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Full Length Research Paper

Development of mapping populations for genetic analysis in yams (*Dioscorea rotundata* Poir. and *Dioscorea alata* L.)

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Progress is being made at the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) to develop molecular tools for marker-assisted selection that would complement and expedite conventional breeding approaches for genetic improvement of yams (*Dioscorea* spp.). F_1 full-sib mapping populations were developed from crossing male and female parents of *Dioscorea rotundata* Poir. and *Dioscorea alata* L. that differ in specific traits of interest towards identification of molecular markers linked to those traits. Success in hybridization was validated based on DNA analysis with SSR markers on agarose gel. Traits for which the populations were developed included multiple tuber production, cooking quality and virus disease resistance in *D. rotundata* and anthracnose disease resistance, cooking quality and tuber oxidation in *D. alata*. Death of plants in the field and rotting of tubers in storage, possibly due to pests, diseases and other environmental factors were encountered, that led to the reduction in sizes of the populations. Low seed multiplication ratio necessitated two to three cycles of tuber multiplication of mapping population genotypes to achieve adequate numbers of seed tubers for field experimentation. These mapping populations are valuable tools for genetic analysis and molecular marker development in yam improvement programmes.

Key words: *Dioscorea alata, Dioscorea rotundata*, hybrid identification, mapping population, simple sequence repeat (SSR) markers, trait mapping, yam, seed multiplication, seed losses.

INTRODUCTION

Yam (*Dioscorea* species of family Dioscoreaceae) is a multi-species, polyploid and clonally-propagated crop that is cultivated for its starchy tubers. Yam is important for food, income and socio-cultural events. The major edible yam species are *Dioscorea rotundata* P., *Dioscorea cayenensis* Lam., *Dioscorea dumetorum* (Kunth) Pax, *Dioscorea alata* L., *Dioscorea bulbifera* L., *Dioscorea esculenta* (Lour.) Burk. *Dioscorea trifida* L. and *Dioscorea nummularia* Lam.

Genetic improvement of yams at the International Institute of Tropical Agriculture (IITA, Ibadan, Nigeria) and its partners in West Africa is focused on *D. rotundata, D. cayenensis* and *D. alata*. *D. rotundata* (2*n* = 40 chromosomes) and *D. cayenensis* (2*n* = 60 and 80 chromosomes) (Dansi et al., 2000, 2001), (together also referred to as *D. cayenensis–rotundata* complex) are indigenous to Africa and represent most of the global yam production. They have the highest market value owing to the superior suitability of their tubers to the preferred food uses for the crop in West Africa. *D. alata,* introduced from Asia to Africa, is the most widely distributed *Dioscorea* species throughout the tropics. It includes accessions with 2*n* = 40, 60 and 80 chromosomes (Abraham and Nair 1991; Gamiette et al., 1999; Malapa et al., 2005; Arnau et al., 2009). Its advantages include high yield potential, ease of propagation (through production of bulbils and reliability of sprouting), early growth vigor for weed suppression and long storability of tubers. These are valuable characteristics for sustainable production but the species has a major limitation in the field - high susceptibility of

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most varieties to a devastating foliar disease, anthracnose, caused by *Colletotrichum gloeosporioides* Penz.

Genetic improvement of yam through conventional breeding alone is difficult and slow. Molecular markers are being developed in *D. alata* and *D. rotundata* for genetic linkage and quantitative trait locus (QTL) analyses to identify markers that are linked to traits of interest and that can be used to aid selection in breeding programs. These analyses require mapping populations.

A mapping population is a population in which recombination of parental alleles can be traced. An appropriate mapping population, together with suitable marker system and the software for analyses of data are the key requirements for a molecular mapping and molecular breeding programme. The type and size of mapping population are very important for QTL analysis, but their choice largely depends on the goal of the mapping project, the species concerned, type of marker system, traits to be mapped and the availability of time and other resources. In choosing the crossing parents, it is recommended that, they should be different in the traits of interest in order to facilitate linkage mapping and segregation analysis (Young, 1994). However, when using mapping populations based on highly heterozygous parents, differences in the trait of interest between the parents is not essential, because many of the genes underpinning the trait still segregate in the population (Barrett et al., 2004). There are several methods for generating a mapping population, but the choice depends on the pollination pattern of the plant species. In open pollinated species, populations suitable for genome mapping can be generated through a cross between two heterozygous genotypes (Grattapaglia and Sederoff, 1994) or between one heterozygous parent and a doubled haploid parent (Bert et al., 1999; Jones et al., 2000). One advantage of the former breeding method is that, it minimizes the risk of inbreeding depression and segregation distortion in the mapping population, both of which can significantly reduce the power and utility of a genome map (Faville et al., 2003). The successful use of a double heterozygous (or double-pseudotestcross) strategy in open pollinated species has been reported in linkage mapping for perennial ryegrass (Faville et al., 2004), white clover (Barrett et al., 2004) *Eucalyptus* (Grattapaglia and Sederoff, 1994) and asparagus (Lewis and Sink, 1996).

The size of the mapping population is important because it determines the resolution of a map and the precision in marker ordering (Young, 1994). The larger the mapping population is, the higher the chance of seeing more recombinants in the study and hence the better the map resolution. However, population size may be limited by several factors including the number of seeds available and the amount of resources available for data collection. Populations less than 50 individuals provide too little mapping resolution to be useful (Young, 1994). If the goal is high resolution mapping or QTL mapping, much larger populations will be required (Young, 1994; Beavis, 1998).

