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Determination of Genomic Constitution of Banana and Plantain by Peroxidase and Glutamate Oxaloacetate Transaminase Enzyme Profiles

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ABSTRACT. *Enzyme polymorphism of peroxidase (PRX) and glutamate oxaloacetate transaminase (GOT) of banana and plantain in Sri Lanka was studied. Peroxidases were found to be the most useful isozymes for discrimination of genomic groups of this crop. Three zones of peroxidase (PRX) were detected on polyacrylamide gels. Polymorphism found in PRX-1 region allowed differentiation of BB genome from AA, AAA, AAAA, AAB and ABB. The results indicated that a band at Rf 0.92 was characteristic of 'A' genome. ABB and BB genomes can be distinguished from AA, AAA, AAAA and AAB by the location of high intensity band at Rf 0.80. Zone width, intensity area and number of bands evident at PRX-3 region can be used to identify AA/AAA, AAAA, AAB, ABB and BB genomes from each other. Although GOT is of little use in differentiation of genomic groups, it can be employed in identifying AA genome from AAA genome.*

INTRODUCTION

Banana and plantain in Sri Lanka exhibit great diversity. Simmonds (1959) has identified 27 distinct cultivars having the genomic constitutions AB, AAA, AAB or ABB. Fertile diploid *Musa acuminata* AA (locally known as '*Unel*' or '*Una-kehel*') and *M. balbisiana* BB (locally known as '*Ati-kehel*') also are reported (Chandraratne, 1951) in Sri Lanka. The genome identification of various cultivars of *Musa*, which includes both banana and plantain by morphological methods has been described by

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Simmonds and Shepherd (1955). This system of genome identification based on morphological description is time consuming and its reliability depends on the long-term field experience. Apart from the confusing vernacular names, the presence of many characters which change with the environment, the lack of adequate herbarium material (Chandraratne and Nanayakkara, 1951) and the absence of male buds in some bananas have made classification of cultivars very difficult. The influence of environmental or maternal effects (Jarret and Litz, 1986a) and high incidence of somatic mutations occurring within specific clones (Simmonds, 1966) further aggravate the situation.

Isozyme analysis has proven useful in elucidating taxonomic grouping and identifying varieties in many crops. Some recent examples include citrus (Hirai, 1986), cassava (Hussain *et al.*, 1987) and apple (Weeden and Lamb, 1985). Analysis of peroxidase isozymes in *Musa* species as well as clones showed lack of polymorphism (Bonner *et al.*, 1974). Rivera (1983) examined peroxidase and polyphenol oxidase isozyme polymorphism and was able to distinguish the BB or BBB group of cooking banana and the wild species. Jarret and Litz (1986b) reported that peroxidase polymorphism can be used to group cultivars (banana and plantain) according to genomic constitution. Espino and Pimentel (1988) showed that shikimate dehydrogenase (SKDH) and glutamate oxaloacetate transaminase (GOT) were of little use in differentiating the BB or BBB group of cultivars from other genomic groups, although malate dehydrogenase (MDH) could be used to distinguish the cultivars with AAB and ABB genome from those with BB or BBB. Peroxidase and superoxidase dismutase have been reported as most useful enzymes for cultivar identification. But, same banding pattern for more than one cultivar was observed in the presented zymograms (Bhat *et al.*, 1992). These conflicting results may cause inaccurate identification of the germplasm (Espino and Pimentel, 1988). This study made an attempt to determine the genomic constitution of various cultivars of banana and plantain in Sri Lanka.

MATERIALS AND METHODS

Samples of unfurled leaf were collected from the banana and plantain germplasm maintained by Horticultural Research and Development Institute and Plant Genetic Resources Centre, Peradeniya, Sri Lanka. Table 1 shows the cultivars used in this study and their genomic grouping according to Simmonds (1959). Two grams of unfurled leaf tissue of each cultivar was

Table 1. Genomic designation of *Musa* cultivars used in isozyme analysis.

Genomic group	Cultivar	No. accessions used in PRX	No. accessions used in GOT
AA	Unel	2	2
AAA	Sapumal anamalu	2	2
	Anamalu	3	2
	Embon	2	2
	William hybrid	3	-
	Gal anamalu	1	-
	Meegon anamalu	1	1
	Sudu kochchi	1	1
	Rathambala	1	-
	Marathamana	1	-
AAAA	Golden banana (IC2)	6	2
AAB	Rath kehel	3	-
	Kitala	1	1
	Wal suwandel	1	1
	Suwandel	1	1
	Ambul	86	3
	Muwanati kehel	3	1
	Kolikuttu	1	1
ABB	Alu kehel	3	1
	Atamuru	3	1
	Seeni kehel	3	2
	Alu mondan	1	-
	Mondan	1	-
BB	Ati kehel	6	3
Total		136	27

PRX - peroxidase; GOT - glutamateoxaloacetate transaminase

ground with 1ml of extraction buffer. The extract was centrifuged (3500 rpm) for 5 min at room temperature with a small amount of (0.2g) Sephadex G 50 to obtain the soluble fraction of the protein. The crude extract was loaded (5-10 μl) into polyacrylamide gel. 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. Then the gels were stained for peroxidase (15-20 min) and glutamate oxaloacetate transaminase (2-3 h) isozymes. The stained gels were washed with tap water, fixed in 7% (v/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 four runs.

