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OCCURRENCE, CHARACTERISTICS AND DIVERSITY OF WILD BANANA *Musa acuminata* AND *Musa balbisiana* IN SRI LANKA

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ABSTRACT

The wild relatives of cultivated banana, *Musa acuminata* Colla (Unel) and *Musa balbisiana* Colla (Etikesel) are diploid species, useful in banana breeding programmes due to the presence of viable seeds and genetic variation. Several field explorations were carried out to investigate their occurrence and characteristics. Passport data and morphological characters were recorded in the field and in the laboratory. Genetic diversity was estimated by SSR analysis of leaf samples collected from different populations. Unel occurred in the areas of elevations from 179 to 1500 m in Knuckles range (Kandy, Matale districts) and Saptakanya range (Nuwara Eliya and Kegalle districts) where shaded and humid climate is prevalent. Etikesel was located in areas of elevations from 179 to 800 m in Kandy, Kegalle, Kalutara, Ratnapura, Nuwara Eliya and Matara districts. Overlapping populations of both species were also observed in Saptha Kanya range and Punugala. Both species did not show clear morphological differences between populations within a species. However, molecular characterization of populations showed distinct genetic variation. Although the genetic variation within a population or closely associated populations was low in Unel, it was substantial in between populations. In contrast, Etikesel showed genetic variation even between closely located populations. The information generated in this paper is useful for conservation and management of wild *Musa*.

KEYWORDS: Characteristics, Genetic diversity, Occurrence, Wild *Musa*.

INTRODUCTION

Banana is an important fruit crop grown world wide having a production of 70,756,347 tones (FAO STAT, 2006) annually over 130 countries, including South Asia and South America. The diploid wild ancestors of cultivated banana; *Musa acuminata* Colla (Unel) and *Musa balbisiana* Colla (Etikesel) are reported to have originated in South East Asia and Western Pacific Region (Wang *et al.*, 2007). Since they have evolved over thousands of years, they are rich in genetic diversity and may possess genes tolerant to abiotic stress and resistance to several pest and diseases. Both of these wild species are distributed in Malayasia, Burma, Assam, Indo-China and the Philippines while, *M. balbisiana* have extended over Sri Lanka, India, Java, Malaya, Burma (Myanmar) and Siam (Thailand) (Chandrarathna, 1951). *M. acuminata* and *M. balbisiana* are comprised of diploid genomes of A and B respectively (Cheesman, 1948). Most of the edible banana does not produce viable seeds, due to triploid (3n) nature resulting from female sterility and pathenocarpy (Chandrarathna and Nanayakkara, 1951).

In the prehistoric Stone Age man's cave sites in Belilena bellandi Pellessa, Kithulgala (Ratnapura District), the seed remains of 'ati-eta kesel' had been found in a carbonized state (Deraniyagala, 1992) which is evidence that the prehistoric people (over 12000 years ago) had consumed the wild bananas. Previous records indicated the occurrence of *M. balbisiana* in jungles with thin canopy and along river banks, in abandoned plantations of Mathugama: Kalutara District, Kuruwita: Ratnapura District, Kadugannawa, Galagedara and Katugastota: Kandy District (Chandraratna, 1951). *Musa acuminata* has been recorded only in Kiripanagala Range. Although Unel occurred overlapping with Etikesel, it prefers shaded (Chandraratna, 1951) and humid conditions.

The value of conservation and use of crop wild relatives have been realized today than ever before. Since 1951, no investigations have been recorded in order to assess the status in respect to occurrence and characteristics of wild banana that exist in Sri Lanka. Further, the assessment of genetic diversity of wild banana populations has not been done before. The genetic diversity of wild populations can be easily estimated by molecular techniques such as microsatellite markers using a small piece of leaf sample (Wang *et al.*, 2007). Information on occurrence, characteristics and genetic diversity of wild banana is useful in taking *in situ* conservation decisions, germplasm collections and banana breeding programmes. Therefore, this investigation was conducted to study the present distribution, characteristics and the diversity of wild banana using morphological and molecular methods.

