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Genetic diversity of Kensington mango in Australia

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Summary. The genetic diversity of Kensington mangoes (*Mangifera indica* L.) was investigated using random amplified polymorphic DNA (RAPD) analysis. DNA was extracted from leaves of 27 'Kensington Pride', 2 'R2E2' and 1 seedling. RAPD analysis with 10 oligonucleotide primers allowed the scoring of 107 markers. The R2E2 trees (20% dissimilarity) and the seedling (10% dissimilarity) were distinct from the Kensington Pride. However, there was very little evidence of significant genetic variation within Kensington Pride selections. Fifteen of the selections were identical in all 107 markers. Only 2 selections, WEAN2 and ML2N1, differed by more than 5%. These plants provide the best options for use in genetic improvement of the Kensington Pride mango. Many of the differences found in Kensington mango orchards may be due to environmental factors not genetic variations.

Introduction

Mango production in Australia has been predominantly based on the cultivar Kensington Pride which currently comprises over 90% of all trees planted. This situation is likely to continue in the foreseeable future because of market preference for this cultivar. Kensington Pride is highly prized for its distinctive flavours when eaten as fresh fruit and its retention of these flavours when processed. However, it does suffer from problems associated with its irregular bearing and variability of fruit type (Beal 1981), as well as susceptibility to pathogens such as bacterial black spot (*Pseudomonas mangiferaeindicae*), anthracnose (*Colletotrichum gloeosporioides*) and stem end rot (*Dothoriella dominicana*) (Muirhead and Grattidge 1984). For this reason, selection within the Kensington Pride cultivar has been undertaken to overcome these problems.

Kensington Pride, first grown in Bowen, is thought to have been introduced from the East Indies or India on the horse trading ships that visited that port in the late 1800s (Stephens 1969). Like many other cultivars derived from South East Asia, Kensington Pride is polyembryonic, i.e. the seeds have nucellar embryos that are genetically identical to the mother plant (Sturrock 1967). Because of this, polyembryonic cultivars have generally been propagated by seed and Kensington Pride is no exception to this rule.

In some polyembryonic mango cultivars, a viable zygotic embryo is also present in the seed (Sturrock 1967). Schnell and Knight (1991) reported that the number of zygotic offtypes in seedling populations can be as high as 64% in the cultivar Golek and as low as 0% in the Israeli cultivar 13-1. Truscott *et al.* (1993) reported offtypes due to zygotic seedlings that most likely originated from self-pollination ranging from 2% in Sabre to 47% in Florigon and Kensington Pride. The greater the genetic diversity, the better the chance of selecting offtypes with desirable traits that can be incorporated into commercial cultivars.

This study investigated the genetic diversity of trees selected for superior characteristics using random amplified polymorphic DNA (RAPD). The information was used to assess their value in a larger project to improve Kensington Pride.

Materials and methods

Sample collection

Samples of Kensington Pride mangoes (*Mangifera indica* L.) for RAPD-polymerase chain reaction (PCR) analysis were taken from a collection of selected superior trees growing in a uniform block on the Queensland Department of Primary Industries' Ayr Research Station. These lines were selected in 1991 and 1992 from the major mango growing districts in Queensland. The program aimed at identifying lines of Kensington Pride that had superior fruit quality, bearing, and pest and disease tolerance. Thirty lines of mango were sampled (Table 1), 27 of them being Kensington Pride selections, 2 the cultivar R2E2 (2,16), and 1 a local seedling (24) showing some commercial promise. Samples for analysis were taken from the leaves of young flushes while still in the cell expansion stage (purple in colour) to maximise DNA content and minimise other unwanted cellular material.

