USE OF A NON MIST PROPAGATION SYSTEM TO VEGETATIVELY PROPAGATE 12 VARIETIES OF *E. grandis* X *E. camadulensis* HYBRIDS

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

I dedicate this thesis to all small scale tree nursery entrepreneurs and operators in rural Kenya.

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LIST OF ABBREVIATIONS

EGC hybrid	Hybrids of *E. grandis x E camadulensis*
IBA	Indole butyric acid
IAA	Indole acetic acid
NAA	Naphthalene acetic acid
ANOVA	Analysis of variance
CS	Clay sub soil
LSA	Leaf surface area
SCS	Sand soil and clay sub soil (1:1)
S2CS	Sand soil and clay sub soil (1:2)
2SCS	Sand soil and clay sub soil (2:1)
SS	Sand soil and clay sub soil (1:1)
s.e.d	Standard error of deviation

ABSTRACT

A series of nursery experiments was carried out to assess the effects of rooting medium (sand and clay subsoil, mixed in the following ratios 1:0, 1:1, 1:2, 0:1 and 2:1 respectively by volume), auxin concentration (0%, 0.6%, 0.8%, and 1% IBA) and leaf area of cuttings (0, 30, 40, 50, 60, 80 and 100cm^2) on rooting success of juvenile cuttings of *Eucalyptus grandis* x *camadulensis* hybrids. The Eucalyptus hybrids (EGC) cuttings were harvested from 5-year-old ramets, and propagated in non mist poly-tunnels, which act as propagation chambers. Among the treatments experimented, EGCs accounted for 8.6% and 14.4% of the total variability in rooting and shooting, respectively. The rest were accounted for by rooting media, IBA concentrations and their respective interactions. Results from logistic analysis carried out individually for all EGCs showed no significant interaction effect of IBA and media on the rooting of the 12 EGC hybrids tested. This indicates that EGC hybrids did not require the application of exogenous IBA for rooting. Cuttings without application of IBA rooted with highest rooting observed in clay sub soil (65.0%). The overall effect of propagation media was significant ($p<0.01$) on rooting and shooting percentage, with clay sub soil having the highest mean rooting (59.9%) and shooting percentages (81.9%) compared to sand soil which had the least mean rooting (12.5%) and shooting percentages (23.0%) among different EGCs. Leaf area had a pronounced effect on rooting and shooting percentage of EGC hybrids, with leaf area of 100cm^2 giving the highest rooting (65.9%) and shooting (78.1%). Leaf area of 0cm^2 gave the lowest rooting (7.5%) and shooting percentages (12.7%) and least number of roots and shoots in sand soil. On the basis of these results, EGC hybrids can be successfully propagated in a non mist poly tunnel. Clay sub soil, in combination with a leaf area of 100 cm^2 and exclusion of the use of IBA is recommended in this propagation system. Unlike mist propagation chambers, non mist poly tunnels are a cost effective method of propagating EGC hybrids and the exclusion of IBA as deduced from results of this study will further reduce production costs. In addition, this method uses materials that are readily available and can be used in rural areas which are without electricity and in limited water supply.

Key words: *E. grandis* x *E. camadulensis* hybrids, Indole Butyric Acid, Rooting media, leaf area

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Eucalyptus trees have played an important role in forestry in Kenya since the beginning of formal forestry management in the 1920s and they have proved to be a popular species for farm forestry (Dyson, 1974). Eucalyptus is widely planted in Kenya and by 1996, the species occupied approximately 17000 hectares (ha) of government forests and about 9000ha of private plantation forest (Oballa and Giathi, 1996). The Eucalyptus hybrid clones (EGC) are a cross between *E. grandis* and *E. camadulensis*. They were introduced into Kenya in 1997 through the Tree Biotechnology Project, a partnership project between Forest Department of the Ministry of Environment and Natural Resources, Kenya Forestry Research Institute (KEFRI) and Mondi Forests of South Africa. The hybrid clones are drought tolerant and are particularly beneficial in aforestation of semi arid regions of Kenya. Depending on the rotation period, they are used for the production of: fuel wood, poles, telecommunication posts, fencing posts, electricity posts, pulp and timber. The Eucalyptus clones are preferred over seed propagated Eucalyptus as they exhibit uniform growth hence reduced felling and logging costs.