The development of mapping populations for yams is, however, not as easy as for other crops. To date, only two mapping populations, one for *D. alata* and the other for *D. rotundata* have been reported for cultivated yams (Mignouna et al., 2002a, b). Apart from the challenges of poor to non flowering, lack of synchrony in flowering of male and female genotypes and the variation in flowering intensity with season and location (Hamadina et al., 2009), population development in yams is also constrained by pests and diseases, soil nutrient deficiencies and other environmental factors that affect the survival of yams both in the field and in storage (Akoroda and Hahn, 1995; Green and Florini, 1996; Manyong and Oyewole, 1997; Manyong et al., 2001). In spite of these constraints, the need for mapping population to facilitate genetic analysis in yam is urgent.

MATERIALS AND METHODS

Selection of crossing parents

Forty(40) accessions of IITA improved lines and landraces of *D. rotundata* and *D. alata* were characterised based on sex, multiple tuber production, oxidation (enzymatic browning), tuber texture, other morpho-physiological and quality traits at the IITA yam breeding unit, Ibadan, Nigeria. Twenty-one (21) *D. alata* accessions were further screened for reaction to various *C. gloeosporioides* isolates based on growth patterns of mycelia and symptom types using detached leaves and whole plant methods. Fourteen (14) accessions of *D. rotundata* were screened in the field and screenhouse for reaction to viruses. Accessions that differ in each trait were selected and used as crossing parents to generate a mapping population for the trait. The ploidy status of selected parents was determined using flow cytometry.

Crossing procedures and seed processing

Six male and six female parents of the selected accessions were grown in separate crossing blocks (about 500 metres apart). Seven bi-parental crosses of genotypes with same ploidy status (4X) were performed through controlled pollination in 2006 for *D. rotundata* and in 2006 and 2008 for *D. alata*. This involved bagging (cotton fabric) of selected female inflorescences before the flowers were opened. When ready for pollination, anthers were excised from male flowers using the fine point of a pin and deposited on a stigma of the female flower. The flowers were enclosed again in the pollination bags for another two weeks to avoid contamination from other pollen through insects. The fruits (capsules), when matured, were harvested and seeds were extracted and processed.

Seedling nursery establishment and seed tuber multiplication

Botanic seeds, ranging from 266 to 1010 per population of the F_1 populations were germinated in Jiffy 7 peat pots and four week old seedlings, ranging from 215 to 777 were transplanted in a seedling nursery at a spacing of 0.4 x 0.5 m for *D. rotundata* and 1 m x 1 m for *D. alata*. Tubers were harvested seven to eight months after seedlings were transplanted and stored at ambient temperature in

an open-air barn. Tubers harvested from the seedling nursery went through one, two or three subsequent cycles of multiplication in order to get enough seed tubers for evaluation of traits. Seed tubers, ranging from 50 to 263 per population of five of the populations [*D. alata* mapping populations 1 (AM1) and 2 (AM2) and *D. rotundata* mapping populations 1 (RM1), 2 (RM2) and 3 (RM3)] were multiplied in the field in 2009 using tuber sett size of 25 to 50 g at a spacing of 50 x 1 m for *D. alata* and 25 x 1 m for *D. rotundata*. *D. alata* mapping populations 3 (AM3) and 4 (AM4) were grown in seedling nursery in 2009 and were in the first stage of tuber multiplication cycle during the 2010 cropping season.

Verification of hybridization between crossing parents

For each of the mapping populations, total deoxyribonucleic acid (DNA) was isolated from the two parents (P1, the female parent and P2, the male parent) and six randomly selected progenies using a modified protocol of the Asian Maize Biotechnology Network (ABIONET Service Laboratory, 2004). About 100 mg of fresh leaf tissue was ground in liquid nitrogen using a mortar and a pestle. The ground tissue was transferred into an eppendorf tube and 500 µl of CTAB buffer (2% CTAB, 1.4 m NaCl, 20 mMEDTA, 100 mm Tris HCl, pH 8, 3% mercaptoethanol) was added. The samples were incubated in 65°C water bath for 45 min, with the tubes gently mixed 3 to 4 times. Tubes were then removed from the water bath, uncapped and allowed to cool at room temperature or on ice for 1 to 2 min. Chloroform-isoamyl alcohol (24:1) 500 µl was added and the content was mixed for about 1 min by inverting the tube and centrifuged at 13000 rpm for 10 min. The supernatant (about 500 µl) was carefully transferred into 1.5-ml fresh microcentrifuge tubes and 500 µl of ice-cold isopropanol was added. The content was mixed by gentle inversion until the white, thread-like DNA appeared and was centrifuged at 13000 rpm for 10 min. The liquid was poured off, the DNA pellet was washed with 500 µl of 70% ethanol, centrifuged at 13000 rpm for 10 min and the ethanol was carefully poured off. The DNA was dried by inverting the tube on a clean paper towel for 20 to 30 min. The DNA was dissolved in 100 µl of 1xTE (high salt) by gently flicking the tube and centrifuged at 13000 rpm for 10 min to collect the solution at the bottom. 1µl of 10 mg/ml RNase was added to 100 µl of DNA and incubated at 37°C for 30 min. DNA was precipitated by adding 1/10 v (10 µl) of 3.0 M sodium acetate and 2 volumes (200 µl) of absolute ethanol and the content was mixed by gentle inversion and centrifuged at 13000 rpm for 10 min to collect the DNA. The liquid was carefully taken out using a 200 micropipette without disturbing the DNA pellet. 300 µl of 70% ethanol was again added to the DNA, centrifuged for 2 min at 13000 rpm and the liquid was poured off. The DNA was dried by inverting tube on a clean paper towel, re-suspended in 100 µl of 1xTE (low salt), centrifuged for 2 min at 13000 rpm and kept at -20°C. The quantity of DNA was subsequently estimated by visual comparison against Lambda (λ) DNA standards loaded at 200, 100, 75, 50, 35, 25, 20, 15, 10 and 5 ng per lane.