DNA extraction

Mango samples to be analysed were collected and stored at 4°C until DNA extraction. The extraction method was modified from that used by Graham *et al.* (1994). Leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Tissue was weighed before DNA extraction and 2 mL of

Table 1. List of the selections used in the RAPD-PCR analysis and the criteria on which trees were originally selected

No.	Accession	Description
1	RORNT	Consistently productive tree, round, highly coloured fruit; reputedly anthracnose resistant; Ayr
2	RAHNT[A]	R2E2 seedling; this cultivar is a monoembryonic seedling of Kent; pollen parent is unknown; Innisfail district
3	REHNT	Consistently prolific yielding; roundish fruit, very good background and blush colour; Townsville district
4	NUCNT	Very large, rounded fruit; tree has very thick leaves, could be polyploid; Horseshoe Lagoon
5	TE1NT	Very large, rounded fruit with thick leaves, possibly polyploid; Horseshoe Lagoon
6	TE2NT	Excellent size and roundish shape for packing, reasonable blush colour; Horseshoe Lagoon
7	MA1NT	Large fruit with bright red blush colour, bears heavily every year; Home Hill district
8	MA2NT	Excellent fruit shape, colour, taste; bears extremely well every year; large old tree reputed to possibly be a seedling from one of the early 'Bowen Special' trees at Bowen; Home Hill district
10	WALNT	Bears well every year, never failed; reasonable blush colour and shape; Tableland/Mareeba
11	BNAN1	Consistently high yielding, with good fruit shape and colour; Tableland/Mareeba
12	KANNT	Reputed to have large fruit, with extremely high yields every year; locally renowned for its consistency and productivity; Abergowrie
13	WI1NT	Large old tree with very roundish fruit; reputedly a seedling from one of the original 'Bowen Specials'; Bowen
14	WI2NT	Huge old tree reputed to be the oldest seedling from the original Bowen Special tree; Bowen
15	WI3NT	Large tree which yields very highly every year; reputedly the progeny of one of the original Bowen Special mango trees; Bowen
16	R2E2[A]	Grafted R2E2; this cultivar is predominantly polyembryonic but is known to produce a small percentage of monoembryonic seed; it is a seedling of Kent with unknown pollen parent
17	Bambaroo	Previously selected from a tree near Ingham and now adopted by many nurserymen in Queensland
18	Spooner	Previously selected from Cape Tribulation because of its capacity to consistently bear high yields of very clean fruit in a high-rainfall area; has been reasonably widely adopted in some quarters of the Queensland mango industry
19	FITN2	Good roundish-ovate fruit shape with good blush colour and excellent background colour; Yeppoon
20	GRONT	Previously selected in southern Queensland because of its reported resistance to bacterial black-spot; has been adopted to some extent by nurserymen
21	KRAN1	Slightly smaller rounded fruit, good blush colour, seems earlier than other trees in the district; Bowen
23	ML2N1	Prolific bearer; never known to have failed; good shape and excellent blush; Bowen
24	ROYN1[A]	Large pleasant tasting fruit, bright red colour, mostly thin monoembryonic seed with high flesh recovery and excellent shelf life; many large fruit per panicle; tree seems to be completely resistant to scale; this tree was the progeny of seed from a highly coloured wild creekside 'banana' mango tree, but fruit is different in shape from parent; Horseshoe Lagoon
25	WEAN2	Late season selection, ripening after other Kensington trees in the district; Tableland/Mareeba
26	GR1N2	Regular bearer, good fruit shape and colour; locally renowned for its consistently high yields; Yeppoon
27	HA2N2	Exceptional taste and flavour; roundish fruit, highly blushed, reputed to have a good shelf-life; Yeppoon
28	VC4N2	High yielding, outstanding blush colour; good size and shape for ease of packing; Horseshoe Lagoon
29	M18N2	Has some resistance to bacterial black-spot; Mount Morgan
30	HA1N2	Outstanding taste; renowned locally for its flavour; rounded-ovate shape with reasonable blush; Yeppoon
33	NU2N2	Very attractive fruit shape and colour, with consistently high yields; Mount Morgan
34	GAMN1	Prolific bearer, good fruit shape and blush colour; Tableland/Mareeba

[A] Not Kensington Pride.

