimplex markers are located in the map manually by two-point analysis using the maximum likelihood method with a maximum r of 0.25.

recombination fractions <0.25 were included in the simplex marker maps (Figs. 1 and 2). With this criterion, spurious linkages should be minimized; however, some nonsimplex markers were mapped ambiguously (indicated by the open box) due to the large standard error of r involving nonsimplex markers. This was the case of linkage group 12 in the 'Regal' map containing two duplex markers, V17.400 and B564.1200, and one simplex marker, P2.600 (Fig. 2). P2.600 is linked tightly with V17.400 and linked with B564.1200 at an estimated 10.9 cM distance. The linkage between V17.400 and B564.1200 showed an r of 0.4, which should not be included in the map because this linkage could be spurious; however, V17.400 was placed into the map because the tight linkage between V17.400 and P2.600 indicated a true linkage between V17.400 and B564.1200. The distance between V17.400 and B564.1200 was estimated by the distance between P2.600 and B564.1200 because the smaller standard error of the shorter distance provided a more accurate linkage estimate.

The main advantage of including nonsimplex markers in the map was the identification of homologous linkage groups that could enable integration of 45 linkage groups into a basic chromosome number of 15 homologous linkage groups and thus be more informative. Homologous linkage groups were identified using nonsimplex markers (da Silva, 1993). For the 'Vardaman' map, three homologous linkage groups were identified (Fig. 1). Homologous groups 1, 2, and 3 each contained two linkage groups. Twelve linkage groups remained unidentified. Similarly, three homologous linkage groups were identified for the 'Regal' map (Fig. 2). Each homologous linkage contained two linkage groups as well. Ten linkage groups remained unidentified.

Using nonsingle dose markers for autopolyploid mapping can enhance mapping efficiency. Six and ten simplex markers in 'Vardaman' and 'Regal', respectively, which could not be mapped using the simplex marker method, were included in the map by using nonsimplex marker mapping. When simplex, duplex, and triplex markers were included, 48 loci out of 97 markers and 46 loci out of 91 markers were mapped in 'Vardaman' and 'Regal', respectively. When only simplex markers were included, 25 loci out of 76 markers and 20 loci out of 67 markers were found linked and were mapped in 'Vardaman' and 'Regal', respectively. Numbers of mapped loci increased ≈17% and 21% in 'Vardaman' and 'Regal', respectively, by including nonsimplex markers.

Hemmat et al. (1994) constructed molecular linkage maps for apple (*Malus* ×*domestica* Borkh.) using a pseudotestcross mapping population. They reported that this mapping population was suitable for RAPD markers, but with RFLP markers, a large proportion of the probes hybridized to multiple fragments, complicating the genetic analysis. More complicated banding patterns would result if the RFLP technique were applied to the hexaploid sweetpotato due to increased chromosome numbers and higher numbers of possible segregation ratios. On the other hand, the average number of RAPD polymorphisms per primer was high in our study, thus increasing mapping efficiency. In addition, the phenotypes of simplex markers in segregating progeny from this mating configuration represent the genotypes of the progenies. Hence, the disadvantage of complete dominance of RAPD markers was eliminated.

The construction of a genetic linkage map using the pseudotestcross mapping strategy was also accomplished in an interspecific cross between *Eucalyptus grandis* and *E. urophylla*

