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Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas* L.) genotypes

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Abstract In this paper we present a method for the generation of randomly amplified polymorphic DNA (RAPD) markers for sweet potato. These were applied to produce genetic fingerprints of six clonal cultivars and to estimate genetic distances between these cultivars. The level of polymorphism within the species was extremely high. From the 36-decamer random primers used, 170 fragments were amplified, of which 132 (77.6%) were polymorphic. Ten primers resulted in no detected amplification. Of the remaining 26 primers for which amplification was achieved, only one did not reveal polymorphism. Six primers used alone enabled the discrimination of all six genotypes. Pattern analysis, which employed both a classification and ordination method, enabled the grouping of cultivars and the identification of primers which gave greatest discrimination among the cultivars.

Key words *Ipomoea batatas* · Sweet potato · RAPDs · Molecular markers · Genetic polymorphism

Introduction

Relative to its importance as a food crop, sweet potato (*Ipomoea batatas* L., 2n = 6x = 90) has received little attention in terms of plant breeding and, in particular, molecular approaches to its genetic improvement. Breeding is traditionally a slow process, due to difficulties associated with self- and group-incompatiblity, absence of flowering in some genotypes, and polyploidy. Genetic markers offer a number of applications for sweet potato genetic improvement. Few morphological markers have been identified and mapped for sweet potato, and although isozymes have been applied to identify relationships between different *Ipomoea* species, there are too-few polymorphic isozymes for serious consideration of markers within *I. batatas*. Randomly amplified polymorphic DNA (RAPD) methodology offers the promise of virtually unlimited markers in plant species (Welsh and McClelland 1990; Williams et al. 1990). The power of RAPD technology lies in the following attributes: (1) no previous knowledge of the genome is required; (2) rapid results are obtained, especially when compared with the procedures involved in restriction fragment length polymorphism (RFLP) analysis; and (3) a universal set of random (arbitrary) primers can be used for genomic analysis of any organism (Welsh and McClelland 1990; Williams et al. 1990). This technology has already been applied to a range of plant species, including rice (Welsh et al. 1990), maize (Welsh et al. 1991), tomato (Klein-Lankhorst et al. 1991), and papaya (Stiles et al. 1993).

In this paper we present a method for the generation and detection of RAPDs in sweet potato. This allowed the generation of genetic fingerprints of six sweet potato cultivars and the estimation of their genetic relationship based on the analysis of the polymorphisms identified. Pattern analysis, based on the joint application of a classification and ordination procedure, was applied to the polymorphic banding patterns obtained from RAPD analysis. This served to assist in summarising the relationships among genotypes and to enhance the ability to identify primers and markers which gave the best discrimination among the cultivars used.

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Materials and methods

Plant DNA extraction

Six sweet potato cultivars, Hawaii, Wan Mun, Beerwah Gold, TIS 2498, Lole and L0323, were grown in the glasshouse for DNA isola-

tion purposes. RAPD analysis was carried out using genomic DNA extracted by one of two methods.

Method 1: leaf tissue (200 mg) was harvested from glasshouse-grown sweet potato plants and ground in a mortar and pestle under liquid nitrogen. The frozen powder was transferred to 10 ml CTAB (hexadecyltrimethylammonium bromide) buffer [50 mM CTAB, 100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl] and incubated at 65 °C for 30 min with occasional shaking. An equal volume of chloroform:isoamylalcohol (24:1 v/v) was added and mixed by inversion, then centrifuged at 5 000 g for 10 min. The aqueous phase was transferred to a fresh tube and re-extracted with an equal volume of chloroform:isoamylalcohol. The aqueous phase was removed to fresh tubes and pelleted in 1 ml 7 M ammonium acetate and 10 ml ice-cold 99% ethanol with centrifugation at 25 000 g for 15 min. The pellet was washed twice in 70% ethanol and resuspended in 200 µl TE (10 mM Tris, 10 mM EDTA, pH 8.0) and the DNA yield was determined fluorometrically (Hoefer TKO 100 mini-fluorometer).