extraction buffer [2% (w/v) hexadecyltrimethylammonium bromide (CTAB, Sigma), 100 mmol Tris-HCl/L, pH 8.0, 1.4 mmol NaCl/L, 20 mmol EDTA/L] was added per gram of sample and mixed by gentle inversion. Samples were then incubated at 55°C for 20 min then centrifuged for 5 min at 15 000 x *g* in a microcentrifuge. The supernatant was collected and 1 volume of chloroform:iso-amyl alcohol (24:1) added and mixed by gentle inversion for 2 min before centrifugation at 15 000 x *g* for 20 s. The upper aqueous phase was collected and 1/10 volume of 7.5 mol/L ammonium acetate and 2 volumes of ice-cold ethanol were added and mixed by gentle inversion before centrifugation at 1500 x *g* for 1 min. The pellet containing DNA was washed twice with 70% (v/v) ethanol, mixing gently by inversion, and dried in a desiccator. It was then dissolved in 500 μL of TE buffer (10 mmol Tris-HCl/L, pH 8.0, 1 mmol EDTA/L, pH 8.0). The DNA was analysed by electrophoresis on a 1% (w/v) agarose gel in TBE buffer (45 mmol Tris-borate/L, 1 mmol EDTA/L, pH 8.0). Each DNA sample was further purified by column filtration. Spectophotometric determination of the quantity and quality of the DNA was performed using the Nucleic Acid Soft-Pac Module on a Beckman Du series 60 spectophotometer.

RAPD-PCR

Oligonucleotide primers (10-mer) of arbitrary nucleotide sequence were obtained from Operon Technologies (Alameda, CA, USA) and used in the following reaction mix to a total volume of 12.5 μL: reaction buffer (10 mmol Tris-HCl/L, pH

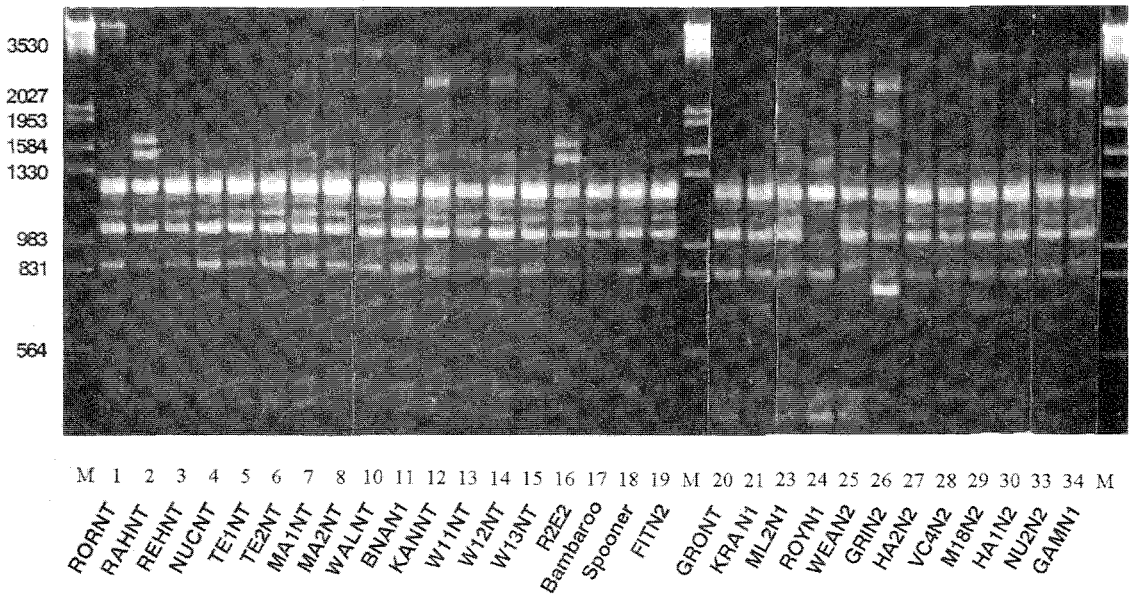

Figure 1. RAPD-PCR patterns for primer OPS-17 of the Kensington Pride mango selections. Lanes marked M are DNA size markers. Numbers on the left hand side represent the number of base pairs. Note the high degree of uniformity amongst selections.

8.3, 50 mmol KCl/L, 1.5 mmol $MgCl_2$/L), 200 μmol/L each of dATP, dTTP, dCTP and dGTP, 0.2 μmol/L of a single 10-mer oligonucleotide, 0.4 units of *Taq* polymerase (Boehringer Mannheium, Melbourne, Australia), and 10 ng of template DNA. The thermal cycling program was run on a Perkin Elmer 9600 Thermal Cycler. All programs had 1 initial cycle with melting at 94°C for 2 min, annealing at 40°C for 30 s and extension at 72°C for 1 min. Negative controls were set up without template and cycled with the other PCR reactions. The RAPD-PCR products were visualised following electrophoresis on a 1.5% (w/v) agarose gel in TBE buffer at 16 V/cm. After staining with ethidium bromide for 40 min and destaining for 10 min in TBE buffer, photographs were taken. They were scored for the presence or absence of each DNA marker across all 30 samples.

