DOCTOR OF PHILOSOPHY

Provenance variation in Maesopsis eminii ENGL

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Award date:
1999

Awarding institution:
University of Wales, Bangor

PROVENANCE VARIATION IN *Maesopsis eminii* ENGL.

A thesis submitted to the University of Wales

by

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B.Sc. (Hons) Agroforestry

in candidature for the degree of *Philosophiae Doctor*

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June 1999

SUMMARY

Variation in seed dimensions, growth and morphological characteristics of seedlings, isozymes, and capacity for vegetative reproduction and propagation was assessed in eleven provenances of *Maesopsis eminii* from Rwanda (2), Tanzania (2), Kenya (4), Uganda (1), Cameroon (1) and Ghana (1).

Provenances differed significantly in seed length and width. Discrimination between provenances using seed length and width was possible, and provenances could be divided into either three or five groups. Examination of more seed characteristics is needed to determine which of the groupings (three or five) is more robust.

Differences between provenances in all growth and morphological characteristics were statistically significant. Discrimination between provenances using growth characteristics identified four groups: Rukara/Kibungo (Rwanda) and Arboretum de Ruhande (Rwanda); Kakamega (Kenya); Kisaina 4E (Kenya); Kisaina 5B (Kenya) and Budongo Forest Reserve (Uganda).

Four groups were also identified when using either morphological traits alone or all seed and seedling characteristics together, but the groups had a different composition. The groups were: Rukara/Kibungo; Arboretum de Ruhande; Kakamega and Kisaina 5B; Kisaina 4E and Budongo Forest Reserve.

Provenances responded positively to pollarding and coppicing, though there were significant differences between them in the length of pollard and coppice shoots. Experiments on grafting were not successful. Vegetative propagation by stem cuttings was successful, but branch cuttings failed to root.

Isozyme data from eight enzyme systems suggest that the Rwandan, Tanzanian and Kenyan provenances are similar genetically. The Ugandan and the Ghanaian provenances appear distinct from the other provenances, but are similar to each other.

Since different approaches identified different provenance groupings, discrimination between *Maesopsis eminii* provenances should be based on a combination of metric, morphological and biochemical traits. These should also be used in further investigations of genetic variation in *Maesopsis eminii*.

Further work is also needed to solve the problem of sporadic seed germination and to increase the success of vegetative propagation by cuttings and grafting.

ACKNOWLEDGEMENTS

I am very grateful to the Swiss Organisation for Development and Cooperation for the financial support which enabled me to undertake and finish my studies successfully. My family and I deeply appreciate their kindness shown during the whole period of my studies.

I would like also to express my sincere gratitude to my supervisor Dr Christine Cahalan for her remarkable supervision and constructive criticism without which this work would not be accomplished. My family and I highly appreciate the help and support she willingly gave to us during my student life in Bangor.

I also owe many thanks to Dr J.B. Hall, lecturer at the University of Wales, Bangor, for his helpful discussion in the early stages of this work and provision of almost all the literature on the species studied. He also reviewed part of this thesis.

Thanks are also addressed to the "Département de Foresterie/Centrale de Graines Forestières (Rwanda)", Tanzania National Seed Centre, Kenya Forestry Centre, Uganda Forest Department (Seed section), Cocoa Research Institute of Ghana and Dr Nouhou Ndam of the Mount Cameroon Project for providing the seed material used in this study.

I acknowledge the useful discussion and assistance provided by various staff members of the University of Wales, Bangor, especially Dr Z. Teklehaimanot, Dr S.J. Retallick, Dr C.J. Gliddon, Dr C. Hill, Mr Bryan, Mr J. Evans, Mrs H. Simpson, Ms J. Hughes, Mr N. Brown and Mr M. Roberts. Help given by my wife A. Nyirandayambaje and my brother-in-law N. Nsengiyumva in data collection is also highly appreciated.

Special thanks to my mother A. Mujawimana, my parents-in-law Z. Senzige and S. Mukabitega for their continual prayers and encouragement. Many thanks to Dr J.K. Gasana, Dr E.B. Rubaduka, Mr A. Kalinganire for their support and useful suggestions regarding the research carried out. It also gives me pleasure to thank members of my house prayer group, particularly Mrs Jean Bailey for constantly praying for a successful completion of my studies and for God protection and blessings of my whole family.

Finally, it is hard for me to find words to express my gratitude and exceptional appreciation of an incessant loving care, support, encouragement and heartfelt company shown by my wife A. Nyirandayambaje throughout my studies. My children P.D. Mugisha, D.S. Igabe and D.R. Mahoro have also shown an extraordinary understanding and perseverance in accepting hardships caused by my studies. To all I say thank you.

DEDICATION

I would like to dedicate this work to my wife Alodie Nyirandayambaje and my children Patrick Dany Mugisha, Delice Sandra Igabe and Didier Raymond Mahoro in recognition of their exceptional support and encouragement during the whole period of my studies.

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CHAPTER 1: INTRODUCTION

1.1 Trees in the tropics: a valuable resource

In the tropics, trees still constitute one of the essential resources for purposes such as building construction, energy production, food and fodder, medicine, soil stabilisation and amelioration, and many other domestic uses. But in certain areas, unfavourable climatic and edaphic conditions (e.g. cold weather in high altitude areas, rocky, shallow, nutrient-degraded and waterlogged soils) make it impossible to grow some potentially useful tree species. In some areas, there may be successful initial establishment, but growth may later become slow, or trees may not grow at all as a result of failure to adapt to local environments. In other areas, wood scarcity may result from over exploitation of valuable tree species, or attack by biotic agents (e.g. termites and other pests) may lead to the degradation of forests or woodlands. On the other hand, economic and demographic factors require an ever-widening use of fast-growing species, among which the light hardwoods are expected to play a major role (Dubois *et al.* 1966).

Following the current rapid tropical forest over-exploitation, destruction and other forms of forest degradation, it is necessary to find ways of studying and preserving tropical forest tree resources (and their associated ecosystems) to prevent their total destruction. As natural forests continue to disappear, one strategy would be to conserve genetic resources, establish plantations for seed production and apply breeding technologies to meet human demands and environmental challenges.

A task for today's forester or tree breeder is to find tree species or provenances which can withstand adverse growing conditions and destructive biotic agents and which can be used by afforestation programmes to satisfy people's needs.

In breeding, the main aim is to choose particular trees with desirable characteristics as a source of seeds or planting stock, in preference to unselected trees or origins likely to be less useful. This selection process is not easy because a tree has its own phenotype for various reasons. Some of the reasons are genetic (i.e. inherent features determined by both male and female parents), while others are environmental (e.g. soil type, water stress, exposure to light or shade, competition with other plants and interference from grazing animals or human activities). Only features influenced by genetic factors will be passed on, so genetic selection means learning how to choose trees so that desirable characters which are strongly inherited are favoured (Longman, 1993).

One of the most widely used approaches in the selection of potentially valuable genetic material is the study of provenances, especially if a species is to be introduced to areas of difficult growing conditions. The main purpose of provenance testing is to assess genetic variation between populations, because this is considered as the most important determinant of the ability of forest tree populations to survive in different environmental conditions. Here, the term provenance is used to denote the geographical area where seed (or other propagules) are collected (Jones and Burley, 1973, cited by Zobel and Talbert, 1984). There are other definitions of a provenance, such that of Callaham (1964, cited by Persson, 1994) who stated that a provenance represents a population, and that of Persson and Persson (1992, cited by Persson, 1994) who asserted that the term has been attributed to everything from a single tree to a geographic region. A more comprehensive definition would be that of Styles (1976, cited by Kalinganire, 1992) who describes a provenance as the geographical area and environment in which parent trees grew and within which their genetic constitution has been developed through artificial and/or natural selection.

The performance of provenances is tested in replicated trials. These are described by Burley (1980 and 1984, cited by Apudo, 1991) as traditional methods for selecting provenances capable of surviving local conditions where they are intended to be grown, and able to meet the needs and aspirations of the local community under local management systems and conditions. Provenance trials provide basic and useful information that can help in the formulation of afforestation policies. Kemp (1993) reported that provenance trials and breeding programmes carried out on some tropical tree species demonstrated that highly significant increases in productivity and other socio-economic benefits could be achieved by selection between and within provenances. It is by careful comparative trials and the application of the results in fast growing plantations that these gains have been achieved.

Provenance trials play an important role in providing information on potential seed sources. Zobel and Talbert (1984) stated that when large quantities of seeds need to be collected, it is a common practice to go back to the original source or provenance which has been tested earlier and which has proven to be suitable. Although the normal way of producing genetically improved seed is the establishment of seed orchards, Zobel and Talbert (1984) assert that is it sometimes necessary to take shortcuts if large quantities of seeds are needed urgently. One of the shortcuts described consists of establishing orchards based only on the phenotype of the parent trees. Later undesirable genotypes are eliminated from the orchard based on the outcome of the progeny test, rather that waiting to establish an orchard only with parents that have already been tested for their genetic merit. There are also cases (e.g. in Rwanda) where provenance trials have been established with the intention of conversion into seed stands/orchards after roguing out poor provenances. This approach is essentially that which Williams and Matheson (1994) describe as a 'provenance/progeny trial selectively thinned to a seedling seed orchard'. However, the success of this approach in seed production is doubtful, since the

flowering and pollen exchange among genotypes in the orchard may not be uniform and equal as usually assumed (Zobel and Talbert, 1984). Some genotypes or clones produce many more flowers or pollen than others. Then, flowering may not synchronize so certain genotypes rarely mate (Zobel and Talbert, 1984). Usually, establishing provenance trials with the aim of confirming the superiority of some provenances over others is followed by selection within the best provenances of seed stands or individual trees.

Though provenance trials are very valuable, they take a long time to give useful results. Moreover, provenance trials usually deal with morphological traits such as leaf or stem colour, as well as the tree size and/or form of direct interest to growers. Koshy (1987) has pointed out that these morphological traits are of limited use as markers and are difficult to score because of the need to germinate seeds and grow plants in uniform conditions. He also pointed out that the determination of the mode of inheritance of these traits requires controlled crosses and analysis of the seedling progeny, and that when their expression involves some kind of dominance, genetic interpretation is difficult. Yeh and El-Kassaby (1980) added that these quantitative traits are subject to the influence of environmental conditions, making it difficult to ascertain the contribution of individual genes and to detect the extent to which they differ between individuals. Morphological traits may be useful as markers in other plants (e.g. cereals), but unfortunately they seem to be of little value in trees (Hattemer, 1991).

Electrophoresis of enzymes is one of the techniques chosen for use in this study because, as pointed out by Hattemer (1991), isozymes are presently the most widely used group of markers. The Ministère de la Coopération et du Développement (1989) has pointed out that, though it is difficult to relate patterns of enzyme variation to variation in quantitative traits of forest trees, the study of enzymatic systems can provide information which is needed when

making the choice of an improvement strategy. This might include, for example, the extent of intraspecific variation, and the organisation of that variability between and within provenances. In addition, genetic distances determined from enzyme data can be used to identify hybrids that potentially have high value. Kemp (1993) considered that electrophoresis can reveal allelic differences which could be linked to valuable traits. Examples given by Kemp (1993) include resistance to insect pests and extreme environmental conditions, and the potential for adaptation to changing environmental conditions.

1.2 Justification and aims of the study

In this study, the species concerned is *Maesopsis eminii* Engl. Reasons to concentrate on *Maesopsis eminii* in this study are its recognised value as a fast growing species, useful for many purposes, its low susceptibility to diseases and pest attacks, and its increasing importance in agroforestry. It is reported in Francis (s.d.) that *Maesopsis eminii* has been widely planted through the humid tropics where it has gained remarkable popularity as a plantation tree grown for its useful wood and other purposes. It has been successfully introduced to many countries such as Malaysia and Indonesia, and tested for adaptability to conditions in several other countries (e.g. Puerto Rico and India). Nevertheless, in Africa, although *Maesopsis eminii* is such an interesting tree species, it is still mainly found growing in natural forests and is rarely grown in forest plantations, except in Uganda and Congo (former Zaire)

One of the reasons why *Maesopsis eminii* and many other indigenous species are not used in afforestation is the reliance on fast-growing, exotic species for which the silviculture is well known and which are therefore easy to manage. It appears, however, that there has not been much research on tropical indigenous species to compare their growth characteristics (with exotics) and eventually establish plantations with those showing satisfactory growth. *Maesopsis eminii*,

for instance, is an indigenous African tree species whose growth can match that of some fast growing *Eucalyptus* species. It can grow 1-3 m per year in height and 1.5-5.5 cm per year in diameter (Hanum and Van Der Maesen, 1997).

Genetic variation between *Maesopsis eminii* provenances constitutes the major part of this study. It is for the reasons stated above that provenances of this species were collected in various parts of Africa and used in greenhouse and laboratory studies. The information obtained will undoubtedly be useful in drawing up improvement/breeding strategies for the species. Before embarking on such programmes, it is necessary to investigate the genetic variability between and within geographic populations under consideration for inclusion in the programmes.

Seeds of *Maesopsis eminii* are reported to loose viability within a few months of collection (Mugasha, 1981). Six months is said to be the maximum storage period, and beyond this seeds do not remain usefully viable (Anon, 1958, cited by Mondal, 1986). Even with proper storage conditions of 4-8°C, the proportion of viable seeds falls rapidly after three months (Yap and Wong, 1983). Seed pretreatments are necessary to boost germination. Leuchars (1957) found that the best pretreatment was to soak seeds in cold water for three days, while Mugasha and Msanga (1987) obtained the highest germination percentage when scarified seeds were soaked in warm water. Watkins (1960, cited by Mugasha, 1981) observed that the best pre-sowing treatment was to soak seeds in cold water for two days. Kurniaty (1987) found the best treatment to be soaking in 20N sulphuric acid for 20 minutes. It was not the aim of this study to examine this subject further, but it will be necessary in future studies to assess the differences in subsequent germination between seed pretreatments and to determine the best among them.

Because of the rapid loss of seed viability during storage, regular tests are needed in order to use viable seeds as soon as possible and to avoid unnecessary and costly storage of dead seeds. One problem is that checking *Maesopsis eminii* seed using normal germination tests in forestry nurseries may be a lengthy procedure. In some cases, germination may be completed in 97-170 days (Leuchars, 1957). Quick and reliable indirect tests of seed viability are needed to replace conventional germination tests. Such indirect tests include X-radiography, liquid flotation (absorption method), the tetrazolium test, the cutting test and the embryo excision method. These methods were used on six of the provenances included in this study.

Provenances were also assessed for growth characteristics. In the event of future selection of outstanding provenances, growth performance would be among the criteria taken into account. The growth of *Maesopsis eminii* has been reported in previous studies. For instance, Mugasha (1980) stated that in Tanzania the species showed a faster growth in plantation than in natural forest and that it displayed an early culmination in diameter and height growth. Mugasha's study was mainly carried out in old *Maesopsis* stands. Good performance of the species has also been reported from outside its natural habitat where early growth has been remarkable (Mugasha, 1980). However, work on provenance variation in the species is very limited, and there is a need to gather information on the better growing ones, which could be used in tree improvement programmes. In the present study, comparative seedling growth was measured in greenhouses for a relatively short period (12 months) to determine whether early performance differed between provenances. Morphological characteristics and derived indices were also assessed on the different provenances. These included branch angle, leaf area, petiole percentage, leaf number, number of first order branches, number of second order branches, length of a selected branch (i.e. the second branch from the tip of the main stem), and leaf shape index. Although the material was young, it

would be possible to correlate the results with performance in field trials should these be carried out. Provenance variation was also examined by electrophoresis of eight enzymes in leaves and seeds.

Since *Maesopsis eminii* seeds loose viability during storage, which may result in a shortage of planting stock when required, it is important to find other means of plant propagation which do not rely on seeds. Once found, the methods could help not only in providing plant material for afforestation programs, but also in breeding programmes. Mugasha (1981), looking at the structure of the tree, suggested that vegetative propagation is not possible for *Maesopsis eminii* unless hormones are used. Despite the claim, this study examined the possibilities of rooting stem and branch cuttings as a way of plant production. Provenance differences in rooting were also assessed.

Coppicing and pollarding constitute alternative methods of wood production where the availability of seeds and other propagation material is a problem. Mugasha (1981) reported that at Amani and Kwamkoro in Tanzania, *Maesopsis eminii* could be regenerated naturally by coppice. In Malaysia, natural regeneration of the species is prolific and the tree coppices readily (Singham, 1981). An assessment of the response of the different provenances to coppicing and pollarding was carried out to determine whether provenances differ in their ability to produce shoots after they have been cut back.

Grafting is a useful vegetative technique which can be used to perpetuate interesting clones which are difficult to maintain by other vegetative means (e.g rooting of cuttings) and/or other asexual methods (Hartmann *et al.*, 1990). In view of the benefits which grafting might offer to breeding programmes, this study also looked at the response of different *Maesopsis eminii* provenances to grafting.

In summary, the objectives of this study (which will be presented in the thesis in the order shown below) were to examine the variation between provenances of *Maesopsis eminii* in:

- seed characteristics (moisture content, size, viability and germination);
- growth (in height and diameter);
- morphological traits;
- isozymes;
- capacity for vegetative propagation (pollarding, coppicing, grafting and rooting).

CHAPTER 2: LITERATURE REVIEW

2.1 *Maesopsis eminii* Engl. : a promising tropical tree

2.1.1 Taxonomy and general description

According to Evrard (1960) and Hanum and Van der Maesen (1997), *Maesopsis eminii* (known under the trade name of 'Musizi'), is a monospecific genus of tropical Africa which belongs to the Rhamnaceae. Because of its chromosome number (2n=18), its protogynous flowers, the form of its ovary and style and the structure of its wood, it has been isolated within the family. Suessenguth (1953) assigns it to one of the five Rhamnaceae tribes Zizipheae but with reservations since there were no close relatives, noting that Engler (1906) had previously favoured a separate tribe or subfamily (Maesopsideae), a decision more recently supported by Johnston (1972).

The species *Maesopsis eminii* became known scientifically in 1895 following the Emin Pasha expedition which passed through the Bukoba region (Tanzania) in 1890. During that expedition, a specimen was collected by Dr F. Stuhlmann and was later (in 1895) described by Adolf Engler.

There were other discoveries of *Maesopsis eminii* specimens at different periods and in various African forests which led to synonyms (Hall, 1997). Currently, the single species is recognized (*Maesopsis eminii)* but has been separated into two subspecies. The large tree with large prominent glandular teeth on the leaves, which occurs in East Africa and was introduced to southeast Asia, is subsp. *eminii.* The small-sized tree with much less prominent glandular teeth on the leaves (only about 1-1.5 mm long) and which occurs from Nigeria to Angola is subsp. *berchemioides* (Pierre) N. Hallé.

Eggeling and Harris (1939), who were concerned with Ugandan populations, (subsp. *eminii*) described *Maesopsis eminii* as a medium-sized to fairly large,

fast-growing, deciduous tree. It can reach a height of 27-36 m and sometimes may grow up to 45 m tall. The diameter can be as much as 120-180 cm (Egli and Kalinganire, 1988).

Morphologically, the tree has a straight and reasonably cylindrical bole with few or no buttresses, and is clean of branches for up to 20 m (Rwamugira, 1991). To the west, subsp. *eminii* rarely exceeds 15 m in height (National Academy of Sciences, 1983; Mondal, 1986). The bark is thick and pale grey to nearly whitish, except where the tree has been growing in heavy shade (Eggeling and Harris, 1939). Appearance in the young and mature stages is often different. In young trees, branches spread perpendicularly to the bole (horizontal branching habit) (Egli and Kalinganire, (1988). Thus, Eggeling and Harris (1939) observed that the crown of young trees looks flat-topped with the surrounding branches generally at a higher level than that of the leading shoot, but that at a mature stage, the tree crown looks rounded. A large number of small, persistent, leafless twigs are a characteristic feature. The leaves are sub-opposite to (near branch ends) alternate, glossy and remotely toothed. The lamina is elliptical to lanceolate, pointed at the apex and with numerous veins. Binggeli (1989) gives a description of the reproductive organs as follows: small and yellow-green flowers composed of five united sepals and five small hooded petals, each almost entirely covering one stamen in small (5 cm long) axillary cymes. *Maesopsis eminii* has a superior unilocular ovary. Each inflorescence usually produces one or two fruits, but exceptionally three to five fruits develop. The fruit is an obovoid drupe (with the proximal end tapering), measuring 20-35 mm x 10-18 mm (Hanum and Van der Maesen, 1997). A soft, fleshy exocarp covers a hard mesocarp and an endocarp. In colour, the fruit is green when immature, turning yellow during maturation and finally becoming purple-black at full maturity.

2.1.2 Reproductive biology

2.1.2.1 Flowering and fruiting

Maesopsis eminii flowers are bisexual. Taylor (1960) reported that in Ghana, flowering began with a flush of new leaves. Trees in Ghana are generally leafless from January to mid-April. Fruits in Ghana appear in November and in December. Observations in the Lushoto Arboretum, Amani and Kwamkoro, Tanzania (Mugasha, 1981) show that *Maesopsis eminii* starts to produce flowers and fruits 4-6 years after planting. However, under natural or invasion conditions, an age of about 10 years for the onset of flowering is more likely (Hall, 1994). At that age fast-growing trees would be about 20 cm in diameter at breast height and the crowns of such trees visible in the forest canopy.

Two flowering and two fruiting periods have been reported in Tanzania: Mugasha (1981) states that *Maesopsis eminii* flowers in March to April and in November with fruits in October to January and June to July. Binggeli (1989) suggests that most of the first crop of fruits falls whilst they are still green, and that they do not appear to contain prolific seeds. Mugasha (1981) claims that flowering occurs in the rainy season but that rainfall does not have any influence on fruit maturity. In contrast, Storrs (1979) notes that it is in the dry season that flowering takes place in Zambia. Karani (1968, cited by Binggeli, 1989) reports only one long annual period of flowering in Uganda, starting at around the end of December and lasting in some areas until the first week of June. There is an overlap of flowering and fruiting periods and young fruits are present by the end of March. Most mature fruits can be seen in June-July though some are already formed in April and May. In Uganda (Entebbe area), falling fruits can be seen from April till December. Binggeli (1989) suggests the possibility of a second flowering period, explaining the green fruits observed in mid-December. In another Ugandan report (Anon, 1958) fruits were reported to be produced from June to October or November, with high

quality seed being available in July and August. In Peninsular Malaysia, Yap and Wong (1983) and Hanum and Van der Maesen (1997) report that *Maesopsis eminii* flowers from February to May and from August to September. After flowering, ovules take two months to mature.

Hall (1994) expresses doubts about the consistent existence of strictly regular flowering and fruiting periods since, in the East Usambaras (Tanzania), for example, a continually equable climate predominates. He emphasises the presence of fruits every month from June to January, raising more doubts about the seasonal fruiting of the species in the East Usambaras. This inconsistency of reported flowering periods suggests that close and thorough monitoring is needed to obtain reliable information on fruit and seed production. There is speculation by Mugasha (1981) that insects are the pollinating agents, but more information is needed to confirm or refute this claim. After pollination, fruits take three to four months to develop.

Maesopsis emini, being deciduous, sheds its leaves at certain times of year, especially in dry season. Binggeli (1989) reports that leaf shedding occurs when there is a severe dry season, and that at Amani it may happen simultaneously with fruit setting. In Rwanda, leaf fall is observed from July to August, while fruits are collected from November to January (Kalinganire 1989). Based on the above information, Hall (1994) suggests that in more regularly humid environments, there is not any regular period in which *Maesopsis eminii* would lose its foliage even when trees are fruiting; leaves persist at the apices of branches where fruits have not yet formed.

Maesopsis eminii produces edible fruits that serve as food for wild animals, especially birds and monkeys. Hanum and Van der Maesen (1997) report bats and rodents as seed disseminating agents. By disseminating seeds, these animals assist in the natural regeneration of the species. Moreau (1935) stresses

that at Amani, hornbills have been particularly efficient dispersal agents for decades. Binggeli (1989) reports blue monkeys, *Cercopithecus mitis*, as another important dispersal agent at Amani. Hall (1994) argues that dispersal of seeds will be restricted to the vicinity of trees and will not extend into open areas since the animals are strictly arboreal. He adds that, due to the extended fruiting period, seeds may be dispersed over long periods.

The conflicting information described above suggests that either flowering and fruiting occur at different periods in different countries/regions or climatic environments, or that there has been inconsistent monitoring of the phenology of the tree. More phenological studies covering a wide range of localities might resolve the contradictions.

2.1.2.2 Seed biology and germination

According to Mugasha (1981), *Maesopsis eminii* seeds cannot be stored for any length of time because their viability is lost within the first few months after collection. However, it is important to point out that the way and conditions in which seeds are collected influence their storability. Yap and Wong (1983) observed that carelessness in picking up fallen seeds from the ground could result in germination varying from 0-72 percent, depending on the time seeds had spent on the ground prior to collection and whether they had been attacked by fungi/insects or not.

Mondal (1986) reported that seeds stored at 4-8°C lost their viability after three months. In Uganda, six months was suggested by Anon (1958) as the maximum period for seed storage; beyond that, viability was lost. In Rwanda, very good progress with seed storage of this species has been reported. Seeds stored in jute sacks placed on raised pallets, in an aerated room at ambient temperature (18-25°C), still gave up to 60% germination after 18 months of

storage (Kalinganire, 1989). Seeds should be protected against rodents and high humidity (Kalinganire, 1989). Observations made by R.Willan (Mugasha, 1981) indicate that seeds collected in August-September and kept under cool storage conditions could successfully germinate in January-March and give high germination percentages. Similarly, a high germination percent was obtained when seeds were stored in a dehumidified room at ordinary room temperature (18-22°C) (Mugasha, 1981).

The germination of *Maesopsi eminii* is epigeal and the speed with which it germinates depends on collection, handling, storage and germination conditions. According to the Uganda Forest Department (1958), seeds take one to two months to germinate and a three-day soak in water before sowing accelerates germination. Similar claims are made by Mugasha (1981), who reported that *Maesopsis eminii* seed start germinating from 33 to 65 days after sowing. Completion of germination takes 97 to 170 days from sowing. Yap and Wong (1983) reported that germination was slow and took 100 to 200 days to complete. Msanga (cited by Mugasha, 1981) found that it was in the fourth week that seeds, kept in a dehumidified room at ordinary temperature, germinated. Three other batches of seeds, one kept in forest floor litter, another in an open store at ambient air temperature (18-22°C) and a third in a fridge (3 ± 1°C) germinated respectively in the fifth, sixth and seventh week after sowing. Generally, however, germination takes 2-6 weeks (Hanum and Van der Maesen, 1997). When the germination period takes as long as reported above, seeds are likely to be attacked by fungi and hence the number of seedlings will fall.

Although it is safe and wise to collect fruits from standing trees, it can be sometimes slow and frustrating where there is a lack of climbing experience or other seed harvesting techniques. It is therefore often easier to collect ripe

fallen fruits, but care must be taken to pick up only freshly fallen ones to avoid disappointing results from those left longer on the ground.

When sown fresh, seeds can give up to 90% germination, but to improve germination of old seeds, Hanum and Van der Maesen (1997) suggest soaking them in water for 1-2 days or in concentrated (20N) sulphuric acid for 20 minutes. Kalinganire and Ndeze (s.d.) recommend soaking seeds in cold water for 48 hours prior to sowing and making sure the water is replaced with fresh water every 12 hours. When dealing with small quantities, keeping seeds in sealed plastic bags after soaking reduces the time spent in the nursery beds as seeds start germinating in the bags. Direct sowing in nursery beds is a good practice for large quantities of seed in order to minimise handling and avoid problems of damage to seeds germinated in the bags. Storing seeds in a germinating hole dug in the ground gives good results at altitudes around 1500 m; storing them in wood shavings seems better at higher altitudes (Kalinganire, 1989).

In Malaysia, several seed pretreatments tried in efforts to boost germination gave poor results. Soaking in cold water for 12 hours and conducting germination in a bag filled with oxygen gave better results than did 24 hours cold water soaking, chipping of seed ends and the untreated control (Yap and Wong, 1983). However, it is difficult to judge these results since different batches of seeds were used for different pretreatments and it is not known whether the batches were collected and stored in the same conditions prior to germination tests. Germination rate was only improved when seeds were air-dried for a short period of up to 10 days, sun-dried for a period of up to five days or oven-dried for three days at 35°C (Yap and Wong, 1983).

2.1.3 Distribution

2.1.3.1 Natural range

Maesopsis eminii has a range which covers much of equatorial and humid Western Africa: from 12° 40′ W, in Senegal, to 34° 52′ E in Kenya and from 13° 00′ N, in Senegal, to 10° 59′ S in the former Zaire (Figure 2.1) (J. B. Hall, personal communication).

Details of the distribution of *Maesopsis eminii* are reported by Mugasha (1981), Mondal (1986) and Hall (1994), the last author concentrating particularly on the distribution in east and equatorial Africa.

2.1.3.2 Introduction and performance outside the natural range

Because of its reputation as a fast growing tropical hardwood species, *Maesopsis eminii* has been introduced to various countries outside its natural range to meet the ever-growing wood demand in tropical areas (Figure 2.2). In Malaysia, plantations of *Maesopsis eminii* were established in 1952 with seeds imported from Bogor, Indonesia (Sandrasegaran, 1966). These seeds were originally imported from East Africa, most probably from Uganda. Interest in the species arose because of its very fast early growth, its ease of handling in the nursery, the various ways by which it can be propagated (e.g. from stumps), its early production of fertile seeds, and its ability to regenerate naturally and by seed and coppice. When compared with species such as *Paraserianthes falcataria* (L.) Fosberg and *Gmelina arborea* Roxb., it was observed that *Maesopsis eminii* had superior silvicultural characteristics (Mitchell, 1963). *Maesopsis eminii* seedlings raised from stumps could be grown at closer initial spacing than *Paraserianthes falcataria* and without requiring special soil conditions necessary for *Paraserianthes falcataria* and *Gmelina arborea.* Also, *Maesopsis eminii* was valued for its ability to suppress undergrowth/weeds because of its spreading crown. In growth terms, *Maesopsis eminii* had a mean

annual volume increment (MAI) of 13.5 m^3, while *Eucalyptus robusta* Sm. and *Paraserianthes falcataria* had values of 9.45 m^3 and 4.05-5.4 m^3 respectively (Mitchell, 1963). Under the best site conditions the performance of *Gmelina arborea* was similar (MAI 14.85 m^3). Competition with weeds such as *Imperata cylindrica* (L.) Raeuschel was considered a limiting factor for the growth of *Maesopsis eminii,* though tolerance of poor sites was noticed (Mitchell, 1963).

In India, *Maesopsis eminii* has been introduced on an experimental basis to provide shade to coffee plants at Karnataka. After 18 years, *Maesopsis eminii* trees were 13 m in height with a diameter at breast height (dbh) of 16 m (Ananthanarayana and Jain, 1982). This gives mean annual height and diameter increments of 0.7 m and 0.8 cm respectively, although these are lower than reported in the natural range (1-3 m and 1.5-5.5 cm per year for height and diameter respectively) (Hanum and Van der Maesen, 1997). During a tour in the Philippines, Sarawak and Fiji, Lowe (1981) noted that *Maesopsis eminii* grew very satisfactorily in plantations. At 17 years trees had reached 40-50 cm dbh.

In the Solomon Islands, *Maesopsis eminii* was among various tree species introduced for monitoring performance through species trial plots. Although in some places trees were damaged by cyclones or destroyed completely, Marten (1975) observed that in other areas *Maesopsis eminii* initially grew very fast and well. In some plots, a few previously damaged trees are reported to have completely recovered.

In Indonesia (West Java), *Maesopsis eminii* was introduced in an attempt to meet local wood demand as a replacement for the *Paraserianthes falcataria* which was being attacked by *Xystrocera festiva* (Warsopranoto *et al.*, 1966). Other places where *Maesopsis eminii* has been introduced include: Central

America (Costa Rica), the Carribean Territories (Puerto Rico) and the Pacific region (including Hawaii and Western Samoa) (National Academy of Sciences, 1983; Francis, s.d; Fenton *et al.* 1977; Hanum and Van der Maesen, 1997; Liogier, 1994; Rwamugira, 1991).

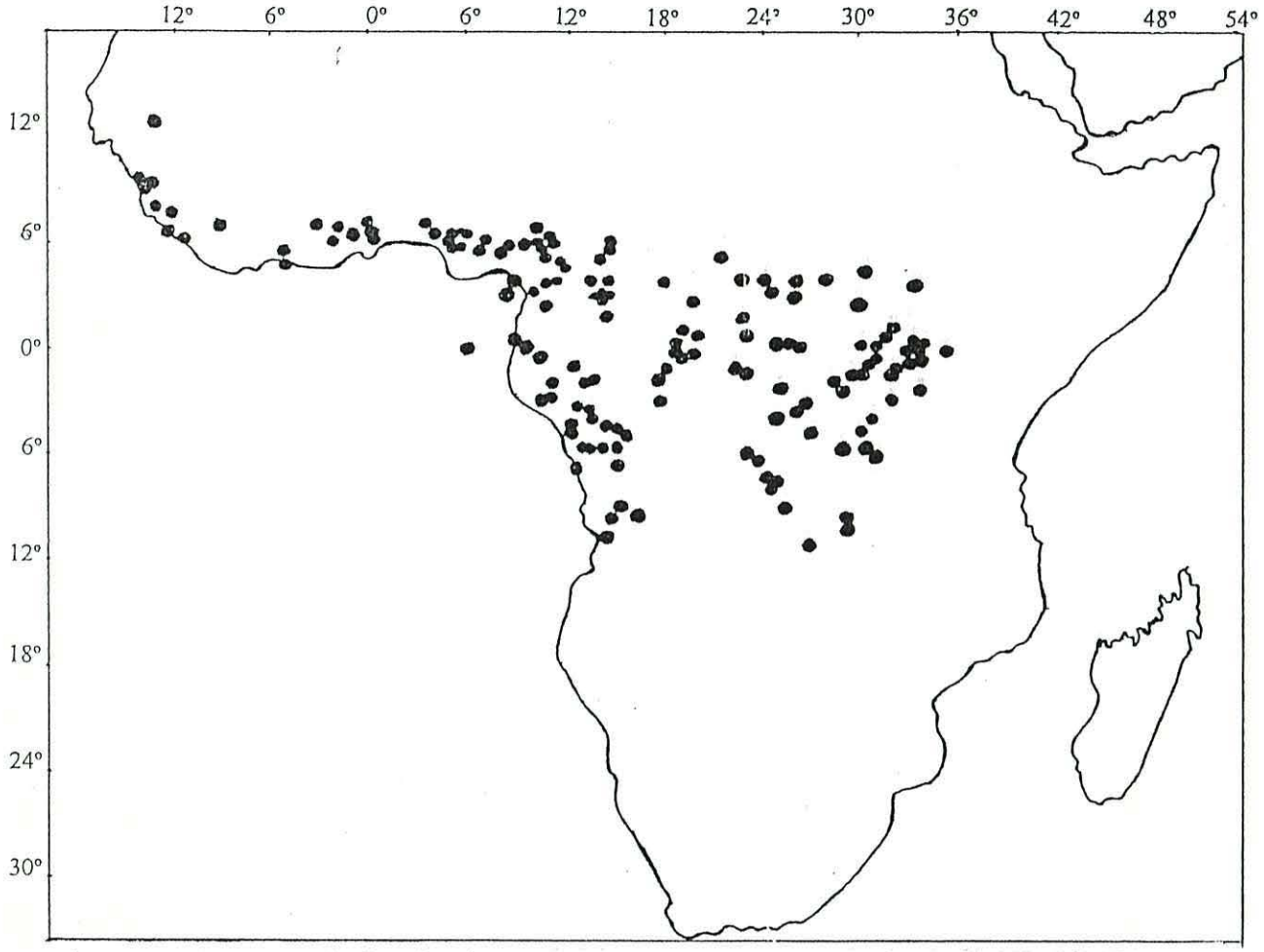


Figure 2.1: Natural distribution of *Maesopsis eminii.* Dots indicate places where the species has been reported. Note that the species is thinly distributed throughout the area indicated. The dots show recorded localities and do not distinguish discrete populations (J.B.Hall, personal communication).

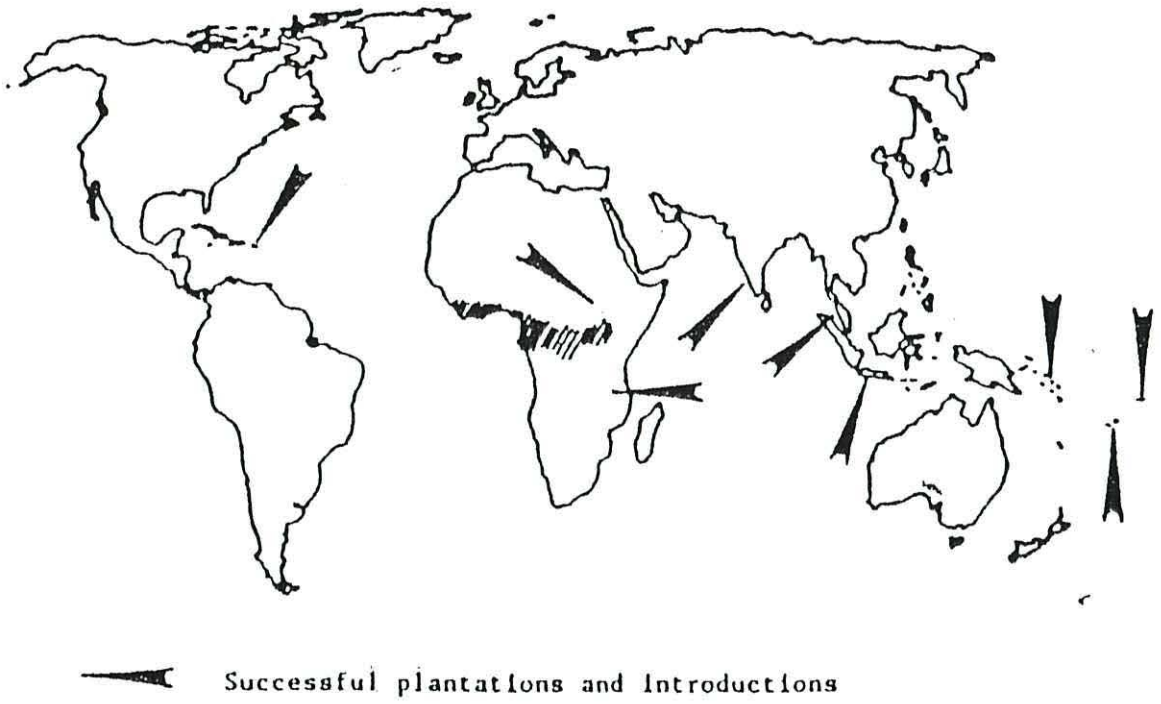


Figure 2.2 Areas of *Maesopsis eminii* introduction (adapted from Francis, s.d)

2.1.4 Environmental requirements

2.1.4.1 Temperature

Information about the temperature regime within the natural range of *Maesopsis eminii* is given by Mondal (1986). In the western part of the range, temperatures are higher than in the eastern. Monthly mean values of daily temperature maxima and minima range respectively from 26°C to 34°C and 19°C to 26°C. The highest monthly maxima are registered in February and March, towards the end of the dry season, while lower values are recorded during the rainy season (in August). The period of January to March is also marked by the highest monthly minima in most parts of this zone.

In the eastern part of the range, monthly mean values of daily temperature maxima and minima are respectively 25°C to 32°C and 15°C to 20°C. The highest monthly maxima are in January and February, but are not associated with a marked dry season except in some parts of Uganda. Monthly minima are highest in April-May. This zone has a more equatorial climate and higher variation in temperature due to greater variation in relief.

2.1.4.2 Altitude

Maesopsis eminii is a plastic species which occurs over a wide range of altitude. Generally, it is found between 100 m and 700 m above sea level, but it grows also at higher altitudes (National Academy of Sciences, 1983). In Uganda, it is reported in areas up to 1100 m elevation (Eggeling, 1947), while in Tanzania, it occurs from 900 m to 1500 m (Mugasha, 1981). In Kenya, it occurs at 1500 m altitude. In West Africa it grows at elevations below 500 m (Mondal, 1986).

2.1.4.3 Rainfall

Mondal (1986) reports considerable variation in rainfall where *Maesopsis eminii* grows. Hanum and Van der Maesen (1997) associate *Maesopsis eminii* with a mean annual rainfall of 1200 mm to 1500 mm. In most areas, there is one distinct dry and one distinct wet season each year, the dry season lasting from one month to five months.

2.1.4.4 Soil

Maesopsis eminii is reported to be tolerant of a moderately wide range of site conditions. It can survive on various soil types which range from medium to light in texture and from neutral to very acidic in reaction. In some areas where it has been introduced (e.g. Malaysia), *Maesopsis eminii* has grown well on what are considered poor soils, where other species did not survive (Streets, 1962). It is noted by Hanum and Van der Maesen (1997) that in Malaysia, *Maesopsis eminii* plantations have been successful on alluvial and granite-derived soils. In Kakamega Forest, Kenya, excellent height growth has been observed in *Maesopsis eminii* planted on soils over Pre-Cambrian sedimentary grit and mudstone with a crumbly, red lateritic earth of considerable depth (Philip, 1961).

The species is intolerant of waterlogged soils and cannot withstand competition in grasslands where *Digitaria* and *Imperata* predominate. Suitable soils include sandy and clay loams, red clay-loams derived from gneiss, granite-derived loams and soils of various texture and derivation such as limestone and volcanic clays (Laurie 1966). The best growth, however, has been recorded on deep, fertile, well-drained, light to medium textured soils (e.g. sandy loam soils), with a neutral to acid pH (National Academy of Sciences, 1983; Egli and Kalinganire, 1988; Kalinganire, 1989; Rwamugira, 1991).

2.1.5 Regeneration, growth characteristics and silviculture

Generally, *Maesopsis eminii* is propagated using seed sown in forestry nurseries. After fruit collection, seeds are separated mechanically from the surrounding pericarp and then dried for several days down to about 10% moisture content (Kalinganire, 1989; Hanum and Van der Maesen, 1997). Good germination is achieved with fresh seeds. However, if it is necessary to store some seed for future use only a short period of storage is advised since seed does not remain viable for long. Before sowing, to improve germination, seeds should be pre-treated. Soaking in cold water for 2-3 days is common practice.

In the nursery, seeds are sown in seedbeds and pricking out is of seedlings aged 4-6 days, just after the expansion of the first two leaves. Seeds pre-germinated in plastic sachettes are pricked out in polyethylene bags after the splitting of the outer parts and after emergence of the radicle. Afterwards, seeds are covered by a thin layer of soil, making sure the radicle is not damaged. In Rwanda, large polyethylene bags are used as seedlings remain in the nursery for 6-9 months. Overall, according to the National Academy of Sciences (1983) and Hanum and Van der Maesen (1997), the period ranges from 2 months to 24 months for seedlings to attain plantable size.

As seedlings rapidly form tap roots, it is advisable to frequently move the pots and prune roots which extend out of them, a process repeated at intervals until seedlings are planted out in the field, when they are 20-30 cm high (Kalinganire, 1989).

Maesopsis eminii regenerates naturally with ease. Seed is spread by birds (hornbills), bats, rodents and monkeys that feed on seeds. In Ghana, Taylor (1960) observed that regeneration was mainly concentrated under mother trees

but survival was dependent on the availability of light. Any time light intensity is sharply increased on the forest floor, *Maesopsis eminii* can start to grow rapidly either from suppressed seedlings or previously ungerminated seeds. In natural forest, *Maesopsis eminii* becomes established in tree fall gaps and its success is the result of abundant seed production, the presence of dispersal agents, the absence of diseases (apart from a fungus, *Meliola maesopsidis*, which infects seedlings), and fast growth rates (Binggeli and Hamilton, 1990). Mugasha (1981) reports that at Amani and Kwamkoro, Tanzania, soon after seed fall, seedlings establish themselves in newly felled areas. In Bukoba, Tanzania, where *Maesopsis eminii* is often grown with banana and coffee plants, a similar pattern of seedling establishment has been observed in non-shaded gaps. It is possible to regenerate the species artificially by direct seeding.

It has been claimed that stumps cannot be used, but Brasnett (1940) confirmed that *Maesopsis eminii* could be established by stumps, as plantations in Busoga, Uganda, demonstrated (Swabey, 1954).

Initial spacing in *Maesopsis eminii* plantations should ensure enough room for the crown to expand, whilst avoiding invasion by weeds. Although *Maesopsis eminii* is a colonising species and after establishment competes successfully with grass, there are certain aggressive grasses such as *Imperata* with which it cannot compete while it is young. However, if clean weeding is carried out in the early years, its rapidly spreading crown will suppress competing vegetation. Clean weeding also reduces the fire risk within the plantation.

Maesopsis eminii is phototropic, growing well in pure plantations and as an enrichment or underplanting species for open woodlands (National Academy of Sciences, 1983). Information on growth in diameter is given by Hall (1994), who infers from Eggeling's work (Eggeling, 1947) in Ugandan forests that

trees with a dbh of 30-50 cm are common in vegetation 30-40 years old. In vegetation aged 50-60 years most *Maesopsis eminii* trees are over 50 cm in diameter and small individuals (i.e. less than 20 cm) are rare. From 60-150 after colonization, *Maesopsis eminii* trees are less prominent, but those which persist often reach or exceed 60 cm dbh. The only known wild Zambian individual (in a block of riparian forest at Kapweshi Forest station) was 70 cm dbh with a 20 m bole (Lawton, 1969).

In Malaysia, first thinning is recommended after the fifth year from establishment to encourage the development of a suitable crown/stem ratio. In one study, after 59 months at a density of 823 trees/ha the timber yield including thinnings (36 m^3/ha) was about 162 m^3/ha. After 9.5 years when the density was reduced in two thinnings to 124 trees/ha, timber yield including three thinnings (36 m^3/ha, 70 m^3/ha and 92 m^3/ha) was about 259 m^3/ha (Sandrasegaran, 1966). Kingston (1974), for a site index of 25, gives a maximum periodic mean annual volume increment of 24 m^3/ha/year for individuals 5-10 years old including contributions from 40 stems/ha removed in thinning during that period, as well as the residual crop (160 stems/ha). For pole, fuelwood and pulp production, a rotation of about eight years is used. After harvesting, stumps may be left to coppice or the site may be replanted with another species, depending on management objectives.

2.1.6 Wood properties and utilisation

The description of *Maesopsis eminii* wood by different authors varies. According to Ananthanarayana and Jain (1982), *Maesopsis eminii* has an olive brown heartwood which becomes reddish-brown when exposed, and a whitish to yellow-brown coloured sapwood. In contrast, Hanum and Van der Maesen (1997) describe it as yellowish green when fresh, changing to a golden and/or dark brown colour, with a sharply defined whitish sapwood 2.5-5 cm thick.

The heartwood is soft and fairly strong with medium to coarse texture. The density is 350-510 kg/m^3 (Kalinganire, 1989; Hanum and Van der Maesen, 1997). The grain is interlocked, producing well-marked strips or ribbon grain on quartersawn surfaces. On flatsawn wood, it is possible to see marked irregularities such as pronounced wavy grain (Kingston, 1974).

Although difficult to finish, wood is easily sawn and works well with machines. There is however a need to use very sharp tools with a cutting angle of 20°. Nailing is also good, but timber has to be supported adequately to prevent chipping in drilling and mortising. Wood stains well but needs careful preparation and filling before varnishing or polishing, and before painting (Eggeling and Harris, 1939).

Seasoning properties are good. Seasoning should be done with precautions as the wood tends to warp. Although it air-seasons quickly, the wood should be stored under cover or piled under moderate drying conditions to avoid the splitting and warping which results from extremely quick drying. Kiln seasoning is advised, being quick and ensuring the wood seasons well and without any tendency to split or check. During felling and storage, the timber tends to split.

When wood is seasoned to 12% moisture content, shrinkage is about 4 cm and 2.5 cm per metre in tangential and radial directions respectively (Anon, 1954). The report also states that during kiln seasoning, wood becomes darkish, changing from a golden brown to a medium greenish brown. The colour of air-seasoned wood is restored after few months of exposure. It is worth mentioning that when seasoned, *Maesopsis eminii* is almost mechanically equal in weight and in strength to red deal (*Pinus sylvestris*), thus making it appropriate for building construction purposes.

Maesopsis eminii provides overall a good general purpose timber for indoor construction including light structural work and internal joinery, millwork, plywood and particle board manufacture, and packaging (Watkins, 1960; Sommerlatte and Sommerlatte, 1990; Seymour, 1993). The timber is also suitable for furniture making and the manufacture of matches and boats (Anon, 1960; Kalinganire, 1989). The wood can also be used in papermaking (Hanum and Van der Maesen, 1997).

Maesopsis eminii wood is not resistant to insects and fungi unless treated with preservatives. Pre-treatment is advised when wood is likely to be used in exposed conditions. The wood is easily treated and very permeable to preservatives. There is no difference in impregnation between sapwood and heartwood.

2.1.7 Other tree-derived products and secondary benefits

Maesopsis eminii fruits contain an edible oil. The seed contains a fatty oil which has not yet been exploited. The kernel has been found (Prabhu and Theagarajan, 1986) to contain 40-45% oil. The main fatty acids are oleic (47.49%), stearic (26.48%) and linoleic (14.79%). Defatted seeds are rich in crude protein (48-51%).