Method 2: DNA was extracted as described by Varadarajan and Prakash (1991), a method which involved plant incubation in the dark for 48 h prior to extraction in an attempt to reduce the amount of polysaccharide isolated from leaf tissues.

Amplification and electrophoresis

Decamer random primers (kits H and U, Operon Technologies, Alameda, California) were used in single-primed PCR reactions to generate polymorphisms. A 25-µl reaction was set up as follows: 400 µM dNTPs (1:1:1:1 mix of dATP, dCTP, dGTP and dTTP), 10 pM primer, 4 mM $MgCl_2$ and 50 ng template DNA in 1 × PCR buffer (Biotech International, Perth, Australia). The reaction was overlaid with mineral oil and the reaction mix heated to 94 °C for 5 min to denature the template DNA. The reaction mix was then held at 72 °C while the *Tth*-plus DNA polymerase (Biotech International, Perth, Australia) was added to the reaction mix. Amplification proceeded on a DNA Thermal Cycler (Perkin-Elmer) for 45 cycles of 1 min at 94 °C, 1 min at 37 °C and 2 min at 72 °C. The total volume of the reaction was loaded onto a 1.2% agarose gel containing ethidium bromide at a final concentration of 0.3 µgml^{-1}. The samples were run at 80 V for 4 h. λ-*Hin*dIII and λ-*Pst*I were the molecular weight markers used to determine the size of the amplified fragments.

Data analysis

The results obtained from the experiments were compiled into a two-way matrix (cultivar × marker) to generate a "finger-print" of each cultivar. Each fragment was identified by its size and the primer used, e.g., OPH5-500 refers to the 500-bp fragment amplified by primer 5 in kit H. The relationships among the cultivars and the markers were investigated by pattern analysis (Williams 1976) employing a combination of complementary classification and ordination procedures. Following Stiles et al. (1993) the similarity between the cultivars was measured as a simple matching coefficient, and the group average (UPGMA) strategy was used to group the cultivars. The relationships among the cultivars was portrayed graphically in a dendrogram. Following the general relationships developed between similarity and dissimilarity measures given by Gower (1966, 1967), an ordination procedure, complementary to the classification, was applied to both cultivars and markers using the singular value decomposition procedure. The relationships among cultivars and markers identified by the ordination were represented on a bi-polt (Gabriel 1971; Kempton 1984) of the first two principal components.

Results

Both methods of DNA extraction yielded good-quality template for PCR amplification with decamer primers. There were no differences in the amplified products obtained using the DNA from the two methods. Method 1 has the considerable advantage that no dark pre-incubation of plant is required, hence this is the preferred extraction protocol for RAPD analysis of sweet potato.

It was found that 26 (72%) of the 36 primers tested generated at least one polymorphic band. The sequences amplified by each primer varied in number (1–12) as well as intensity, with a range of amplified products from 100 bp to 2000 bp; a total of 170 bands was observed. With ten primers (OPH-1, 2, 4, 7, 8, 10, and 14, OPU-2 and 4) no amplification was obtained with any cultivar. OPU-8, was the only primer which resulted in amplification and yielded no polymorphisms. In total, 132 polymorphic bands were detected (77.6% of the total number amplified), varying from 1 to 10 per primer, with a mean of 3.7 polymorphisms per primer (or 4.9 if only those primers which gave amplification are included) over the six cultivars (Table 1). Six primers (OPU-1, 5, 6, 7, 14, 17), when used alone, gave a unique fingerprint for all six cultivars tested in this study.