Data analyses

The qualitative data was analysed using NTSYS-pc Taxonomy and Multivariate Analysis System (Exeter Software, Setauket, New York, USA). Similarity indexes were calculated using SIMQUAL (similarity for qualitative data) by simple matching coefficient:

$$F = (a + d)/[(b + c) + (a + d)] \quad (1)$$

where $a + d$ are the total number of matches between 2 samples; and $b + c$ are the total number of non-matches between 2 samples.

The use of similarity coefficients for qualitative data was discussed by Sokal and Sneath (1963). From the similarity matrix Sequential, Agglomerative, Hierarchical and Nested (SAHN) clustering was performed using the unweighted pair-group method, arithmetic average (UPGMA) algorithm (Sneath and Sokal 1973). A UPGMA phenogram was plotted to show the genetic relationships and distance of each provenance.

Results

RAPD analysis with 10 primers (Table 2) produced 107 markers. Seven of the 10 primers were informative showing genetic variation between several of the samples; notably these were largely in selections that were not Kensington Pride. The gel patterns for the primer OPS-17 are shown in Figure 1. Scoring of 107 markers allowed calculation of similarity coefficients and construction of a dendrogram which is presented in Figure 2.

The 2 non-Kensington lines, R2E2 and its polyembryonic seedling RAHNT, were the most distinct, with a 20% dissimilarity. The other non-Kensington seedling ROYN1 was also very different (10% dissimilarity). Many of the Kensington Pride selections could not be distinguished. Fifteen of the selections did

Table 2. List of operon primers used

Informative refers to genetic variation between several samples

Primer name	Primer sequence	Informative
OPS-11	5'- AGTCGGGTGG -3'	Yes
OPS-12	5'- CTGGGTGAGT -3'	No
OPS-17	5'- TGGGGACCAC -3'	Yes
OPT-12	5'- GGGTGTGTAG -3'	Yes
OPU-8	5'- GGCGAAGGTT -3'	No
OPU-13	5'- GGCTGGTTCC -3'	No
OPU-17	5'- ACCTGGGGAG -3'	Yes
OPV-2	5'- AGTCACTCCC -3'	Yes
OPV-3	5'- CTCCCTGCAA -3'	Yes
OPW-6	5'- AGGCCCGATG -3'	Yes