MATERIALS AND METHODS

Several field surveys were carried out in the Kandy, Matale, Kegalle, Kalutara, Ratnapura, Nuwara Eliya and Matara Districts based on literature and personal contacts and Plant Genetic Resources Centre (PGRC) passport data. During the survey, eighteen Unel and 20 Etikesel populations were located. Photographs were taken of those species which showed the prominent morphological characters. Passport data and some possible morphological characters were recoded using the specific forms. In addition, samples of bunches including male buds were collected for characterization using descriptors for banana (IPGRI – INIBAP/ CIRAD, 1996). For molecular analysis, tender leaf materials were collected from each population and stored at -20°C in the PGRC Molecular Biology Laboratory. DNA extraction and purification was carried out using modified CTAB procedure for *Musa spp.* (Samarasinghe *et al.*, 2001). DNA was quantified using 0.8 % (w/v) agarose gels and UV spectrophotmeter (Biomate 3) and diluted to 50 ng/ µl for PCR. The PCR amplification reactions were carried out in the reaction mixture containing 10 ng/ µl of template DNA, 2.5 mM $MgCl_2$, 0.2 mM each dNTPs, 10 pM/ µl of each forward and reverse primers, 0.025 u/µl Taq DNA polymerase and 1x buffer (Promega, USA). *Musa* SSR primers (Table 1) such

as AGMI101/102, AGMI103/104, AGMI95/96, MaSSR24, MaSSR9 and MaSSR18 (used only for *M. balbisiana*) were used (Kaemmer *et al.*, 1997). Polymerase Chain Reactions (PCR) were performed using Eppendorf Master Cycler Gradient programmed to an initial denaturation temperature at 94°C 2 min. followed by touch down cycles starting from 30 sec. at 94°C for denaturation; 30 sec. 70°C and 30 sec. at 72°C, decreasing 3°C per cycle down to 58°C for AGMI103/104, 95/96, MaSSR24, 9 and decreasing 3°C per cycle down to 55°C for AGMI101/102 and MaSSR18; Then 35 cycles of 30 sec. at 94°C; 30 sec. at 55°C (except for AGMI101/102 and MaSSR18) or 30 sec. at 52°C (for AGMI101/102 and MaSSR18) for annealing; 30 sec. of 72°C , terminating with 5 min. at 72°C final extension. Amplification of the PCR products were confirmed on 0.8% w/v agarose gels. Four microlitres of denatured PCR products (mixed with 50% v/v Poly Acrylamide Gel Electrophoresis (PAGE) loading dye) were run along with 1kb ladder (Sigma, USA) on 10% 0.4mm thickness denaturing polyacrylamide gel containing 42% (w/v) of urea in adjustable height nucleic acid sequencing system (CBS).

Table 1. Details of *Musa* SSR primers (Kaemmer *et al.*, 1997) used for PCR.

Primer	*Sequence 5'---------- 3'*	*Annealing temperature (°C)*	*Product size (bp)*
MaSSR 9a (F) MaSSR 9b (R)	ATGTCGCTTCGGACCAGA GCAGGACGAAGAACTTACC	55°C	162
MaSSR 24a (F) MaSSR 24b (R)	GAGCCCSTTAAGCTGAACA CCGACAGTCAACATACAATACA	55°C	172
MaSSR 18a (F) MaSSR 18b (R)	CGTCACAGAAGAAAGCACTTG CCTCTCCATCGTCATCAATC	55°C	100-200
AGMI 95 (F) AGMI 96 (R)	ACTTATTCCCCCGCACTCAA ACTCTCGCCCATCT`TCATCC	55°C	220
AGMI 101 (F) AGMI 102 (R)	TGCAGTTGACAAACCCCACACA TTGGGAAGGAAAATAAGAAGATAGA	52°C	189
AGMI 103 (F) AGMI 104 (R)	ACAGAATCGCTAACCCTAATCCTCA CCCTTTGCGTGCCCCTAA	55°C	181

Electrophoresis was carried out at 1550 V, 60 W for 3 hours in 0.5 x TBE after pre heating the gel for 1 hour at the same power levels. Gels were sliver stained using the procedure described by Samarasinghe *et al.* 2001 with a modification of using 0.3 % (w/v) NaOH instead of $NaHCO_3$ as the developer. The bands which appeared on the gel were photographed using a digital camera, SSR loci and alleles were identified and were scored. SSR data were analysed using computer software POPGENE 1.31 and SPSS Version 10 and dendograms were constructed.

