PROPAGATION OF *PARINARI CURATELLIFOLIA* PLANCH. EX BENTH AND *UAPACA KIRKIANA* MUELL. ARG BY SEEDS AND STEM CUTTINGS

BY

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN FORESTRY AT THE SOKOINE UNIVERSITY OF AGRICULTURE

1997

ABSTRACT

Two experiments on propagation of *Parinari curatellifolia* and *Uapaca kirkiana* were carried out at the National Tree Seed Programme (NTSP), Iringa Zonal Tree Seed Centre during December, 1995 - June, 1996 with the aim of determining efficient techniques of enhancing the germination of *P. curatellifolia* seeds and to see whether *P. curatellifolia* and *U. kirkiana* could be raised vegetatively through stem cuttings.

The *P. curatellifolia* seeds were subjected to different pre-sowing treatments involving the physical scarification to the seedcoats, soaking in hot water and concentrated sulphuric acid at various storage periods. For vegetative propagation of both *P. curatellifolia* and *U. kirkiana*, the possibility of using either basal or terminal parts of the stem cuttings and influence of different concentrations of Indole-3-Butyric Acid hormone were tested. The results indicated the existence of serious seed dormancy problems which limit the rate of germination in *P. curatellifolia* seeds. Only pre-sowing seed treatments by complete and partial removal of the seedcoats gave germination at all the storage periods with the former giving better mean cumulative germination of 34%, germination energy of 30%, germination value of 1.4, complete, total and differential dormancy periods of 27.8, 67.6 and 40.6 days respectively. The storage period enhanced early germination in seeds stored for at least 30 days with the combined effect of seed storage for 60 days and pre-sowing treatment by complete removal of the seedcoats giving early germination (21.3 days). The results for the vegetative propagation indicated a potential of raising both species by stem cuttings with the basal parts combined with hormone application of 375 ppm for *P.*

curatellifolia and 125 ppm for *U. kirkiana*. The best establishment performances of sprouting, rooting, root number and root length means of 46.7%, 41.3%, 5.4 and 50.8 mm for *P. curatellifolia* and 62.9%, 53.3%, 5.2 and 58.8 mm for *U. kirkiana* respectively were obtained.

It is concluded that, both tree species have a potential of being raised through seeds and stem cuttings. When seeds are preferred in raising *P. curatellifolia* plants, they need to be pre-treated by completely removing the seedcoats to improve the permeability of the seeds to air and water and in overcoming the mechanical resistance to embryo growth. The storage of seeds for 60 days before sowing allows the maturation of the embryos and thus in enhancing their early germination. Since the germination of 34% obtained in the present study is still low and sporadic, other types of dormancies like chemical and physiological or a combination of the two are also suspected to play a role. While the immediate application of the obtained results is being recommended, further research is required to confirm the identity of the other types of dormancies still associated with the *P. curatellifolia* seeds in order to resolve them and raise the overall seed germination and improved efficiency and effectiveness of the vegetative propagation technology by stem cutting.

DECLARATION

I PATRICK PASCHAL LUSINDE MWANG'INGO, do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my own original work and has never been submitted for a degree award in any other university.

Signature . . . . . .

Date 13 June 1997

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ACKNOWLEDGEMENT

I am indebted to many people and organisations who made possible the completion of this study. My deepest gratitude goes to, Prof. L.L.L, Lulandala my supervisor, not only for his tireless supervision, valuable guidance, constructive criticisms and encouragement in developing the proposal and finally the dissertation, but also in encouraging me to pursue further studies rather than being satisfied at the level I reached before.

My sincere thanks are also being extended to the following: The African Network for Agroforestry Education/International Centre for Research in Agroforestry (ANAFE/ ICRAF) for the financial assistance, without which, this study would not have been carried out; Tanzania Forestry Research Institute (TAFORI), my employer, who granted me the study leave; the administration of the National Tree Seed Programme (NTSP) for permitting the use of laboratory facilities for this study.

My sincere appreciations become incomplete without mentioning Mr H.P, Msanga for his valuable assistance in getting in touch with some of the literature and in experimental designing; Dr A.G, Mugasha who read the first draft of my dissertation and gave his valuable comments; Mr M.K, Shellimoh, Mr N.J, Muhunzi and all workers of the NTSP (Iringa Zone) for their co-operation and prompt assistance; Miss H. Yusufu, who assisted me right from the time of material collection, processing and in ensuring that the experiments were tended as they were prescribed. Finally but not least my deepest thanks are being extended to all those people who have in different ways contributed towards the successful completion of this study.

DEDICATION

To all those people who devoted their money, time and moral support to enable me reach the level I have attained.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANAFE	African Network for Agroforestry Education
C/N	Carbon/Nitrogen ratio
DMRT	Duncan Multiple Range Test
FAO	Food and Agricultural Organisation of the United Nations
GE	Germination Energy
GP	Germination Percentage
GV	Germination Value
IAA	Indole Acetic Acid
IBA	Indole Butyric Acid
ICRAF	International Centre For Research in Agroforestry
ISTA	International Seed Testing Association
NAA	Naphthalene Acetic Acid
NTSP	National Tree Seed Programme
SDGS	Sum of Daily Germination Speed
P	Probability level
RH	Relative Humidity
TAFORI	Tanzania Forestry Research Institute

CHAPTER 1

1.0 INTRODUCTION

Over the past few years increasing emphasis in agroforestry research has been placed on the increase in the number of multipurpose trees as much as possible with special emphasis on indigenous fruit trees (Willan, 1983; Nair, 1993; Prins and Maghembe, 1994). This has been important because some of these species have been playing a greater role as food sources and thus in improving the nutrition standards of the community (FAO, 1982; Saka and Msothi, 1994). Other important reasons are that, some of the few existing species such as *Leucaena leucocephala* which people used to depend on have already been threatened by insect/pests such as Leucaena psyllid (*Heteropsylla cubana*) attack (Palmer *et al.*, 1989) and moreover, they can not fit in all ecological zones (Hines and Eckman, 1993; Nair, 1993). These also have necessitated the identification of more species so as to provide a wide range of choice which will provide alternatives once other species perform poorly.

Among the most useful indigenous tree species that have been identified to be desirable for incorporation in agroforestry systems include *Parinari curatellifolia* Planch. ex Benth and *Uapaca kirkiana* Muell. Arg (Hans *et al.*, 1978; Hines and Eckman, 1993; Storrs, 1995). Their usefulness range from food sources as fruits to provision of timber which is used for various purposes (Willan, 1983; Mwamba *et al.*, 1992; Mbuya *et al.*,

1994; Storrs, 1995). However, like many other indigenous tree species, their usefulness have been constrained by limited knowledge regarding important aspects in their propagation (Willan, 1983; Hines and Eckman, 1993; Prins and Maghembe, 1994).

Seeds of *P. curatellifolia* present difficulties in germination. Rarely they have been found to germinate and most trees that are seen in many areas have been regenerating through root suckers and coppicing. This has contributed to the difficulty of enabling farmers to have trees of the species in farms where they had not existed before apart from their willingness to have them (Willan, 1983; Hines and Eckman, 1993). Trials to germinate seeds of the species have given very poor results. Without any pre-treatment less than three percentage germination in an erratic manner after three months have been obtained (Mwang'ingo, 1993). Mbuya *et al.* (1994) reported that even after six months the attained germination is still low. It is postulated that the observed difficulty in germination, is associated with dormancy problems. The seed coat is tough, thick and woody which might be presenting a barrier to embryo growth and permeability to air and water (FAO, 1982; Hines and Eckman, 1993; Mbuya *et al.*, 1994; Mwang'ingo, 1993). Furthermore seeds of such nature are likely to be associated with embryo dormancy and thus need to be collected and sown at the period when further development of the embryo after fruits/ seeds are shade from the trees have taken place (Bhagat *et al.*, 1992).

Seeds of *U. kirkiana* are recalcitrant in nature and thus they are faced with storage problems making them difficult to store due to rapid loss of viability (Willan, 1983;

Mbuya *et al.*, 1994). Soon after collection germination is over 70% (Mbuya *et al.*, 1994) but after four weeks the germination tends to fall almost to nil even under cold storage; and thus storage of seeds is not advised (Willan, 1983; Ngulube and Kananji, 1989; Msanga (personal communication)). This makes seeds of *U. kirkiana* unavailable at the right time for nursery activities, and in years of poor seeding, this phenomenon presents a serious constraint. Ezumah (1986) and Maithan *et al.* (1989) noted that seeds exhibiting recalcitrant features can not be stored for a longer period of time due to rapid loss of viability. In some cases even with elaborate and expensive treatments, the maximum storage period achieved is still short. This has forced investigations on other techniques of propagating such trees.

Although the use of vegetative materials like cuttings is thought to assist in propagation of both species (Hines and Eckman 1993; Mbuya *et al.*, 1994) very little information is available quantifying the success of these techniques. Bearing in mind the importance of the trees as among the most important suppliers of food resources with high nutritive values and many other uses, all the mentioned problems call for research on identification of proper propagation techniques that would allow easy raising of these useful fruit trees. Solving the problems associated with germination of *P. curatellifolia* seeds could be a step toward enabling raising of the species artificially. This could latter allow farmers to raise the species and incorporate it on their farms. Also the findings are expected to contribute to the possible use of the species as a multipurpose tree in agroforestry systems (Hans *et al.*, 1978; Hines and Eckman, 1993 and Mbuya *et al.*, 1994). Furthermore they can provide light to the people involved in nursery activities as seed dormancy tends to increase the rearing period of seedlings, tying the nursery space and thus increasing the cost of production unnecessarily (Mugasha and Msanga, 1987).

The use of vegetative propagation as possible means of raising the trees is important as it has been serving as a good tree propagation alternative once the use of seeds seems to be not promising in many species (Adriancer and Brison, 1955; Kijkar, 1992b). This technique could also be important in duplicating the genotype of the trees, thus perpetuating the desired characters of any single plant which can not be achieved by use of seeds (Hartmann and Kester, 1989). Furthermore unnecessary competition in gathering fruits for seed extraction with local people who are interested with selling in the market could be reduced through this technique.

The main objectives of this research were therefore, to find out efficient techniques that could enhance the germination of *P. curatellifolia* seeds and to see whether *P. curatellifolia* and *U. kirkiana* trees could be raised vegetatively. The specific objectives were:

(i) To evaluate the effect of various seed pre-sowing treatments on the germination of *P. curatellifolia seeds.*

(ii) Determine the effect of storage time (sowing at different time intervals) from the date of collection on germination of *P. curatellifolia* seeds to see whether the problem of seed germination is due to delayed embryo maturation or otherwise.

(iii) To develop vegetative propagation technique of both *P. curatellifolia* and *U. kirkiana* by use of stem cuttings with a view of;

- Assessing the effect of positions of stem cuttings on sprouting, rooting ability (number of cuttings that could form roots), root number and root length of the species.
- Determining the effect of different hormone concentration on the success of propagating the species.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Description of the species

2.1.1 *Parinari curatellifolia* Planch. ex benth

This is an evergreen sclerophyllous species which belongs to the family *Chrysobalanaceae*. The tree is a medium sized one ranging from 7.5 to 15 m and occasionally reaching up to 20 m in height (FAO, 1982). The bole is usually short (about 3 m) with greyish-black, lenticellate, rugose and deeply fissured bark in older specimen. The globular rounded crown is densely branched with erect branches. Leaves are simple and alternate, entire, oblong-elliptic, obtuse with a rounded or slightly attenuate base, coriaceous, shining on the upper surface, lower surfaces are covered with a greyish or reddish tomentum and possess parallel veins. The inflorescence is a terminal panicle bearing many white-pink flowers, born on long pedicels which are thickened toward the top and tomentose. The flowering period is very variable depending on the locality (FAO, 1982; Willan, 1983; Mbuya *et al.*, 1994; Storrs, 1995). In Zambia, Storrs (1995) found that flowering takes place between July and October while in Mufindi District (Iringa, Tanzania) flowering has been observed to start in March, few months after the fruiting period ends (Mwang'ingo, 1993). The ripe fruits are reddish-brown, ovid drupe with a fibrous skin bearing greyish lenticels. The seeds are hard and woody about 2.1 - 3 cm long and 1.1 - 2.5 cm in diameter with kernels enclosed in wool like material. The

number of seeds per kilogram is about 250 - 350 (FAO, 1982; Mbuya *et al.*, 1994; Storrs, 1995).

The tree is indigenous in Tanzania and is abundant in the greater west *Brachystegia* forest, ranging from Uvinza to Ufipa. Dense stands are found in Njombe and Iringa (FAO, 1982; Hines and Eckman, 1993). It covers a wide range of altitudes from zero to 1900 m above sea level. The climatic pattern is also variable with rainfall ranging from 401 to 2373 mm (Willan, 1983). The species does well in light yellowish-brown to reddish-yellow gritty, sandy clay loams and red to dark red, friable clays with laterite horizon (Morgan, 1972).

P. curatellifolia is a multipurpose one and is very desirable to be incorporated in agroforestry systems. It bears among the best tropical wild fruits that are edible by man rich in vitamin "C" and can be processed into juice, jam and brew. The fruits are widely sold in the markets. The kernels too are edible and have high oil content that can be extracted and used as food and in making paint or varnish. The tree also serves for medicinal purposes (infusion against fever, application to fractures and vermoesen say it is important as a bait to trap animals such as antelope), fuelwood and charcoal. Its wood is hard and is used for making railway sleepers, mining timber, canoes, mortar and house building. Fodder can too be provided by the tree (FAO, 1982; Hines and Eckman, 1993; Mbuya *et al.*, 1994; Storrs, 1995). In Isanga village (Mbeya), the tree has been left in many farms to provide shade to the banana and coffee crops (Willan, 1983).