Maesopsis eminii is suitable for growing in combination with crops requiring shade. Anon (1947) included *Maesopsis eminii* among species suitable for coffee shade, noting that while shading causes a reduction of coffee yield, it serves as an efficient yield regulator. Sreenivasan and Dharmaraj (1991) suggest that it can best be used for shade by planting the border areas of coffee plantations; this prevents damage to coffee plants from the natural fall of trees and also facilitates felling and extraction of timber at rotation end (approximately 30 years). In Africa, *Maesopsis eminii* is widely retained in

home gardens where it is naturally abundant for shade, and in India it is often planted as shade for tea and cardamom plantations as well as for coffee (Hanum and Van der Maesen, 1997). In Cameroon and in Congo (formerly Zaire), *Maesopsis eminii* has been recommended as a shade species for cocoa plantations.

Maesopsis eminii leaves may be used as animal feed (Mahyuddin *et al.*, 1988). The dry matter content of the leaves is about 35% of fresh weight. Proximate analysis gives 26% crude protein, 3.6% fat, 5% ash, 20% crude fibre and 45% carbohydrate. Phenols and tannin are present (Hanum and Van der Maesen, 1997).

2.1.8 Diseases and pests

Young trees may suffer from cankers caused by *Fusarium solani* and other fungi. Early symptoms are the appearance of brownish patches on the young bark, which spread rapidly, followed by severe cracking and blackening and curling of the bark. Exudation of a slimy, yellowish-white fluid follows. Leaves show no visible symptoms except in the last stages of a severe attack, when they may become yellowish and fall. There is variation in the final effects of the cankers depending on the degree of the attack. Experience in Uganda is that very few trees are killed and only young trees (1-2 years) are affected (Brown, 1964). In Tanzania, however, seven year-old trees have been attacked.

Environment is believed to affect the incidence of cankers. Ofong (1974) concluded that trees growing on hilly and valley sites with poor soils tend to develop a weak bark, making them vulnerable to canker attack, and suspected nutrient deficiencies, competition, a thick undergrowth at the tree base (which created a humid micro-climate), and the activity of rodents to be factors contributing to susceptibility. Earlier studies by Brown (1964) suggested that

infection could only take place through damaged bark. The pathogen is spread by insects, particularly diptera and coleoptera, feeding at the edge of active cankers (Mugasha, 1981). On poor sites, especially those which are waterlogged, problems arise from bacterial wilt (National Academy of Sciences, 1983). In Peninsular Malaysia, a new fungus (*Cylindrocladium scoparium* Morgan) has been isolated from *Maesopsis eminii* seeds and found to be pathogenic to seedlings of other tree species such as *Dipterocarpus grandiflorus* and *Pinus caribaea* (Lee and Manap, 1982).

In Africa, wood borers such as *Monohammus scabiosus* Qued. cause damage to planted trees. Affected trees break at the point of attack, usually about 3 m from the ground. Breaking results from the weakening caused by two channels, one in the cambial area and one in the pith (Bredo, 1933). Measures to contain the parasite entail preventive distempering of stems with a toxic substance. Larvae are in parts of the plant spread over the ground surface and it is therefore advisable for broken stems to be destroyed (e.g. by burning) before imagos develop. Other control options are the insertion of minor quantities of carbon tetrachloride or carbon sulphate into the stem through holes in the bark. *Toxoptera odinae*, an Asian aphid whose main hosts are trees and shrubs, has been identified on *Maesopsis eminii* in Burundi (Remaudiere and Autrique, 1984).

Damage in nurseries and to forest plantations from generalist insect pests, notably termites, and vertebrates, has also been reported. Squirrels feed on the bark (Sandrasegaran, (1966). In nurseries, seedlings are subject to attack by rodents (Rwamugira 1991). Despite all the diseases and pests reported above, *Maesopsis eminii* does not, overall, seem to have very serious destructive agents. Personal observations in areas planted with the species in Rwanda revealed no diseased trees and only minor signs of insect defoliation.

2.2 Genetic variation in trees

An understanding of the fundamental basis of tree variability depends on adequate taxonomic knowledge as well as knowledge of genetics and breeding systems (Hawkes, 1976). The investigation of many woody plant species has revealed that the physiological characteristics exhibited by an individual are under the control of the specific genetic information it carries, and are modified by its environment. The genetic information carried by individual trees is as much a factor of forest production as their environment (Hattemer and Melchior, 1993). In general, the two factors are considered equally important because the phenotype is the result of the interaction between the genetic make up of an individual tree and its environment. As a rule, the genetic information carried by an individual differs from that of all other individuals belonging to the same species. This variation is of fundamental importance for biological processes in an ecosystem (Hattemer and Melchior, 1993).

Although genetic variation has been surveyed in a wide range of species, including conifers and some tropical and temperate angiosperms, the existing forestry data base is largely derived from the study of conifers in the north temperate zone (Ledig, 1986; Zobel and Talbert, 1986 cited in National Academy of Sciences 1991). Although intensive breeding programmes of some tropical trees have been conducted in the past, it seems that no regional or local investigations were carried out on the patterns of genetic variability in most of the species concerned. Implicit in selection schemes often employed for tropical trees is the assumption that the pattern of variation in natural populations of these trees is similar to that of temperate zone trees (Bawa, 1976). However, it is difficult to justify this assumption because of the lack of information on the differences and similarities between the two groups in their genetic systems and population dynamics. Bawa (1976) argued that in temperate zones, most conifers which have been used in improvement

programmes are monoecious, anemophilous, widely outcrossed and usually grow in high density stands. He described tropical trees as having a wide variety of breeding systems, as being somewhat or solely zoophilous and normally having a low population density. Given these differences, it is possible that there would be differences in patterns of genetic variation between temperate and tropical tree species. Bawa (1976) reported that tropical trees, unlike those of temperate zones, appear to show higher levels of differentiation between populations as a result of factors such as reduced gene flow, variation in selection intensity over small geographical distances, and genetic drift as a result of low population density.

Despite the very different reproductive mechanisms of many tropical species, a preliminary analysis revealed that levels of genetic variation within populations are similar to those observed for temperate tree species (National Academy of Sciences, 1991). Generally, the results of enzyme studies have shown only little genetic differentiation between populations of forest trees, so that to a large measure, the genetic variation of a species may be represented by only a few populations (Hattemer and Melchior, 1993). It has been asserted that while this may be valid for enzyme loci, it is not necessarily true for other genetic markers or phenotypic attributes that are under genetic control. Hamrick and Loveless (1986) and Buckley *et al.* (1988, cited by Hattemer and Melchior, 1993) have concluded that, with the above reservation, tree species in tropical and temperate zones may have similar patterns of variation. However, some other studies, such those done on Southeast Asian and Australian *Acacia* species, lead to the conclusion that tropical tree species may differ more or less in their patterns of genetic variation from northern temperate trees species (Moran *et al.*, 1989, cited by Hattemer and Melchior, 1993). With such conflicting information, more comparative research on tree species from both tropical and temperate areas is required.

2.2.1 Reasons for estimating genetic variation

One of the most fundamental requirements for improved management of forest tree diversity is the understanding of the genetic variation within and between tree species (National Academy of Sciences, 1991). Furthermore, development of reliable strategies for conservation and use of tree species needs a thorough knowledge of the genetic architecture of tree populations. Without such information, it is not possible to make effective plans for the management of genetic resources of tropical trees. It is normally advised that for the conservation of genetic resources it is necessary to maintain the broadest possible genetic base in the species as a whole, by conserving as wide as possible a range of provenances over the natural geographical and ecological range (Kemp, 1993). This is important because, according to Schultz (1990, cited by Hattemer and Melchior, 1993), genetic variation is generally used indirectly by exploiting the extensive natural variation in yield-determining characteristics. In the first stages of exploitation, it is necessary, for a given species, to explore its natural range and identify areas to which it has been introduced. It is also imperative to determine its taxonomic status and to study its breeding system. While it is acknowledged that such information is available for most temperate tree species, the same is not true of tropical species. From several hundred to a few thousand potentially valuable tree species, particularly in the tropics, are not yet widely used either for commercial purposes or for rural development. Little is known about their attributes, status, or distribution (Burley and Von Carlowitz, 1984, cited by the National Academy of Sciences, 1991).

Therefore, when studying genetic variation in tree species, it is necessary to gather information on their genetic make up, their reproductive system, population variation and distribution in cases where these are little understood (National Academy of Sciences, 1991).

Once the information is gathered, variation in useful attributes can be exploited. Once interesting provenances have been identified, several breeding techniques can be used to provide high quality genetic material to substitute for natural stands and to meet particular objectives. At the level of the gene, allelic differences could be the basis of valuable traits, for example resistance to pests or to severe environmental stress; both are of great potential value for adaptation to changing environmental conditions and for future use (Kemp, 1993). Moreover, information on genetic variation of trees can allow the rapidly advancing engineering techniques to produce new combinations of genetic attributes capable of leading to the creation of better plantations in terms of production or other desired characteristics. This can also contribute to the best use of difficult sites, including those which have been subject to severe deforestation and degradation. It is encouraging to note that, for the few tropical tree species which have been the subject of provenance trials and breeding programmes, important increases in productivity and related economic and social benefits have been achieved, based on selection from the very diverse provenances sampled (Kemp, 1993).

2.2.2 Methods for measuring genetic variation in tree species

Genetic variation is often considered to be essential for the survival of species. It is common for the individuals of species to express different characteristics as a result of variation between genotypes and/or the environments in which they grow. In order to assess genetic variation within and between populations of a species, there is a need to use appropriate experimental methods. There should normally be a consensus on the use of parameters in studies of genetic variation so that comparisons can be made with ease. Appropriate management of genetic resources can then be implemented, based on the information provided by research.

Various methods have been used to determine levels of variation among and within specific populations. Broadly speaking, depending on the objectives of the research, the genetic variation in tree populations is assessed using the approaches described below.

2.2.2.1 Provenance testing

According to the National Academy of Sciences (1991), provenance testing is done by collecting seeds from different populations and assessing variation in performance in plants grown under uniform environmental conditions at one or more planting sites. In general, sampling is done in populations from different geographic and climatic zones. The parameters assessed normally include height, diameter and yield, but it is also common practice to gather information on morphological characteristics to check for any variability in the provenances under investigation. Puttonen (1997) has reported that the main advantage of morphological assessment is its simplicity of application on a large scale and the fact that measures are usually quantitative, thus allowing statistical analysis with parametric methods. He also asserts that it is usually a non-destructive method which allows repeated measurements. In this respect, as the growth and morphology of a plant are the result of the integration of a range of biological processes, the analysis of a plant's development over time may allow these underlying processes to be interpreted. Moreover, comparative growth and development studies with other genotypes or provenances can be made, or the influence of various environmental factors on growth and development ascertained (Hunt, 1982).

2.2.2.2 Phenolics

Use of plant phenolics in chemotaxonomy derives from Bate-Smith's research in the 1960s on the taxonomic distribution of the different types of plant tannins and other phenolics using advanced chromatographic techniques

(Forrest, 1994). The fact that those compounds, especially the flavonoids, are highly diversified in plants makes them particularly useful in the study of taxonomic variation. However, the difficulties encountered in the extraction of compounds from plants constitute a major problem in using plant phenolics, because different extraction approaches can lead to different results. In cases where comparisons are qualitative, this is less important, but for quantitative studies, there is a need to standardise the extraction method for the material concerned. Some of the problems are caused by the presence of high levels of polyphenol oxidases and related enzymes in many tissues, and precautions need to be taken to minimise their degradative effects during the extraction process and when collecting material from the plant (Forrest, 1994).

Kleiner (1991, cited by Forrest, 1994) has reported that the techniques used in the handling and storage of samples can also have significant effects on their phenolic levels. Other important alterations and interconversions of phenolics and phenolic glycosides may occur during freeze-drying or oven-drying of samples (Lindroth and Pajutee, 1987, cited by Forrest, 1994). Techniques employed in the estimation of complex phenolics (e.g. tannins) can also be critical, particularly when analysing taxonomically diverse material.

Nevertheless, Forrest, (1994) points out that phenolics have been useful for determining taxonomic variation in tree species. In conifers, for instance, these compounds can provide valuable taxonomic information for the separation of species, the study of hybrids, and may even act as clonal markers (Niemann, 1979, cited by Forrest, 1994). Despite the problems encountered in the use of phenolics, they have been used successfully not only as taxonomic markers but also in the study of pest and disease resistance in forest trees (Forrest, 1994).

2.2.2.3 Terpenes

The use of these compounds has been reviewed by Baradat *et al.* (1991). Terpenoids include a large number of compounds which are widely distributed in the plant and animal kingdoms. Baradat *et al.* (1991) have reported that although the biosynthetic pathways leading to the formation of terpenes are known, not all the enzymes catalysing the reactions involved have been isolated and well defined. Despite this, terpenes are reported to have made useful contributions to the study of geographic variability of coniferous species because of their high discriminatory power. In many instances, the distinguishing ability of terpenes has been used to identify varieties of a single species. Another quality of terpenes is that they may be used simultaneously with isozymes to study mating patterns in natural populations or in seed orchards (Baradat *et al.*, 1991).

2.2.2.4 Isozymes

Assessing genetic variation using this approach involves collecting tissues from individuals of particular provenances or populations, maceration of samples and separation of isozymes by electrophoresis. Gels are scored for the presence and absence of particular bands. According to Hunter (1996), 'variation may represent functionally similar forms of enzymes (isozymes) or variants of enzymes which are different allelic forms of the same gene locus (allozymes)'. Identification of alleles and calculation of allele frequencies allows examination of the variation among populations or provenances under investigation.

Among the various groups of molecules employed in studies of genetic variation, isozymes are currently considered the most widely used. This claim is made, for example, by Hunter (1996), who sees electrophoresis as the fundamental tool for all molecular genetic techniques. As pointed out by

Hattemer (1991), since codominance is prevalent at the loci regulating isozymes, it is also possible to detect alleles in the heterozygous condition. Consideration of many isozyme loci has often shown the presence of numerous alleles in a wide range of populations. However, Hattemer (1991) has also stressed the fact that it is also common to encounter individuals with identical isozyme genotypes, and considers this situation beneficial because it provides replicates of particular genotypes.

2.2.2.5 Methods based on the analysis of DNA variation

The methods commonly used to detect DNA variation are those known as restriction fragment analysis, random amplification of polymorphic DNA and analysis of simple sequence repeats. When restriction fragment methods are used to analyse DNAs from two genetically dissimilar individuals, it is usual to observe polymorphism in the restriction or hybridization patterns resulting from variability in the number of recognition sites between the individuals.
The term restriction fragment length polymorphism (RFLP) has been coined to describe this variation (Botstein *et al.* 1980). The molecular basis of RFLPs is the loss or gain of a restriction site or of a short region of the genome due to a point or length mutation, or inversion (Szmidt and Wang, 1991).

a) Restriction fragment length polymorphism (RFLP)

In this method, restriction enzymes which recognise specific nucleotide sequences cut DNA strands within these sequences. This results in the production of DNA fragments that are electrophoretically separated and scored as bands on the gel. If similar size fragments reflecting the same cleavage sites are produced by two individuals, the analysis will assume that individuals involved are closely related.

b) Random amplification of polymorphic DNA (RAPDs)

This method uses purified DNA and the polymerase chain reaction (PCR) to isolate random DNA fragments. This is followed by electrophoresis and staining of nucleic acids from which bands are later visualised and scored. Exploratory or investigative diagnostic bands from parental or outside groups form the basis for comparisons within and between populations (Hunter, 1996). Use of the PCR technique means that even very small samples of DNA can be analysed (Hunter, 1996).

c) Simple sequence repeat (SSR) polymorphisms

In this technique, electrophoresis is used to visualise the DNA banding patterns of short, highly repetitive sequences of DNA (microsatellites). This is done after using the polymerase chain reaction (PCR). Variability in the banding patterns arises from the number of simple nucleotide repetitions that an individual carries (Hunter, 1996).

d) DNA sequencing

DNA sequencing is another molecular method used in the analysis of genetic variation. In this approach, polymerase chain reaction or cloning techniques are used to isolate specific DNA (gene) fragments. For sequences which differ in length by at least one base pair, separation on an electrophoretic sequencing gel is done by size (Hunter, 1991). Following separation, the sequence of bases along the fragments is determined, and sequences are aligned so that each site along the length is compared between individuals. Variability in sequences gives the information for calculating measures of genetic relatedness and subsequent phylogenetic analysis (Hunter, 1996).

In summary, genetic variation assessment based on morphological traits has been widely practised in the past, and this approach remains critical. As

reported by the National Academy of Sciences (1991), there are significant population differences in genetically based traits of direct survival value that are demonstrated by provenance trials. But Mitton (1978, cited by Dubbeldam, 1997) emphasises that in morphological studies, there is a danger of environmental bias and a risk of heterozygosity 'hiding' variation. There is also a general lack of correlation between results obtained by different analytical methods. For example, it has been observed that in many species, no correlation exists between morphological variation detected by provenance research and the level of genetic differences revealed by variation in isozymes (Namkoong and Kang, 1990). On the other hand, as reported by Hamrick (1983), a good correlation has been observed in some studies where patterns of variation in quantitative traits and isozymes are similar. Isozyme studies conducted in the past in forest trees were rarely accompanied by parallel work on variation in quantitative traits known to contribute to the fitness of plants.
In future, isozyme and morphometric studies should be conducted in parallel to determine the extent to which isozyme data can be used as indicators of morphological variation or to monitor changes in gene frequency caused by environmental or ecological events (National Academy of Sciences, 1991).

For intending users, the merits, shortcomings, and main applications of the main biochemical markers have been reviewed and summarised by Forrest (1994), and are shown in Table 2.1.

2.2.3 Gel electrophoresis of enzymes in genetic variation assessment

As explained above, there exist various methods for assessing the genetic variation in forest tree population, but one of the simplest and most commonly used techniques is gel electrophoresis. As described below, it has been of particular importance in genetic analyses which use catalytic proteins (enzymes) as markers of variation in the underlying genes.

2.2.3.1 Enzymes: their structure and function

Since enzymes are described as any catalytically active protein, it seems important, for a better understanding of their role in population genetics, to give an account of their structure and function. Proteins are large molecular weight substances (10^4-10^5) consisting of amino acids joined by the α carboxyl group of one acid and the α amino group of another, forming a polypeptide. A protein may be composed of one or many polypeptide chains. Each polypeptide is produced by translation and transcription of information contained in DNA. As a result, the sequence and amino acid composition of any given polypeptide chain is a fairly direct manifestation of genetic information (Feret and Bergmann, 1974).

Palmer (1981) describes proteins as macromolecules with molecular weights of at least several thousand daltons. Of the three main protein classes described by Feret and Bergmann (1974), it is the proteins which are catalytically active (i.e. enzymes) that are mainly used in genetic studies. Each enzyme is specific in character and acts on particular substrates to produce a particular product or products. According to Palmer (1981), enzymes are divided into six main classes:

- oxidoreductases (oxidation/reduction reactions);
- transferases (transfer of an atom or group between two molecules);
- hydrolases (hydrolysis reactions);
- lyases (removal of a group from a substrate);
- isomerases (isomerisation reactions);
- ligases (the synthetic joining of two molecules).

An 'enzyme system' is a category of protein molecules possessing the same or nearly the same catalytic function. The enzymes may be encoded by different loci or may be present in different organisms (Bergmann, 1991).

When enzymes are used in electrophoresis, they are referred to as isozymes or allozymes. These two terms are often used synonymously but their meanings are slightly different. Bergmann (1991) describes isozymes as electrophoretically detectable variants of an enzyme system in the same species which have different structures but an identical or nearly identical function. An isozyme is any of more than one form of the same enzyme encoded by different gene loci, while an allozyme is described as any of more than one variant of an enzyme encoded by different alleles at the same gene locus (Bergmann, 1991).

Table 2.1 Merits, shortcomings and main applications of biochemical markers. After Forrest (1994).

MARKER	ADVANTAGES	DISADVANTAGES	APPLICATIONS
Phenolics	Low cost, rapid Ease of analysis(some) Ecological importance Ubiquitous, diverse	Environmental and temporal variation Within-plant variation Problems of extraction and estimation(some) Complexity(tannins)	Taxonomic markers at various levels; Population variation, introgression; Clonal and hybrid identity(some spp.); Resistence markers.
Terpenes	Ease of extraction and analysis Low environmental variation High data content Multiple resin systems High infraspecific variation Ecological importance	Within-plant variation Temporal variation Limited to conifers(largely) Complexity of data	Population variation, introgression; Origin, cultivar, and clonal identity; Resistence and juvenility markers
Isozymes	All spp. and tissues Small samples required Environmentally stable Allozymes codominant Simple inheritance Highly polymorphic	Few systems only Toxic consumables Variation underestimated Variation with method Within-plant variation Biased genome sample	Mating systems and outcrossing; Comparative population variation estimates; Gene-flow, introgression, hybridization; Genetic and linkage mapping; Crossing authentication; clonal markers
RFLP	All spp. and tissues Unlimited in number Environmentally stable Codominant inheritance Samples much of genome Several genomes available	Expensive and slow Technically complex Radioactive probes Pure DNA required, and large samples	Phylogeny and diversity at various levels; Genetic linkage maps; **QTLlinkages?** **cpDNA** Higher level phylogeny; Introgression and infraspecific variation; Seedlot certification (some spp.) **mtDNA** Population gene-flow; High level phylogeny **nDNA** Population diversity; genotype identity; Variation at various taxonomic levels **rDNA** Introgression and hybrid analysis; infraspecific and population variation; Somatic mutation markers; High level phylogeny **VNTR** Clonal and genotype identity; Mating systems, paternity analysis;Population structure
RAPD	All spp. and tissues Unlimited in number Environmentally stable Genome saturation No radioactivity Fast and fairly simple No sequences required Small impure samples	Dominant markers DNA contamination problems Spurious priming Low reproducibility variation with DNA concentration, polymerase, etc. Expensive consumables Uncertain band identity	Clonal, cultivar and origin identity; Population diversity;spp.relations (some); Paternity analysis; Genome mapping, **QTL linkages?**

2.2.3.2 Electrophoresis: general principles and procedures

Electrophoresis has been defined as the migration of particles under the influence of an electric field (Richardson *et al.* 1986; Sargent and George, 1975). Andrews (1986) elaborated by stating that electrophoresis is simply concerned with the movement of ions through a medium, so that the factors that affect this process are applicable to various kinds of electrophoresis. These types of electrophoresis may be either free-solution based (e.g. moving boundary electrophoresis) or based on a supporting medium such as starch, polyacrylamide, paper or cellulose acetate.

The structures of most molecules dealt with in electrophoresis include cation and anion groups. The net charge on such molecules will depend upon the pH of its environment, so that pH will influence the mobility of the molecule (Andrews, 1986). High migration rates are obtained at low ionic strengths while the slower ones are attributed to high ionic strengths. Practically, slower rates are better than higher rates because they result in sharper zones of separation. On the other hand, higher ionic strength results in an increase in temperature, decreased viscosity of the medium and a change in electrical resistance (Andrews, 1986). The choice of the ionic strength of the buffer therefore influences the magnitude of the electric current used. The size and sign of the charge on molecules will also influence the rate and direction of movement.

Normally, samples need to be supported by a medium that also provides a passage for the electric current. There is a wide range of media available, including agarose, cellulose acetate, paper, starch and acrylamide. Depending on the laboratory equipment available and other technical factors, electrophoresis can be carried out vertically or horizontally. Some of the materials used to make gels (e.g. starch) act as a molecular sieve because they

form a very regular lattice that prevents the migration of enzyme molecules to a greater or a lesser extent depending on their overall size and three dimensional shape (Wickneswari and Norwati, 1992). In electrophoresis, the separation of enzymes is therefore influenced by three main factors:

- the differences in electric charge on particles, causing them to migrate at different rates;
- particle size and shape differences which affect their movement depending on the matrix (molecular sieve) used and
- the pH of the environment (buffer) surrounding the molecules.

According to Hoelzel (1998), the whole process of electrophoresis involves five stages described below.

a) Extraction

There exist many different approaches for the extraction and purification of enzymes. The choice of one or another depends on the material (tissue) being used and the types of enzyme being investigated. In plants including forest trees, the extraction of enzymes from leaf tissues involves tissue maceration, a process that can also lead to the release of undesirable chemical substances. Of the various substances released, tannins (phenols and quinones) are reported to have the capability to denature and to precipitate enzymes, and to possibly cause subsequent loss of enzyme activity in plant leaf enzyme preparations (Feret and Bergmann, 1974). There are several ways by which enzyme denaturation can be prevented. By inactivating phenoloxydase enzymes or reducing oxidized phenols as they are formed, it may be possible to limit phenol oxidation and the accumulation of quinones. Some studies reported in Feret and Bergmann (1974) used sodium hydrosulphite to prevent oxidation, while others used ascorbic acid to reduce oxidized phenolics as they appear.

Phenol oxidation can also be prevented by using additives such as cysteine, 2-mercaptobenzothiazol, mercaptoethanol, polyvinylpyrrolidone (PVP) and diethyldithiocarbamate (DIECA). To limit the adverse effects of tannins on enzymes, various substances such as dipolyethyleneglycol of sorbitan monooleate (Tween 80), dithiothreitol (DTT), mercaptoethanol and cysteine can be used. Most enzymes are very sensitive to temperature, and once samples have been taken, they must be frozen as quickly as possible (below -70°C for most enzymes) in order to prevent enzyme degradation. A very few enzymes such as lactate dehydrogenase are stable and can be stored for decades (Hoelzel, 1998). Extracted and centrifuged leaf samples can be stored for two weeks in the freezing compartment of a domestic refrigerator (-10°C) without loosing their enzymatic activity (personal observation).

b) Separation

To achieve good separation of the particles and fix the substances permanently at the positions to which they have migrated after an electrophoretic run, electrophoresis is carried out in a stabilising medium such as paper, starch or cellulose acetate. Although these media act as sieves that separate molecules according to their size and shape, separation is basically influenced by the charge on the molecule's surface. Under normal electrophoretic conditions, although a few enzymes move towards the negative terminal, most move towards the positive terminal. Electrophoresis can be done vertically or horizontally with the vertical gels providing slightly sharper resolution (Hoelzel, 1998).

c) Staining

After an electrophoretic run, the gels or gel slices are normally placed in a staining solution. Just before staining, it may be necessary to immerse the gels in a buffer solution to adjust the pH. To visualise the enzymes, a specific

staining solution has to be used for the enzyme system under investigation. Such enzyme activity staining (ENACTS) is used to locate the relative positions of the multiple forms of enzymes with common catalytic activity after they have been resolved by means of gel electrophoresis (Kananji, 1988). One to three hours are required for the zymogram (stained gel) to develop. This process takes place at room temperature or at 37°C in a dark incubator. According to Feret and Bergmann (1974), two main enzymatic reaction types are used in zymogram development. The first one is based on the oxidation-reduction processes of all NAD and NADP dependent dehydrogenase systems. The dye system used in the reaction is based on the reduction of tetrazolium salt to formazan. The second reaction type includes non-specific methods that help in the visualisation of esterases, phosphatases, etc.

d) Interpretation

Staining will normally be followed by scoring and the interpretation of zymograms. When electrophoresis, including the staining and development of zymograms, has been carried out properly, it is often possible to detect varying numbers of bands arising from the enzymatic action on gels. When distinguishing the various isozymes, it is a common practice to use letters or numbers referring to their migrating distance, and designation normally proceeds from the anode to the origin (cathodal end of the gel). Researchers have also, in the past, distinguished two genetically defined isozymes and their corresponding alleles as s or f to mean a slow (s) or a fast (f) migrating band in the zymogram. Others prefer to rely on the relative mobility (Rm) to designate isozyme bands. Usually a marker dye is used as a standard and the calculation of the mobility of individual isozyme is done by dividing the distance between the origin and the band by the distance to which the marker dye has migrated.

The ability to recognise differences in banding patterns for different enzymes helps in understanding the genetic basis of the observed phenotype. By understanding the genetic basis of differences in banding pattern, it becomes easier to formulate predictions about the inheritance of the genes responsible for that variation in the population (Hoelzel, 1998).

e) Application

The genetic information derived from electrophoresis can be used in various fields depending on the nature of the data. Some of the applications have been reported by Hoelzel (1998), and are listed below.

- **Population analysis**. Analyses in this field examine the variability at 20-100 loci to determine levels of variation within and among populations. In the same way, by using algorithms, populations with the most or the least similarity can be determined and a dendrogram constructed to illustrate relationships between them.
- **Systematics**. These analyses deal with the determination of systematic relationships within a group of taxa (generally species within a genus or family) based on percent shared alleles at individual loci.
- **Parental analysis**. Using electrophoretic data, putative parents can be identified in certain circumstances.
- **Mixed stock analysis**. Partition of a mixed population into its constituents or contributions of parental populations to a second generation mixed population can be made using electrophoretic data.
- **Linkage**. These analyses make it possible to assign the positions of isozyme coding loci along chromosomes.
- **Isolate identification**. Discrimination of individuals or clones from one another can be achieved using a combined genotype over a number of loci.

- **Hybrid identification**. Variability based on one locus with different alleles fixed in different species allows unequivocal recognition of interspecies hybrids as heterozygotes.

2.2.3.3 Isozymes as genetic markers

The choice of isozymes as genetic markers in various studies results from the many advantages they offer over other methods (e.g. use of morphological or other markers such as terpenes). The advantages given by Bergmann (1991) include the following.

- Most enzyme systems are not influenced by environmental factors.
- Many isozyme patterns remain stable during the development of individuals (ontogeny).
- Genetic interpretation is usually relatively simple because the band patterns on zymograms are not complicated.
- In general, isozymes do not exert pleiotropic or epistatic effects on individuals. Pleiotropy is the condition in which a single gene exerts simultaneous effects on more than one character, while epistasis is concerned with the suppression of gene expression by one or more other genes.
- Since the expression of isozymes is codominant, homozygous and heterozygous genotypes for a given locus can be identified easily.

The numbers and kinds of enzyme systems, or more precisely the numbers of gene loci which can be scored, are usually limited by time and financial considerations, not by biological factors.

Isozymes are different molecular forms of the same enzyme. These distinct molecular forms are the result of mutations which alter the base sequence of DNA molecules. The modification that arises in this sequence may cause an

alteration in the amino acid sequence of polypeptide chains (Wickneswari and Norwati, 1992). The protein or enzyme formed from DNA translation after the mutation will be slightly different from the one produced before the mutation. Electrophoresis can detect those changes if they have resulted in the alteration/modification of the net charge and/or the shape of the enzyme molecules.

Phenotypic characteristics for which variation in expression clearly implies differences in the controlling genotype are reported to be under strict genetic control and are sometimes referred to as genetic traits. Most morphological, physiological and chemical characteristics are not genetic traits, since not only is their genetic control unclear (such traits are often under polygenic control), but they are also strongly influenced by environmental factors (Bergmann, 1991). Because of this, it is difficult to correlate phenotypic variation with the underlying genetic variation. However, isozymes generally act as genetic traits and the patterns of many enzyme systems are not affected by environmental variation (Bergmann *et al.* 1989). The fact that polyptetides (i.e. enzyme constituents) constitute primary products of genes in the DNA, and thus reflect the information contained in their DNA coding regions, justifies the use of isozymes as reliable genetic markers. Therefore, isozymes can be used to detect genetic differences at the DNA level if it is assumed that differences in the electrophoretic mobility of isozymes reflects differences in the coding genes/alleles (Bergmann, 1991).

Although electrophoresis is such a widespread and reputable method in the determination of genetic variation, it has some limitations. According to Hoelzel (1998), electrophoresis does not detect all genetic variability in structural genes. Reasons include the low number of enzyme markers and the highly restricted group of structural genes they represent. Moreover, many other important proteins (e.g. structural proteins) and water-insoluble or cell

structure-bound enzymes have not been included in enzyme surveys (Bergmann, 1991). As a result, the estimations of genetic differences and heterozygosity may be biased, as the samples are not representative. Other shortcomings of isozyme gene markers are reviewed by Bergmann (1991), and described below.

Bergmann (1991) stresses that genetic variation within populations may be overestimated if a particular type of enzyme system has been selected for use in population genetic studies.

Other problems may arise from the difference in the amount of genetic variability between the two groups of enzyme systems (group-I and group-II) used in genetic studies. For one and the same individual or species, using a mixture of enzymes from the two groups may lead to heterogeneous results arising from the different numbers and types of enzyme loci used in different investigations.

Amino acid substitutions which change the electrophoretic mobility of enzyme molecules make up only a proportion of the nucleotide substitutions in the DNA. It has been estimated that only approximately 30% of amino acid substitutions result in a charge shift distinguishable with normal electrophoretic methods. More sophisticated analytical methods (e.g. sequential electrophoresis) would be needed to detect the estimated additional 20-30% of substitutions which result in conformational changes (Ramshaw *et al.* 1979, cited by Bergmann, 1991). Electrophoretic studies therefore underestimate levels of genetic variation in most cases. Allelic variation at polymorphic loci may also be underestimated if a single isozyme band in zymograms obscures other bands of similar mobility.

At the technical level, the lack of standard 'portable' protocols appropriate to different species suggests that the usefulness of enzyme markers is not as great as sometimes claimed.

2.2.3.4 Measures of genetic variation derived from electrophoresis

When analysing the genetic structure of populations, it is advisable to use a technique or method which can discriminate between individual genotypes at a level as close as possible to the DNA (Brown and Weir 1983). Hubby and Lewontin (1966, cited by Brown and Weir, 1983) have suggested four criteria for methods used for measuring genetic variability in plant populations.

- Allelic expression should be distinguishable in individuals.
- The effect of each allelic substitution should be locus-specific, and distinguishable from substitutions at other loci.
- All bases substitutions should be detectable.
- Loci should be sampled at random, irrespective of their function or likely level of polymorphism.

They considered that isozyme techniques meet the criteria more closely than the other methods available at the time. According to Hoelzel (1998), polymorphism (P) and heterozygosity (H) are the two parameters generally used to assess variation. Other measures are also widely used, and the commonly derived parameters are described below.

a) *Allele frequency*

Allele frequencies are defined by Richardson *et al.* (1986) as the relative frequencies of the various alleles at a particular locus. These should be distinguished from the genotype frequencies which represent the proportions of various genotypes in a given population. It is however possible to use the

frequencies of the various alleles at a locus to determine the relative frequency of genotypes that would be expected in an idealised, randomly mating population.

Allele frequency is calculated as follows.

Consider a gene with two alleles, f and s. The f allele codes for a fast-moving form of an enzyme, and the s allele for a slow-moving form. The frequency of the f allele, Pf, is given by

$$Pf = \frac{\text{number of f alleles in the population sample}}{\text{total number of alleles in the population sample}} .$$

The frequency of the s allele, Ps, is given by

$$Ps = \frac{\text{number of s alleles in the population sample}}{\text{total number of alleles in the population sample}} .$$

Note that $Pf + Ps = 1$.

While allelic frequencies are the basic parameters of population genetics, Koshy (1987) criticises them as inadequate indices of genetic diversity. He asserts that further calculations are needed to produce other, more useful, measures of genetic diversity, some of which are described below.

b) Percentage polymorphic loci (P)

Brown and Weir (1983) describe this statistic as a rough guide to the level of genetic variation in a sample. It is calculated as

$$P = \frac{\text{number of polymorphic loci}}{\text{total number of loci}} \times 100 \quad .$$

A polymorphic locus is arbitrarily defined but usually a locus is classified as polymorphic when the frequency of the commonest allele (at that locus) is equal to or less than 0.95, or sometimes 0.99 (Kananji, 1988). Despite the wide inclusion of this statistic in most isozyme studies, it is criticised for its dependency on sample size and on the number and types of enzymes surveyed, in addition to the problems related to the definition of polymorphism (Brown and Weir 1983). The amount of polymorphism is a useful measure of variation for certain purposes, but as stated above, it suffers from arbitrariness and imprecision, the number of variable loci observed depending on how many individuals are examined.

c) Average number of alleles per locus (A)

This measure has the advantages of concentrating on the 'allelic richness', an important constituent of diversity, defined as the number of distinct alleles detected in a sample of a particular size (Brown and Weir, 1983; Koshy, 1987). It is calculated as

$$A = \frac{\text{total number of alleles in a sample}}{\text{total number of loci sampled}} \quad .$$

This statistic is often criticised because it is affected by sample size and because all alleles are counted equally, without taking their biological importance into consideration. To alleviate these shortcomings, Kananji (1988) asserted that it is sometimes preferable to calculate the effective number of alleles (ne), defined by Crow and Kimura, (1970) as follows.

$ne = 1/\Sigma x_i^2$, where x_i represents the frequency of the i-th allele at a locus.

d) Average heterozygosity (H)

The amount of heterozygosity observed in a population is considered to be a simple measure of genetic variation and is usually reported for a single locus or as an average over several loci (Weir, 1996). Heterozygosity is a good measure of variation because it estimates the probability that two alleles taken at random from the population are different. Heterozygosity is commonly expressed as 'average heterozygosity' per individual (H) and is calculated by first determining the proportion of observed heterozygotes at each locus (H_o) as

$$H_o = \frac{\text{no. of heterozygotes at a given locus}}{\text{no. of individuals at that locus}} .$$

Average heterozygosity (H) is then calculated as

$$H = \frac{\Sigma H_o}{\text{no. of loci}} .$$

To achieve a reliable estimate of the average expected heterozygosity, Nei and Roychoudhury (1974, cited by Kananji, 1988) suggested sampling a large number of loci rather than a large number of individuals per locus.

e) Genetic identity (I) and genetic distance (D)

Genetic distance is a measure of gene diversity between populations expressed as a function of genotype frequency (Hoelzel, (1998). Ways of calculating those statistics have been proposed by Nei (1975) and are elaborated in Hoelzel (1998).

Consider two populations, X and Y, in which multiple alleles are segregating at a locus. Let x_i and y_i be the frequencies of the i-th alleles in X and Y,

respectively. The probability of identity of two randomly chosen alleles at a single locus (j_k) is

$j_x = \Sigma x_i^2$ for population X and $j_y = \Sigma y_i^2$ for population Y ,

where x_i and y_i are the frequencies of the i-th alleles at a given locus in populations X and Y respectively.

The probability of identity of two alleles at the same locus, one taken from population X and one from population Y (J_{xy}), is

$j_{xy} = \Sigma x_i y_i$.

The normalized identity between populations X and Y with all loci considered is

$I = J_{XY}/(J_X J_Y)^{1/2}$,

where J_X, J_Y and J_{XY} are the arithmetic means of j_x, j_y and j_{xy} respectively taken over all loci (monomorphic included).

The genetic distance (D) between populations X and Y can be calculated as

$D = - \log_e I$ (Nei, 1972).

f) Genetic differentiation (D_{ST}, $\bar{D}_m$, R_{ST}, G_{ST})

The procedure to calculate these measures of genetic differentiation is as follows (as presented by Nei, 1975, Cahalan, 1983 and Hoelzel, 1998).
First, using isozyme data, the probability of gene identity for each population is determined. For a single locus, gene identity (homozygosity) in the x-th population is given by J_s, calculated as

$J_s = \Sigma p_{ix}^2$,

where p_{ix} is the frequency of the i-th allele at a single locus in population x.

Gene diversity (H_S) (heterozygosity) is given by

$H_S = 1 - J_S$.

Gene identity for the total population ensemble (J_T) is given as

$J_T = \Sigma \bar{p}_i^2$,

where $\bar{p}_i$ is the mean frequency of the i-th allele over populations.

Gene diversity for the total population ensemble (H_T) is given as

$H_T = 1 - J_T$.

The average gene diversity between populations (D_{ST}) is calculated as

$D_{ST} = H_T - \bar{H}_S$,

where $\bar{H}_S$ is the arithmetic mean of the within-population heterogeneities.

D_{ST} measures the absolute amount of gene differentiation and takes into account the level of heterosigosity within populations. When the measurement does not take into account the level of heterosigosity within populations, the interpopulational gene diversity $\bar{D}_m$ is given as

$\bar{D}_m = sD_{ST}/(s-1)$

Where s is the number of populations.

Interpopulation gene diversity relative to that within populations (R_{ST}), is defined as

$R_{ST} = D_m/\overline{H_S}$, and gene differentiation relative to the total population group (G_{ST}) is calculated as

$G_{ST} = D_{ST}/ H_T$.

2.2.3.5 Brief overview of studies using isozyme markers

According to Bergmann (1991), the pioneer work in the detection of electrophoretic enzyme variability in mammals was done by Hunter and Markert (1957) and later by Markert and Moller (1959), who proposed the use of the term isozyme. Some years later, Lewontin and Hubby (1966) and Harris (1966) used the technique in their investigations on *Drosophila* and human populations respectively. Following other successes in the use of isozymes, studies were extended to agricultural plants. Early reports of variation in enzyme systems included those for amylase and malate dehydrogenase in barley (Frydenberg and Nielson, 1965; Yue, 1966). Other work by Beckman *et al.* (1964), Schwartz and Endo (1966) and Brown and Allard (1969a,b) revealed the presence of different forms of dehydrogenases, esterases, phosphatases and catalases in corn (*Zea mays*). The broad bean (*Vicia faba* L.) was found to show variation in tyrosinase (Robb *et al.* 1965) while the bean (*Phaseolus vulgaris* L.) showed variation in peroxidase and phosphatase (Racusen and Foote, 1966).

Isozymes were also used to verify taxonomic relationships. By concentrating on peroxidases, Chu and Oka (1967, cited by Feret and Bergmann, 1974) distinguished two varieties of rice. Steward *et al.* (1965), Coleman *et al.* (1966), Gerloff *et al.* (1967) and Hadacova and Sahulka (1967) are some of the

researchers who investigated the changes in isozymes during plant growth and development.

Genetic control of isozyme variation was examined in several early investigations on *Triticum* spp., *Hordeum* spp., *Zea* spp., *Avena* spp. and *Phaseolus* spp. (Feret and Bergmann, 1974). Schwartz (1960) published the first results of studies on the genetic control of plant enzymes, in which he found phosphatase synthesis in maize endosperm to be under the control of the Sh gene on chromosome 9. Later, Shwartz *et al.* (1965) demonstrated the genetic control of dimeric esterases in the same species.

More details of similar studies can be found in books such as those edited by Miksche (1974) and Tanksley and Orton (1983).

2.2.3.6 Isozyme studies in forest tree populations

According to Feret and Bergmann (1974), investigations of isozymes in forest trees started shortly before 1970. Studies ranging from simple screening of species and provenances to biochemical studies have been carried out. Some of these studies are briefly reviewed below.

Early work includes that of Lewis and Cech (1969, cited in Feret and Bergmann, 1974), who demonstrated the existence of electrophoretic variation of enzymes and proteins in *Prunus serotina* Ehrh. In that study, four types of isozymes (esterases, amino peptidases, phosphatases and peroxidases) were resolved.

Other studies were carried out to characterise proteins in different tree species. One such study, carried out by Durzan (1966), made a comparison between *Pinus strobus* L., *Picea glauca* (Moench) Voss, and *Pinus banksiana* Lamb. He

noticed differences between embryo and female gametophyte tissue proteins. The work by Bartels (1971) allowed isolation of gene markers in *Picea abies.* Several gene loci controlling leucine amino peptidase and esterase isozymes were identified.

Fowler and Morris (1977) conducted a survey of genetically determined enzyme mobility differences in 297 megagametophytes of red pine (*Pinus resinosa* Ait.) from five widely separated geographical sources. They observed a consistent and reproducible enzyme banding patterns in five of the seven isozyme systems essayed. There was no variation in band mobility in any of these systems. Another investigation was carried out by Copes (1978) to examine differences in isozyme activities in compatible and incompatible Douglas-fir graft unions. He found peroxidase bands with relative mobility (Rm) values of 0.77 to 0.80 and esterase bands with Rm values of 0.50 to 0.56 in tissues of incompatible Douglas-fir graft unions. In a study of enzyme variation in beech (*Fagus sylvatica* L.), Thiebaut *et al.* (1982) found peroxidase enzymes to be monomeric, while the glutamate-oxaloacetate transaminases were dimeric.

Yeh and O'Malley (1980) studied enzyme variation patterns in 11 natural populations of Douglas fir (*Pseudotsuga menziesii* (Mirb) Franco) and found a high degree of inter-locus variation in heterozygosity within populations. Wheeler and Guries (1982) worked on lodgepole pine (*Pinus contorta* Dougl.) in a study of population structure, genic diversity and morphological variation. Their results showed moderate heterozygosities (0.10 - 0.14) and low standard errors (0.02 - 0.04) for all populations. The distribution of isozyme variation indicated that there is very little population differentiation in this species. Nikolic and Tucic (1982) worked on black pine (*Pinus nigra* Arnold), using isozyme variation to assess genetic variability within and among populations. Nei's statistics indicated that heterogeneity within populations was higher than

heterogeneity between populations or subspecies. Differences in allelic frequencies were statistically significant among the localities examined, but no clear geographic pattern was evident.

Steinhoff *et al.*'s (1983) work on isozyme variation dealt with *Pinus monticola.* On average, 65% of the loci per stand were polymorphic and expected heterozygosity was 18%. With electrophoretic techniques, it was possible to assign the populations involved to two groups.

In red pine, Simon *et al.* (1986) conducted an electrophoretic study on the genetic structure of six populations comprising 124 trees. They did not observe any differences among individuals in the number and mobility of esterases, acid phosphatases, superoxide dismutase or peroxidases.

Rajora and Zsuffa (1990) used isozyme techniques to study genetic divergence in three species of *Populus*, namely *Populus deltoides* Marsh, *P. nigra* L., and *P. maximowiczii* Henry. They found that the three species were genetically distinct from each other. Later, Rajora (1990) used the same *Populus* species, their interspecific hybrid *P.* x *canadensis* Moench and F1 progeny of controlled crosses in further investigations. He found genes and alleles that could effectively be used to discriminate between the three *Populus* species and their interspecific hybrids. Three years later, Rajora and Dancik (1992) worked on exactly the same *Populus* species. This time eleven enzymes were assayed in fifty-four individual clones and the purpose was to compare isozyme inheritance in leaves and in root tips. A total of 33 genes coding for 11 enzyme systems were observed. 87 to 91% of the genes expressed in leaf tissue of the three *Populus* species and one interspecific hybrid were also expressed in root tips. Isozymes of 26 loci expressed identically in leaves and in root tips. In *Salix* spp. (section Longifoliae), patterns of genetic variation assessed by enzyme electrophoresis suggested that the section could be divided into four

major categories: *Salix interior, S. taxifolia,* the *Exigua* group, and *S. melanopsis* (Brunsfeld *et al.*, 1991). Isozymes were also used by Comps *et al.* (1991) to examine genetic differentiation in beech. These studies showed that European beechwoods from different geographic locations are genetically distinct. Possible causes were given, including selection, mating system and historical factors. In Germany, different populations of *Fagus sylvatica, Quercus robur* L., and *Quercus petraea* Liebl. were assessed for genetic variability by Müller-Starck and Ziehe (1991). There was great intrapopulation and relatively little interpopulation variation in the populations studied. The two oak species showed greater genetic variation within and between populations than beech. The assessment of genetic variation in beech populations in relation to environmental stress suggested that large heterozygosities and genetic diversities are associated with greater adaptive abilities. Similar patterns of genetic variation in Mediterranean oak (*Quercus ilex* L.) populations were reported by Lumaret and Michaud (1991), who found that most of the diversity was within rather than between populations. They asserted that this kind of diversity might be due to various characteristics of the mating system.

Genetic variability has been assessed in three geographical sources of south-western ponderosa pine (Yow *et al.*, 1991). Measures of genetic diversity showed that there were no differences between select trees (trees of outstanding phenotype) and their neighbours. Similarly, no differences in isozyme frequencies were detected between the two groups (select and neighbour trees), but there were differences between the three populations studied. Approximately 2% of the total variation was attributed to differences among geographical locations (Yow *et al.*, 1991). Using poplar species and their hybrids, Müller-Starck (1991) conducted a study to verify the genetic control and mode of inheritance of 15 enzyme systems. He concluded that 12 enzyme systems were controlled genetically by at least 18 polymorphic gene loci.

Cedar (*Cedrus* spp.) species have been subjected to electrophoretic analysis using extracts taken from dormant vegetative buds (Panetsos *et al.*, 1992). The results showed that in the four species studied, heterozygosity in *Cedrus brevifolia, C. libani* and *C. atlantica* was high in contrast to the low value in *Cedrus deodara.* Furthermore, it was shown that there were clear differences between *C. brevifolia, C. deodara* and the *C. atlantica, C. libani* group (Panetsos *et al.*, 1992).

Differences in genetic diversity and structure were investigated in sugar maple by Foré *et al.* (1992). Forty eight individuals were studied, and no significant difference in heterozygosity was observed between the cohorts of trees. In eastern hemlock (*Tsuga canadensis* (L.) Carr.), isozyme variation was investigated by Zabinski (1992) using 17 populations collected throughout the species' natural range. For the six enzyme systems essayed, one out of 10 loci was polymorphic and two alleles at the polymorphic locus (cytochrome oxidase) were observed in 15 populations.

Genetic variation within and genetic differentiation between four populations of *Taiwania cryptomerioides* Hay. was investigated by Lin *et al.* (1993). They found that eight out of fifteen loci examined were polymorphic and that within population differences accounted for 94.7% of total variation. Geographic distances and Nei's genetic distances were reported to be positively related except for one population which appeared to be genetically distinct from the others.

Some isozyme studies have been very useful in clonal identification. Miyazaki and Sakai (1969, cited by Feret and Bergmann, 1974) reported success in the identification of *Cryptomeria japonica* clones. Cheliak and Pitel (1984) were also able to identify different trembling aspen clones using isozymes. Bergmann (1987), using isozyme electrophoresis, was able to characterise

multiclonal aspen cultivars. Relying on five enzyme systems, it was possible to distinguish 19 out of the 20 clones under investigation. Electrophoresis was used to assay isozyme variants of 19 enzyme systems coded by 28 genetic loci in seeds and vegetative bud tissues of coastal Douglas fir (*Pseudotsuga* var. *menziensii* (Mirb.) Franco) (Adams *et al.*, 1989). By comparing isozyme band patterns in bud tissues from seed orchard clones with expected patterns from megagametophyte analysis, it was possible to confirm that 20 of the 27 loci were expressed in dormant vegetative tissues. Mendelian inheritance was confirmed for isozymes at 27 loci (Adams *et al.*, 1989).