RESULTS AND DISCUSSION

Occurrence of *Musa acuminata* and *Musa balbisiana*

Musa acuminata was found rarely in areas of elevations from 179 to 1500 m in Knuckles and Sapthakanya mountain ranges and suburbs where shaded and humid climate is prevalent. In Knuckles, scattered small Unel populations were located in Rangala, Coberts Gap, Duckwary Estate (Elagolla): Kandy district; Rattota, Gammaduwa and Karagahathenna: Matale district (Fig. 1 and Table 2). In Sapthakanya Mountain range, small populations were located in Neelawathukanda, Kottallena: Nuwara Eliya district and in Halgolla Estate (Poonagala) in Kegalle district (Fig. 1 and Table 2). It was important to note that almost all the Unel populations observed, had a few clumps and a few suckers/plant.

In contrast, *Musa balbisiana* was found to be distributed in open and humid places elevated from 179 to 800 m in several districts. The locations were: Poonagala, Halgolla in Kegalle district; Ginigathhena, Pitawala, Norton, Laxapana, Mandaram Nuwara in Nuwara Eliya district; Deniyaya and Mulatiyana in Matara district and Galkonawatthe, Kiriella in Ratnapura district (Fig. 1 and Table 3). It was interesting to note that, overlapping populations of both species were also observed in Punugala and Sapthakanya Range (Fig. 1) as mentioned previously (Chandraratna, 1951) which can facilitate inter-specific hybridization between *M. acuminata* and *M. balbisiana.*

Table 2. *Musa acuminata* populations traced in different locations.

No.	*Address/ Village*	*No.*	*Address/ Village*
1	Gammaduwa Swarnawahini	10	Rangala - Coberts Gap Road Side
2	Near Tea Estate, Gammaduwa Road Side	11	Elagolla, Duckwary Estate
3	29/4 (C.N.), Laggala, Pallegama	12	Elagolla, Duckwary Estate
4	25/5, Enasal Watta, Laggala road.	13	Duckwary Estate, Galabada road
5	Rattota	14	Saptha Kanya hilly range, Kottallena
6	Rattota	15	Saptha Kanya hilly range, Kottallena
7	Hellena Gala, Coberts Gap (Thangappuwa to Loolwatta)	16	Neelawathukanda
8	Hellena Gala, Coberts Gap (Thangappuwa to Loolwatta)	17	Neelawathukanda
9	Coberts Gap, Near Banglow	18	Wavilthalawa, Halgolla estate

Table 3. *Musa balbisiana* populations traced in different locations.

No.	*Address/ Village*	*No*	*Address/ Village*
1	29/2, Rantileka, Galkonawatthe	11	Yaddala road, Nugekapaputhana
2	Kawitikanda, Kiriella, Rathnapura	12	Laxapana, Hangarapitiya
3	Halgolla Estate, Halgolla	13	Hangarapitiya, Hatton
4	Galagedarawatta, Kalanigama	14	Kudapana, Gonbaddala
5	Samanala power station, Kelanigama	15	Ihalawththa, Koratuwa
6	Denigastenna, Ambatalawa	16	16/2 bridge, Halgolla Estate, Halgolla
7	Ytiberiya, Pitawala	17	Gannoruwa, Peradeiya
8	Halgolla Estate, Dodawaththa, Malalpola	18	Sinharaja Division, Deniyaya
9	Halgolla Estate, Dodawaththa, Malalpola	19	Suduhumpala road, Kandy
10	Laxapana power house	20	Udagabbila, Mandaram Nuwara

