Tropical Agricultural Research Vol. 9 1997, 147-157

# Biodiversity of Fruit and other Tree Species in the Huruluwewa Watershed with Emphasis on their Economic Utility

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***ABSTRACT.*** *This study attempted to determine species and genetic diversity in the Huruluwewa watershed considering basic information on native tree species with emphasis on fruit and other tree species of future economic utility.*

*This study was carried out in different phases, namely, "reconnaissance survey" leading to specific information of the Huruluwewa watershed to determine the species diversity and "Identification of genetic diversity of fruit tree species" through starch gel electrophoresis techniques. Few groups of fruit tree species showing close similarities were used to establish the "Finger printing" through starch gel electrophoresis to identify the genetic diversity.*

*The existing high degree of species diversity in the Huruluwewa watershed can be categorized into different groups based on their economic utility. These are fruit tree species, medicinal plants, firewood species, timber trees, ornamental plants food crops, shade trees, cover crops and fodder crops. Different banding patterns in the starch gel electrophoresis established for fruit tree species with close morphological similarities, indicated the genetic diversity within and among fruit tree species in the watershed.*

## INTRODUCTION

A major part of the Huruluwewa watershed lies in the dry zone with a very narrow portion spreading into the intermediate zone in Sri Lanka. The total area of watershed is about 30,000 ha and its base is at Lenadora, proximity to the city of Dambulla. The Yan Oya runs along the watershed and through

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which Mahaweli water flows to Huruluwewa. The watershed can be categorized into two major parts based on the type of vegetation and the pattern of land use. A typically layered structure is distinguishable in the watershed with an upper canopy of moderately large trees, an under storey or sub canopy of smaller pole-sized species, and definite shrubs and field layers (Nisbet, 1961).

A majority of native tree species in the Huruluwewa watershed has not been clearly studied. This is an obstacle for the use of such species in multiplication and crop improvement programmes and in some cases, *ex situ* conservation. Hence, studying the biodiversity in the Huruluwewa watershed provides a valuable foundation to protect species from rapid extinction and uncontrolled human exploitation. On the other hand, preserving of species diversity will help to confront the challenge posed by the possible effect of global warming (Geiser and Sommer, 1982).

Phenological characters of leaves, stems, flowers, fruits and branching habits provide details in identification of different species and varieties or cultivars of plants. However, some plants in different groups show close similarity to each other. Hence, differentiation and identification of such plants using only morphological characters is difficult. Genetic characters will help to realize the diversity of such plants. Starch gel electrophoresis is one of the reliable techniques to explain such genetic diversity. Thus, this technique, an isoenzyme analysis, is a useful tool for studying the taxonomy, genetics, evolution, physiology and biochemistry of plants (Brewer, 1970).

The broad objective of this study was to compile basic information on native tree species in the Huruluwewa watershed with emphasis on fruit and other tree of future economic value. The specific objectives were to determine the species diversity in the watershed using morphological characters and the genetic diversity of fruit tree species in the watershed using electrophoresis techniques.

## MATERIALS AND METHODS

### Phase 1: Reconnaissance survey

The data collection format provided a general framework for the survey. It consisted of two parts. The first was intended to assemble a general description of the Huruluwewa watershed and background information relevant

to home garden systems and cultivation practices. The second dealt with the identification of plant species. Plants from each species, representing a wide range of habitats, were collected in twenty selected areas in the watershed (Figure 1). Fruit tree species, plants with valuable medicinal properties, nut and forest trees, shrubs and herbaceous plants were identified. Voucher specimens (Herbarium) of each species was prepared for reference.