Vegetative propagation by rooting stem cuttings is a simple and comparatively less expensive method for clonal multiplication of genetically superior trees. Its use is rapidly increasing and presents attractive investment opportunities. Whereas sexual reproduction by seeds provides opportunity for variation, vegetative propagation aims at the production of identical superior trees, with desirable characteristics (Wiesman and Jaenicke, 2002). In addition to this, breeding of tree species for genetic gains takes years or even decades and seed raised plantations of any species is bound to exhibit variations in growth and other characteristics (Thirunavoukkarasu and Gurumurti, 1998).

A major breakthrough was made by transferring clonal propagation technology of genetically superior Eucalyptus hybrid clones and seedlings into Kenya, from South Africa. The aim was to provide superior clonal material to the rural/urban communities in East Africa through the integration of improved forestry biotechnologies into traditional propagation systems. However, the propagation techniques currently used in the clonal nursery at Karura are directly adopted from South Africa. These techniques use synthetic rooting media in mist propagators, raising production costs, making this system unaffordable by the rural poor. It has

therefore been found necessary to develop and optimise vegetative propagation techniques that are cheaper, adaptable to the local Kenyan environment and which can be used in rural areas without electricity and limited water supply.

This study seeks to develop a vegetative protocol for clonal Eucalyptus hybrids, by assessing some of the factors likely to affect their rooting such as leaf area, different auxin concentration and growth medium in the Kenyan environment, in a non mist propagation system.

1.2 Problem statement

There is an acute shortage of wood products in Kenya, with the forest cover standing at approximately 1.7% of closed canopy forest (Diro, 2006). This is far below the global standards of 10% for environmental stability. The Eucalyptus hybrids are fast growing with a plant to polling duration of 4 to 10 years compared to 30 years for indigenous trees and can be used to meet the current demand for wood products which far outstrips the supply.

However, despite various attempts to propagate the fast growing Eucalyptus hybrids, their vegetative propagation has not been optimised. This is because of

inherently poor rooting in some of the clonal varieties (Kanyi, 2005), coupled with the high cost infrastructure used to propagate the Eucalyptus hybrids, making the technology unaffordable by the rural poor. The technology used to propagate the Eucalyptus hybrids adopted from South Africa involves the use of mist chambers. This requires the use of electricity and a reliable source of water. The rooting media used is synthetic further increasing the production costs. A cheaper, simple and farmer friendly technology such as the non mist poly tunnels could be used to make this technology adoptable by the rural poor.

1.3 Justification

There is a need to reduce pressure on the indigenous forests due to their long rotation periods, scarcity of seeds and their over exploitation. This can be achieved by creating alternative sources of wood and other forest products by planting of faster growing trees by rural communities to provide wood for domestic use as well as increase forest cover outside designated forest reserves.

Eucalyptus trees are already accepted in Kenya and their commercial propagation is growing rapidly due to increased demand of wood products. In Kenya, wood supplies 70% of the total energy and is mainly supplied by fast growing tree species on farm (Mutitu *et al*, 2004). Eucalyptus trees from Mondi forest in South

Africa are fast growing and are a commercial source of fuel and other wood products at house hold levels.

The current demand of Eucalyptus hybrids far outstrips the supply in Kenya. This has created a need to simplify and upscale the propagation of Eucalyptus hybrids to meet the current demand for the trees which stands at 80 million seedlings annually. Vegetative propagation in tunnels is a simple farmer friendly method which can upscale the propagation of Eucalyptus species throughout Kenya and considerably reduce the cost of Eucalyptus production.. In order for this to be achieved, understanding the factors that affect rooting of cuttings is paramount.

The purpose of this study is to assess the factors that affect rooting of EGC hybrids, i.e. leaf area, auxin concentration and media in developing a vegetative propagation protocol for the hybrid clones, using a non mist propagation system (tunnels). This will set a guideline on the appropriate leaf cut size, media and auxin concentration to use while propagating EGC in tunnels. With this understanding, rooting success will improve therefore increasing the number of trees available to farmers for planting and eventually increasing the forest cover.

1.3 Objectives of the study

1.3.1 General objectives

The main objective of this study is to develop a cheaper propagation protocol for Eucalyptus hybrid clones in Kenya, using a non mist propagation system.