2.1.2 *Uapaca kirkiana* Muell. arg

This is a much branched evergreen or semi-deciduous tree which belongs to the family *Euphobiaceae*. It is a small to medium-sized tree growing to a height of 13 m with a dense rounded crown. The bark of the tree is dark grey or grey brown with vertical fissures. Leaves of the species are simple, alternate or in whorls confined at the end of branchlet. They are shiny dark green, thick and course, usually about 7 - 27 cm long and 4 - 16 cm wide with woolly hairs below and on veins. Tips of leaves are notched.

The species has male and female flowers borne on different trees. All flowers are greenish-yellow, inconspicuous, growing from older wood. Flowering of the species occurs during the rain season between December and April while fruiting takes place between August and December. The fruits are rounded, rusty green berry measuring up to 3 cm in diameter, turning rusty yellow when ripe, sweet pulpy flesh surrounding 3 - 4 seeds. The number of seeds per kilogram is about 2500. (Willan, 1983; Mbuya *et al.*, 1994; Storrs, 1995). Seeds of the species are recalcitrant in feature having good germination within the first four weeks after extraction. Beyond this period, the viability is rapidly reduced almost to nil. As such storage of the seeds is not advised (Willan, 1983; Ngulube and Kananji, 1989; Mbuya *et al.*, 1994).

This tree is a well known fruit tree found in Burundi, Zaire, Mozambique, Zambia and South Africa. In Tanzania it is found in extensive pure stands in deciduous woodland, wooded grassland and along streams and is co-dominant and dominant in *Isoberlinia-Brachystegia-Parinari* open to closed Miombo woodland. Geita, Iringa, Njombe,

Ruvuma and Mbeya are rich in this species (Willan, 1983; Mbuya *et al.*, 1994). Altitudinally it is found between 800 - 1900 m above sea in association with Miombo woodland species (Willan, 1983). It prefers sand and loam soil with overlying murram in areas with rainfall of 508 - 1270 mm (Morgan, 1972).

This tree is of social importance and worth cultivating, due to its multiple uses and can easily be incorporated in agroforestry systems (Mwamba *et al.*, 1992; Storrs, 1995). It bears edible fruits that are rich in ascorbic acid (1.8 mg/gm) (Watt and Breyer-Brandwijk, 1962), total free sugar (8.5 %) (Sufi and Kaputo, 1977), and are sold widely in the market. The fruits can be processed into jam, eaten raw and made to produce refreshing drink and brew. It is an important famine food. Other uses include firewood, charcoal, timber (for general purposes) and poles. Further it serves for medicinal purposes, bee forage and shade (Willan, 1983; Mwamba *et al.*, 1992).

Another important feature of the tree is that it performs well even in poor, stony, rocky soils that are not good for agriculture. For that reason even the waste land can be rehabilitated using this species (Mbuya *et al.*, 1994).

2.2 Propagation of tree species

Tree species can be propagated either through sexual (using seeds) or asexual (using vegetative material) propagation (Adriancer and Brison, 1955; Hartmann and Kester, 1989; Kijkar, 1992b; Kantari 1993a).

2.2.1 Propagation by seeds

A seed is a reproductive structure that bears heritable factors from a sperm cell and an egg cell. It is the most efficient and economical method for most species provided genetic variability can be controlled within acceptable limits (Hartmann and Kester,1989). In some species it is the only important means because vegetative methods are either not possible or uneconomical to use. Also the juvenility growth phase habit obtained from seedlings in some species and in addition some variability between seedlings is useful (Adriancer and Brison, 1955; Hartmann and Kester, 1989). Furthermore seeds are easier to transport far from the area where they are collected without loosing viability easily, than other vegetative means such as cuttings which can subject the material to undesired effect such as drying during transportation (Willan, 1986; Hartmann and Kester, 1989; Bewley and Black, 1978).

Trees have been propagated by seeds with varying degrees of success (Willan, 1986). Some have been presenting difficulties unless they are handled and treated in one way or another. There are many problems that are associated with propagation by seeds and among the most important ones include difficulties in seed germination (dormancy problems) (Nikolaeva, 1977; Willan, 1986; Meyer and Poljakoff-Mayber, 1989; Bewley and Black, 1994) which reduces their efficient use and rapid loss of viability (Willan, 1983; Kijkar, 1992a; Mbuya *et al.*, 1994) which make them difficult to store for future propagation operations.

Since there was no experiments which involved propagation or storage of *U. kirkiana* seeds, little literature on it has been included.

2.2.1.1 Seed dormancy problems and their possible remedy

In order for viable seeds to germinate, they need to be placed in an environment that favours their germination. These include adequate water supply, suitable temperatures, good composition of gases and in some seeds light may be of importance (Meyer *et al.*, 1973; Nikolaeva, 1977). However it has been observed that, there are many other tree seed species which don't germinate at all or delay to germinate and do so irregularly apart from being supplied with conditions that are favourable for germination. Such seeds are termed dormant and they tend to germinate only after some sort of pretreatment has been done to overcome the dormancy (Goor and Burney, 1968; Willan, 1986; Mugasha and Msanga, 1987; Sadhu and Kaul, 1989; Meyer and Poljakoff-Mayber, 1989).

Seed dormancy presents various problems to nurserymen and other seed users. The long period involved in raising the seedlings needs more labour and tends to tie up the nursery space unnecessarily. As such the cost of seedling production in the nurseries is increased (Mugasha and Msanga, 1987). Furthermore seeds tend to germinate irregularly resulting into patch nurseries with stock of variable age and sizes. This has a problem in the field

establishment as small pieces of land spread over time will be required to be planted at different times. Also mechanization and the use of chemicals such as pre-emergencies to control weeds may be a problem to such fields (Nwoboshi, 1982). Thus removal of dormancy is very important to ensure that seeds do germinate uniformly and at a rapid rate.

Many authors favour to classify seed dormancy into exogenous (seedcoat/pericarp) dormancy, endogenous (morphological /physiological) dormancy and a combination of the two (Waering and Saunders, 1971; Nikolaeva, 1977). In dry tropics dormancy associated with seedcoat/ pericarp is the most common in many seeds (Willan, 1986).

(a) Exogenous dormancy

This is associated with seedcoat or pericarp and can be in terms of physical, chemical or mechanical dormancy. In Physical dormancy the seedcoat/pericarp tend to be impermeable to water or gases and sometimes to both (Nwoboshi, 1982). Without imbibition and gaseous exchange to take place, renewal of embryo growth and germination are impossible (Bewley and Black, 1994; Meyer and Poljakoff-Mayber, 1989). This is common especially in many legumes such as Leucaena, Albizia, Ceratonia, and some *Acacia spp* (Amen, 1963; Willan, 1986, Duangpatra, 1991). The impermeability of the seedcoats to water and gases are normally caused by the hard seedcoats or presence of cutinized layers of the seedcoat. In seeds with small openings

(strophiolar cleft) that permit the entry of water and gases, for example in *Trigonella arabica*, the entry may be plugged with cork-like filling consisting of suberin thus rendering the seed impermeable (Black and Wearing, 1959; Meyer and Poljakoff-Mayber, 1989; Bewley and Black, 1994).

To overcome this type of dormancy, the methods used are designed at softening, puncturing, wearing away or splitting the seed coat to render it permeable to water and air. Some of the methods that have been in use include rubbing the seed coat with sand paper, filing, drilling a small hole, partial removal of the seedcoat and cracking the testa (Small *et al.*, 1977; Willan, 1986; Tietema *et al.*, 1992). Sometimes chipping the fruits of some species have been found to be useful (Nwoboshi, 1982).

Maghembe and Msanga (1988), showed that partial removal of the seed coat at the radicular end and complete removal of the seedcoat were able to overcome the problem of impermeability in *Trichilia emetica* seed. Germination was raised from 40% for untreated to 90%. Onyekwelu (1990) working with germination of *Tetrapleura tetraptera* seeds, reported that giving a shallow cut at one side or both of the seedcoat could enhance germination by improving the permeability. Germination was raised up to 73% compared with low percentage for untreated seeds. It has been observed that during these processes other changes may be induced such as sensitivity of seeds to light or temperatures which in some seeds are crucial.

Other techniques that have been reported to remove the problem of physical dormancy include soaking of seeds in cold or hot water for various periods. This tend to weaken the seed coat or melt the suberin material and thus rendering it permeable (Meyer and Poljakoff-Mayber, 1989; Duangpatra, 1991). Hot water treatment is effective for seeds of most leguminous species and few others with physical dormancy. Larsen (1964) and Patanath (1982), cited by Willan (1986) reported this to be useful in *Acacia mearnsii*, *A. melanoxylon*, *A. procera* and *Albizia omara.*

Chemical dormancy is associated with the presence of inhibitors in the pericarp or seedcoat. Some of the main inhibitors that have been identified in many fruits and plant tissues include B-inhibitor complexes (di-nitrophenols, salycic and oxybenzoic acids), abscisic acid, Coumarin, cyanide, azide, fluoride and hydroxylamine (Guan *et al.*, 1988; Krawiarz (1973) cited by Khan, 1977; Brookman-Amisah (1976) cited by Willan, 1986; Meyer and Poljakoff-Mayber, 1989). Guan *et al.* (1988) observed that abscisic acid was interfering with the germination of seeds in *Corms officinalis.* Brookman-Amisah (1976) cited by Willan (1986) observed coumarin to be existing in *Terminalia ivorensis.* These inhibitors tend to interfere with normal metabolic activities by interfering certain pathways such as respiration of tissues which are essential for initiation of growth (Khan, 1977).

To overcome chemical dormancies, pre-treatments are usually aimed at leaching away the inhibitors. Liquid pretreatments such as water and peroxides have been found to

tackle this problem. For example water was used by Brookman-Amisah (1976), cited by Willan (1986) to leach Coumarin in *Terminalia ivorensis* seeds, hence allowing germination to take place.

Mechanical dormancy is associated with the resistance of the seedcoat or pericarp to the growth of the embryo. Usually the seed coats of these seeds are tough, thick and sometimes woody. Thus great pressure is required by the seed embryos to break and germinate (Duangpatra, 1991). This tendency has been reported by many authors such as Sadhu and Kaul (1989) in *Robinia pseudo-acacia*, Msanga and Kalaghe (1992) in *Vangueria infausta* and Yadav (1992) in *Tectona grandis*.

To overcoming mechanical dormancy imposed by the seed coat to the embryo growth, some of the methods used in solving the physical dormancy can be used. Normally splitting, cutting or wearing away the seedcoat have been in use. Splitting the seed coat at the radicular end was reported by Msanga and Kalaghe (1992) in *Vangueria infausta* to have improved germination to 70% from 47% for untreated seeds. Similarly Sadhu and Kaul (1989), reported that rupturing the seedcoat at the radicular end in *Robinia pseudo-acacia* did overcome the mechanical resistance of the seedcoat and germination was raised to 100% from a low 25% in the control.

The chemicals such as sulphuric acid to crack and wear away the seedcoats have also been in use (Sagwal, 1986, Tietema *et al.*, 1992). Sadhu and Kaul (1989) working with

Robinia pseudo-acacia managed to remove the hard seed coat dormancy and an improvement to 78% from 25% in the control was obtained. Similarly Sagwal (1986) obtained a germination of 87.6% compared to 49.6% of the control in *Erheitia accuminata* when seeds were soaked in concentrated sulphuric acid for ten minutes. Chamshama and Downs (1984) noted similar improvement in *Acacia tortilis* seeds when soaking in the acid was done for one hour.

Other methods to overcome the mechanical dormancy include soaking of seeds in water (Willan, 1986; Tietema *et al.*, 1992). Sagwal (1986) used this in *Erheitia accuminata* while Yadav (1992) working with teak seeds recommended soaking to be done in running water for 144 hours to facilitate germination. Also alternate soaking and drying of teak seeds for 48 hours was found to improve germination by weakening the coat.

(b) Endogenous dormancy

This type of dormancy is normally related with the morphology or physiology of the seeds (Willan, 1986; Meyer and Poljakoff-Mayber, 1989; Duangpatra, 1991). Morphological dormancy is mostly attributed by the incomplete maturation of the embryo by the time seeds or fruits are shed from the trees (Nwoboshi, 1982). Such seeds may need more time to complete the differentiation of cells which must precede any germination (Duangpatra, 1991).

To overcome morphological dormancy, Nwoboshi (1982) recommended seeds to be collected and sown at the time when maturation has taken place. The time required for maturation in most woody perennials vary from one to three months, though for certain species five to six months may be necessary. This time allows the embryos which have not completed their development to mature and the anatomical changes to occur to the extent that allows germination (Hartmann and Kester, 1989; Meyer and Poljakoff-Mayber, 1989).

Bhardwaj and Chakraborty (1994) while working with the germination of *Terminalia bellicia* and *T. chebula* seeds observed that sowing seeds 50 days from the date of collection improved germination. In few species, maturation can be gained in dry storage. Sometimes a period of moist warm pretreatment is necessary before the embryo develop sufficiently for germination to take place (Willan, 1986).

Physiological dormancy on the other hand is associated with decreased activity of the embryo. Furthermore, there are biochemical changes which must take place in the seeds before germination could commence. However little knowledge is available concerning the biochemical changes which need to take place (Nikolaeva, 1977; Meyer and Poljakoff-Mayber, 1989).

In overcoming physiological dormancy, where it is suspected to occur, the period of afteripening may be necessary (Nwoboshi, 1982). During this period, the composition of

the storage material in the seed may alter, the permeability of the seed coat may change and substances promoting germination may appear or the inhibitory ones may disappear (Meyer and Poljakoff-Mayber, 1989). Other common methods that have been in use include cold stratification. In this treatment the use of "cold moist" is adapted. This has been found to trigger off biological changes which transform complex food substances into simpler forms utilized by the embryo when it renews growth at germination (Nikolaeva, 1969). Maithan *et al.* (1990), showed that stratification of *Aesculus indica* for 30 days at 2-4 °C increased germination to 78% from 12% of the control.