The 60 well agarose gels were prepared using 250 ml of 0.5xTBE buffer (Tris Borate-EDTA, pH 8.0) and 0.8% (w/v) agarose. DNA samples were mixed briefly by flicking the tube and then centrifuged at 13000 rpm for 1 min. 2 µl of DNA solution plus 2 µl of 6x loading dye (15% ficoll (w/v); 0.25% bromophenol blue; 0.25% xylene cyanol FF) were loaded onto the gel and run at 80 V for 2 to 3 h. The gel was stained in 2 ml ethidium bromide solution (500 µl /ml) for 30 to 60 s and then de-stained by placing in fresh tap water (about 2 ml) for 20 to 30 min. Quantities were estimated by the eye against the λ DNA standards and based on these estimations, DNA solutions of 5 ng/µl were made with sterile (autoclaved) water for each genotype of the two parents (P1 and P2) and six randomly selected progenies from each of the mapping populations.

One hundred and forty-five (145) simple sequence repeat (SSR) primer pairs, 45 from genomic origin (Mignouna et al., 2003; Tostain et al., 2006; Tamiru et al., unpublished) and 100 from expressed sequence tags (Satya et al., 2007), were screened for amplification and polymorphism in the parents of each of the mapping populations. Primers that were polymorphic between parents of each of the populations were used to genotype the two parents and six randomly selected progenies of the respective populations.

Polymerase chain reaction (PCR) amplifications were conducted in a 20 µl reaction volume (96 well plate formats) containing 20 ng (4 µl) of genomic DNA, 2.0 mM magnesium chloride, 10x PCR buffer (Invitrogen), 0.2 mM of each dNTP, 2.0 µM each of forward and reverse SSR primer and 0.06 U of Platinum Taq DNA polymerase (Invitrogen). PCR was carried out using iCyclers (Bio-Rad, Hercules, Calif., USA) programmed for one cycle at 94°C for 4 min, followed by 34 cycles of 94°C for 30 s, 51°C for 1 min and 72°C for 1 min. After the 34 cycles, the samples were held at 72°C for 8 min (final extension step) and then stored at 4°C. The PCR products were electrophoresed in 2% SFR agarose (AMRESCO®, AMRESCO Inc. Solon Industrial Pkwy, USA) gel at 80 V for 2 to 3 h. The gels were next stained with ethidium bromide for 30 to 60 s, de-stained for 20 to 30 min and then observed under a UV transilluminator.

A problem of amplification and reproducibility of PCR products was encountered with DNA from three parental genotypes - TDa 01/00081, TDa 95/00328 and TDr 97/00917. The DNA extraction protocol was modified and used to re-extract DNA from those genotypes.

RESULTS

The morpho-agronomic characteristics of the parents of the mapping population are listed in Table 1 and their pedigree information is shown in Table 2. Traits for which the mapping populations were developed are shown in Table 3. The results from the crosses are shown in Table 4. Fruit set ranged from 14% in cross TDa 01/00081 x TDa 01/00039 to 56% in TDa 95/00328 x TDa 95-310. Percent seed set was highest (65%) in TDr 97/00793 x TDr 95/01932 and lowest (6%) in TDa 01/00081 x TDa 01/00039 and was generally higher in *D. rotundata* than in *D. alata*. The number of seeds harvested ranged from 266 in TDa 01/00081 x TDa 87/01091 to 1010 in TDr 97/00793 x TDr 95/01932.

Percent loss of progenies was 5 to 57% in the seedling nursery and 7 to 86% in storage (Table 5). The number of tubers produced per progeny was small during the first cycle of tuber multiplication, but was higher in *D. alata* populations than in populations of *D. rotundata* (Table 5). The mean tuber number per genotype was 11 and 12 in populations AM1 and AM2, but was less than 10 in RM1, RM2 and RM3.

The result of SSR primer (marker) analysis confirming successful hybridization of the mapping population parents is shown in Table 6 and Figure 1. Out of the 145 markers surveyed across the seven mapping populations (AM1, AM2 AM3, AM4, RM1, RM2 and RM3), nine were polymorphic between the parents of each of the populations. The number of markers used to genotype the populations ranged from two (AM3 and RM1) to four (AM1). Populations AM2, AM4, RM2 and RM3 were

Table 1. Characteristics of *D. rotundata* and *D. alata* mapping population parents.

Parent	Morphological and agronomic characteristics
TDr 02/00076	Produce multiple tubers, susceptible to virus
TDr 95/01932	Produce single or fewer tubers, moderately resistant to virus
TDr 97/00793	Produce multiple tubers, non-oxidising
TDr 97/00917	Moderately resistant to virus, good cooking quality
TDr 00/00380	Moderately resistant to virus, poor cooking quality
TDa 01/00081	Non-oxidising, produce bulbils, good cooking quality, moderately resistant to anthracnose
TDa 87/01091	Oxidises, resistant to aggressive strain (SGG) of *C. gleosporioides*, produce bulbils, high yielding
TDa 92-2	High yielding, poor tuber shape, purplish fleck flesh colour, oxidises, susceptible to anthracnose, bulbils
TDa 01/00039	Oxidising, resistant to anthracnose, cooks well, produce bulbils
TDa 95/00328	High yielding, poor tuber shape, oxidises, resistant to moderately virulent (FGS) of *C. gleosporioides*
TDa 95-310	Purplish fleck flesh colour, purplish petiole colour, susceptible to anthracnose, produce bulbils

Table 2. Pedigree of *D. rotundata* and *D. alata* accessions that were used as crossing parents in developing mapping populations.