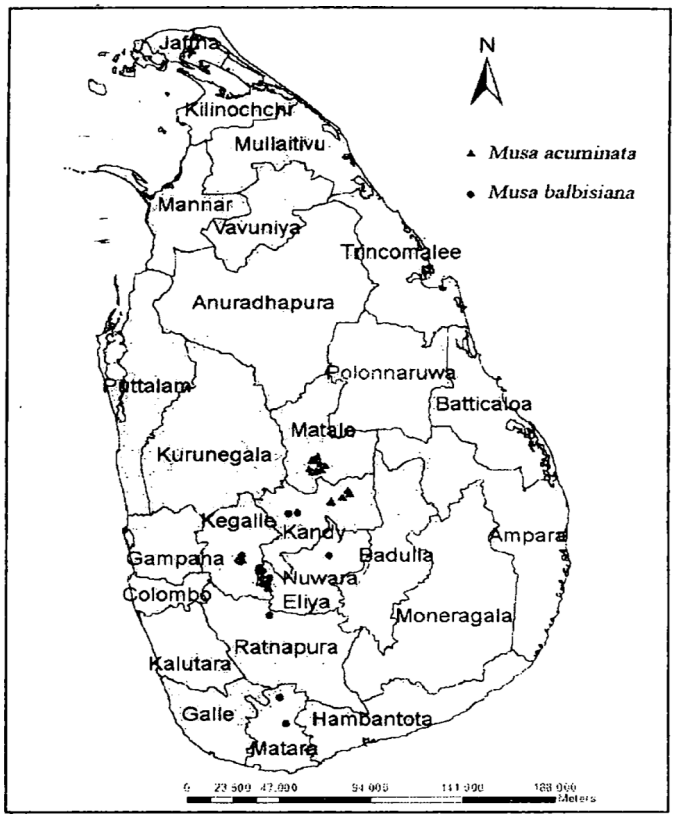

Figure 1. Distribution of *Musa acuminata* (Unel) and *Musa balbisiana* (Etikesel).

Even though *Musa balbisiana* has been previously recorded in Kadugannawa, Balana in Kandy district (Chandrarathna, 1951) it was not possible to locate such occurrences of the species in the present study. Mulatiyana population has not been traced in their natural habitats, and at present they are found only in some home gardens, which are grown for obtaining the unopened flower for cooking. Further studies have shown that the reduction of populations was attributed to deforestation, urbanization, expansion of agricultural lands, climate changes, porcupine damages etc. The observed populations were located in protected areas as well as in unprotected areas mainly in private tea and rubber estates.

The wild banana populations lie in unprotected as well as protected areas and are under a threat of declining. Hence, there is an urgent need for *in situ* conservation while assessing the risk category of wild banana which has been initiated by the Conservation of Crop Wild Relatives through Enhanced Information Project.

Characteristics of *Musa acuminata* and *Musa balbisiana*

The cultivated type Seeni Kesel fruits rarely posses a few seeds that may be mistakenly identify as Etikesel. However, Etikesel or Unel fruits are filled with large number of seeds which can be detected easily (Fig. 2 J and K). Both Unel and Etikesel show clearly distinctive morphological characters (Table 4 and Fig. 2), which are useful in correct identification of the two species. Characters such as peduncle hairiness, distance between lateral veins, bract dehiscence, bract curling and corrugations on free tepal were the new observations made, in addition to the previous records (Chandraratna, 1951). Morphological characteristics of populations did not show clear differences between populations within a species even though they were geographically far apart.

Table 4. Morphological characteristics of *Musa acuminata* and *Musa balbisiana*.

No.	*Character*	*Musa acuminata* (Unel)	*Musa balbisiana* (Etikesel)
1	Leaf habit	Erect (Fig. 2 A)	Drooping (Fig. 2 B)
2	Mature plant Height (Fig. 2 A, B)	Usually 3 – 5 m	Usually up to 7 - 8 m
3	Pseudostem color	Large brown blotches or slight	More or less heavily marked with brown or black blotches
4	Petiole channel	Margin opened and erect (Fig. 2 D)	Margin enclosed, curved inward (Fig. 2 C)
5	Lateral vein distance	Wide (Fig. 2 E)	Narrow (Fig. 2 E)
6	Peduncle	Pubescence (Fig. 2 H)	Glabrous (Fig. 2 H)
7	Bract color (Fig. 2 O)	Purple to violet outer surface of the bract; light red paling to base on inner surface of the bract	Purple on outer surface and dark crimson/ pink outer surface of the bract
8	Bract apex (Fig. 2 N)	Acute	Obtuse
9	Bract curling	Bract reflex and roll back after opening (Fig. 2 F)	Bract lift but do not roll (Fig. 2 G)
10	Bract dehiscent	Yes (Fig. 2 F)	No (Fig. 2 G)
11	Male flower color (Fig. 2 Q)	Creamy white	Variably flushed with pink
12	Free tepal of male flower (Fig. 2 P)	Corrugated below tip	Rarely corrugated
13	Fruit diameter (Fig. 2 K)	Around 2 – 2.5 cm	Around 3 cm
14	Seeds	Irregularly angulated (Fig. 2 L)	Globuse (Fig. 2 M)
15	Ovules (Fig. 2 K)	Two regular rows in each loculus	Four irregular rows in each loculus