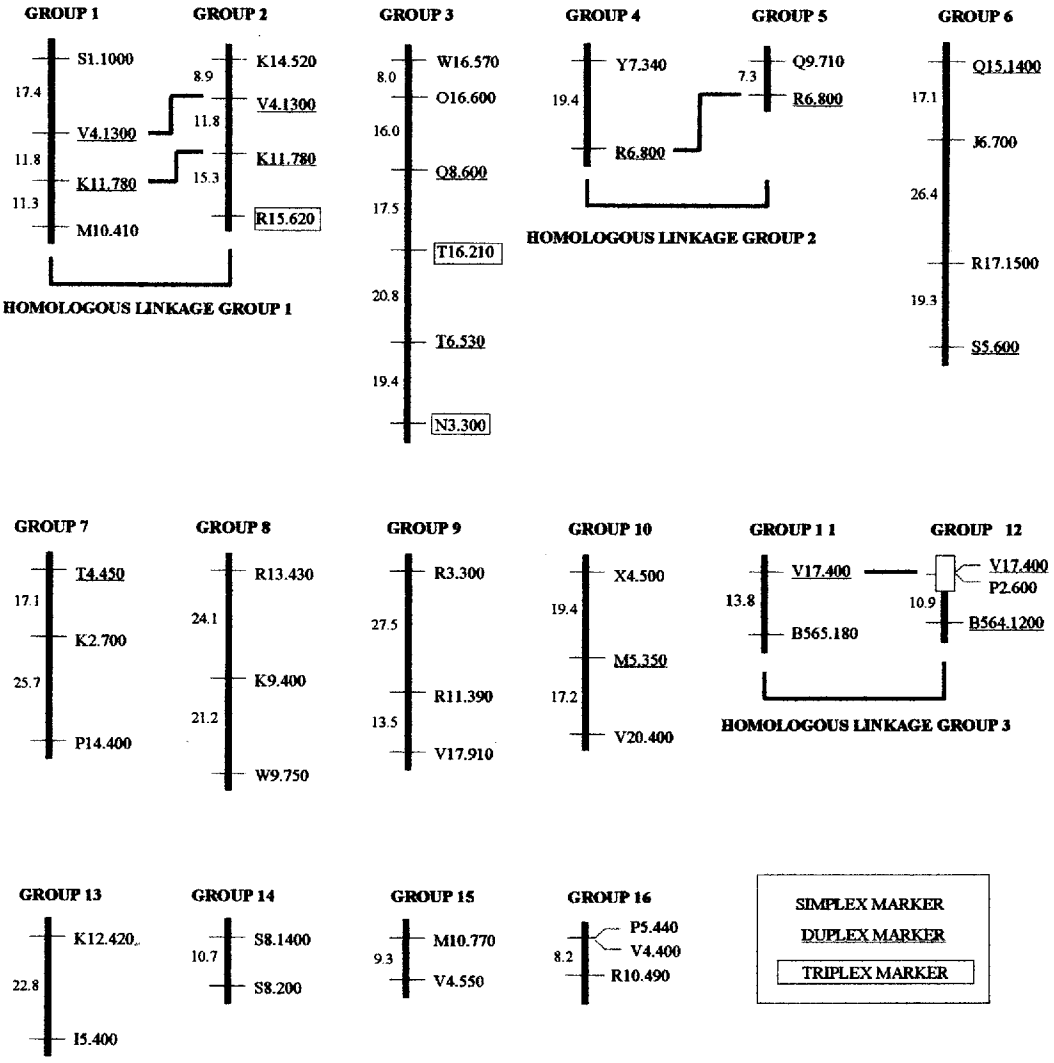

Fig. 2. Partial genetic linkage map of the hexaploid sweetpotato 'Regal'. Estimated map distances are shown on the left (in cM) using a Kosambi mapping function. Letters and numbers on the right indicate the randomly amplified polymorphic DNA (RAPD) marker locus designated by primer number and approximate molecular weight in base pairs. A box on the chromosome indicates the distance estimated ambiguously (see text). Putative homologous groups are indicated by the lines between two doses of a duplex marker located in two linkage groups. Simplex markers are located in the map by MAPMAKER V.3 with a minimum LOD of 4.0 and a maximum r of 0.25. Nonsimplex markers are located in the map manually by two-point analysis using the maximum likelihood method with a maximum r of 0.25.

Table 3. Gametic series for hexaploid species involving two linked loci segregating for dominant randomly amplified polymorphic DNA markers.