1.3.2 Specific objectives

i) Determine the IBA concentration for the rooting of Eucalyptus hybrids

ii) Identify the suitable media for the rooting of Eucalyptus hybrids

iii) Determine the most appropriate leaf area for the rooting of Eucalyptus hybrids

iv) Determine whether EGC hybrids are amenable to non mist propagation system

1.4 Hypotheses

i) Auxin concentration (IBA) has an effect on rooting of Eucalyptus hybrids

ii) leaf area has an effect on the rooting of Eucalyptus hybrids clones

iii) Rooting media has an effect on the rooting of Eucalyptus hybrids clones

iv) EGC hybrids are amenable to non mist propagation system

1.5 Significance and anticipated output

The present study will elucidate the factors that affect rooting ability, the most important of which are: leaf area, auxin concentration, and rooting medium. The results of this study will be used to develop a propagation protocol recommending the best leaf area, media and IBA concentration to use while propagating the 12 EGC hybrids in the non mist tunnels, in order to achieve highest rooting success. Understanding of these factors will provide a sound, cost-effective system for commercial clonal propagation that is affordable and adoptable by the rural poor. This will in turn increase the number of trees available for planting, increasing the forest cover in Kenya.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 General review of the genus Eucalyptus

The genus Eucalyptus belongs to the Myrtaceae family and comprises of about 400 species which are native to Australia (Poynton, 1979). All Eucalyptus species bear perfect flowers and each tree blooms serially, leading to self-fertilization. In a South African seed orchard, selfing occurred with a frequency of 10 to 38 percent, and caused 10 recognizable detrimental abnormalities, and depressed the height growth of out planted seedlings 8 to 49 percent compared to crossed progenies (Hodgson, 1976).

Eucalyptus grandis grows 43 to 55 m tall and 122 to 183 cm in diameter (Hall *et al,* 1970). Its form is excellent with tall, straight, clean boles up to two-thirds of the total height. The bark is thin and deciduous. It grows in temperatures ranging from 2 to 10° C and mean maximums near 29° C. Rainfall averages should not be less than 900mm. This species grows best on moist, well-drained, deep, loamy soils of alluvial or volcanic origin. Clay soils are acceptable if they are well drained (Poynton, 1979). *Eucalyptus camadulensis* grows in temperatures ranging from 29 to 35 °C and rainfall 450-900 mm per year (Jacobs, 1976). The tree grows best in alluvial, silty soils of good depth, but can also grow in sands overlaying

clay moist sub soil. It grows to a height of between 24-37m and a diameter of 90 cm. When it occurs in dense stands, its bole maybe free of branches to a fair height. Its timber is termite resistant (Willis, 1972).

The *Eucalyptus grandis* and *camadulensis* were crossed to produce the Eucalyptus *grandis* x *camadulensis* hybrids. This was done by stimulating the two varieties to be crossed to flower simultaneously followed by artificial crossing. Combining the drought tolerance of *E. camadulensis*, and the excellent tall, straight, clean boles of *E. grandis*, through artificial pollination, produces a hybrid tree with all of these desirable characteristics.

2.2 Importance of vegetative propagation of Eucalyptus hybrids

Some potential advantages (Libby, 1985) of clonal forestry include: -the ability to rapidly capture a greater proportion of the additive and non-additive genetic variation that can be achieved by breeding; the mass production of those rare individuals which have two or more favourable characteristics which are usually negatively correlated; the ability to select and utilize greater genetic diversity than is normally found in a single progeny; the ability to use clones that are well adapted to a particular site ; the greater simplicity and flexibility of managing sets of stock plants than in seed orchards; the shorter period, compared to seed orchards, between selection and production; the increasing superiority of clones

passing through multiple-trait selection programmes; and the ability to use mature tissues.

2.3 Vegetative propagation of tropical trees

Following research to understand the factors affecting rooting ability in tropical hardwoods, vegetative propagations techniques are now available that are applicable to almost all species (Leaky 1991). According to Wachira (1997), Chaperon was the first to achieve mass propagation by cuttings on a semi-industrial scale in 1978. A study was carried out in Kenya (Indieka, 2007) with the objective of testing three vegetative propagation techniques on tropical tree species: Macro propagation, direct *in vitro* shoot multiplication and direct somatic embryogenesis on *Melia volkensii.* The effect of fungicide pretreatment, surface sterilisation and plant growth regulators Indole acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) at 0, 2, 4, 6, 8, and 10g l^{-1} on leafy cuttings in a low cost mist propagator were evaluated. The results indicated that *M. volkesii* is amenable to propagation by rejuvenated leafy stem cuttings.