Electrophoresis methods have also been applied to rain forest trees. The studies reported include that of Gan and Robertson (1981), who investigated isozyme variation between *Shorea leprosula, S. ovalis,* and *Xerospermum intermedium.* They found a high level of intra-population genetic variation in *S. leprosula* and in *X. intermedium* but not in *S. ovalis,* which showed uniform isozyme patterns. A few years later, Ashton *et al.* (1984) carried out a study on ten rain forest species of *Shorea* to compare electrophoretic and morphological parameters. There was an overall consistency between the grouping of species based on weighted and unweighted morphological criteria, and the grouping based on the zymograms.

Balasubramanian (1981) used leaf peroxidases to distinguish clones of teak (*Tectona grandis).* Results of polyacrylamide disc gel electrophoresis revealed differences between plus trees and comparison trees. *Eucalyptus* is also one of the tropical genera to which isozyme research has been applied. Though few tropical species have been studied, there is considerable variability among them. For example, *Eucalyptus caesia, E. cloeziana, E. delegatensis, E.grandis,* and *E. saligna* were all found to be very variable, with an average population genetic diversity per variable locus of 25% (Moran and Bell, 1983).

Isozyme research has also been successfully applied to tropical *Acacia* species by Wickneswari and Norwati (1992), who point out that important areas of application in these species would be in determining the genetic structure of natural populations and verification of hybrids between species.

In another study involving eleven provenances of *Faidherbia albida,* isozyme analysis allowed definition of their relative geographical ranges. Ibrahim (1996) identified two isozyme loci coding for superoxide dismutase (SOD-1 and SOD-2) which showed two different patterns over the geographical range of the species: the Sahelian range and the Southern distribution range. However, the two patterns were found to be overlapping in the Central distribution range (north-eastern Ethiopia).

It is also possible to use isozymes to trace the origin and follow the evolution of tree species. This is shown by the studies carried out by Villani and Pigliucci (1991), who worked on several populations of chestnut (*Castanea sativa* L.) from Italy and Turkey, which are considered to be the centre of origin of the species. Italian samples showed little genetic differentiation between populations. Morphologically, they found that the evolution of fruits and leaves were distinctly separate, and suggested that chestnut differentiation in the Mediterranean basin was the result of both artificial and natural selection. A strong differentiation was noticed in Turkish populations, which were characterized by a continuous cline running in an east-west direction, and a genetic discontinuity reflected in the similarity between western Turkish and Italian populations.

From this brief review, it can be seen that isozymes still constitute a powerful tool in forest genetics, particularly in determining the population structure of a species. Moreover, it is clear that they can play an important role in the determination of species boundaries and in phylogenic reconstruction, where

genetic markers are used as taxonomic traits to determine the evolutionary relationships of a group of several species. Although other markers (e.g. DNA markers) have been discovered, research based on isozymes is still being published, thus justifying the interest and importance of these biochemical markers in forest tree genetics.

2.3 Seed propagation of forest trees

Forest seeds constitute the primary source of planting material of forest tree species. In most afforestation programme worldwide, propagation of trees has been done using seeds, whose genetic and physiological quality is a determining factor in the establishment of healthy forest plantations. While it is recognised that the genetic constitution of seeds determines attributes such as growth rate, quality and health of future trees, and the stability of plantations, careful collection and handling can also play an important role in maintaining their viability. There are many factors which have to be monitored to ensure that seed viability is maintained. Some of the factors affecting seed viability are inherent in seeds themselves and determine the way they respond to treatment during and after collection, and the conditions in which they have been stored. During storage, seeds may be susceptible to rapid deterioration if drying has not been done to the required moisture content. There are, however, other contributing factors to the loss of viability during storage. Because seed viability is sensitive to internal and external factors, there is a need to handle seeds in appropriate ways (which depend on the species) during storage and sowing.

2.3.1 Moisture content and its influence on seed viability, storage and germination of seeds

The influence of moisture content on seeds will depend on the type (species) of seeds being handled. The so called orthodox seeds can be dried down to a

moisture content of about 5% without losing their viability, while recalcitrant seeds cannot survive drying below a relatively high moisture content (often about 20 - 25%) (Albrecht, 1993). It is evident that special care must be taken to dry seeds to optimum moisture content so that viability is not lost. Stubsgaard (1989, cited by Albrecht, 1993) states that the higher moisture content of recalcitrant seeds means that they are at risk of damage caused by high temperatures, fungi and animals, and of suffocation, physiological breakdown and overheating, especially if they are not properly stored. However, it is not only moisture content that affects seed viability. The length of time for which seeds can maintain their viability is influenced not only by their quality at the time of collection, but also by their treatment between collection and storage, and the conditions (e.g. temperature, relative humidity) in which they are stored. Even if the best storage conditions are provided, one will end up with poor germination after storage if seeds have already lost some of their viability before storage (Willan, 1985). Storage is normally not required when seeds are to be sown immediately after collection, but may sometimes be necessary for a period of time to insure sufficient stocks for future plantings. Nevertheless, control of seed moisture content is apparently the most important factor in seed longevity and storage (Hartmann *et al.*, 1990), and it is evident that poorly dried (and poorly stored) seeds will have reduced germination capacity. It is necessary to know the optimum moisture contents to which seeds should be dried and to provide appropriate storage conditions in order to maximise their germination capacity.

2.3.2. Seed size and its relevance to seed germination and seedling development

Within a given species, seed size can have an effect on germination and on the development of seedlings. This is because the size of the seed tends to be related to the size of food reserves (Halley and Soffe, 1988), which in turn can

dictate the type of plant produced in term of vigour, quality or other seedling characteristics. Larger seeds tend to perform better than smaller ones, probably due to the larger embryo and/or larger endosperm that inevitably encourage the development of larger seedlings with larger roots and shoots. According to Willan (1985), larger seeds contain greater food reserves, and are likely to germinate better and produce initially more vigorous seedlings. For instance, as reported by Goor and Barney (1976, cited by Willan, 1985), experiments with *Eucalyptus citriodora* showed that larger seeds had higher germination rates than medium-sized seeds, which in turn had higher rates than small seeds. Observations made by Turnbull (1983, cited by Willan, 1985) indicate that the effect of seed size on the growth of *Eucalyptus* seedlings lasted for 8-14 weeks after sowing, but he claims that other factors may subsequently become more important.

Based on the fact that small seeds may not be completely developed and may have reserves insufficient to give a normal plant, seed technologists carry out grading to sort seeds according to their size. One of the aims of grading is to ensure a greater uniformity of a seed lot, since variation in the size and weight of seeds is a result of both the seed genotypes and environmental influences (Albrecht, 1993).

However, when seeds are collected from different trees, it is not necessarily true that small seeds will give poor seedlings, because different trees growing on the same site can produce very different forms and sizes of seed. Also, old trees usually produce smaller seeds than younger ones (Suszka *et al.*, 1996). It is clear that it would be risky to concentrate collections on big seeds only because this may result in eliminating seed from old trees which, as the result of careful selection, maybe of great genetic and ecological value. The best way to preserve the variability (as suggested by Suzka *et al.*, 1996) is to collect seeds tree by tree and then eliminate the smallest ones from each tree. After

grading, the best (biggest) seeds from each tree would be mixed despite the heterogeneity in seed sizes.

In view of the effects seeds may have on germination and subsequent seedling development, it is sensible, when dealing with a given tree species, to ascertain the validity of assertions stated above to make the best use of seeds (e.g. to avoid unnecessarily discarding seeds due to their sizes).

2.3.3 Direct methods of testing seed viability

Direct methods of checking the viability of seeds consist of carrying out germination tests either in laboratories or in forest nurseries. Germination tests are so widely accepted that most people consider seed germination and viability to be one and the same (Copeland and McDonald, 1995). Despite their widespread acceptance, germination tests give just an estimate of, and have certain limitations as universal measures of, seed quality. On the other hand, germination data can be a good indicator of viability if these limitations are recognised. Germination tests carried out in laboratories give information on the viability of seeds by estimating the maximum number of seeds that can germinate in optimum conditions. However, Willan (1985) claims that results obtained under ideal controlled conditions in the laboratory do not reflect performance in the field nursery, where it is impossible to control all the environmental conditions in which seeds germinate. This is the reason why, when seed germination is carried out in the laboratory, it is advisable to use correction factors to estimate the field germination one is likely to get under given conditions.

Whether germination tests are carried out in field nurseries or in laboratories, it is possible to get information on seed viability from them, provided tests are conducted in known environmental conditions.

2.3.4 Indirect methods of testing seed viability

Many trees, especially those of the tropical rain forest, produce recalcitrant seeds or seeds with similar characteristics. Carrying out a germination test for such seeds according to ISTA (International Seed Testing Association) rules takes time (several weeks or even months), during which the rest of the seedlot loses viability (Pancel, 1993). Thus people often resort to indirect, quick methods of estimating seed viability such as those described below.

2.3.4.1 X-radiography test

The determination of seed quality by radiography methods has been practised over several decades and the studies of Simak and Gustafsson (1953, cited by Willan, 1985) are some of those which highlighted the use of the x-ray technique as an analytical technique in tree seed biology. The x-ray method presents several advantages: it is possible to detect empty seeds, mechanically damaged seeds or those showing signs of abnormal development in their internal structures. Moreover, though the x-ray test is not a viability test as such, the information it provides can be used to assess seed viability because it is capable of revealing morphological deformities which indicate the structural potential for viability (Copeland and McDonald, 1995). One x-ray technique, the x-ray contrast method, is very useful for distinguishing viable from nonviable seeds. It involves the use of various metallic salts that, in penetrating into seeds, can help to distinguish live from dead tissues.

Some of the merits of the x-ray technique are that it is a rapid and non-destructive method. It gives a quick indication of abnormal morphology or mechanical damage that might impair germination capacity (Copeland and McDonald, 1995). On the other hand, even when contrast agents are used, it is sometimes not easy to distinguish viable seeds from the dead ones, and this is

the reason why the technique is generally used as a complement to other tests of viability (Suzka *et al.*, 1996).

2.3.4.2 Liquid flotation test (absorption and density methods)

This approach for separating viable from nonviable seeds is normally one of the processes involved in the seed cleaning carried out after seed extraction. It is based on the fact that sound seeds may be distinguished from empty ones by several characteristics, one of which is their specific gravity (Willan 1985). Empty seeds float on the surface of a liquid of appropriate specific gravity, and can be separated from those that sink to the bottom and which are the full seeds (Suszka *et al.*, 1996). There exist two general methods by which the two categories of seeds can be separated: the absorption and density methods.

a) Absorption method

In this method, seeds are poured into water. Both full and empty seeds normally float for a certain period of time, which can vary from a few minutes to several hours depending on the nature of the seeds. Thereafter, full seeds absorb water, get heavier and sink at the bottom of the container. Those which sink are taken as viable seeds, but it is usually advisable to verify this by formal germination tests. Willan (1985) claims that this method can be very useful in separating full and empty seeds whose density differences are very small.

b) Density method

This method uses liquids of known specific gravity. For a given type of seed, the liquid used should have a specific gravity lying between that of the full and the empty seed. Willan (1985) has indicated that the specific gravity of the liquids used is usually below 1.0 (but is sometimes adjusted), such that the full seed sinks and the empty seed floats.

2.3.4.3 Staining tests

These tests provide a quick estimation of viability for species whose seeds take a very long time to germinate. Suszka *et al.* (1996) describe two widely practised approaches in routine seed viability assessment: the indigo-carmine and the tetrazolium tests. They also indicate that the first method is particularly used in Eastern Europe (e.g. Poland and Romania). In this method, seeds to be assessed are generally moistened by imbibition for 24 hours in distilled water after removing the seed coat or other seed coverings. Embryos are then extracted from seeds and immersed for a given period of time (usually 1-2 hours) in a solution of indigo-carmine diluted at 1 in 2000 at 20°C in the dark. Dead tissues are recognised by staining in blue, while living ones do not stain.

According to Copeland and McDonald (1995), the tetrazolium test is a method developed in Germany in the early 1940s by Professor Georg Lakon, who started the experiment with selenium salts and later used tetrazolium salts to separate live from dead seeds. It is nowadays recognised as an accurate method of estimating seed viability, and is popular in many seed laboratories because it can be completed in few hours. Knudsen (1982, cited by Willan, 1985) acknowledges the existence of satisfactory tetrazolium results in some countries (e.g. Denmark), but stresses that the interpretation of results is difficult and therefore requires experienced staff. Justice (1972, cited by Willan, 1985) has expressed doubts about its practical use in routine testing. He recognises a number of problems, including:

- poor staining of some seeds;
- the need to cut or dissect seeds to allow thorough examination of stained parts;

- tetrazolium results do not agree well with the results of germination tests in some cases, particularly when dealing with seedlots which have low germination;
- lack of uniformity in interpreting staining and difficulty in categorising the different degrees of staining;
- the time needed to test 400 seeds (according to ISTA rules).

These points may justify the assertion by Suszka *et al.* (1996) that this test cannot indicate the true germination capacity of a seed lot. Nevertheless, Willan (1985) claim that, with a well trained staff carrying out the preparation of seeds and the evaluation of staining, the tetrazolium test gives satisfactory results.

To use the tetrazolium test, Suszka *et al.* (1996) suggest following the same procedure as in the indigo-carmine test, except that the extracted embryos are soaked in a different solution (2,3,5 triphenyl tetrazolium chloride or bromide pH 6.5-7.5) for a period of 10-24 hours in the dark. Viable tissues stain red, while dead or damaged ones stay intact or change slightly to a light-red colour.

2.3.4.4 Cutting test

The cutting test is one of the simplest methods for estimating seed viability. It is mostly applied to large seeds, as small ones would pose handling difficulties. To conduct the test, seeds are normally cut longitudinally using a knife or pincers; it is sometimes easier to remove them from their outer (hard) coverings before cutting. For some seeds (e.g. acorns) longitudinal and transversal cuts are necessary to identify any signs of necrosis which can affect viability, particularly when it happens at the base of the cotyledons close to the radicle (Suszka *et al.*, 1996). Healthy seeds are recognised by their white, creamy, brilliant appearance (Suszka *et al.*, 1996); more importantly, if a seed has a

well developed embryo and a normal endosperm colour, it has a good chance of germinating (Willan, 1985). Those which appear milky, unfirm, mouldy, decayed, shrivelled or rancid-smelling are considered dead (Bonner, 1974, cited by Pancel, 1993). Willan (1985) claim that the cutting test is not very reliable, since it may be easy to identify the characteristics of dead seeds as described by Bonner, but difficult to identify dying, freshly dead or freshly injured seeds which look like sound seeds. On the other hand, Suszka *et al.* (1996) affirm that the method is reliable not only for viability testing but also for separating empty, abnormal or parasite-damaged seeds from well developed ones in good quality seed lot. However, they also assert that it often over-estimates the number of healthy seeds in middling quality lots. In practice, the cutting test is mostly used either at the end of a germination test to check whether ungerminated seeds are still viable, or to estimate the size and assess the maturity of the seed crop before collection (Willan, 1985).

2.3.4.5 Embryo excision test

The embryo excision test is an excellent way of assessing seed viability and can greatly reduce the time required to estimate the viability of dormant seeds (Copeland and McDonald, 1995). It is particularly useful in determining the viability of seeds which usually germinate slowly or which manifest dormancy caused by the seed coat and/or embryo (ISTA, 1991). The advantages of the test include the rapidity with which results are obtained (5-14 days) from observations of the condition of the incubated embryo and other tissues exposed following excision. Most importantly, Willan (1985) state that results from the excised embryo test are similar to those from germination tests. However, previously germinated seeds and samples containing any dry germinated seeds should not be tested by this method. During the assessment, embryos which turn green and/or grow and those which remain fresh/firm are classified as viable; non-viable ones decay and give off an unpleasant smell.

Copeland and McDonald (1995) point out some of the drawbacks of the excised embryo test, for example that it requires a high degree of skill and that it takes much time to extract embryos from seeds to avoid damaging them. They also claim that the only obvious embryo damage is that which occurs in the root-shoot axis, while other forms that might hinder normal germination remain undetected. Although the test requires ability, patience and time in extracting embryos, it is often used as an alternative to normal germination tests particularly when seeds require more than a 60 day germination test (ISTA, 1991). Copeland and McDonald (1995) consider that the test is particularly valuable for dormant seeds whose germination may take six months.

2.4 Vegetative propagation

Propagation of forest trees is usually done by seeds, but for some species it can be done more conveniently or more economically by vegetative methods. Since vegetative reproduction does not take place naturally in many tree species, appropriate techniques/methods and facilities have been developed to increase the success of vegetative propagation. There is an ever increasing use of controlled environment facilities (e.g. greenhouses), rooting hormones, mist propagation and others which have helped to produce a wide range of genetic materials by vegetative propagation (Hartmann *et al.*, 1990). Although there are different methods of vegetative propagation, not all of them can be applied to every tree because of genetic differences between species, the differentiation of the tree and its developmental and physiological stage (Muhs, 1992). According to Muhs (1992), vegetative propagation methods can be classified into two main groups: heterovegetative (e.g. grafting) and autovegetative (e.g. rooting of cuttings). The difference between the two lies in their range of application, problems of incompatibility with heterovegetative methods on the one hand, and problems of rooting capacity with autovegetative methods on the

other hand. There are therefore merits and shortcomings associated with each group of methods which must be taken into consideration when attempting to use any method of vegetative propagation.

2.4.1 Importance of vegetative propagation in tree improvement

According to Muhs (1992), while sexual reproduction generates genetic variation, which is balanced by natural and artificial selection to enhance population adaptability, vegetative reproduction is mostly concerned with the maintenance of the genotype best adapted to a particular growing site. In addition, he states that vegetative reproduction is an advantage on marginal sites (e.g. high altitude areas), or in the propagation of artificially selected trees (with a particular combination of desirable traits).

The main reasons for carrying out vegetative propagation have been summarised by Lindgren (1977), as follows.

- Conventional breeding can only utilise a limited part of the available genetic variance. By using vegetative propagation, it is possible to utilise additive and dominance variances.

- Concentration on the very best genotypes is possible. Vegetative propagation can produce numerous plants which are genetically identical to a single parent plant. As pointed out by Forbes and Watson (1992), this means that a successful plant, with genetic characteristics well suited to its environment, will produce equally well-adapted offspring in subsequent generations.

- In areas where there are no reliable sources of good (high quality) seed, selected clones can play an important role in forest plantations.

- In circumstances where there is a negative correlation between cone/fruit production and wood production, vegetative propagation may replace production of plants by seeds. Production gains may be indirectly achieved in this way.

- In genetic improvement programmes, vegetative propagation can be a fast and flexible way of achieving set objectives, as the genetic gains achieved in breeding can quickly be applied in practical forestry.

On this last point, Forbes and Watson (1992) have also asserted that, since vegetative propagules are usually larger than seeds, it is possible to produce a sizeable plant more quickly. The larger vegetative material establishes itself quickly, and is better able to compete with vegetation already established for light, water and minerals. Such rapid early growth may shorten the period of growth between planting and harvesting, giving the forester an opportunity to harvest large sized trees/timber on a short rotation.

Another advantage is that vegetative propagation allows mass multiplication of selected or superior trees (the superiority of which should be tested), and the propagation of trees for conservation (Muhs, 1992).

Lindgren (1977) has also pointed out some of the dangers which may be associated with vegetative propagation, and particularly with cuttings. He claims that 'often genes are favourable when they are rare, but disadvantageous when common (frequency dependent selection)'. For genotypes, the same thing may happen with the effect that 'genotypes may be raised to an unfavourable frequency' during selection. Loss of climatic adaptation for some tree species (e.g. Norway spruce) may be an example of this effect. Forbes and Watson (1992) have also pointed out that the lack of genetic recombination in vegetatively produced plants may lead to reduced variability and therefore

reduced adaptability to changing conditions. Changes in the environment or an outbreak of pests to which a species is susceptible could result in the total destruction of the whole forest plantation, as they will not contain any resistant individuals. The last, perhaps minor, disadvantage of vegetatively propagated materials is that they are produced in much lower numbers than plants raised from seeds. Comparing the costs, the general view is that vegetative propagation is less economical (more expensive per plant) than propagation of the same plant by seed. It is still done because of the high value placed on vegetatively propagated materials, which constitute their main economic advantage in most species (Hartmann *et al.*, 1990).

2.4.2 Coppicing and pollarding

Broadleaves and a very small number of conifers (e.g. *Araucaria araucana)* have the ability to regenerate themselves from cut stumps. The term 'coppice' is used to describe a forest crop raised from shoots produced from the cut stumps (also known as stools) of the previous crop, and coppicing is the operation of regenerating crops in this way (Evans, 1992). In coppicing, when planted trees reach maturity or the required size, they are felled, and the next crop arises from vigorous shoots (coppice shoots) which sprout from the stumps. It is advisable to make cuts as close to the ground as possible, with a smooth sloping cut to prevent collection of water on the cut surface (Redhead and Hall, 1992). Moreover, cutting near the ground will provide stability for the growing poles against strong winds. Physiologically, coppice shoots originate from two main sources: dormant buds located on the stump side, or adventitious buds arising from the cambial layer around the edge of the cut surface (callus shoots) (Evans, 1992). Many shoots will normally grow from the stumps, and the number of shoots kept will be determined by the management objective of the coppice forest. For example, if the management goal is the production of large poles/trees, only one or two strong shoots will be

left to grow for the whole rotation and constitute the final crop. For firewood or small size material, keeping many shoots would be necessary. Generally, coppicing may be repeated several times, but in practice three or four coppice crops are considered ideal because of the death of stumps over the course of successive fellings (Evans, 1992). The species and the condition of the stumps are the main factors determining the ability to coppice. Most broadleaved species, including many of those from the tropics (e.g. *Eucalyptus* spp., *Albizzia* spp.) have exceptional coppicing capacity. It should be noted that even among genera with a reputation for prolific coppicing, there are species that do not coppice well. Examples given by Evans (1992) include *Eucalyptus deglupta* and *Acacia mangium*. Ryan and Bell (1989, cited by Evans, 1992) have pointed out that differences between provenances of the same species may also affect the ability to coppice. Benefits of the coppice system have been given by Matthews (1989) and are summarised below.

- Simplicity in application, as reproduction is usually more certain and cheaper than when using seed.
- Rapid initial growth and production of straighter and cleaner poles than those produced from seeds.
- Shorter rotations as compared with high forest crops.
- Higher yield of the first crop as compared to seedling crops.
- High conservation value due to the variety of habitats provided by different stages of worked coppice.

On the other hand, Matthews (1989) also identifies some disadvantages which should be considered before opting for the coppice system. These include the following.

- The material produced is comparatively small in size.

- Rapid exhaustion of the soil nutrients (especially when rotations are short), since coppice consists largely of vigorous young shoots which need more nutrients than older trees.
- Susceptibility to damage by frost and animals.
- Aesthetically it is less preferable than most form of high forest due to its monotonous appearance.

Nevertheless, the coppice system has been practised extensively in developing countries to meet their ever-increasing wood demand, caused mainly by a rapid population increase. In addition to building poles, the system has been particularly useful in the provision of the fuel wood that serves as the main source of energy in most parts of these countries. Matthews (1989) reported that in tropical (developing) countries, fuel wood is collected from unmanaged and managed forests, from trees growing outside forests in rows and hedges bordering roads, from village woodlots and from scattered trees in fields and around houses. This could be the reason for the wide and still continuing practice of the coppice system in those regions.

Pollarding is an old practice of lopping the top of a tree at 2.5 to 4.0 m above ground level, allowing new shoots to grow at the point of cutting (Read, 1991). Cutting stimulates the production of many shoots that are trimmed off periodically at intervals of one or more years to provide different kinds of wood material. For example, the wood from these shoots can be used for fuel, small size timber, basket making material, fencing hurdles and fascines, while the foliage can be used as fodder (Matthews, 1989; Read, 1991). Pollarding is not generally done in forests, but mainly in wood-pasture systems where trees are combined with grassland or heath (Rackham, 1991) or in agroforestry systems. Compared to coppicing, the advantage of pollarding is that it is possible to use a silvipasture system where animals such as cattle or sheep can successfully be kept with no danger of browsing young shoots. In practice, pollarding is labour

demanding, making it less widely practised than coppicing. Although pollarding is reported to be disappearing from European landscapes, it is still a practice commonly found throughout the dry tropical regions (Matthews, 1989). Countries given as examples include Ethiopia, Sudan, Pakistan and northern Nigeria, where 12 year-old *Azadirachta indica* trees are pollarded and regularly lopped for fuel wood. Treating about 30 trees in this way has allowed an average rural family to be self sufficient in fuel wood for 15 years (Spears, 1983 quoted in Matthews, 1989). These few examples illustrate how pollarding can still solve some wood scarcity problems in marginal areas.

2.4.3 Cuttings

Propagation by cuttings is done by inserting a piece of stem, root or leaf into a growth medium to encourage the formation of roots and shoots and then a plant. Chemical substances (hormones) may be used to promote root and/or shoot formation. Although this technique is mostly used in the production of ornamental and fruit trees, it is also used to produce clones of forest tree species with particular productive qualities or which suit certain environmental conditions. Longman (1993) has reported that more than 80% of tropical forest trees so far tested can be rooted as leafy stem cuttings. Production of plants from cuttings has many advantages, some of which are given by Hartmann *et al.* (1990). The method is simple, less costly and quicker than other vegetative propagation methods such as grafting, budding and micropropagation. It avoids the problems of heterovegetative methods such as grafting, where the graft union may fail, or where problems of incompatibility may arise. Using a few stock plants, it is possible to produce many new plants, which usually do not differ genetically from the parent plant. Vegetative propagation by cuttings results in plants which are not subject to the variation originating sometimes from the variability in seedling rootstocks of grafted plants. There exist different types of cuttings, and their classification is based on the part of the

plant from which they are taken. Hartmann *et al.* (1990) describe four broad types. Stem cuttings consist of shoot segments with lateral or terminal buds, and are grown in conditions allowing adventitious roots to develop and produce independent plants. For some species, the success of rooting will depend on several factors including the ripeness of wood and the time of the year when the cuttings are taken. Root cuttings give satisfactory results when taken from young stock plants at a time when there are plenty of food reserves (e.g. in winter). Preparation of root cuttings when needed in large numbers is quite time consuming and difficult, and one has to be extremely careful to maintain the correct polarity (to avoid planting them upside down). Leaf cuttings consist of a leaf blade or a leaf blade and petiole; both adventitious roots and shoots are developed at the base of the leaf and these form a new plant. Leaf-bud cuttings consist of a leaf blade, petiole and a short piece of stem with the attached axillary bud. These cuttings are particularly important for species where a detached leaf is necessary to initiate adventitious leaves. The shoot develops from the axillary bud at the base of the petiole.

Cuttings, and to a lesser extent other vegetative materials, can help solve many problems in forestry. Zobel and Talbert (1984) list the following.

- Genotype preservation using clone banks.
- Multiplication of specially selected genotypes for particular purposes (e.g. seed orchards or breeding orchards).
- Evaluation of genotypes and their interaction with the environment through clonal testing.
- Securing maximum genetic gains when used for regeneration in planting programs.

2.4.4 Grafting

Grafting is one of the vegetative propagation methods providing opportunities to combine the potential benefits from two or more plants with different characteristics. As described by Hartmann *et al.* (1990), this is achieved by joining two pieces of living plant tissue together in such a way that they will unite and subsequently grow and develop as one plant. The two pieces of living plant tissue joined together are commonly termed stock and scion. Hartmann *et al.* (1990) describe a scion as a short piece of detached shoot containing several dormant buds, which, when united with the stock, comprises the upper portion of the graft and from which will grow the stem or branches, or both. The stock (rootstock) is the lower portion of the graft, which develops into the root system of the grafted plant. It may be a seedling, a rooted cutting, or a layered plant. Forbes and Watson (1992) have provided a physiological account of how grafting works. The union of the graft is made possible through the mixing of callus tissue produced by both stock and scion in response to cutting. Initially, the callus tissue is undifferentiated but after few days, cambial cells form in the callus adjacent to the cambia of stock and scion. Division of these cells follows, producing phloem and xylem towards the outside and inside of the stem respectively. Finally, there is a joining of the new vascular tissues to those of the stock and the scion to form a link enabling the flow of the water and nutrients between them. For grafting to be successful, the stock and the scion need not be genetically identical, but should be genetically similar, either belonging to the same species or to closely related species. Forbes and Watson (1992) point out that, though the pieces grafted together function as a single plant, there is no interchange of genetic information between them.

There are good reasons why grafting is performed as a means of vegetative propagation of plants, some of which are important in forestry. According to Hartmann *et al.* (1990), grafting can assist in preserving clones that cannot be

easily maintained by cuttings, layering, division, or other asexual methods. According to Longman (1993), grafting is particularly used for the production of clones to be planted in seed orchards, and has been successful for many tropical and subtropical tree species such as *Cedrela* spp., *Cordia* spp., *Pinus* spp. and *Tectona* spp. In grafting, it is possible to benefit from the use of particular rootstocks. There are situations where trees have desirable bole and crown characteristics but poor root systems for growing in certain environmental conditions. On the other hand, some trees (in the same genus) may have a strong root system but poor bole and crown quality. In such cases, grafting provides an opportunity to combine the desired qualities of the scion and stock. For example, good rootstocks can provide an opportunity to grow trees in unfavourable conditions such as dry or shallow soils, waterlogged areas, or areas where trees are prone to attack by soil-borne insect and fungi. In other cases, Forbes and Watson (1992) point out that the large root system of the stock may enables more rapid growth of the scion than would be possible by growing it on its own roots as a rooted cutting. According to them, controlling the growth and development of the scion is probably the most important reason for grafting. There are many other advantages of grafting which are more relevant to horticulture and which are therefore not discussed here.

CHAPTER 3: PROVENANCE VARIATION: SEED CHARACTERISTICS

3.1 Introduction

In forest practice, seeds constitute the material mostly used to produce plants for afforestation purposes. Successful plantations are the result of high quality seeds. This is because, among other influencing factors, the genotypes of the seeds determine the growth rate, quality and health of future trees and the stability of a plantation (Albrecht, 1993). Genetic variability in different seed characteristics often determines the performance of plants in terms of growth and adaptation to particular environmental conditions.

Seeds produced from populations or provenances represented by a small number of individuals are often characterised by inbreeding deficiencies in terms of viability and/or performance of seedlings in the field. It is known that seedlots derived from self-pollinated trees contain a high proportion of non-viable or empty seeds, and that their progenies are often lacking in vigour (Wilcox 1983, cited by Albrecht, 1993). The need to collect seeds from populations composed of a large number of different genotypes is therefore evident. Selecting good seed sources for collection, followed by proper seed handling, can have a positive effect on established plantations in terms of growth, volume production, adaptation to various marginal environmental conditions, disease resistance, and other characteristics. For a given species, better results will be obtained by the collection and use of seed of the provenance with qualities best suited to the area where the species is to be grown.

This chapter deals with provenance variation in seed characteristics of *Maesopsis eminii*. Measurements were made of the size, moisture content, germination (rate and final percentage) and viability of seed from six

provenances. Seeds of different provenances are likely to exhibit characteristics determined by their different genotypes. Seed size (i.e. seed length and width) variation is a result of genetic factors and environmental influences (Albrecht, 1993). In some cases, it may be possible to use seed size to distinguish between provenances, or to identify seedlots.

Seed moisture content influences germination rate and percentage, and should be measured prior to tests of seed germination. Provenance variation in seed germination may reflect differences in seed moisture content, or the environmental conditions under which seed was formed, rather than genetic differences between provenances.

Indirect tests of seed viability are needed in *Maesopsis eminii,* since conventional germination tests take up to 179 days to complete. The indirect tests used in this study were the cutting test, the tetrazolium test, the water absorption method, the embryo excision method and the x-ray (radiography) method.

3.2 Material and methods

3.2.1 Provenances: source and handling of seeds

Details of the provenances used in some or all parts of the study are shown in Table 3.1. Apart from plants of provenance PL- Amani/Kwamkoro (Tanzania), which were donated later, it was initially intended to use ten *Maesopsis eminii* provenances (collected from three different geographical areas in Africa) in all experiments. However, only eight provenances were available for the study of seed characteristics described in this chapter. This was due to the late arrival of seed of the Ghana (I-Onwamdua) and Cameroon (J-Mount Cameroon) provenances.

The seeds of these late-arriving provenances were picked from the ground or collected directly from trees and were not used in the moisture content assessment because they were still fresh (seed of the other provenances had been dried before dispatch to the UK). In addition, it was not possible to include these provenances in the seed size assessment because the fruit was still firmly attached to the hard seed coat.

Table 3.1 Details of *Maesopsis eminii* provenances.

Code and name of provenance	Region of seed collection	Country of seed collection	Latitude	Longitude	Altitude (m)
A Rukara – Kibungo	Kibungo	Rwanda	2° 09′ S	30° 33′ E	1400
B Arboretum de Ruhande	Butare	Rwanda	2° 35′ S	29° 44′ E	1706
C Amani/Kwamkoro	Tanga	Tanzania	5° 07′ S	39° 05′ E	800-900
D Bunyala	Kakamega	Kenya	0° 20′ N	34° 45′ E	1600
E Kakamega	Kakamega	Kenya	0° 17′ N	34° 47′ E	1600
F Kisaina-5B	Kakamega	Kenya	0° 17′ N	34° 47′ E	1600
G Kisaina-4E	Kakamega	Kenya	0° 17′ N	34° 47′ E	1600
H Budongo Forest Reserve	Budongo	Uganda	1° 45′ N	31° 25′ E	1050
I Onwamdua	Nkawkaw	Ghana	6° 35′ N	0° 45′ W	Unknown
J Mount Cameroon	Dikolo Forest	Cameroon	4° 13′ N	9° 10′ E	< 50
PL Amani/Kwamkoro*	Tanga	Tanzania	5° 07′ S	39° 05′ E	800-900

* Plants donated by P. Binggeli of the University of Ulster, Coleraine.

All seeds were ordered either from forest seed centres or collected by private individuals working in research institutions. Details of seed collection dates were only available for three of the eight seedlots (the two Rwandan and the Ugandan provenances). There was no information on seed storage conditions since collection. The two Rwandan provenances were supplied by the Département de Foresterie/Centrale de graines forestières of ISAR (Institut des Sciences Agronomiques du Rwanda). Rukara seeds were collected from scattered trees in the Kibungo area, a region with semi arid/dry conditions. The Arboretum de Ruhande provenance was gathered from two plots of 0.25 ha each (with about 200-300 trees per plot) in the 200 ha Rwandan Arboretum.

The Tanzanian provenance (C-Amani/Kwamkoro), which unfortunately did not germinate, was collected from Amani forest in Tanga and supplied by the Tanzania National Seed Centre. Fortunately, a few plants (PL-Amani/Kwamkoro), grown from seeds collected from the same area (Amani forest), were donated by the University of Ulster, Coleraine, and were used to represent the Tanzanian provenance in isozyme studies (Chapter 5).

Four Kenyan provenances were supplied by the Kenya Forestry Seed Centre but only three were used in the study, as one of them (Bunyala) did not germinate. Seeds were probably drawn from old stocks, as even the remaining Kenyan provenances did not germinate satisfactorily.

The single Ugandan provenance came from Budongo Forest Reserve and was supplied by the Uganda Forest Department, Seed Section.

The Ghanaian provenance was obtained through the Cocoa Research Institute in Ghana. Seeds were collected in the locality of Nkawkaw, in the Eastern Region of Ghana, not from a forest or plantation as such, but from two trees (10-12 m tall) on an abandoned cocoa farm.

The provenance from Cameroon came from Dikolo Forest. Some seeds were picked up from the ground near two parent trees, while others were directly collected from three trees. All trees were located on the eastern foothills of Mount Cameroon.

Apart from the provenances from Ghana and Cameroon, which were sown immediately after they arrived at the University of Wales, Bangor, all other seeds were kept in a cold store (running at 5°C) until they were needed for experiments.

3.2.2 Seed moisture content

Seed moisture content was measured in the laboratories of the School of Agricultural and Forest Sciences, University of Wales, Bangor, in February 1996. Representative samples were drawn by thoroughly mixing each seedlot (provenance) to insure homogeneity. As seeds for the different provenances were provided in small quantities, mixing and sampling were done by hand. Four samples of 100 grams each were taken for each provenance.

Sampling was followed by labelling and an immediate transfer of seed lots in moisture-proof plastic bags to the testing laboratory. As seeds were taken from cold storage, they remained in the bags for about two hours to let them reach the laboratory temperature, reducing the risk of an increase in moisture content by direct exposure to warmer or moist air (both of which cause moisture condensation on seeds) in the laboratory. Seeds were removed from plastic bags, placed in aluminium foil trays and wet weight determined on a balance weighing to four decimal places. The temperature of the laboratory at the moment of weighing was about 23°C. The oven-drying method, the most common for both official testing under ISTA rules and for routine testing, was used in this study. In this method, seeds have to be dried for 17 ± 1 hours at a

low constant temperature of 103°C (see Willan, 1985 and International Seed Testing Association, 1991). After the wet weight measurements, seeds in their aluminium foil trays were placed in an oven so that air could circulate easily within it, thus allowing seeds to dry thoroughly. At the end of the oven drying, seeds were placed in a desiccator for half an hour for cooling to room temperature. Dry weight measurements were taken immediately after cooling. The calculation of moisture content was made using the formula:

$$\text{moisture content \%} = \frac{\text{original weight} - \text{oven dry weight}}{\text{original weight}} \times 100$$

3.2.3 Seed size

For each provenance, seed size measurements were carried out on four samples of 25 seeds each, taken from plastic storage bags after a thorough hand mixing. Sample preparation and seed measurements were carried out in laboratories of the School of Agricultural and Forest Sciences, University of Wales, Bangor, in March 1996. Sampling was done by hand after a thorough hand mixing. Provenances were labelled and placed in separate plastic bags prior to being measured. Measurements were made of seed length and width using digital callipers. The length of seed was taken as the distance from the point of attachment of the seed on the branch to the point opposite it. Width was measured half way along the length of the seed.

3.2.4 Seed germination and viability

3.2.4.1 Germination of seeds: sampling, pretreatment and sowing

Germination tests of the different provenances were carried out in greenhouses at the Pen-y-ffridd field station of the University of Wales, Bangor, during the period March-July 1996. Two greenhouses operating at temperatures of 20°C and 25°C were used for the study. Temperature was regulated by hot water

circulating through steel pipes along the greenhouse walls. A 16-hour supplementary illumination period (from 7 am to 9 pm) was supplied by several 400 watts vapour lamps, suspended at about 1.5 m above the greenhouse benches. Benches were covered with black capillary matting to help maintain moisture levels in plant pots. The greenhouse roof was automatically vented for aeration. The sowing medium was John Innes No.1 compost, which was used to fill seed trays and was moistened before sowing seeds. In each greenhouse, each provenance was represented by eight trays (replicates) of 50 seeds each, making a total of 400 seeds per provenance. Trays were arranged on one bench in a randomised block design with eight blocks. Each block consisted of eight trays representing the eight provenances.

To insure that samples were representative, hand mixing and sampling of seeds was done in the same way as for seed moisture content measurements (section 3.2.2). Before seeds were sown, they were soaked for three days in cold water. Seeds were sown in trays at a depth of about 2cm, and were watered immediately after sowing. Watering was done twice a day (morning and evening), with additional watering if necessary to keep seeds moist, continuing until the end of the germination period. Germinated seeds were periodically transferred from germinating trays to plastic pots (in the same greenhouse), and seedlings were left to grow on for further investigations. The potting medium was the same as that used for germination tests. Results were used to calculate final percentage germination and rate of germination (i.e. time to 50% of final germination.

3.2.4.2 Tests of seed viability: sampling, seed preparation and testing

All tests were carried out in one of the laboratories of the School of Agricultural and Forest Sciences, University of Wales, Bangor in March 1996. Mixing and sampling of samples were done by hand as described in section

3.2.2. For each provenance, four separate samples of 25 or 50 seeds each were randomly taken from plastic storage bags for use in each test.

a) Cutting test

This test was performed using a small handsaw and a pair of pliers to break the seed coat and expose the internal parts of the seed for inspection and detection of abnormalities. The four samples taken per provenance consisted of 25 seeds each. For each seed, the endosperm was inspected for its colour and the embryo for its state of development. If the endosperm and embryo were normal, then the seed was classified as viable. Following Bonner (1974, quoted by Willan, 1985), seeds with embryos that were unfirm, decayed, rancid-smelling, shrivelled, mouldy or milky and empty seeds (without embryos) were classified as nonviable. Percentage of full seeds was calculated for each provenance.

b) Tetrazolium test

For this test, a 1% tetrazolium solution was used. It was prepared according to the method given by International Seed Testing Association (1991). Two solutions were needed:

- for solution 1, 9.078g of potassium di-hydrogen orthophosphate (KH_2PO_4) were dissolved in 1 litre of distilled water;
- for solution 2, 9.472g of di-sodium hydrogen orthophosphate (Na_2HPO_4) were dissolved in 1 litre of distilled water.

Then:

- two parts of solution 1 were mixed with three parts of solution 2 to make a buffer;

- 1g of tetrazolium salt was dissolved in 100ml of the buffer solution (to make a 1% tetrazolium solution).

After drawing samples (four samples of 25 seeds each per provenance), seeds were soaked in cold water for three days for conditioning. This facilitated removal of external seed coats to expose internal seed parts as well as helping to activate enzymes and food reserves, and allowing thorough absorption of the tetrazolium by all parts of the embryo. As in the cutting test, a small handsaw and a pair of pliers were used to carefully break the seed coat. The internal thin but tough seed coat was also removed to get access to the embryos, which were then removed. Embryos were placed in tetrazolium solution for staining. During the test, samples were covered with black plastic sheets and placed in cupboards, since the reaction that occurs within the tissues is light sensitive. Seeds were left to stain for a period of 24 hours. Thereafter, they were washed with water, ready for immediate evaluation (drying out can change the appearance of the stain). Red- stained and fully turgid embryos were classified as viable, while unstained or poorly stained ones were counted as non-viable.

c) Water absorption method

This method also used four samples of 25 seeds each for each provenance. Drawing of the samples followed the same procedure as in the other indirect tests. Four 1 litre glass bottles were filled with cold tap water in which seeds were immersed for five hours. After that period, seeds absorbed water and separated into two layers: heavy seeds, assumed to be full and therefore viable, sank and stayed at the bottom of the bottles, and light seeds, assumed to be empty and therefore non-viable, floated. The two layers of seeds were sown separately at Pen-y-ffridd field station (25°C greenhouse) using the same types of tray and medium as those used for germination test (section 3.2.4.1). Seeds

were periodically inspected for germination. A final evaluation of germination was done at the end of the germination period.

d) Embryo excision test

The test was performed on four samples of 25 seeds each per provenance with some spare seeds to replace those injured during excision. A small handsaw, a pair of pliers and scalpels were used to extract embryos from other seed parts. The working area and dissecting instruments were cleaned with a 70% ethanol solution to prevent infection by pathogens and contamination of healthy embryos. Seeds were previously soaked in cold tap water for three days to help soften the outer coat. Extracted embryos were kept on blotting paper under moist conditions while preparing the growing environment. The four replicates of 25 excised embryos each (for each provenance) were placed on moistened blotting paper, laid in petri dishes and incubated in a growth cabinet providing 24 hour illumination and at a constant temperature of 20°C. The condition of the embryos was inspected daily. Petri dishes were regularly sprayed with a fungicide to prevent growth of fungi that could interfere with embryo development. For most embryos, growth started after 14-16 days incubation, and after 28 days (the time of final evaluation), those embryos with an elongated hypocotyl and radicle were classified as viable.

e) X-ray method

Samples consisting of 60 seeds per provenance were sent to the seed testing laboratory of the Forestry Commission Research Division (now Agency), Alice Holt Lodge, for x-ray examination. The equipment used was a Faxitron cabinet X-ray system (detailed specifications of the model are not available). Seeds were x-rayed using the direct radiography (x-radiography) method at 17.5 kV, 3 mA, 60 seconds, and 56 cm focus film distance. Photographs were taken on Kodak Industrex M x-ray films. The whole sample of each provenance was

exposed to radiography at once (i.e. not by replication) for financial reasons. A screen viewer was used to examine radiographic images and evaluate the condition of seeds and classify them into full or empty/diseased/damaged seeds. After the evaluation, seeds were pretreated (three days cold tap water soak) and sown at Pen-y-ffridd field station (25°C greenhouse) in trays filled with John Innes No.1 compost.

3.2.5 Data analysis

Analysis was performed using Minitab statistical packages. Data for seed moisture content, seed length and seed germination were normally distributed and were subjected to analysis of variance. Data on seed width had a slightly skewed distribution, but analysis of variance was also applied since the very few outliers were not expected to affect the outcome of the analysis. However, results from seed width data should be treated with caution. Where differences between provenances were significant, Tukey's tests were applied (at the 95 % level). Principal component analysis (PCA) of seed size (i.e. length and width) data was used to group provenances on the basis of their seed dimensions, and the degree of discrimination between provenances (using squared distances) was done using discriminant function analysis (DFA). Results of the seed viability tests were compared with final seed germination percentages to determine the extent to which the viability tests overestimated or underestimated germination.

Paired t-tests were carried out on data from the water-absorption and x-ray tests and the corresponding final seed germination percentages.

3.3 Results

3.3.1 Seed moisture content

Provenances C (Amani/Kwamkoro, Tanzania) and D (Bunyala, Kenya) failed to germinate, and only results for the remaining six provenances are presented here. Mean values of seed moisture content of the six provenances are presented in Table 3.2. The differences in seed moisture content between provenances were statistically significant ($P \leq 0.05$) (see Table A.1 in appendix A). Tukey pairwise comparisons indicated that, of a total of 15 pairwise comparisons, nine (i.e. 60%) were significantly different (Table 3.3). The range of moisture content was from 10.50% to 12.54%, the lowest moisture content being for the Kisaina 4E (Kenya) and the highest for the Iroro/Kakamega (Kenya) provenance.

Table 3.2 Seed moisture content of six *Maesopsis eminii* provenances.

Provenances		Moisture Content (%)
A Rukara-Kibungo	RWANDA	11.47
B Arboretum de Ruhande	RWANDA	11.93
E Iroro –Kakamega	KENYA	12.54
F Kisaina 5B	KENYA	12.06
G Kisaina 4E	KENYA	10.50
H Budongo Forest Res.	UGANDA	11.53

Table 3.3 Results of Tukey's pairwise comparison test for significance of differences in seed moisture content between six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	NS					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	*	NS	NS			
G-Kisaina 4E-Kenya	*	*	*	*		
H-Budongo Forest Reserve- Uganda	NS	NS	*	NS	*	

* $P \leq 0.05$; NS not significant.

A plot displaying the relationship between seed moisture content and final germination percentage is shown in Figure 3.1. The correlation between those two parameters was low and negative (–0.124, not significant), implying an inverse relationship, but there is little sign in Figure 3.1 of a linear relationship.

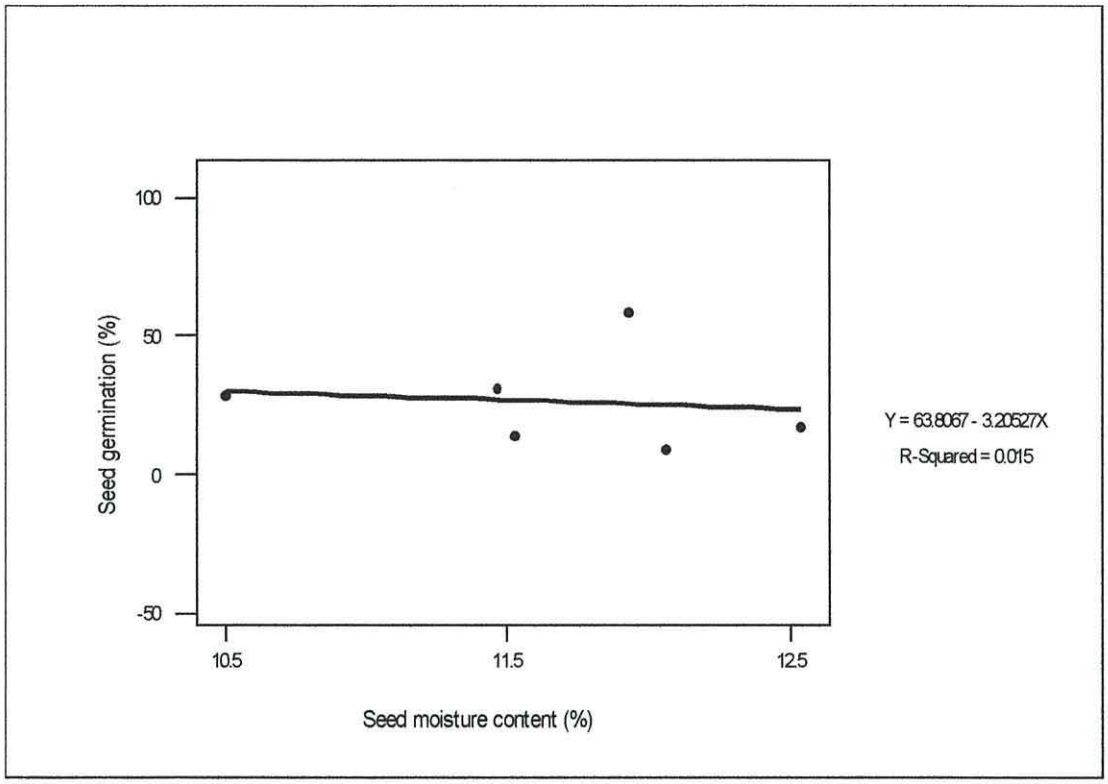


Figure 3.1 Relationship between seed moisture content and final germination percentage in six *Maesopsis eminii* provenances.