Also the use of chemicals such as gibberellic acid, citric acid, hydrogen peroxide and Indole acetic acid (IAA) have been found to be useful. IAA was shown to bring a significant effect on the germination of *Spruce* seeds (Viendra, 1990). Also soaking the same seeds in kinetin (1.5×10^{-5} Mole concentration) improved the germination to 52.5% from 34.5% in the control when it was done for 72 hours (Viendra, 1992).

(c) Combined dormancy

Sometimes a combination of exogenous and endogenous dormancies does occur together in the same seed (Meyer and Poljakoff-Mayber, 1989; Bewley and Black, 1994). A number of species in the *Rosaceae* family possess a combination of physical or mechanical and physiological dormancy (Gordon and Rowe, 1982). For example in *Tillia cordata* seeds, it was observed that physical and deep physiological dormancies do occur together (Willan, 1986).

In this case breaking of one type of dormancy may be insufficient to enhance germination unless it is followed by the second pretreatment. Balcos (1989) demonstrated that, in *Pittosporum resinifera* seeds, physical scarification and water soaking alone gave no germination because seeds were accompanied by strong growth inhibitors which were not removed by these treatments. Similarly Marshal (1981) cited by Willan (1986) reported the same in *Fraxinus pennsylvanica* seeds.

To overcome the problem of combined dormancy, then the method to be used needs to be adjusted accordingly. Gordon and Rowe (1982) recommended the use of a combination of warm moist followed by cold moist pretreatments in a number of species in *Rosaceae* family.

2.2.2 Vegetative propagation

This is a reproductive technique involving the use of vegetative plant parts such as stems, roots, bulbs, leaves and tissue culture to multiply plants rather than using seeds (Adriancer and Brison, 1955; Hartmann and Kester, 1989; Kijkar 1992a; Kantari, 1993a). The methods include the use of cuttings, grafting, budding, layering and use of specialized stems and roots (Mahlstede and Harber, 1957). The method to be used depends on the response of the plant as plants respond differently to the method used. While others can be propagated better by one method, others have several. For those with more than one, the economy of the method and the usefulness or value of the plant produced are considered in the choice (Hartmann and Kester, 1989).

Many advantages of vegetative propagation have been advocated and some of the most important ones include; maintenance of clones as it involves mitotic cell division that duplicates the genotype thus perpetuating the desired character of any single plant; multiplication of plants that produce seeds which are difficult to germinate but can be propagated more economically by vegetative parts; propagation of plants that produce seedless fruits or unviable seeds such as is the case in some orange varieties and figs; avoidance of long juvenile period which may tie up the nursery space unnecessarily (Adriancer and Brison, 1955; Krishnamoorthy, 1981; Hartmann and Kester, 1989; Kantari, 1993b). Other advantages of vegetative propagation include production of uniform stock as some rootstock are useful as they can resist drought, cold, parasites and diseases and other external influences; control of growth form (undesirable traits); combination of clones in the same plant by budding or grafting (Adriancer and Brison, 1955).

2.2.2.1 Vegetative propagation by stem cutting

Compared with other vegetative propagation techniques such as budding, grafting, layering and tissue culture which are rather difficult and require special technical knowledge, the use of cuttings is the simplest, easier and cheapest (Krishnamoorthy, 1981; Kantari, 1993a). Cuttings are made from vegetative portions of the plants such as stems, modified stems (rhizomes, tubers, corms and bulbs), leaves or roots. In forestry the most widely used type of cutting is the stem cutting (Adriancer and Brison, 1955;

Kantari, 1993a). Moreover, of the stem cuttings used (hardwood, semi-hardwood, softwood and herbaceous), hardwood and occasionally semi-hardwood are frequently used. The rest are mostly used in ornamental plants (Hartmann and Kester, 1989). Hardwood cuttings are easy to prepare and are not readily perishable. They may be shipped safely over long distances and require little or no special equipment during rooting (Mahlstede and Harber, 1957).

2.2.2.2 Factors influencing the success of stem cuttings in propagation

To be successful in the use of stem cuttings, formation of roots is the critical factor as the shoot is already existing. There are several factors that can be of importance for root formation to take place. Some of the most important ones include: state of cutting material to be used (physiology of the donor), juvenility factor or phase change (age of the stock), position of the stem from which the cuttings are made, time in a year at which the cuttings are collected, treatment of cuttings and exogenous hormone application and control of environmental conditions during rooting (Priston *et al.*, 1953; Mahlstede and Harber, 1957; Krishnamoorthy, 1981; Hartmann and Kester, 1989; Kijkar, 1992b; Kantari, 1993a).

(a) State of the cutting material to be used (physiology of the donor)

Physiologically the cutting material to be used should preferably have a high content of carbohydrate (Mahlstede and Harber, 1957; Kantari, 1993a). The nutrition of the plant

stock exerts a strong influence on the development of roots and shoots from the cutting (Priston *et al.*, 1953; Hartmann and Kester, 1989; Kijkar 1992b). Generally initial carbohydrate content provides the cutting with adequate energy reserves for optimal rooting under conditions of limited photosynthesis. This has been observed in most *Citrus*, *Dipterocarps*, and *Hibiscus* species (Jouhari and Rahman, 1959; Stoltz and Hess, 1966). According to Hartmann and Kester (1989) selecting suitable cuttings as far as carbohydrate content is concerned can be done by determining stem firmness although this can be confused with maturity. However most cuttings from previous season growth collected at the end of the dormant period or before active growths such as flowering resumes can meet this requirement.

The mineral nutrition is also of importance and a moderate level of nitrogen is best for rooting. A very low level leads to reduced vigour while high levels cause excessive vigour and the cuttings become too soft which could lead to rotting (Kantari, 1993b).

(b) Juvenility factor or phase change (age of the stock)

Stem cuttings taken from the plants in the juvenile development phase often form new roots much more readily than those taken from the plants in the adult phase (Sax, 1962; Libby *et al.*, 1972; Libby and Hood, 1976; Kantari, 1993a). Thaiman (1941) cited by Hartmann and Kester (1989) while working with certain coniferous and deciduous species and Gupta *et al.* (1993) with *Dalbergia sissoo* known to root with extreme difficult also observed this behaviour.

Kijkar (1992a) observed that 90 % of hybrid *Acacia* cuttings formed roots when the stem cuttings were taken from two months old plants while older stocks gave less favourable results. This was described as due to the fact that, in older stock the tissues were too hard which caused low rooting. Also it is postulated that, the reduced rooting potential as plant ages may also be a result of lowering phenolic levels which are believed to act as auxin co-factors or synergist in root initiation (Girouard, 1967). However in some species, the difficulty to root is not only due to age of the stock but also due to natural presence of some anatomical barriers or inhibitors and absence of some rooting co-factors (Hess, 1965).

Nowadays with additional information on rejuvenation techniques which assures the availability of juvenile shoots, it has been possible to propagate vegetatively even the hard to root species (Kijkar, 1992b). This technique is done either through felling the donor to allow young shoot to come out, hedging the trees or severe pruning (Libby *et al.*, 1972; Kantari, 1993a).

(c) Position of the stem from which the cuttings are made

Normally cuttings taken from basal portion of any single shoot, root better than those from the upper part of the shoot (Srivastava and Mangil, 1981; Singh *et al.*, 1984). However in others such as *Shorea macrophylla* (Lo, 1985) rooting was found to be better with cuttings from the middle. In *Azadirachta spp* (Kijkar, 1992b) and *Carallia brachiata* (Kumar *et al.*, 1993) only terminals were found to give good rooting.

The better performance of basal cuttings compared with those from other parts within a stem cutting was explained by Viart (1979), Orourke (1940) cited by Hartmann and Kester (1989) as due to uneven distribution of inhibiting substances in different parts of shoots. Their concentrations generally decrease with the position away from the terminal where most of them are manufactured. Furthemore the number of preformed root initials in some plants decrease from the base to the tip of the shoot. Consequently the rooting of basal portion of such shoots tend to be considerably higher than those of terminal parts.

Another reason for better performance of basal cuttings than those of terminals was explained as due to differences in the nutritional status of the cuttings (Viart, 1979; Haissig, 1986). Cuttings with higher C/N ratio contain more food materials especially carbohydrates than those with lower C/N ratio. This ratio is generally lower at the top than at the base of a single stem cutting from which the cuttings are made. This also could explain why basal cuttings in some plants tend to perform better than terminals.

Poor performance with basal cuttings compared with terminals have however been observed in some species (Lo, 1985; Kumar *et al.*, 1993). Hartmann and Kester (1989), advocated that the better performance of the terminal cuttings may be explained by the possibility of higher concentration of endogenous root- promoting substances arising in the terminal buds. Furthermore the better performance of terminal cuttings in some species had been explained as due to juvenility factor (Lo, 1985; Kijkar, 1992b). Under

this aspect terminal cuttings are younger and posses cells which are less differentiated than basal cuttings. Due to this, more cells are capable of becoming meristematic and form roots easily as they are not much specialized.

(d) Time in a year at which the cuttings are collected

Whether cuttings are made when the donor is at flowering or vegetative growth, can influence rooting potential. For easy to root species, cuttings at any period of the year does not influence the results, but this may be important in difficult to root species (Nanda and Anand, 1970; Joshi *et al.*, 1992; Kantari, 1993a).

Taking cuttings prior to flowering or active growth starts in most cases results into better performance. This is probably due to low levels of flower stimulus antagonistic to rooting or by eliminating competition for materials necessary for both rooting and flower formation and high levels of food content at that time (Johnson (1970) cited by Hartmann and Kester, 1989; Kantari, 1993a). Joshi *et al.* (1992) reported that, poor rooting in some species depends on the period at which cuttings are collected. It was explained that, during certain times in the year the food reserve may be too low to initiate rooting or dormancy in plants may render rooting difficult. Further Nand and Anand (1970) reported that, levels of endogenous regulator substances and nutritional status of plants is high in some periods of the year. As such cuttings harvested in this period may need less application of hormones and other rooting co-factors to initiate

rooting compared to when these regulators fall in certain periods of the year. Hartmann and Kester (1989), Kantari (1993a) and Forestry Commission (1995) recommended that, for deciduous species, cuttings should be collected in early winters during leafless period while for evergreen species with one or more flushes of growth per year, cuttings can be obtained at various times in relation to such flushes (i.e. after flushes of new growth but before flowering commence) to get reasonable rooting. At these periods the levels of endogenous regulators and nutritional status of the parent stock is high.

(e) Treatment of the cuttings and exogenous hormone application

Collection and handling of the cuttings and rooting hormone application can affect rooting in various ways. Leach (1977) and Kantari (1993a) proposed collection of the cuttings to be done in the morning on cloudy days to maintain their turgidity and be kept in cool moist place. Usually dehydrated cuttings give poor rooting. As such removal of leaves, shoot tips and vegetative buds to discourage vegetative growth and promote root initiation can be of importance. Furthermore the free upper ends of the cuttings are recommended to be sealed with petroleum jelly or dipped in molten paraffin wax to discourage water loss from the cuttings by evaporation via the free cut surfaces (Krishnamoorthy, 1981; Aminah, 1991; Gupta *et al.*, 1993).

Auxins such as Indole Butyric Acid (IBA), Naphthalene Acetic Acid (NAA) and Indole Acetic Acid (IAA) and presence of other rooting co-factors are believed to influence

adventitious root formation in stem cuttings. As such plants with high amount of these auxins and rooting co-factors tend to root more easily than those with low amounts (Farmer, 1966; Hansen *et al.*, 1978; Hineslay and Blazich, 1980; Hartmann and Kester, 1989). Hormones are important in cell division and elongation in plants. When they are applied to the cuttings, root primodia originate by cell division of the phloem parenchyma or pericycle. The root primodia initiated emerges through the cortex. Generally hormones hasten the initiation of roots, the number of roots formed on the cuttings, root length and the ability of the cuttings to be rooted over a wide period of time (Krishnamoorthy, 1981; Hartmann and Kester, 1989).

Among the hormones that have been in use, IBA and NAA are particularly very effective as these are not inactivated in plants. Furthermore IBA has shown good response with most plants and is non toxic over wide range of application (Krishnamoorthy, 1981; Hartmann and Kester, 1989; Kijkar, 1992a). The need to apply a certain amount of external hormone to accelerate rooting in cuttings depends on the amount of auxins and rooting co-factors present in a particular plant (Nanda and Anand, 1970). Even the plants that were thought to be impossible to propagate vegetatively had been found to respond well with external hormone application. Hartmann and Kester (1989) however cautioned that, the response of cuttings to exogenous hormone application is not universal. As such cuttings of some difficult to root species still root poorly after treatment with Auxins.

Many plants have shown good responses with application of exogenous hormones such as IBA (Puri and Shamet, 1988; Gupta *et al.*, 1993; Bhardwaj *et al.*, 1993; Bhagat and Arun, 1993). Some of these include *Acacia* hybrid (*A. mangium x A. auriculiformis*) (Kijkar, 1992a), *Perrila frutescens* (Badola *et al.*, 1993), *Woodfordia floribunda*, *Coriaria napalensis* and *Debregeasia hypoleuca* (Chauhan *et al.*, 1994). In *Dipterocarps*, the use of IBA, NAA, and IAA to improve rootability showed little evidence in increasing rooting rate. However an increase in the number of roots with better developed systems was observed (Kantari, 1993a; b).