Parent of mapping population	Pedigree
TDr 02/00076	(TDr 93-1 x TDr 95-204) x TDr 00-2-5
TDr 95/01932	TDr 86/00609 (HS)
TDr 97/00793	TDr 93 - 23 (HS)
TDr 97/00917	TDr 89/01892 x IN94R–2
TDr 00/00380	(TDr 87/00571 × TDr 91/00200) x (TDr 95 - 235 × IN94R – 31)
TDa 01/00081	(TDa 95/00328 × TDa 98-150) x (TDa 95/00270 × TDa 291)
TDa 87/01091	OP of A19-165-445
TDa 92-2	Landrace
TDa 01/00039	(TDa 95/00328 × TDa 98 – 150) x (TDa 95/00270 × TDa 291)
TDa 95/00328	TDa 92-2 x TDa 85/00257
TDa 95-310	Landrace

HS, Half-sib ; OP, open pollination ; TDa 93-23, landrace 'Obiaoturugo' collected from Obinagu in Ohaozara LGA, Ebonyi State Nigeria; IN94R-2 ,landrace collected from Benin Republic; TDa92-2, landrace 'Werededede' collected from Sagbe in Ibadan, Oyo State Nigeria; Tda 95-310, landrace 'Brazo Fuerte' collected from Bouake, Côte d 'Ivoire.

genotyped by three markers each. All the markers were marker Dab2E07 that was specific to population AM1. Marker Dab2Co5 was informative in all the populations. All the markers were amplified in all the six selected progenies from each of the populations, except for markers Dcay 245 and Dab2Co5 that failed to be amplified in two genotypes of population AM2 and one genotype of AM4, respectively. The number of bands (alleles) amplified at each marker locus ranged from 2 to 5 and the size of alleles ranged from 100 to 550 base pairs. In the RM2 population, one of the alleles of parent 1 did not amplify in the progeny. In all the populations, the genotype of each of the six progenies showed a combination of their parental alleles.

DISCUSSION

Seven F_1 full-sibs mapping populations, three of *D. rotundata* (RM1, RM2 and RM3) and four of *D. alata* (AM1, AM2, AM3 and AM4) were developed. The sizes informative in more than one population except for (number of genotypes) of the mapping populations ranged from 50 in population AM2 to 283 in population AM4. Losses of 5 to 57% in the field and 7 to 86% in storage were encountered that led to the reduction in the size of the populations. These losses were mainly in the form of plant death in the field and tuber rot in storage which may have been caused by pests and diseases or other environmental conditions. Losses of about 30% in storage and also during processing due to pre-harvest invasion or infection by pathogens and insect pests, damage during harvest and transit and unfavorable physical factors of the environment (especially temperature and humidity) have been reported (Akoroda and Hahn, 1995, Green and Florini, 1996). The loss of genotypes in the field and in storage warrants an alternative method of conserving yam genotypes, for instance, in tissue culture, especially for those of map-ping populations, in order to prevent the population or the individual progenies from going into extinction. Tuber multiplication is another constraint in the development and utilization of mapping

Table 3. Bi-parental crosses and mapping populations for traits analyses in *D. rotundata* and *D. alata*.

Cross	Mapping population	Trait
TDr 02/00076 (P1) × TDr 95/01932 (P2)	*D. rotundata* mapping population 1 (RM1)	Multiple tubering/virus
TDr 97/00793 (P1) × TDr 95/01932 (P2)	*D. rotundata* mapping population 2 (RM2)	Multiple tubering
TDr 97/00917 (P1) × TDr 00/00380 (P2)	*D. rotundata* mapping population 3 (RM3)	Cooking quality
TDa 01/00081 (P1) × TDa 87/01091 (P2)	*D. alata* mapping population 1 (AM1)	Cooking quality
TDa 01/00081 (P1) × TDa 01/00039 (P2)	*D. alata* mapping population 2 (AM2)	Oxidation
TDa 92 - 2 (P1) × TDa 01/00039 (P2)	*D. alata* mapping population 3 (AM3)	Anthracnose
TDa 95/00328 (P1) × TDa 95 – 310 (P2)	*D. alata* mapping population 4 (AM4)	Anthracnose

Table 4. Data on bi-parental crosses for generating mapping populations in *D. rotundata* and *D. alata*.

Species	Cross	Code name	Number of Flowers ollinated	Number of fruits collected	% fruit set	Expected number of seeds	Number of seed obtained	% seed set	Crossing year
D. rotundata	TDr 02/00076 (P1) × TDr 95/01932 (P2)	RM1	517	240	46	1440	613	43	2006
	TDr 97/00793 (P1) × TDr 95/01932 (P2)	RM2	597	258	43	1548	1010	65	2006
	TDr 97/00917 (P1) × TDr 00/00380 (P2)	RM3	513	219	43	1214	744	57	2006
D. alata	TDa 01/00081 (P1) × TDa 87/01091 (P2)	AM1	251	100	40	1506	266	18	2006
	TDa 01/00081 (P1) × TDa 01/00039 (P2)	AM2	2016	285	14	12096	707	6	2006
	TDa 92 - 2 (P1) × TDa 01/00039 (P2)	AM3	207	118	57	1242	461	37	2008
	TDa 95/00328 (P1) × TDa 95 – 310 (P2)	AM4	359	202	56	2154	473	22	2008

Table 5. Size of mapping population, percent loss of genotype in nursery and during storage, and number of tubers multiplied in the field in 2009. Populations AM3 and AM4 were in seedling nursery in 2009 and are in the multiplication field this year.