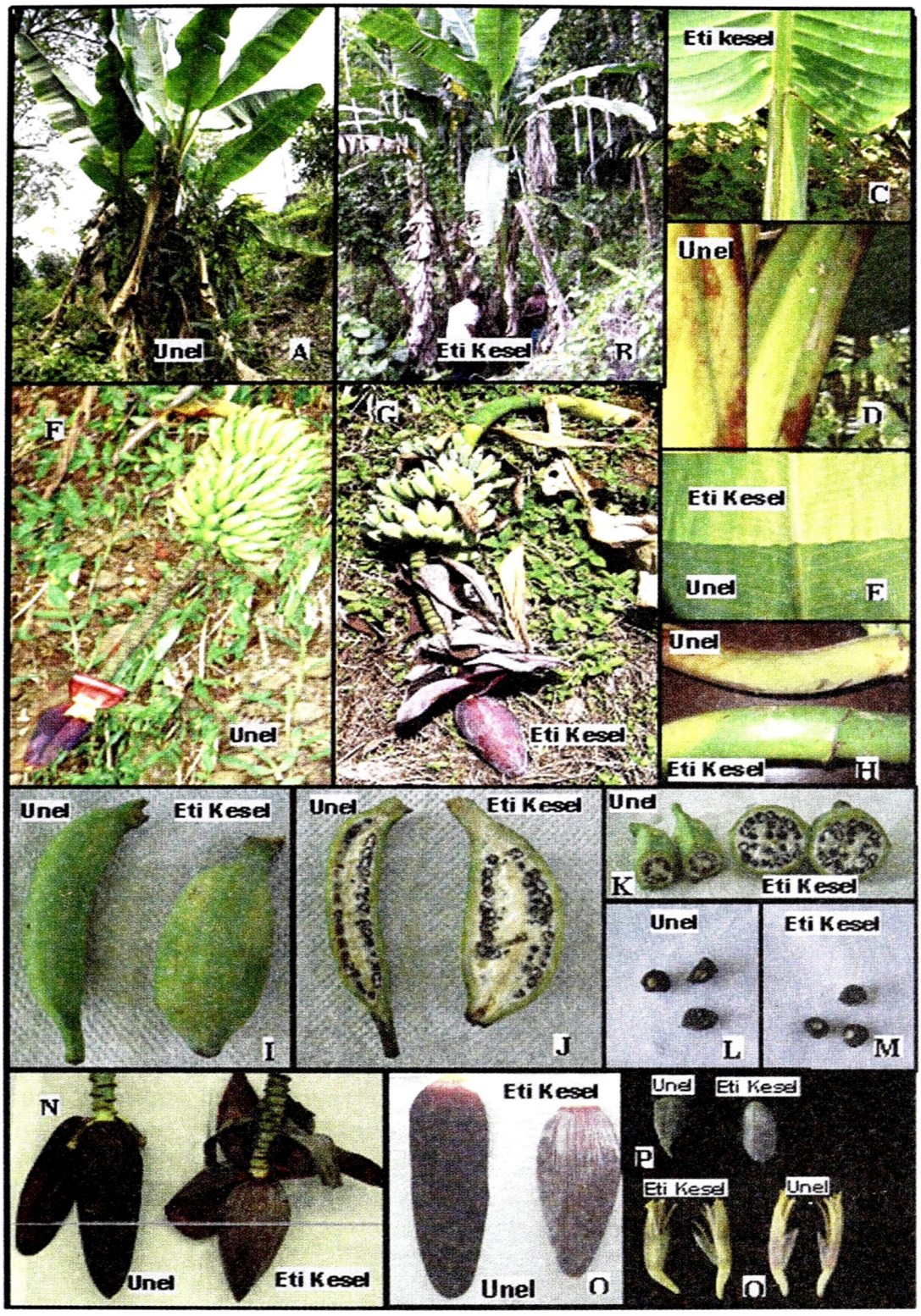

Figure 2. Morphological characteristics of M. acuminata and M. balbisiana. A, B - Leaf habit, C, D-Petiole channel, E-Lateral vein distance, F, G - Bunch, H - Peduncle, I, J, K - Fruit, L, M - Seeds N - Male Inflorescence, O Bract, Q - Male flower, P - Free tepal, Male flowers.