In Cameroon, (Tchoundjeu *et al*, 2002) studies were conducted to assess the effects of rooting medium (sawdust, sand and a 50:50 mixture of sand and sawdust), auxin concentration (0, 50, 100, 150, and 200 µg IBA), and leaf area (0, 5, 10, 20, and 25 cm^2) on rooting success of juvenile cuttings of *P. africana.* It was

concluded that *P. africana* is amenable to vegetative propagation. In another experiment, provenance variation in rooting ability was examined in *Racosperma* spp (Accacia) using leafy stem cuttings of *R. auriculiforme* and *R. mangium* taken from 5-month-old ortets (Khasa *et al* 1995). The cuttings were set in a 1:1 peat moss and vermiculite mixture and were rooted in a propagator. The application of 0.8% (IBA) hormone significantly increased ($P < 0.05$) number of roots per cutting, root length, root dry weight, and rooting percentage, but marginally decreased number of shoots

These experiments have intensively investigated the effects of leaf size, auxin concentrations and media, amongst other parameters, on the rooting of various tree species, and have been largely successful. However, there still remains a great need to develop vegetative propagation protocols for EGC hybrids in Kenya, and to make this information and methods more widely available to farmers, scientists and partners involved in EGC propagation.

2.4 Vegetative propagation of Eucalyptus hybrids

Good rooting requires a propagation environment that minimizes physiological stresses. For survival, the cuttings must be able to maintain water balance by replenishing the water lost in transpiration. This can be achieved by use of a mist Chamber (Plate 1) where moisture is provided by an intermittent mist-like spray (Murray and Thompson, 1988; Sharma and Gurumurti, 1986). Another system is

the use of poly-tunnels made of rigid covering such as fiber glass, polycarbonate or acrylic sheets as propagation chambers (Tinus and McDonald, 1979). Such tunnels, when placed on the moist soil with the rooting materials inside, create a very high humid environment inside the tunnel through condensation processes, which helps in keeping the cuttings in moist condition (Plate 2). The poly-tunnels are inexpensive, simple systems highly appropriate for areas without reliable sources of piped water or electricity (Murray and Thompson, 1988).

Plate 1: Mist chamber

Misting of cuttings in progress

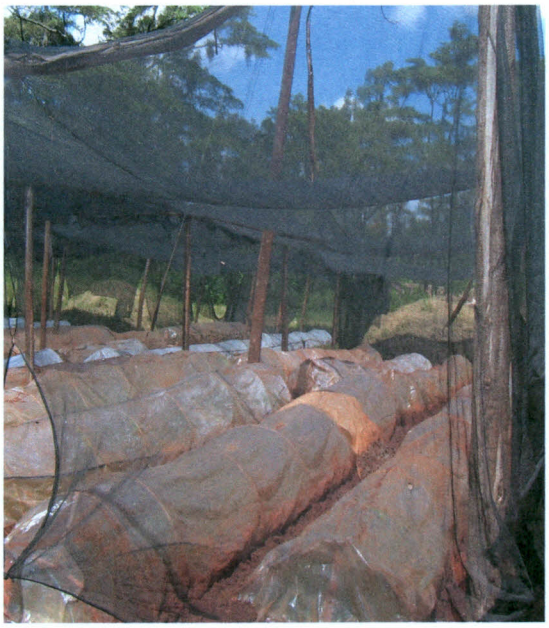

Plate 2: Poly tunnels

Non mist propagation system

The requirement of auxins to induce rooting in tree species is already known (Nanda *et al.*, 1968). Rooting is enhanced by an auxin application of about 40µg of IBA per cutting and a leaf area of about 50 cm^2. The latter optimises the balance

between photosynthesis and transpiration (Leakey *et al.*, 1982). The most commonly used rooting stimulators are IAA and IBA. NAA is used for root induction, but the compound is more toxic than IAA and IBA. IAA is unstable to both heat and light. This leaves IBA as the preferred auxin for clonal propagation of trees. The principal role of IBA is to favour conjugation between endogenous IAA and amino acids which leads to the synthesis of the specific protein necessary for the formation of root initials (Ryugo and Breen, 1974).

Generally, cuttings should be taken from healthy mother plants free from water, nutrient or other stresses. Cleanliness should be maintained in the working and growing environment (Warrick, 2003).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Description of the study area

The study was conducted in the Tree Biotechnology Program nursery located at the Kenya Forest Service headquarters along Kiambu road, in Karura forest, Kiambu District with a longitude of 36^{0}6W and latitude of 36^{0}6E (Survey of Kenya, 1999). The area is characterised by reliable, bimodal rainfall and the soils are rich, red clay, classified Humic Nitisols (Woomer *et al.,* 1998).