Mapping population	Number of plant transplanted in seedling nursery	Number of Plants survived in seedling nursery	Genotype loss in seedling nursery (%)	Genotype loss in storage (%)	Population size (number of genotypes)	Number of tuber sets per genotype planted in multiplication		Number of tubers per genotype harvested in multiplication	
						Range	Mean	Range	Mean
RM1	416	396	5	56	175	2 - 49	12	1 - 47	9
RM2	777	420	46	35	263	1 - 10	2	1 - 8	8
RM3	518	450	13	69	109	1 - 24	5	1 - 18	4
AM1	215	190	12	24	144	1 - 108	12	1 - 119	12
AM2	518	345	33	86	50	2 - 58	9	1 - 74	11
AM3	322	140	57	7	130				
AM4	349	267	23	0	283				

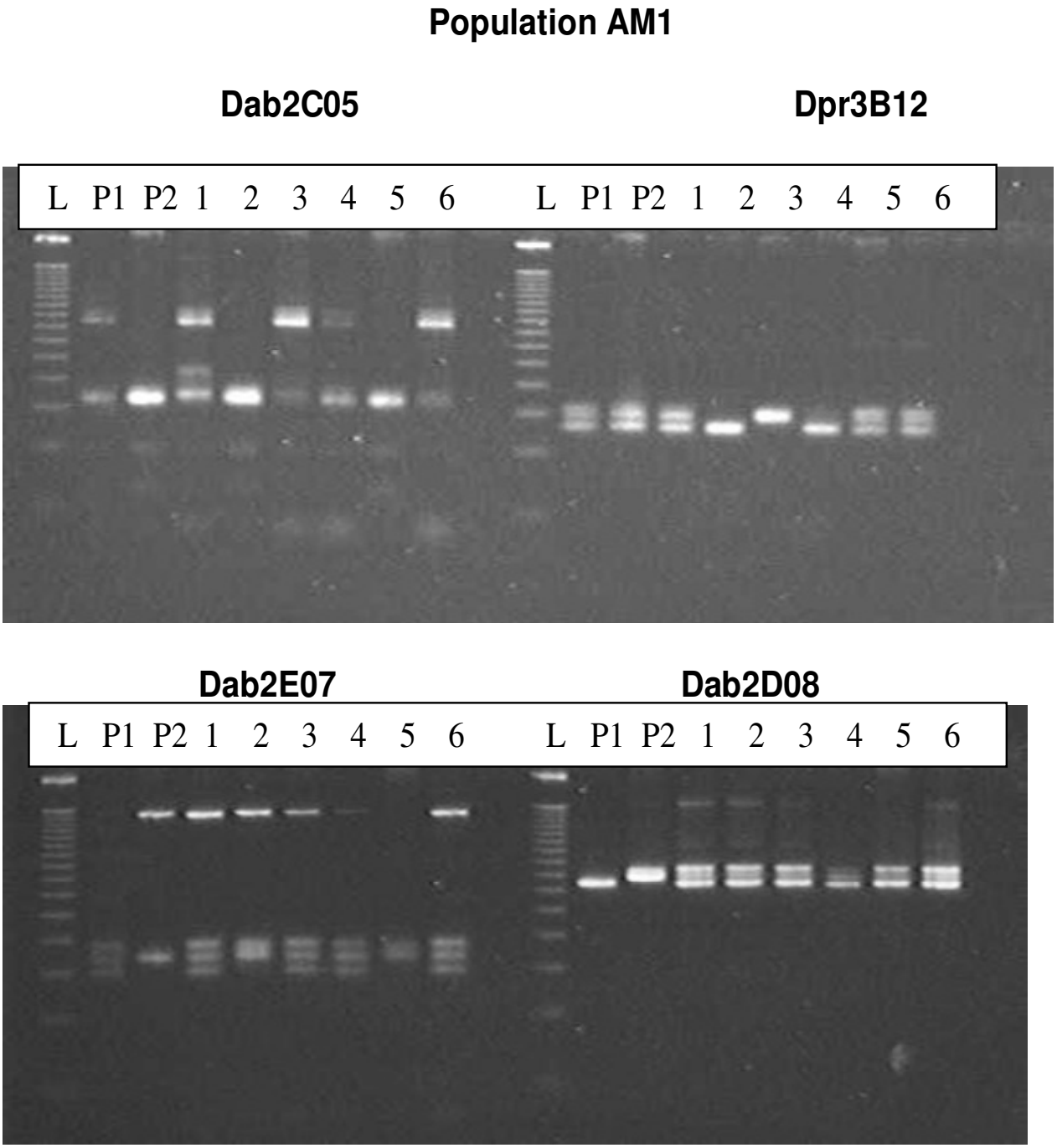
populations in yams. Seed multiplication ratio, which is some cereals (1:300) prevents the immediate use of a mapping population. For instance, during the first year of seed tuber multiplication, the numbers and sizes of very low in yams (less than 1:10) when compared with tubers produced per progeny were too small to establish a field trial especially for *D. rotundata* populations. Tuber increase was relatively higher in *D. alata* populations

Table 6. Segregation pattern of parental alleles in six selected progenies of seven mapping populations of *D. alata* (AM1, AM2, AM3 and AM4) and *D. rotundata* (RM1, RM2 and RM3) using nine SSR markers (Dab2C05, Dpr3B12, Dab2E07, Dab2D08, DPr3F12, Dcay 223, Dcay 245, Dcay 405 and YM-13).