Genetic diversity of wild banana
***Musa acuminata* (Unel)**

In the dendogram, (Fig. 4) it was clear that, SSR analysis of populations of both the species showed distinct genetic variation at DNA level even though morphological variation could not be observed.

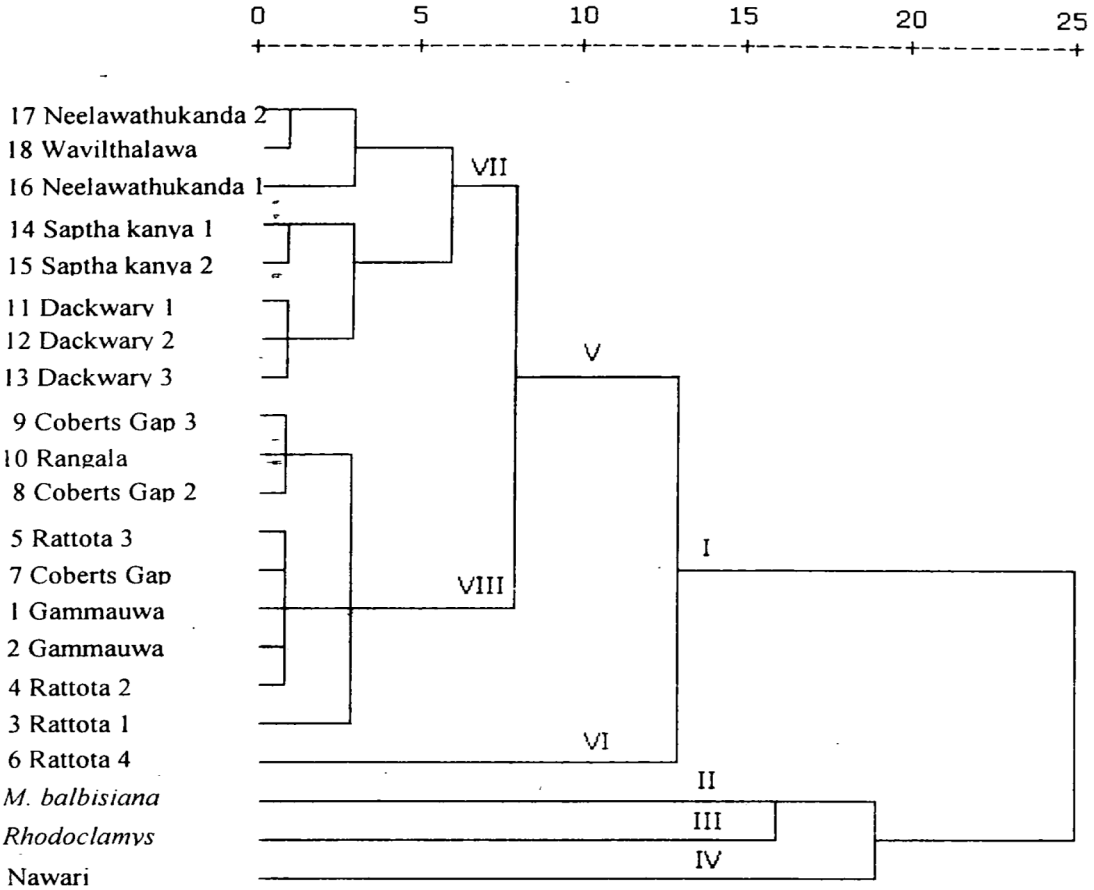

Figure 4. Dendogram of *Musa acuminata* molecular diversity based on hierarchical cluster analysis using SPSS software average linkage - between groups.

There was a genetic variation between Unel populations of Knuckles and Saptakanya with few exceptions. In general, diversity within closely located populations is lower than that of distantly located populations. In this analysis several closely located populations showed commonly shared SSR alleles which led to identify them as duplicates. The possible reason may be that Unel is naturally cross pollinated (Chandraratna, 1951) and gene exchange may take place. Pollinators are bees and small bats, and they may carry pollens to greater distance.

Collections from Duckwary Estate (in Knuckles range) were more related to Saptakanya (Nuwara Eliya district) populations. However, a collection from Rattota 4 was different from the other Unel populations

collected from Rattota area. These results revealed the deviations from the expected results irrespective of the locations that they have collected. Therefore, further studies are needed to confirm the results.

