South African Avocado Growers' Association Yearbook 1992. 15:70-71

Application of the isozyme technique for identification of avocado cultivars

Michéle Truscott and Elizabeth A Lewis
Citrus and Subtropical Fruit Research Institute, Private Bag X11208, Nelspruit 1200, RSA

INTRODUCTION

Cultivar and race identification of avocado in South Africa is currently limited to horticultural characteristics. The problem with such an identification system, however, is that variations may be due to environmental conditions and not a true expression of genetic variation.

Cultivars of fruit trees are vegetatively propagated to ensure a uniform crop with fruit of consistent quality. Unequivocal identification of the propagation material is essential for assuring the desired properties. Furthermore, in many cases morphological assessment of flowering and fruiting material is not possible because of the season, immaturity of material or because the material of interest is a rootstock (Paterson *et al,* 1991).

Although morphological markers occasionally may be useful in cultivar identification, there are instances where such markers are unsatisfactory. It is therefore important to have reliable markers to unambiguously identify cultivars.

The study of isozymes may provide new approaches to some objectives which have proven difficult to achieve with classical techniques. Isozymes are multiple molecular forms which are specific proteins and they have been used successfully as genetic markers for cultivar identification in several crops, including avocado (Goldring *et al,* 1985), grape (Arulsekar and Parfitt, 1986), loquat (Degani and Blumenveld, 1986) and mango (Degani *et al,* 1990). The avocado is an important economic crop and the subject of intensive breeding programmes aimed at the improvement of fruit quality and productivity. Such improvements can be obtained by hybridizations, designed to combine complementary traits of two cultivars or selections (Degani and Gazit, 1984). Offspring from self and cross-pollinations may be differentiated by using appropriate isozymes as specific markers, due to the fact that the electrophoretic pattern is a reflection of the genotype.

MATERIALS AND METHODS

Leaf and cotyledon samples were taken from the avocado collection at the CSFRI, Nelspruit. Electrophoresis was performed using starch gels (Sigma starch hydrolyzed). The composition of the gel and the electrode buffers used were described by Degani *et al,* (1986). For isozymic analyses 6 x 4 mm pieces of leaves and cotyledons were crushed thoroughly in extraction buffer (Degani and Gazit, 1984) and the extracts

absorbed onto Whatman 3MM paper wicks. After loading, the gels were placed in a 4°C incubator and run at 25 mA for 30 min. The wicks were then removed and electrophoresis was continued at constant current and 300 V limit for a period that depended on the buffer used. When the run was complete, gels were sliced horizontally to yield slices 1 mm thick and subsequently were stained for phosphoglucose isomerase (PGI; EC 5.3.1.9) (Goldring *et al,* 1985), phosphoglucomutase (PGM; EC 2.7.5.1} (Sotos *et al*, 1983V leucine aminopeptidase (LAP; EC 3.4.11.1) (Degani *et al,* 1986), malate dehydrogenase (MDH; EC 1.1.1.37) (Torres and Bergh, 1980) and alcohol dehydrogenase (ADH; EC 1.11.1.7) (Torres, 1974).

TABLE 1 Genotypes of cultivars of *Persea americana*

Cultivar	Isozyme			
	PGM1	LAP2	MDH1	ADH2
Bacon	FS	FF	FF	FF
Duke 7	SS	FS	FS	FF
Edranol	FS	FS	SS	FF
Esther	FF	FF	SS	FF
Ettinger	FS	FS	SS	FF
Fuerte	FS	FF	FS	FF
Hass	FS	FF	SS	FF
Gwen	FF	FF	SS	FF
Pinkerton	FF	FF	FS	FF
Rincon	FF	FF	FF	FF
Rinton	–	–	–	FF
Ryan	FF	–	SS	FF
Teague	SS	FF	FF	FF
Thille	FF	FF	SS	FF
Thomas	FS	–	FS	FF
Topa topa	SS	FF	FS	FF
Whitsell	FS	FS	SS	FF
Wurtz	FS	FF	SS	FF

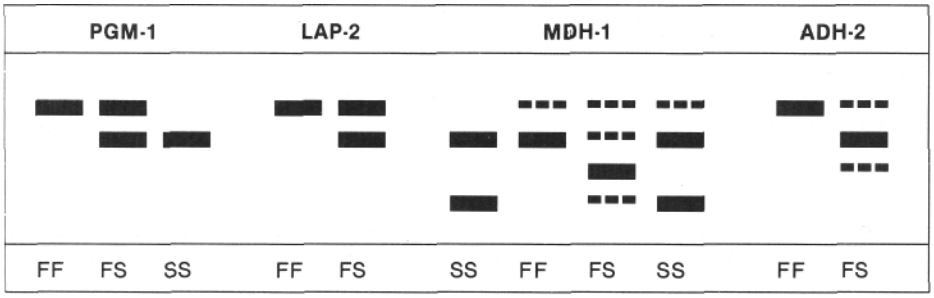

Fig 1 Schematic representation of electrophoretic patterns of PGM, LAP, MDH (leaf) and ADH (cotyledon) in avocado.