The Tree Biotechnology Program nursery is involved in the propagation and distribution of Eucalyptus hybrids from South Africa to all parts of Kenya on a commercial basis.

3.2 Propagation system and propagation of cutting

3.2.1 Sources of explants

The vegetative propagules (ex-plants) were obtained from 5-year-old mother plants (Plate 3). The EGC hybrids, which comprise of 12 varieties (GC 3, GC 15, GC 10, GC 14, GC 15, GC 167, GC 522, GC 581, GC 584, GC 642, GC 796 and GC 785) were obtained from South Africa as rooted cuttings and were planted as

young plantlets (ramets).

Plate 3 : Mother plants of variety GC 167

3.2.2 Construction of the tunnels

The cuttings were planted in tunnels as in the guidelines set in the Tree Growers Handbook (Tea Research Foundation of Kenya, 1986). The beds for the tunnel were marked out measuring 1m x 4m. A wooden frame was constructed around the measured out perimeter. This frame is the area in which the potted pots were arranged. Hoops to support the polythene sheeting were placed every 1m along the bed. The hoops were made of sticks collected from *Lantana camara* as they were readily available in the nursery. They were slightly curved so that rainwater easily run off the surface of the polythene. The hoops were placed 30cm above the top of the potted pots.

Four holes were dug at the Four Corners of the bed, 1m away from the perimeter of the bed. Posts were put into the 4 holes, 4m above the ground. Wooden frames were then constructed across and along the four posts. They acted as a support frame for the 50% black shade net, which was high enough for someone to walk around. The shade net covered the top and sides of the bed. Once the cuttings were planted into the pots, the tunnel was sealed by covering the sides of the polyethene sheet that is stretched across the *Lantana camara* hoops with soil.

3.2.3 Preparation of cuttings

Coppices were harvested from the mother plants described in 3.2.1 when they attained maturity using secateurs. Maturity was determined by checking for a color change of the coppices from green to red. The harvested coppices were collected in a bucket half filled with water to minimise transpiration losses. The harvested coppices were taken to an enclosed area sheltered from wind and sun, where they were cut into cuttings using scissors. In general, only one cutting was made from each shoot stem, taking care to avoid shoots with branches as well as stems, which were either too hard or too soft. This was achieved by making cuttings from the middle portion of the shoots.

3.2.4 Monitoring

Once planted in the respective media, cuttings the cuttings were closely monitored through daily routine checks, for incidence of drying of cuttings or fungal infection. It was also carried out to check for the ideal heavy condensation on the inner surface of the polythene sheet that cuts out a clear view of the cuttings inside. This is an indication that there is sufficient amount of water in the media. The tunnel was opened for watering and spraying with fungicides once after every two weeks. The fungicide was changed every time the tunnel is opened. The fungicides used were benlate, milraz and thiovit. Humidity and temperature readings of the tunnel were recorded three times a day.

3.2.5 Experimental design

The experimental design used in this experiment was the Randomised Complete Block Split Plot Design. In this study, the whole units were the EGC hybrids, the sub units were media, leaf surface area and IBA concentration. This is further explained in section 3.3 under the propagation experiments.

The split plot experimental design was chosen as it allows for experiments with a factor requiring relatively large amounts of experimental material (whole units) along with a factor requiring relatively little experimental material (sub -unit) in

the same experiment as was the case in this study. This helped in reducing costs of setting up the experiment.

3.3 Propagation experiments

3.3.1 Assessing the effect of different rooting media and IBA concentrations on the rooting of different EGC hybrids.

The harvested coppices were made into cuttings. The leaves were trimmed to an area of 50 cm^2 using a template cut from graph paper. Each cutting had a pair of leaves and one bud and had a mean length of 8 cm and a mean diameter of 2.5-4mm. Cuttings were left in water with 0.5 g/l of Benlate fungicide for a few minutes, removed, followed by dipping the base of the cutting in a talc powder containing IBA before being planted into pots.

Four concentrations of IBA were tested in this experiment: 0%, 0.6%, 0.8%, and 1% weight by weight. The 2 cm bottom of the stem base of each cutting was treated with 0.02g of the different mentioned auxin concentrations. The cuttings were then inserted in specific rooting media in potted tubes measuring 6" long and 3 1/4'' in diameter, in the tunnel. Five rooting media comprising of a mixture of sand (S) and clay subsoil (CS), mixed in the following ratios 1:0 (SS), 1:1 (SCS), 1:2 (S2CS), 0:1 (CS) and 2:1 (2SCS) respectively by volume were evaluated. 20

cuttings were tested per IBA concentration and media. Table 3.1 illustrates the number of cuttings per media and IBA concentration.