The gel was prepared to contain acrylamide 4.5% in the stacking gel and 7.8% in the separating gel. Protein extraction buffer (pH 7.5) was made with tris-hydrochloric acid (1.2% w/v), glycerol (20% w/v), polyvinyl pyrrolidon (4% w/v), dithiothreitol (0.15% w/v), $MgCl_2$ (0.4% w/v) and distilled water.

The electrode buffer (pH 8.3) contained tris-hydroxymethyl amino methane (3% w/v), glycine (1.4% w/v) and distilled water. Peroxidase stain contained 3-amino-9 ethyl carbozole (25 mg), N,N-dimethylformamide (25ml), 1M acetate buffer, (pH 4.65, 5ml), 0.1M $CaCl_2$ solution (1ml), distilled water (40ml) and 7% H_2O_2 solution (1ml). Gel was stained for glutamate oxaloacetate transaminase with D-L aspartic acid (80mg), ketoglutaric acid (50mg), 0.5M tris-HCl buffer (pH 8.5, 40ml), pyridoxal 5 phosphate (1mg) and fast blue BB salt (80mg).

RESULTS AND DISCUSSION

Three regions labelled as PRX-1, PRX-2 and PRX-3 (Figures 1 and 2) were identified on gels stained for peroxidase activity. Fast migrating band at Rf 0.92 was characteristic of the cultivars of AAAA, AAA, AA, AAB and ABB genomic groups, but was not present with the BB genome. The presence of a doublet of bands with Rf 0.87 and Rf 0.85 was common to all accessions of AAAA, AAA, AA, AAB, ABB and BB genomes. Another doublet was resolved at Rf 0.81 and Rf 0.80 for ABB and BB genomic groups. The staining intensity of peroxidase was very high for the band at Rf 0.80 of ABB and BB genomic groups compared to that of AA, AAA, AAAA or AAB. The band at Rf 0.81 was more prominent in BB than ABB, but, was not present in AA, AAA, AAAA and AAB genomic grous (PRX-1).

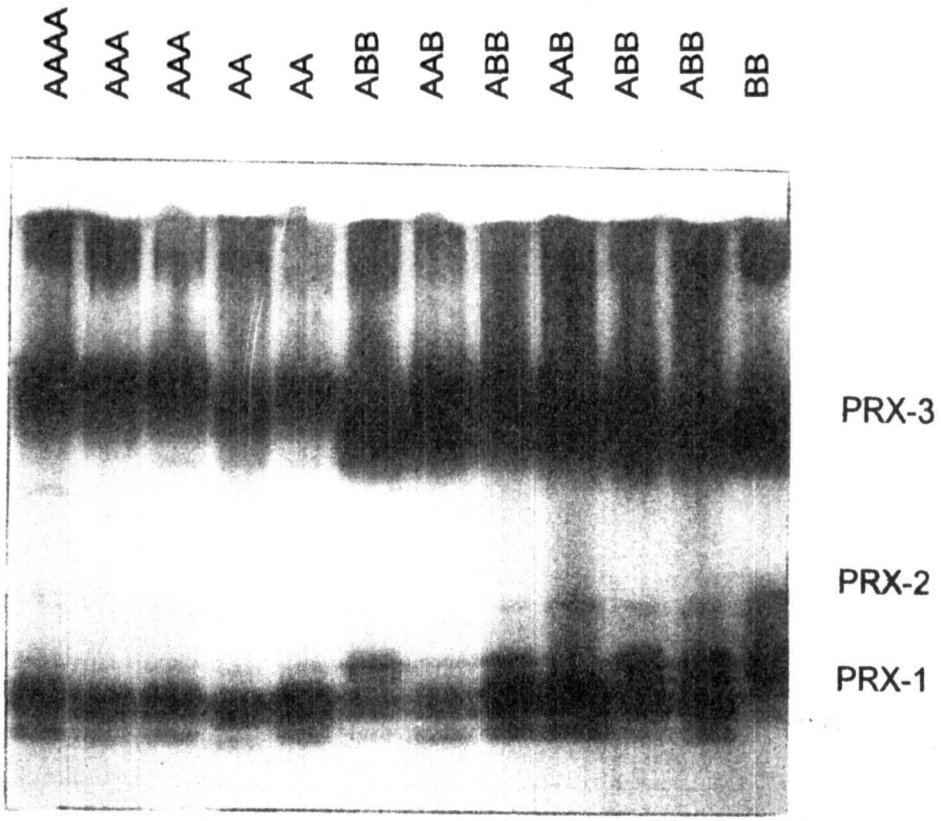

Figure 1. Zymogram of Peroxidase.

