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ISOZYMES REVEAL GENETIC RELATIONSHIPS IN BANANA GERMPLASM

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ABSTRACT

Isozyme variation was analyzed in a collection of banana cultivars to clarify genetic relationships among clones and to determine the affinities of Hawaiian bananas of early Polynesian introduction to other cultivated types. Enzymes were extracted from fresh young leaf tissues in modified Bousquet buffer at 4° C and separated by starch gel electrophoresis. Polymorphic enzymes, including ACO, MDH, PGI, and PGM, were well resolved in a continuous histidine citrate (pH 6.5) buffer system after 7 hr of electrophoresis at 15 V/cm. Isozyme variation was analyzed by cluster analysis, and the resulting groups generally corresponded well with the classification of Simmonds (1959) based on morphology. Three groups of Hawaiian cultivars were evident in this study. They were distinct from other clones and corresponded completely with a traditional classification, based on morphology, which distinguished 'Iholena', 'Maia Maoli' and 'Popoulu' groups. Cluster analysis placed the Hawaiian bananas squarely among the AAB cultivars, supporting Simmond's estimation of the genomic constitution of 'Maia Maoli' and 'Popoulu' cultivars, but contradicting his assignment of 'Iholena' bananas to the AAA group.

INTRODUCTION

Genetic variation in cultivated bananas is attributable to different ploidy levels and to interspecific hybridization between *Musa acuminata* and *M. balbisiana*, as well as to normal intraspecific variability. Genetic relationships among banana cultivars have traditionally been expressed in terms of the number and type of genomes (A for *M. acuminata* and B for *M. balbisiana*) responsible for each phenotype (Simmonds and Shepherd, 1955). A combination of cytogenetic and morphological investigations is necessary to obtain such information, and accurate classification of unknown cultivars in the field requires considerable experience. In recent years, new methods have been applied to measure and describe variation in banana germplasm. Morphological data have been subjected to principal coordinate analysis (Simmonds and Weatherup, 1990). Jarrett and Litz (1986a, 1986b) have demonstrated the existence of allozymes that serve as markers for the presence of the B genome and have shown that members of the Cavendish group of commercial cultivars are indistinguishable on the basis of their isozyme profiles. A more extensive chemotaxonomic study, involving an analysis of isozyme and flavonoid polymorphism in wild diploids and cultivars, has been published recently (Horry, 1989). All of these investigations have largely confirmed the traditional scheme based on morphology.

Isozyme electrophoresis has been a particularly popular technique, because of its convenience and power. However, with the exception of a genetic distance analysis performed on diploid M. acuminata and M. balbisiana populations (Horry, 1989), no estimation of genetic relationships among banana cultivars has been derived from isozyme data. Relationships between cultivars common in Hawaii are sometimes obscure, and much synonymy may exist. Our objective was to survey the isozyme variation in available banana cultivars and determine relationships among clones by cluster analysis. We were particularly interested to investigate the relationship of Hawaiian cooking bananas to other, more widely distributed cultivars. The Hawaiian cultivars, including the 'Iholena', 'Maia Maoli' and 'Popoulu' groups, are early introductions made in prehistoric times by colonizing Polynesians (Pope, 1926). On the basis of morphological evidence, Simmonds (1959) classified the 'Iholena' group with the AAA bananas and the 'Maia Maoli' and 'Popoulu' groups with the AAB bananas.

MATERIALS AND METHODS

Banana plants were grown at the Wailua Experiment Station, Kauai. The accessions included in the survey are listed in Table 1.

Tissue was taken from the youngest fully expanded leaf and ground at 4 C in modified Bousquet et al. (1987) extraction buffer containing 0.1M tris, 0.2M sucrose, 0.0005M EDTA (disodium), 0.005M dithiothreitol, 0.012M cysteine HCl, 0.025M ascorbic acid, 1% polyethylene glycol, 0.02M sodium metabisulfite, 0.005M DIECA, 2% Tween 80, 10% dimethylsulfoxide, 0.1% bovine serum albumin, 1% 2-mercaptoethanol and PVPP (8.0g/100ml of buffer). Extracts were subjected to electrophoresis in hydrolyzed potato starch gels (12%) made with buffer containing 0.065M histidine (free base) and 0.007M citric acid (anhydrous). Tray buffer was 0.016M histidine and 0.002M citric acid. Both buffers were adjusted to pH 6.5 before use.