3.3.2 Seed size

Mean values of seed length and seed width are presented in Table 3.4. The differences in seed length between provenances were statistically significant ($P \leq 0.05$) (see Table A.2 in appendix A). Tukey pairwise comparisons indicated that, of a total of 15 pairwise comparisons, 12 (i.e. 80%) were significantly different in seed length (Table 3.5). The highest and lowest values for seed length were 24.72 mm and 18.71 mm for provenances Rukara–Kibungo/Rwanda) and Budongo Forest Reserve/Uganda respectively.

Of the total variation in seed length, almost half (49.5%) was accounted for by provenances, and the other half (50.5%) by seeds within provenances (Table 3.6).

Table 3.4 Summary statistics (mean, standard deviation, and coefficient of variation) for seed length and width of six *Maesopsis eminii* provenances. Full names of provenances are given in Table 3.1.

Provenance	Seed characters measured					
	Seed length			Seed width		
	Mean (mm)	Standard deviation (mm)	Coefficient of variation (%)	Mean (mm)	Standard deviation (mm)	Coefficient of variation (%)
A Kibungo Rwanda	24.72	2.195	8.88	12.47	0.695	5.61
B Arboretum de Ruhande Rwanda	21.67	2.192	10.11	11.78	0.837	7.10
E Kakamega Kenya	20.11	2.061	10.24	11.69	1.014	8.67
F Kisaina 5B Kenya	20.74	1.537	7.41	11.88	1.015	8.54
G Kisaina 4E Kenya	20.56	1.541	7.49	11.35	1.300	11.45
H Budongo Forest Res. Uganda	18.71	1.586	8.47	10.36	0.793	7.65
Overall Mean	21.09	1.852	8.766	11.58	0.94	8.17

Table 3.5 Results of Tukey's pairwise comparison test for significance of differences in seed length between six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	*					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	*	*	NS			
G-Kisaina 4E-Kenya	*	*	NS	NS		
H-Budongo Forest Reserve- Uganda	*	*	*	*	*	

* $P \leq 0.05$; NS not significant.

Table 3.6 Amount of variation accounted for by provenances and by seeds within provenances in two seed characteristics of *Maesopsis eminii.*

Seed characters	Variation accounted for by provenances %	Variation accounted for by seeds within provenances %
Length	49.5	50.5
Width	29.5	70.5

For seed width, results of analysis of variance also showed significant ($P \leq 0.05$) differences between provenances (see Table A.3 in appendix A). As for seed length, provenance Rukara- Kibungo (Rwanda) had the highest value (12.47 mm) and the Ugandan provenance the lowest (10.36 mm) (Table 3.4). Of a total of 15 pairwise comparisons made with Tukey's test, 11 pairs (73 %) were significantly different (Table 3.7). Differences within provenances accounted for more than twice (70.5%) the amount explained by differences between provenances (29.5%) (Table 3.6).

Table 3.7 Results of Tukey's pairwise comparison test for significance of differences in seed width between six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	*					
E-Kakamega-Kenya	*	NS				
F-Kisaina 5B-Kenya	*	NS	NS			
G-Kisaina 4E-Kenya	*	*	NS	*		
H-Budongo Forest Reserve-Uganda	*	*	*	*	*	

* $P \leq 0.05$; NS not significant.

PC1 and PC2 scores derived from principal component analysis of seed dimensions (i.e. length and width) are shown in Figure 3.2. On the basis of seed dimensions, the provenances could (arguably) be classified into five groups (Figure 3.2, and see discussion in section 3.4).

Group 1 A (Rukara/Kibungo, Rwanda);

Group 2 B (Arboretum de Ruhande, Rwanda);

Group 3 E (Kakamega, Kenya) and F (Kisaina 5B, Kenya);

Group 4 G (Kisaina 4E, Kenya Kenya);

Group 5 H (Budongo Forest Reserve, Uganda).

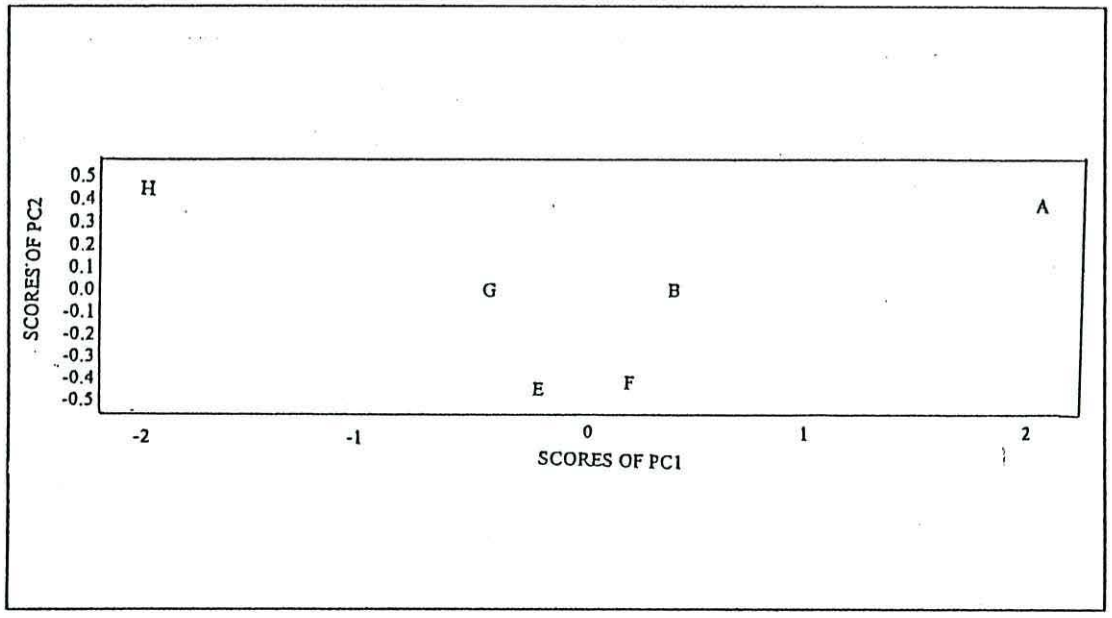


Figure 3.2 Results of principal component analysis of seed size (length and width) of six provenances of *Maesopsis eminii*. Scores of PC1 and PC2 accounted for 92.1% and 7.9% of the total variability respectively.

Discriminant function analysis showed that the overall proportion of correctly classified measurements based on seed dimensions (i.e. length and width) was only moderate (64.4 %) (Table 3.8). However, squared distances (Table 3.9) between seed dimensions indicate considerable discrimination, since the

number of pairs of provenances for which discrimination was possible was 14 out of 15 (93.3%).

Table 3.8 Summary of results of classification using seed length and width of provenances by discriminant analysis.

Suggested group/provenance	True group (provenance)					
	A	**B**	**E**	**F**	**G**	**H**
A	**22**	2	0	0	0	0
B	0	**11**	2	2	1	0
E	0	3	**10**	2	4	1
F	0	4	8	**14**	5	0
G	0	2	2	2	**8**	1
H	0	0	0	2	4	**20**
Total N	22	22	22	22	22	22
N correctly classified	**22**	**11**	**10**	**14**	**8**	**20**
Proportion of correctly grouped/ classified measurements	**100%**	**50%**	**45.5%**	**63.6%**	**36.4%**	**90.9%**
Overall proportion of correctly grouped/classified measurements	**64.4%**					

Table 3.9 Squared distances (top line) and test statistic values (bottom line) between provenances, generated by discriminant function analysis of seed dimensions (seed length and width).

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda		9.540 51.222*	21.915 17.665*	17.494 93.925*	18.223 97.843*	40.867 219.414*
B-Arboretum-Rwanda			2.637 14.158*	1.667 8.950*	1.624 8.716*	13.014 69.874*
E-Kakamega-Kenya				0.476 2.555	1.097 5.889*	7.909 42.464*
F-Kisaina 5B-Kenya					1.860 9.987*	11.916 63.978*
G-Kisaina 4E-Kenya						5.545 29.774*
H-Budongo For. Uganda						

NB. Small squared distances between pairs of provenances indicate similarity, while larger ones indicate dissimilarity.

* Pairs for which discrimination between provenances is possible. In 93.3% of cases, the test statistic values exceed the critical value at $p \leq 0.05$ (i.e. 3.23), suggesting that discrimination using seed dimensions is justified.

3.3.3 Seed germination

Results of seed germination tests of the six provenances sown in the two growing environments (i.e. greenhouse 1= 20°C and greenhouse 2 = 25°C) are illustrated in Figure 3.3. Temperature had no significant effect on final germination percentage; the difference between the two growing environments was less than 1%. There were significant ($p \leq 0.05$) differences in final germination percentage between the different provenances (see Table A.4 in appendix A). Differences between provenances accounted for 79% and differences between replicates for 17.9% of the total variation in final germination percentage. Germination ranged from 58.8% to 9%, with only the Arboretum de Ruhande provenance showing a final germination above 50%.

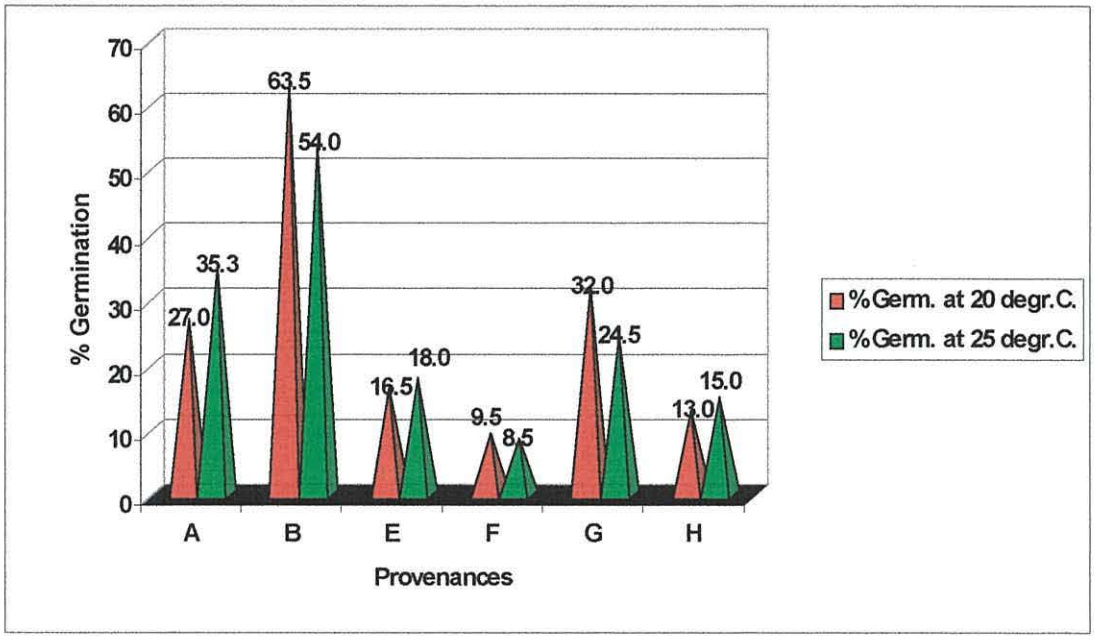


Figure 3.3 Final percentage germination of seed of six *Maesopsis eminii* provenances at two temperatures (20°C and 25°C). Codes for provenances as in Table 3.1.

Tukey's tests showed that 73% of provenance pairs differed significantly in final germination percentage (Table 3.10). There was also a significant ($P \leq 0.05$) provenance x temperature interaction in final germination percentage (Table A.4, appendix A). However, the interaction accounted for only 2.6% of the total variation in seed germination, and was caused mainly by the poorer germination of provenances B (Arboretum de Ruhande, Rwanda) and G (Kisaina 4E, Kenya) at 25°C (Figure 3.4).

Table 3.10 Results of Tukey's pairwise comparison test for significance of differences in germination (mean final germination percentage of seeds sown at at 20°C and 25°C) of six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	*					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	*	*	NS			
G-Kisaina 4E-Kenya	NS	*	*	*		
H-Budongo Forest-Uganda	*	*	NS	NS	*	

* $P \leq 0.05$; NS not significant.

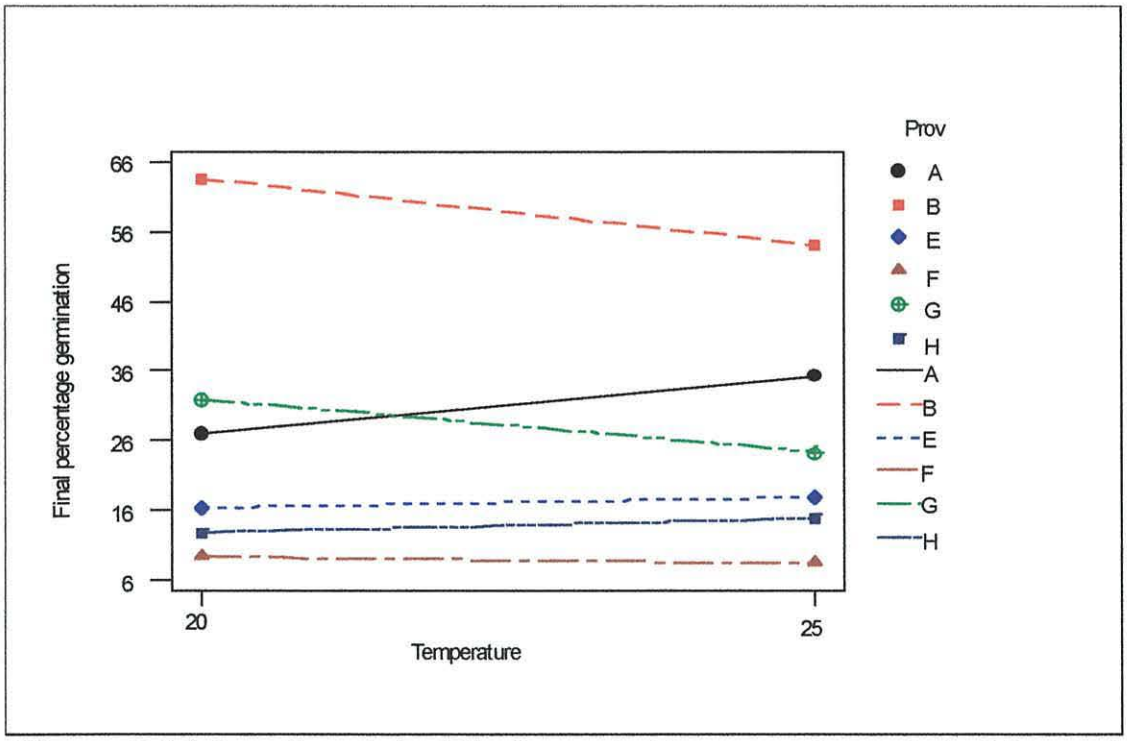


Figure 3.4 Interaction plot of final germination percentage of six Maesopsis eminii provenances sown at two temperatures (20°C and 25°C). Codes for provenances as in Table 3.1.

Although the growing environment (temperature) did not affect the final germination percentage, it had an effect on the speed of germination. Seeds germinated 14 days earlier in the 25°C than in 20°C greenhouse, and on average, germination was complete 42 days earlier at 25°C than at 20°C. Details of seed emergence and germination period are presented in Table 3.11.

The rates of germination (i.e. the number of days required to attain 50% of final germination) derived from germination curves (Figures 3.5 and 3.6 for 20°C and 25°C respectively) are presented in Table 3.12. At 20°C, the time to 50% of final germination varied from 55 to 89 days (the lowest value being for the Kisaina 5B (Kenya) provenance). At 25°C it varied from 33 to 49 days and the Rukara/Kibungo (Rwanda) provenance had the lowest value.

Table 3.11 Time (days) between seed sowing and emergence, and germination period, for six provenances of *Maesopsis eminii* at two temperatures (20°C and 25°C).

Provenance	20°C				25°C			
	Seedling emergence	Minimum period for germination to complete	Maximum Period for germination To complete	Average Period for germination to complete	Seedling emergence	Minimum period for germination to complete	Maximum Period for germination to complete	Average period for germination to complete
	(days after sowing)	(days)	(days)	(days)	(days after sowing)	(days)	(days)	(days)
A Kibungo Rwanda	42	95	137	110	28	49	69	61
B Arboretum Rwanda	40	102	132	123	25	45	69	52
E Kakamega Kenya	44	74	137	99	33	43	81	59
F Kisaina 5B Kenya	53	64	113	85	36	43	78	57
G Kisaina 4E Kenya	50	97	137	123	37	69	104	82
H Budongo F. Uganda	53	74	133	99	38	49	102	77
Average (for all prov.)	**47**	**84**	**132**	**107**	**33**	**50**	**84**	**65**

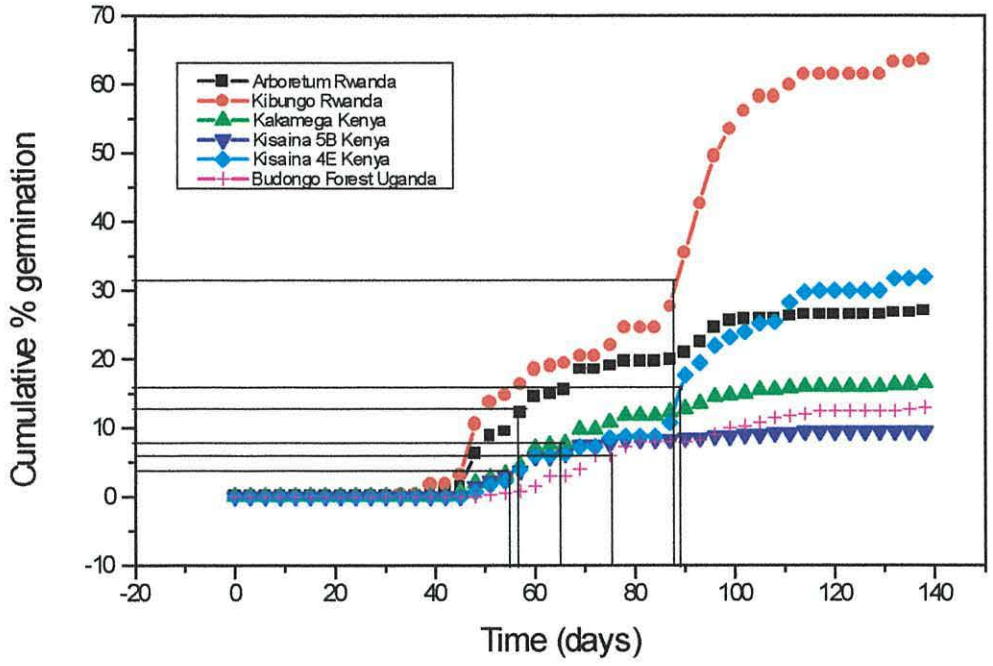


Figure 3.5 Germination curves for six *Maesopsis eminii* provenances at 20°C. (Horizontal lines show the values of 50% of final germination and vertical lines the times taken to reach these values).

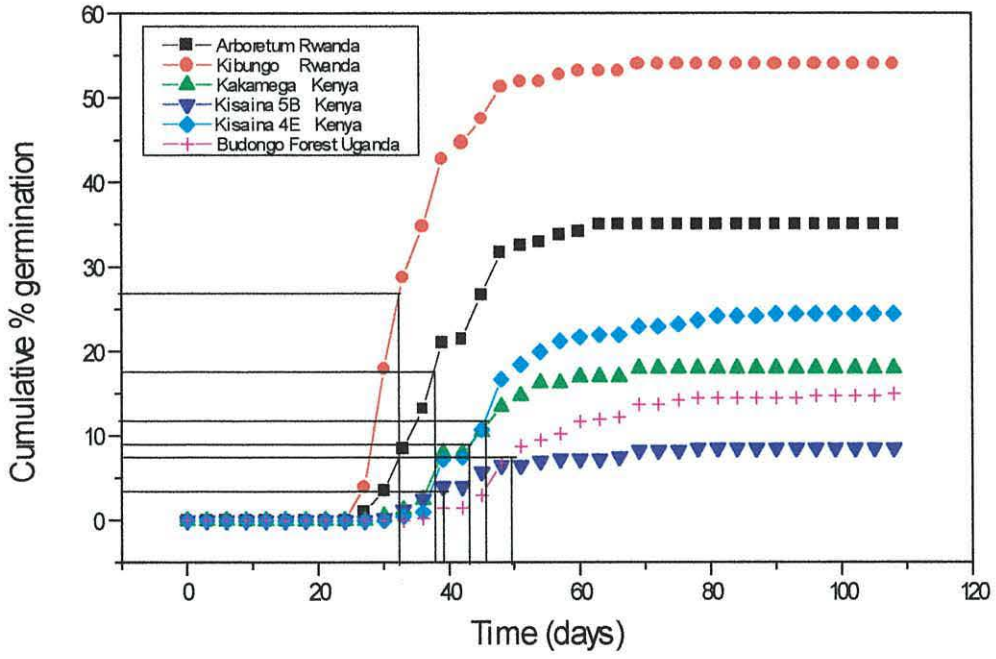


Figure 3.6 Germination curves for six *Maesopsis eminii* provenances at 25°C. (See Figure 3.5 for details).

Table 3.12 Rate of germination (time to 50% of final germination) for six Maesopsis *eminii* provenances at two temperatures (20°C and 25°C).

Number of days to 50% of final germination		
Provenance	**20°C**	**25°C**
A- Rukara/Kibungo RWANDA	88	33
B- Arboretum de Ruhande RWANDA	57	38
E- Kakamega - KENYA	66	43
F- Kisaina 5B - KENYA	55	39
G- Kisaina 4E - KENYA	89	46
H- Budongo Forest Reserve UGANDA	76	49

3.3.4 Seed viability

Detailed results of the seed viability tests for the six *Maesopsis eminii* provenances are presented in Table 3.13. The tests gave different results from the seed germination tests (section 3.3.3). Some of them suggested higher seed viability (i.e. x-ray, cutting and water absorption methods) while others (i.e. embryo and tetrazolium tests) suggested lower viability for individual provenances. Figure 3.7 shows the extent to which seed viability tests underestimated or overestimated final seed germination percentage determined in tests carried out in greenhouse conditions.

Final germination percentages of sinkers (water absorption method) and full seeds (x-ray method) are presented in Table 3.14. Paired t-tests indicated that there were significant differences ($P \leq 0.05$) between results of viability tests and corresponding germination percentages (see Tables A.5 and A.6 in appendix 1). Overall, there was a considerable overestimation of viability, as

only 35.2% of sinkers (water absorption method) and 12.5% of full seeds (x-ray method) germinated.

Table 3.13 Results of viability tests for seeds of six *Maesopsis eminii* provenances.

PROVENANCE	S E E D V I A B I L I T Y T E S T S				
	Cutting test full seeds (%)	**Tetrazolium test fully stained seeds (%)**	**Water absorption method**	**Embryo excision**	**X-ray method**
			Sinkers (%)	**Growing embryos (%)**	**Full seeds (%)**
A Rukara/Kibungo Rwanda	81.8	12.9	17.0	0.0	95.0
B Arboretum de Ruhande Rwanda	72.7	3.6	3.0	30.1	95.0
E Kakamega Kenya	66.7	10.3	34.0	0.0	96.6
F Kisaina 5B Kenya	50.0	4.2	42.0	0.0	91.6
G Kisaina 4E Kenya	75.0	21.7	55.0	3.8	95.0
H Budongo Forest Reserve Uganda	80.0	16.6	26.0	0.0	96.6
Overall mean	**71.0**	**11.5**	**29.5**	**5.6**	**95.0**

Table 3.14 Final seed germination percentage of sinkers and floaters (water absorption method) and full seeds (x-ray method) in six *Maesopsis eminii* provenances.

PROVENANCE	WATER ABSORPTION METHOD		X-RAY METHOD
	SINKERS	FLOATERS	FULL SEEDS
	Germinated seeds (%)	Germinated seeds (%)	Germinated seeds (%)
A Rukara/Kibungo Rwanda	64.7	39.8	20.0
B Arboretum de Ruhande Rwanda	66.7	51.5	40.0
E Kakamega Kenya	20.6	13.6	5.0
F Kisaina 5B Kenya	23.8	13.8	1.7
G Kisaina 4E Kenya	16.4	11.1	5.0
H Budongo Forest Reserve Uganda	19.2	21.6	3.4
Overall mean	35.2	25.2	12.5

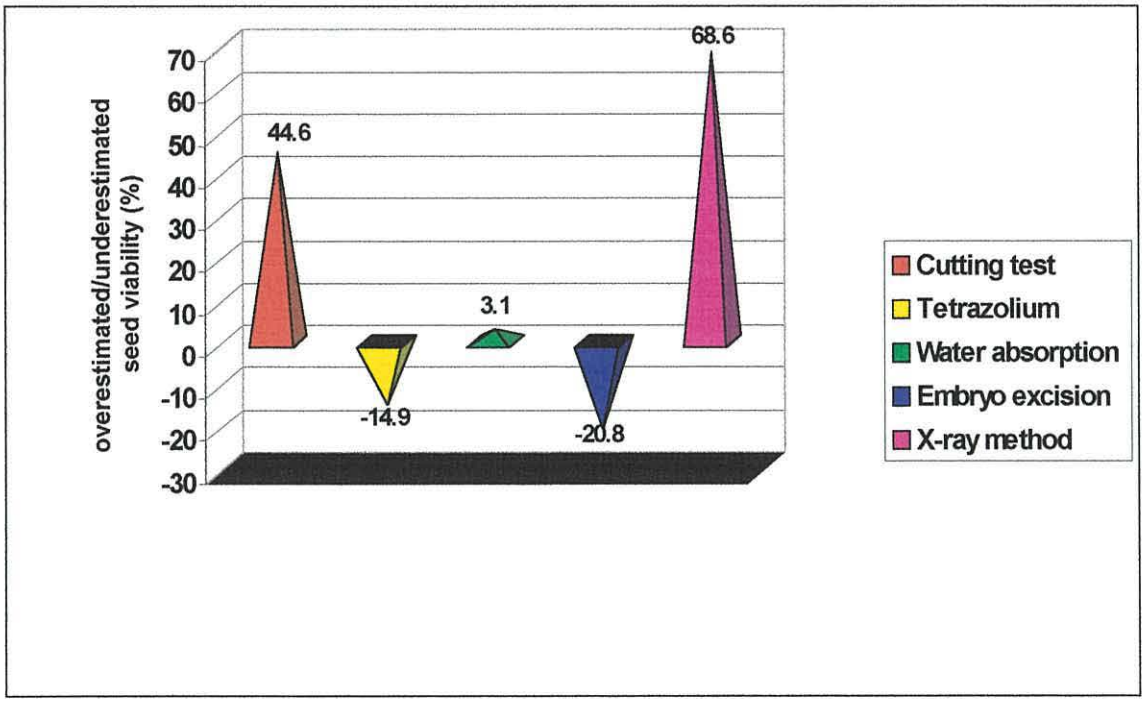


Figure 3.7 Percentage by which seed viability tests overestimated or underestimated final seed germination percentage.

3.4 Discussion

Seed moisture content was found to differ significantly between provenances. The range of the moisture contents displayed by different seed provenances suggests that seeds were handled differently (especially during drying and storage) in their countries of origin or that they changed moisture content during transport to the UK. This is possible because of their hygroscopic nature; when detached from the parent tree, for example, they lose or gain moisture content to or from the surrounding atmosphere. Dry seeds surrounded by moist air will gain moisture (Willan, 1985).

For orthodox seeds, moisture content is probably the single most important factor determining seed longevity (Holmes and Buszewicz, 1958, cited by Willan, 1985). This is the reason why it is necessary to reduce the moisture content, which results in the reduction of respiration and a subsequent slowing down of seed ageing, in order to store such seeds. A moisture content of 4 to 8% is considered safe for most orthodox seeds (Willan, 1985). For the *Maesopsis eminii* seeds used in this study, the lowest moisture content was 10.50%.

Moisture content values were determined, not for use in the genetic differentiation of the provenances, but to check for their effect on seed germination. It has been reported by Bhumibhamon (1980) that moisture content is among the factors which can significantly contribute to the deterioration of seed viability, as it influences the metabolic rate of seeds. Moreover, Baldwin and Holmes (1955) pointed out that the viability of unsatisfactorily dried seeds is more affected by their moisture content than the temperature of storage. In this study, the correlation coefficient between those two parameters was low and negative (–0.124, not significant) implying that moisture content and germination are weakly but negatively correlated (i.e. an

increase in moisture content of seeds results in a decrease in germination percent). However, no obvious linear relationship between variables was observed (Figure 3.1). The range of moisture contents of the different provenances was probably not wide enough to show satisfactorily its effect on germination. Although it appears that seeds are more likely to loose their viability at higher moisture contents, the effect was minimal.

In previous studies such that of Yap and Wong (1983), there was no linear relationship between seed moisture content and germination percentage, but the latter tended to decrease at higher moisture contents. Yap and Wong (1983) did not calculate the correlation coefficient between germination percentage and moisture content, but based on the data they present, its value is -0.535, a non-significant ($P > 0.05$) and negative correlation similar to the one calculated in this study.

The poor germination of seeds in this study may have been caused by the length of time seeds were stored before the tests. As reported by various authors (see section 2.1.2.2), *Maesopsis eminii* seeds do not maintain their viability for long. Perhaps the Rwandan provenances gave better results than others because they were stored for a short period (3-4 months) before sowing. In the Ugandan provenance, germination decreased from 70% (as indicated on the papers accompanying the seedlot) to 14% (mean final germination percentage obtained in these experiments) after eight months of storage.

The temperature of the growing environment (i.e. greenhouse) had a significant effect on the speed of seed germination. Seeds sown in the 25°C greenhouse germinated faster than those sown at 20°C. Similar effects of temperature on seed germination have been reported in other seed studies, such as that on *Melia volkensii* seeds carried out by Milimo and Hellum (1990). In this study

the highest rate of germination was observed at 37°C and the lowest at 25°C. The rate of germination increased with increasing temperature. Other results were reported by Corbineau and Come (1986). They found that the optimal germination temperature for *Shorea roxburghii* and *Hopea odorata* seeds was in the range 30°C-35°C, and that below 30°C germination became slower. Although the temperatures used for *Maesopsis eminii* seeds in this study and those used for other studies are different, there is a general trend of an increase of germination speed (rate) as the temperature of the growing environment increases. However, there must be a temperature limit or optimum for all species, and indeed some of seeds in Corbineau and Come's (1986) studies failed to germinate or germinated poorly at temperatures above 37°C. A thorough investigation of the effect of temperature regime(s) on the germination of *Maesopsis eminii* seeds would be very informative.

Seed dimensions were found to be statistically different between provenances. The two Rwandan provenances had the greatest length, while the Ugandan provenance had the lowest. All Kenyan provenances had seed lengths which were less than those of the Rwandan provenances but greater than those of the Ugandan provenance. The same trend was observed for seed width except in one Kenyan provenance (Kisaina 5B). There is no obvious explanation for these differences in seed dimensions, but genetic factors are likely to be important. There is speculation in the literature that seed size is highly heritable. For example, Harper (1977, cited by Ibrahim, 1996) suggests that seed size is not only heritable but also strongly affected by natural selection. Variation in seed size may also be attributed to maternal effects. Chaisurisri *et al.* (1992) reported that of the seed and seedling characteristics assessed in their study, most of the variation in seed size was due to maternal genetic influences. Roach and Wulf (1987, cited in Ibrahim, 1996) reported that the maternal environment strongly affects traits early in the life cycle of plants.

The superior seedling height of Rwandan provenances (Chapter 4) may partly be due to a higher seed germination rate, which resulted in early production of seedlings, but their larger seed size (i.e. length) could also have contributed to a certain extent. Harlan *et al.* (1976) stated that seedling vigour is closely related to seed size and the energy stored in the endosperm. If it could be shown (in further studies) that larger *Maesopsis eminii* seeds produce larger seedlings, then selection could be directed at choosing large-seeded provenances which would presumably produce vigorous plants capable of growing fast (and giving early timber yields), or competing/surviving in harsher environmental conditions.

In discriminating between provenances on the basis of seed dimensions (seed length and width), five groups were identified. However, it is also possible to interpret Figure 3.2 as showing three groups: provenance A (Rukara/Kibungo, Rwanda) in group 1, provenances B (Arboretum de Ruhande, Rwanda), E (Kakamega, Kenya), F (Kisaina 5B) and G (Kisaina 4E, Kenya) in group 2, and provenance H (Budongo Forest Reserve, Uganda) in the third group.

It is difficult to explain these groups without knowing the historical events surrounding the movement of seed from one place to another in this seemingly restricted geographical area. According to Palmblad (1968), seed size is one of the least plastic traits of trees and can be used in some cases to identify seed collections of unknown provenances. In this study, however, only two seed characteristics (seed length and width) were assessed, making it difficult to decide whether the five or the three groups is correct. In further studies, more seed characteristics such as seed weight, colour and form should also be assessed to confirm or invalidate the grouping suggested in this study. Discrimination on the basis of growth and morphological characteristics

(Chapter 4) can also provide information to help determine which group (s) different provenances belong to.

Of the tests of seed viability, the x-ray method, the cutting test and the water absorption method overestimated final germination percentage, while the tetrazolium and embryo excision tests underestimated it (Figure 3.7). While it was possible in some tests (e.g. water absorption and x-ray methods) to assess seed viability indirectly and then use the same seeds for conventional germination tests to check the reliability of the viability tests, it was impossible in others (i.e. the cutting, tetrazolium and embryo tests) because seeds were destroyed during the tests. Another issue of concern is that, in some tests, seed viability assessment was based on theoretical assumptions. For instance, in the water absorption method, seeds which sank (sinkers) to the bottom of the container after absorbing water were assumed to be viable, while the floating ones (floaters) were classified as non-viable. Similarly, in the cutting test, seeds with normal embryos, full and free from insect attack, were counted as viable, while the non-viable ones were assumed to be those which were empty, attacked by insects or which had damaged embryos. For the x-ray method, seeds which appeared full and normal on x-ray photographs were taken as viable, and the empty or apparently diseased ones as non-viable. However, such classification or categorisation may be unreliable since a full or sinking seed is not necessarily viable and a floating one is not necessarily non-viable. For the water absorption and x-ray methods, totally different results (low germination) were obtained when the same seeds were sown to verify whether the tests predicted correctly the germinability of seeds. For each of the two methods, a paired t-test indicated that there was a significant difference ($P \leq 0.05$) between results of the indirect (viability) test and germination test.

When compared with the overall final seed germination percentage, the water absorption method gave, overall, a good estimate of germination percentage (Figure 3.7). To some extent, the embryo excision and tetrazolium tests could be said to have also given a reasonable appreciation of seed viability. However, when germination tests of sinkers and floaters were carried out, the water absorption method appeared misleading. Seeds which it classed as viable (i.e. sinkers) gave comparatively low germination percentages, and those classed as non-viable (floaters) often germinated (see section 3.3.4). In the embryo excision test, embryos would either grow or not, thus giving a clear visual appreciation of viability. However, the test is not easy to carry out (it needs very careful handling to avoid damaging embryos) and fungal attack can prevent embryos from growing, thus giving a misleading impression of seed viability. For the tetrazolium test, stained embryos (though difficult to assess) could with little hesitation be accepted as viable, as staining is a good sign of viability. Use of the tetrazolium test needs training and experience in the assessment of seed viability. The cutting test and the x-ray method appear to be very unreliable for estimating *Maesopsis eminii* seed viability, and this is true for all provenances. As far as efficiency is concerned, it has been shown that the x-ray method is usually quicker and easier to perform and interpret than the cutting test (Simak, 1990). According to the same author, the x-ray method has the advantage of being non-destructive so that seeds analysed by the x-ray method can be used in subsequent germination tests. In contrast, the cutting test does not offer the possibility of using the seeds for germination tests. To check its reliability, germination has to be performed on a different seed sample, and the frequency of empty seeds can vary considerably among replicates of a sample (Gordon and Wakeman, 1978, cited by Simak, 1990). Therefore, germination capacity calculated on the basis of full seeds determined by the cutting test is less reliable than that based on the x-ray method. According to Chaichanasuwat *et al.* (1990), there are cases where the x-ray method is

efficient in predicting the germination capacity of certain tropical species (e.g. *Peltophorum pterocarpum)*. In this study, however, the low germination of seeds which appeared to be full and therefore viable seeds under x-rays suggests that it would be a serious mistake to rely on the x-ray method to assess *Maesopsis eminii* seed viability. The flotation method, though reported to be an effective method of seed separation for a number of forest tree species (Simak, 1973, cited by Haines and Gould, 1983), did not work for *Maesopsis eminii* seeds since a good proportion of germinable seeds floated. This may have been due to the fact that the density difference between full and empty seeds was very small (in each provenance considered individually). As a result and given that separation on the basis of the type of flotation principle used in the water absorption method is of rather limited application (Simak 1973, cited by Haines and Gould, 1983), it is not recommended for use in the determination of *Maesopsis eminii* seed viability.

CHAPTER 4: PROVENANCE VARIATION: SEEDLING GROWTH AND MORPHOLOGICAL CHARACTERISTICS

4.1 Introduction

Genetic variation is considered the most important determinant of the ability of forest tree populations to survive in temporarily and spatially heterogeneous environmental conditions (Müller-Starck and Ziehe, 1991, cited by National Academy of Sciences, 1991). In order to choose populations or provenances from which to select the best performing individuals, identification and assessment of potential seed sources is essential. The most practical approach is often to describe geographic variation patterns and select at this level first, and then select within the best geographic zones afterwards. Genetic variation is measured in various ways, one being the assessment of morphological characteristics/traits which may discriminate between different populations.

Provenance testing is performed by collecting seeds from different populations and assessing variation in performance (e.g. height, diameter, yield) in plants raised from these seeds under uniform environmental conditions at one or more planting sites (National Academy of Sciences, 1991). As geographic variation in environmental conditions has resulted in great genetic diversity in species with wide distributions, it should be possible to identify provenances of a given species which possess useful attributes for afforestation programs in particular areas.

Provenance variation in the species used in this study (*Maesopsis eminii*) is not well understood. Provenances from central and east Africa are thought to be closely related, and there appears to be a clear distinction between them and the west African provenances which are characterised by their relatively small size. It would be very useful to identify morphological features which could be used to distinguish between provenances, and to determine the extent of provenance

variation in characters of interest to growers and users of *Maesopsis eminii*. This chapter presents the results of a greenhouse study of provenance variation in selected growth and morphological characteristics of *Maesopsis eminii* seedlings.

The growth and morphological characteristics assessed on seedlings were:

- total height;
- root collar diameter;
- number of first order branches;
- number of second order branches;
- branch angle (second branch on main stem);
- number of leaves on the main stem;
- number of leaves (second branch on main stem);
- branch length (second branch on main stem);
- leaf area (first leaf on second branch);
- petiole percentage (first leaf on second branch);
- leaf shape index (first leaf on second branch);
- leaf area (largest leaf on second branch);
- petiole percentage (largest leaf on second branch);
- leaf shape index (largest leaf on second branch).

4.2 Material and methods

4.2.1 Provenances

Detailed information on the provenances used in the assessment of growth and morphological characteristics is shown in Table 3.1 (Chapter 3). Only six of the provenances (A, B, E, F, G, H) listed in Table 3.1 were used in the study described in the following sections.

4.2.2 Experimental details

Experiments were carried out at the Pen-y-ffridd field station of the University of Wales, Bangor. Seedlings came from the germination tests carried out in two greenhouses (see Chapter 3).

During the experiment, the temperature in the greenhouses was kept constant and was regulated by circulating hot water in metal pipes arranged along the greenhouse walls. Lighting was provided from 7 am to 9 pm by high pressure sodium vapour lamps of 400 watts each, suspended about 1.5 m above the benches. These benches were about 1 m high and supported by metal frames. A capillary matting was spread over the benches to help retain moisture in the potting medium after watering.

As soon as seeds germinated (see Chapter 3), they were pricked out into plastic pots filled with John Innes No.1 compost and then placed on different benches in the same greenhouse. Fifty four (54) seedlings per provenance were retained in each greenhouse. Seedlings were watered twice a day (morning and evening). At the end of every week, fumigation was done to control pests. Also, each time seedlings were attacked by insects, an insecticide was sprayed. From two months after potting until the end of the experiment, seedlings were fed weekly with liquid fertiliser (Vitax/Vitafeed 111 19:19:19). The experiment was carried out during the period April 1996 to January 1997.

In each greenhouse, the experimental design adopted was a randomised complete block design with two blocks (benches) of six plots (provenances) and 27 plants per plot, making a total of 54 seedlings per provenance in each greenhouse.

Initial height and collar diameter were measured immediately after potting. The final height and collar diameter growth, and morphological measurements were taken on potted seedlings after 10 months of growth in the greenhouses. Height from the root collar to the tip of the main shoot was measured with a ruler to the nearest centimetre. Root collar diameter was measured with a micro-calliper to the nearest millimetre. The number of leaves on the main stem was determined by counting and recording all living leaves present on the main stem of each seedling. The number of first order branches was obtained by simply counting all branches on the main stem. The same procedure was done for second order branches by, this time, counting all branches on all first order branches. Branch angle was taken as the angle between a first order branch and the main stem. It was measured on the second branch from the top of the main stem using a protractor. The number of leaves on this branch was counted and the length of the branch was measured. For leaf area, two leaves were taken: the first (nearest to the main stem) and the largest leaves on the same sample branch (i.e. the second branch from the top of the main stem). The two leaves were measured separately using a portable area meter (type Li-COR-3000A) fitted with a transparent belt conveyor. The same leaves were used to estimate the petiole percentage (i.e. the percentage of the entire leaf length taken up by the petiole).

This was calculated as

$PP = 100 \times P/(L+P)$, where

PP = petiole percentage

P = petiole length

L = leaf length (from base to tip excluding the petiole) .

Leaf shape index was calculated for the same two leaves as

$$LSI = \frac{W3 - W1}{W2}, \quad \text{where}$$

W1, W2 and W3 are the widths of the leaf outline measured at 1/4, 1/2 and 3/4 of the lamina length from the apex.

4.2.3 Data analysis

Before data on seedling growth were analysed, mean monthly height and root collar diameter increments were calculated as (final height or diameter – starting height or diameter)/10.

Data were subjected to analysis of variance using balanced anova (for complete data sets) or general linear model (GLM) (for unequal data sets) procedures in Minitab. These methods are not usually used for class/count data such as those for number of branches and number of leaves. These data were analysed using the chi-square test.

Apart from leaf area (first leaf on second branch) data, which were not entirely normal due to the presence of a few outliers, data on all traits assessed in the experiment, including numbers of branches and leaves, were normally distributed. As leaf area (largest leaf on second branch) data were normal and analysis of variance was applied to them, it was also decided to use the same method for analysis of leaf area of the first leaf on the second branch. Analysis of variance is a robust technique even when data are not entirely normal. Following analysis of variance, where differences were statistically significant, a Tukey's pairwise comparison was performed to determine which pairs of provenances were significantly different. Where appropriate, correlation

analyses were also performed between variables. Principal component analysis was used in an attempt to reduce and simplify data and reveal combinations of characteristics that could be used to distinguish between provenances. Discriminant function analysis (DFA) was used to check the classification of provenances using assessed traits and to determine the precision with which the traits could be used to allocate individual trees to provenance. Distance measures and the F test statistic were also used to assess the statistical validity of the classification system.

4.3 Results

Means, standard deviations and coefficients of variation of all growth and morphological characteristics are presented in Table 4.1. Comparisons of provenance means for each of the characteristics are presented in Figures 4.1 - 4.14. Analysis of variance and chi square tests showed that provenance differences in growth and morphological characteristics were statistically significant ($P \leq 0.05$), except for the number of leaves on the main stem, the number of first order branches and the number of leaves on the second branch (see Tables B.1-B.14 in appendix B). Temperature also had an effect on most of the characteristics assessed. Thus, there were statistically significant ($P \leq 0.05$) differences between temperatures (greenhouses) for most characteristics except the leaf shape index of the first leaf on the second branch (see Table B.7 in appendix B). The provenance x temperature interaction was statistically significant ($P \leq 0.05$) for six of the characteristics assessed (excluding count data, which were analysed using the chi square test).

Growth characteristics (height and diameter growth) and some morphological characteristics (i.e. the number of leaves on the main stem and the number of first order branches) had higher values in the 25°C greenhouse. The rest of the

characteristics had higher values in the 20°C greenhouse, except for number of leaves on the second branch where three provenances had higher values in the 25°C greenhouse, and three other provenances in the 20°C greenhouse. For leaf shape index (first leaf on the second branch), two provenances had the same values at both temperatures, two had higher values in the 20°C greehouse and the remaining two had higher values in the 25°C greenhouse (Figures 4.1-4.14). For almost all the growth and morphological characteristics, the two Rwandan provenances (Arboretum de Ruhande and Rukara/Kibungo) had the highest values, while the provenance from Budongo Forest Reserve and two of the Kenyan provenances (Kisaina 5B and Kisaina 4E) had the lowest values. The Kakamega (Kenya) provenance had intermediate values for most of the characteristics.