Mapping population	SSR Marker	Number of allele in parental genotype	Size of allele (base pair)				Parent 1 allele	Parent 2 allele	Number of progeny with allele 'a'	Number of progeny with allele 'b'	Number of progeny with allele 'c'	Number of progeny with allele 'd'
			Allele 'a'	Allele 'b'	Allele 'c'	Allele 'd'						
RM1	Dab2C05	3	150	170	190		a,b and c	a and b	2	6	4	
	Dcay 223	4	350	370	400	500	a and c	b and d	6	0	2	6
RM2	Dab2C05	4	120	150	170	200	a, b and c	b and c	3	3	3	4
	DPr3B12	2	140	200			a and b	a	6	0		
	Dcay 223	4	370	420	450	550	a, c and d	b and d	2	4	1	6
RM3	Dab2C05	2	190	220			a	a and b	6	6		
	Dcay 405	2	270	300			a	a and b	6	5		
	YM-13	4	220	250	290	300	b and c	a,b and d	3	3	1	1
AM1	Dab2C05	2	100	300			a and b	a	2	4		
	Dpr3B12	3	120	150	160		a, b and c	a and b	3	2	1	
	Dab2E07	3	100	120	150		a, b and c	b	4	5	3	
	Dab2D08	2	300	350			a and b	b	5	1		
AM2	Dab2C05	4	150	200	220	400	a,b,c and d	a,b and c	5	6	6	5
	DPr3F12	3	150	170	220		b and c	a,b and c	1	6	5	
	Dcay 245	2	300	350			a	b	1	3		
AM3	Dab2C05	4	170	250	400		a,b and c	a and b	6	2	3	
	Dab2D08	2	320	370			a and b	a	6	4		
AM4	Dab2C05	4	160	180	220	400	a,b and c	a,b,c and d	3	5	5	2
	DPr3B12	2	170	190			b	a and b	5	6		
	DPr3F12	2	170	190			b	a and b	5	5		

(more than 10 tubers per genotype) than in those of *D. rotundata* (fewer than 10 per genotypes). The relatively large quantity of tubers produced per progeny in populations AM1 and AM2 suggests that, enough planting materials may be available to establish field trial in *D. alata* after one or two cycles of multiplication. In the case of *D. rotundata*, two to three cycles of seed tuber multiplication may be required before field experimentation.

Yam is an open-pollinated crop and there is a chance for cross pollination with pollen from unwanted male plant to occur if hybridization is not properly controlled. Contamination by pollen from an unknown parent will lead to spurious analysis. Genetic analysis using mapping populations requires the determination of the genotype of the parents in order to trace recombination of parentalalleles in the population. To confirm that mapping population progenies were the true hybrids of the male and female crossing parents, DNA from six progenies and the two parents (P1 and P2) of each of the seven populations were analyzed with SSR markers. The results indicated that, the parental alleles recombined in the progenies of each of the populations (Table 6 and Figure 1) confirming that the crosses were successful. The male parent (P2) allele, either in the form of hetero-zygote or homozygote was present in all the six samples of each population



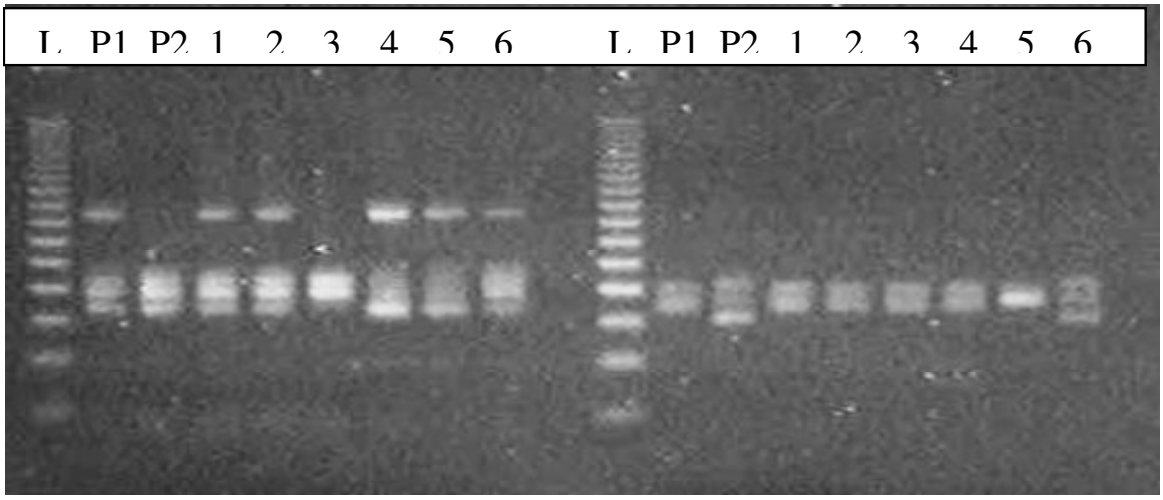
indicating that, all the six F_1 progeny were hybrids. The failure of markers Dcay 245 and Dab2Co5 to be amplified in two progenies of population AM2 and AM4 revealed the difficulty of isolating quality DNA from a population of yam genotypes using the same protocol. This may be due to the high heterozygosity as a result of the out-crossing nature of yams. It has earlier been highlighted that, a single DNA isolation protocol for heterozygous species may not allow optimal DNA yield and that even closely related species may require different DNA extraction protocols (Loomis, 1974; Weishing et al., 1995). More than one DNA extraction protocol for parental genotypes TDa 01/00081, TDa 95/00328 and TDr 97/00917 was used before achieving reproducible DNA amplification in the genotypes.