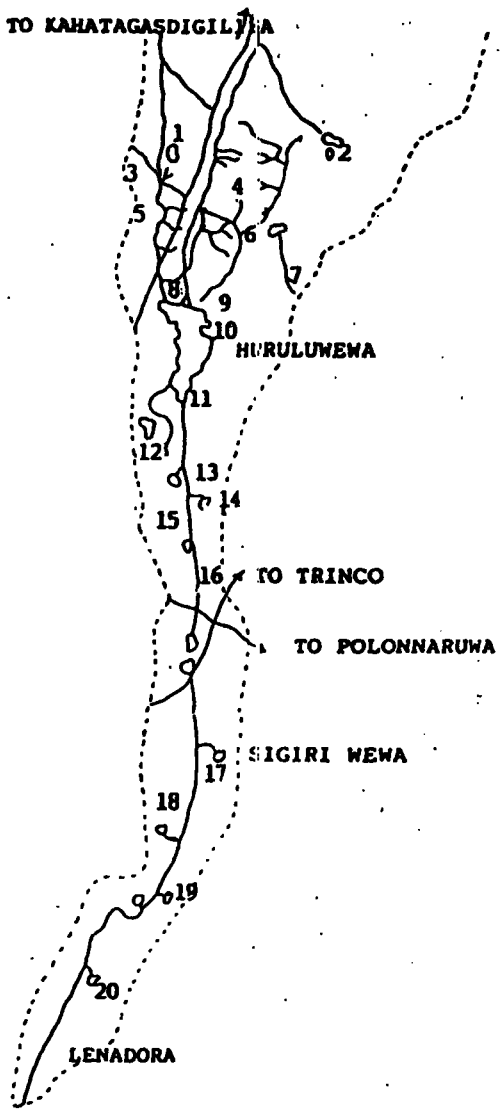
**Phase 2: Identification of genetic diversity**

Identification of morphological characters of fruit tree species and the establishment of "finger printing" of such species through starch gel electrophoresis were covered in this phase of the research study.

Morphology of some fruit trees were closely similar to each other specially during their vegetative phase although they are of different plants. Such plants were examined carefully and they were grouped into seven categories to conduct the starch gel electrophoresis so that the genetic diversity in each group could be identified (Table 1).

Tender and immature shoots were detached from each tree as they can be ground easily when extracting the cell sap. Besides high enzymatic activities and a greater number of banding patterns can be achieved with leaf materials (Nehara *et al.*, 1991). A sample of 0.5 g was crushed with 1 ml of extraction solution or buffer under cool condition followed by centrifugation at 4500 rpm for 20 min at 4°C, as described by Peiris (1986). The clear supernatant of each sample was stored at 10°C, prior to being used for electrophoresis. Hydrolyzed potato starch 25 g and 220 ml of the gel buffer were mixed together and heated vigorously in a vacuum filter flask to prepare the gel under investigation, as described by Weeden (1982). Gels were cast in a rectangular gel mould and allowed to cool for one hour. After cooling gels were covered with a plastic wrap to prevent desiccation and left to set overnight at room temperature.

Heavy filter paper wicks were used to absorb samples of thawed supernatants for insertion into a slit cut 4.0 cm from the cathodal edge of a gel. A wick having a dye marker was placed at one edge of the gel to determine the rate of protein migration. The gel was then placed on the electrode buffer tray in a refrigerator. The wicks were removed after 15 minutes of electrophoresis at 50 mA or 350 $V_{max}$. After inserting a straw between the cathodal end of the




1. Kinugonawewa
2. Kokawewa
3. Ulpathgama
4. Galenbindunuwewa
5. Maradankalla
6. Garandiyaulpotha
7. Bamunottuwa Kanda
8. Hurulukanda
9. Padikaramaduwa
10. Horowbindunuwewa
11. Halmillawa-Madawala
12. Mahadivulwewa
13. Ritigala
14. Meegaswewa
15. Puwakpitiya
16. Habarana
17. Sigiriya
18. Welangolla
19. Angunawelpelessa
20. Walgamwewa