Table 4.1 Mean, standard deviation and coefficient of variation (%) for growth and morphological characteristics of six *Maesopsis eminii provenances.*

Provenance	Location (Greenhouse)	Height (mean monthly growth) (cm)	Diameter (mean monthly growth) (mm)	No. of leaves on main stem	No. of first order branches	No. of 2nd order branches	Branch angle (degrees)	No. of leaves on 2nd branch	Length of 2nd branch (cm)	Leaf area (1st leaf on 2nd branch)	Petiole % (1st leaf on 2nd branch)	Leaf shape index (1st leaf on 2nd branch)	Leaf area (largest leaf on 2nd branch)	Petiole % (largest leaf on 2nd branch)	Leaf shape index (largest leaf on 2nd branch)
A (Rukara/Kibungo	20 °C	6.24	1.01	30.35	9.19	4.74	67.48	13.96	27.83	17.46	8.93	-0.38	41.55	5.87	-0.41
Rwanda)	25 °C	8.57	1.26	39.33	12.63	2.24	66.22	14.15	21.90	7.71	6.94	-0.39	21.80	5.55	-0.37
Mean		**7.41**	**1.14**	**34.84**	**10.91**	**3.49**	**66.85**	**14.06**	**24.87**	**12.59**	**7.94**	**-0.38**	**31.67**	**5.71**	**-0.39**
Std. Deviation		**0.11**	**0.02**	**0.27**	**0.19**	**0.35**	**0.85**	**0.39**	**0.71**	**0.53**	**0.20**	**0.02**	**0.96**	**0.15**	**0.01**
Coeff. Variation		**1.51**	**1.77**	**0.77**	**1.72**	**9.95**	**1.26**	**2.76**	**2.85**	**4.21**	**2.48**	**3.99**	**3.04**	**2.57**	**3.21**
B (Arboretum de	20 °C	6.03	1.00	29.90	9.19	6.56	65.04	13.23	25.91	20.47	8.57	-0.44	41.62	6.43	-0.43
Ruhande - Rwanda	25 °C	9.22	1.37	38.56	13.57	7.17	64.35	12.37	21.87	8.82	6.80	-0.41	25.33	5.60	-0.39
Mean		**7.62**	**1.18**	**34.23**	**11.38**	**6.86**	**64.70**	**12.80**	**23.89**	**14.64**	**7.68**	**-0.43**	**33.48**	**6.01**	**-0.41**
Std. Deviation		**0.11**	**0.02**	**0.27**	**0.19**	**0.35**	**0.85**	**0.39**	**0.72**	**0.53**	**0.20**	**0.02**	**0.96**	**0.15**	**0.01**
Coeff. Variation		**1.48**	**1.72**	**0.79**	**1.67**	**5.11**	**1.32**	**3.06**	**2.99**	**3.64**	**2.58**	**3.57**	**2.88**	**2.46**	**3.08**
E (Kakamega	20 °C	5.26	0.86	28.98	6.87	3.67	71.37	12.70	23.13	18.38	8.99	-0.38	39.45	6.35	-0.39
Kenya)	25 °C	8.07	1.27	37.91	9.87	3.65	67.70	12.48	20.98	10.74	6.89	-0.38	27.45	5.00	-0.35
Mean		**6.67**	**1.07**	**33.44**	**8.37**	**3.66**	**69.54**	**12.59**	**22.06**	**14.56**	**7.94**	**-0.38**	**33.45**	**5.68**	**-0.37**
Std. Deviation		**0.11**	**0.02**	**0.27**	**0.19**	**0.35**	**0.85**	**0.39**	**0.71**	**0.53**	**0.20**	**0.02**	**0.96**	**0.15**	**0.01**
Coeff. Variation		**1.69**	**1.88**	**0.80**	**2.25**	**9.50**	**1.22**	**3.08**	**3.21**	**3.63**	**2.48**	**4.05**	**2.86**	**2.58**	**3.37**
F (Kisaina 5B	20 °C	5.36	0.89	29.00	6.49	3.94	71.70	13.39	23.05	17.01	8.81	-0.37	39.54	5.81	-0.43
Kenya)	25 °C	6.37	1.18	37.97	8.29	2.50	66.12	12.35	19.44	9.95	6.87	-0.41	25.55	4.97	-0.37
Mean		**5.86**	**1.03**	**33.49**	**7.39**	**3.22**	**68.91**	**12.87**	**21.25**	**13.48**	**7.84**	**-0.39**	**32.55**	**5.39**	**-0.40**
Std. Deviation		**0.14**	**0.03**	**0.34**	**0.24**	**0.44**	**1.07**	**0.49**	**0.90**	**0.67**	**0.25**	**0.02**	**1.21**	**0.19**	**0.02**
Coeff. Variation		**2.42**	**2.45**	**1.01**	**3.23**	**13.71**	**1.56**	**3.83**	**4.23**	**4.97**	**3.18**	**5.01**	**3.73**	**3.45**	**3.99**
G (Kisaina 4E	20 °C	4.82	0.81	27.83	6.78	3.15	70.76	12.89	22.98	18.84	8.34	-0.40	42.23	5.31	-0.48
Kenya)	25 °C	7.11	1.26	37.41	10.44	2.30	67.07	13.48	21.64	10.33	6.62	-0.40	28.31	4.55	-0.38
Mean		**5.96**	**1.04**	**32.62**	**8.61**	**2.72**	**68.92**	**13.19**	**22.31**	**14.58**	**7.48**	**-0.40**	**35.27**	**4.93**	**-0.43**
Std. Deviation		**0.11**	**0.02**	**0.27**	**0.19**	**0.35**	**0.85**	**0.39**	**0.71**	**0.53**	**0.20**	**0.02**	**0.96**	**0.15**	**0.01**
Coeff. Variation		**1.88**	**1.95**	**0.82**	**2.18**	**12.77**	**1.23**	**2.94**	**3.17**	**3.62**	**2.64**	**3.85**	**2.72**	**2.99**	**2.91**
H (Budongo Forest	20 °C	4.70	0.91	27.56	6.00	2.96	69.74	12.48	22.02	17.53	8.06	-0.45	42.01	5.33	-0.44
Reserve - Uganda)	25 °C	6.70	1.20	36.82	7.82	2.26	65.20	13.48	20.25	9.18	6.78	-0.42	26.44	5.03	-0.39
Mean		**5.70**	**1.05**	**32.19**	**6.91**	**2.61**	**67.47**	**12.98**	**21.14**	**13.36**	**7.42**	**-0.43**	**34.22**	**5.18**	**-0.41**
Std. Deviation		**0.11**	**0.02**	**0.27**	**0.19**	**0.35**	**0.86**	**0.40**	**0.72**	**0.54**	**0.20**	**0.02**	**0.97**	**0.15**	**0.01**
Coeff. Variation		**1.99**	**1.95**	**0.85**	**2.78**	**13.58**	**1.28**	**3.05**	**3.42**	**4.03**	**2.70**	**3.60**	**2.85**	**2.89**	**3.07**

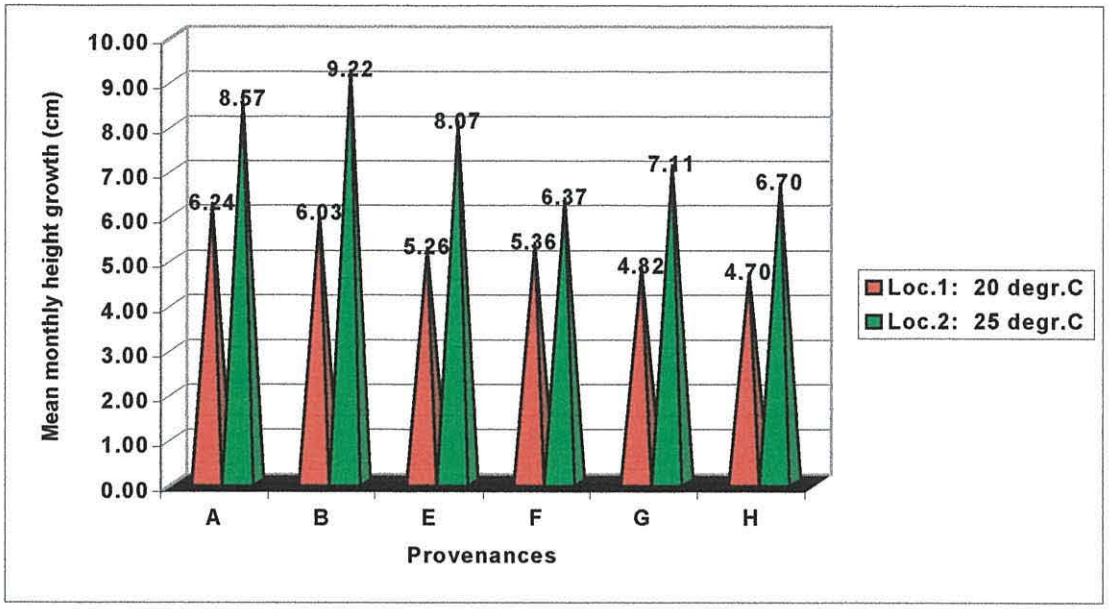


Figure 4.1 Mean monthly seedling height growth (cm) for six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

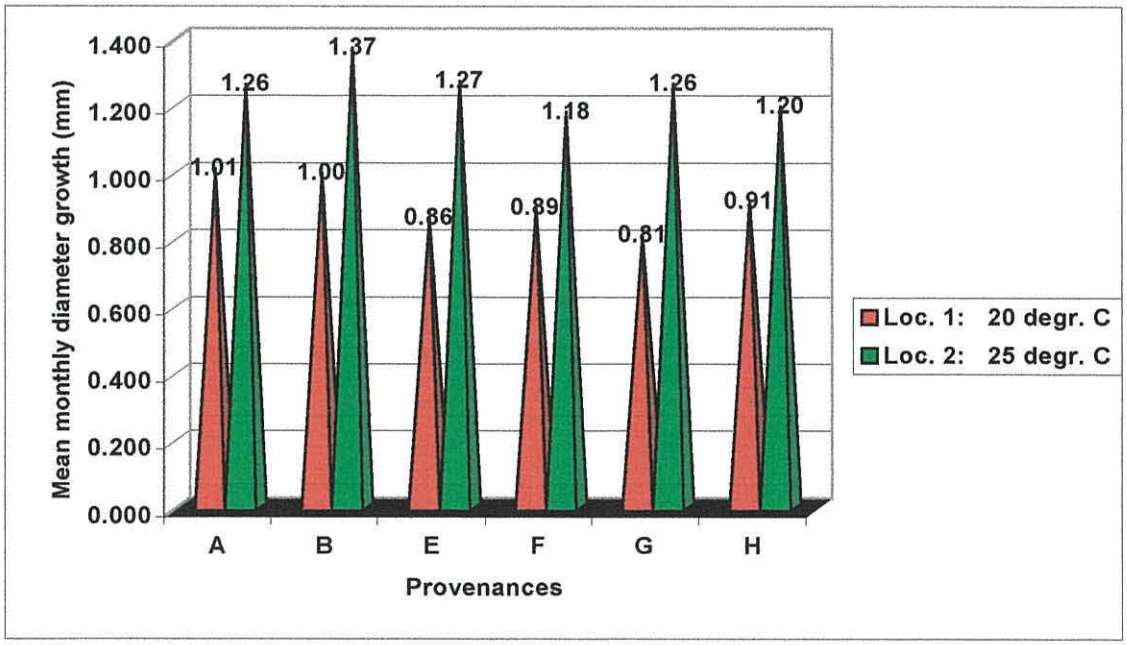


Figure 4.2 Mean monthly seedling diameter growth (mm) for six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

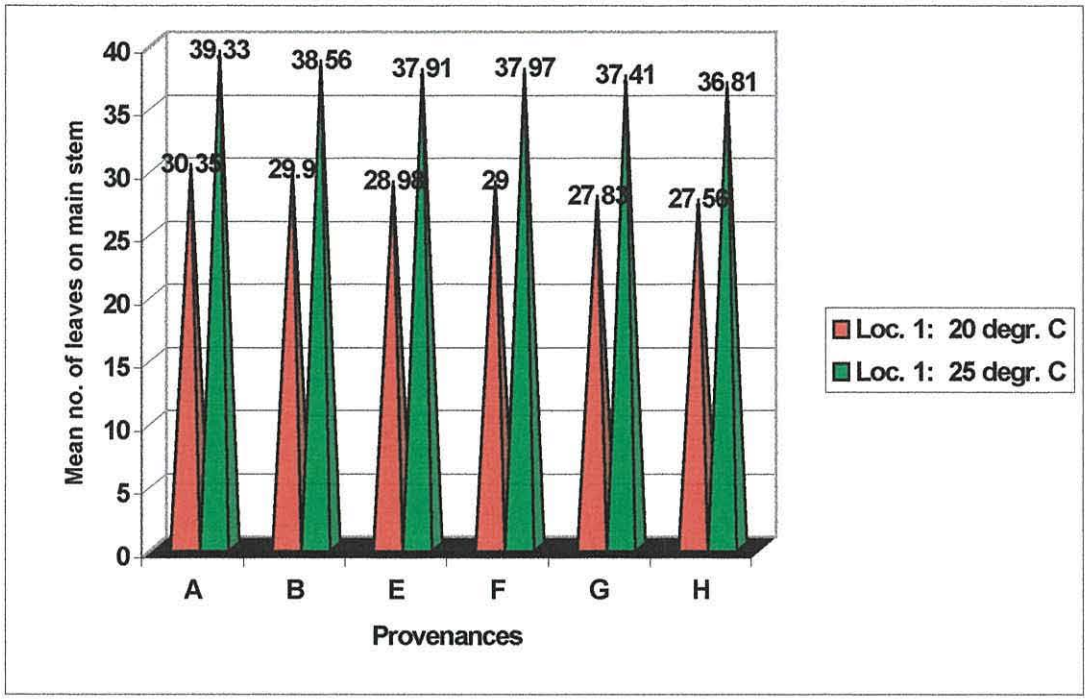


Figure 4.3 Mean number of leaves on the main stem of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

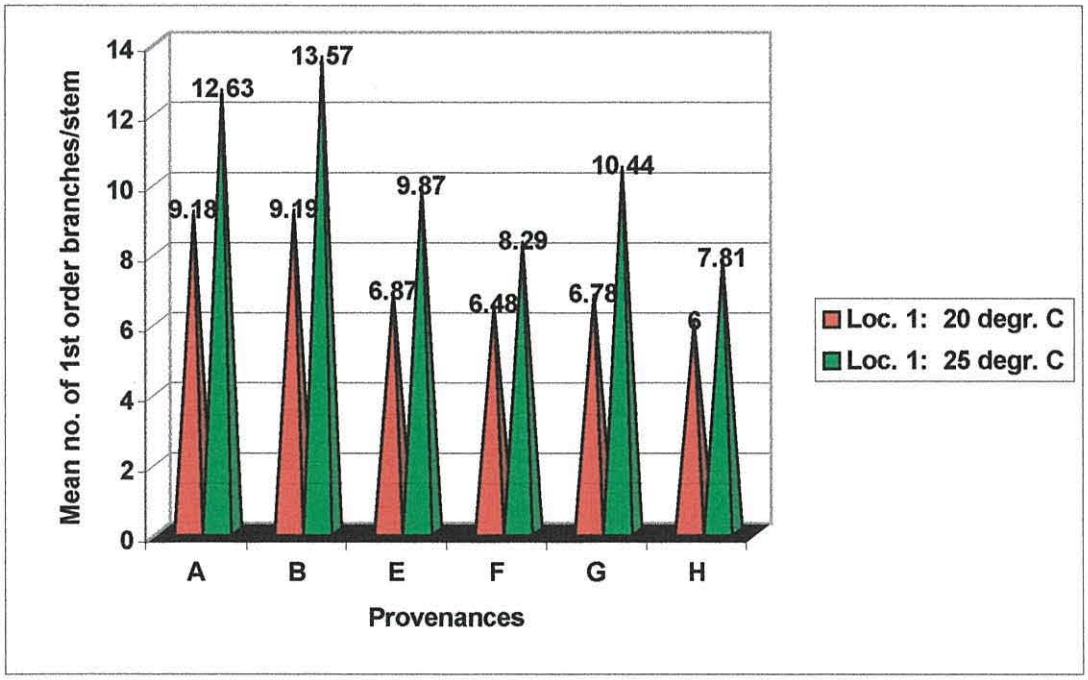


Figure 4.4 Mean number of first order branches on the main stem of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

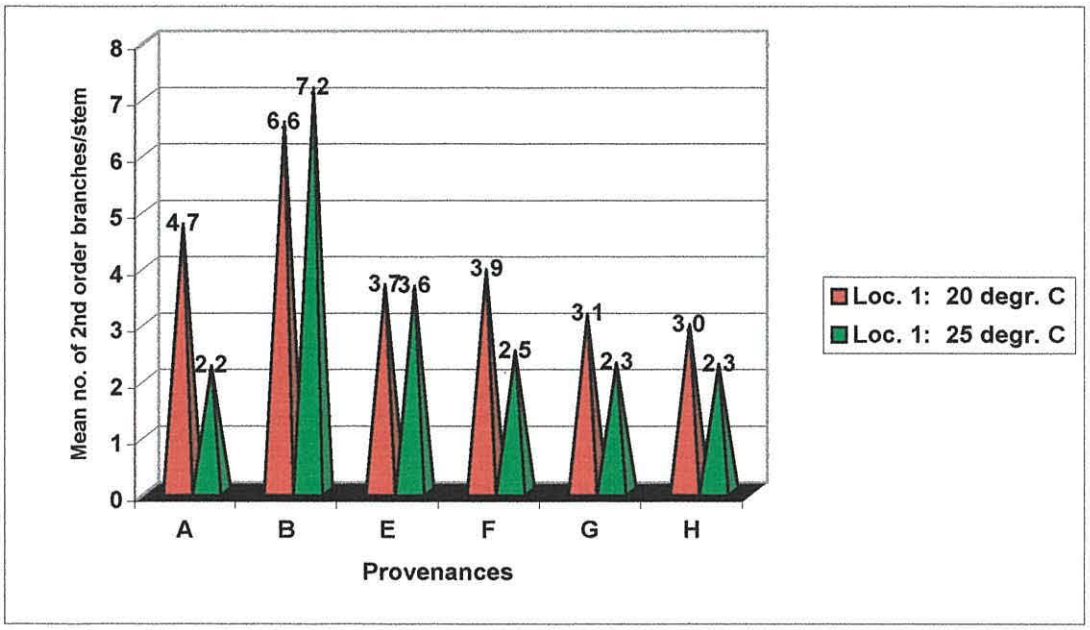


Figure 4.5 Mean number of second order branches on the main stem of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

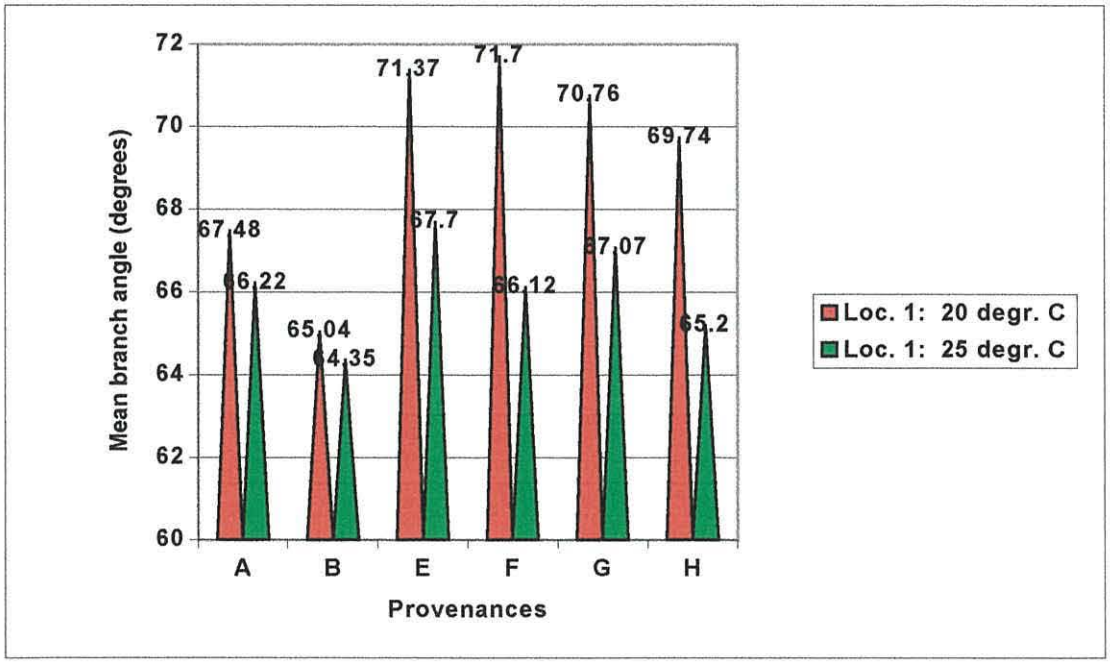


Figure 4.6 Mean branch angle (degrees) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

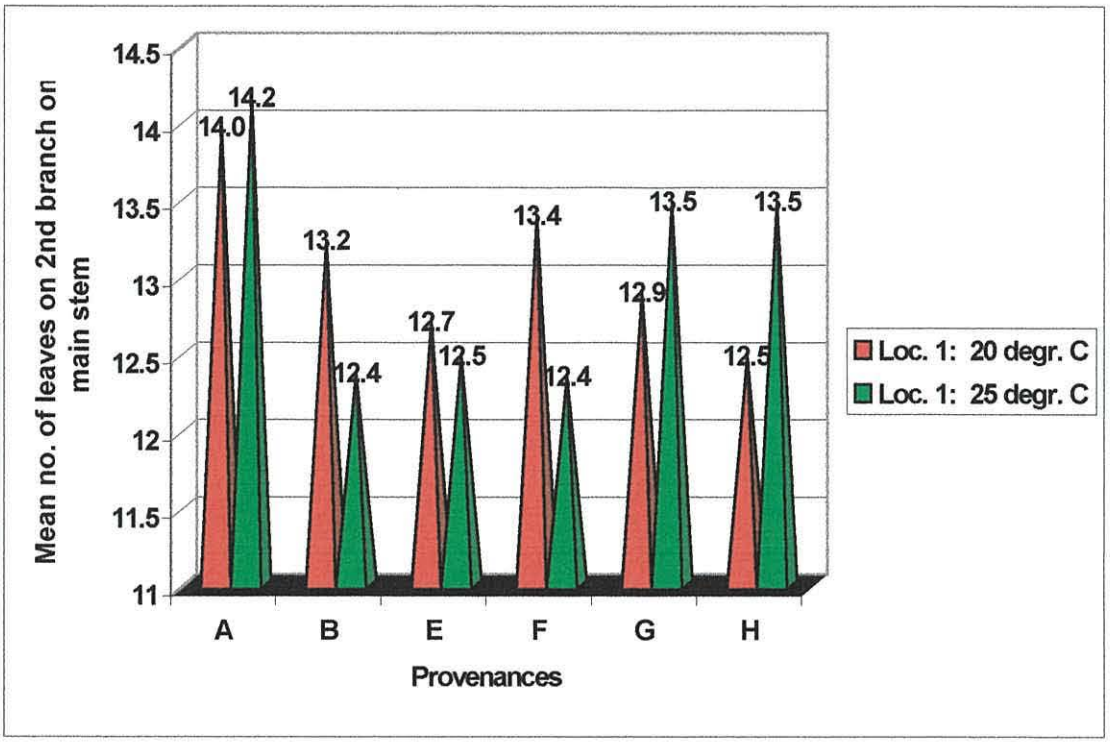


Figure 4.7 Mean number of leaves (second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

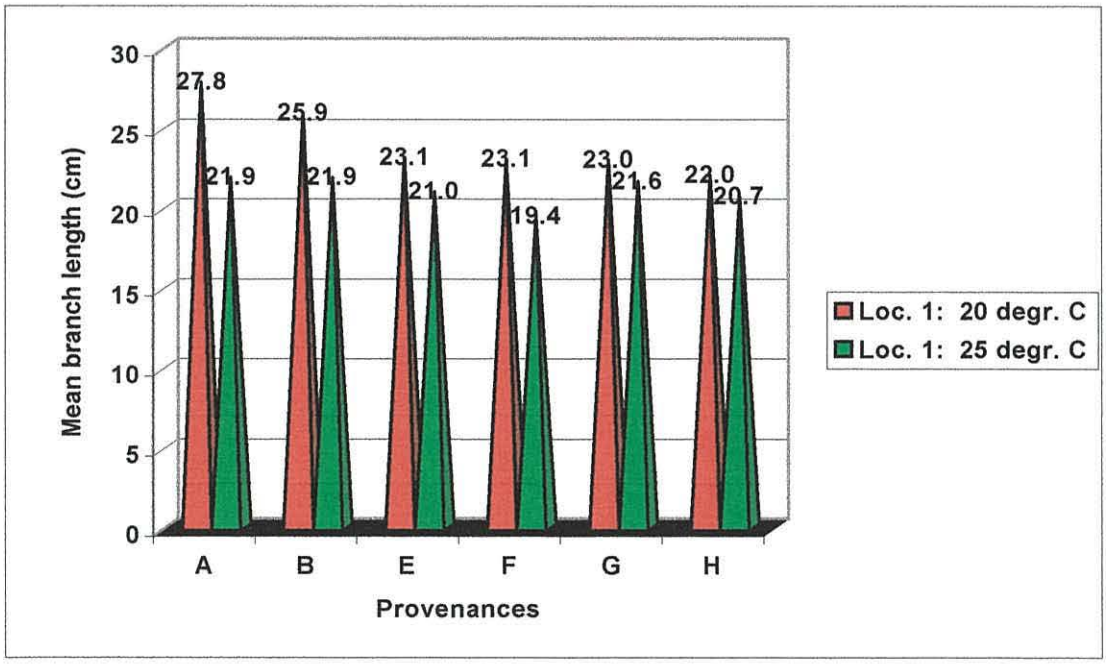


Figure 4.8 Mean branch length (cm) (second from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

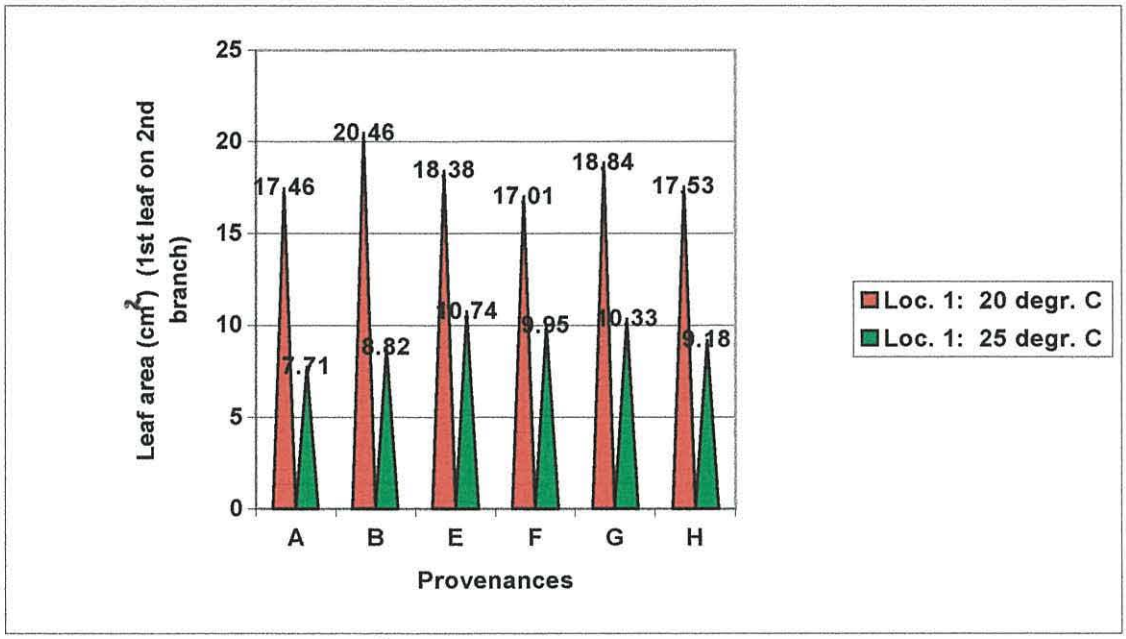


Figure 4.9 Leaf area (cm^2) (first leaf on second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1

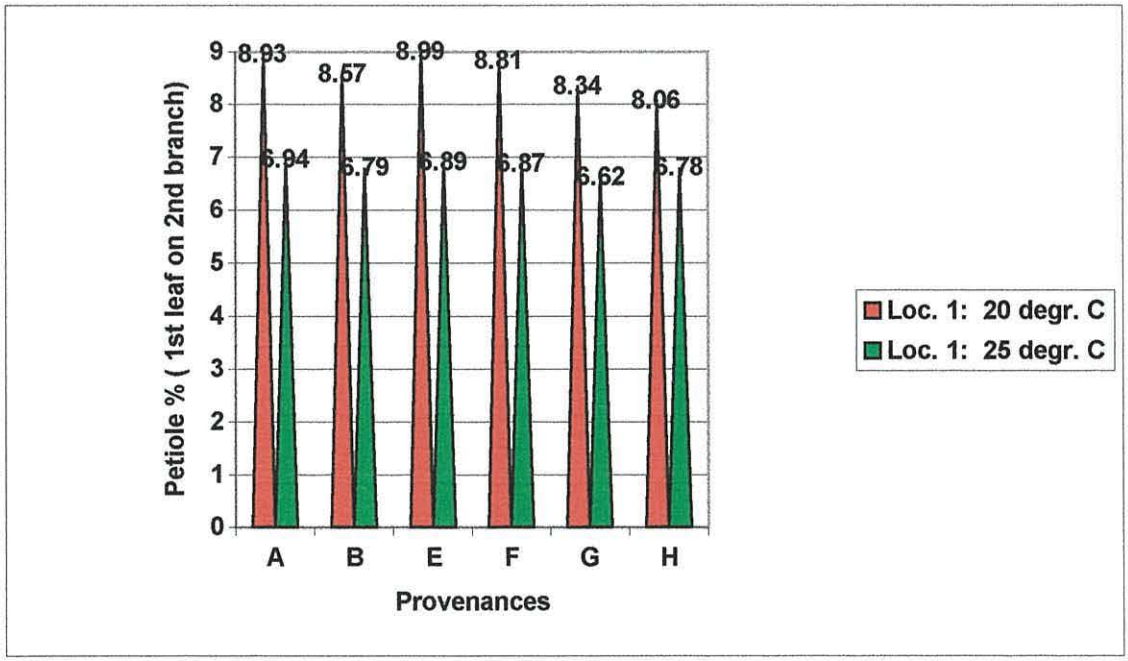


Figure 4.10 Petiole percentage (first leaf on second branch from the top of the codes as main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

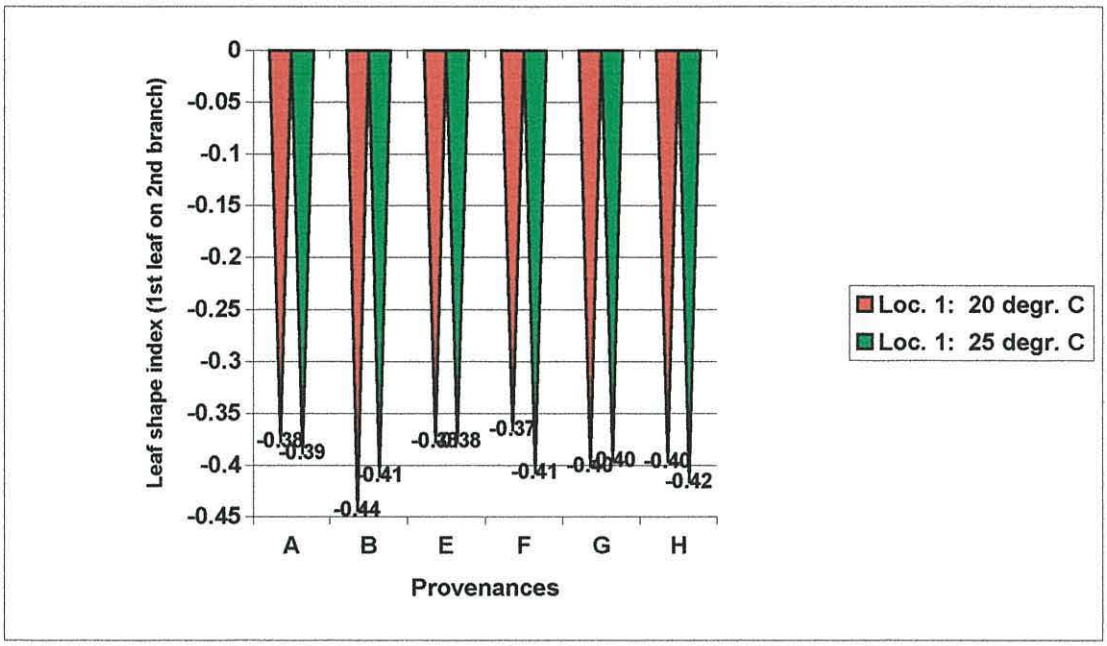


Figure 4.11 Leaf shape index (first leaf on second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures. Provenance codes as in Table 4.1.

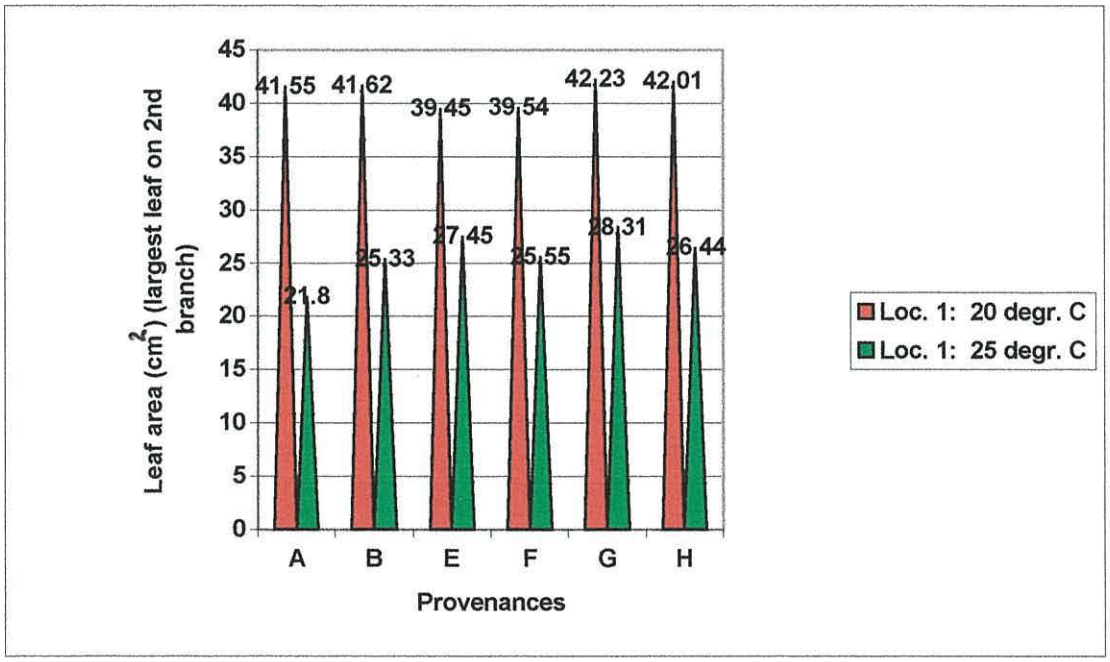


Figure 4.12 Leaf area (cm^2) (largest leaf on second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

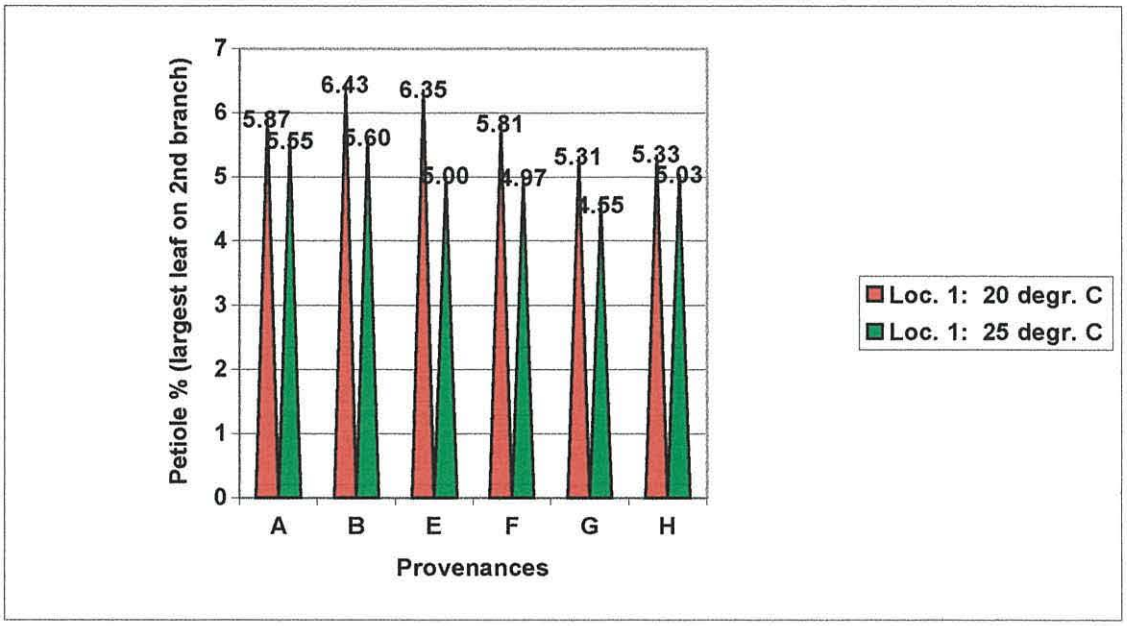


Figure 4.13 Petiole % (largest leaf on second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

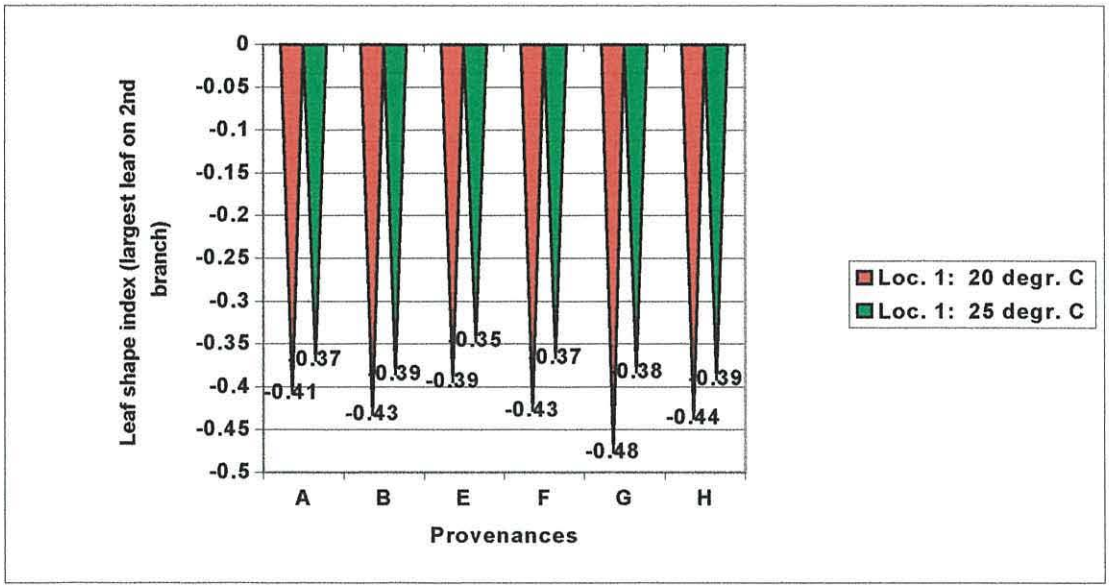


Figure 4.14 Leaf shape index (largest leaf on second branch from the top of the main stem) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C). Provenance codes as in Table 4.1.

The amount of variation accounted for by provenance, temperature, the provenance x temperature interaction and trees within provenance is shown in Table 4.2. The percentage of the total sum of squares accounted for by provenances ranged from 1.3% (for leaf area of the largest leaf on the second branch) to 17.1% (for mean monthly height increment). The variation accounted for by temperature (i.e. greenhouse) ranged from 0% (for the leaf shape index of the first leaf on the second branch) to 55.5% (for the leaf area of the first leaf on the second branch). The amount of variation accounted for by provenance x temperature interactions was low and ranged from 0.7% (for petiole percentage of the first leaf on the second branch) to 2.8% (for mean monthly height increment). The variation accounted for by plants within provenance was generally high, ranging from 38.3% (for mean monthly diameter increment) to 96.8% (for leaf shape index of the first leaf on the second branch). Over all growth and morphological characteristics, variation accounted for by provenance, temperature, the provenance x temperature interaction and plants within provenance was 5.5%, 26.2%, 1.7% and 66.6% respectively.

Table 4.2 Amount of variation accounted for by provenance, temperature, provenance x temperature and trees within provenance of ten growth and morphological characteristics in six *Maesopsis eminii* provenances (count data not included).

CHARACTERISTICS	PERCENTAGE VARIATION ACCOUNTED FOR BY:			
	PROVENANCE	TEMPERATURE (Greenhouse)	PROV X TEMPERATURE	TREES WITHIN PROVENANCES
1. Height increment	17.1 ***	41.0 ***	2.8 ***	39.1
2. Diameter increment	5.5 ***	53.8 ***	2.4 ***	38.3
3. Branch angle	6.3 ***	5.4 ***	1.7 NS	86.6
4. Branch length (2^{nd} branch on main stem from top)	5.6 ***	7.4 ***	2.3 **	84.7
5. Leaf area of the 1^{st} leaf on 2^{nd} branch from top	1.9 ***	55.5 ***	1.5 ***	41.1
6. Petiole % of the 1^{st} leaf (near stem) on the 2^{nd} branch	1.6 *	27.6 ***	0.7 NS	70.1
7. Leaf shape index of the 1^{st} leaf on 2^{nd} branch	2.3 *	0.0 NS	0.9 NS	96.8
8. Leaf area of the largest leaf on the 2^{nd} branch	1.3 **	53.4 ***	1.4 **	43.9
9. Petiole % of the largest leaf on the 2^{nd} branch	9.5 ***	9.2 ***	2.4 **	78.9
10. Leaf shape index of the largest leaf on the 2^{nd} branch	4.0 ***	8.3 ***	0.9 NS	86.8

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; NS not significant

Results of pairwise comparisons made using Tukey's test are given in Tables 4.3–4.12. For characteristics which showed significant provenance x temperature interactions, data from each greenhouse were treated separately

and Tukey's tests performed on each temperature data set. For some characteristics, there were no significant differences between provenances at one of the temperatures. In such cases, the results of Tukey's test are shown only for the temperature where there were differences. At 20°C, the percentage of significantly different pairs ranged from 6.7% (for petiole percentage of the first leaf on the second branch) to 53.3% (for monthly seedling diameter growth). At 25°C, the range was from 13.3% (for petiole percentage of the largest leaf on the second branch) to 66.7% (for monthly seedling height growth).

For characteristics which did not show significant differences between the two growing temperatures (i.e. $P > 0.05$ for provenance x temperature interactions), data were combined and Tukey's tests performed on mean values over the two temperatures. For these comparisons, the range of percentage of pairs showing significant differences was from 13.3% (for the leaf shape index of the first leaf on the second branch) to 33.3% (for branch angle).

Table 4.3 Results of Tukey's pairwise comparison test for significance of differences in monthly seedling height growth (cm) between six *Maesopsis eminii* provenances growing at 20°C and 25° C. Provenance codes as in Table 4.1.

	20° C						25° C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda												
B-Arboretum de Ruhande-Rwanda	NS						NS					
E-Kakamega-Kenya	*	*					NS	*				
F-Kisaina 5B-Kenya	*	NS	NS				*	*	*			
G-Kisaina 4E Kenya	*	*	NS	NS			*	*	*	NS		
H-Budongo Forest Reserve-Uganda	*	*	NS	NS	NS		*	*	*	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.4 Results of Tukey's pairwise comparison test for significance of differences in monthly seedling diameter growth (mm) between six *Maesopsis eminii* provenances growing at 20°C and 25° C. Provenance codes as in Table 4.1.

	20° C						25° C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda												
B-Arboretum de Ruhande-Rwanda	NS						*					
E-Kakamega-Kenya	*	*					NS	*				
F-Kisaina 5B-Kenya	*	*	NS				NS	*	*			
G-Kisaina 4E Kenya	*	*	NS	NS			NS	*	NS	NS		
H-Budongo Forest Reserve-Uganda	*	NS	NS	NS	*		NS	*	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.5 Results of Tukey's pairwise comparison test for significance of differences in branch angle between six *Maesopsis eminii* provenances. Provenance codes as in Table 4.1.

Provenance	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	NS					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	NS	*	NS			
G-Kisaina 4E Kenya	NS	*	NS	NS		
H-Budongo Forest Reserve-Uganda	NS	*	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.6 Results of Tukey's pairwise comparison test for significance of differences in branch length (second branch on the main stem) between six *Maesopsis eminii* provenances at 20°C. Provenance codes as in Table 4.1.

	20 °C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	NS					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	*	NS	NS			
G-Kisaina 4E Kenya	*	*	NS	NS		
H-Budongo Forest Reserve-Uganda	*	*	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.7 Results of Tukey's pairwise comparison test for significance of differences in leaf area (first leaf on second branch on the main stem) between six *Maesopsis eminii* provenances growing at 20°C and 25°C. Provenance codes as in Table 4.1.

	20° C						25° C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda												
B-Arboretum de Ruhande-Rwanda	*						NS					
E-Kakamega-Kenya	NS	NS					*	*				
F-Kisaina 5B-Kenya	NS	*	NS				*	NS	NS			
G-Kisaina 4E Kenya	NS	NS	NS	NS			*	NS	NS	NS		
H-Budongo Forest Reserve-Uganda	NS	*	NS	NS	NS		NS	NS	*	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.8 Results of Tukey's pairwise comparison test for significance of differences in petiole % (first leaf on second branch of the main stem) between six *Maesopsis eminii* provenances at 20°C. Provenance codes as in Table 4.1.

	20°C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	NS					
E-Kakamega-Kenya	NS	NS				
F-Kisaina 5B-Kenya	NS	NS	NS			
G-Kisaina 4E Kenya	NS	NS	NS	NS		
H-Budongo Forest Reserve-Uganda	NS	NS	*	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.9 Results of Tukey's pairwise comparison test for significance of differences in leaf shape index (first leaf on second branch of the main stem) between six *Maesopsis eminii* provenances. Provenance codes as in Table 4.1.

Provenance	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	*					
E-Kakamega-Kenya	NS	*				
F-Kisaina 5B-Kenya	NS	NS	NS			
G-Kisaina 4E Kenya	NS	NS	NS	NS		
H-Budongo Forest Reserve-Uganda	NS	NS	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.10 Results of Tukey's pairwise comparison test for significance of differences in leaf area (largest leaf on second branch of the main stem) between six *Maesopsis eminii* provenances at 25°C. Provenance codes as in Table 4.1.

	25°C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	*					
E-Kakamega-Kenya	*	NS				
F-Kisaina 5B-Kenya	NS	NS	NS			
G-Kisaina 4E Kenya	*	NS	NS	NS		
H-Budongo Forest Reserve-Uganda	*	NS	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.11 Results of Tukey's pairwise comparison test for significance of differences in petiole % (largest leaf on second branch of the main stem) between six *Maesopsis eminii* provenances growing at 20°C and 25°C. Provenance codes as in Table 4.1.

	20° C						25° C					
Provenance	**A**	**B**	**E**	**F**	**G**	**H**	**A**	**B**	**E**	**F**	**G**	**H**
A-Kibungo-Rwanda												
B-Arboretum de Ruhande-Rwanda	NS						NS					
E-Kakamega-Kenya	NS	NS					NS	NS				
F-Kisaina 5B-Kenya	NS	NS	NS				NS	NS	NS			
G-Kisaina 4E Kenya	NS	*	*	NS			*	*	NS	NS		
H-Budongo Forest Reserve-Uganda	NS	*	*	NS	NS		NS	NS	NS	NS	NS	

* $P \leq 0.05$; NS not significant

Table 4.12 Results of Tukey's pairwise comparison test for significance of differences in leaf shape index (largest leaf on second branch of the main stem) between six *Maesopsis eminii* provenances. Provenance codes as in Table 4.1.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum de Ruhande-Rwanda	NS					
E-Kakamega-Kenya	NS	*				
F-Kisaina 5B-Kenya	NS	NS	NS			
G-Kisaina 4E Kenya	*	NS	*	NS		
H-Budongo Forest Reserve-Uganda	NS	NS	*	NS	NS	

* $P \leq 0.05$; NS not significant

Provenance x temperature interactions for growth and morphological characteristics are shown in Figures 4.15–4.20. Significant provenance x temperature interactions were found in six out of ten characteristics subjected to analysis of variance (see Table 4.2). No plots are presented for characteristics which did not show a provenance x temperature interaction or for count data (since they were not subjected to analysis of variance).

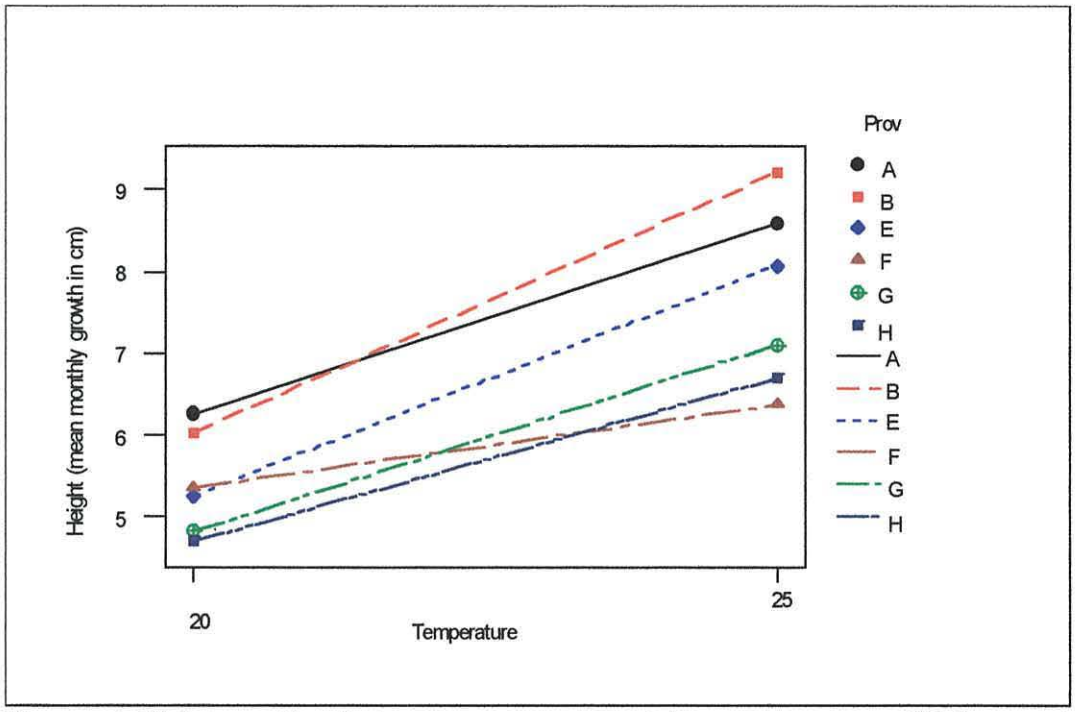


Figure 4.15 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in mean monthly height growth (cm). Provenance codes as in Table 4.1.

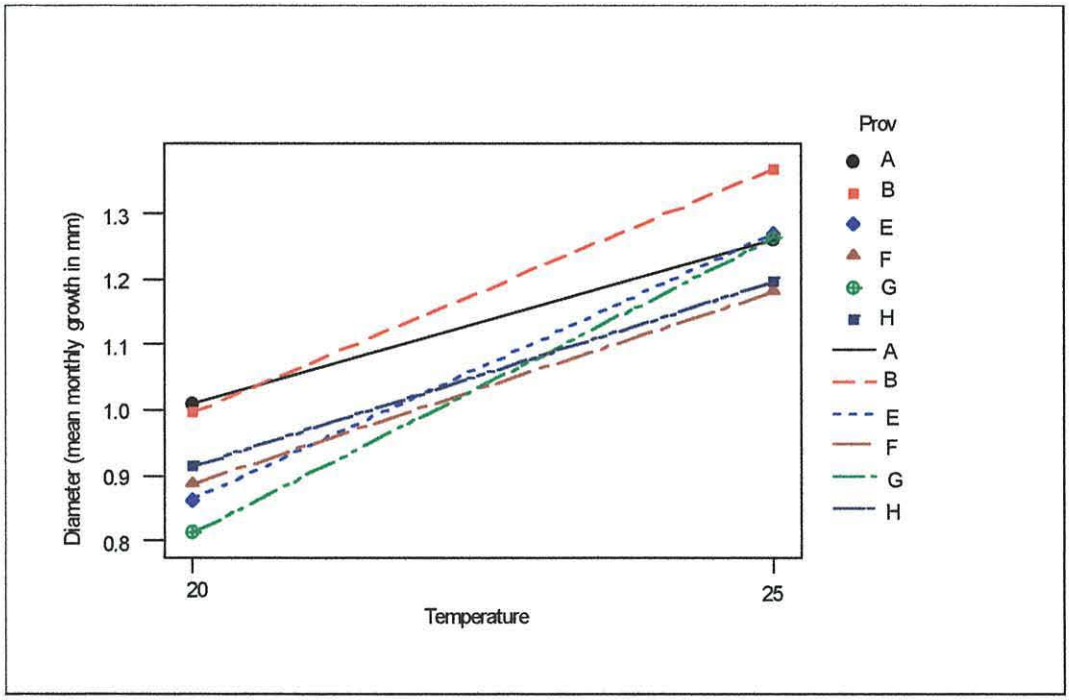


Figure 4.16 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in mean monthly diameter growth (mm). Provenance codes as in Table 4.1.