Conclusions

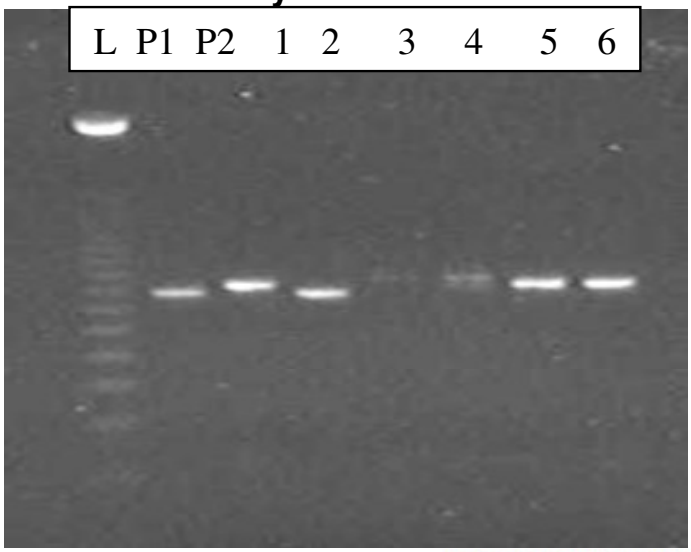
Seven F_1 full-sibs mapping populations in yams, three in *D. rotundata* for virus disease, multiple tuber production,