**Figure 1.** Randomly selected areas used in the Huruluwewa watershed.

**Table 1. Fruit species in Huruluwewa watershed selected for electrophoretic techniques.**

| Group | Common name (Sinhala name) | Botanical name |
|---|---|---|
| 1 | Mas–Mora | *Euphoria longana* |
| | Atta–Mora | *Euphoria longana* |
| 2 | Mas–Weera | *Drypetus sepiaria* |
| | Atta–Weera | *Drypetus sepiaria* |
| 3 | Lokku–Beli | *Aegle marmelos* |
| | Polli–Beli | *Aegle marmelos* |
| 4 | Sulu–Pera | *Psidium guajava* |
| | Rathu–Pera | *Psidium guajava* |
| | Ambul–Pera | *Psidium guajava* |
| 5 | Kirinelli | *Averrhoa spp.* |
| | Kamaranga | *Averrhoa carambola* |
| | Bilin | *Averrhoa bilimbi* |
| 6 | Weli–Anoda | *Annona squamosa* |
| | Gaja–Anoda | *Annona spp.* |
| | Katu–Anoda | *Annona muricata* |
| 7 | Pani–Dodam | *Citrus sinensis* |
| | Ambul–Dodam | *Citrus aurantium* |
| | Heen–Naran | *Citrus crenatifolia* |
| | Deni | *Citrus aurantifolia* |
| | Nahtharan | *Citrus megaloxylocarpa* |
| | Jarna–Naran | *Citrus reticulata* |
| | Kenda–Naran | *Citrus reticulata* |
| | Marmalate | *Citrus spp.* |

gel and the ebonite strip of the form, electrophoresis was continued for 3–4 h at 5°C. The gel was then sliced horizontally in four or more sections and the slices were carefully placed in individual staining trays. Three isozyme stain

recipes, namely, Glutamate Oxaloacetate Transaminase (GOT), Phospho–glucoisomerase (PGI), Phospho-glucomutase (PGM) were used to stain the gel. The bands on the gel were traced on paper to distinguish the genetic differences in each plant after the staining procedure was completed.

## RESULTS AND DISCUSSION

### Reconnaissance survey

The Huruluwewa watershed is composed of 3 major types of lands, namely forests, marshy lands and non–forested lands used for shifting cultivation and home gardening. The major and common habitats identified in the area are forests, paddy fields, low lands, high lands, rocky areas and other water logging pockets. Other minor habitats such as plants under or on the rocks, plants on other trees and plants in decomposing layers were also found in some areas.

In Huruluwewa watershed, 389 species including some rare plants with valuable medicinal properties such as Bim–kohomba (*Munronia pumila*), Dimi–bidju (*Rivina humilis*) and many multi–purpose plants and large tree species were identified. Therefore, species diversity in Huruluwewa watershed can be categorized into different groups based on their economic utility. Out of such species, 90% have medicinal properties and they can be used specially for indigenous medicines in various diseases, injuries, snake bites and other ailments. Some of them can have potential values in the future. Nine percent of plants identified can be used as fruit tree species. Plants used for timber, food and firewood are the other common uses identified. Some plants are also used as shade trees, ornamental plants, vegetables, fodders and cover crops. Some are identified as weeds. However, some of the species are used occasionally as they have specific values. Using such plants for religious activities, production of unfermented toddy, carving activities, production of gummy substances and stains, obtaining of fibre, wrapping purposes and extraction of insecticides and oil are some of specific values identified (Table 2).

Most plants identified under one category of major economic utility can fall into other categories indicated in Table 2 as well. Therefore, most of species collected in the Huruluwewa watershed have multiple uses. Such multi–purpose species will serve the people in this area in many ways to improve their living standards.

**Table 2. Species diversity in Huruluwewa watershed categorized according to their major economic utility.**

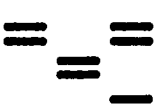
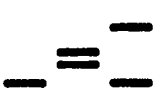
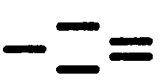
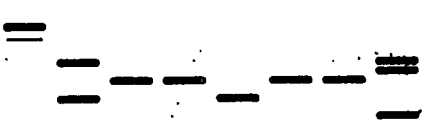
| Major Economic Utility | Number of Species |
|---|---|
| Medicinal plants/trees | 233 |
| Firewood | 91 |
| Timber | 50 |
| Food (Leafy vegetables/ fruit vegetables/root crops) | 41 |
| Fruit trees | 37 |
| Ornamental | 34 |
| Shade trees | 27 |
| Cover crops | 9 |
| Fodder crops | 5 |

**Identification of genetic diversity**

Genetic diversity refers to the variation of alleles and genotypes between and within species. The diversity within a species indicates the ability to adapt to changes in environment, agricultural practices, or to the presence of new pests and diseases (Saouma, 1993).

Each fruit species was identified and morphological characteristics determined on the basis of the results of the reconnaissance survey. During this study, some plants showed close similarity to each other making their differentiation and identification difficult by morphological characterization alone (Table 1).