The amount of hormone to be applied as reported earlier is variable depending on how easy or difficult the species is to rooting (Hartmann and Kester, 1988). Kijkar (1992b) working with propagation of *Azadirachta spp* using stem cuttings, observed that dipping terminal shoots in IBA at 100 ppm for five minutes was enough to give better results of about 92.5% rooting compared with 65% rooting of the control. Joshi *et al.* (1992) working with *Dedregeasia hypoleuca* spp observed that soaking stem cuttings in 100 mg/litter of IBA for 24 hours improved rooting (67.7%), root number (29.9) and root length (20.1 cm) compared with 26% rooting, 7.4 root number and 10.1 cm root length of the control. In *Woodfordia floribunda*, however higher concentrations of IBA (400 - 700 mg/litter) were required to promote rooting. On average mean rooting of 31% was achieved with 700 mg/litter compared with 18.4% and 23.8% of control and 100 mg/litter respectively. Gupta *et al.* (1993) reported 100 ppm of IBA applied for 24 hours was effective in promoting rooting of *Tectona grandis* stem cuttings.

(f) Control of environmental conditions during rooting

Control of environmental conditions is important in rooting of stem cuttings otherwise much variability in rooting and in some cases, no rooting will take place (Kijkar, 1992b; Kantari, 1993a). Some of the most important environmental conditions to control include water relation, temperature, light and rooting medium.

Low water potential of the cutting is always associated with low level of rooting (Leach, 1977). Maintenance of leaves in the cuttings, may encourage loss of water and reduce the water content of the cutting to an extend that may cause death before root formation takes place. Should the leaves be maintained, then the vapour pressure of the water in the atmosphere surrounding the leaves should be maintained nearly equal to that in the intercellular spaces within the leaves (Aminah, 1991). Kijkar (1992a) used the mist spray chamber to obtain optimal conditions for producing rooted cuttings of various species. A condition with a relative humidity above 80% and under 30 ^{o}C temperature was obtained which was ideal for rooting hybrid *Acacia*. A similar structure was used by Kijkar (1992b) in raising cuttings of *Azadirachta spp*. In advanced nurseries where the cuttings are raised, the areas are equipped with mist spray equipments which provide a constant watering to maintain high relative humidities (Kantari, 1993b). However where advanced facilities are lacking frequent watering is recommended to supply enough water to the cuttings.

Day time air temperatures of about 21 to 27°C with night temperatures of about 15°C are satisfactory for rooting cuttings of most species. However, some species root better at lower temperatures (Hartmann and Kester, 1989; Kijkar, 1992b). High temperatures promote development of buds in advance of roots which can increase water loss. Kantari (1993b) working with *Hopea odorata*, used two layers of black shade net about 30 cm apart to maintain favourable temperatures for rooting cuttings. Kijkar (1992a) used a 75% nylon shading net to prevent temperatures from getting too high. This also helped to maintain a high relative humidity in the chamber used to propagate hybrid *Acacia* cuttings.

Evidence from rooting studies shows that, light influences formation of adventitious roots in stem cuttings. Many plants root better at low intensity than at high intensity (Eliasson, 1971; Hartmann and Kester, 1989). The reasons for this is not well known but Eliasson (1971) theoreted that levels of certain natural growth inhibitors are higher in plant tissues grown in the light than in etiolated tissue. It is thought that, even rooting of stem cuttings where the bases are in the dark within a rooting medium stimulates rooting due to etiolation effect. Kantari (1993b) used the black shade net to keep 70 - 80% shade. This accomplished low light intensity, favourable temperatures, high relative humidity and favourable tissue development.

The rooting medium needs to accomplish three main functions; to hold the cuttings in place during the rooting period, to provide moisture to the cuttings and to permit

penetration of air to the bases of cuttings. Thus a medium needs to be of light weight, loose structure, hold water well, easily available and inexpensive. Kijkar (1992b) used coconut husk to root *Azadirachta* and *Acacia* hybrid cuttings and was found to be the best. However due to unavailability of the material in some places, river sand also performs equally well with many species.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Location of the study site

The experiments were conducted in Iringa at the Zonal Seed Centre which is located about 3.5 km west of Iringa road junction on the Dar-es-salaam - Mbeya Highway within the Iringa municipality at about $35^{0}41'$ E, $7^{0}46'$ S and 1640 m above sea level. The area has a mean rainfall of 670 mm per annum with mean daily temperatures of 19.2^{0}C (East Africa Meteorological Department, 1975). The station was selected because it possesses laboratory facilities which were necessary for the experiments such as seed processing machines and germination rooms. Furthermore the station is close to the site from where experimental materials were collected, a consideration which was important in order to minimize some of the possible undesirable effects such as over heating of the fruits and drying of the cuttings due to transportation over longer distances.

The experimental materials were collected in Mufindi District ($34^{o}38'$ E, $8^{o}35'$ S and 1750 m above sea level) which is about 100 km from the Iringa Zonal Tree Seed Centre (the study site). This area receives a rainfall of about 610 mm per year. The vegetation type is mostly Miombo woodland with *Isoberlinia* and *Brachystegia* species being dominant.

3.2 Experimental procedures

There were two experiments that were carried out in this study: Propagation by seeds of *P. curatellifolia* in which the effects of seed pre-sowing treatments and storage periods were tested and evaluation of the potential of using stem cuttings in propagating both *P. curatellifolia* and *U. kirkiana*. There was no test which involved the germination of *U. kirkiana* seeds as these are known to have good germination characteristics although they are faced with storage problems due to their recalcitrant nature (Willan, 1983; Mbuya *et al.*, 1994; Msanga, personal communication).

3.2.1. Experiment 1: The effect of seed pre-sowing treatments and storage period on the germination of *P. curatellifolia seeds*

3.2.1.1 Seed collection and Processing

Ripe fresh fruits of *P. curatellifolia* which had fallen within the previous 12 hours were collected from the ground in the following morning from trees growing naturally in the natural forest in Mufindi District Tanzania. Prior to fruit collection in early December, 1995, the area was carefully surveyed, suitable trees from which fruits were to be collected were identified and the ground under the selected trees was cleared of all fleshly fallen fruits. To get representation of the species, the fruits were collected from 20 trees which physically appeared healthy and vigorous (Mugasha and Msanga, 1987; Maghembe and Msanga, 1988). After collection the fruits were transported to the Iringa Zonal Tree Seed Centre the same day.

In the following morning, the fruits were soaked for 24 hours in tap water to allow easy removal of the skin and pulp. To avoid fermentation of the fruits water was changed after every six hours. Then the skins and pulp were separated from the seeds through the maceration process. The seeds that were found floating were discarded as they were considered empty or damaged and thus unsuitable. The rest were sun dried to attainment of moisture content of about 10% which is safe for storage of seeds to be used at different sowing phases of the experiment (Willan, 1986). Seeds of almost the same size (1.5 - 2.0 cm in diameter) were selected and used in this study. This is because seeds of different sizes have different germination ability (Dunlap and Barnet, 1983; Msanga, 1992). Prior to sowing, the viability of seeds was tested using the cutting test method as described in the International Seed Testing Association Rules (ISTA, 1985). After these processes, seeds were considered ready for the germination experiment.

3.2.1.2 Seed pre-sowing treatments and storage periods

There were two major factors which were investigated: seed pre-sowing treatments and seed storage period. In the seed pre-sowing treatments study, *P. curatellifolia* seeds were divided into nine groups and each group received a different pre-sowing treatment. The following treatments were involved:

(i) Partial removal of the seedcoat (T_1). This involved partial filing of the seedcoat at the radicular end to reduce its thickness.

(ii) Complete removal of the seedcoat (T_2). Here kernels were extracted from the hard cover of seeds and sown.

(iii) Soaking entire seeds in concentrated sulphuric acid for different times (T_3 =15 and T_4 = 30 minutes).

(iv) Splitting the seedcoat almost into two halves (ie leaving the embryo on one side). Then soak in concentrated sulphuric acid for different times (T_5 = 0 no soaking, T_6 = 15 and T_7 = 30 minutes).

(v) Soaking entire seeds in hot water (T_8). This involved bringing water to boiling point and then pouring in a container with seeds and left to cool for 12 hours.

(vi) Control (T_9). Only removal of skin and pulp was involved.

For the seed storage periods factor, seeds of *P. curatellifolia* were divided and sown at different period from the date of collection. The main intention of this test was to determine the length of period, from the date of collection, which would be required to store seeds before sowing that could be appropriate to give optimal germination capacity. Three levels of this factor were involved.

(i) Sowing seeds soon after collection, processing and air drying (S_1),

(ii) Sowing seeds after storing for 30 days (S_2),

(iii Sowing seeds after storing for 60 days (S_3).

To get a sample of seeds to be sown at a given period, a sample of about 1500 seeds were drawn from the seed lot, divided into nine groups and subjecting each group to the corresponding pre-sowing treatment. The rest were packed in a cotton bag and stored at room temperature (ie 19.2^{0}C at the NTSP laboratory).

3.2.1.3 Experimental design

The experiment involved a (9 x 3) factorial combination of nine seed pre-sowing treatments and three seed storage periods. Each combination was replicated three times which were then assigned to plots in shelves in a germination room in a completely randomized block design. The shelves in a room represented the blocks and each block contained one of the replicates of each of the treatment combination. In total there were 81 observation plots each containing 50 seeds, involving a total of 4050 seeds for the whole experiment. This kind of design has been used in seed testing before (Maghembe and Msanga, 1988; Msanga and Kalaghe, 1992).

Seeds were germinated in a germination room where the temperature and relative humidity were controlled. The relative humidity of 70 - 80% and temperature of about 25 ± 3°C were maintained. This is the optimal condition for germination of most tropical tree seeds (Msanga and Kalaghe, 1992). The aluminium pans were used and each one represented one observation plot of a particular treatment combination. The aluminium pans were filled with sterilized sifted river sand as a germination medium. In each pan seeds were spread evenly without touching each other and covered on top with sand equal to the thickness of the seeds themselves (about 2 cm). Sand was then moistened with water to supply enough moisture and re-watering was done only when it was necessary.

3.2.1.4 Data collection

A seed was considered to have germinated when the shoot apex from the embryo was visible on the surface of sand in accordance with the ealier recommended procedure (Bhagat *et al.*, 1992). All germinated seeds were counted and recorded everyday for each observation plot. In recording, the number of germinated seeds were expressed as a percentage of the total seeds that were sown. At the termination of the experiment, all seeds which did not germinate were tested for their viability by using a cutting test to see whether they were still alive or dead. Alive together with germinated seeds were again expressed as a percentage of the total seeds that were sown for each observation plot as end viability percentage. Another important aspect that was recorded is the dormancy period i.e. complete dormancy (time taken by seeds to start germinating), total dormancy (time taken to complete germination) and the differential dormancy (total dormancy minus complete dormancy). Each sowing phase was terminated after 90 days.

3.2.1.5 Data analysis

Seed germination attributes which were analyzed were cumulative germination percentages (GP), germination values (GV), germination energy (GE), dormancy periods and end viability percentages. To calculate the germination values, the method proposed by Djavanshir and Pourbeik (1976) was used.

$$GV = SDGS/N \times GP/10.$$

where:

DGS = Daily germination speed,

N = Number of counts since commencement of germination,

GP = Cumulative germination percentage at the termination of experiment,

10 = A constant,

SDGS = Sum of individual daily germination speeds till the end of germination.

For germination energy a criterion used by Winters and Ford-Robertson (1983) was adapted which considers germination energy as a percentage of seeds which germinate up to the time of peak germination (i.e. when daily germination speed within 24 hours is the highest). To bring normality before statistical analysis was done cumulative germination, germination energy and end viability percentages were transformed into arcsine angle values as proposed by Freeze (1967) and Snedecor and Cochran (1989). Dormancy periods were subjected to square root values as proposed by Gomez and Gomez (1983). These values were then subjected to analysis of variance using SPSS/PC+ statistical software to see whether there existed any significant differences among the treatments. Where significant differences were observed, a Duncan Multiple Range Test (DMRT) as described by Gomez and Gomez (1983) was used to separate the differing treatment means. It should be noted that, treatments were declared to be significantly different at $P \leqslant 0.05$.

3.2.2. Experiment 2: Propagation of *P. curatellifolia* and *U. kirkiana* using stem cuttings

This experiment involved the use of stem cuttings in propagating the two species. Stem cuttings of about 10 - 15 mm in diameter and 40 cm long from tree branches containing several nodes were collected in early February, 1996 from healthy mother trees growing in natural woodland in Mufindi District. This was done before resumption of flowering. Only stem cuttings of the previous season's growth were used.

To reduce desiccation, collection of the cuttings was done early in the morning, wrapped in damp hessian sacks and transported in polythene bags to the experimental site the same day. At the site the cuttings were stored in the germination room (RH 70 - 80%, Temp. 25 ± 3°C) and in the evening they were dipped in different hormone concentrations to be tested. Insertion in the rooting media was then done the following morning.

3.2.2.1 Treatment of stem cuttings

Two factors were investigated in this study: Position of the cuttings and hormone treatments. Position of cuttings involved two levels, lower part of the stem cutting (basal (B)) and upper part of stem cutting (terminal (T)). For hormone treatments, Indole-3-Butyric acid was used at seven hormone concentration levels (0 ppm (H_0) as control, 125 ppm (H_1), 250 ppm (H_2), 375 ppm (H_3), 500 ppm (H_4), 625 ppm (H_5) and 750 ppm (H_6) were tested.

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To accomplish these, each stem cutting harvested was sectioned into two parts of about 20 cm long each (basal and terminal) so that each part contained at least two nodes. Then each cutting part (3 - 5 cm of its basal ends) was dipped for 12 hours in different concentrations of the hormone. Control sets were dipped in distilled water. Upper cut ends of the cuttings were sealed with petroleum jelly to reduce water loss through the free cut surfaces (Aminah, 1992; Gupta et al, 1993).

3.2.2.2 Experimental design

For each tree species, a 2 x 7 factorial combination of two cutting positions (basal and terminal) and seven hormone concentration levels (0, 125, 250, 375, 500, 625 and 750 ppm) was used. There were three replications of each of the 14 treatment combinations which were arranged in a completely randomized design. For each experimental unit (treatment combination replicate) there were 25 cuttings involving a total of 1050 cuttings for each of the two species.