The RAPDs generated were used to determine the genetic distances between the six cultivars. The relationship of these six cultivars, as identified by the classifica-

Table 1 Number of polymorphic amplification products produced by each primer. The number of distinct discrimination patterns represents how many of these markers gave a distinct discrimination (i.e., were not in the same group after truncation at the 48-group level) among the six cultivars. Unique discrimination patterns are those which were only observed once among the 132 polymorphic markers

Primer	Primer sequence (5′-3′)	Polymorphic markers	Distinct discrimination patterns	Unique discrimination patterns
OPH-3	AGACGTCCAC	5	5	2
OPH-5	AGTCGACGCC	2	2	0
OPH-6	ACGCATCGGA	3	1	0
OPH-9	AGTCGTCCCC	5	5	2
OPH-11	CTTCCGCAGT	6	4	1
OPH-12	ACGCGCATGT	5	3	1
OPH-13	GACGCCACAC	4	2	0
OPH-15	AATGGCGCAC	2	2	0
OPH-16	TCTCAGCTGG	2	2	0
OPH-17	CACTCTCCTC	4	3	0
OPH-18	GAATCGGCCA	3	2	1
OPH-19	CTGACCAGCC	3	3	0
OPH-20	GGGAGACATC	4	4	1
OPU-1	ACGGACGTCA	9	9	1
OPU-3	CTATGCCGAC	9	4	0
OPU-5	TTGGCGGCCT	10	9	1
OPU-6	ACCTTTGCGG	8	8	1
OPU-7	CCTGCTCATC	7	4	0
OPU-11	AGACCCAGAG	1	1	0
OPU-12	TCACCAGCCA	4	4	0
OPU-13	GGCTGGTTCC	5	5	0
OPU-14	TGGGTCCCTC	8	6	1
OPU-15	ACGGGCCAGT	2	2	0
OPU-16	CTGCGCTGGA	7	5	2
OPU-17	ACCTGGGGAG	9	7	1
OPU-18	GAGGTCCACA	5	5	1

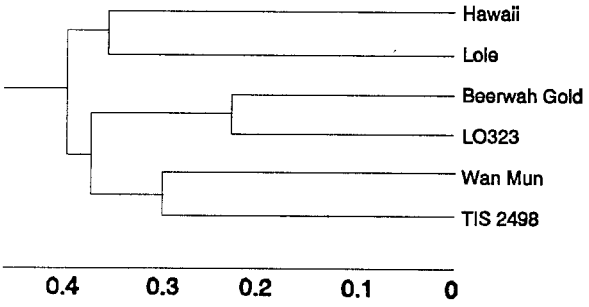

Fig. 1 Dendrogram of six sweet potato cultivars determined from 132 RAPD markers. Dissimilarity between cultivars was measured using a simple matching coefficient with clustering performed by the UPGMA strategy

tion, has been represented as a dendrogram (Fig. 1). It can be seen that the two most-closely related cultivars were Beerwah Gold and LO323 (0.24 dissimilarity), while the most distant were Beerwah Gold and Hawaii (0.41 dissimilarity). This reveals a relatively high degree of genetic variability within the species.

Ordination of marker scores and cultivar scores as a bi-plot (Fig. 2) reveals both the relationships among cultivars and the discrimination power of the individual markers. The relationships among the cultivars identified by the ordination reflected the grouping-association identified by classification (Fig. 1). The ordination of marker scores assisted inspection of the way in which the markers contributed to the discrimination among the six cultivars. As an example, it can be seen that U-16-6 was effective in discriminating between the group of four cultivars, Wan Mun, TIS 2498, Hawaii and Lole, and the group of two cultivars, LO323 and Beerwah Gold. The ordination also reveals that many markers gave identical discrimination as they plotted to the same position, a result of giving the same pattern of presence/absence among the six cultivars. The 132 markers actually give only 48 unique patterns of discrimination, meaning that 48 different patterns of bands (of the possible $2^6 = 64$ possible patterns) were observed. Clustering of the marker data using the group-average strategy with truncation at the 48-group level allowed rapid identification of the frequency of particular patterns of discrimination (Fig. 3). Sixteen markers revealed unique discrimination patterns, while one particular pattern (a band present only in cv Lole) was replicated ten times by nine different primers. In many cases, a particular pattern was replicated by two or three different markers.