**Table 3. Results of the electrophoretic analysis (using GOT).**

| Group | Plant Species | Banding Patterns and Relative Distances of Migration |
|---|---|---|
| 1 | Mas-Mora<br>Atta-Mora |  |
| 2 | Mas-Weera<br>Atta-Weera |  |
| 3 | Podi-Beli<br>Loku-Beli |  |
| 4 | Sudu-Pera<br>Rathu-Pera<br>Ambul-Pera |  |
| 5 | Kamaranga<br>Kirinelli<br>Bilin |  |
| 6 | Weli-Anoda<br>Gata-Anoda<br>Katu-Anoda |  |
| 7 | Pani-Dodam<br>Ambul-Dodam<br>Heen-Naran<br>Dehi<br>Naththaran<br>Jama-Naran<br>Konda-Naran<br>Marmalade |  |

Preliminary screening for 3 enzymes by electrophoresis indicated that one enzyme, Glutamate Oxaloacetate transaminase (GOT) could be used to characterize the 7 selected groups of plant species as distinguished by isozyme bands (Table 3).

The results (Table 3) explains the genetic diversity of each plant group. Mas–Mora (*Euphoria longana*) and Atta–Mora (*Euphoria longana*) in the group 1 and Mas–Weera (*Drypetus sepiaria*) and Atta–Weera (*Drypetus sepiaria*) in the group 2 were two genetically different plant species or varieties of the same species. Each plant type in each group showed different banding patterns.

Podi–beli (*Aegle marmelos*) and Loku–Beli (*Aegle marmelos*) in group 3 showed some genetic differences as they had two different banding patterns. Therefore, Podi–Beli (*Aegle marmelos*) and Loku–Beli (*Aegle marmelos*) may be two different varieties of the same species.

Rathu–pera (*Psidium guajava*), Sudu–pera (*Psidium guajava*) and Ambul–pera (*Psidium guajava*) in group 4 had different banding patterns and different relative distances of their migrations. Therefore, they may be three different varieties of the same species.

Although Kamaranga (*Averrhoa carambola*), Kirihelli (*Averrhoa spp.*) and Bilin (*Averrhoa bilimbi*) are similar in morphological characteristics, they are three genetically different plant species or groups.

In the sixth group, banding patterns of each plant type and relative distance of their migrations were different. Therefore, Weli–Anoda (*Annona squamosa*), Gata–Anoda (*Annona spp.*), and Katu–Anoda (*Annona muricata*) are genetically different.

In group 7, similar banding patterns were obtained in Heen–Naran (*Citrus crenatifolia*), Dehi (*Citrus aurantifolia*), Jama–Naran (*Citrus reticulata*) and Konda–Naran (*Citrus reticulata*), indicating that they are genetically very similar to each other. However, Peni–Dodam (*Citrus sinensis*), Ambul–Dodam (*Citrus aurantium*), Naththaran (*Citrus megaloxylocarpa*) and Marmalade (*Citrus spp*) are genetically different with different banding patterns while being genetically different from Heen–Naran (*Citrus crenatifolia*), Dehi (*Citrus aurantifolia*), Jama–Naran (*Citrus reticulata*) and Konda–Naran (*Citrus reticulata*), as well

Therefore, among fruit plants showing close similarities to each other a great genetic diversity could be seen in the Huruluwewa watershed even in the preliminary studies conducted with few number of fruit plants.

## CONCLUSIONS

Species and genetic diversity existing in the Huruluwewa watershed are utilized by the people in the area for different purposes. Hence, species diversity can be categorized into different groups based on their economic utility. However, the extinction of some fruit tree species and some species with valuable medicinal properties like Bim–kohomba (*Munronia pumila*) and Dimi–bidju (*Rivina humilis*) is indicated. On the other hand, multi–purpose tree species provide many services to the people in the Huruluwewa watershed improving their living standards.

Established "Finger Printing" by starch gel electrophoresis technique for some of the fruit tree species could be utilized to identify and differentiate species with close genetic similarities to some extent, generalizing the existing genetic diversity in the Huruluwewa watershed. This study indicates the maintenance of existing diversity and identification of the potential value of Huruluwewa watershed area which is rich both in species and genetic diversity.

However, future studies should include all the plant and tree species in the Huruluwewa watershed in the identification of whole genetic diversity although it is a very difficult task.

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