Table 3.1: Number of cuttings per media and IBA concentration

Media	IBA Concentration				Total
	0.0%	0.6%	0.8%	1.0%	
SCS	20	20	20	20	80
SS	20	20	20	20	80
S2CS	20	20	20	20	80
2SCS	20	20	20	20	80
CS	20	20	20	20	80
Total	100	100	100	100	400

Key:

Clay sub soil (CS) and sand soil (SS) mixed in the following ratios: 1:0 (SS), 1:1 (SCS), 1:2 (S2CS), 0:1 (CS) and 2:1 (2SCS)

This structure was repeated for the 12 EGCs and replicated three times. At IBA level, each concentration had a total of 80 explants per replicate, for the three replicates. The experiment was laid in the constructed tunnels each constituting a replicate whose overall structure was 12 varieties x 5 levels rooting media x 4

levels of IBA concentrations x 20 cuttings per IBA concentration (12x5x4x20) x3. A total of 14,400 explants were used.

The experiment was set in a split- split plot design with each treatment replicated three times. EGC hybrids was the main treatment, rooting media sub treatment nested within the EGCs and IBA concentration was the sub -sub treatment nested within the rooting media. Figure 3.1 illustrates an excerpt of the split-split plot design used in this experiment.

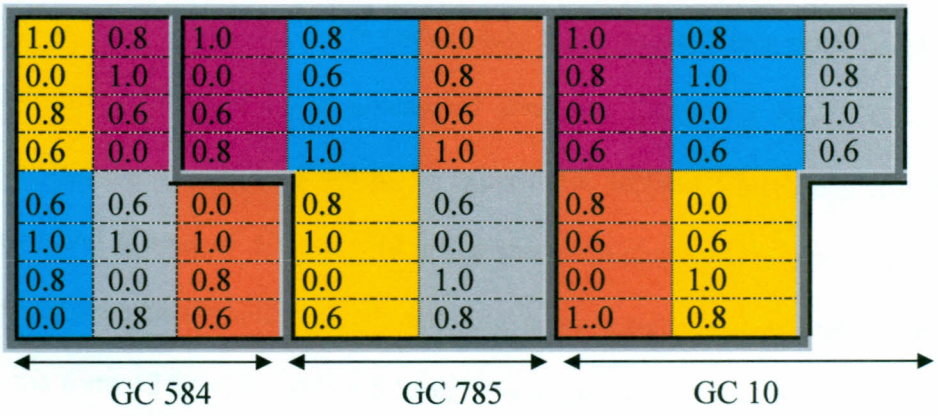

Figure 3.1: Illustration of split-split plot design showing different rooting media and IBA concentrations per Eucalyptus hybrid variety

Key: Rooting media used:

1:0=sand soil alone (SS)
1:1=sand soil and clay sub soil(SCS)
1:2= sand soil and clay sub soil(S2CS)
0:1=clay sub soil (CS)
2:1= sand soil and clay sub soil(2SCS)

GC 584, GC 785 and GC 10 = three of the 12 Eucalyptus hybrid varieties tested in this study. IBA concentrations: 0=0%, 0.6=0.6%, 0.8=0.8%, 1.0=1.0% (W/W).

3.3.2 Assessing the effect of different rooting media and leaf surface area on the rooting of EGC hybrids

Five leaf areas: 0, 30, 40, 50, 60, 80 and 100cm^2 were assessed. The leaf areas were trimmed to the respective size using templates cut from a graph paper. Each cutting had a pair of leaves and one bud and had a mean length of 8 cm and a mean diameter of 2.5-4mm. Cuttings were left in water with 0.5 g/l of Benlate fungicide for a few minutes, removed, followed by dipping the base of the cutting in a talc powder containing 0.8% IBA before being planted into pots. A total of 700 cuttings were used for a given leaf area and media for each EGC. The different rooting media used are as in experiment section 3.3.1. Each leaf area treatment had a total of 60 explants in the three replicates. The experiment was laid in constructed tunnels each constituting a replicate whose overall structure was in the form 12 EGC hybrids x 5 levels rooting media x 7 levels of leaf surface area and 20 cuttings per leaf area and media (12x5x7x20) x3. A total of 25,200 explants were used in setting up this experiment as illustrated in table 3.2.