Discussion

The level of genetic polymorphism within sweet potato is quite high, with 3.7 polymorphisms revealed per primer over a small number of cultivars. This compares favourably with other species including *Arabidopsis thaliana* (0.3), soybean (0.5), maize (1.0) (Tingey and del Tuto 1993), and wheat (0.38) (He et al. 1992). The level of dissimilarity between the two most-distant cultivars (0.41 between Beerwah Gold and Hawaii) demonstrates that the level of genetic variation within the species is

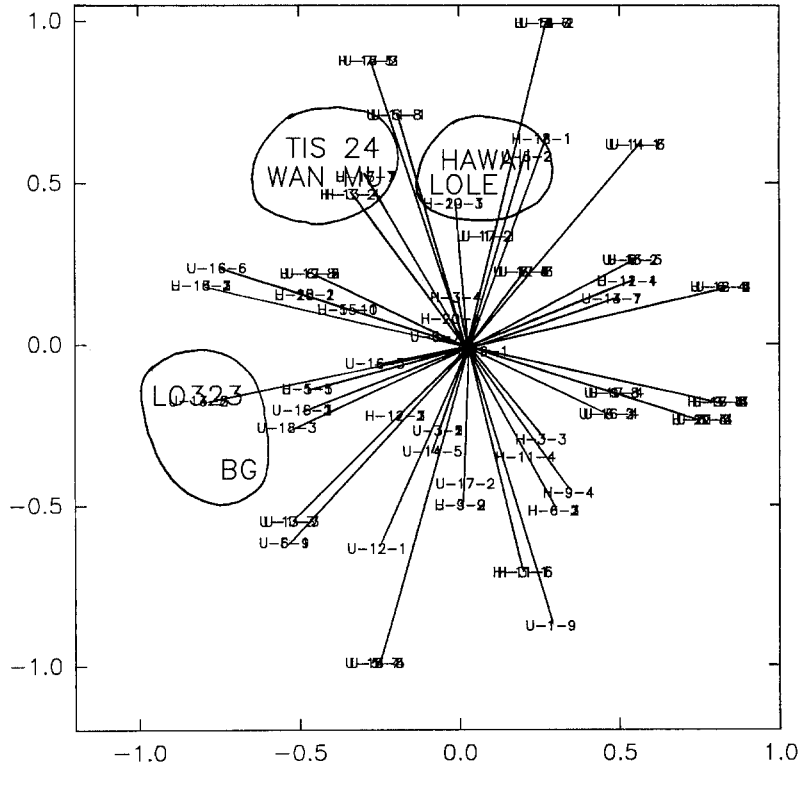

Fig. 2 Bi-plot of relationships among sweet potato cultivars and RAPD markers identified by ordination of the first two principal components as described in Materials and methods

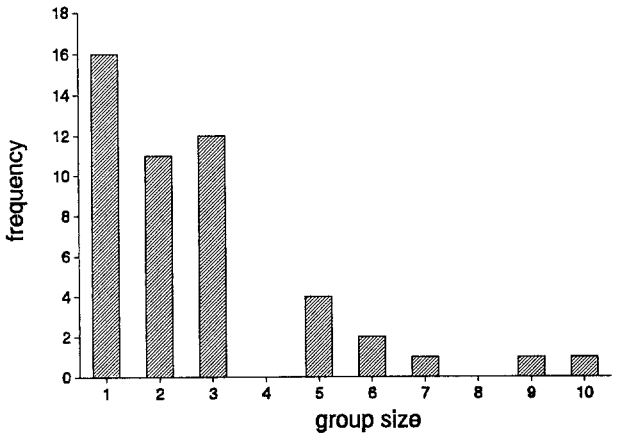

Fig. 3 Frequency distribution of the occurrence of particular patterns of discrimination across the six sweet potato cultivars. Forty-eight groups or distinct patterns of discrimination were identified by clustering. Group size refers to the number of times a particular pattern was replicated. Hence, for example, 12 particular patterns of discrimination (groups) were identified with three markers giving each pattern

substantial, and suggests that the genetic base of domesticated sweet potato germplasm is quite broad. This augurs well for the potential of marker-assisted breeding methodology in sweet potato improvement.