Locus A	Locus B	Gametic series
Simplex	Simplex	$(AB)_1(ab)_5$ or $(Ab)_1(aB)_1(ab)_4$
Simplex	Duplex	$(AB)_1(aB)_1(ab)_4$ or $(Ab)_1(aB)_2(ab)_3$
Simplex	Triplex	$(AB)_1(aB)_2(ab)_3$ or $(Ab)_1(aB)_3(ab)_2$
Duplex	Duplex	$(AB)_2(ab)_4$ or $(AB)_1(Ab)_1(aB)_1(ab)_3$ or $(Ab)_2(aB)_2(ab)_2$
Duplex	Triplex	$(AB)_2(aB)_1(ab)_3$ or $(Ab)_1(AB)_1(aB)_2(ab)_2$ or $(Ab)_2(aB)_3(ab)_1$
Triplex	Triplex	$(AB)_3(ab)_3$ or $(AB)_2(Ab)_1(aB)_1(ab)_2$ or $(AB)_1(Ab)_2(aB)_2(ab)_1$ or $(Ab)_3(aB)_3$

Table 4. Hexaploid phenotypic segregation proportions for linked and nonlinked loci and general gametic proportions of the most informative gametic series involving coupling linkage phase in F_1 pseudotestcross populations.

Gametic series	Phenotypic class	Phenotypic proportions		General gametic proportions
		Linked	Nonlinked	
1 and 2[z] Asymmetrical coupling	M_1M_2	0.5	0.4	0.5–0.2r
	M_1m_2	0	0.1	0.2r
	m_1M_2	0.3	0.4	0.3 + 0.2r
	m_1m_2	0.2	0.1	0.2 – 0.2r
2 and 2 Double coupling	M_1M_2	0.8	0.65	$0.8 - 0.4r + 0.2r^2$
	M_1m_2	0	0.15	$0.4 - 0.2r^2$
	m_1M_2	0	0.15	$0.4 - 0.2r^2$
	m_1m_2	0.2	0.05	$0.2 - 0.4r + 0.2r^2$
1 and 3 Asymmetrical coupling[y]	M_1M_2	0.5	0.475	0.5 – 0.5r
	M_1m_2	0	0.025	0.5r
	m_1M_2	0.45	0.475	0.45 + 0.5r
	m_1m_2	0.05	0.025	0.05 – 0.05r
2 and 3 Asymmetrical coupling	M_1M_2	0.8	0.7625	$0.8 - 0.1r + 0.05r^2$
	M_1m_2	0	0.0375	$0.1r - 0.05r^2$
	m_1M_2	0.15	0.1875	$0.15 + 0.1r - 0.05r^2$
	m_1m_2	0.05	0.0125	$0.05 - 0.1r + 0.05r^2$

[z]1, 2, and 3 = simplex, duplex, and triplex, respectively.
[y]This gametic series was excluded for linkage estimation due to the low power of χ^2.

(Grattapaglia and Sederoff, 1994). The efficiency of this mapping strategy was proportional to heterozygosity of the species. Sweetpotato is considered to be a highly heterozygous plant due to self-incompatibility. Therefore, the pseudotestcross strategy should provide a high mapping efficiency in this crop; however, the genetic divergence of a specific cross contributes to the efficiency as well. Since an intraspecific cross was used in our study, the mapping efficiency was not as high as in the Grattapaglia and Sederoff (1994) report in which an interspecific cross was used. The level of genetic divergence could be determined by the amount of markers segregating in a 3:1 ratio produced by heterozygous loci in both parents (Grattapaglia and Sederoff, 1994). In the interspecific cross of *Eucalyptus* spp., only 1.9% of the markers showed a 3:1 segregation ratio compared to our study in which 18% of the markers segregated in a 3:1 ratio. Therefore, sweetpotato appears to be highly heterozygous.

Literature Cited

Agresti, A. 1990. Building and applying loglinear models: Categorical data analysis. Wiley, New York, NY.

Al-Janabi, S.M., R.J. Honeycutt, M. McClelland, and B.W.S. Sobral. 1993. A genetic linkage map of *Saccharum spontaneum* L. 'SES 208'. Genetics 134:1249–1260.

Bernatzky, R. and S.D. Tanksley. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898.

Bishop, E.T., C. Canning, and M. Skolnick. 1983. The number of polymorphic DNA clones required to map the human genome, p. 181–200. In: B.S. Weir (ed.). Statistical analysis of DNA sequence data. Marcel Deckker, New York.

Chang, C., J.L. Bowman, A.W. DeJohn, E.S. Lander, and E.M. Meyerowitz. 1988. Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 85:6856–6860.