Musa Balbisiana (Etikesel)

The dendogram (Fig. 5) derived from similarity analysis between the Etikesel populations showed that, out of 9 populations sampled, 8 were different from each other.

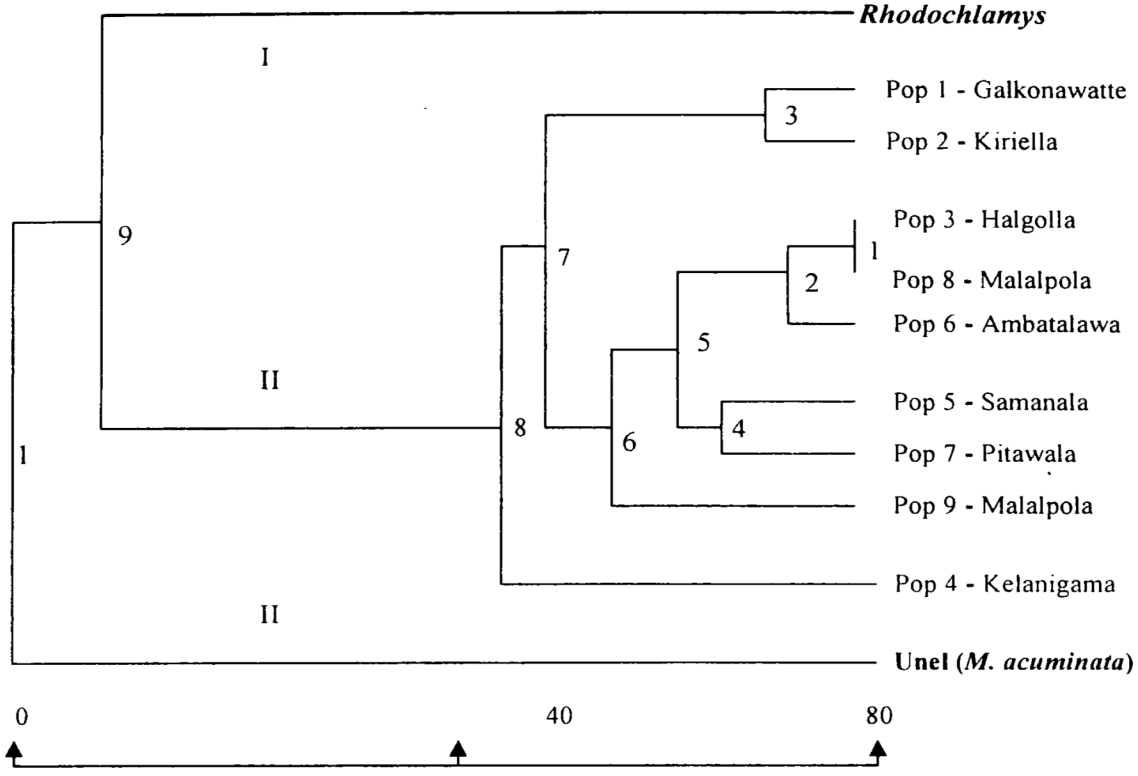

Figure 5. Dendogram of *Musa balbisiana* based Nei's genetic distance, 1972.

Sample collected from Kalanigama was uniquely different from the rest of the Etikesel samples. Only one duplicate (3 – Halgolla and 8 – Malalpola) was identified in this analysis. Other populations showed genetic difference even when they were closely located. The reason may be that Etikesel shows a high degree of selfing (Chandraratna, 1951) to generate homozygous genotypes. The information generated by this work is useful to identify populations for *in situ* conservation in protected and unprotected areas, collecting genotypes for *ex situ* conservation and evaluation for desirable traits.

CONCLUSIONS

Occurrence of Unel (*Musa acuminata*) is restricted to Knuckles and Saptakanya Mountain ranges and its existence is endangered. In contrast, Etikesel (*Musa balbisiana*) is more adaptable to diverse environmental conditions and therefore is distributed in several districts but populations are gradually declining. Unel and Etikesel can be identified and distinguished using morphological characters as described in this paper.

SSR analysis of both the species exhibited a genetic diversity among populations of each species. Unel showed genetic similarity between geographically close populations. In contrast, Etikesel exhibited genetic variation between closely located populations. The information generated by this work is very useful for the conservation and management of wild banana.

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