We had planned to use the information obtained on the genetic distances between cultivars to identify possible cross-compatibilities. If a relationship could be established between the degree of RAPD polymorphism and cross compatibility, the empirical fashion of crossing, given the self- and cross-incompatibility within sweet potato (Martin 1967; Williams and Cope 1967), may be avoided. Such information would enable us to perform progeny tests on putative transgenic sweet potato plants. However, of the cultivars which could be induced to flower, all were found to be not only self-incompatible, but cross-incompatible with lines most distant genetically, and no seed set was obtained. One possible explanation for this is that the cultivars used here were from the same compatibility group, although this is unlikely since they originate from breeding/selection programs on three continents, and include orange-, white- and purple-skinned types, and orange and white flesh. Further work may lead to the identification of specific polymorphisms which delineate self-incompatible groups.

The ordination bi-plot was found to be very useful in interpreting the discrimination power of individual markers. Markers plotting to the same co-ordinates can be quickly identified in this manner, which is an extremely useful tool in determining not only which markers, but also which primers, reveal the highest levels of polymorphism. For example, primers OPU-1, OPU-3 and OPU-17 each reveal nine polymorphic bands. However, only in the case of OPU-1 does each marker plot to different co-ordinates on the bi-plot, which means the discrimination pattern is different for each band. Primers OPU-3 and OPU-17 reveal four and seven distinct discrimination patterns respectively, which means that the amount of information gained from these primers is somewhat less than that gained from amplification with OPU-1. Four of the nine bands amplified by OPU-3 gave an identical discrimination pattern, which was, in fact, a band present in Hawaii only. Three of the remaining bands amplified by OPU-3 gave another discrimination pattern, and were present only in LO323 and TIS 2498. In all, 12 of the 26 primers which revealed polymorphism replicated at least one pattern of discrimination. When two distinct primers gave rise to identical discrimination patterns (i.e, amplified markers which plotted to the same co-ordinates on the bi-plot) it is highly unlikely that the same sequence was amplified, particularly as the primer sequences were very different and the band was usually of different size. This may not always be the case when similar discrimination patterns were amplified by the one primer, as in the case of OPU-3. In some species, such as wheat, there is good evidence that many of the RAPD markers are from highly-repetitive sequence DNA (Devos and Gale 1992). It could be postulated that some of the bands which give identical discrimination patterns from a single primer may be from such highly-repetitive sequence DNA, where multiple homologous sites exist. Indeed, there is some evidence that some of the low-molecular-weight bands are in fact a subset of one of the larger fragments. This was found to be the case in one instance where an amplified band was isolated from the agarose gel and multiple products were detected when amplification was repeated using that DNA as a template (data not shown). Alternatively, similar discrimination patterns could be the result of hexaploidy, with different amplification products arising from different donor progenitors. Further work is required to elucidate this.

Other possible uses of RAPD analysis in sweet potato include the testing of plants propagated in vitro for genetic integrity, phylogenetic studies within the genus *Ipomoea*, a survey of germplasm collections and the identification of core collections. Self-incompatibility problems will hinder the speed of application of molecular markers to sweet potato improvement. Classical mapping populations, such as F_2, BC and recombinant inbreds, will not be available. However, the level of polymorphism apparent in this initial screen demonstrates that RAPD markers have great potential for marker-assisted breeding in sweet potato for difficult-to-select traits such as weevil resistance and culinary/nutritional quality.

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