Washed river sand was used as a rooting medium. This sand was filled in polyethylene tubes (20 cm flat diameter, 20 cm in height) in which cuttings were inserted. Prepared rooting bed made of bricks (200 cm wide, 1500 cm long and 100 cm high was made and in it experimental units were separated by wooden boards. Thereafter cuttings forming the same treatment unit were inserted in one of these prepared chambers. To control high temperatures, the rooting bed was constructed under tree shade and thatched on top with grasses. To maintain adequate moisture in the bed, watering was done two times a day.

Light intensity was controlled by covering on top (75 cm) from the bed wall with a black translucent polythene sheet. This also helped to maintain high relative humidity and prevent algae growth (Eliasson, 1971; Kantari, 1993b).

3.2.2.3 Data collection

The data collected and used as a criterion for successful performance of the cuttings included sprouting and rooting percentages; number of roots per cutting and mean root length. For sprouting and rooting percentages, the number of cuttings that formed at least one shoot/root were expressed as a percentage of total cuttings that were in each experimental unit. For the number of roots, roots formed for each cutting in a plot were counted and recorded and later a mean root number per cutting in an experimental plot was determined. For root lengths, each root in a cutting was measured to the nearest millimetre and an average root length per cutting in a plot found and recorded. All these activities were done after 90 days from the set up of the experiment (at its termination).

3.2.2.4 Data analysis

To test differences in sprouting, rooting success for the treatments, analysis of variance using SPSS/PC+ statistical software was done. Where significant differences were observed a Duncan Multiple Range Test was used to separate the differing treatment means. Before statistical analysis was done all percentages were transformed into arcsine angle values. Again treatments were declared to be significantly different at $P \leq 0.05$.

CHAPTER 4

4.0 RESULTS

4.1 The effect of seed pre-sowing treatments and storage periods on the germination of *P. curatellifolia* seeds

The overall results of the experiment on the effect of pre-sowing treatments and storage periods on the germination of *P. curatellifolia* seeds are presented in Table 1. It should be noted that, only seeds that received pre-sowing treatments by partial removal of the seedcoat at the radicular end by filing (T_1) and those with complete removal of the seedcoat (T_2) germinated at all the three storage periods. The rest of the pre-sowing treatments gave no germination at all the storage periods. Thus parameters involving cumulative germination percentage, germination energy, germination value and dormancy periods (complete dormancy, total dormancy, and differential dormancy) were analyzed only for treatments that gave germination. The end viability percentages, however, were analysed for both treatments with and without initial germination by inspection of the ungerminated seeds.

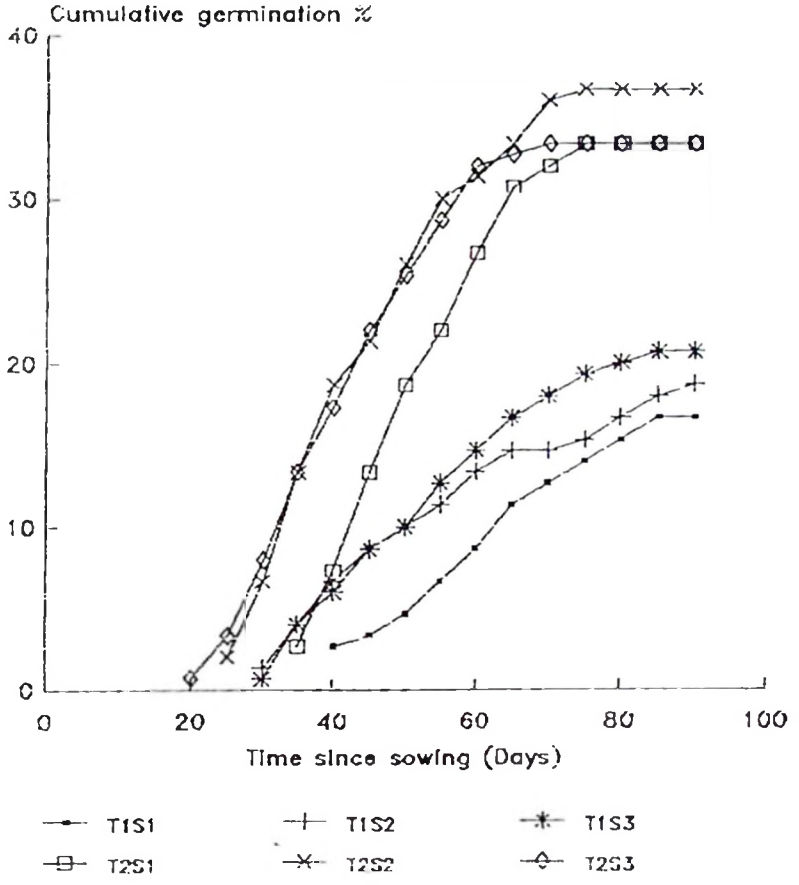
4.1.1 Germination pattern of seeds

The germination behaviour of seeds was slow and sporadic (Figure 1). The best treatment (T_2) sown after storage for 60 days started its germination after 21.3 days and continued

Table 1: Effect of seed pre-sowing treatments and storage periods on the germination of *P. curatellifolia* seeds.

Seed pre-sowing treatment	Storage period	Cumulative germination percentage
T_1	S_1	16.6
	S_2	18.3
	S_3	21.3
T_2	S_1	32.6
	S_2	36.7
	S_3	33.2
T_3	S_1	0.0
	S_2	0.0
	S_3	0.0
T_4	S_1	0.0
	S_2	0.0
	S_3	0.0
T_5	S_1	0.0
	S_2	0.0
	S_3	0.0
T_6	S_1	0.0
	S_2	0.0
	S_3	0.0
T_7	S_1	0.0
	S_2	0.0
	S_3	0.0
T_8	S_1	0.0
	S_2	0.0
	S_3	0.0
T_9	S_1	0.0
	S_2	0.0

slowly to 64.3 days after sowing. This behaviour was more pronounced in seeds which received partial removal of the seedcoat at the radicular end T_1. For example seeds which received pre-sowing treatment T_1 and sown in the first phase delayed to start germination (37 days) and continued slowly in a sporadic manner to 82.3 days.



Key
T_1S_1 = Seeds with partial removal of the seedcoat after storage for 0 days
T_1S_2 = Seeds with partial removal of the seedcoat after storage for 30 days
T_1S_3 = Seeds with partial removal of the seedcoat after storage for 60 days
T_2S_1 = Seeds with complete removal of the seedcoat after storage for 0 days
T_2S_2 = Seeds with complete removal of the seedcoat after storage for 30 days
T_2S_3 = Seeds with complete removal of the seedcoat after storage for 60 days

Figure 1: Effect of seed pre-sowing treatments and storage periods on germination response of *P. curatellifolia* seeds.

4.1.2 Effect of seed pre-sowing treatments and storage periods on cumulative germination percentage, germination energy and germination value

The mean results of the experiment on the above parameters are presented in Table 2 and analysis of variance in Appendix 1a. There were significant differences in cumulative germination percentage, germination energy and germination value between pre-sowing

Table 2: Effect of seed pre-sowing treatments and storage periods on cumulative germination percentage, germination energy percentage, and germination value of *P. curatellifolia* seeds.

Treatment factor	Cumulative germination %	Germination energy %	Germination value
Pre-sowing			
T_1	18.7 (1.5) b	15.3 (4.3) b	0.4 (0.2) b
T_2	34.2 (2.0) a	30.3 (5.8) a	1.4 (0.5) a
Storage period			
S_1	24.1 (3.9) a	22.4 (9.3) a	0.7 (0.5) a
S_2	27.0 (4.7) a	21.8 (10.8) a	1.0 (0.7) a
S_3	27.0 (3.4) a	22.9 (8.9) a	0.9 (0.7) a
Pre-sowing x Storage period			
T_1S_1	16.6 (1.7) a	15.2 (1.8) a	0.2 (0.1) a
T_1S_2	18.3 (3.7) a	13.6 (3.7) a	0.4 (0.2) a
T_1S_3	21.3 (1.7) a	17.2 (2.4) a	0.5 (0.1) a
T_2S_1	32.6 (3.5) a	30.6 (3.5) a	1.1 (0.2) a
T_2S_2	36.7 (2.5) a	31.3 (1.8) a	1.6 (0.2) a
T_2S_3	33.2 (4.6) a	29.1 (5.3) a	1.4 (0.4) a

Note: For each factor and Interactions, means followed by the same letter in each column are not significantly different at $P \leq 0.05$. Standard errors are in parenthesis.

treatments (all P = 0.0001). The storage periods had no significant differential influence on all the three parameters assessed. The influence of the interactions between pre-sowing treatments and storage periods, however, had no significant differential effect (P = 0.602) (Appendix 1a). In all three parameters tested, seeds in which pre-sowing treatment by complete removal of the seedcoat (T_2) gave highest mean cumulative germination percent, germination energy and germination value.

4.1.3 Effect of seed pre-sowing treatments and storage periods on seed dormancy (complete, total and differential)

The results of the effect of seed pre-sowing treatments and storage periods on seed dormancy are presented in Table 3. It should be noted that complete seed dormancy was significantly affected by seed pre-sowing treatments (P = 0.001), seed storage periods (P = 0.0001) as well as the interactions between the two (P = 0.01) (Appendix 1b).

Note that seeds that were subjected to complete removal of seedcoat (T_2) started to germinate earlier than those with partial removal of seedcoat at the radicular end. For the storage times, seeds sown after storage for 30 (S_2) and 60 (S_3) days started to germinate earlier than those sown soon after extraction and drying (S_1). The seeds which had been stored for at least 30 days and the seedcoats completely or partially removed started to germinate earliest with storage for 60 days and complete removal of the seedcoats leading (ie at 21.3 days after sowing). The pre-sowing treatments also significantly influenced both

Table 3: Effect of seed pre-sowing treatments and storage periods on seed dormancy (Days) of *P. curatellifolia* seeds.

Treatment factor	Complete dormancy	Total dormancy	differential dormancy
Pre-sowing			
T_1	33.5 (1.2) a	82.7 (1.4) a	49.1 (1.6) a
T_2	27.8 (2.3) b	67.6 (1.4) b	40.6 (1.4) b
Storage period			
S_1	36.3 (1.0) a	76.7 (2.6) a	40.4 (2.3) b
S_2	29.1 (1.4) b	75.4 (3.9) a	48.0 (2.7) a
S_3	26.7 (2.8) b	72.8 (4.4) a	46.1 (1.9) a
Pre-sowing x storage period			
T_1S_1	37.0 (0.9) a	82.3 (1.7) a	45.3 (1.5) a
T_1S_2	31.0 (1.5) ab	84.0 (2.1) a	52.9 (3.3) a
T_1S_3	32.6 (1.7) ab	81.9 (3.7) a	49.3 (2.1) a
T_2S_1	35.6 (2.3) a	71.3 (1.4) a	35.7 (0.4) a
T_2S_2	27.3 (1.9) ab	67.3 (1.5) a	43.3 (1.2) a
T_2S_3	21.3 (1.0) b	64.3 (2.6) a	42.9 (1.8) a

Note: For each factor and Interactions, means followed by the same letter in each column are not significantly different at $P \leq 0.05$. standard errors are in parenthesis.

the total and differential dormancies ($P = 0.0001$). The storage period on the other hand, significantly influenced the differential dormancy ($P = 0.003$) but impartial on the total dormancy ($P = 0.269$). In both cases the interactions had no significant differential effect (i.e. $P = 0.308$ for total dormancy and $P = 0.540$ for differential dormancy) (Appendix 1b). Note also that seeds which received complete removal of the seedcoat T_2 had significantly shorter total and smaller differential dormancy periods than those with partial removal of the seedcoat. Also storage periods S_1 had a significantly shorter differential dormancy period.

4.1.4 Effect of seed pre-sowing treatments and storage periods on the end viability percentage for treatments with and without initial germination

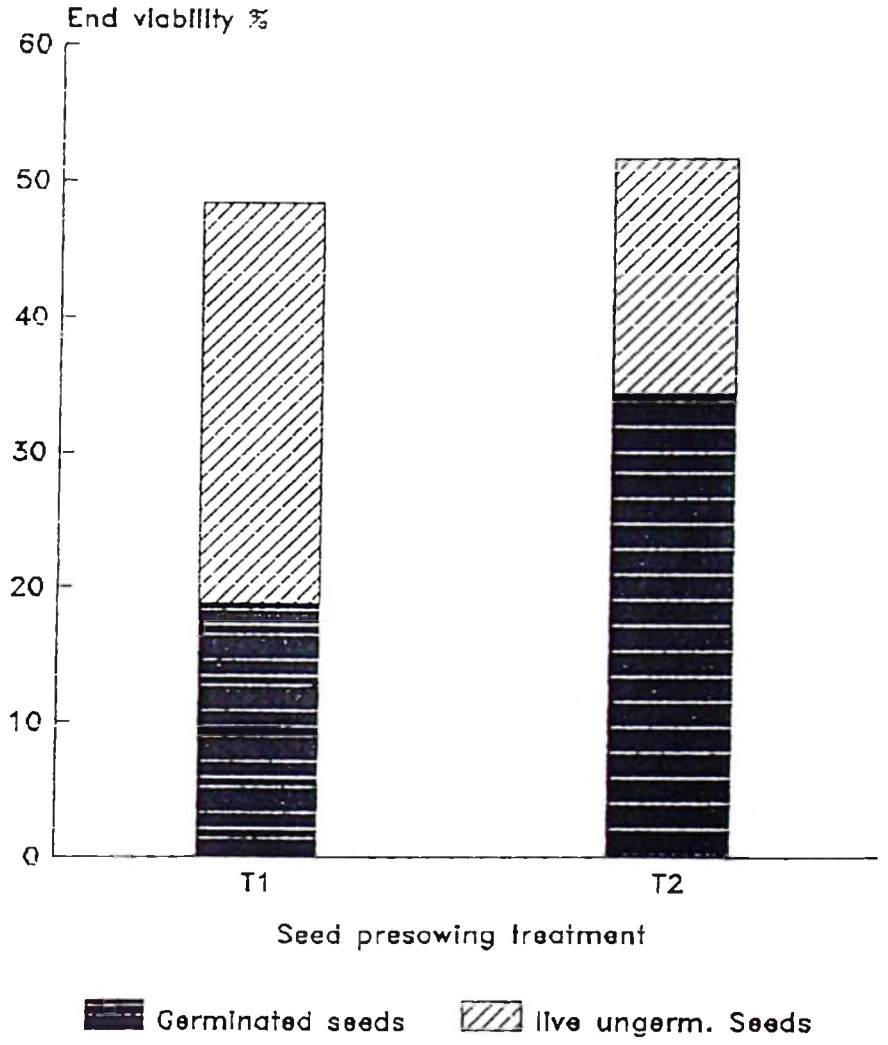
The results on the effect of pre-sowing treatments and storage periods on the end viability percentage for treatments with and without initial germination are presented in Table 4 and in Figure 2. For treatments with initial germination, neither the seed pre-sowing treatments (P = 0.583) nor the storage periods (P = 0.334) had a significant impact on end viability percentage. Their interactions were also found to be insignificantly different (P = 0.732). For treatments without initial germination, only seed pre-sowing treaments significantly influenced the end viability percentage (P = 0.0001). Neither the storage periods (P = 0.876) nor their interactions with seed pre-sowing treatments (P = 0.716) had a significant influence (Appendix 1c).