In PRX-2 region, a band located at Rf 0.71 was monomorphic for all 6 genomic groups tested. A single band at Rf 0.49 was present in accessions of AA, AAA and AAAA genomic groups. A band at Rf 0.55 was observed only with AAAA genome. (These two bands were not clearly reproduced on the photograph due to the low intensity of the bands). PRX-3 region with slow mobility was stained as a single broad zone of activity when the sample was 10 μl/well. This zone was resolved into 2 bands for AAAA, AAA and AA genomic groups, 3 bands for AAB and ABB genomic groups and a single band for BB genomic group when the sample loaded was 5μl/well. However zone width and intensity of different areas within the zone varied according to genomic group. The observations from PRX-3 region are summarized in Table 2 and Figure 2. Peroxidase polymorphism can be used to group accessions of *Musa* into genomic constitution (Jarret and Litz, 1986b). Jarret and Litz (1986a,b) reported that a doublet of Rf 0.85 and 0.82 was in

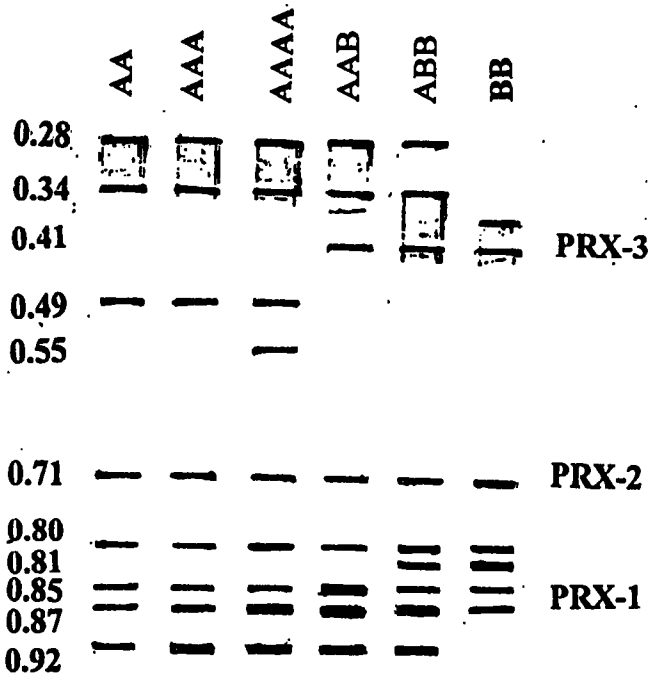

Figure 2. Zymogram of Peroxidase in *Musa* (Zymotypes of various genomic groups).

Table 2. Genomic group and polymorphism in PRX-3 loci.

Genomic group	Band position Rf value	zone width Rf value	High intensity area within the zone
AA, AAA, AAAA	0.28 0.34	0.28 - 0.36	0.28 - 0.34
AAB	0.28 0.34 0.41	0.28 - 0.43	0.28 - 0.34
ABB	0.28 0.34 0.41	0.28 - 0.43	0.35 - 0.42
BB	0.41	0.36 - 0.43	0.40 - 0.42

M. acuminata clones, whereas, a doublet at Rf 0.75 and 0.71 was characteristic of *M. balbisiana*. The isozyme patterns in the present study differed from those of Jarret and Litz (1986b) by having a broad zone (PRX-3) in hybrid genome too, and from Bhat *et al.*, (1992) with respect to mobility of bands (doublet) which were polymorphic to all genomic groups. The results of the present study suggest two important regions, PRX-1 and PRX-3 for genome identification of *Musa*.

In the present study, the doublet at Rf 0.81 and 0.80 in PRX-1 region was common to ABB and BB genomes. The doublet at Rf 0.87 and 0.85 was found with all genomic groups (AA, AAA, AAAA, AAB, ABB and BB). The BB genome did not produce a band at Rf 0.92 though it was evident in other genomic groups (AA, AAA, AAAA, AAB and ABB). This indicated that the band at Rf 0.92 was characteristic of 'A' genome and that the band with higher intensity at 0.80 typified 'B' genome. Plants with ABB and BB genome groups can be distinguished from those with AA, AAA, AAAA and AAB genome groups due to the high intensity of the band at Rf 0.80. Thus banding patterns of PRX-1 region help to identify ABB and BB genomic groups of *Musa* germplasm, although, Bhat *et al.*, (1992) reported a doublet (at fast mobility region) which was polymorphic for all the genomic groups.

