J. Natn. Sci. Coun. Sri Lanka 1998 **26** *(2):125-131*

SHORT COMMUNICATION

DIFFERENTIATION OF A & B GENOME OF BANANA AND PLANTAIN (*MUSA* SPP.) BY ESTERASE ENZYME

A.S.U. LIYANAGE*, M.M.C. MANAWAPREMA and M.H. MENDIS
Plant Genetic Resources Centre, P.O. Box 59, Gannoruwa, Peradeniya.

(Received: 30 July 1997; accepted: 05 June 1998)

Abstract: Esterase isozyme variation detected by polyacrylamide gel electrophoresis was used to investigate the genomic constitution of the local cultivars of bananas and plantains and their wild relatives (*Musa* spp.). A high degree of polymorphism was prevalent for isozymes allozymes of esterase in accessions belonging to the AA, AAA, AAAA, AAB, ABB and BB genomic groups. One band (Rf. 0.37) of the zymograms was observed to be unique and monomorphic for all the genomic groups tested. The intensity, width and Rf value (0.37) of this band was identified. A unique doublet (Rf 0.37 and 0.43) was found to be common to all accessions of AAB, ABB and BB. The band at Rf 0.43 is specific to cultivars with B genome. Therefore cultivars with only A genome (diploid, triploid or tetraploid) can be readily distinguished from those with B genome or hybrid genome (A & B) by absence of the particular band with Rf 0.43.

Key words: Esterase, genomes, isozymes, *Musa*, zymograms.

INTRODUCTION

Banana and plantain (*Musa*) are the most important fruit crops of Sri Lanka. Due to the great diversity and number of local names for a particular cultivar, cultivated varieties are innumerable. A comparative account of the morphological features of 29 cultivars of banana in Sri Lanka has been published.[1,2] Subsequently Simmonds[3] proposed the genomic constitution (AA, AAA, AB, AAB or ABB) for 27 banana varieties of Sri Lanka. According to a report of IBPGR[4] the main cultivated group of banana and plantain has AA, AAA, AB, AAB or ABB genomic constitutions. In addition, AAAB, AABB and AAAA genomic groups are also found.

Although some morphological descriptor lists are available, precise identification of even the most widely grown cultivars are difficult or impossible due to multiple vernacular names for some clones, lack of herbarium materials and absence of male buds of some plantains. Further difficulties arise when classifying them according to the genomic group only by morphological characters, due to influence of environment[2] and high incidence of somatic mutation within specific clones.[6]

* Corresponding author

Hence a classification based upon molecular and biochemical characters rather than morphological characters has been suggested for this crop.[5,7]

The use of isozymes as genetic markers for varietal identification has received considerable attention and some progress in this direction has been reported for banana. Different experimental procedures have been reported in the attempts at establishing possible relationships within some varieties or species and for the detection of varietal or cultivar specific isozyme polymorphism.[7-10] But so far, genome specific isozyme polymorphism has been elucidated only with peroxidase and Glutamate Oxaloacetate Transaminase. Liyanage *et al.*[11] reported that peroxidase was the most useful isozyme for discrimination of genomic groups of this crop. The present work describes experimental protocols developed for the detection of esterase isozymes polymorphism and elucidates its application for genome specific identification of banana and plantain of Sri Lanka.

METHODS AND MATERIALS

Plant materials for this study were collected from the banana germplasm maintained by the Horticultural Research and Development Institute and Plant Genetic Resources Centre (PGRC), Peradeniya, Sri Lanka. Wild species, *Musa acuminata* (AA) (locally known as 'Unel' or 'Unakehel') was collected through field exploration in Yatiyantota area of Sri Lanka in 1995. Fertile diploid *M. balbisiana* BB (locally known as 'Eti-Kehel') was obtained from Exploration unit of PGRC.

A total of 150 accessions belonging to the genomic groups[1,3,11]ABB, AAB, AAA, AAAA, BB, AA were sampled for analysis of esterase isozymes.

As the apparatus mini-slab-gel electrophoresis Kit (ATTO Corp.) was chosen. The gel for this kit is small sized, easy to handle and needs only a small amount of chemicals. The extract, polyacrylamide gels and electrode buffer were prepared as described earlier.[11] Electrophoresis was carried out using a constant electric current (20 mA D.C.) for 3-4h until the tracking dye (bromophenol blue) reached the end of the gel. Subsequently gels were stained for esterase (30-40 min) isozyme. The stained gels were washed with tap water, fixed in 7% (w/v) acetic acid solution and subjected to photography. The Rf value for bands was calculated and zymograms were constructed using the average Rf value of at least two runs.