Note that, although the cumulative germination percentage in treatment T_1 was statistically lower than that of treatment T_2 (Table 2) and although T_1 has a slightly higher value (Table 4), their end viability percentages do not differ significantly. Naturally, the percentage contribution of live ungerminated seeds on the end viability percentage was more in treatment T_1 than in T_2 (Figure 2 and Appendix 1d). Also note from Table 4 that, in treatments without initial germination, seed pre-sowing treatment T_3 was having the highest end viability percentage and treatment (T_8) which involved soaking seeds in hot water was having the lowest possibly indicating that, more of the soaked seeds imbibed water to initiate embryo development but later died due to their inability to break out the hard seedcoats.

Table 4: Effect of seed pre-sowing treatments and storage periods on the end viability percentage of *P. curatellifolia* seeds for treatments with and without initial germination.

Treat. Factor	End viability % (Treatments with initial germination)	End viability % (Treatments without initial germination
Pre-sowing		
T_1	49.1 (3.8) a	
T_2	52.3 (3.8) a	
T_3		76.6 (2.5) a
T_4		64.6 (2.7) b
T_5		69.9 (2.7) ab
T_6		60.4 (3.4) bc
T_7		45.7 (2.4) c
T_8		31.9 (2.0) d
T_9		76.4 (3.0) a
Storage period		
S_1	47.3 (4.2) a	60.9 (7.8) a
S_2	56.8 (4.1) a	60.6 (7.7) a
S_3	47.9 (5.2) a	62.0 (6.2) a
Pre-sowing x storage period		
T_1S_1	47.3 (4.6) a	
T_1S_2	47.3 (7.5) a	
T_1S_3	56.7 (6.5) a	
T_2S_1	56.8 (6.4) a	
T_2S_2	43.2 (7.6) a	
T_2S_3	50.9 (7.3) a	
T_3S_1		73.1 (6.4) a
T_3S_2		81.5 (2.4) a
T_3S_3		74.8 (2.9) a
T_4S_1		65.0 (5.2) a
T_4S_2		65.8 (6.8) a
T_4S_3		62.7 (2.9) a
T_5S_1		69.5 (3.5) a
T_5S_2		71.4 (1.7) a
T_5S_3		68.7 (8.3) a
T_6S_1		60.2 (6.1) a
T_6S_2		53.4 (6.7) a
T_6S_3		67.4 (2.4) a
T_7S_1		42.6 (4.8) a
T_7S_2		45.3 (3.5) a
T_7S_3		49.2 (4.7) a
T_8S_1		31.2 (3.5) a
T_8S_2		29.2 (3.5) a
T_8S_3		35.2 (4.0) a
T_9S_1		81.4 (5.7) a
T_9S_2		73.9 (6.9) a
T_9S_3		73.7 (5.2) a

Note: For each factor and interactions, means followed by the same letter in each column are not significantly different at $P \leq 0.05$. standard errors are in parenthesis.



Key

T_1 = Seeds with partial removal of the seedcoat at the radicular end.

T_2 = Seeds with complete removal of the seedcoat.

Figure 2: Percentage contribution of germinated and live ungerminated seeds on the end viability percentage of *P. curatellifolia* seeds in treatments with initial germination.

4.2 The effect of position of the cuttings and hormone concentrations on sprouting, rooting, root number and root length of *P. curatellifolia* and *U. kirkiana* stem cuttings

The results of the effect of position of the cuttings and different hormone concentrations application on the sprouting, rooting, root number and root lengths of stem cuttings of *P. curatellifolia* are presented in Table 5 and those for *U. kirkiana* are presented in Table 6. The trends of responses to hormone application for *P. curatellifolia* and for *U. kirkiana* are given in Figure 3 and Figure 4 respectively.

It should be noted that application of different levels of the hormone concentrations had significant effect on rooting percentage (P = 0.044), root number (P = 0.001) and root length (P = 0.0001) of *P. curatellifolia* cuttings but did not significantly influence their sprouting ability (P = 0.365). On the other hand, the position of the stem cuttings had a significant influence on sprouting (P = 0.004), rooting percentage (P = 0.0001) and root length (P = 0.010) but had no differential influence on the root number (P = 0.052). The interaction between the two factors had significant influence only on sprouting percentage (P = 0.042), rooting percentage (P = 0.005) and root number (P = 0.002) but not on the root length (P = 0.300) (Appendix 2a). Overall, hormone concentration 375 ppm (H_3) and its interactions with basal position of the stem cuttings with 46.7%, 41.3%, 5.4, and 50.8 mm for sprouting, rooting, root number, and root length respectively gave the best performance.

Table 5: Effect of hormone concentrations and position of the cuttings on sprouting, rooting, root number and root length of *P. curatellifolia* stem cuttings.

Treat. Factor	Sprouting %	Rooting %	Root number	Root length (mm)
Hormone				
H_0	32.5 (2.4) a	24.4 (2.8) ab	3.1 (0.3) c	34.4 (3.3) c
H_1	31.1 (5.8) a	26.4 (2.7) ab	4.9 (0.4) ab	41.2 (1.5) abc
H_2	28.3 (5.0) a	19.1 (2.4) b	4.0 (0.3) abc	47.7 (4.8) a
H_3	37.7 (3.8) a	29.9 (5.5) a	4.5 (0.5) ab	48.0 (2.9) a
H_4	34.3 (4.0) a	25.9 (4.8) ab	4.9 (0.6) a	43.9 (3.4) ab
H_5	28.3 (3.9) a	18.7 (3.4) b	3.6 (0.4) bc	38.2 (4.0) bc
H_6	30.2 (4.7) a	20.0 (3.8) b	3.3 (0.3) c	32.3 (2.0) c
Position				
B	35.6 (1.9) a	27.8 (1.9) a	4.3 (0.3) a	43.9 (2.8) a
T	28.0 (1.7) b	19.2 (1.7) b	3.8 (0.2) a	37.8 (1.8) b
Horm. x Pos.				
BH_0	30.6 (3.5) bc	21.3 (2.7) cdef	3.3 (0.6) cd	40.0 (3.9) a
BH_1	26.5 (3.6) bc	23.7 (4.6) bcdef	4.2 (0.5) bcd	43.3 (2.0) a
BH_2	34.5 (4.7) abc	22.5 (3.6) cdef	3.6 (0.3) cd	55.8 (2.5) a
BH_3	46.7 (4.8) a	41.3 (3.5) a	5.4 (0.1) ab	50.8 (4.4) a
BH_4	41.3 (3.5) ab	35.9 (3.9) ab	6.0 (1.8) a	46.5 (6.1) a
BH_5	33.1 (5.8) abc	25.2 (2.7) bcde	4.2 (0.5) bcd	35.3 (7.8) a
BH_6	37.2 (5.8) abc	26.4 (5.6) bcd	3.2 (0.6) cd	35.5 (2.8) a
TH_0	34.6 (3.5) abc	27.8 (4.6) bcd	2.8 (0.1) d	28.8 (2.9) a
TH_1	36.0 (2.3) abc	29.3 (2.6) abc	5.6 (0.6) ab	39.1 (1.6) a
TH_2	22.4 (3.7) c	15.9 (2.3) def	4.4 (0.6) bc	39.6 (6.6) a
TH_3	29.1 (4.9) bc	19.6 (4.6) cdef	3.5 (0.6) cd	45.3 (4.1) a
TH_4	27.8 (4.6) bc	17.1 (3.5) cdef	3.7 (0.1) cd	41.4 (3.8) a
TH_5	23.7 (4.6) c	13.0 (3.4) f	2.9 (0.2) cd	41.0 (3.3) a
TH_6	23.7 (4.6) c	14.3 (3.6) ef	3.4 (0.2) cd	29.1 (1.6) a

Note: For each factor and interactions, means followed by the same letter in each column are not significantly different at $P < 0.05$). Standard errors are in parenthesis.

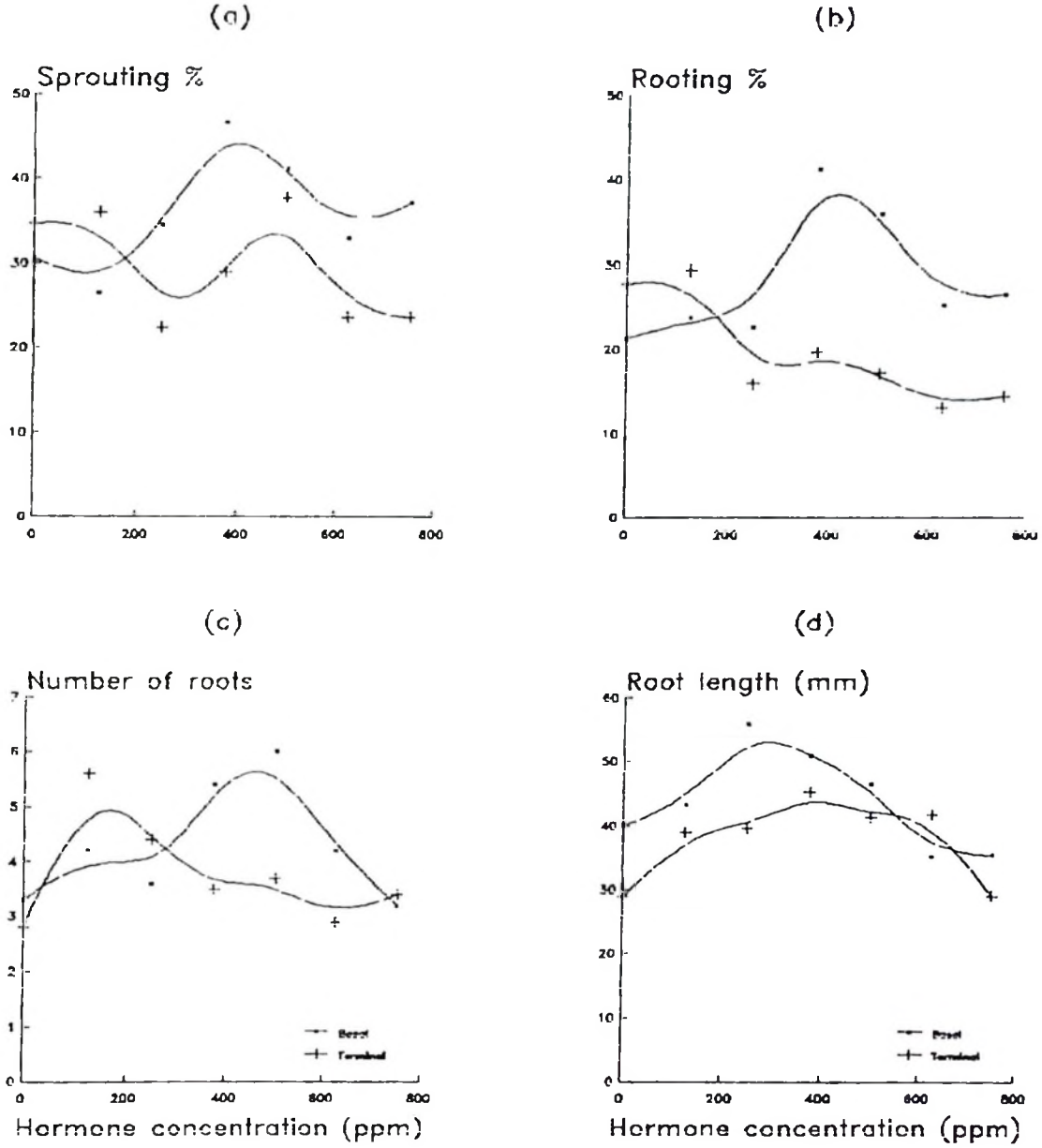
Table 6: Effect of hormone concentrations and position of the cutt rooting, root number and root length of *U. kirkiana* stem

Treatment	Sprouting %	Rooting %	Root nun
Hormone			
H_0	37.8 (3.3) a	28.4 (3.3) ab	3.5 (0.4)
H_1	46.3 (8.4) a	37.7 (7.7) a	4.6 (0.3)
H_2	46.6 (3.8) a	37.8 (3.9) a	4.2 (0.3)
H_3	36.3 (5.3) a	29.3 (5.1) ab	4.8 (0.5)
H_4	37.6 (6.1) a	24.2 (3.8) b	3.7 (0.3)
H_5	37.7 (4.1) a	21.0 (2.7) b	3.4 (0.2)
H_6	30.5 (2.6) a	24.2 (3.5) b	3.6 (0.3)
Position			
B	45.8 (2.9) a	34.3 (2.8) a	4.3 (0.2)
T	31.1 (1.8) b	23.5 (1.8) a	3.6 (0.1)
Horm. x Pos.			
BH_0	42.6 (5.8) a	30.5 (4.9) a	4.0 (0.5)
BH_1	62.9 (6.8) a	53.3 (4.8) a	5.2 (0.3)
BH_2	52.0 (4.6) a	43.9 (4.0) a	4.7 (0.3)
BH_3	45.2 (7.3) a	38.6 (3.5) a	5.4 (0.9)
BH_4	49.3 (7.0) a	30.3 (5.7) a	4.1 (0.4)
BH_5	37.2 (4.8) a	22.5 (3.6) a	3.5 (0.5)
BH_6	31.8 (4.6) a	23.5 (4.0) a	3.5 (0.6)
TH_0	33.3 (3.4) a	26.4 (4.5) a	3.0 (0.3)
TH_1	30.3 (5.9) a	23.3 (6.3) a	3.9 (0.2)
TH_2	41.2 (4.8) a	31.8 (4.6) a	3.7 (0.3)
TH_3	27.8 (3.9) a	20.7 (5.7) a	4.3 (0.7)
TH_4	26.6 (3.5) a	18.5 (2.5) a	3.4 (0.5)
TH_5	30.2 (6.9) a	19.6 (4.6) a	3.4 (0.3)
TH_6	29.2 (3.6) a	25.0 (4.8) a	3.7 (0.4)

Note: For each factor and Interactions, means followed by the same letter i significantly different at $P \leq 0.05$). standard errors are in parenthesis.