Gels were stained to visualize aconitase (ACO), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI) and phosphoglucomutase (PGM) according to procedures reported by Soltis et al. (1983). The stained gels were scored for the presence or absence of 45 different electromorphs, including six for ACO, 14 for MDH, 11 for PGI, and 14 for PGM. No attempt was made to provide a genetic interpretation of the banding patterns.

Cluster analysis of the binary isozyme data was performed with the assistance of NTSYS-pc software, version 1.21 (Applied

TABLE I. Banana clones surveyed by isozyme electrophoresis and conclusions regarding genome constitution compared with conclusions obtained from morphological analysis (Simmonds, 1959).

Cultivar	Genome Constitution on Basis of: Morphology	Isozyme Survey
1. Eslesno	AAB	AAB
2. Poovan	"	"
3. Lele 1 (Iholena)	AAA	"
4. Lele 2 (")	"	"
5. Kapua (")	"	"
6. Ulaula (")	"	"
7. Williams	"	AA, AAA
8. Kahiki hai (Maia maoli)	AAB	AAB
9. Ihou 1 (")	"	"
10. Ihou 2 (")	"	"
11. Maia maoli (")	"	"
12. Kaualau (")	"	"
13. Manaiula (")	"	"
14. Eleele (")	"	"
15. Malei (")	"	"
16. Nou (")	"	"
17. Dwarf Horn Plantain	"	"
18. Ulaula - P (Popoulu)	"	"
19. Hua moa (")	"	"
20. Kai'o (")	"	"
21. Eka (Maia maoli)	"	? (mislabeled?)
22. Golden Beauty	AAAA	AAAA
23. Lacatan	AA	AA, AAA
24. *Musa balbisiana*	BB	BB
25. Neali	"	"
26. Ice Cream	ABB	ABB
27. Paka	"	"
28. Monthan	"	"
29. Largo	"	"
30. Saba	"	"
31. Cocos	AAA	AA, AAA
32. Valery	"	", "
33. Robusta	"	", "
34. Hamakua	"	", "
35. Dwarf Cavendish	"	", "
36. Double Bunch	"	", "
37. KHT Kom	"	", "
38. Pisang Raja (mislabeled?)	AAB	" "
39. Tuu Gia	AA	", "
40. Golden Aromatic 1	AAA	", "
41. Golden Aromatic 2	"	?
42. Colorado Blanco	"	AA, AAA
43. Cuban Red	"	", "
44. Berangan	AA	", "
45. Lady Finger	AA, AB	AB
46. Brazilian	AAB	AAB
47. Dwarf Apple	"	"
48. Walha	"	"
49. Go Sai Heong	?	"
50. Kantung	?	"
51. Father Leonore	AAB	"

Biostatistics Inc., Setauket, NY). Similarity coefficients were computed using both a simple matching coefficient and Jaccard's coefficient.

RESULTS AND DISCUSSION

There were no important differences between the phenograms generated through use of the Jaccard coefficient as opposed to the simple matching coefficient in the cluster analysis. The phenogram in Fig. 1 utilized simple matching coefficients, and it shows that the clustering of banana cultivars on the basis of isozyme phenotypes corresponds closely with major genome categories as determined by morphological and cytogenetic data (Simmonds, 1959). This result is in accord with the findings of others who have used chemotaxonomic approaches to study the relationships between banana clones (Jarrett and Litz, 1986a, 1986b; Horry, 1989). The close correspondance between the results of isozyme and morphological surveys indicates that isozyme markers are as useful as morphological traits for indicating the genomic constitution of banana clones. In the PGI system, the fastest moving (most anodal) band was a consistant marker for the presence of the B genome.