In staining the gels, β-naphthylacetate 2% w/v and fast blue RR salt (0.1% w/v) in 0.1M phosphate buffer (pH 7.2) was used.

RESULTS

The isozyme profiles of each cultivar or variety or species was observed and differences and similarities within the banding patterns were compared. In the esterase zymograms for 7 varieties (Anamalu, Sapumal anamalu, Waduru anamalu, Rathambala, William hybrid, Bin kehel, Marathamana) with AAA genome, a total of 47 different bands (according to Rf value) were observed for 34 accessions.

One band of zymograms was observed to be unique and monomorphic for all accessions with this genomic constitution (AAA). The intensity, width and Rf value (0.37) of this band were identical. This band was also found in zymograms of the 3 accessions with AAAA genomic constitution (Fig. 1 and 2). Zymograms of *Musa acuminata* ("Una kehel or Unel") also exhibited this band.

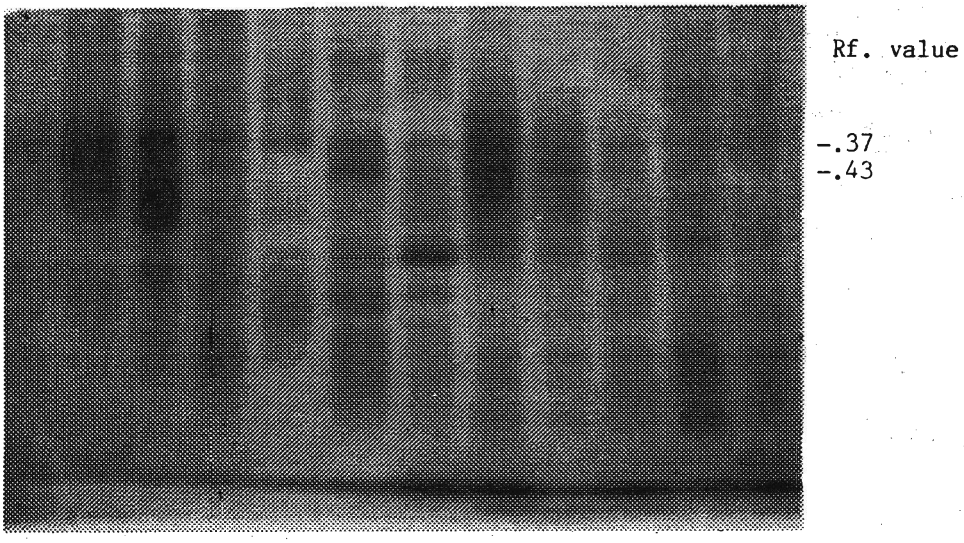

Figure 1: Example of resolution achieved for enzyme variants of esterase.

A unique doublet (Rf 0.37 and 0.43) was found to be common to all 80 accessions of 7 varieties with AAB genome. This doublet was also present in esterase zymograms of 24 accessions of 5 varieties with ABB genome. A total of 23 different bands was observed for accessions of this genomic group. The same doublet (Rf 0.37 and 0.43) was present in zymograms of *M. balbisiana* (Eti-kehel). Cultivars of Sudu kochchi, Embon and Rat kehel had a band specific to Rf 0.37 (of the doublet).