For the case of *U. kirkiana*, it was revealed that different levels of hormone concentrations had significant effect only on rooting percentage (P = 0.011), root number (P = 0.033) and root length (P = 0.010) but did not significantly influence their sprouting ability (P = 0.054). On the other hand the position of the stem cuttings had a significant influence on all four parameters (ie sprouting (P = 0.0001), rooting percentage (P = 0.0001), root number per cutting (P = 0.009) and root length (P = 0.0001)). With the exception of root lengths (P = 0.032), the interactions between different levels of hormone concentrations and position of the stem cuttings did not significantly influence the sprouting percentage (P = 0.155), rooting percentage (P = 0.920) and number of roots per cuttings (P = 0.633) (Appendix 2b). Overall, hormone concentration 125 ppm (H_1) and its interactions with the basal position of the stem cuttings with 62.9%, 53.3%, 5.2, and 58.8 mm for sprouting, rooting, root number, and root length respectively resulted into the best performance (Table 6).

The trend of response of stem cuttings with respect to sprouting, rooting, root number per cutting and root length per root following hormone application differed for the two species (Figure 3 and 4). For *P. curatellifolia* basal cuttings responded to higher hormone concentrations better than terminal cuttings in all parameters except for root length whose response at hormone concentrations higher than 450 ppm fell below that of terminal cuttings. This rate of increase was directly positive up to 375 ppm (H_4) hormone concentration, beyond which a decline in the trend of responses was observed as presented in Figure 3. However, at lower hormone concentrations (ie < 150 ppm), terminal cuttings responded better to hormone application than basal cuttings for the sprouting, rooting and root number.

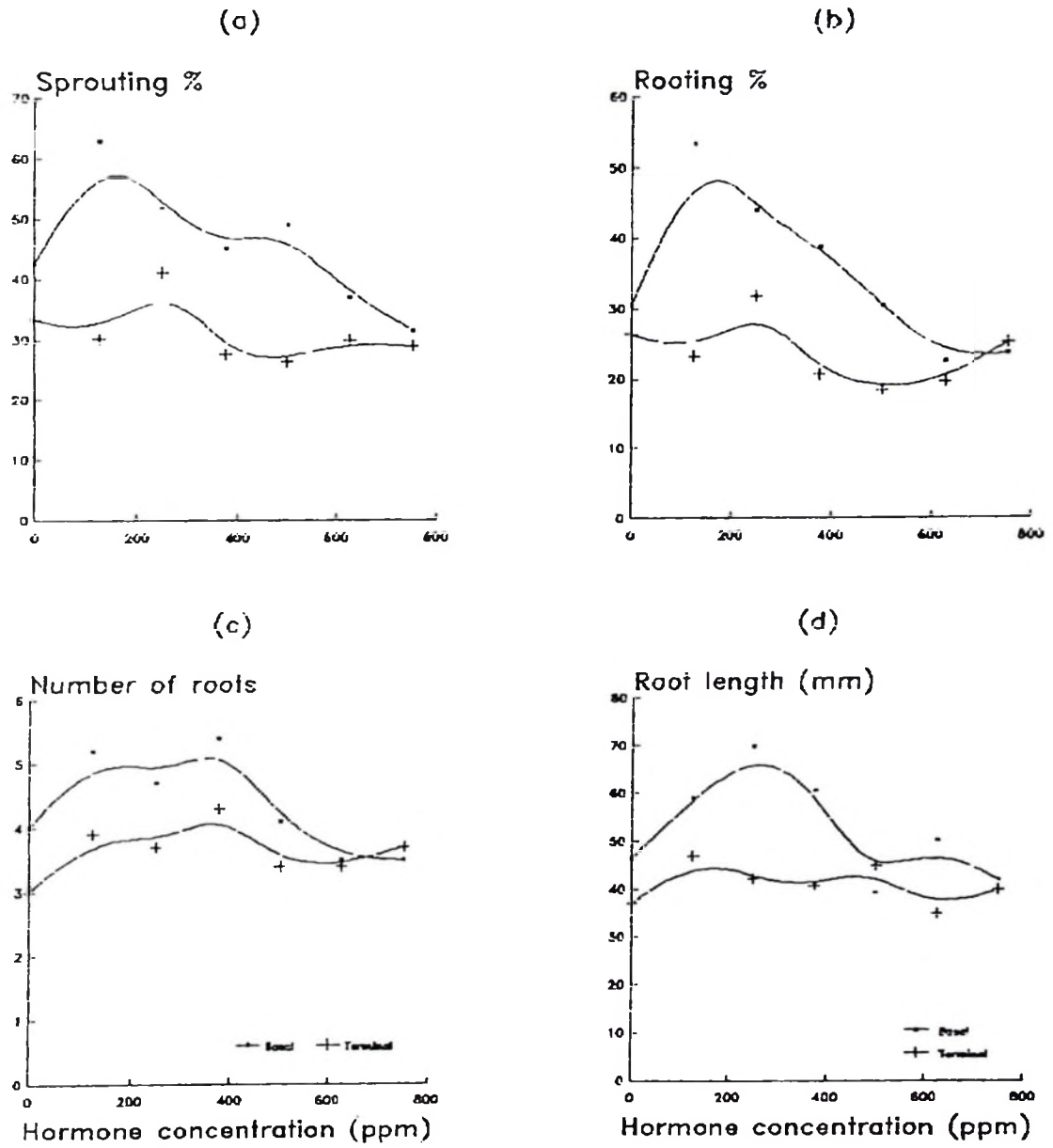


Key:

B = Basal position

T = Terminal position

Figure 3: Effect of hormone concentrations on sprouting, rooting, root number and root length responses of *P. curatellifolia* stem cuttings.



Key:

B = Basal position

T = Terminal position

Figure 4: Effect of hormone concentrations on sprouting, rooting, root number and root length responses of *U. kirkiana* stem cuttings.

In the case of *U. kirkiana*, increasing hormone concentrations improved the response especially for basal cuttings in respect of all parameters assessed up to 125 ppm (H_1) although root length reached its peak at 250 ppm (H_2). Beyond these limits a sharp decline in response was observed. Terminal cuttings showed no clear pattern of response (Figure 4).

Overall the response of the basal position of the stem cuttings for both species was more pronounced compared with the terminal position of the cuttings

CHAPTER 5

5.0 DISCUSSION

5.1 The effect of seed pre-sowing treatments and storage periods on germination of *P. curatellifolia* seeds

5.1.1 Effect of seed pre-sowing treatments

The results of the effect of pre-sowing treatments on the germination of *P. curatellifolia* seeds are presented in Tables 1, 2, 3, 4 and Fig.1 and 2. It was noted that, only two seed pre-sowing treatments which involved complete removal of the seedcoat (T_2) and partial removal of the seedcoats at the radicular end (T_1) had germination both in terms of cumulative germination percentage, germination energy, germination value and dormancy periods. The rest of the pre-sowing treatments gave no germination. However, they had an influence on the end viability percentage. These results are in general agreement with those findings reported by Msanga and Kalaghe (1992) and Sadhu and Kaul (1989) who used a similar method to test the germination of *Vangueria infausta* and *Robinia pseudo-acacia* respectively. Others who found similar results include Maghembe and Msanga (1988) with *Trichilia emetica*, and Onywekwelu (1990) with *Tetrapleura tetraptera* seeds.

Even the removal of the seedcoats (either partially or completely) only partially overcomes the problem of dormancy inherent in *P. curatellifolia* seeds possibly due to

improved permeability of the seeds to moisture and air together with overcoming the mechanical resistance imposed by the seedcoats to the embryo growth. These improvements are important in seed germination as they allow reserved food materials to dissolve, respiration to take place and reduce or remove the mechanical obstruction normally imposed by the seedcoats on the embryo growth during germination (Black and Wearing, 1959; Amen, 1963; Small *et al.*, 1977; Nikolaeva, 1977; Nwoboshi, 1982; Bewley and Black, 1978, 1994; Willan, 1986; Meyer and Poljakoff-Mayber, 1989; Duangpatra, 1991).

The ineffectiveness of the treatments which involved soaking entire or half split seeds in concentrated sulphuric acid and in hot water contrast inversely with the findings reported by Chamshama and Downs (1984) with *Acacia tortilis*, Sagwal (1986) with *Erheitia accuminata*, and Sadhu and Kaul 1989) with *Robinia Pseudo-acacia* who recommended the use of sulphuric acid to weaken the tough seedcoats of such species to improve the germination. These findings are also in inverse contrast with those reported by Patanath (1982) in Willan (1986), Willan (1986), Duangpatra (1991) and Tietima *et al.* (1992) who recommended soaking seeds in cold or hot water for varied periods in some species. The reasons for the failures of especially the acid, to enhance the germination are not clearly known. It could possibly, however, be due to either being insufficiently severe to damage the seedcoats to allow in moisture and air, and to remove their mechanical resistance to embryo growth on one hand or being too severe leading to the damage of the embryos (Sagwal, 1986; Tietema *et al.*, 1992).

Although the two pre-sowing treatments (i.e. complete (T_2) and partial (T_1) removal of the seedcoats) improved germination of *P. curatellifolia* seeds, there was a general inferiority in germination characteristics in seeds where partial removal of the seedcoats was employed compared to those in which complete removal was done. This inferiority could possibly be explained by the presence of the remaining seedcoats after filing at the radicular end which provided some mechanical resistance to embryo growth. Furthermore the permeability of water and gases as is the case for other similar species (Meyer and Poljakoff-Mayber, 1989; Bewley and Black, 1994), could not have been improved sufficiently enough to allow easy initiation of embryo growth as also reflected by the higher contribution of live ungerminated seeds to the end viability percentage (Figure 2). This also could be the reason why seeds in treatment T_1 (partial removal of seedcoats) showed germination patterns whose end was not clearly defined. A Similar behaviour was observed by Msanga and Kalaghe (1992) in *Vangueria infausta* seeds, which necessitated complete removal of the seedcoats in order to get optimal germination.

The high percentage of ungerminated viable seeds observed at the termination of the study in seeds which had received a 15 minutes treatment with concentrated sulphuric acid contrasts inversely with the earlier findings (Chamshama and Downs, 1984). Possibly this is an indication that, the acid treatment is insufficient to overcome the impermeability to water and gases and mechanical resistance to the embryo growth imposed by the tough woody seedcoats of *P. curatellifolia* seeds.

The low end viability percentage observed in treatments with seedcoats almost half sp\ and soaked in concentrated sulphuric acid for 30 minutes (T_7) and those immersed in hot water and left to cool for 12 hours (T_8), could possibly be due to the fact that the treatments only partially managed to overcome the physical barriers of the seedcoats to the entrance of water and gases, but totally failed to remove the mechanical resistance. As such water and gases were absorbed which caused most of the failed to germinate embryos to rot. Similar behaviour had been observed in other species by Gordon and Rowe (1982), Balcos (1989) and Marshal (1981) cited by Willan (1986). On the other hand, the acid treatments on half splited seedcoats could have been too severe leading to the damage of the embryos and thus accounting for the failure in their germination as had similarly been found earlier on (Sagwal, 1986; Tietema *et al.*, 1992).

5.1.2 Effect of seed storage periods

This factor appeared to be important only on dormancy periods (Table 3). Seeds that were sown after 30 and 60 days of storage started to germinate earlier than those sown immediately after collection and processing. This delayed germination could possibly be due to the fact that, the seeds of this tree species require some time of rest after the fruits ripen and shed from the trees to allow the embryos to complete their maturation processes in order to be able to germinate (Nwoboshi, 1982; Meyer and Poljakoff-Mayber, 1989; Duangpatra, 1991). Similar results were reported earlier by Bhardwaj and Chakraborty (1994) in *Terminalia bellicia* and *Terminalia chebula* seeds. Regardless of the

improvements in permeability and reduced mechanical resistance to the embryo growth which may be rendered by various pre-treatments, they may be insufficient to initiate early germination in the seed embryos that have not yet attained full maturity. On the basis of the present results, full maturity of the embryos in *P. curatellifolia* is reached after 30 days of fruit ripening and shedding and sowing of seeds after 60 days of storage accompanied by complete removal of the seedcoats gives early germination.