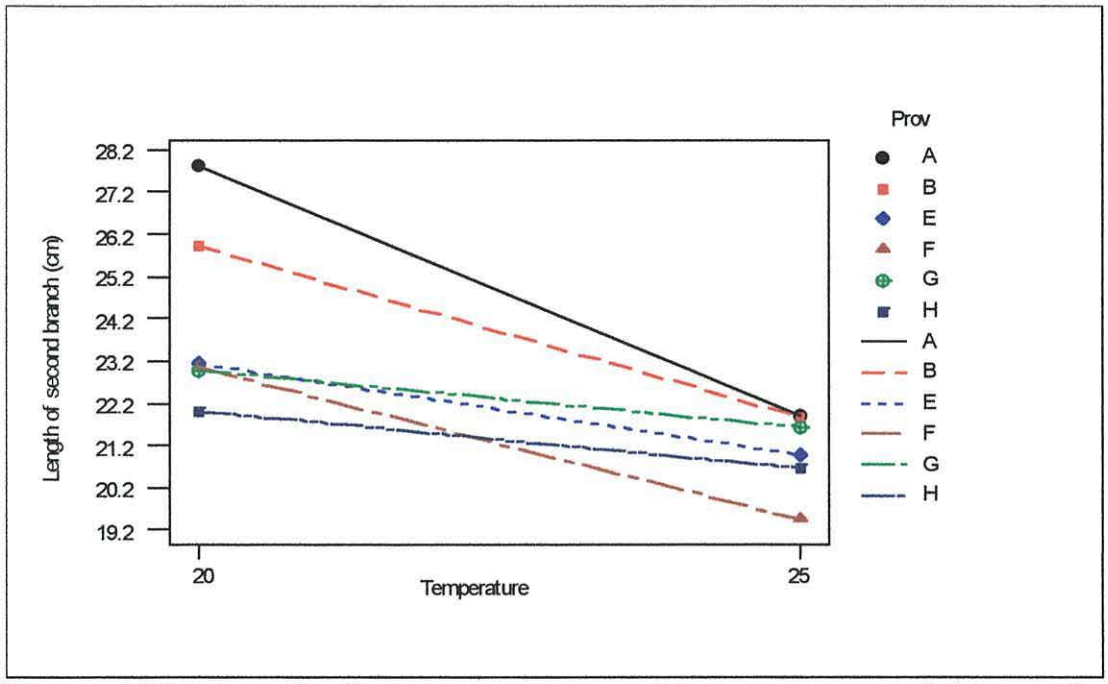


Figure 4.17 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in branch length (second branch on the main stem). Provenance codes as in Table 4.1.

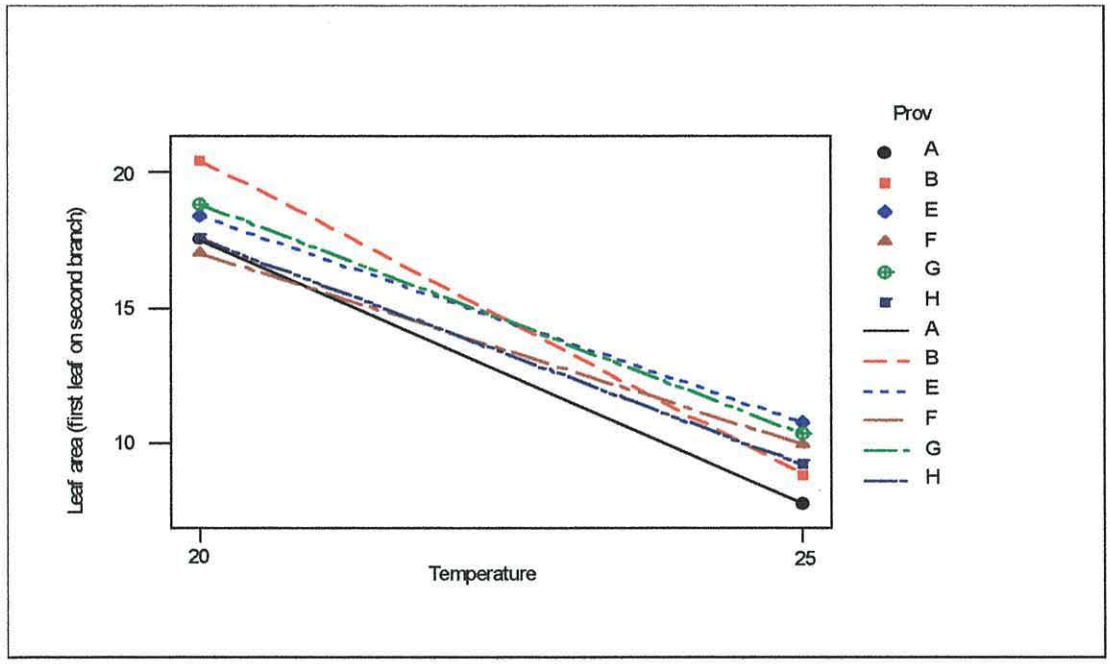


Figure 4.18 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in leaf area (first leaf on second branch on the main stem). Provenance codes as in Table 4.1.

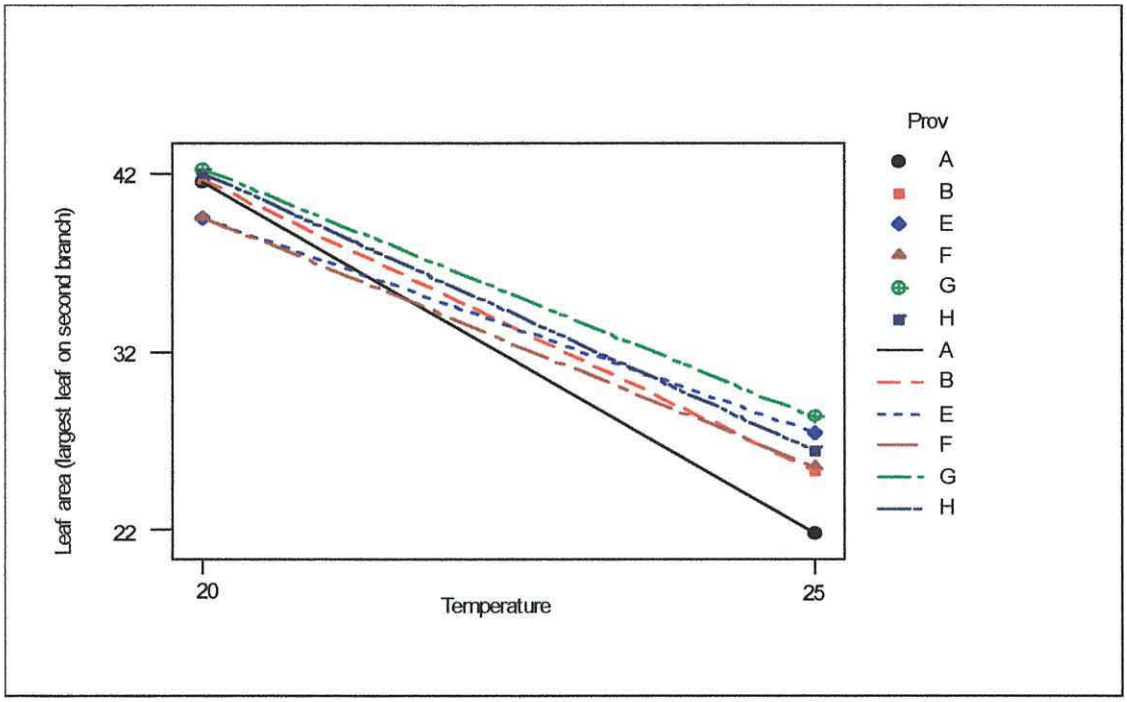


Figure 4.19 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in leaf area (largest leaf on second branch on the main stem). Provenance codes as in Table 4.1.

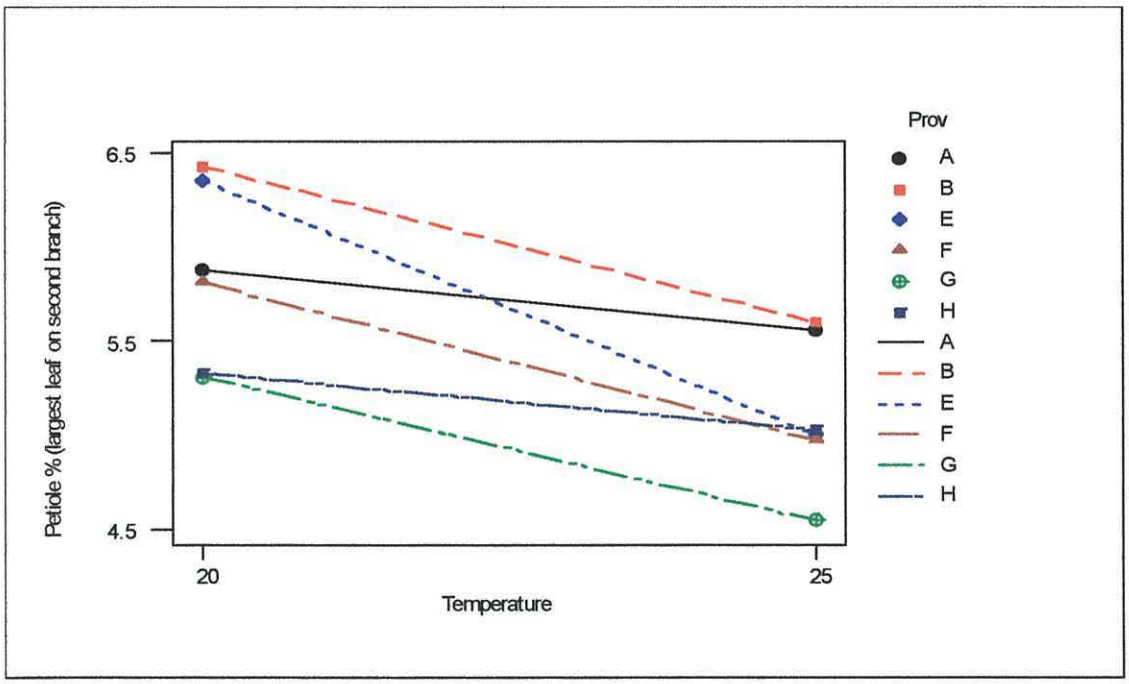


Figure 4.20 Interaction between provenance (six provenances of *Maesopsis eminii*) and temperature (20°C and 25°C) in petiole % (largest leaf on second branch on main stem). Provenance codes as in Table 4.1.

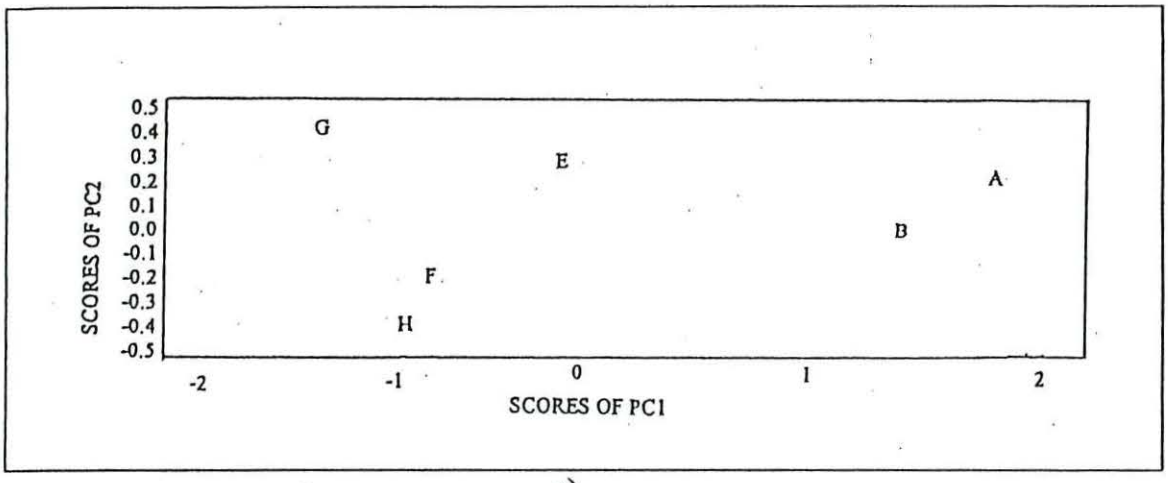
Results of principal component analysis (plots of PC1 and PC2 scores for growth characteristics, PC1, PC2 and PC3 scores for morphological characteristics, and PC1, PC2 and PC3 scores for all seed, growth and morphological characteristics) are presented in Figure 4.21 (a-g). Selection of components was based on the general guideline that selection of principal components should be such that they account for at least 80% to 90% of the total variability in the data (Gardiner, 1997).

Figure 4.21 (a), based on growth characteristics alone, shows four distinct groups of provenances.

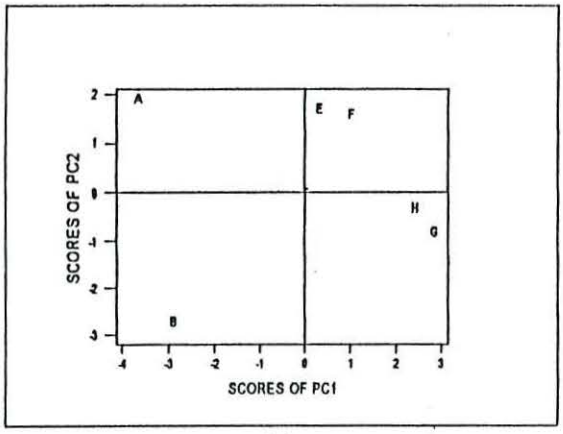
Group 1	A Rukara/Kibungo, Rwanda
	B Arboretum de Ruhande, Rwanda
Group 2	E Kakamega, Kenya
Group 3	G Kisaina 4E, Kenya
Group 4	F Kisaina 5B, Kenya
	H Budongo Forest reserve, Uganda

Figure 4.21 (b-d), based on morphological characteristics only, and Figure 4.21 (e-g), based on all seed and seedling characteristics, show different provenance groupings. As in Figure 4.21 (a), four provenance groups were identified in Figure 4.21 (b-g), but with the following composition.

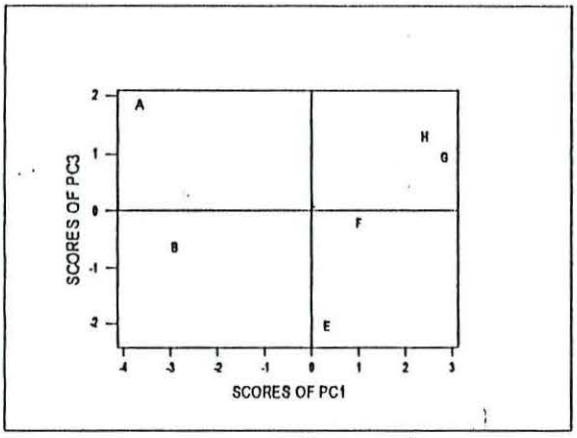
Group 1	A Rukara/Kibungo, Rwanda
Group 2	B Arboretum de Ruhande, Rwanda
Group 3	E Kakamega, Kenya
	F Kisaina 5B, Kenya
Group 4	G Kisaina 4E, Kenya
	H Budongo Forest Reserve, Uganda


SCORES OF PC2
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
G
E
A
B
F
H
-2
-1
0
1
2
SCORES OF PC1

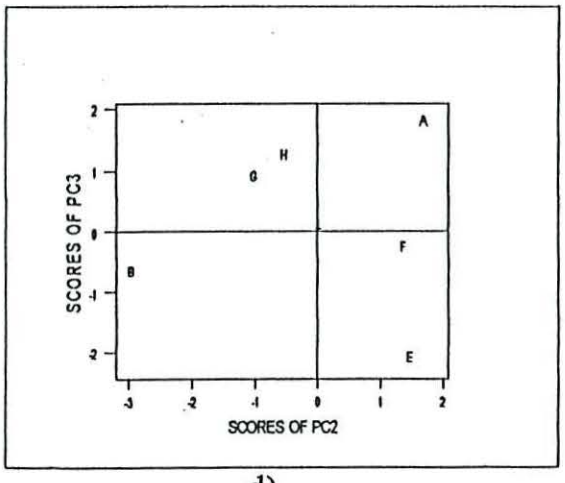
a)


SCORES OF PC2
A
E
F
H
G
B
SCORES OF PC1

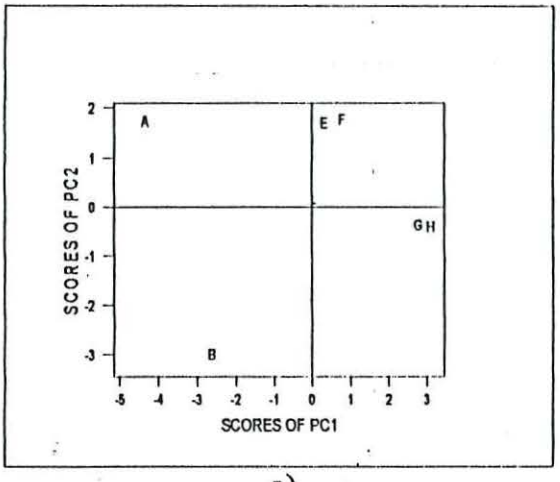
b)


SCORES OF PC3
A
H
G
F
B
E
SCORES OF PC1

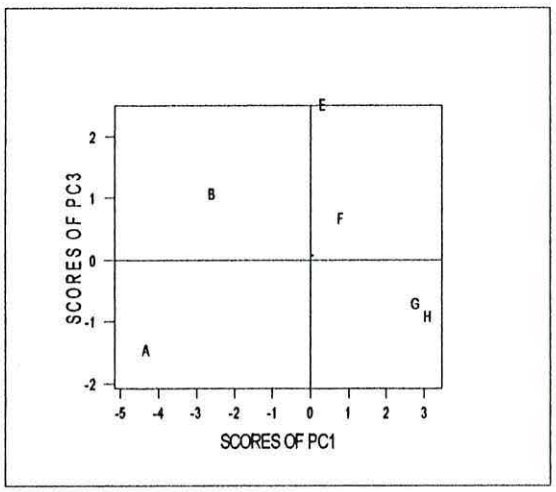
c)


SCORES OF PC3
A
H
G
F
B
E
SCORES OF PC2

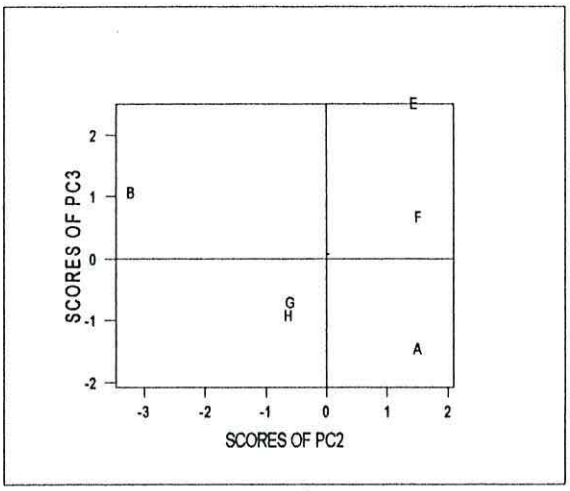
d)


SCORES OF PC2
A
E F
G H
B
SCORES OF PC1

e)



f)



g)

Figure 4.21 (a-g) Plots of first, second and third components from principal component analysis of seed and seedling characteristics of six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

a) Seedling growth characteristics (mean monthly height and diameter growth). Scores of PC1 and PC2 accounted for 93% and 7% of the total variance respectively.

b-d) Seedling morphological characteristics. Of the total variance, scores of PC1, PC2 and PC3 accounted for 46.2%, 28.2%, and 17.1% respectively. Three principal components were needed to reach the target of 80-90% of the total variance accounted for. Pairwise combinations of PCs were made to determine which pair best explains the structures and patterns in measured characteristics.

e-g) Seed size, seedling growth and morphological characteristics. Of the total variance, scores of PC1, PC2 and PC3 accounted for 54.4%, 22.2%, and 13.8% respectively. Three principal components were again needed to account for 80- 90% of the total variability.

Discriminant function analysis showed that the overall proportion of correctly classified provenances was low (37.9%) when growth characteristics alone (i.e. height and diameter) were used, moderate (70.5%) when morphological

characteristics were used and high (86.4%) when all seed, growth and morphological characteristics were considered together (Tables 4.13-4.15).

Squared distances and F test statistics (Tables 4.16-4.18 for (1) growth, (2) morphological characteristics and (3) seed, growth and morphological characteristics) indicated a reasonable number of pairs of provenances for which discrimination was possible. Discrimination possibilities were least (67%) when only growth characteristics were used, high (86.7%) when morphological characteristics were used, and very high (93.3%) when all seed and seedling characteristics were analysed together.

Table 4.13 Summary of classification of provenances by discriminant analysis (seedling growth characteristics only).

Suggested group/provenance for correctly and/or misclassified measurements	**True group (provenance)**					
	A	**B**	**E**	**F**	**G**	**H**
A	**10**	6	3	0	3	4
B	7	**14**	3	1	0	0
E	4	0	**6**	2	4	3
F	0	0	2	**11**	4	8
G	0	0	4	2	**5**	3
H	1	2	4	6	6	**4**
Total N	22	22	22	22	22	22
N correctly classified	**10**	**14**	**6**	**11**	**5**	**4**
Proportion of correctly grouped/classified measurements	**45.5%**	**63.6%**	**27.3%**	**50%**	**22.7%**	**18.2%**
Overall proportion of correctly grouped/classified measurements	**37.9%**					

Table 4.14 Summary of classification of provenances by discriminant analysis (seedling morphological characteristics only).

Suggested group/provenance for correctly and/or misclassified measurements	**True group (provenance)**					
	A	**B**	**E**	**F**	**G**	**H**
A	**20**	0	1	2	3	0
B	2	**22**	0	1	0	0
E	0	0	**13**	5	1	1
F	0	0	6	**10**	2	3
G	0	0	1	1	**13**	3
H	0	0	1	3	3	**15**
Total N	22	22	22	22	22	22
N correctly classified	**20**	**22**	**13**	**10**	**13**	**15**
Proportion of correctly grouped/classified measurements	**90.9%**	**100%**	**59.1%**	**45.5%**	**59.1%**	**68.2%**
Overall proportion of correctly grouped/classified measurements	**70.5%**					

Table 4.15 Summary of classification of provenances by discriminant analysis (seed, seedling growth and seedling morphological characteristics).

Suggestedgroup/provenance for correctly &/or misclassified measurements	**True group (provenance)**					
	A	**B**	**E**	**F**	**G**	**H**
A	**22**	1	0	0	0	0
B	0	**21**	0	0	0	0
E	0	0	**17**	4	1	0
F	0	0	2	**16**	2	0
G	0	0	3	2	**18**	2
H	0	0	0	0	1	**20**
Total N	22	22	22	22	22	22
N correctly classified	**22**	**21**	**17**	**16**	**18**	**20**
Proportion of correctly grouped/classified measurements	**100%**	**95.5%**	**77.3%**	**72.7%**	**81.8%**	**90.9%**
Overall proportion of correctly grouped/classified measurements	**86.4%**					

Table 4.16 Squared distances (top line) and test statistic values (bottom line) between provenances, generated by discriminant function analysis of seedling growth characteristics.

Provenance	A	B	E	F	G	H
A-Rukara/Kibungo Rwanda		0.259 1.394	0.971 5.212*	4.084 21.927*	2.750 14.765*	3.061 16.436*
B-Arboretum de Ruhande Rwanda			2.234 11.997*	6.376 34.235*	4.697 25.217*	5.056 27.148*
E-Kakamega Kenya				1.122 6.025*	0.459 2.464	0.674 3.618*
F-Kisaina 5B Kenya					0.164 0.880	0.091 0.489
G-Kisaina 4E Kenya						0.090 0.488
H-Budongo Forest Uganda						

NB. Small squared distances between pairs of provenances indicate similarity, while larger ones indicate dissimilarity.

* Pairs for which discrimination between provenances is possible. In 67% of cases, the test statistic values exceed the critical value at $P \leq 0.05$ (i.e. 3.23), suggesting that discrimination using growth characteristics is possible.

Table 4.17 Squared distances (top line) and test statistic values (bottom line) between provenances, generated by discriminant function analysis of seedling morphological characteristics.

Provenance	A	B	E	F	G	H
A-Rukara/Kibungo Rwanda		9.838 6.656*	7.985 5.402*	12.256 8.292*	6.848 4.633*	12.540 8.484*
B-Arboretum de Ruhande Rwanda			17.619 11.920*	23.945 16.201*	18.038 12.204*	25.306 17.122*
E-Kakamega Kenya				1.694 1.146	5.107 3.456*	5.040 3.410*
F-Kisaina 5B Kenya					4.442 3.005*	1.936 1.309
G-Kisaina 4E Kenya						3.241 2.193*
H-Budongo Forest Uganda						

NB. Small squared distances between pairs of provenances indicate similarity, while larger ones indicate dissimilarity.

* Pairs for which discrimination between provenances is possible. In 86.7% of cases, the test statistic values exceed the critical value at $P \leq 0.05$ (i.e. 2.09), suggesting that discrimination using morphological characteristics is possible.

Table 4.18 Squared distances (top line) and test statistic values (bottom line) between provenances, generated by discriminant function analysis of seed, seedling growth and morphological characteristics.

Provenance	A	B	E	F	G	H
A-Rukara/Kibungo Rwanda		19.678 8.697*	32.097 14.186*	34.689 15.332*	26.887 11.883*	55.775 24.650*
B-Arboretum de Ruhande Rwanda			23.373 10.330*	32.328 14.288*	23.514 10.392*	41.623 18.396*
E-Kakamega Kenya				3.494 1.544	6.362 2.812*	14.024 6.198*
F-Kisaina 5B Kenya					7.078 3.128*	15.631 6.908*
G-Kisaina 4E Kenya						10.329 4.565*
H-Budongo Forest Uganda						

NB. Small squared distances between pairs of provenances indicate similarity, while larger ones indicate dissimilarity.

* Pairs for which discrimination between provenances is possible. In 93.3% of cases, the test statistic values exceed the critical value at $P \leq 0.05$ (i.e. 2.06), suggesting that therefore there is sufficient evidence to indicate that discrimination using a combination of characteristics is possible.

4.4 Discussion

Provenance variation has been extensively investigated in forest tree species. In the present study, the variation found between the six *Maesopsis eminii* provenances in growth and morphological characteristics suggest the existence of a considerable amount of genetic variability at the early (seedling) stage of development. According to Perks and McKay (1997), it is unlikely that differences among provenances result from variation in the greenhouse environment, since this is usually uniform to ensure that seedlings are exposed to essentially the same growing conditions. However, it is possible that the differences in growth and morphological characteristics could be related to the time of seedling emergence and/or seed size.

In both height and diameter growth, seedlings of both Rwandan provenances were distinctly faster growing than the other provenances. Kenyan provenances had lower height and diameter growth, while the Ugandan provenance had the lowest. The superiority of the Rwandan provenances may reflect both their genotype and their rapid seed germination and emergence, especially in the 25°C greenhouse. On average, the Rwandan provenances, Rukara/Kibungo and Arboretum de Ruhande, emerged 35 and 33 days respectively after sowing, compared to the three Kenyan and the Ugandan provenances whose average seed emergence dates were 39, 45, 44 and 46 days respectively after sowing.

The difference in height and diameter growth could also be explained by the sporadic germination of seeds. In this study, the minimum and the maximum periods for germination to complete were 7 and 12 weeks in the 25°C greenhouse, compared with 12 and 19 weeks in the 20°C greenhouse. Mugasha and Msanga (1987) reported similar sporadic and prolonged germination (up to 12 weeks) of *Maesopsis eminii*, while Mondal (1986) reported even a longer period for the completion of *Maesopsis eminii* seed germination (14 to 25

weeks). Mugasha and Msanga (1987) assert that the sporadic and prolonged germination of *Maesopsis eminii* is an example of an adaptation characteristic of some tropical tree species, and allows seed germination to be spread over a long period to increase the chances of seedling survival under highly fluctuating environmental conditions.

To reduce errors which might have resulted from measuring plants of different ages and sizes, the dates on which germinated seeds were pricked out into plastic pots were recorded. It was in fact the growth rates over the period spent in the plastic pots that were used in comparisons.

Seed size may also have influenced the height and diameter growth of provenances. The two Rwandan provenances, which had the most rapid growth in height and diameter, also had the greatest seed size. Seeds were, on average, 24.7 mm long and 12.4 mm wide in the Rukara/Kibungo provenance, and 21.7 mm long and 11.8 mm wide in the Arboretum de Ruhande provenance. The Ugandan provenance, which had the slowest growth, also had the smallest seeds (18.7 mm long and 10.4 mm wide on average). Harlan *et al.* (1976) suggest that seedling vigour is closely related to seed size and energy stored in the endosperm and that this may determine the ability of seedlings to survive in areas where seedling vigour is important. This assertion supports claims made by Stebbins (1974) that natural selection for increased seed size was of prime importance in habitats where species survival or successful adaptation depended on seedling vigour. Foster and Janson (1985) have confirmed the existence of a relationship between large seed size and successful establishment in shaded plant communities. They stressed the greater importance of large seeds in shaded habitats than in open ones. Fast growing seedlings such as those of the Rwandan provenances may be better able to survive in competitive environments or to withstand damage from wild animals. The production of

shorter seedlings by the small-seeded Ugandan provenance is a characteristic which could also be explained by natural selection. Ibrahim (1996), working on *Faidherbia albida* provenances, reported that the shortest seedlings were produced from small-seeded provenances. He suggested that the small-seeded habit evolved under the harsh environmental conditions (i.e. a short rainy season of low and erratic rainfall and a long, hot and dry season) of Sahelian Africa. It is, however, surprising that the Ugandan provenance of *Maesopsis eminii* had the smallest sized seed, given that they were collected in the Budongo Forest Reserve where environmental conditions are not as extreme as those in the Sahel. The impression is that rainfall and other environmental conditions for growth in Budongo Forest Reserve are sufficient not to confer an evolutionary advantage on the production of small seeds. It should also be remembered that even the relatively small seeds of the Budongo provenance are much larger than the seeds of *Faidherbia albida.*

The differences in height growth between provenances were significant, but the amount of variation accounted for by provenances was low compared with the variation accounted for by seedlings within provenances (Table 4.2). Since there is considerable variation within provenances, there is a possibility of identifying good individual genotypes by progeny testing. Selecting for height would be useful since total height is considered to be a characteristic indicating growth superiority (Brown and Goddard, 1961). If individuals with fast juvenile height growth are likely to develop more rapidly later, as asserted by Daniels *et al.* (1979), it may be possible to select for growth at the seedling stage.

A similar pattern was observed for diameter: the proportion of the total variation due to provenances was very low and that due to seedlings within provenances was relatively high. As for height, selecting within provenances

should allow the identification of good genotypes. As reported by Philip (1983), diameter growth is extremely slow during the early stages of growth and early differences in diameter may be very important.

In general, plants grown in the 25°C greenhouse showed higher rates of height and diameter growth. This shows the influence the temperature had on growth and suggests that growth of *Maesopsis eminii* would be affected (slowed down) if it were planted in areas characterised by low temperatures. The highest proportion of the total variation in height and diameter growth was accounted for by location (greenhouse). Selecting the right place/site for planting *Maesopsis eminii* is therefore important if the aim is to achieve high rates of growth.

The assessment of morphological characteristics showed that some provenances had high values for several characteristics and others had low values for the same characteristics. In ranking the different provenances according to the values of different characteristics, the following summary gives a general picture.

The Rukara/Kibungo (Rwanda) provenance had the highest values for number of leaves on the main stem, number of leaves on the second branch, and branch length. This provenance and Kakamega (Kenya) had also the highest value for petiole percentage of the first leaf on the second branch (Table 4.1).

The Arboretum de Ruhande (Rwanda) provenance had the highest values for height and diameter growth, number of first order branches, number of second order branches, leaf area (first leaf on the second branch) and petiole percentage (largest leaf on the second branch) (Table 4.1).

The Rukara/Kibungo (Rwanda) and Kakamega (Kenya) provenances had the highest values for petiole percentage (first leaf on second branch). The Kakamega provenance had the widest branch angle. The Kisaina 4E (Kenya) provenance had the highest leaf shape index and leaf area (largest leaf on second branch) (Table 4.1).

Higher values for most morphological characteristics were generally recorded in the Rwandan provenances, while the lowest were mainly found in the Ugandan and Kisaina 5B (Kenyan) provenances. Seedlings of the Rwandan provenances were the first to be produced as a result of early seed germination and emergence. This suggests that the high values were the result of the large seedling sizes of the Rwandan provenances. The low values for the Ugandan provenance may be linked to the small size of its seeds and/or to the slow germination of seeds.

Although there were significant differences in morphological characteristics between provenances, the amount of variation accounted for by provenances in most of the characteristics was very low. Most of the variation in morphological characteristics was accounted for by differences between plants within provenances (Table 4.2).

For some morphological characteristics, such as leaf areas of the first and largest leaves on the second branch, the highest proportion of the total variation was accounted for by the growing environment (Table 4.2). For all provenances, the higher total number of leaves on the main stem was recorded in the 25°C greenhouse, while the higher leaf areas for all provenances were observed in the 20°C greenhouse. This suggests that the higher temperature in the 25°C greenhouse has stimulated more growth and hence the production of more leaves on main stems. It is surprising that the number of leaves on second

branches was not influenced in the same way, but it is suspected that excessive shedding of leaves in the 25°C greenhouse may be the cause. In contrast, the temperature in the same greenhouse caused a reduction in leaf area compared with that observed in the 20°C greenhouse.

Since much of the variation in morphological characteristics is accounted for by plants within provenances (as was also observed for height and diameter growth), there is an opportunity for selection within provenances for characteristics of interest. Leaf shape (and size) are under strong genetic control (Parkhurst and Loucks, 1972). Differences in leaf shape and size affect energy and gas exchange processes and may affect the ability to develop large leaf areas under plantation conditions. The high variation in leaf shape indices observed within provenances could therefore be usefully exploited. However, since leaf shape (and size) are also under strong developmental control (Mitchell *et al.*, 1992), it should be borne in mind that leaf morphology may change over time, and selection for that characteristic should take this into consideration.

The variation in branch angle observed within provenances offers the possibility of selection for branch-related characteristics useful for many purposes. As pointed out by Kramer and Kozlowski (1979), forest geneticists are interested in the effects of branch angles of trees on wood quality. They state that conifers with narrow or acute branch angles tend to prune less readily and have larger knots than those with wide branch angles. This would not perhaps be very relevant to *Maesopsis eminii* since it is a self-pruning species, but branch angles are important in other respects. Kramer and Kozlowski (1979) found that differences in crown form of some tree species are associated with variation in branch angles. For *Maesopsis eminii*, selection for branch angle and hence crown form could be done to meet requirements for specific

purposes such as crop shading (e.g. in coffee plantations) or agroforestry practices since this species is already among those used in crop associations.

Discrimination between the different provenances on the basis of height and diameter growth produced four groups (Figure 4.21a), in contrast to discrimination based on seed length and width, which gave five groups (Figure 3.2). Plots resulting from principal component analysis of morphological characteristics (Figure 4.21 b-d) and of all seed and seedling traits (Figure 4.21 e-g) gave exactly the same number of provenance groups (i.e. four groups), with the same provenances in each group. The two Rwandan provenances formed two distinct groups, the two Kenyan provenances (Kakamega and Kisaina 5B) formed the third group, and one Kenyan (Kisaina 4E) and the Ugandan (Budongo Forest Reserve) provenances formed the fourth group. The different groupings resulting from the use of different seed and seedling characteristics suggests that it is necessary to identify reliable diagnostic characteristics for assessing similarities/differences between provenances. Alternatively, genetic markers could be used to countercheck the discrimination made separately by seeds, growth, morphological and the combination of all seed and seedling characteristics to determine which combinations are likely to have the greatest discriminating power.

These early observations obtained from greenhouse performance may not reflect differences in mature trees. Nevertheless, the apparent provenance differences in growth (height and diameter) and in morphological characteristics may provide a basis for the initial stages of a tree improvement programme in which superior individuals could be selected from within the best performing provenances.

CHAPTER 5: PROVENANCE VARIATION: ISOZYMES

5.1 Introduction

Electrophoresis of enzymes is one of the techniques used by forest geneticists to examine genetic variation between and within populations of tree species. Busov (1995) considers that electrophoretic methods have considerable potential for distinguishing between populations, mainly when discrimination is not possible by morphological traits.

Distinguishing between populations (seed origins) of *Maesopsis eminii* would be of a great practical importance because of its wide geographic distribution. Although it is a species with a high concentration in eastern Africa, its natural distribution extends from south to western Africa. It has also been introduced in many parts of the world (see Chapter 2).

It is reported that a clear distinction (on the basis of tree size) can be made between east-central and western African populations. However, stands of *Maesopsis eminii* in the east-central geographic zone (which constitute most of the provenances used in this study) look so similar that discrimination on the basis of morphological and/or phenological characteristics alone may be difficult. Therefore, a study of electrophoretically detectable isozyme patterns, most of which reflect genetic variation (Bergmann 1987), was carried out to determine whether they can be used to reliably differentiate between *Maesopsis eminii* provenances.

The study includes only two provenances from the western part of the distribution of *Maesopsis eminii*. However, even this initial study of mainly Central-East African provenances could provide useful information for developing conservation, breeding and/or management strategies for *Maesopsis eminii*. If the discriminating power of isozymes could be demonstrated in

Maesopsis eminii, a wider investigation involving more provenances (representing the whole natural distribution of the species) could follow. Studies of the genetic structure of individual populations might also be possible because, as pointed out by Hamrick and Godt (1989), there is evidence that high levels of genetic variation exist among individuals within populations of many forest tree species.

5.2 Material and methods

5.2.1 Provenances

Out of eleven provenances originally provided for use in this study, nine were used in electrophoresic analysis. These were A = Rukara/Kibungo (Rwanda), B = Arboretum de Ruhande (Rwanda), E = Kakamega (Kenya), F = Kisaina-5B (Kenya), G = Kisaina-4E (Kenya), H = Budongo Forest Reserve (Uganda), I = Onwamdua (Ghana), J = Mount Cameroon (Cameroon) and PL = Amani/Kwamkoro (Tanzania). Detailed information on provenances used in isozyme studies is presented in Table 3.1 (Chapter 3).

Provenances C = Amani (Tanzania), J = Mount Cameroon (Cameroon) and K = Bunyala (Kenya) were originally excluded from isozyme studies because their seeds did not germinate and hence there were no plants from which leaf material could be collected. A later attempt to use seeds rather than leaves in isozyme studies of the Cameroon provenance was successful. However, seeds displayed both negatively and positively charged enzymes on zymograms, in contrast to the negatively charged enzymes which were the only ones to be resolved from leaves of the other provenances. Comparison of leaf-resolved and seed-resolved enzymes of all provenances was not possible within the time of the study, but seed enzymes of the Arboretum de Ruhande provenance were also examined. A comparison between this provenance and the one from Cameroon, based on seed enzymes, is presented in this chapter, together with a

comparison of seven provenances whose seed did germinate and one provenance (PL) whose plants were provided by P. Bingelli of the University of Ulster, Coleraine.

Plants previously used in the greenhouse study of provenance variation in growth and morphological characteristics (Chapter 4) provided leaf tissue for the main isozyme study. Thirty plants of each provenance were sampled. In addition, all plants of the Ghanaian provenance (I, seven plants), and the Tanzanian provenance (PL, 13 plants) were sampled.

5.2.2 Sample collection and preparation

Whole leaves were collected from each plant, labelled and placed immediately in a portable thermos cool box to prevent enzyme denaturation and to facilitate transportation to the laboratory. Sample leaves were in an intermediate stage of growth (i.e. not young or mature) and were chosen from the first three branches from the top of the plant. This was done because very young leaves did not show any enzyme activity on zymograms and mature ones were difficult to grind. It was also suspected that mature leaves would contain high concentrations of some secondary plant metabolites (e.g. tannins), which could cause rapid deterioration of the enzymes when the leaves were homogenised. Young adult leaves often contain fewer secondary metabolites than older leaves (Wickneswari and Norwati, 1992). Leaves were taken to the laboratory immediately after collection, cleaned in cold tap water to remove any impurities and dried with a soft tissue. 100-150 mg of leaf tissue were taken from cleaned leaves and placed in a 5 ml test tube. 0.9 ml of extraction buffer was added to the tube, and the sample was then ready for grinding. Grinding was done by hand using a small plastic rod until all leaf segments were completely ground. Samples were then centrifuged for five minutes at 6000 rpm in a microcentrifuge. After centrifuging, samples were immediately placed

in a freezer (-10°C) for a period between one and four days, while preparing gels and buffers for an electrophoretic run.

For provenances J (Mount Cameroon), which failed to germinate, and B (Arboretum de Ruhande), re-run for comparison with seed isozymes of the Cameroonian provenance, twenty five seeds per provenance were used. The outer hard coat and inner seed coverings were removed before placing seeds in test tubes (one seed per tube) for grinding. Grinding, centrifuging and storage of seed samples was done following the same procedure as that used for leaf samples.

5.2.3 Buffers and gel preparation

5.2.3.1 Extraction buffer

Buffer additives are normally used to improve extract quality. They may include phenol-complexing agents such as polyvinylpyrrolidone (PVP), phenoloxidase inhibitors (e.g. diethyldithiocarbamate-DIECA), various antioxidants and reducing agents (e.g. mercaptoethanol, dithiothreitol), enzyme-stabilizing products such as sucrose and detergents such as Triton X100. Various combinations of these products are usually tried to find the optimal buffer for the enzymes under consideration. The extraction buffer which worked for enzymes dealt with in this study was prepared as follows.

Tris	1.212g
Sucrose	17.12g
Ascorbic acid	0.118g
Cysteine	0.106g
Dithiothreitol	0.015g
EDTA	0.002g
Triton X100	1ml

PVP	4g
2-mercaptoethanol	0.25ml
Distilled water to	100ml

5.2.3.2 Running gel buffer and gel preparation

In order to obtain desirable gel handling properties and adequate isozyme separation and clarity, it is necessary that a gel is properly prepared. Using an appropriate buffer is an important step into achieving this objective. Since there is no universal running gel buffer for all types of enzymes, it is necessary to survey the existing buffer systems in order to find the most appropriate for the enzymes being studied. In this study, the running gel buffer which gave best handling properties and resolution of bands consisted of 22.72 g of Tris dissolved in 500 ml of distilled water. The buffer was adjusted to pH 8.9 using hydrochloric acid (HCl).

Polyacrylamide gels were prepared using acrylamide (15 g), bis-acrylamide (0.39 g), N, N, N'N'-tetramethylethylenediamine (TEMED) (0.19 ml), running gel buffer (250 ml) and ammonium persulphate (0.175 g). The above quantities were enough to make three gels. A flat, two-piece perspex mould was used in the preparation of gels. The gel solution was pipetted into the mould, which was sealed with a rubber band/gasket and clamped together with eight strong clips. The gels set in about twenty minutes, after which they were kept in a fridge (4°C) until electrophoresis started.

5.2.3.3 Electrode buffers

Because the most important influence on the quality of electrophoresis results is often the electrode buffer chosen (Wendel and Weeden, 1989), a survey was

made to find the most appropriate buffer for the enzymes studied. Four types of electrode buffer were used because not all enzymes responded satisfactorily to any one buffer. The buffers used were as follows.

a) Buffer 1 (for MDH, ME, SDH, GLUDH)

Tris	18.80 g
Glycine	85.56 g
Distilled water	3 litres
pH adjusted to 8.3 using HCl	

b) Buffer 2 (for EST)

Tris	24.22 g
Citric acid	10.07 g
Distilled water	10 litres
pH adjusted to 7.5	

c) Buffer 3 (for ACP and ALP)

Tris	24.22 g
Citric acid	10.07 g
EDTA	3.70 g
Distilled water	10 litres
pH adjusted to 7.5	

d) Buffer 4 (for GOT)

Tris	44.00 g
Citric acid	10.07 g
Distilled water	10 litres
pH adjusted to 8.3	

5.2.4 Gel loading and running

Electrophoresis was carried out according to the procedure described by Cahalan (1983) and Kananji (1988).

Four buffer tanks of approximately 1 litre capacity each were used at a time. They were filled with appropriate electrode buffers and connected to two LKB 2103 power packs for the supply of electric current. Each power pack had two channels which made it possible to supply simultaneously the current to two tanks. The cooling system was switched on a few hours before the electrophoresis run to bring the temperature of the apparatus down to 4°C. The cooling was ensured by a 3:1 mixture of water-ethylene glycol which was pumped around a closed circuit through the base plates on which gels were placed. It was necessary to keep the apparatus cooled to 4°C because bands are usually sharpest if electrophoresis is performed at that temperature (Wendel and Weeden, 1989). To start the electrophoresis run, a gel was removed from the fridge and placed on the base plate of a tank with the sample pockets nearest the cathodal end. Using a pipette, the pockets (15 in total per gel) were filled with gel buffer and the power (100 volts) was switched on for 30 minutes. According to Hames (1981, cited by Kananji, 1988), it is necessary to provide this pre-electrophoresis period in order to remove excessive persulphate ions from the gel, since their presence can cause denaturation of native proteins. Contact between the gel and the electrode buffer was through

wicks cut from Vileda car cloths. After 30 minutes, the power was switched off and absorbent papers were carefully used to remove the remaining gel buffer from pockets. Samples, which were removed 30 minutes earlier from the freezer to allow them to thaw before the electrophoresis run, were pipetted into pockets. The power was switched on again, first for 30 minutes at 50 volts and then for a further 90 minutes at 250 volts. The type of apparatus used for electrophoresis is shown in Figure 5.1.

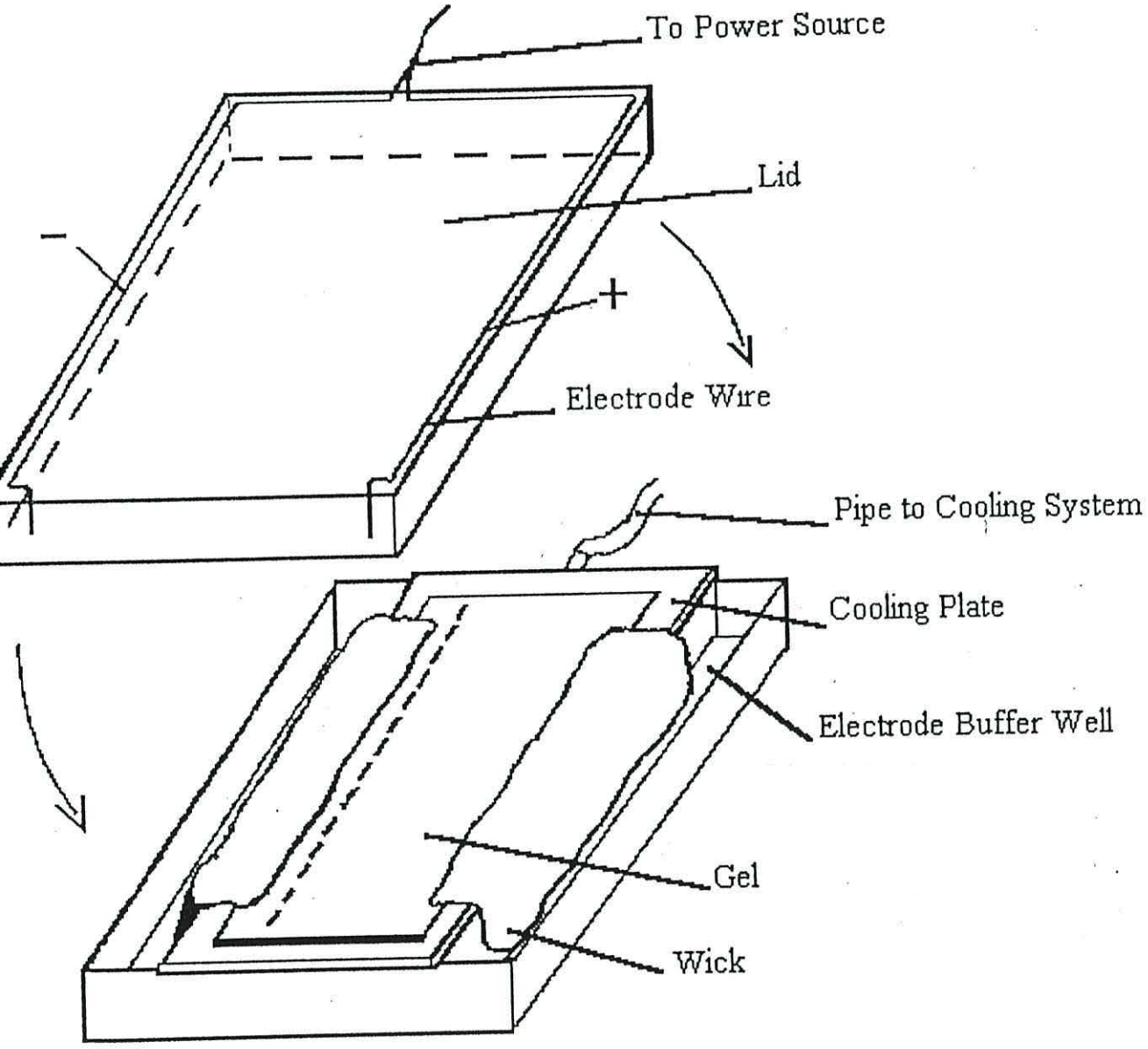


Figure 5.1 Electrophoresis apparatus. Reproduced from Dubbeldam (1997).