Slow mobility region (PRX-3) was described by Bhat *et al.*, (1986) as the least polymorphic irrespective of the genomic group; but, according to the present results, the two bands at Rf 0.28 and 0.34 (broad zone) were characteristic of 'A' genome. Plants with AAAA genomic group can be identified from those with AA or AAA genome due to the presence of a polymorphic band at Rf 0.55 (PRX-3). The single band at Rf 0.49 was monomorphic for AA, AAA and AAAA genomic groups. AAB and ABB genomic groups can be distinguished from each other by the location of broad intense areas of the PRX-3 region. The highest intensity zone in PRX-3 region varied according to the genome. When only 'A' was present in the genome it was concentrated only between Rf 0.28 -0.34. In AAB, it extended from 0.28 to 0.35. In ABB the intensity was between 0.35-0.42, and in BB at 0.42 (Table 2). A single band at Rf 0.41 was detected in the broad zone of the BB genomic group and can, therefore, be considered as characteristic of BB genome.

Three loci of Glutamate oxaloacetate transaminase (GOT) activity were evident on polyacrylamide gels (Figure 3). GOT-1 located at Rf 0.53 was monomorphic. No discernible pattern was associated with the different genomic groups. Similar results have been reported by Jarret and Litz (1986

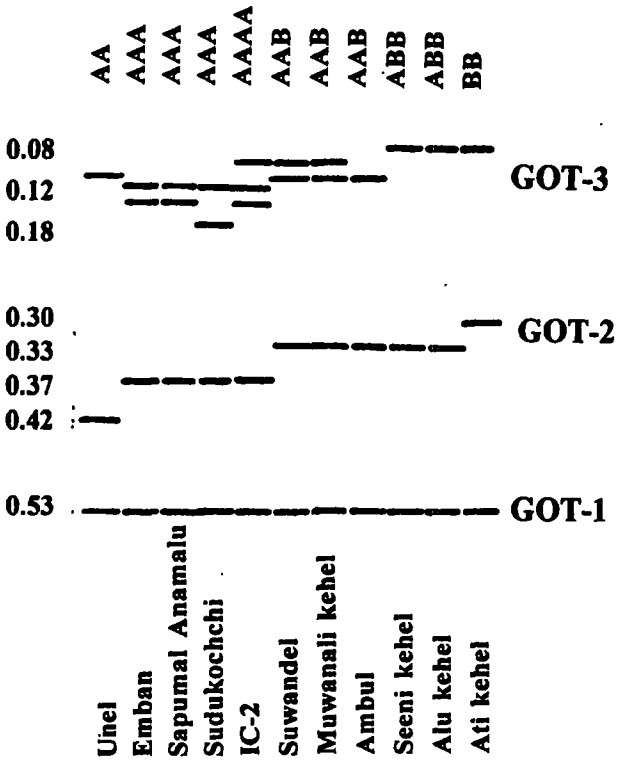

Figure 3. Schematic illustrations of GOT zymogram of *Musa* cultivars (various genomic groupings).

b) and Espino and Pimentel (1988). GOT-2 was resolved into bands with Rf values from 0.30 to 0.42 for different genomic groups. A band at Rf 0.42 was unique to AA genome. GOT-2 was monomorphic for (Rf 0.37) within the *acuminata*-derived clones (cultivars). This loci were monomorphic and located at Rf 0.33 for AAB and ABB genomic groups. A single band of activity was observed by Espino and Pimentel (1988) from this region, while Jarret and Litz (1986b) reported additional doublets when *M. balbisiana* genome was present. In GOT-3 zone, 3 bands from AAAA genome, two bands from AAA genome and one from AA genome were observed. Polymorphism at this locus allowed differentiation within the *acuminata* types: Eg. '*Sudukochchi*' was distinguishable from '*Sapumal anamalu*' and '*Embon*'. Presence of a single band was evident at Rf 0.12 for AA genome

('Unel'). BB/ABB genomic groups exhibited a single broad band at Rf 0.08. However, present results indicated that GOT is of little use in differentiating *Musa* in to genomic groups except for distinguishing AAA group from AA.

CONCLUSIONS

Isozyme technique provides additional or more accurate results for genomic identification of banana and plantain in Sri Lanka. Two polymorphic loci for peroxidase isozymes were identified and analyzed. Genomic constitution of *Musa* using peroxidase isozymes can be determined with greater precision than the more subjective analysis of complex morphological features. Glutamate oxaloacetate transaminase is of limited use for genomic identification of this crop, although polymorphism of the GOT-3 loci can be used for clonal identification to some extent.

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