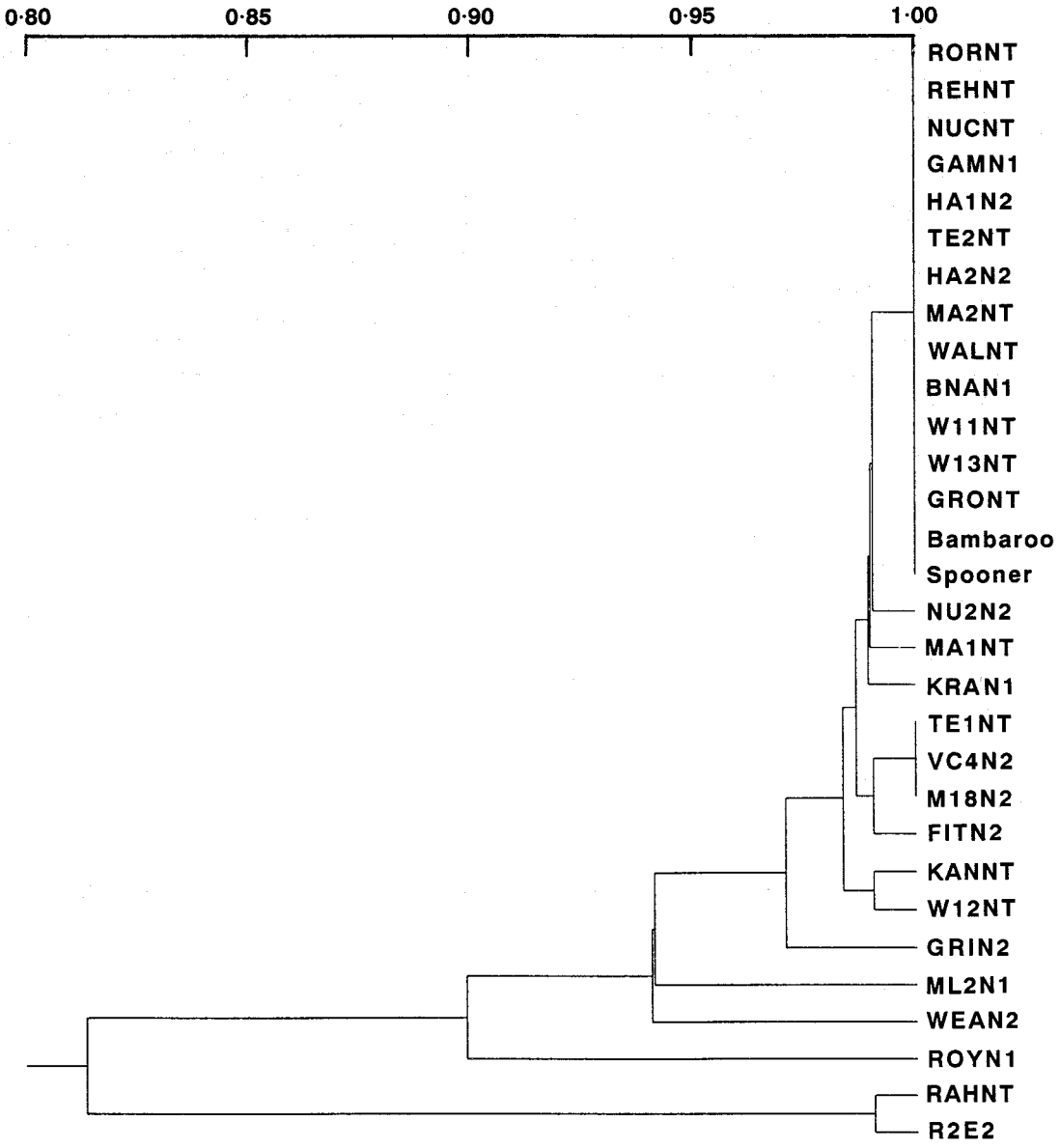

Figure 2. Dendrogram of genetic distances between the selections of Kensington Pride and other mangoes based on the unweighted pair-group method, arithmetic average (UPGMA). Analysis uses 107 RAPD-PCR molecular markers.

not differ in a single marker of the 107 scored. Two selections, WEAN2 and ML2N1, displayed differences (5% dissimilarity) suggesting that they might represent valuable genetic resources for Kensington Pride improvement.

Discussion

Greater genetic dissimilarity between the tested trees was expected because of the large variation seen in tree habit and fruit type in commercial orchards. Trees used in the analysis were selected because of their desirable but distinct traits compared with the general population. These results suggest that the variations seen in Kensington Pride orchards reflect environmental rather than genetic differences. Ongoing field evaluation of the selected trees under uniform soil and management conditions will help confirm these results.

Previous investigators (Truscott 1993; Schnell and Knight 1991; Knight 1970) found greater genetic variation in polyembryonic seedling populations.

Truscott (1993) found 12% of zygotic offtypes in Kensington Pride seedlings. These levels were not evident in this study. The low occurrence of genetically different trees in this study may reflect the small sample size, but also suggests that the traditional method of propagating seedlings by selecting only the 3 or 4 most vigorous from each germinated seed is an effective way of avoiding the weaker zygotic seedling.

The occurrence of only small numbers of genetic variants amongst these selections suggests that little may be gained from selecting existing trees within Kensington Pride, and the introduction of genes from other cultivars by crossing may be the best way to improve the cultivar.

This genetic analysis has proven to be a valuable tool in the selection and improvement of Kensington Pride, by identifying true genetic variants and by providing powerful information on which to base selection decisions. It enables selection based on known genetic differences rather than those implied by phenotypic variants, thereby identifying trees which are the most likely to give genetic traits useful in improving the cultivar as a whole. In tree crops where breeding and selection programs can take decades, genetic testing of this type can reduce some of the lengthy field testing of lines.

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