5.2.5 Staining

Four Perspex stain trays were available to stain four gels (from the four tanks) separately. After the electrophoretic run, gels were removed from the tanks, placed in trays and pre-prepared stains poured over them. After an incubation of about 20 minutes, enzyme bands could be visualised and scored using a light box. In total, eight enzyme systems could be interpreted: malate dehydrogenase (MDH), malic enzyme (ME), sorbitol dehydrogenase (SDH), glucose dehydrogenase (GLUDH), esterase (EST), acid phosphatase (ACP), alkaline phosphatase (ALP), and glutamate oxaloacetate transaminase (GOT). After band scoring and/or photographing, gels were discarded.

Stains and stain buffers for the different enzyme systems were prepared as suggested by Cheliak and Pitel (1984) and Trathan (1983).

5.2.5.1 Stains

MDH	L-malic acid	0.67 g
	Na_2CO_3	0.53 g
	NAD	20 mg
	NBT	20 mg
	PMS (added after 30 minutes)	3 mg
	0.01 M Tris - HCl pH 8.00	50 ml
	Incubate at 37°C until blue bands appear.	
ME	L-malic acid	0.67 g
	Na_2CO_3	0.53 g
	NADP	20 mg
	NBT	20 mg

	PMS (added after 30 minutes)	3 mg
	0.01 M Tris – HCl pH 8.00	50 ml
	Incubate at 37°C until blue bands appear.	

SDH	Sorbitol	0.50 g
	NAD	20 mg
	NBT	20 mg
	PMS (added after 30 minutes)	3 mg
	0.01 M Tris – HCl pH 8.00	50 ml
	Incubate at 37°C until blue bands appear.	

GLUDH	Glucose	250 mg
	NAD	1.5 ml
	NBT	0.5 ml
	PMS	0.5 ml
	0.2 M Tris – HCl pH 8.0	25 ml
	Incubate in the dark at 37°C until blue bands appear.	

EST	Fast blue RR	50 mg
	α-napthylacetate	20 mg
	β-napthylacetate	20 mg dissolved in:
	Acetone	1 ml
	0.01 M Tris – HCl pH 7.23	50 ml
	Incubate at 37°C until red and black bands appear.	

ACP	Fast blue RR	50 mg
	Na-α-napthyl phosphate	50 mg
	0.05 M acetate-acetic acid pH 5.60	50 ml
	Incubate at 37°C until brown bands appear.	

ALP	Fast blue RR	50 mg
	β-napthyl phosphate	50 mg
	$MgSO_4.10H_2O$	123 mg
	0.01 M Tris – HCl pH 7.50	50 ml
	Incubate at 37°C until red bands appear.	

GOT	Fast blue RR	150 mg
	α- ketoglutaric acid	100 mg
	L- aspartic acid	100 mg
	Pyridoxal-5-phosphate	5 mg
	0.01 M Tris – HCl pH 7.50	50 ml

5.2.5.2 Stain buffers

a) 0.05 M acetate-acetic acid pH 5.60

1 M acetic acid	56 ml
1 M NaOH	50 ml
Distilled water to	1 litre

b) 0.01 M Tris – HCl pH 7.23

Tris	12.10 g
Distilled water to	1 litre
1 M HCl to required pH	

c) 0.01 M Tris – HCl pH 7.50

Tris	12.10 g
Distilled water to	1 litre
1 M HCl to required pH	

d) 0.01 M Tris – HCl pH 8.00

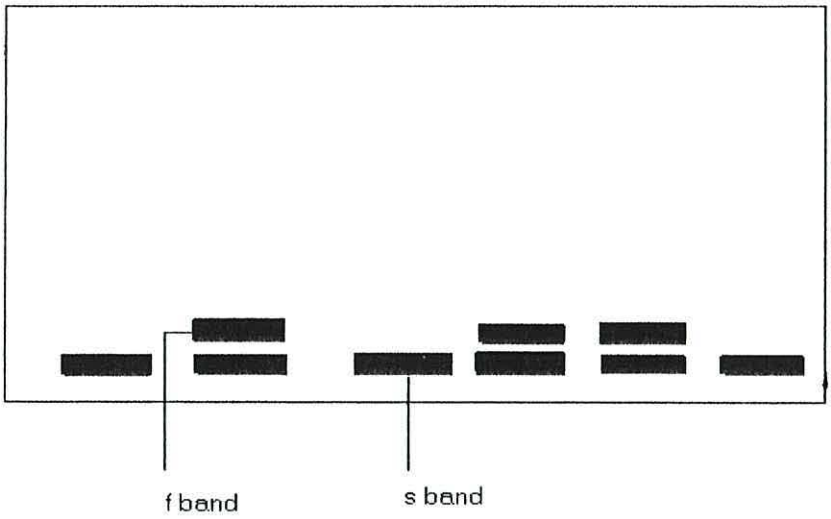
Tris	12.10 g
Distilled water to	1 litre
1 M HCl to required pH	

e) 0.2 M Tris – HCl pH 8.00

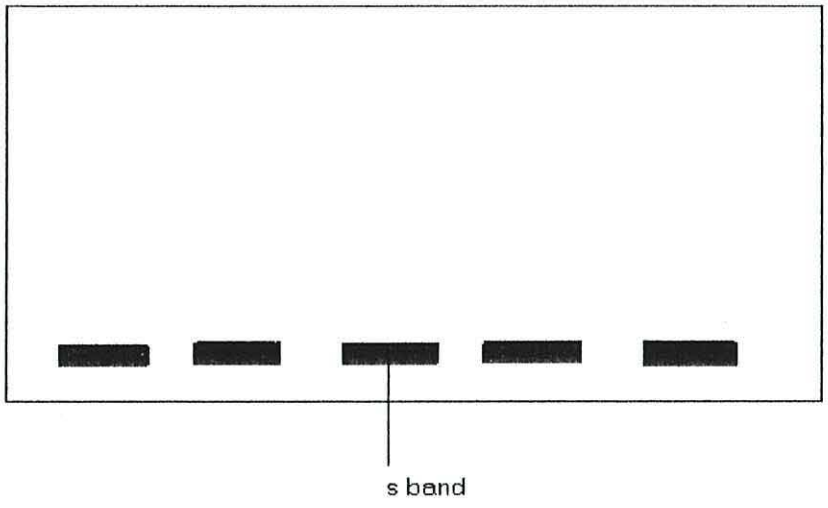
Tris	24.22 g
Distilled water to	1 litre
1 M HCl to required pH	

5.2.6 Zymogram survey and band scoring

After the development of the zymogram, various bands arising from the enzymatic action were observed. Enzymes essayed in this study displayed only one locus on each gel. These loci were mostly monomorphic; for the few polymorphic loci, scoring of bands was done by assigning the letters s (slow) for bands nearest to the origin (cathode) and f (fast) for those near the anodal end. For a monomorphic locus, all bands were considered slow and assigned the letter s as they were very near to the origin. Illustrations of typical zymograms are given in Figure 5.2.



(a) Polymorphic locus showing slow (s) and fast (f) bands (alleles)



(b) Monomorphic locus showing only slow (s) bands (alleles)

Figure 5.2 Typical zymograms.

5.2.7 Measures of variability

As the purpose of the isozyme study was to discriminate between provenances, only appropriate measures of variability were determined. Three measures of genetic variability within provenances were calculated: average number of alleles per locus (A), percentage of loci polymorphic per provenance (P), and average heterozygosity (H). Genetic identity (I) and genetic distance (D) were calculated for all pairs of the eight provenances from which leaf samples were taken. Genetic differentiation in the group of eight provenances was estimated by calculating D_{ST}, $\overline{D}_m$, R_{ST} and G_{ST}. The formulae used in calculations of the above measures are described in Chapter 2. UPGMA (unweighted pairs group method using arithmetic averages) clustering (Sneath and Sokal, 1973) based on genetic distances was used to produce a dendrogram showing the relationships between provenances.

5.3 Results

The enzyme systems assayed produced a total of eight loci (Table 5.1). MDH, ME, GLUDH, SDH, GOT and EST could be scored quite easily. For ACP and ALP the resolution was not good but scoring was still possible. All enzymes were inferred to be under the control of single gene loci. A locus was considered polymorphic if at least one individual in a one sample of 30 plants (of one provenance) was heterozygous at that locus. Using this criterion, seven out of eight loci were found to be polymorphic, with the number of loci polymorphic in any one provenance varying between three and seven.

The number of alleles per locus (A), percentage of polymorphic loci (P) and average heterozygosity (H) for each provenance are shown in Table 5.2. Table 5.3 shows the allele frequencies for all loci scored.

Table 5.1 Enzyme systems scored and number of alleles observed in nine provenances of *Maesopsis eminii.*

Enzyme	Category of enzyme	Number of alleles
Glutamate oxaloacetate transaminase (GOT)	Polymorphic	2
Esterase (EST)	Polymorphic	2
Malate dehydrogenase (MDH)	Polymorphic	2
Acid phosphatase (ACP)	Polymorphic	2
Alkaline phosphatase (ALP)	Polymorphic	2
Glucose dehydrogenase (GLUDH)	Polymorphic	2
Malic enzyme (ME)	Polymorphic	2
Sorbitol dehydrogenase (SDH)	Monomorphic	1

5.3.1 Average number of alleles per locus (A)

The average number of alleles per locus for the eight provenances (i.e. those from which leaf samples were taken) differed considerably. Values ranged from 1.13 to 1.63 for Nkawkaw (Ghana) and Kisaina 4E (Kenya) provenances respectively, with an average of 1.34 for all provenances. The Arboretum de Ruhande also had a high average number of alleles per locus (1.50) in comparison to the rest of provenances (Table 5.2).

5.3.2 Percentage polymorphic loci (P)

Differences between provenances in percentage polymorphic loci were relatively large. The highest percentage (63%) was found in Kisaina 4E (Kenya) provenance, while the lowest (13%) was in the Nkawkaw (Ghana) provenance. A value of 50% for the Arboretum de Ruhande provenance is also

high compared to the values for other provenances. The overall average for all provenances was 34% (Table 5.2).

5.3.3 Average heterozygosity (H)

Estimates of the average heterozygosity varied from 0.071 (for Budongo Forest Reserve provenance) to 0.262 (for the Arboretum de Ruhande provenance) with an average of 0.158 for all provenances. The PL (Amani/Kwamkoro, Tanzania) and G (Kisaina 4E, Kenya) provenances also showed high average heterozygosity values compared to the rest (Table 5.2).

Table 5.2 Number of alleles per locus (A), percentage polymorphic loci (P), and average heterozygosity (H) in nine provenances of *Maesopsis eminii*.

Provenance	Number of Plants	Number of alleles per locus (A)	Percentage of polymorphic loci (P)	Average heterozygosity (H)
Leaf samples				
A-Rukara /Kibungo Rwanda	30	1.38	38	0.112
B-Arboretum de Ruhande Rwanda	30	1.50	50	0.262
E-Kakamega- Kenya	30	1.25	25	0.129
F-Kisaina 5B- Kenya	30	1.33	33	0.085
G-Kisaina 4E- Kenya	30	1.63	63	0.204
H-Budongo Forest Reserve – Uganda	30	1.25	25	0.071
I - Nkawkaw - Ghana	7	1.13	13	0.152
PL- Amani/Kwamkoro Tanzania	13	1.25	25	0.250
Mean	**25**	**1.34**	**34**	**0.158**
Seed samples				
B- Arboretum de Ruhande Rwanda	30	1.11	11	0.044
J- Mount Cameroon Cameroon	8	1.11	11	0.032
Mean	**19**	**1.11**	**11**	**0.038**

Table 5.3 Allele frequencies at eight enzyme loci in *Maesopsis eminii.* (letters f and s represent the fast and the slow alleles respectively).

Provenance	Allele	E N Z Y M E							
		GOT	EST	MDH	ACP	ALP	GLUDH	ME	SDH
Leaf samples									
A	f	0.27	0.18	0.03	0.00	0.00	0.00	0.00	0.00
Rukara/Kibungo Rwanda	s	0.73	0.82	0.97	1.00	1.00	1.00	1.00	1.00
B	f	0.48	0.33	0.07	0.17	0.00	0.00	0.00	0.00
Arboretum de Ruhande Rwanda	s	0.52	0.67	0.93	0.83	1.00	1.00	1.00	1.00
E	f	0.30	0.22	0.05	0.00	0.00	0.00	0.00	0.00
Kakamega Kenya	s	0.70	0.78	0.95	1.00	1.00	1.00	1.00	1.00
F	f	0.42	0.05	0.03	0.05	0.05	0.00	0.00	0.00
Kisaina 5B Kenya	s	0.58	0.95	0.97	0.95	0.95	1.00	1.00	1.00
G	f	0.35	0.25	0.05	0.35	0.00	0.10	0.17	0.00
Kisaina 4E Kenya	s	0.65	0.75	0.95	0.65	1.00	0.90	0.83	1.00
H	f	0.22	0.93	0.05	0.00	0.00	0.00	0.00	0.00
Budongo Forest Reserve Uganda	s	0.78	0.07	0.95	1.00	1.00	1.00	1.00	1.00
I	f	0.07	0.57	0.07	0.00	0.00	0.00	0.00	0.00
Nkawkaw Ghana	s	0.93	0.43	0.93	1.00	1.00	1.00	1.00	1.00
PL	f	0.50	0.50	0.08	0.00	0.00	0.00	0.00	0.00
Amani/Kwamkoro Tanzania	s	0.50	0.50	0.92	1.00	1.00	1.00	1.00	1.00
Seed samples									
B	f	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arboretum de Ruhande Rwanda	s	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00
J	f	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mount Cameroon Cameroon	s	0.86	1.00	1.00	1.00	1.00	1.00	1.00	1.00

5.3.4 Gene identity (Js), gene diversity (Hs) and Jxy values.

These measures were calculated for each locus and provenance and were used in the calculation of genetic distances between provenance pairs. Details are given in Tables 5.4 (for Js and Hs) and 5.5 (for Jxy).

5.3.5 Genetic identity (I) and standard genetic distance (D)

Nei's (1972) genetic identity (I) and standard genetic distance (D) were calculated for pairwise comparisons of eight *Maesopsis* provenances as shown in Tables 5.6 and 5.7 respectively. Genetic identities ranged from 0.883 (Kisaina 5B versus Budongo Forest Reserve) to 0.999 (Rukara/Kibungo versus Kakamega, and Budongo Forest Reserve versus Nkawkaw). For genetic distances, the values ranged from 0.001 (Rukara/Kibungo versus Kakamega and Budongo Forest Reserve versus Nkawkaw) to 0.124 (Kisaina 5B versus Budongo Forest Reserve). A dendrogram based on the matrix of genetic distances between provenances is presented in Figure 5.3.

Values of genetic identity and genetic distance for the pair of provenances J (Mount Cameroon) and B (Arboretum de Ruhande) assessed using seed samples were 0.997 and 0.003 respectively.

Table 5.4 Gene identity (Js, top line) and gene diversity (Hs, bottom line) for eight loci in nine provenances of *Maesopsis eminii*. Mean values Js and Hs were calculated over all eight loci.

PROV	E	N	Z	Y	M	E		$\bar{Js}$	$\bar{Hs}$
	GOT	EST	MDH	ACP	ALP	GLUDH	ME	SDH		
Leaf samples										
A										
Rukara/	0.606	0.705	0.942	1.000	1.000	1.000	1.000	1.000	**0.907**	**0.093**
Kibungo	0.394	0.295	0.058	0.000	0.000	0.000	0.000	0.000		
Rwanda										
B										
Arboretum de										
Ruhande	0.501	0.558	0.870	0.718	1.000	1.000	1.000	1.000	**0.831**	**0.169**
Rwanda	0.499	0.442	0.130	0.282	0.000	0.000	0.000	0.000		
E										
Kakamega	0.580	0.657	0.905	1.000	1.000	1.000	1.000	1.000	**0.893**	**0.107**
Kenya	0.420	0.343	0.095	0.000	0.000	0.000	0.000	0.000		
F										
Kisaina 5B	0.513	0.905	0.942	0.905	0.905	1.000	1.000	1.000	**0.896**	**0.104**
Kenya	0.487	0.095	0.058	0.095	0.095	0.000	0.000	0.000		
G										
Kisaina 4E	0.545	0.625	0.905	0.545	1.000	0.820	0.718	1.000	**0.770**	**0.230**
Kenya	0.455	0.375	0.095	0.455	0.000	0.180	0.282	0.000		
H										
Budongo										
Forest	0.657	0.870	0.905	1.000	1.000	1.000	1.000	1.000	**0.929**	**0.071**
Reserve	0.343	0.130	0.095	0.000	0.000	0.000	0.000	0.000		
Uganda										
I										
Nkawkaw	0.870	0.510	0.870	1.000	1.000	1.000	1.000	1.000	**0.906**	**0.094**
Ghana	0.130	0.490	0.130	0.000	0.000	0.000	0.000	0.000		
PL										
Amani	0.500	0.500	0.853	1.000	1.000	1.000	1.000	1.000	**0.857**	**0.143**
Kwamkoro	0.500	0.500	0.147	0.000	0.000	0.000	0.000	0.000		
Tanzania										
Seed samples										
B										
Arboretum de	0.680	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.960**	**0.040**
Ruhande	0.320	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
Rwanda										
J										
Mount	0.755	1.000	1.000	1.000	1.000	1.000	1.000	1.000	**0.969**	**0.031**
Cameroon	0.245	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
Cameroon										

Table 5.5 J_{XY} values (means of eight loci) for pairs of eight provenances of *Maesopsis eminii.*

PROVENANCE	B	E	F	G	H	I	PL
A-Rukara /Kibungo Rwanda	0.857	0.899	0.896	0.817	0.847	0.882	0.862
B-Arboretum de Ruhande Rwanda		0.853	0.851	0.788	0.823	0.837	0.836
E-Kakamega- Kenya			0.888	0.811	0.847	0.877	0.860
F-Kisaina 5B- Kenya				0.811	0.810	0.851	0.849
G-Kisaina 4E- Kenya					0.769	0.795	0.782
H-Budongo Forest Reserve Uganda						0.899	0.860
I – Nkawkaw - Ghana							0.858
PL- Amani/Kwamkoro Tanzania							

Table 5.6 Nei's (1972) genetic identity (I) for pairs of eight provenances of *Maesopsis eminii.*

PROVENANCE	B	E	F	G	H	I	PL
A-Rukara /Kibungo Rwanda	0.984	0.999	0.993	0.980	0.910	0.965	0.969
B-Arboretum de Ruhande Rwanda		0.988	0.987	0.986	0.922	0.957	0.991
E-Kakamega- Kenya			0.993	0.981	0.917	0.968	0.975
F-Kisaina 5B- Kenya				0.979	0.883	0.935	0.966
G-Kisaina 4E- Kenya					0.902	0.949	0.963
H-Budongo Forest Reserve Uganda						0.999	0.954
I – Nkawkaw - Ghana							0.969
PL- Amani/Kwamkoro Tanzania							

Table 5.7 Nei's (1972) standard genetic distance (D) for pairs of eight provenances of *Maesopsis eminii*.

PROVENANCE	B	E	F	G	H	I	PL
A-Rukara /Kibungo Rwanda	0.016	0.001	0.007	0.020	0.094	0.036	0.031
B-Arboretum de Ruhande Rwanda		0.012	0.013	0.014	0.081	0.044	0.009
E-Kakamega- Kenya			0.007	0.019	0.087	0.033	0.025
F-Kisaina 5B- Kenya				0.021	0.124	0.067	0.035
G-Kisaina 4E- Kenya					0.103	0.052	0.038
H-Budongo Forest Reserve Uganda						0.001	0.047
I – Nkawkaw - Ghana							0.031
PL- Amani/Kwamkoro Tanzania							

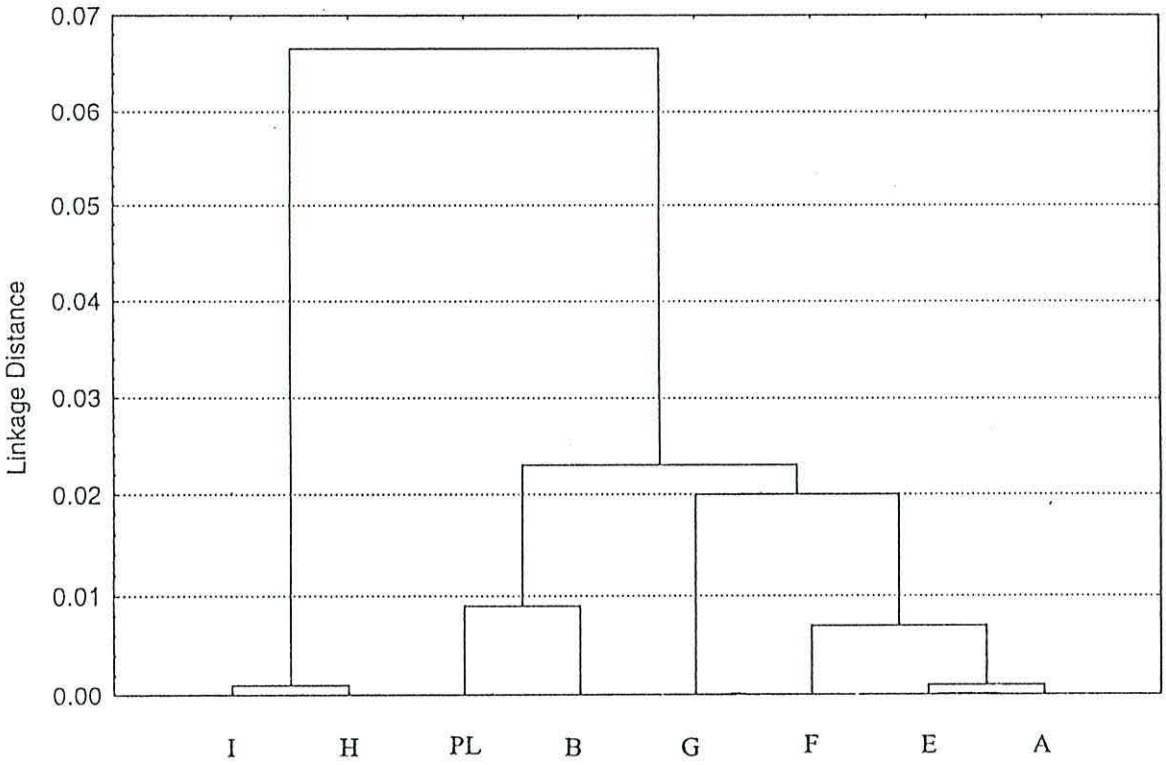


Figure 5.3 UPGMA dendrogram based on Nei's genetic distance for pairs of eight provenances of *Maesopsis eminii.*

Key to provenances.

A = Rukara/Kibungo (Rwanda)
B = Arboretum de Ruhande (Rwanda)
E = Kakamega (Kenya)
F = Kisaina 5B (Kenya)
G = Kisaina 4E (Kenya);
H = Budongo Forest Reserve (Uganda)
I = Nkawkaw (Ghana)
PL = Amani/Kwamkoro (Tanzania).

5.3.6 Genetic differentiation

Various measures of genetic differentiation among the eight provenances of *Maesopsi eminii,* based on the eight scored enzymes, are presented in Table 5.8.

Table 5.8 Measures of genetic differentiation among eight provenances of *Maesopsis eminii* for eight loci.

LOCUS	MEASURES OF GENETIC DIFFERENTIATION			
	D_{ST}	$\overline{Dm}$	R_{ST}	G_{ST}
GOT	0.036	0.041	0.102	0.082
EST	0.137	0.156	0.468	0.291
MDH	0.001	0.001	0.007	0.006
ACP	0.028	0.032	0.311	0.214
ALP	0.001	0.001	0.053	0.044
GLUDH	0.002	0.002	0.111	0.089
ME	0.006	0.007	0.205	0.152
SDH	0.000	0.000	0.000	0.000
Mean	**0.026**	**0.030**	**0.157**	**0.110**

Definitions of the above measures as given by Nei (1975) are as follows.

- D_{ST} is a measure of the absolute amount of gene differentiation and takes into account the level of heterozygosity within populations.
- $\overline{D}_m$ is a measure of interpopulation gene diversity which does not take into account the level of heterozygosity within populations.
- R_{ST} is a measure of interpopulation gene diversity relative to that within populations.
- G_{ST} is a measure of gene differentiation relative to the total population group. G_{ST} is also defined as the proportion of variation among populations.

5.4 Discussion

Electrophoresis of enzymes has proved to be a successful method of detecting geographic heterogeneity in many tree species. In this study, the enzymes scored showed moderate levels of variability in eight *Maesopsis eminii* provenances. Only one locus for each enzyme could be detected on stained gels. MDH, ACP, ALP, GLUDH, ME and SDH enzymes were marked by a deficiency of heterozygotes. Typically, in a sample of fifteen plants (the number of samples on a single gel), only one or two heterozygotes could be observed. Deficiency in heterozygotes could be the result of Wahlund effect - sampling of distinct subpopulations within a population. It could also be the result of mis-scoring or could be due to the presence of null alleles. Null alleles may not be eliminated by selection, and their frequency in a population may be sufficient to cause an apparent deficiency of heterozygotes (Hoare and Beamont, 1995, cited by Wilding, 1996). There were more heterozygotes at the GOT and EST loci. Provenances B (Arboretum de Ruhande, Rwanda) and G (Kisaina 4E, Kenya) had the highest values for the mean number of alleles per locus (A), percentage of polymorphic loci (P), and average heterozygosity (H) Table 5.2). The average heterozygosity of the PL (Amani/Kwamkoro, Tanzania) provenance was also high, but the mean number of alleles per locus and the percentage of polymorphic loci were lower than those of provenances B and G. The lowest values for the mean number of alleles per locus and percentage of polymorphic loci were found in the Ghanaian provenance, and the lowest average heterozygosity in the Ugandan provenance (Table 5.2).

The overall average heterozygosity obtained for the eight *Maesopsis eminii* provenances was 0.158. This value is low when compared with that reported by Ibrahim (1996) for *Faidherbia albida* (H = 0.346) or that reported by Playford (s.d., cited by Ibrahim, 1996) for *Acacia melanoxylon* (H = 0.209). However, it is slightly higher than the value reported by Hamrick and Loveless (1986) for

tropical rain forest trees (H = 0.111) or that reported for dicotyledons (H = 0.113) by Koshy (1987). The value falls within the range of average heterozygosities reported for some *Acacia* species, for example *Acacia crassicarpa* (H = 0.131 - 0.150) and *Acacia auriculiformis* (H = 0.126 - 0.166) (Moran *et al.*, 1989). High isozyme variability is often associated with a wide geographic distribution, mating systems characterised by high level of outcrossing, seed dispersal by animals, a large effective population size and a long life span (high longevity) (Ledig, 1986; Hamrick and Godt, 1989). As *Maesopsis eminii* meets these conditions, it is surprising that it shows relatively little isozyme variation. It could be that the rate of outcrossing in *Maesopsis eminii* is not as high, and/or that effective population sizes are lower, than is generally assumed. The slightly greater variation found in the Arboretum de Ruhande, Amani/Kwamkoro and Kisaina 4E provenances might suggest that environmental factors or historical events (e.g. introductions from other parts of the natural range) have played a role in reducing genetic variation in the other *Maesopsis eminii* provenances. The gene diversity parameters estimated for this group of eight *Maesopsis eminii* provenances suggest that the level of genetic differentiation between provenances is rather low (Table 5.8).

The range of genetic distances for *Maesopsis eminii* (0.001 – 0.124) falls into the range observed for other woody perennials (0 – 0.333) as reported by Hamrick (1978, cited by Cahalan, 1983). Hartl (1980) suggests that D (genetic distances) values greater than 0.05 indicate greater genetic differentiation than is generally found between populations of a single species. Table 5.7 shows that distances between the Ugandan provenance (Budongo Forest Reserve) and the rest (with the exception of the Nkawkaw and Amani/Kwamkoro provenances from Ghana and Tanzania respectively) are greater than this value of 0.05. This suggests that the Ugandan provenance is genetically distinct from all the other provenances except the ones from Ghana (Nkawkaw) and

Tanzania (Amani Kwamkoro). The high genetic distances between provenances Kisaina 5B (Kenya) and Nkawkaw (Ghana), and provenances Kisaina 4E (Kenya) and Nkawkaw (Ghana) also suggest dissimilarity.

The UPGMA dendrogram (Figure 5.3) based on Nei's genetic distances suggests that the three Kenyan provenances (E-Kakamega, F-Kisaina 5B and G-Kisaina 4E) are very similar. This is not surprising since the three stands are located in the same region (Kakamega), and suggests that provenances F and G might have been established from seeds/material collected in the Kakamega Forest Reserve. The similarity of one of the Rwandan provenances A (Rukara/Kibungo) to Kenyan provenances (Kakamega particularly), would suggest that there has been an extensive exchange of material within the sub-region. However, there are no historical documents to support this suggestion.

Provenances B (Arboretum de Ruhande) and PL (Amani/Kwamkoro) appear to be very similar genetically. This similarity can perhaps be explained by the fact that Kibungo (Rwanda), where seeds used to establish *Maesopsis eminii* experimental plots at Ruhande Arboretum were collected (Département de Foresterie, ISAR, 1987), and Bukoba, the probable source area for the seed taken to Amani (Hall, 1994), are very close border regions.

Provenance H (Budongo Forest Reserve) and I (Nkawkaw) appear very similar and distinct from all other provenances. It is possible that the provenance from Ghana was established with seeds from Budongo Forest Reserve through collaboration (exchange or movement of forest material) between territories under the same colonial rule.

Genetic differentiation relative to the total group of provenances (G_{ST}) was 0.110. This value is lower than that reported for temperate zone species (G_{ST} =

0.246 ± 0.015) and species with a similar reproductive mode (G_{ST} 0.213 ± 0.027) (Hamrick and Godt, 1989). El-Kassaby and Yanchuk (1995) suggest that low G_{ST} values may be indicative of extensive gene flow (via pollen and/or seeds), factors which reduce the effect of geographic isolation of breeding regions and thus reduce the extent of genetic divergence. The low G_{ST} value of 11% for *Maesopsis eminii* indicates that most of the genetic diversity (89%) occurred within provenances. According to Lumaret and Michaud (1991), the fact that most of the diversity is found within, rather than between, provenances suggests that gene flow between populations has an important influence on genetic variation in this species.

The R_{ST} value found for *Maesopsis eminii* (0.157) is within the range of 0.02 – 0.53 for outbreeding plant species reported by Brown (1979, cited by Cahalan, 1983).

D_{ST} measures the absolute amount of gene differentiation and takes into account the level of heterozygosity within populations. D_{ST} for the group of eight *Maesopsis eminii* provenances was 0.026. D_{ST} values of 0.02 and 0.01 for eastern and western Turkish provenances of *Castanea sativa* were reported by Villani and Pigliucci (1991).

The fourth measure of genetic differentiation calculated in this study, $\overline{D}_m$, is perhaps the best measure of divergence (Nei, 1975). $\overline{D}_m$ is a measure of the absolute amount of genetic differentiation which does not take into account the level of heterozygosity within populations. The value for the eight *Maesopsis eminii* provenances was 0.030. $\overline{D}_m$ values for local populations of other species include those for humans (0.014), house mouse (0.015), *Drososphila equinoxialis* (0.026), horseshoe crab (0.006), and *Lycopodium lucidulum*

(0.027) (Nei, 1975). The degree of gene differentiation among *Maesopsis eminii* provenances therefore appears to be relatively high. However, Cahalan (1983) and Schoen (1982, cited by Cahalan, 1983) have reported high $\overline{D}_m$ values in species such as *Primula vulgaris* ($\overline{D}_m$ = 0.046) and *Gilia achilleifolia* ($\overline{D}_m$ values ranging between 0.076 and 0.183), which suggest that gene differentiation in *Maesopsis eminii* is moderate compared with differentiation in some other plant species.

Although genetic distance measures provide additional information, not absolute yardsticks for making taxonomic decisions (Rosenblatt and Waples, 1986, cited by Wilding, 1996), they have contributed to a picture of genetic variability among the *Maesopsis eminii* provenances studied, which may be used for developing future conservation and breeding strategies for the species.

CHAPTER 6: PROVENANCE VARIATION: VEGETATIVE REPRODUCTION AND PROPAGATION

6.1 Introduction

Maesopsis eminii is usually propagated by seeds, and no any other means of propagating the species has been systematically investigated. Therefore, to ensure that *Maesopsis eminii* plants are available to growers, a regular supply of seeds is required. Normally, this is not a problem since *Maesopsis eminii* seeds are produced in most years. However, as reported by Mondal (1986), *Maesopsis eminii* seeds lose their viability within a few months of collection, making it difficult to store them for future planting. Thus, despite the many advantages offered by the species, its use could be limited by seed shortages in bad seed years with adverse consequences on planting programs.

This suggests that alternative ways of reproducing trees and producing planting stock should be investigated in order to ensure a continuous supply of services offered by the species. Coppicing, pollarding, grafting and vegetative propagation by cuttings are the approaches investigated in this chapter. A short investigation by Geddes (1993) indicated that *Maesopsis eminii* has the ability to coppice from cut stumps. This study deals with provenance variation in the capacity for vegetative reproduction, which could be exploited by selecting trees which respond well to coppicing and pollarding, and which are amenable to vegetative propagation by cuttings.

Coppicing of *Maesopsis eminii* would be very useful in many tropical countries where rotation periods (for tropical species) are normally short, and provide regular, large quantities of small roundwood material. For instance, trials with *Eucalyptus saligna* and *E. maidenii* in Rwanda have shown that coppicing is a practical way of producing (say) firewood and large poles from the same plantation (Evans, 1992). It is possible that coppicing of *Maesopsis*

eminii could also be practised in agroforestry (e.g. in alley cropping systems). With pollarding, which is rather like coppicing at a high level, it is possible to produce one large diameter pole-length from the bole while continuing to obtain regular supplies of firewood (Evans 1992). Trees which showed good pollarding could also be used in agroforestry and especially in sylvipasture, as the regrowth that develops on boles is out of reach of livestock browsing. Successful vegetative propagation by cuttings could not only be used to supplement planting material in the case of seed shortages, but also for multiplying genetically superior stock for use in clonal plantations. Clones (though not dealt with in this study) offer the advantages of genetic uniformity and the immediate availability of superior individuals for seed orchards and for plantations (Bhatnagar, 1973).

6.2 Material and methods

6.2.1 Provenances

Details of the provenances used for pollarding, coppicing, grafting and rooting of cuttings are shown in Table 3.1 (Chapter 3). Out of the 11 provenances in Table 3.1, only six (A, B, E, F, G, H) were used in the study described in the following sections.

6.2.2 Experimental details

Experiments on pollarding, coppicing and grafting were carried out at the Pen-y-ffridd field station of the University of Wales, Bangor. Seedlings used in these experiments had been grown in the 25°C greenhouse, and had previously been assessed for growth and morphological characteristics (Chapter 4). Greenhouse conditions (operating temperature, lighting, benches) were as described in section 4.2.2 (Chapter 4). Seedlings were regularly watered and fed with a balanced liquid fertiliser (Vitax - Vitafeed 111 – 19:19:19). Weeding of potted seedlings and fumigation were carried out whenever necessary.

Seedlings were arranged in a randomised complete block design; details of the number of blocks, plots and seedlings are given in Chapter 4 (section 4.2.2).

6.2.2.1 Pollarding

This experiment was carried out in March 1997 and seedlings were 12 months old at the time when pollarding was done. Seedlings were cut at a height of 20 cm above the root collar (about 1/2 of the total height) using a pair of sharp secateurs. All branches and leaves on the remaining stumps were also removed. The cut seedlings remained in their positions on benches. Watering, feeding (with the same Vitafeed 111 liquid fertiliser), weeding and fumigation continued as before the pollarding. After four months, the cut stumps (pollards) were assessed for regrowth. The total number of shoots was counted using a tally device and the length of the longest shoot on each stump (which was always the shoot nearest the apex) measured using a ruler. Data on shoot length were subjected to an analysis of variance to test for significant difference between provenances.

6.2.2.2 Coppicing

After assessing shoot regrowth on pollards, the plants were cut again in July 1997 for the coppice experiment. The plants were then about 16 months old. Cuts were made at about 3 cm above the root collar using a pair of secateurs. Caring for the stumps continued as before. As in the pollarding experiment, assessment for regrowth was done after four months. The total number of shoots was counted using the same tally device and the length of the longest shoot per stump (i.e. again the shoot nearest the apex) measured using a ruler. Data on shoot length were analysed using analysis of variance methods.

6.2.2.3 Grafting

This experiment was carried out in December 1997/January 1998 when seedlings were 21-22 months old. Only five provenances: A (Rukara/Kibungo), B (Arboretum de Ruhande), E (Kakamega), G (Kisaina 4E) and H (Budongo Forest Reserve) were used because there were not enough plants of the F (Kisaina 5B) provenance to be included in the experiment. Whip grafting was the technique used. Cuts were made at the top of the stock and at the bottom of the scion. The stock and the scion were then inserted into each other, with the tongues interlocking, taking care to match the cambium layers. Budding rubbers were used to hold the scion and the stock securely together and help unification, and the joins were covered by polythene sachets to protect them from desiccation. Sachets and rubbers were removed after 3-4 weeks, when shoot growth started. Thirty rootstocks (five groups of six rootstocks) per provenance were used, and each group received six scions from a single provenance, including the provenance forming the rootstocks. This provided thirty grafts per provenance, making a total of 150 grafts for all the five provenances used. Grafted plants were arranged on benches in a randomised block design in the 20°C greenhouse.

6.2.2.4 Rooting of cuttings

The experiment on rooting of cuttings was carried out in a heated glasshouse at Treborth Botanical Gardens, University of Wales, Bangor, in March 1997. The glasshouse contained two mist propagation benches, but only one was used. The bench was 4 - 5 m long, 1 m wide and about 0.25 m deep. It was supported by a metal frame elevated about 0.80 m from the ground. Misting was done through nozzles supported by water pipes raised about 50 cm above the bench and was controlled by four electronic leaf sensors inserted into the propagation medium. The mist propagation unit provided moisture to the cuttings at regular intervals, ensuring a film of water was maintained on the leaves. Heat to the

propagation medium was provided by thermostatically controlled warming cables laid in the medium. To monitor the temperature of the bench, five thermometers were inserted in the rooting medium at regular intervals along the bench. During the experiment, the temperature of the medium ranged from 28°C to 34°C (average 30°C). The rooting medium was a 1:1 mixture of perlite and grit that was evenly spread over the bench. The air temperature in the mist-propagation unit was maintained at 20°C. Supplementary lighting was provided by 400 watt high presssure sodium vapour lamps situated approximately 1 m above bench level.

Plants used to produce cuttings were the ones grown in the 20°C greenhouse at Pen-y-ffridd field station and which had previously been assessed for growth and morphological characteristics. They were 13 months old at the time cuttings were taken. Four types of cuttings were used in this experiment. Three of them were collected from main stems at three positions (basal, medial and distal), while the fourth was collected from branches. For each provenance, 20 taller plants were selected to provide cuttings. As each plant provided three leafless stem cuttings (see above), a total of 60 stem cuttings were prepared for each provenance. Three branch cuttings with a reasonable amount of lignification were also prepared from each plant, making a total of 60 branch cuttings for each provenance. All the cuttings were taken using a pair of sharp secateurs, labelled, wrapped in previously moistened, water absorbent papers and immediately placed in pre- moistened polythene bags. Cuttings were 15 cm long on average but medial and particularly distal cuttings were occasionally longer (± 5 cm longer) than basal ones in order to maintain a constant number of nodes (four nodes) per cutting. They were stored overnight in a cold store (5°C) at Pen-y-ffridd field station and then transported the next morning to Treborth Botanical Gardens.

Experiments on stem and branch cuttings were set up on the same mist bench, adjacent to each other. A completely randomised design was used for both stem and branch cutting experiments. Cuttings were directly inserted into the rooting medium at a depth of 3-4 cm. After three months, cuttings were assessed for rooting. The percentage of cuttings rooted was subjected to analysis of variance to check for significant differences between provenances. The number of roots per rooted cutting was counted and provenance means compared; statistical analysis was not possible because of the low number of roots per cutting and the non-normality of the data. Cuttings were then potted up.

6.3 Results

6.3.1 Pollarding

The percentage of stumps sprouted in each of the six *Maesopsis eminii* provenances is shown in Table 6.1. The length (cm) of the longest shoot and the number of shoots per pollard stump sprouted are shown in Figures 6.1 and 6.2 respectively. Analysis of variance showed that provenance differences in the length of the longest shoot were statistically significant ($P \leq 0.05$) (see Table 6.2 and Table C.1 in appendix C). The two Rwandan provenances had the greatest shoot length while the three Kenyan provenances had the lowest. The Ugandan provenance performed well, with shoots which almost reached the lengths of the Rwandan provenances. In contrast, the three Kenyan provenances produced more shoots per stump than the other provenances. The two Rwandan provenances had the lowest numbers of shoots.

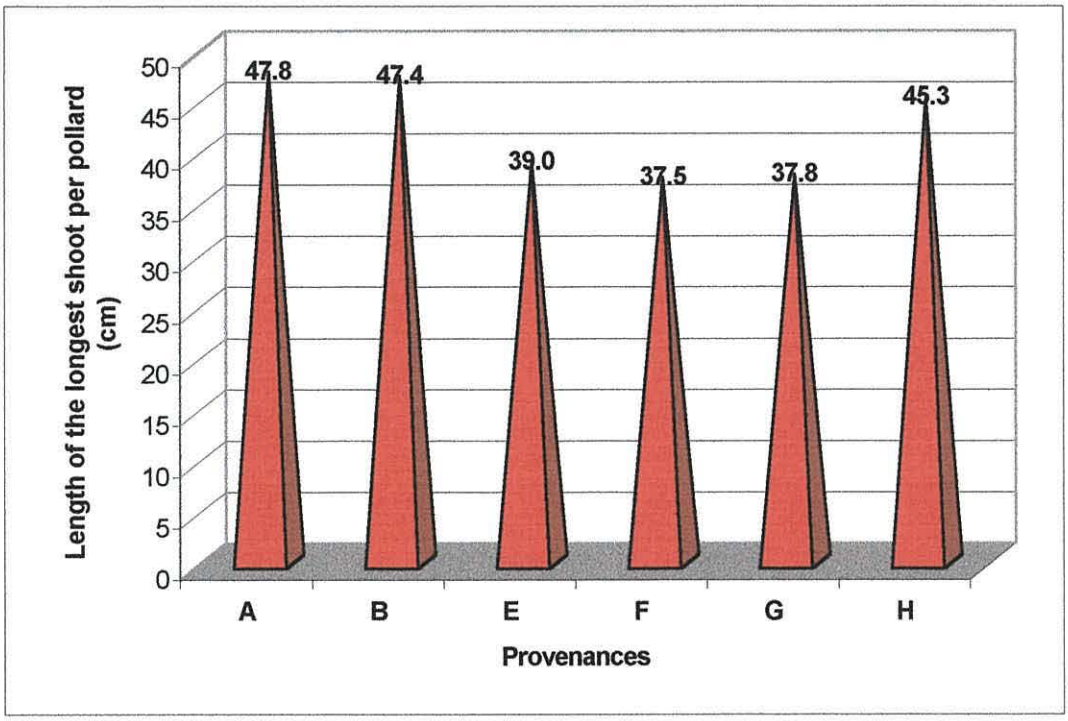


Figure 6.1 Length (cm) of the longest shoot per pollard stump in six provenances of *Maesopsis eminii.* Provenance codes as in Table 4.1.

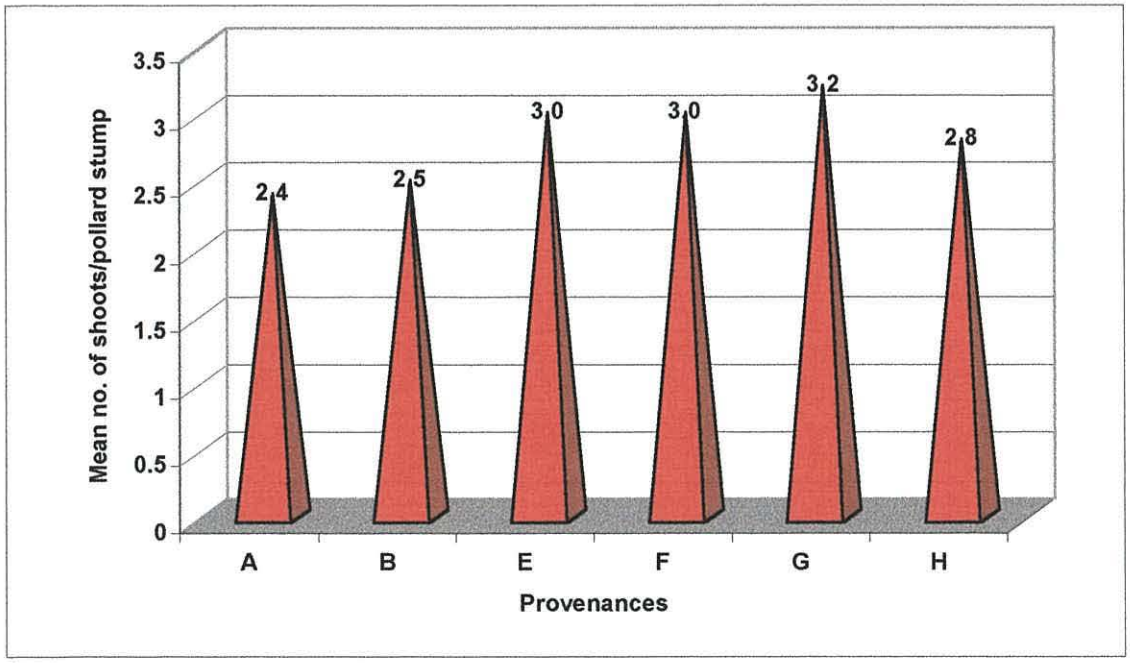


Figure 6.2 Mean number of shoots per pollard stump of six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

The amount of variation accounted for by provenance, block, the provenance x block interaction and trees within provenance in the length of the longest shoot is shown in Table 6.2. The greatest proportion of the variation was accounted for by trees within provenance (78%). Results of pairwise comparisons made using Tukey's test are given in Table 6.3. The percentage of significantly different pairs in the length of the longest shoot is 60%.

Table 6.1 Percentage of pollard stumps sprouted in six *Maesopsis eminii* provenances.

Provenances	**Block 1 (25°C)**	**Block 2 (25°C)**	**Average for the two blocks**
	Stumps sprouted (%)	**Stumps sprouted (%)**	**Stumps sprouted (%)**
A - Rukara/Kibungo Rwanda	100	88.9	94.4
B - Arboretum de Ruhande Rwanda	100	85.2	92.6
E - Kakamega Kenya	85.2	100	92.6
F - Kisaina 5B Kenya	92.6	100	96.3
G - Kisaina 4E Kenya	81.5	100	90.7
H - Budongo Forest Reserve Uganda	100	100	100

Table 6.2 Percentage variation accounted for by provenance, block, provenance x block and trees within provenance in length of the longest shoot of six *Maesopsis eminii* provenances.

PARAMETER	**PERCENTAGE VARIATION ACCOUNTED FOR BY:**			
	PROVENANCE	**BLOCK**	**PROVENANCE X BLOCK INTERACTION**	**TREES WITHIN PROVENANCE**
Length (cm) of the longest shoot per pollard	15.4 ***	0.03 NS	6.4 ***	78.17

Stars indicate significance of differences identified in analysis of variance.
*** $P \leq 0.001$; NS not significant

Table 6.3 Results of Tukey's pairwise comparison test for significance of differences in the length of the longest shoot per pollard stump of six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	NS					
E-Kakamega-Kenya	*	*				
F-Kisaina 5B-Kenya	*	*	NS			
G-Kisaina 4E-Kenya	*	*	NS	NS		
H-Budongo Forest Reserve- Uganda	NS	NS	*	*	*	

* $P \leq 0.05$; NS not significant.

6.3.2 Coppicing

The percentage of stumps sprouted in each of six *Maesopsis eminii* provenances is shown in Table 6.4. The length (cm) of the longest coppice shoot and the number of shoots per coppice stump sprouted are shown in Figures 6.3 and 6.4 respectively. Analysis of variance showed that provenance differences in the length of the longest shoot were statistically significant ($P \leq 0.05$) (see Table 6.5 and Table C.2 in appendix C). Kisaina 4E provenance (Kenya) had the longest coppice shoot and produced the highest number of coppice shoots per stump. The most striking result is the failure of one of the Rwandan provenances (i.e. Arboretum de Ruhande) to produce coppice shoots.

Table 6.4 Percentage of coppice stumps sprouted in six *Maesopsis eminii* provenances.