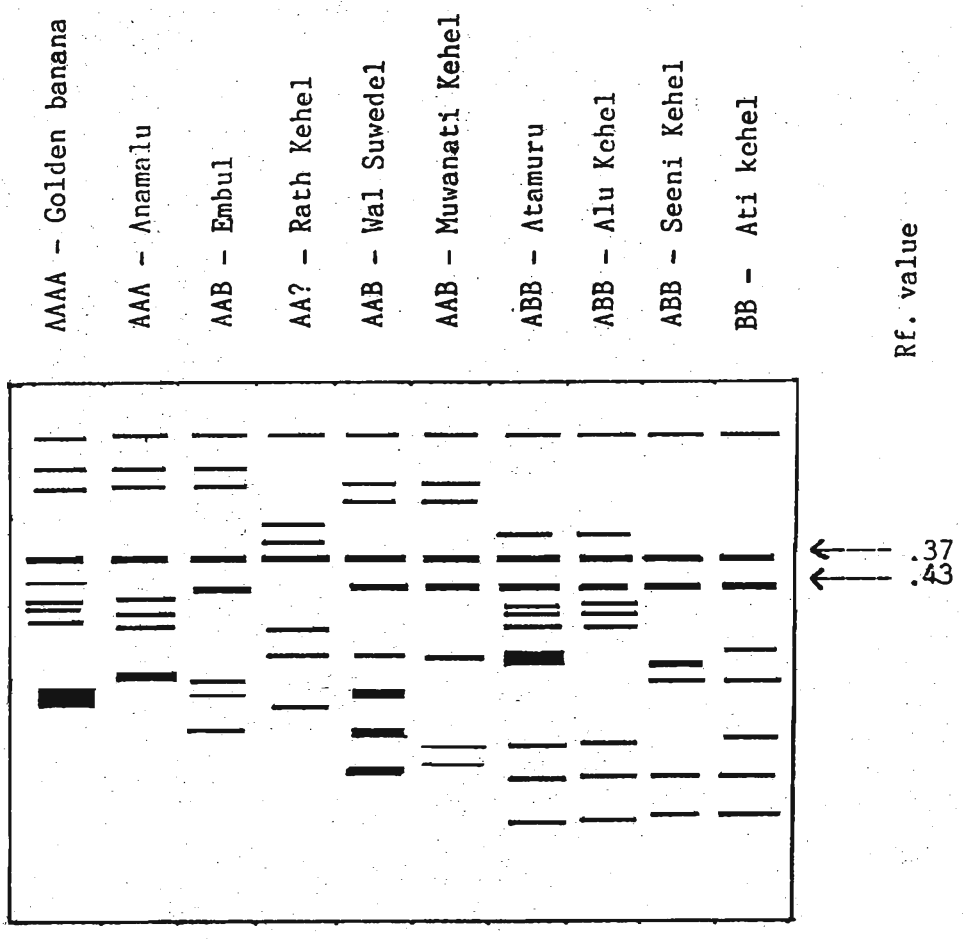

Figure 2: Zymogram of esterase in *Musa* (Zymotypes of various cultivars and wild *balbisiana*).

DISCUSSION

About 50% of cultivated bananas have originated as somatic mutations[12]and hence genetic diversity within a genome group is very limited. The genetic base of this crop especially within the *acuminata* types is reported to be much narrower than morphology suggests.[5] The edible bananas originated primarily from *M. acuminata* and *M. balbisiana*. The system of classification presently adopted is the system based on the work of Simmonds & Shepherd[13]where a scoring method is used to indicate the relative contribution of the two wild species to the genetic constitution of a given cultivar. Mature plants are needed for this kind of classification and one has to wait 12 - 18 months in order to observe morphological characters.

Although classification of banana and plantain (of Sri Lanka) according to morphological characters into genome groups has been done, some cultivars are still left out with an unknown genomic constitution.

Isozyme techniques have been used to solve a number of problems in plant breeding and systematics, to establish genetic purity, to locate duplicate accessions and to identify cultivars and somaclonal variants.[14,15] The technique has been used for clonal identification and for quantification of genetic diversity.[7,8,16,17] The identification of genomic groups of banana and plantain through isozyme analysis offers more precision than through morphological descriptors alone.

Analysis of 35 clones of *M. acuminata* Colla, representing eight putative sub-species and *M. balbisiana* Colla for 8 enzyme systems[5] and of 24 clones of banana and plantain for 5 enzyme systems[7] has been reported. It was mentioned that a total of 30 bands was resolved on individual gels of esterase zymograms. However their results were not reported for discussion. In another study, 44 cultivars belonging to the genome groups AB, AAB, ABB and AAA have been analysed for isozyme polymorphism.[9,10] A high degree of polymorphism has been observed for isozymes of esterase, acid phosphatase, and catalase[9] and of peroxidase and superoxide dismutase[10] in 44 cultivars of Indian bananas and plantains.

However, clear correspondence between banding patterns and genomic groups has not been reported in these studies. In the present study cultivars with B genome (AAB or ABB or BB) had a characteristic doublet band (Rf 0.37 & 0.43) in their zymograms while those with A genome (AA, AAA, AAAA) had a single band (Rf 0.37).