The Hawaiian banana cultivars showed no isozyme variation within the three major groups, and the 'Popoulu' (Fig. 1, #18-20) and 'Maia Maoli' (Fig. 1, #8-16) groups were themselves closely allied, sharing about 98% of the electromorphs. The 'Iholena' (Fig. 1, #3-6) group was more distinct, having only 88% of the electromorphs in common with the other two groups. However, the Hawaiian bananas are embedded convincingly in the cluster of AAB clones, and it seems conclusive that all of them, including the 'Iholenas' that Simmonds placed in the AAA category, are in fact AAB types. Of the other AAB clones, 'Dwarf Horn Plantain' (Fig. 1, #17) is most similar to the Hawaiian groups, sharing about 95% of the electromorphs with the 'Popoulu' and 'Maia Maoli' groups.

The uniformity of the AAA Cavendish cultivars (Fig. 1, #7,32-36) is apparent, as previously noted (Jarrett and Litz, 1986b). Surprisingly, the ABB group, including 'Saba', 'Largo', 'Monthan', 'Ice Cream' and 'Paka' (Fig. 1, #26-30), shares only about half of the electromorphs with M. balbisiana (Fig. 1, #24-25).

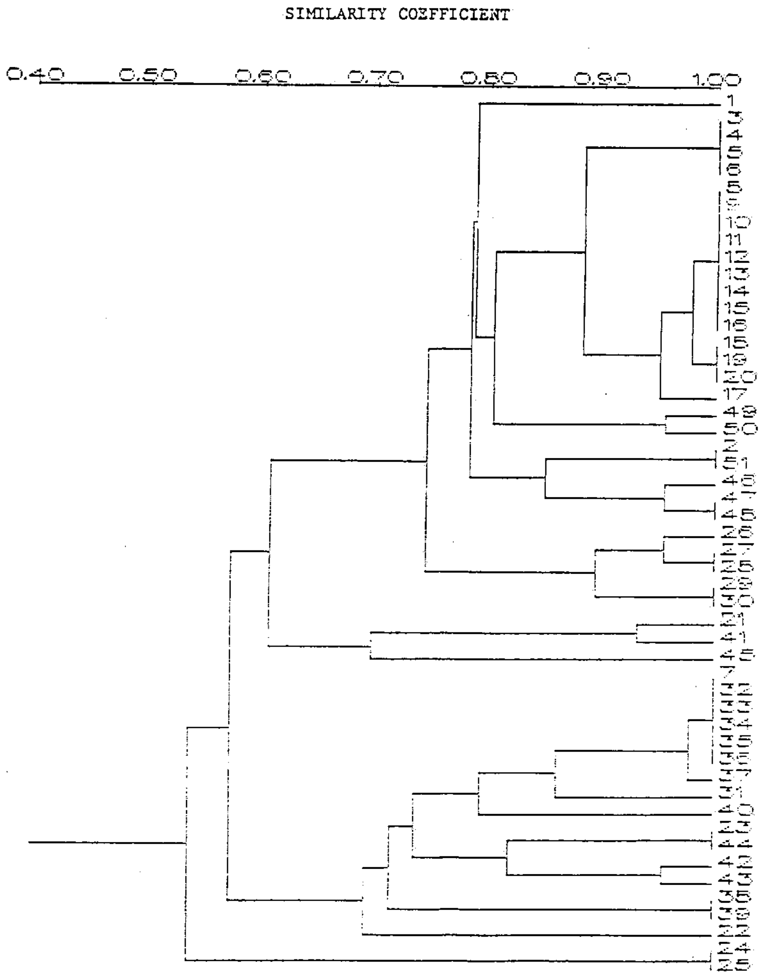

Fig. 1. Phenogram showing relationships in isozyme banding patterns among 51 banana and plantain clones from a *Musa* germplasm collection at Kauai, Hawaii. Cluster analysis was based on simple matching coefficients generated from 45 isozyme electromorphs.

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