Population AM2

Dab2C05 **DPr3F12**

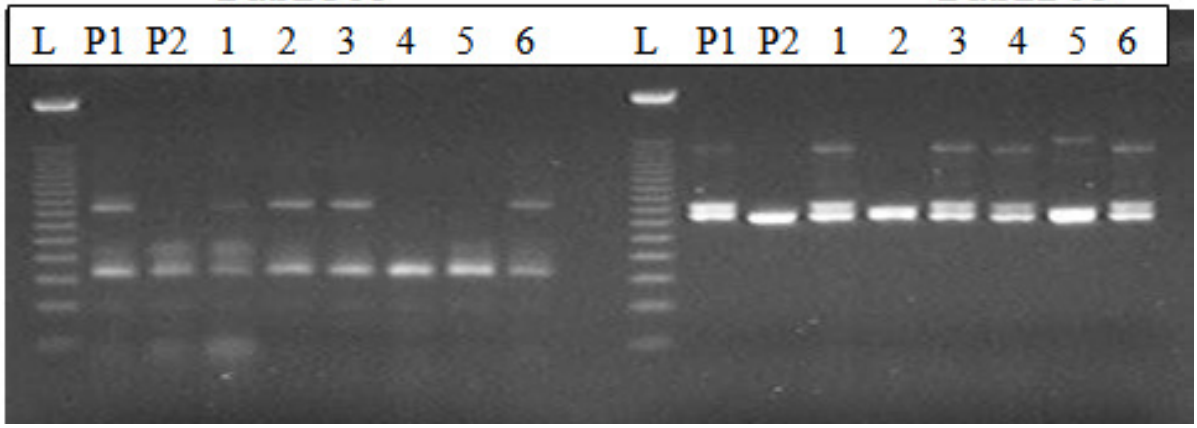


Dcay 245



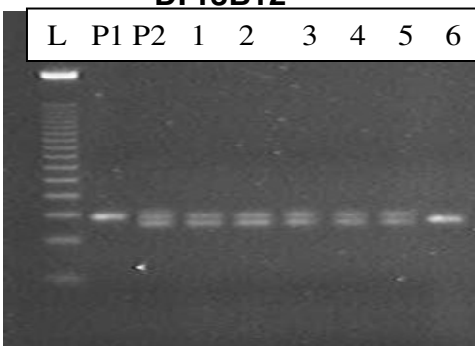
Population AM3

Dab2C05 **Dab2D08**

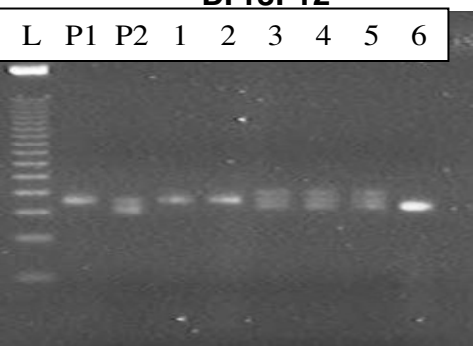


Population AM4

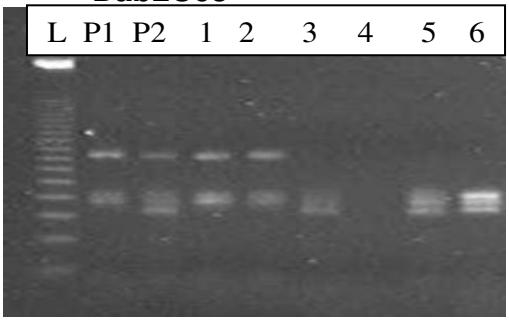
DPr3B12



DPr3F12

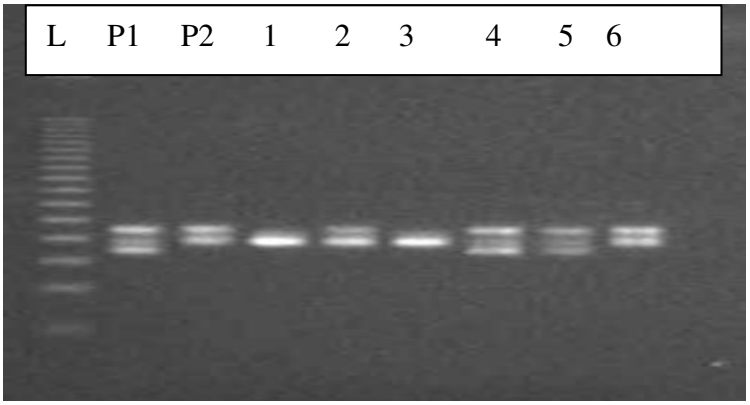


Dab2C05

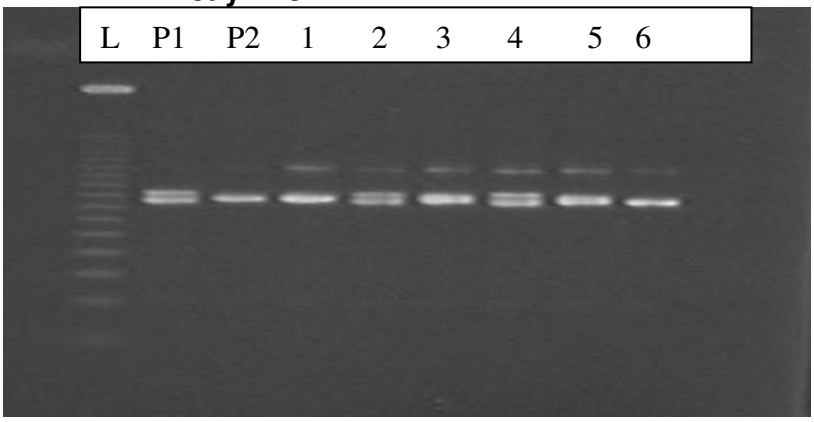


Population RM1

Dab2C05



Dcay 223



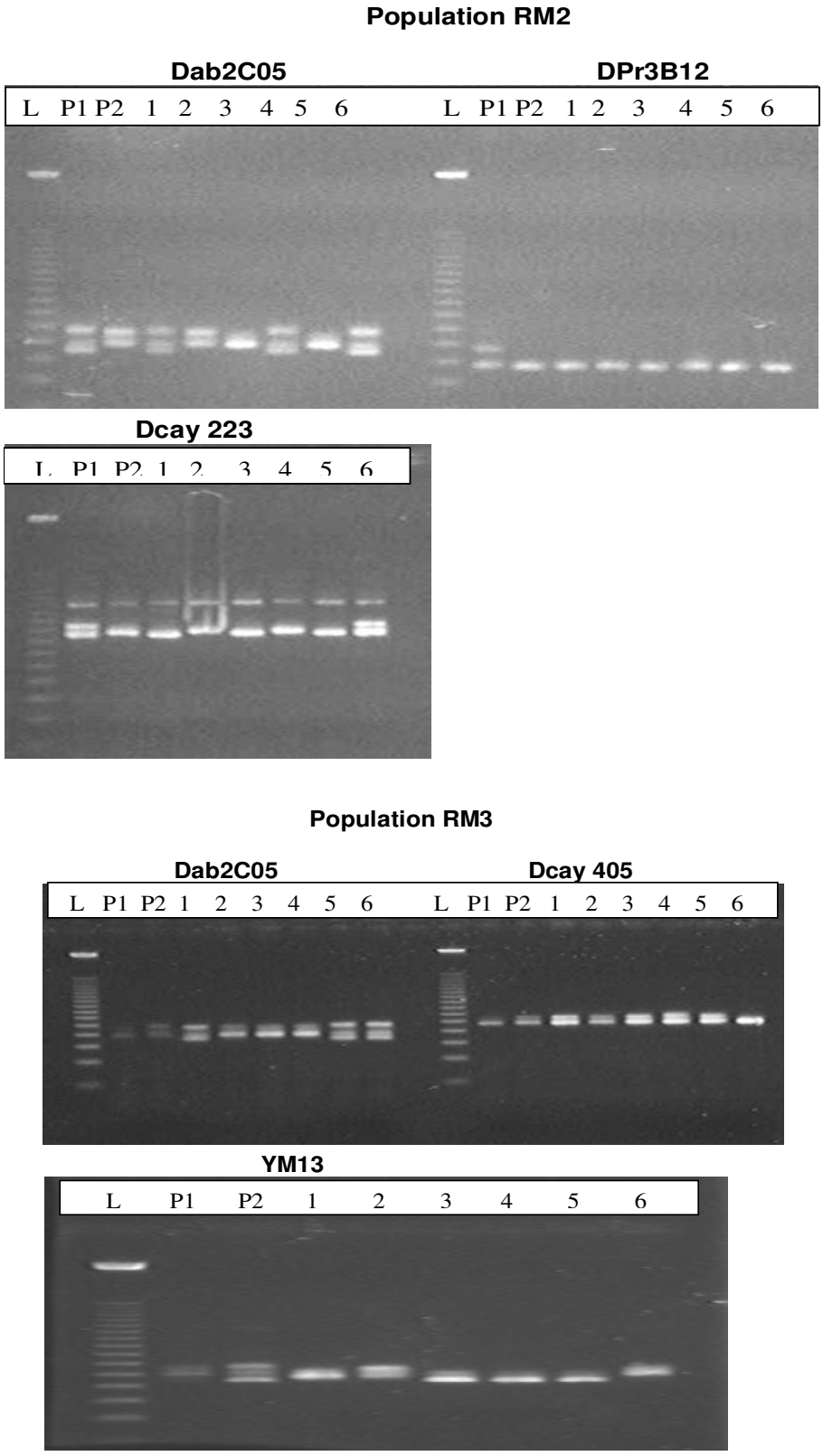


Figure 1. Agarose gel photos showing banding pattern of DNA from six hybrid genotypes (1, 2, 3, 4, 5 and 6) and their two parents (P1 and P2) of mapping populations AM1, AM2, AM3, AM4, RM1, RM2 and RM3, using nine SSR markers (Dab2C05, Dpr3B12, Dab2E07, Dab2D08, DPr3F12, Dcay 223, Dcay 245, Dcay 405 and YM-13). L is 50 bp ladder.

cooking quality or tuber texture and four in *D. alata* for anthracnose disease, cooking quality and oxidation were developed. The number of genotypes per population ranged from 50 to 283. Death of plants in the field and rotting of tubers in storage possibly due to pests, diseases and other environmental factors were encountered that led to the reduction of the size of the populations. SSR marker analysis of DNA from parents and six randomly selected progeny of each of the populations indicated that, parental crosses were successful and the progeny were true hybrids. It is recommended that, the populations can be maintained in the field and also in tissue culture (*In vitro*) for long term conservation.

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