Therefore varieties with only A genome (diploid, triploid or tetraploid) can be readily distinguished from those with B genome or hybrid genomes (A x B) by the absence of the particular band with Rf 0.43. From the present study, it was also clear that only *acuminata* (A) genomes are found in Sudu kochchi and Embon in their genomic constitution and Ratkehel, which was grouped under AAB,[3] has only A genomes. However, it was not possible to distinguish the cultivars with AAB genome from those with ABB genome.

CONCLUSIONS

The detection of esterase isozymes polymorphism can be applied to some extent to genome-specific identification of banana and plantain of Sri Lanka. One polymorphic loci for esterase isozyme was identified and analyzed. Using this loci, the cultivars with B or A & B genome were readily distinguished from the cultivars having only A genome. Also polymorphism of the esterase isozyme can be used for clonal identification with a greater precision.

Acknowledgement

The authors are grateful to the staff of the HORDI, directed by Dr. S.D.G. Jayawardena, for supplying most of the samples for this study. The assistance given by Mr. U. Siriyadasa and Ms. S.M. Amarasinghe is also appreciated.

We especially thank Dr. V.A. Sumanasinghe of the University of Peradeniya for his many valuable comments.

References

1. Chandraratna M.F. (1951). The origin of cultivated races of banana. *Indian Journal of Genetics and Plant Breeding* **11**: 29-33.

2. Chandraratna M.F. & Nanayakkara K.D.S.S. (1951). Cultivated varieties of banana in Ceylon. *Tropical Agriculturist* **107**: 70-91.

3. Simmonds N.W. (1959). *Bananas*. 1st ed. Longmans, London.

4. IBPGR. (1983). *Descriptor list for bananas*. IBPGR, Rome.

5. Jarret R.L. & Litz R.E. (1986). Enzyme polymorphism in *Musa acuminata* Colla. *Heredity* **77**: 183-188.

6. Simmonds N.W. (1962). *The evolution of the bananas*. Longman, London, 170.

7. Jarret R.L. & Litz R.E. (1986 b). Isozyme as genetic markers in banana and plantains. *Euphytica* **35**: 539-549.

8. Rivera F.N. (1983). Protein and isozyme banding patterns among Philippine cooking bananas and their wild parents (*Musa* species). *Paradisiaca* **6**: 7-12.

9. Bhat K.V., Bhat S.R. & Chandel K.P.S. (1992). Survey of isozymes polymorphism for clonal identification in *Musa*. Esterase, acid phosphatase and catalase. *Journal of Horticultural Science* **67**(4): 501 - 507.

10. Bhat K.V., Bhat S.R. & Chandel K.P.S. (1992). Survey of isozyme polymorphism for clonal identification in *Musa* II. Peroxidase, superoxide dismutase, shikimate dehydrogenase and malate dehydrogenase. *Journal of Horticultural Science* **67**(6): 737-743.

11. Liyanage A.S.U., Sumanasinghe V.A., Mendis M.H. & Ganashan P. (1995). Determination of genomic constitution of banana and plantain by peroxidase and glutamate oxaloacetate transaminase enzyme profiles. *Tropical Agricultural Research* **7**: 237-246.

12. Simmonds N.W. (1966). *Bananas*. 2nd ed. Longmans, London.

13. Simmonds N.W. & Shepherd K. (1955).Taxonomy and origin of the cultivated bananas. *Journal of Linner Society of Botany, London* **55**: 302-312.

14. Hussain A., Bushuk W., Rmirez H. & Roca W. (1987). Identification of cassava (*Manihot esculenta* Crantz) by electrophoretic patterns of esterase isozyme. *Seed Science Technology* **15**: 19-22.

15. Scowcroft W.R. (1984). Genetic variability in tissue culture. Impact on germplasm conservation and utilization. *Technical report*, IBPGR, Rome.

16. Bonner J.W., Warner R.M. & Brewbaker J.L. (1974). A chemosystematic study of *Musa* cultivars. *Horticulture Science* **9**: 325-328.

17. Horry J.P. (1985). Mise an point d'une technique de caraterisation de queloues genotype de bananiers (*Musa* sp.) cultives *in vitro* par electrophorese des esterases. *Fruits* **40**: 785-788.