Although the results of the present study indicated the best germination performance to be attained in seeds initially subjected to a storage period of at least 30 days and to complete removal of the seedcoats the results can still be considered low. Furthermore the sporadic nature of germination was not much improved and a relative good number of seeds (17.5%) which did not germinate still remained viable at the termination of the experiment (i.e. 90 days).

This suggests that, the seeds of *P. curatellifolia* were still having some unknown problems that were not associated with the impermeability and mechanical resistance of the seedcoats not even with morphological dormancy. This phenomenon was described by Nwoboshi (1992), Meyer and Poljakoff-Mayber (1989), Bewley and Black (1994) and Willan (1986) as a combined dormancy effect where by removal of one or two types of dormancy may be insufficient to give optimal germination. Similar results were reported by Gordon and Rowe (1982) in a number of Rosaceae family seeds, Balcos (1989) on *Pithosporus resiniferum* and Marshal (1981) cited by Willan (1986) in *Fraxinus*

pennsylvanica seeds. They are therefore suspected to be accompanied by c emical or physiological dormancies or a combination of the two (Willan, 1986; Me, d Poljakoff-Mayber, 1989) which need to be removed. The treatments in the present stuo did not address them as they were not suspected and therefore not focused.

5.2 The effect of position of the cuttings and hormone concentrations on propagation of *P. curatellifolia* and *U. kirkiana* stem cuttings

5.2.1 Effect of position of the stem cuttings

The results of the effect of position of the cuttings on the propagation of *P. curatellifolia* and *U. kirkiana* through stem cuttings are presented in Tables 5, and 6. Both species responded well to propagation when basal position of the stem cuttings was used. These results are in agreement with the findings of many other scientists such as Srivastava and Mangil (1981) and Singh *et al.* (1984). However they are in contrast with the findings of Lo (1985), Kijkar (1992b) and Kumar *et al.* (1993) who found that only terminal cuttings had good rooting ability.

The better performance of basal cuttings compared with those of the terminals could possibly be due to an uneven distribution of inhibiting substances in different parts of the shoots as reported by Viart (1979) and Orourke (1940) cited by Hartmann and Kester (1989). They reported that most of inhibitory substances are produced in the apical parts

and thus terminal cuttings possess more of these substances than bas‹ ‘tings. These substances inhibit root initiation as observed in many plant species (Viart, 19‘ ›rourke (1940) cited by Hartmann and Kester, 1989). Furthermore the observation that, ‹ number of preformed root initials in some plants decrease from the base to the tip of the shoot and consequently causing better performance of basal than terminal cuttings (Viart, 1979; Orourke (1940), cited by Hartmann and Kester, 1989) could possibly also explain the observed results.

Another possible reason for the differences in the performance could partly be due to the differences in the nutritional status of the cuttings (Viart, 1979; Haissig, 1986). Cuttings with higher C/N ratios root better than cuttings with lower ratios because the former possess higher food reserves especially carbohydrates. The ratios generally decrease from the base to the top of any single plant shoot from which the cuttings are made. Thus basal cuttings are better in nutrition than terminal cuttings and thus perform better.

The theory that terminal cuttings tend to perform better than basal cuttings taken from the same shoot due to juvenility and less lignification (Lo, 1985 and Kijkar, 1992b) and possibility of higher concentration of endogenous root-promoters arising in the terminal buds (Orourke (1940) cited by Hartmann and Kester (1989) seem to be uncertain to play a role for *P. curatellifolia* and *U. kirkiana* species.

5.2.2 Effect of hormone concentrations

The results of the effect of hormone concentration treatments on the propagation of *P. curatellifolia* and *U. kirkiana* through stem cuttings are presented in Tables 5, 6 and Fig. 3 and 4. With the exception of sprouting for both species, hormone application influenced positively rooting percentage, root number as well as root length. Similar findings on the responses of stem cuttings to hormone application have been reported by many other authors such as Puri and Shamet (1988), Gupta *et al.* (1993), Bwardwaj *et al.* (1993), Bhagat and Arun (1993), Kijkar (1992a), Badola (1993) and Chauhan *et al.* (1994) with other tree species. Indole-3-Butyric Acid (IBA) in the presence of other rooting co-factors is believed to influence adventitious root formation in stem cuttings. Generally hormones hasten cell division and elongation in plants and they initiate root formation, increase the number of roots formed on the cuttings, increase root length and the ability of the cuttings to be rooted over a wide range of periods (Nand and Anand, 1970; Krishnamoorthy, 1981; Hartmann and Kester, 1989). The findings appear to tally well with the results of both *P. curatellifolia* and *U. kirkiana* species in the present study.

The level of hormone that stimulated vegetative growth differed for the two species. While higher concentrations of IBA (ie 375 ppm) promoted good performance of *P. curatellifolia* stem cuttings (Fig. 3), *U. kirkiana* appeared to perform better when lower concentrations (i.e. 125 ppm) were applied (Fig. 4). Possible reasons for these differences could be the fact that some plants possess naturally higher amounts of these auxins and rooting co-factors than others. As such they tend to root more easily than those with low

amounts (Hansen *et al.*, 1978; Hartmann and Kester, 1988; Farmer, 1966; Hineslay and Blazich, 1980). It appeared that *P. curatellifolia* tree species possess less amounts of these hormones which necessitates application of higher concentrations from external sources. This observation is in agreement with the findings by Bhagat and Arun (1993) in *Ficus glomerata* species stem cuttings. However, higher concentrations beyond 375 ppm for sprouting, rooting and root length and 500 ppm for root number depressed the response. This could possibly be due to toxic effects at higher levels of the hormone.

On the other hand, the requirement for lower concentrations of the hormone in the stem cuttings of *U. kirkiana* suggests that the species is possibly an easy to root one. Hartmann and Kester (1989) recommended the use of lower concentrations on such species to, also, reduce unnecessary cost. These findings are in general agreement with those reported by Kijkar (1992b), Gupta *et al.* (1993) and Chauhan *et al.* (1994) who obtained good results with as low concentration as 100 ppm in *Azadirachta*, *Coriaria nepalensis* and *Tectona grandis* tree species respectively. Chauhan *et al.* (1994) further obtained favourable responses with as low as 50 ppm in *Debregeasia hypoleuca* stem cuttings. In all cases it was again observed that applying hormone concentrations, especially beyond 250 ppm, retarded the responses of the parameters assessed possibly due to toxic effects at high levels for the species.

As noted in the results, the interactions between basal cuttings and hormone concentrations of 375 ppm for *P. curatellifolia* and 125 ppm for *U. kirkiana* gave the best results, suggesting that these combinations are more effective in propagating these species through stem cuttings.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results obtained and the subsequent discussions, the following main conclusions can be drawn: For propagation of *P. curatellifolia* through the use of seeds,

- The seedcoats play a significant role in limiting the germination of seeds by restricting the entry of gases and water which are essential for germination. They also provide physical and mechanical barriers to the embryo growth.
- Only seed pre-sowing treatments by partial and complete removal of the seedcoats overcome some of the dormancies exercised by the seedcoats with the latter being more effective. The rest of the treatments as listed in the methodology are not effective.
- Even with the pre-sowing treatment by complete removal of the seedcoats which gave the best results, the highest germination attained was still low with a mean of 34.2%. It is suspected that, seeds of *P. curatellifolia* are also accompanied by other types of dormancies which together with mechanical and physical dormancies hindered the attainment of higher germination.
- A storage period of at least 30 days play a significant role in enhancing early germination of *P. curatellifolia* seeds. This is possibly due to enhanced maturation of the embryos during storage.

In the case of propagation of *P. curatellifolia* and *U. kirkiana* tree species through the use of stem cuttings:

- There is a potential of propagating both species through the use of stem cuttings.
- Basal cuttings perform better than terminal cuttings for both tree species.
- External application of hormone (IBA) is useful in promoting propagation of both species by stem cuttings with 375 ppm and 125 ppm for *P. curatellifolia* and *U. kirkiana* respectively being the most effective.

6.2 Recommendations

- The propagation of *P. curatellifolia* can be done through the use of seeds. They need to be collected and stored at least for 30 days before sowing out in order to allow full maturation of the embryos to take place. The seeds should be sown after the seedcoats have been completely removed.
- The still very low seed germination percentage obtained with the treatments of the present study suggests that the problems associated with the dormancies in *P. curatellifolia* seeds are not completely resolved. It has attempted to solve the physical, mechanical and morphological based seed dormancies. Therefore It is recommended that, more research is needed to resolve the suspected chemical and physiological dormancies in order to improve the overall germination of the seeds of this tree species.

On propagation of *P. curatellifolia* and *U. kirkiana* through the use of stem cuttings it is recommended that;

- Stem cuttings can be used as alternative propagation techniques for *Parinari. curatellifolia* and *U. kirkiana* tree species. Whenever this technique is adopted basal cuttings should be preferred in combination with hormone concentrations (IBA) of 375 ppm for *P. curatellifolia* and 125 ppm for *U. kirkiana.*
- Further research is required to confirm the nursery results in the field. Also research on other factors such as time in the year at which the cuttings can be collected and juvenility of the parent tree is important to improve the results achieved in the present study.

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APPENDICES

Appendix 1a:

Analysis of variance for the effect of seed pre-sowing treatments and storage periods on cumulative germination percentage, germination energy and germination value of *P. curatellifolia* seeds.

Parameter	Source of variation	Df	Mean squares	F value	Probability level (P)
Cumulative germination %	Pre-sowing (P)	1	463.09	33.84	0.0001
	Storage period (S)	2	7.27	0.53	0.601
	P X S	2	7.75	0.53	0.602
	Error	12	13.68		
Germination energy	P	1	481.74	30.61	0.0001
	S	2	0.81	0.05	0.950
	P X S	2	6.82	0.43	0.660
	Error	12	15.74		
Germination value	P	1	4.58	33.36	0.0001
	S	2	0.16	1.15	0.350
	P X S	2	0.42	0.30	0.740
	Error	12	0.14		

Appendix 1b:

Analysis of variance for the effect of seed pre-sowing treatments and storage periods on seed dormancy (complete, total and differential dormancy) of *P. curatellifolia* seeds.

Parameter	Source of variation	Df	Mean squares	F Value	Probability level (P)
	Pre-sowing (P)	1	1.20	20.92	0.001
Complete	Storage period (S)	2	1.18	20.71	0.0001
dormancy	P X S	2	0.40	6.97	0.010
	Error	12	0.057		
	P	1	3.42	64.00	0.0001
Total	S	2	0.08	1.47	0.269
dormancy	P X S	2	0.07	1.30	0.308
	Error	12	0.05		
	P	1	1.84	33.67	0.0001
Differential	S	2	0.54	9.79	0.003
dormancy	P X S	2	0.03	0.65	0.540
	Error	12	0.05		

Appendix 1c:

Analysis of variance for the effect of seed pre-sowing treatments and storage periods on the end viability percentage of *P. curatellifolia* seeds for treatments with and without germination.

Parameter	Source of variation	Df	Mean squares	F Value	Probability level (P)
Treatments with initial germination	Pre-sowing (P)	1	14.72	0.32	0.583
	Storage period (S)	2	55.61	1.20	0.334
	P X S	2	14.83	0.32	0.732
	Error	12	31.12		
Treatments without initial germination	P	6	878.32	29.91	0.0001
	S	2	3.92	0.13	0.876
	P X S	12	21.39	0.73	0.716
	Error	42	29.36		

Appendix 1d:

T-test for the analysis of percentage contribution of live ungerminated seeds on the end viability percentage of *P. curatellifolia* seeds in treatments T_1 and T_2.

Parameter	Value
T-value	3.60
Degree of freedom	16
2-Tail probability	0.002
Standard error for T_1	1.92
Standard error for T_2	1.29

Appendix 2a:

Analysis of variance for the effect of hormone concentrations and position of the cuttings on sprouting, rooting, root number and root length of *P. curatellifolia* stem cuttings.

Parameter	Source of variation	Df	Mean square	F Value	Probability level (P)
Sprouting	Hormone (H)	6	25.90	1.14	0.365
	Position (P)	1	225.41	9.94	0.004
	H x P	6	58.19	2.56	0.042
	Error	28	22.68		
Rooting	H	6	50.74	2.53	0.044
	P	1	355.95	17.79	0.0001
	H x P	6	579.82	3.99	0.005
	Error	28	20.01		
Roots number	H	6	3.27	5.19	0.001
	P	1	2.61	4.13	0.052
	H x P	6	2.92	4.62	0.002
	Error	28	0.63		
Root length	H	6	230.57	4.28	0.0001
	P	1	393.15	7.03	0.010
	H x P	6	68.63	1.27	0.300
	Error	28	54.83		

Appendix 2b:

Analysis of variance for the effect of hormoneconcentrations and position of the cuttings on sprouting, rooting, root number and root length of *U. kirkiana* stem cuttings.

Parameter	Source of Variation	df	Mean square	F Value	Probability level (P)
Sprouting	Hormone (H)	6	77.10	2.39	0.054
	Position (P)	1	789.01	24.52	0.000
	H x P	6	55.16	1.71	0.155
	Error	28	32.18		
Rooting	H	6	104.43	3.44	0.011
	P	1	495.22	16.32	0.000
	H x P	6	62.30	2.05	0.920
	Error	28	30.35		
Root number	H	6	19.63	2.72	0.033
	P	1	5.44	7.75	0.009
	H x P	6	0.51	0.72	0.633
	Error	28	0.70		
Root length	H	6	235.28	3.52	0.010
	P	1	1386.33	20.75	0.000
	H x P	6	182.40	2.73	0.032
	Error	28	66.80		