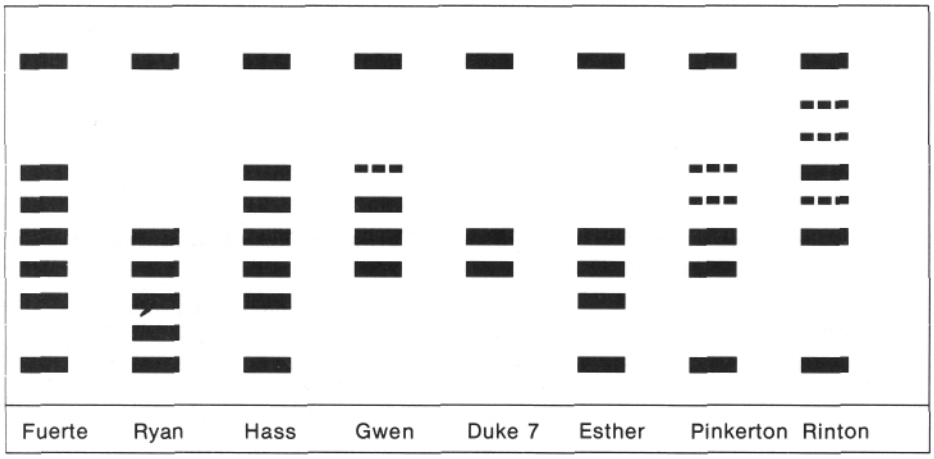

Fig 2 Schematic representation of electrophoretic patterns of PGI extracted from leaves of avocado cultivars. The dotted lines indicate weakly stained isozymes.

RESULTS AND DISCUSSION

Isozyme results obtained for avocado cultivars at the CSFRI are being compared to reported work (Degani and Gazit, 1984; Degani *et al,* 1986, 1989; Torres, 1984; Torres and Bergh, 1978,1980; Torres *etal,* 1978), to ensure that cultivars match those in other countries. Cultivars that have been tested are listed in Table 1. According to literature (Torres and Bergh, 1980), genes which specify the slower or slowest anodally migrating isozyme set are designated the number 1 and the genes which code for successively faster migrating sets are called 2. The alíele which specifies the slowest anodally migrating isozyme within a set is generally called S and for faster migrating alíeles, F (Figure 1).

Zymograms of the dimeric enzyme phosphoglucose isomerase in avocado showed complex patterns characteristic of duplicated enzymic genes. Genetic analysis of PGI in the cultivars Fuerte, Hass, Ryan, Gwen, Duke 7, Ettinger, Edranol, Pinkerton and Rinton was conducted by comparing zymograms of leaf tissue. The zymograms of these cultivars are depicted in Figure 2. The PGI patterns from leaf extracts of the nine avocado cultivars showed no variability in PGI-1 and a high variation for PGI-2. Each variety had a unique PGI pattern except for Fuerte ana Hass. It -was, therefore, possible to distinguish between Pinkerton and Rinton using the PGI system. These two cultivars were the subject of some confusion in 1989. Variability in the PGI enzyme system can thus be used for cultivar identification and for the determination of the pollen parent in avocado crosses (Goldring *et al,* 1985).

CONCLUSIONS

In future studies, correlations between isozyme markers and horticulturally significant characters, such as disease resistance, need to be determined for the expansion of breeding programmes. Isozymes can serve as markers that are associated or linked to these genes. Further uses of isozymes in a breeding programme, besides characterization and identification of cultivars, include the possibility to determine the

pollen parent effect of different avocado cultivars during fruit development and retention (Degani and Gazit, 1984). The parentage of cultivars and the progeny from a self-pollination event, as opposed to cross-pollination, can be determined. These areas of research still need to be examined, once "fingerprinting" of the avocado cultivars has been completed.

REFERENCES

ARULSEKAR, S & PARFITT, D E 1986. Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. *Hort Sci,* **21(4),** 928 - 933.

DEGANI, *C &* BLUMENVELD, A 1986. The use of isozyme analysis for differentiation between loquat cuitivars. *Hort Sci,* 21, 1457 - 1458.

DEGANI, C, EL-BATSRI, R & GAZIT, S 1990. Enzyme polymorphism in mango. *J Amer Soc Hort sci,* **115(5),** 844 - 847

DEGANI, C & GAZIT S, 1984. Selfed and crossed proportions of avocado progenies produced by caged pairs oí complementary cultivars. *Hort Sci,* **19(2),** 258-260.

DEGANI, C, GOLDRING, A & GAZIT, S 1989. Pollen parent effect on outcrossing rate in "Hass" and "Fuerte" avocado plots during fruit development. *J Amer Soc Hort Sci,* **114(1),** 106-111

DEGANI, C, GOLDRING, A, GAZIT, S & LAVI, U 1986. Genetic selection during the abscission of avocado fruitlets. *Hort Sci,* **21(50),** 1187 - 1188.

GOLDRING, A, ZAMIR, D & DEGANI, C1985. Duplicated phosphoglucose isomerase genes in avocado. *Theor Appl Genet,* **71,** 491 - 494

PATERSON, A H, TANKSLEY, S D & SORRELLS, M E1991 DNA markers in plant improvement. *Adv Agron,* **46,** 39 - 90

SOLTIS, D E, HAUFLER, C H, DARROW, D C & GASTONY, G J 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Amer Fern J,* **78(1),** 9 - 27

TORRES, A M 1974. Sunflower alcohol dehydrogenase ADH-1 genetics and dissocationrecombination. *Biochem Genet* **11,** 17 - 24

TORRES, A M & BERGH, B O 1978. Isozymes as indicators of outcrossing among "Pinkerton" seedlings. *Calif Avo Soc Yrb,* **62,** 103 - 110.

TORRES, A M & BERGH, B O 1980. Fruit and leaf isozymes as genetic markers in avocado. *J Amer Soc Hort Sci,* **105,** 614 - 615

TORRES, A M, DIEDENHOFEN, U, BERGH, B O & KNIGHT, R J 1978. Enzyme polymorphisms as genetic markers in avocado. *Amer J Bot,* **65(2),** 134 - 139.