Chakravarti, A., L.K. Lasher, and J.E. Reefer. 1991. A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182.

da Silva, J.A.G. 1993. A methodology for genome mapping of autopolyploids and its application to sugarcane (*Saccharum* spp.). PhD diss., Cornell Univ., Ithaca, N.Y.

da Silva, J.A.G., M.S. Sorrells, W.L. Burnquish, and S.D. Tanksley. 1993. RFLP linkage map and genome analysis of *Saccharum Spontaneum*. Genome 36:782–791.

de Winton, D. and J.B.S. Haldane. 1931. Linkage in the tetraploid *Primula sinensis*. J. Genet. 24:121–144.

Gawel, N.L. and R.L. Jarret. 1991. A modified CTAB DNA extraction procedure of *Musa* and *Ipomoea*. Plant Mol. Biol. Rpt. 9:262–266.

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

Helentjaris, T., D.F. Webber, and S. Wright. 1986. Use of monosomics to map cloned DNA fragments in maize. Proc. Natl. Acad. Sci. USA 83:6035–6039.

Hemmat, M., N.F. Weeden, A.G. Manganaris, and D.M. Lawson. 1994. Molecular marker linkage map for apple. J. Hered. 85:4–11.

Hulbert, S.H., T.W. Ilott, E.J. Legg, S.E. Lincoln, E.S. Lander, and R.W. Michelmore. 1988. Genetic analysis of the fungus *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958.

Jansson, R.K. and K.V. Raman. 1991. Sweetpotato pest management a global perspective. Westview Press, Boulder, Colo.
Jones, A. 1965. Cytological observations and fertility measurements of sweetpotato. Proc. Amer. Soc. Hort. Sci. 86:527–537.
Kennedy, L.S. and P.G. Thompson. 1991. Identification of sweetpotato cultivars using isozyme analysis. HortScience 26:300–320.
Lander, E.S., P. Greon, J. Abrahumson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181.
Magoon, M.L., R. Krishnar, and K.V. Bai. 1970. Cytological evidence on the origin of sweetpotato. Theor. Appl. Genet. 40:360–366.
Mather, W.B. 1964. Principles of quantitative genetics. Burgess Publishing Co., Minneapolis.
McCouch, S.R., G. Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush, W.R. Coffman, and S.D. Tanksley. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815–829.
Nam, H.G., J. Giraudat, B. Den Boer, F. Moonan, W.D.B. Loos, B.M. Hauge, and H.M. Goodman. 1989. Restriction fragment length polymorphism linkage maps of *Arabidopsis thaliana*. Plant Cell 1:699–705.
Nishiyama, I. 1982. Autohexaploid evolution of the sweet potato, p. 263–274. In: R.L. Villareal, and T.D. Griggs (eds.). Sweet potato. Proc. 1st Intl. Symp. AVRDC Publ. 82–172, Taiwan, China.
Plucknett, D.L. 1991. Forward. In: R. K. Jansson, and K.V. Raman (eds.). Sweetpotato pest management a global perspective. Westview Press, Boulder, Colo.
Shiotani, I. 1988. Genomic structure and the gene flow in sweetpotato and related species, p. 61–73. In: Exploration maintenance and utilization of sweetpotato genetic resources. Intl. Potato Center, Lima, Peru.
Sorrells, M.E. 1992. Development and application of RFLPs in polyploids. Crop Sci. 32:1086–1091.
Tanksley, S.D. 1983. Molecular markers in plant breeding (review). Plant Mol. Biol. Rpt. 1(1):3–8.
Ting, Y.C. and A.E. Kehr. 1955. Meiotic studies in sweetpotato [*Ipomoea batatas* (L.) Lam]. J. Hered. 44:207–211.
Ting, Y.C., A.E. Kehr, and J.C. Miller. 1957. A cytological study of the sweetpotato plant *Ipomoea batatas* and its related species. Amer. Naturalist 101:197–203.
Wu, K.K., W. Burnquish, M.E. Sorrells, T.L. Tew, P.H. Moore, and S.D. Tanksley. 1992. The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor. Appl. Genet. 83:294–300.
Yu, K.F. and K.P. Pauls. 1993. Segregation of random amplified polymorphic DNA markers and strategies for molecular mapping in tetraploid alfalfa. Genome 36:844–851.