Provenances	Block 1 (25°C)	Block 2 (25°C)	Average for the two blocks
	Stumps sprouted (%)	Stumps sprouted (%)	Stumps sprouted (%)
A - Rukara/Kibungo Rwanda	44.4	18.5	31.5
B - Arboretum de Ruhande Rwanda	0.0	0.0	0.0
E - Kakamega Kenya	59.3	7.4	33.3
F - Kisaina 5B Kenya	57.1	22.2	39.7
G - Kisaina 4E Kenya	22.2	11.1	16.7
H - Budongo Forest Reserve Uganda	55.6	63.0	59.3

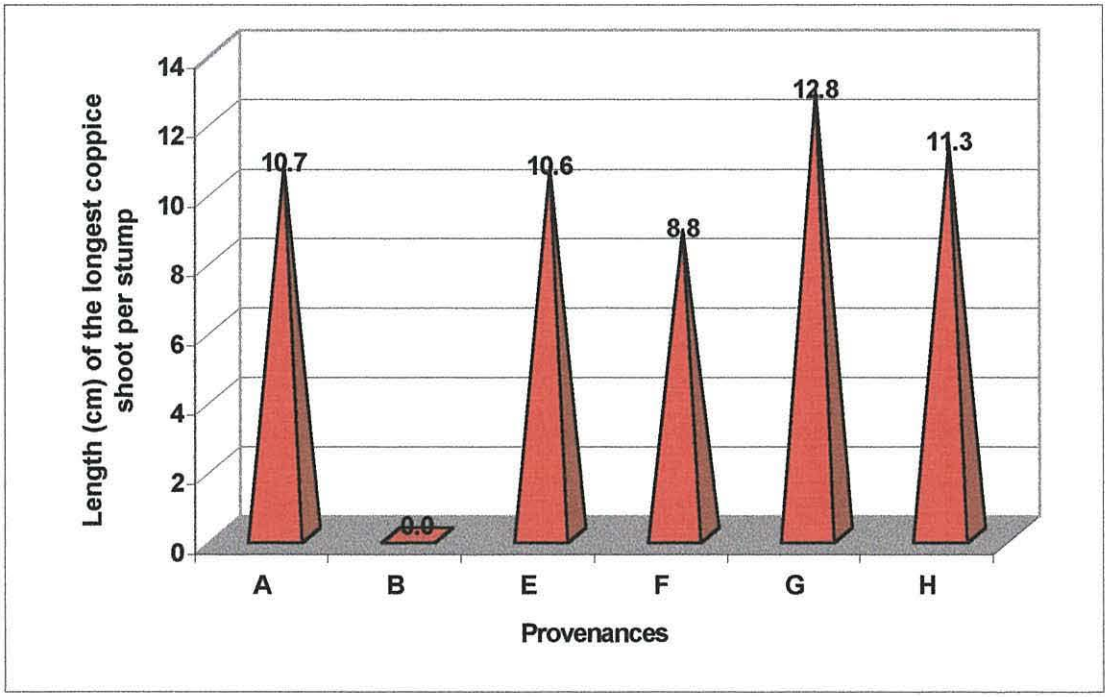


Figure 6.3 Length (cm) of the longest coppice shoot per stump in six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

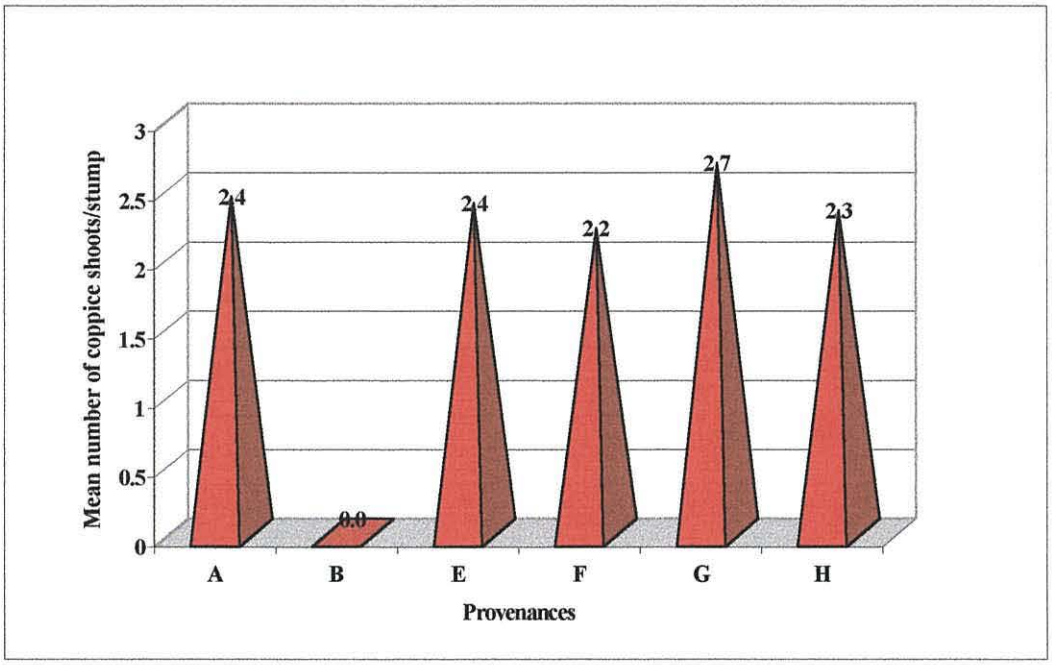


Figure 6.4 Mean number of coppice shoots per stump in six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

Most of the variation in the length of the longest shoot was accounted for by provenances (59.6%). The percentage variation accounted for by trees within provenance was also relatively high. More details are given in Table 6.5. Results of pairwise comparisons made using Tukey's test are given in Table 6.6. The percentage of significantly different pairs in the length of the longest coppice shoot is 33%. However, it should be noted that the significantly different pairs were those between the Arboretum de Ruhande provenance (which did not produce any coppice shoots) and the rest.

Table 6.5 Percentage variation accounted for by provenance, block, provenance x block and trees within provenance in the length of the longest coppice shoot per stump of six *Maesopsis eminii* provenances.

PARAMETER	PERCENTAGE VARIATION ACCOUNTED FOR BY:			
	PROVENANCE	BLOCK	PROVENANCE X BLOCK INTERACTION	TREES WITHIN PROVENANCE
Length (cm) of the longest coppice shoot per stump	59.6 ***	0.002 NS	5.35 **	35.04

Stars indicate significance of differences identified in analysis of variance.
** $P \leq 0.01$; *** $P \leq 0.001$; NS not significant

Table 6.6 Results of Tukey's pairwise comparison test for significance of differences in the length of the longest coppice shoot per stump of six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	*					
E-Kakamega-Kenya	NS	*				
F-Kisaina 5B-Kenya	NS	*	NS			
G-Kisaina 4E-Kenya	NS	*	NS	NS		
H-Budongo Forest Reserve- Uganda	NS	*	NS	NS	NS	

* $P \leq 0.05$; NS not significant.

6.3.3 Grafting

No results for the grafting experiment are presented because none of the 150 grafts was successful.

6.3.4 Rooting of cuttings

Percentages of basal, medial and distal cuttings rooted and the number of roots per rooted cutting of the six *Maesopsis eminii* provenances are shown in Figures 6.5, 6.6 and 6.7 respectively. Figure 6.8 shows the average percentage rooting and the number of roots per rooted cutting for each provenance. There are no results for branch cuttings because none of them rooted.

Analysis of variance showed that provenance differences in the percentage of basal cuttings rooted were statistically significant ($P \leq 0.05$) (see Table 6.7 and Table C.3 in appendix C). Two Kenyan provenances (Kakamega and Kisaina 4E) had the highest percentage of rooted cuttings (80%), while the Ugandan provenance (Budongo Forest Reserve) had the lowest percentage (40%). However, pairwise comparisons made using Tukey's test did not show any pairs of provenances which were significantly different. The highest number of roots per rooted basal cutting was also recorded in two Kenyan provenances (5.8 and 5.7 for Kisaina 5B and Kakamega respectively). The Ugandan provenance had the lowest number of roots per rooted basal cutting.

Differences between provenances in the percentage of medial cuttings rooted was also statistically significant ($P \leq 0.05$) (see Table 6.7 and Table C.4 in appendix C). The highest percentage of medial cuttings rooted was found in the Rwandan provenances (80% and 60% for Rukara/Kibungo and Arboretum de Ruhande respectively). Pairwise comparisons using Tukey's test are shown in Table 6.8. Only 20% of pairs are statistically different. The Arboretum de Ruhande had the highest number of roots per rooted medial cutting (4.1), followed by Kisaina 4E provenance (3.0). The Ugandan provenance had the lowest number (1.0).

For distal cuttings, the two Rwandan provenances again performed well, though the percentage of rooted cuttings was low compared to that observed in basal and medial cuttings. The two Rwandan provenances (and the Kisaina 4E provenance from Kenya) also had the highest number of roots per rooted distal cutting. The Budongo Forest Reserve provenance (Uganda) had the lowest percentage of distal cuttings rooted and the lowest number of roots per rooted distal cutting. However, no significant differences were found between the provenances in the percentage rooting of distal cuttings (see Table 6.7 and Table C.5 in appendix C).

There were statistically significant ($P \leq 0.05$) differences between provenances in the overall percentage of cuttings rooted when calculations were based on the total of the three cutting categories (i.e. basal, medial and distal) (see Table 6.7 and Table C.6 in appendix C). The Rukara/Kibungo (Rwanda) provenance showed the best rooting (62%). The Arboretum de Ruhande (Rwanda) and Kakamega (Kenya) provenances gave almost the same percentage of rooted cuttings (48% and 50% respectively). The Budongo Forest Reserve (Uganda) provenance had the lowest rooting capacity (22%). Pairwise comparisons based on Tukey's test are shown in Table 6.9; only about 7% of pairs are statistically different. Overall, the number of roots per rooted cutting was almost the same in all provenances except for the slightly higher number in the Arboretum de Ruhande provenance and the lower number in the Budongo Forest Reserve provenance.

The amount of variation accounted for by provenances and by trees within provenances in the percentage rooting of the three types of cutting is shown in Table 6.7.

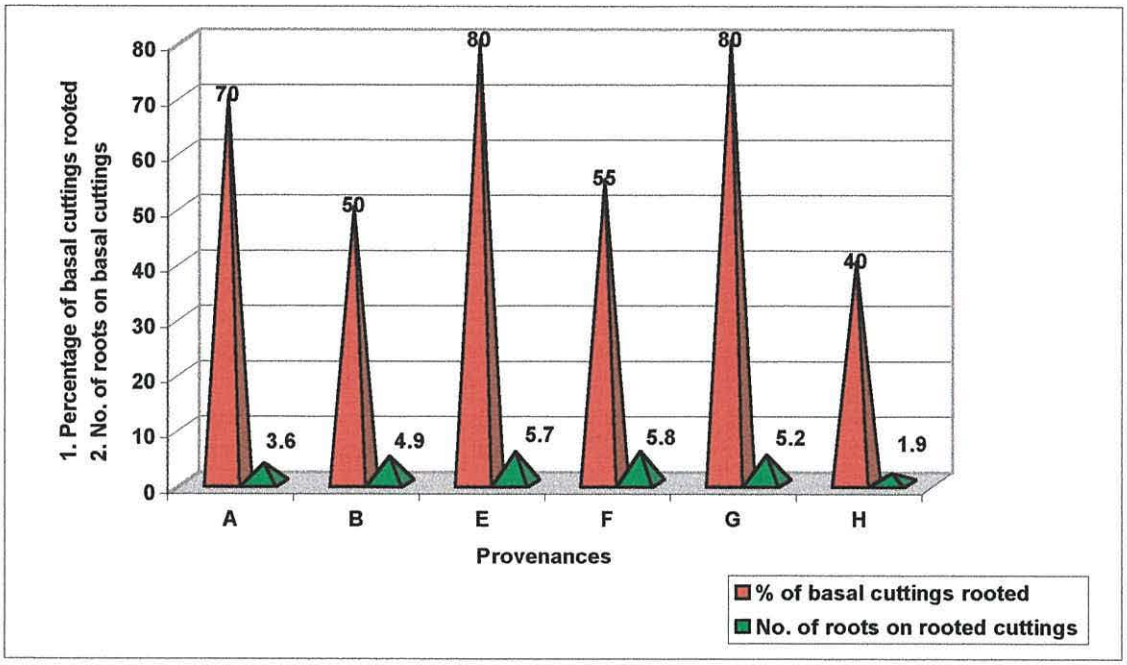


Figure 6.5 Percentage of basal cuttings rooted and mean number of roots per rooted basal cutting for six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

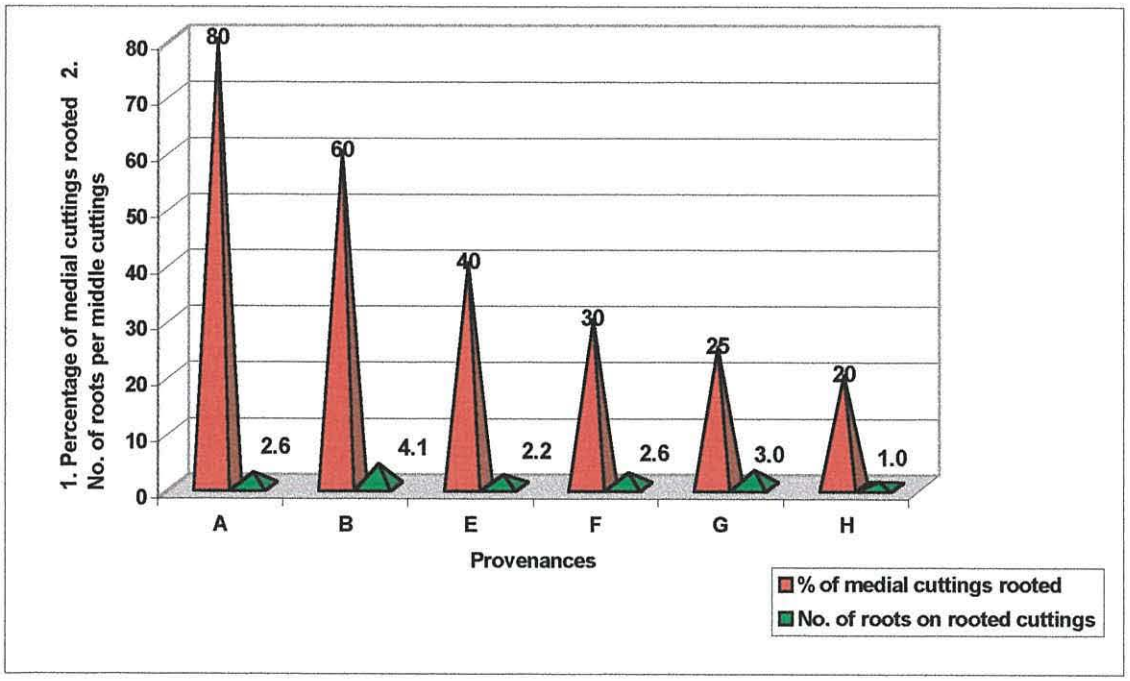


Figure 6.6 Percentage of medial cuttings rooted and mean number of roots per rooted medial cutting for six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

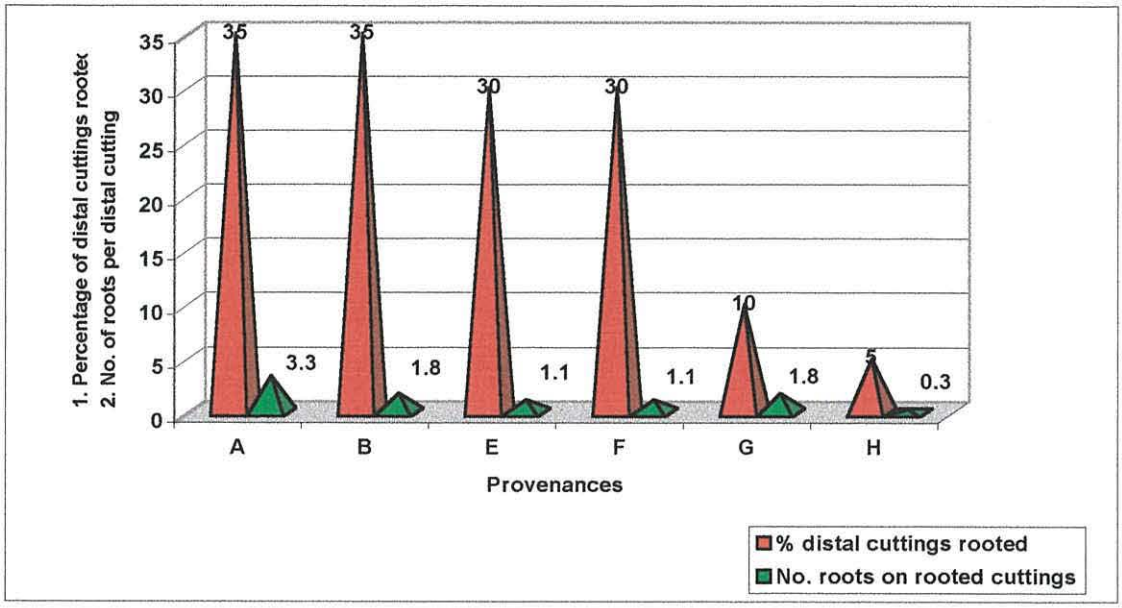


Figure 6.7 Percentage of distal cuttings rooted and average number of roots per rooted distal cuttings for six provenances of *Maesopsis eminii*. Provenance codes as in Table 4.1.

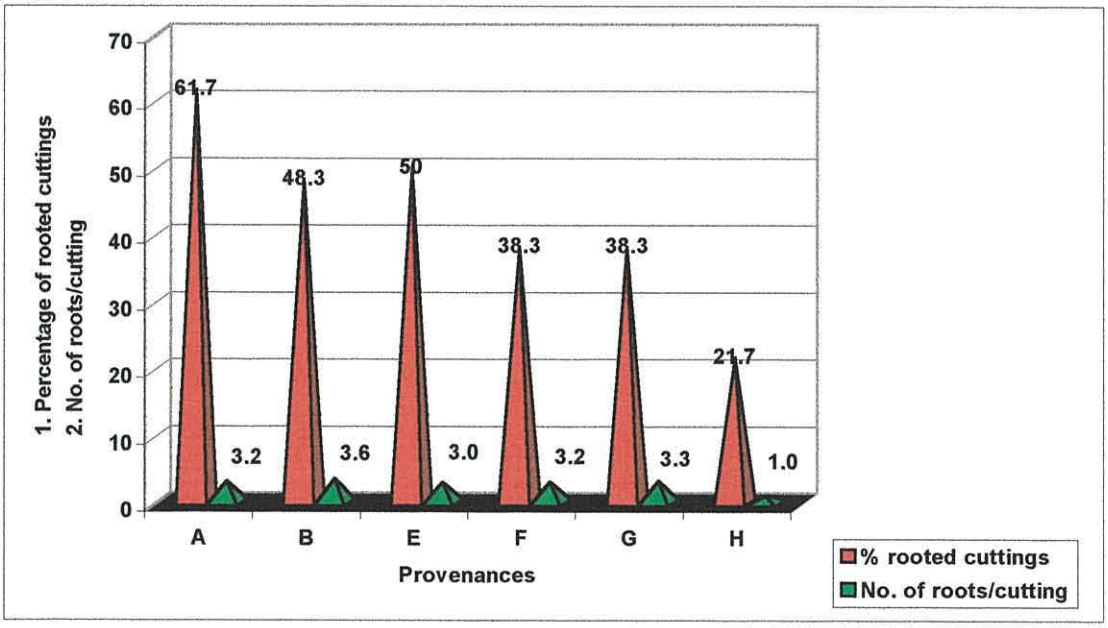


Figure 6.8 Percentage of rooted cuttings and mean number of roots per rooted cutting for six provenances of *Maesopsis eminii* (all categories of stem cuttings, i.e. basal, medial and distal are included). Provenance codes as in Table 4.1.

Table 6.7 Amount of variation accounted for by provenances and by trees within provenances in percentage rooting of three types of cutting in six *Maesopsis eminii* provenances.

Type of cutting	Variation accounted for by provenances %	Variation accounted for by trees within provenances %
Basal cuttings	45.3*	54.7
Medial cuttings	55.3**	44.7
Distal cuttings	24.2 NS	75.8
All cuttings	17.4*	82.6

Stars indicate significance of differences identified in analysis of variance.
* $P \leq 0.05$; ** $P \leq 0.01$; NS not significant

Table 6.8 Results of Tukey's pairwise comparison test for significance of differences in the percentage rooting of medial cuttings of six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	NS					
E-Kakamega-Kenya	NS	NS				
F-Kisaina 5B-Kenya	*	NS	NS			
G-Kisaina 4E-Kenya	*	NS	NS	NS		
H-Budongo Forest Reserve- Uganda	*	NS	NS	NS	NS	

* $P \leq 0.05$; NS not significant.

Table 6.9 Results of Tukey's pairwise comparison test for significance of differences in percentage rooting of all categories of cuttings (i.e. basal, medial and distal) of six *Maesopsis eminii* provenances.

Provenance	A	B	E	F	G	H
A-Kibungo-Rwanda						
B-Arboretum-Rwanda	NS					
E-Kakamega-Kenya	NS	NS				
F-Kisaina 5B-Kenya	NS	NS	NS			
G-Kisaina 4E-Kenya	NS	NS	NS	NS		
H-Budongo Forest Reserve- Uganda	*	NS	NS	NS	NS	

* $P \leq 0.05$; NS not significant.

6.4. Discussion

In the pollarding experiment, the greatest shoot lengths (47.8 cm and 47.4 cm) were recorded in the Rwandan provenances which, as shown previously (Chapter 4), also had high seedling diameter and height growth rates. However, it was also observed that pollard shoot length in the Ugandan provenance, whose average seedling diameter and height growth rates were among the lowest, was also high (45.3 cm). This suggests that the size (i.e. root collar diameter) of the pollard stumps did not influence pollard shoot growth. This observation is supported by studies on *Maesopsis eminii* coppicing carried out by Geddes (1993). He reported that the vigour with which stumps produced coppice shoots was not related to stump size. The three Kenyan provenances, which had the lowest pollard shoot length, produced the highest number of shoots per pollard stump. It appears, therefore, that provenances which have long pollard shoots have low numbers of shoots and vice versa, as observed in the Rwandan and Kenyan provenances respectively.

Overall, all *Maesopsis eminii* provenances responded well to pollarding (depending on provenance, 92.6% to 100% of pollard stumps sprouted). However, it is not known whether the response to pollarding would be the same in mature trees. Normally, pollarding commences once the stem reaches about 10 cm in diameter (Evans, 1992). Investigations are needed to determine whether results reported here would be consistent with the pollarding ability of older trees. The restricted use of pollarding in forestry today is reflected in the lack of experimental studies and findings which could be compared with the results obtained in this experiment.

In the coppice experiment, the greatest shoot length and the highest number of shoots per stump were found in the Kisaina 4E (Kenya) and Ugandan provenances. The Arboretum de Ruhande provenance, which performed well in

the pollarding experiment, did not produce any coppice shoots. Some of the sprouting shoots showed visible signs of scorching several days after feeding. It may also be that stumps were exhausted after the pollarding experiment, and that they could not therefore produce many shoots. Compared to pollarding, coppicing produced a low percentage of sprouted stumps (30.1% versus 94.4% on average for the coppicing and pollarding experiments respectively). The average number of coppice shoots on stumps which did sprout does not seem to differ much between this study and Geddes's (1993) experiment. Results were 2.5 to 3 shoots per stump for Geddes's (1993) experiment against 2.2 to 2.7 shoots per stump in this study. The percentage of stumps producing coppice shoots was low compared to the results published by Geddes (1993). He found that 75% of *Maesopsis eminii* stumps displayed signs of coppice regrowth, while in the study described here, the percentage of sprouted stumps ranged from 0% to 59.3% between provenances. It is possible that this low percentage of sprouted stumps was the result of damage caused by the liquid fertiliser during feeding.

The results reported here show that *Maesopsis eminii* can be propagated vegetatively by shoot cuttings. The rootability was moderate to high, especially for the basal and medial cuttings. One of the most important physiological factors affecting rootability of cuttings is thought to be the age of the ortet or "parent" plant (Hong, 1973; Wright, 1976). For example, cuttings from pine trees more than three years of age showed a severe decrease in rooting ability (Yim 1962, cited by Hong, 1973), though pines are among the most difficult trees to root (Hong, 1973). The medium to high rooting percentage observed in *Maesopsis eminii* provenances could therefore be explained by young age (12 months) of the parent plants when cuttings were taken.

Rooting varied considerably depending on the type of cuttings used. A general observation was that basal cuttings rooted better than medial and distal cuttings for all provenances except those from Rwanda, in which medial cuttings rooted better than basal and distal ones. The trend was therefore for rooting percentage (rootability) to decrease linearly from basal to distal cuttings. Similar results for *Khaya ivorensis* A. Chev. (African mahogany) were reported by Tchoundjeu and Leakey (1996). They found that nodal cuttings from basal nodes had greater rooting percentages (84.2% on average) than those taken from apical nodes (59.6%). There was also a weak, but significant, positive relationship between cutting length and rooting ability in their study. The cuttings of *Maesopsis eminii* used in this study had almost equal lengths, and it was therefore not possible to test the relationship. It is worth noting that basal cuttings in some *Maesopsis eminii* provenances achieved almost the same rooting percentage as those of *Khaya ivorensis*, whose rooting was enhanced by IBA (concentration 8-300 μg).

Normally, when a constant number of nodes is maintained, rooting is better in apical cuttings (Leakey, 1983). In this study, basal cuttings (± 15 cm long) had a higher rooting percentage than medial and distal cuttings, which were slightly longer (± 5 cm longer to maintain the constant four nodes). However, basal cuttings had the largest diameter of the three types, suggesting that cutting stem volume may in fact be more critical than length (Leakey *et al.* 1993, cited by Tchoundjeu and Leakey, 1996). Tchoundjeu and Leakey (1996) claim that the cuttings with a greater stem volume probably provide a larger storage reservoir for current assimilates prior to the formation of the new roots. This could explain the higher rooting percentage associated with basal (larger) cuttings. The importance of cutting storage volume in influencing rooting capacity was

also demonstrated in stem cuttings of *Eucalyptus grandis* (Hoad and Leakey, 1993).

Other reasons for the superior rooting capacity of basal cuttings have been suggested by Hartmann *et al.* (1990). They consider that both carbohydrates and nitrogen are important factors in rooting but that optimal rooting of cuttings is achieved if the amount of nitrogen is moderate while carbohydrates are kept at adequate levels. Hackett (1970, cited by Hartmann *et al.*, 1990) stressed that it is important to maintain the nitrogen metabolism of stock plants and prevent the stimulation of shoot development by high nitrogen levels, since newly forming roots would be at a competitive disadvantage with rapidly developing shoots for carbohydrates and other nutrients. Keeping nitrogen fertilisation to moderate levels would reduce shoot growth and allow the accumulation of carbohydrate for use by developing roots (Hartmann *et al.*, 1990). It has been suggested that those regions of the shoot that are known to have a high carbohydrate content should be selected for the production of cuttings. Tukey and Green (1934, cited by Hartmann *et al.*, 1990) found that in rose (*Rosa* spp.) shoots of the type used for cuttings, nitrogen content increased from the base of the shoot to the tip, suggesting that basal portions were low in nitrogen and high in carbohydrates, a situation which would favour good rooting. As basal cuttings in *Maesopsis eminii* gave the highest rooting percentages, a similar gradient may exist in the main stem of young plants of this species. Provenances Kakamega (Kenya), Kisaina 4E (Kenya) and Rukara/Kibungo (Rwanda) may have a more pronounced gradient of nitrogen/carbohydrate, since they gave the highest rooting percentages of basal cuttings (80%, 80%, and 70% respectively).

With few exceptions, central and basal parts of hardwood cuttings make the best cuttings (Hartmann *et al.*, 1990). The rooting percentage of distal (tip)

cuttings for all provenances was generally low, and it is probable that an unfavourable nitrogen/carbohydrate balance (see above) put distal cuttings at a disadvantage compared with medial and basal cuttings. Moreover, Haissig (1965, cited by Hartmann *et al.*, 1990) pointed out that the number of preformed root initials in woody stems decreases markedly from the base to the tip of the shoot. As a result, the rooting capacity of apical cuttings is expected to be lower than that of basal ones. In hardwood cuttings, the tip portions of shoots, which are usually low in stored foods, are generally discarded (Hartmann *et al.*, 1990). However, this is not always the case, because there are instances where distal cuttings have given better results than those taken at lower positions on the stem. Experiments on *Parkia biglobosa* for example have demonstrated that stem cuttings with the terminal node rooted best (Teklehaimanot, 1997).

Differences in the number of roots per rooted cutting between provenances were not large. Based on the claims made by Haissig (1965, cited by Hartmann *et al.* (1990) of a decrease in the number of preformed root initials in woody stems from the base to the tip of shoots, it would be expected that the highest number of roots would be produced by basal cuttings. In this study, the highest number of roots was clearly obtained in basal cuttings, and there was a general decrease in root number towards distal cuttings, as predicted by Haissig's hypothesis.

All branch cuttings failed to root. There was a low incidence of invasion of the mist bench by unidentified algae during the rooting experiment, but this was probably not the cause of the failure of branch cuttings to root, since stem cuttings (which did root) were adjacent to branch cuttings on the same bench. Stem cuttings also suffered from the algal invasion, but their growing shoots were the only parts which appeared affected. It may be that the temperature in

the mist propagation unit was too high, leading to a wilting of branch cuttings, but on the other hand high temperatures cause frequent mist applications and reduce wilting. The most probable cause of rooting failure in branch cuttings is that they were very tender, insufficiently lignified, with small diameters and therefore volumes (capacities) insufficient to hold the amounts of food reserves needed to promote rooting. This idea is supported by Leakey and Storeton-West (1992, cited by Pinkett, 1994) who stated that cutting volume determines the capacity of a cutting to store assimilates produced both pre- and post-severance. The fact that cuttings with small volumes may become saturated with starch, which can inhibit photosynthesis and subsequent rooting (Pinkett, 1994), may also explain the failure of the small *Maesopsis eminii* branch cuttings (± 5 mm diameter) to root.

To sum up, the study showed that rooting response varied considerably with the stem position from which cuttings were taken. Also, at this early stage of growth, differences in the rootability of provenances was clear, giving foresters a chance to select easily-rooting provenances and use the technique for plant production. Once the rooting technique is mastered, *Maesospis eminii* cuttings could also, as suggested by Kiang *et al.* (1973), be used in genetic improvement programmes for the species, as individual trees with the best combination of desirable characteristics can be multiplied without changing their genetic makeup.

CHAPTER 7: GENERAL DISCUSSION

1. With a few exceptions, the objectives of this study have been achieved. Studies of seed characteristics, seedling growth, morphological traits, isozymes and vegetative reproduction and propagation revealed considerable differences within and between provenances of *Maesopsis eminii*. In the isozyme studies, it was not possible to compare the Cameroonian provenance with all the others because seeds did not germinate to provide the material (leaves) used for electrophoresis. Attempts were successfully made to use the ungerminated seeds in isozyme studies, but comparisons with other provenances were not possible because electrophoresis of seed extracts resolved positively and negatively charged enzymes, and many loci, while electrophoresis of leaf extracts detected only negatively charged enzymes and relatively few loci. The only comparison possible was between the Cameroonian and the Arboretum de Ruhande (Rwanda) provenances, since both leaf and seed extracts of the Arboretum de Ruhande provenance were assayed to check for differences and similarities between zymograms of leaf and seed extracts. If zymograms had been similar, this would have justified inclusion of the Cameroonian provenance in UPGMA clustering based on isozymes; unfortunately this was not the case. In future electrophoresis studies, it may be worth trying to use seed extracts, as this would avoid dependence on seedlings that may not be available due to the rapid loss of seed viability.

2. The germination tests conducted during this study have confirmed the difficulty of achieving uniform germination of *Maesopsis eminii* seeds. Seeds of all provenances germinated sporadically and it took a long time (up to 132 days for some provenances) for germination to complete. This resulted in considerable variation between and within provenances in many of the size and form parameters assessed. Perhaps the problems are

physiological and appropriate seed pretreatments would reduce the period over which seeds germinate.

3. Although germination tests take a long time to complete, they give the most reliable indication of germination capacity. Moreover, they are practically easy to carry out. As observed in this study, viability tests need skills in the preparation of seeds (e.g. in embryo excision tests) and/or in distinguishing viable or dead seeds (e.g. in tetrazolium tests), and some give very misleading information on seed viability (e.g. the cutting test, the x-ray and water absorption methods). However, if the necessary skills are mastered, such methods offer a very good way of assessing seed viability in a very short time. For *Maesopsis eminii*, the excised embryo and tetrazolium tests gave estimates of seed viability which were closer to the results of conventional germination test than the other methods investigated, despite the technical and evaluation difficulties mentioned above. The water absorption method appeared to give results which were similar to those of the conventional germination test, but was found unreliable when seeds assessed as viable failed to germinate.

4. The grafting experiment failed completely. The experiment was not designed to assess different grafting techniques in order to determine the most appropriate for routine use in grafting *Maesopsis eminii*. The objective was to assess the response of the different provenances to one grafting technique chosen on the advice of experienced plant propagators. It may be that the technique was simply not appropriate for *Maesopsis eminii*. It is also felt that the environment (open benches in a heated greenhouse) was not favourable for grafting, and the handling of the grafts may have caused damage. A more comprehensive experiment to test various grafting techniques and post-grafting environments should be carried out,

particularly if breeding programmes including clonal seed orchards are envisaged.

5. It was found that *Maesopsis eminii* could successfully be propagated from stem cuttings. In preparing cuttings, basal and medial portions of stems should be selected as they showed a higher rooting percentage than distal cuttings in all provenances. However, the effect of cutting size and rooting environment on the quality of rooted cuttings should be investigated further before producing cuttings for widespread use in afforestation or breeding programmes.

6. Analysis of data on seed, growth and morphological characteristics revealed variation between and mostly within provenances. This is a pattern which is observed in many widespread, outcrossing plant species, and means that there is an opportunity to select good genotypes within provenances through progeny and/or clonal trials.

   Seed characteristics may reliably be used in the identification or discrimination of *Maesopsis eminii* provenances, but this could be done with more confidence if it were based on more seed diagnostics than were considered in this study. For instance, seed colour, dry weight and form/shape are characteristics that could be used for identification or discrimination. Using seedling growth (diameter and height) characteristics alone for provenance identification is likely to be unreliable, since growth traits can be influenced by environmental factors, resulting in mis-identification of provenances. For instance, principal component analysis (PCA) based on growth characteristics identified provenance groupings (Figure 4.21a) which were different from those derived from PCA based on morphological characteristics alone (Figure 4.21b-d) or of seed, growth and

morphological characteristics (Figure 4.21e-g). It appears that morphological characteristics could also be used to discriminate between provenances, but this should be checked in older trees. The plants used in this study were very young, and their parts may not have been fully developed.

It should be pointed out that the Ghanaian provenance was not assessed for seed, growth or morphological characteristics (see Chapters 3 and 4).

7. The isozyme study showed moderate to high levels of genetic variation. Measures of genetic differentiation (G_{ST}, D_{ST}, D_m and R_{ST}) were within the ranges found for other widespread, outcrossing tree species. Genetic distances between populations were generally low. However, the Ghanaian and Ugandan provenances appeared distinctly different from the other provenances and similar to one another. For other provenances, suggestions based on isozyme data are that one of the Rwandan provenance (Arboretum de Ruhande) is similar to the Tanzanian (Amani/Kwamkoro) provenance and that the other Rwandan provenance (Rukara/Kibungo) is similar to those from Kenya, particularly to the Kakamega provenance. The three Kenyan provenances appear to be very similar, as would be expected.

   It could be deduced from the isozyme groupings that the Ghanaian provenance is descended from the *Maesopsis eminii* population in Budongo Forest Reserve. There are no historical documents to hand to support this assertion, but during colonial time, forestry activities at Budongo Forest Reserve were so renowned that forest material could have been passed to Ghana (which, like Uganda, is an English speaking country) by foresters of the British colonial territories.

It may be true that the Arboretum de Ruhande provenance is descended from *Maesopsis eminii* populations in Tanzania. In fact seeds used to establish the experimental plot in the Arboretum de Ruhande were collected from the region of Kibungo (Rwanda), a region which shares borders with the Tanzanian town of Bukoba, where *Maesopsis eminii* is concentrated. According to Kalinganire (1989), *Maesopsis eminii* in Kibungo might have been introduced from Bukoba in the 15th century by Rwandans who frequently visited Bukoba due to the presence in that region of a royal descendant from Rwanda. The same historical events would be expected to apply to the Rukara/Kibungo provenance, due its proximity to Bukoba, and the closeness of Rukara/Kibungo to the Kenyan provenances is puzzling.

The similarity of the Kisaina 5B and Kisaina 4E provenances to the Kakamega provenance seems reasonable and suggests that they descended from a population in Kakamega Forest Reserve. To sum up, a general picture derived from the isozyme study is that the Ugandan and the Ghanaian provenances are very similar and form one group while the rest (the Rwandan, Kenyan and Tanzanian provenances) form another group. It appears that there has been an extensive exchange or movement of *Maesopsis eminii* material (seed, plants) within these east and central African countries.

8. Provenance groups identified by PCA of seed, seedling growth and morphological characteristics (together) (Figure 4.21e-g) were different from groups identified by UPGMA clustering based on genetic distances (Figure 5.3). This is rather confusing but, as pointed out by Ashton *et al.* (1984), such situations are likely to be common because genetic variation in morphological characters is almost invariably complex in origin and involves an indefinite number of loci. Morphology may also be affected by

environmental variation of uncertain magnitude. By contrast, the differences revealed by electrophoresis are usually under simple genetic control and are almost unaffected by environmental variation (Ashton *et al.*, 1984). Isozymes are selectively neutral and as a result, variation does not usually reflect variation in adaptive morphological traits (Eriksson, 1995).

9. In situations such as this, where patterns of isozyme and adaptive variation are different, problems arise when sampling to determine the extent and pattern of the genetic resources used for breeding and, in some cases, which are in need of conservation.

   Various researchers have expressed their preferences for the traits which should be used in such sampling. A comprehensive review of the various publications is given by Eriksson (1995). Libby and Crichfield (1987, cited by Eriksson, 1995) suggested that all types of traits - morphological, metric and biochemical - should be used. Ledig (1988) and Barnes and Burley (1990), both cited by Eriksson (1995), prefer to use biochemical markers for the determination of population structure. Schaal *et al.* (1991, cited by Eriksson, 1995) did not find any reliable way of comparing the various methods used for estimating genetic variation for gene conservation purposes, but seemed to prefer the use of biochemical markers. They were, however, concerned about the adequacy of biochemical markers genes for representing the entire genome.

   In *Pinus ponderosa*, isozyme genotypes were found not to be good predictors of metric traits (Linhart *et al.*, 1989). No strong relationship was found between metric traits and biochemical markers in *Pinus lambertiana* (Eriksson, 1995). In his studies on *Picea abies* and *Pinus sylvestris*, Eriksson (1995) also found that there were considerable differences

between provenances in growth and stem quality traits. Differences among provenances in metric traits were large compared to the small differences revealed by isozyme data. These few examples clearly show that patterns of variation in molecular markers and morpho-metric traits can be very different, as was also observed in the *Maesopsis eminii* provenances investigated in this study.

In his concluding remarks, Eriksson (1995) suggested that sampling for genetic conservation is most efficiently implemented if there is prior knowledge of the pattern of variation in metric traits. In view of the different groupings of *Maesopsis eminii* provenances given by analysis of seed, growth and morphological characteristics and of isozyme data, sampling for gene resources in this species should be based on morphological, metric and biochemical variation, as suggested by Libby and Crichfield (1987, cited by Eriksson, 1995).

Based on the results obtained in this study, suggestions for future work on *Maesopsis eminii* include:

- looking at more provenances from a wide geographical range, including both natural and introduced provenances;
- using a more powerful technique capable of revealing all (or more) genetic variability (e.g. look at DNA variation using RFLP or AFLP methods);
- establishing field trials of as many provenances as possible;
- finding appropriate grafting techniques to use in breeding programmes;
- looking at ways of improving rooting of cuttings;
- solving the problems of sporadic seed germination.

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APPENDICES

Appendix A

Table A.1 Analysis of variance of moisture content of six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
PROV	5	4.8102	0.9620	47.08	<0.001
Error	6	0.1226	0.0204		
Total	11	4.9328			

Table A.2 Analysis of variance of seed length of six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
Prov	5	2049.60	409.92	116.45	<0.001
Error	594	2091.04	3.52		
Total	599	4140.63			

Table A.3 Analysis of variance of seed width of six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
Prov	5	230.819	46.164	49.80	<0.001
Error	594	550.661	0.927		
Total	599	781.480			

Table A.4 Analysis of variance of final seed germination percentage of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	SS	MS	F	P
Prov	5	25800.2	5160.0	74.46	<0.001
Temp	1	26.0	26.0	0.38	0.542
Prov*Temp	5	861.2	172.2	2.49	0.038
Error	84	5821.5	69.3		
Total	95	32509.0			

Table A.5 Results of paired t-test for comparison of viability (sinkers in the water absorption method) and percentage of germinated seeds.

Variable	N	Mean	StDev	SE Mean	T	P-Value
D = Sinkers - Germinated	6	22.17	16.73	6.83	3.25	0.023

Table A.6 Results of paired t-test for comparison of viability (full seeds as detected by the x-ray method) and percentage of germinated seeds.

Variable	N	Mean	StDev	SE Mean	T	P-Value
D = Full seeds - Germinated	6	52.17	8.75	3.57	14.60	<0.001

Appendix B

Table B.1 Analysis of variance of seedling height growth of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	346.14	346.57	69.31	51.32	<0.001
Temp	1	831.03	750.25	750.25	555.47	<0.001
Prov*Temp	5	57.67	57.67	11.53	8.54	<0.001
Error	588	794.18	794.18	1.35		
Total	599	2029.02				

Table B.2 Analysis of variance of seedling diameter growth of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	1.8513	1.8310	0.3662	16.78	<0.001
Temp	1	18.0187	17.1543	17.1543	785.92	<0.001
Prov*Temp	5	0.8136	0.8136	0.1627	7.45	<0.001
Error	588	12.8342	12.8342	0.0218		
Total	599	33.5178				

Table B.3 Analysis of variance of branch angle of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	1662.51	1660.93	332.19	8.61	<0.001
Temp	1	1420.41	1525.24	1525.24	39.52	<0.001
Prov*Temp	5	420.10	420.10	84.02	2.18	0.055
Error	589	22729.36	22729.36	38.59		
Total	600	26232.37				

Table B.4 Analysis of variance of branch length (second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	1055.23	1051.47	210.29	7.76	<0.001
Temp	1	1391.08	1374.14	1374.14	50.73	<0.001
Prov*Temp	5	431.43	431.43	86.29	3.19	0.008
Error	589	15954.95	15954.95	27.09		
Total	600	18832.69				

Table B.5 Analysis of variance of leaf area (first leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	401.5	382.9	76.6	5.09	<0.001
Temp	1	11976.7	11330.0	11330.0	752.80	<0.001
Prov* Temp	5	329.8	329.8	66.0	4.38	0.001
Error	588	8849.6	8849.6	15.1		
Total	599	21557.7				

Table B.6 Analysis of variance of petiole percentage (first leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	28.809	27.002	5.400	2.59	0.025
Temp	1	483.441	472.205	472.205	226.15	<0.001
Prov*Temp	5	10.801	10.801	2.160	1.03	0.396
Error	588	1227.738	1227.738	2.088		
Total	599	1750.789				

Table B.7 Analysis of variance of leaf shape index (first leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	0.17421	0.17585	0.03517	2.80	0.017
Temp	1	0.00121	0.00393	0.00393	0.31	0.576
Prov* Temp	5	0.07094	0.07094	0.01419	1.13	0.344
Error	588	7.39848	7.39848	0.01258		
Total	599	7.64484				

Table B.8 Analysis of variance of leaf area (largest leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	826.8	804.5	160.9	3.27	0.006
Temp	1	35086.4	33759.6	33759.6	685.31	<0.001
Prov*Temp	5	927.5	927.5	185.5	3.77	0.002
Error	586	28867.2	28867.2	49.3		
Total	597	65707.9				

Table B.9 Analysis of variance of petiole percentage (largest leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	82.343	82.283	16.457	14.19	<0.001
Temp	1	79.683	78.253	78.253	67.46	<0.001
Prov*Temp	5	19.956	19.956	3.991	3.44	0.004
Error	588	682.058	682.058	1.160		
Total	599	864.040				

Table B.10 Analysis of variance of leaf shape index (largest leaf on second branch from top) of six *Maesopsis eminii* provenances growing at two temperatures (20°C and 25°C).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	0.227889	0.233152	0.046630	5.57	<0.001
Temp	1	0.466590	0.458071	0.458071	54.67	<0.001
Prov*Temp	5	0.053751	0.053751	0.010750	1.28	0.270
Error	588	4.926722	4.926722	0.008379		
Total	599	5.674952				

Table B.11 Chi square test for differences in the number of leaves on the main stem of six *Maesopsis eminii* provenances (mean numbers for temperatures 20°C and 25°C were used).

Provenances	Mean number of leaves on main stem
A-Rukara/Kibungo-Rwanda	34.84
B-Arboretum de Ruhande-Rwanda	34.23
E-Kakamega-Kenya	33.44
F-Kisaina 5B-Kenya	33.49
G-Kisaina 4E-Kenya	32.62
H-Budongo Forest Reserve-Uganda	32.19
Mean = Expected value	33.47

$\chi^2 = ((34.84-33.47)^2 + (34.23-33.47)^2 + (33.44-33.47)^2 + (33.49-33.47)^2 + (32.62-33.47)^2 + (32.19-33.47)^2)/33.47 = 0.144$

d.f. 6-1 = 5 Tabulated value @ 0.05 = 11.07 $P > 0.05$

Table B.12 Chi square test for differences in the number of first order branches of six *Maesopsis eminii* provenances (mean numbers for temperatures 20°C and 25°C were used).

Provenances	Mean number of first order branches
A-Rukara/Kibungo-Rwanda	10.907
B-Arboretum de Ruhande-Rwanda	11.383
E-Kakamega-Kenya	8.370
F-Kisaina 5B-Kenya	7.389
G-Kisaina 4E-Kenya	8.611
H-Budongo Forest Reserve- Uganda	6.907
Mean = Expected value	8.928

$\chi^2 = ((10.907-8.928)^2 + (11.383-8.928)^2 + (8.370-8.928)^2 + (7.389-8.928)^2 + (8.611-8.928)^2 + (6.907-8.928)^2)/8.928 = 1.883$

d.f. 6-1 = 5 Tabulated value @ 0.05 = 11.07 $P > 0.05$

Table B.13 Chi square test for differences in the number of second order branches of six *Maesopsis eminii* provenances (mean numbers for temperatures 20°C and 25°C were used).

Provenances	Mean number of first order branches
A-Rukara/Kibungo-Rwanda	3.49
B-Arboretum de Ruhande-Rwanda	6.86
E-Kakamega-Kenya	3.66
F-Kisaina 5B-Kenya	3.22
G-Kisaina 4E-Kenya	2.72
H-Budongo Forest Reserve-Uganda	2.61
Mean = Expected value	3.76

$\chi^2 = ((3.49-3.76)^2 + (6.86-3.76)^2 + (3.66-3.76)^2 + (3.22-3.76)^2 + (2.72-3.76)^2 + (2.61-3.76)^2)/3.76 = 12.39$

d.f. 6-1 = 5 Tabulated value @ 0.05 = 11.07 $P \leq 0.05$

Table B.14 Chi square test for differences in the number of leaves on the second branch of six *Maesopsis eminii* provenances (mean numbers for temperatures 20°C and 25°C were used).

Provenances	Mean number of leaves on the second branch
A-Rukara/Kibungo-Rwanda	14.06
B-Arboretum de Ruhande-Rwanda	12.80
E-Kakamega-Kenya	12.59
F-Kisaina 5B-Kenya	12.87
G-Kisaina 4E-Kenya	13.19
H-Budongo Forest Reserve-Uganda	12.98
Mean = Expected value	13.08

$\chi^2 = ((14.06-13.08)^2 + (12.80-13.08)^2 + (12.59-13.08)^2 + (12.87-13.08)^2 + (13.19-13.08)^2 + (12.98-13.08)^2)/13.08 = 0.45$

d.f. 6-1 = 5 Tabulated value @ 0.05 = 11.07 $P > 0.05$

Appendix C

Table C.1 Analysis of variance of length of the longest shoot per pollard stump of six *Maesopsis eminii* provenances.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	5398.0	4637.2	927.4	9.17	<0.001
Block	1	9.5	0.4	0.4	0.00	0.949
Prov*Block	5	2254.7	2254.7	450.9	4.46	0.001
Error	271	27395.6	27395.6	101.1		
Total	282	35057.8				

Table C.2 Analysis of variance of length of the longest coppice shoot per stump of six *Maesopsis eminii* provenances.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Prov	5	3969.49	3773.72	754.74	40.48	<0.001
Block	1	0.10	12.82	12.82	0.69	0.409
Prov*Block	5	356.36	356.36	71.27	3.82	0.003
Error	125	2330.62	2330.62	18.64		
Total	136	6656.57				

Table C.3 Analysis of variance of percentage of basal cuttings rooted in six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
Prov	5	5550	1110	2.98	0.039
Error	18	6700	372		
Total	23	12250			

Table C.4 Analysis of variance of percentage of medial cuttings rooted in six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
Prov	5	10750	2150	4.45	0.008
Error	18	8700	483		
Total	23	19450			

Table C.5 Analysis of variance of percentage of distal cuttings rooted in six *Maesopsis eminii* provenances.

Source	DF	SS	MS	F	p
Prov	5	3483	697	1.15	0.371
Error	18	10900	606		
Total	23	14383			

Table C.6 Analysis of variance of percentage of rooted cuttings in six *Maesopsis eminii* provenances. All categories of stem cuttings (i.e. basal, medial, distal are included).

Source	DF	SS	MS	F	p
Prov	5	11094	2219	2.78	0.024
Error	66	52633	797		
Total	71	63728			