Table 3.2: Number of cuttings per media and leaf area

Leaf Area (cm^2)	Media					Total
	SCS	SS	S2CS	2SCS	CS	
0	20	20	20	20	20	100
30	20	20	20	20	20	100
40	20	20	20	20	20	100
50	20	20	20	20	20	100
60	20	20	20	20	20	100
80	20	20	20	20	20	100
100	20	20	20	20	20	100
TOTAL	140	140	140	140	140	700

Key:

Clay sub soil (CS) and sand soil (SS) mixed in the following ratios: 1:0 (SS), 1:1 (SCS), 1:2 (S2CS), 0:1 (CS) and 2:1 (2SCS). Leaf area of $0cm^2$ means all the leaves of the cuttings were cut off.

This structure was repeated for the 12 EGCs and replicated three times. This experiment was also set in a split- split plot design with each treatment replicated three times. EGC hybrids was the main treatment, rooting media sub treatment nested within the EGC hybrids and leaf surface area was the sub -sub treatment nested within the rooting media as illustrated in Figure 3.2.

100	100	30	80	0	0	80	100	30	100
50	30	60	0	30	50	100	80	100	0
30	80	80	60	50	60	50	40	80	80
80	40	0	50	100	40	30	0	50	30
60	60	50	30	80	80	0	60	60	40
0	50	40	40	60	30	40	50	0	50
40	0	100	100	40	100	60	30	40	60

GC 3 GC 796

Figure 3.2: Split-split plot design showing different rooting media and leaf surface areas per Eucalyptus hybrid variety

Key:

Rooting media combinations

1:0=sand soil alone (SS)
1:1=sand soil and clay sub soil(SCS)
1:2= sand soil and clay sub soil(S2CS)
0:1=clay sub soil (CS)
2:1= sand soil and clay sub soil(2SCS)

GC 3 and GC 796 are two out of the 12 Eucalyptus hybrid clones tested in this study.

3.3.3 Assessing the effect of different leaf surface area and IBA concentrations on the rooting of EGC hybrids

The different leaf surface areas and IBA concentrations are as described in section 3.3.1 and 3.3.2. In this case, the media used was clay sub soil. Consequently, this experiment was also set in a split- split plot design with each treatment replicated three times. EGCs were the main treatment; leaf surface area sub treatment nested within the EGCs and IBA concentrations were the sub -sub treatment nested

within the leaf surface area. Figure 3.3 is an excerpt of the split-split plot design used in this experiment.

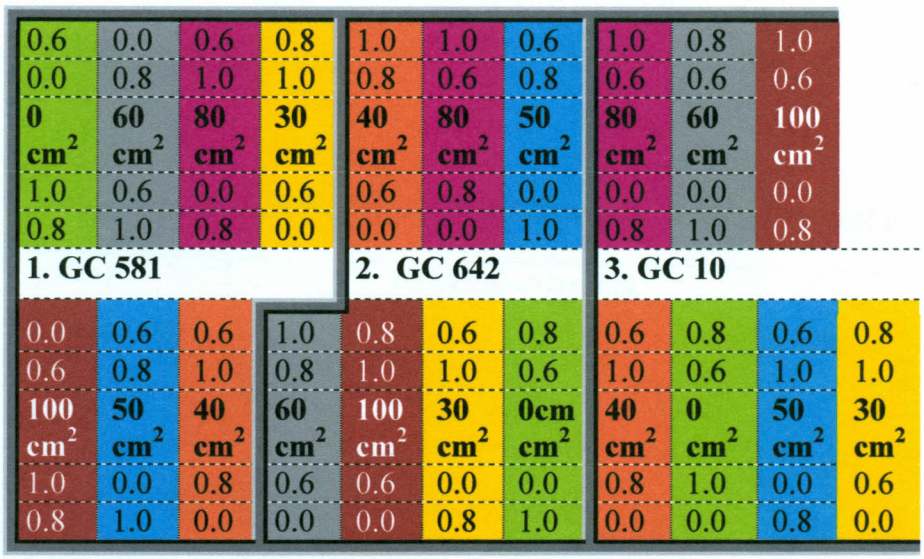

Figure 3.3: Split-split plot design showing different leaf surface areas and IBA concentrations per Eucalyptus hybrid variety

Key:

Leaf area

At IBA level, each concentration had a total of 80 explants in the three replicates. All explants used were rooted in clay sub soil. The experiment was laid in constructed tunnels each constituting a replicate whose overall structure was in the form 12 EGC hybrids x 7 levels of leaf surface area x 4 levels of IBA

concentrations x 20 cuttings per leaf area and IBA concentration (12x7x3x20) x3 replicates. A total of 20,160 explants were used in setting up this experiment as illustrated in Table 3.3.

Table 3.3: Number of cuttings per IBA concentration and leaf area

Leaf Area (cm^2)	**IBA concentration**				**Total**
	0.0%	**0.6%**	**0.8%**	**1.0%**	
0*	20	20	20	20	80
30	20	20	20	20	80
40	20	20	20	20	80
50	20	20	20	20	80
60	20	20	20	20	80
80	20	20	20	20	80
100	20	20	20	20	80
TOTAL	140	140	140	140	700

*Treatments with 0 cm^2 had all the leaves cut off

The structure in table 3 was repeated for the 12 EGCs and replicated three times. Overall, 59,760 explants were used in the three experiments.

3.4 Description of Data and methods of analysis

3.4.1 Measurements

In the three experiments, presence or absence of rooting/shooting was assessed after the first seven days and continued at an interval of one week (7 days) till the 6th week. Where rooting had occurred the number of cuttings rooted and their number of roots and lengths was recorded. Any rooting was visible as the media was potted in clear polyethene tubes. Where rooting had occurred, the cutting was carefully removed from the polyethene tube, the media gently removed from the roots. The number of roots and their lengths were recorded. The number of cuttings shooted, the number of emerging shoots and their lengths were also recorded. The uprooted cuttings were then replanted. Temperature and humidity readings were taken three times a day.

3.4.2 Data analysis

Descriptive data analysis based on cumulative rooting percentage of EGCs shoot cuttings was calculated for each rooting media, IBA, leaf surface area to identify the pattern of rooting as influenced by each factor used in the experiments. Homogeneity of variance for cumulative rooting percentage was evaluated using a Barleit test (Snedecor and Cochran, 1980) where the data was found to be

normally distributed, hence no further transformation was done. Pearson correlation analyses were performed to assess the interrelation of the factors that potentially affect the rooting ability of the cuttings. Comparisons of each rooting media, IBA, and leaf surface area were carried out. A prior and orthogonal contrast was used to compare difference in rooting among the potential factors that would be associated with rooting ability (Steel and Torrie, 1980). Significance levels of ($p< 0.01$ and $p <0.05$) were used for both main effects and interactions.

Overall, the experiments set generated data that was classified as success or failure to root under different factors. This was classified as binary data hence further inferential statistical analyses on rooting under each factors was carried out using logistic regression model.

3.4.3 Development of Logistic regression model

To assess and predict the extend at which each rooting media, IBA, leaf area and number of buds contributed to the rooting ability of different EGC hybrids cuttings, logistic analysis was employed as in Appendix I. This model overcomes most of the problems associated with linear probability models and provides parameter estimates that are symptomatically consistent and computationally easier to use (Pindyck and Rubinfield, 1981).

MS Excel for windows 2003 together with Genstat were used in data management. All analyses were performed using Genstat release 8.11 statistical software (Genstat, 2005) employing the General Linear model (GLM) procedures.

CHAPTER FOUR

4.0 RESULTS

Mean relative humidity inside the propagators differed only slightly between the three experiments. In experiment 3.2.1 temperature inside the propagator ranged between 9-30 ^{0}C with an average of 25.6^{0}C. Humidity readings ranged from 80-97% with an average of 93.5%. Media temperature ranged from 18-35^{0}C with an average of 31.9 ^{0}C. In experiment 3.2.2 temperature inside the propagator ranged between 9-31 ^{0}C with an average of 23.2^{0}C. Humidity readings ranged from 80-96% with an average of 84.4%. Media temperature ranged from 18-33^{0}C with an average of 30.2 ^{0}C. In experiment 3.2.3 temperature inside the propagator ranged between 9-29 ^{0}c with an average of 27.5^{0}C. Humidity readings ranged from 81-97% with an average of 88.0%. Media temperature ranged from 17-34^{0}c with an average of 31.4 ^{0}C.

4.1 Effect of different rooting media and indole butyric acid (IBA) concentrations on the rooting of different EGC hybrids

EGCs hybrids had very minimal rooting in different rooting media and IBA concentrations after 7 days of the experiment. Results from further assessment of rooting after every after seven days up to the 40th day when the experiment was terminated showed that there was increased rooting after 14 days, which gradually increased minimally till the termination period (Figure 4.1).

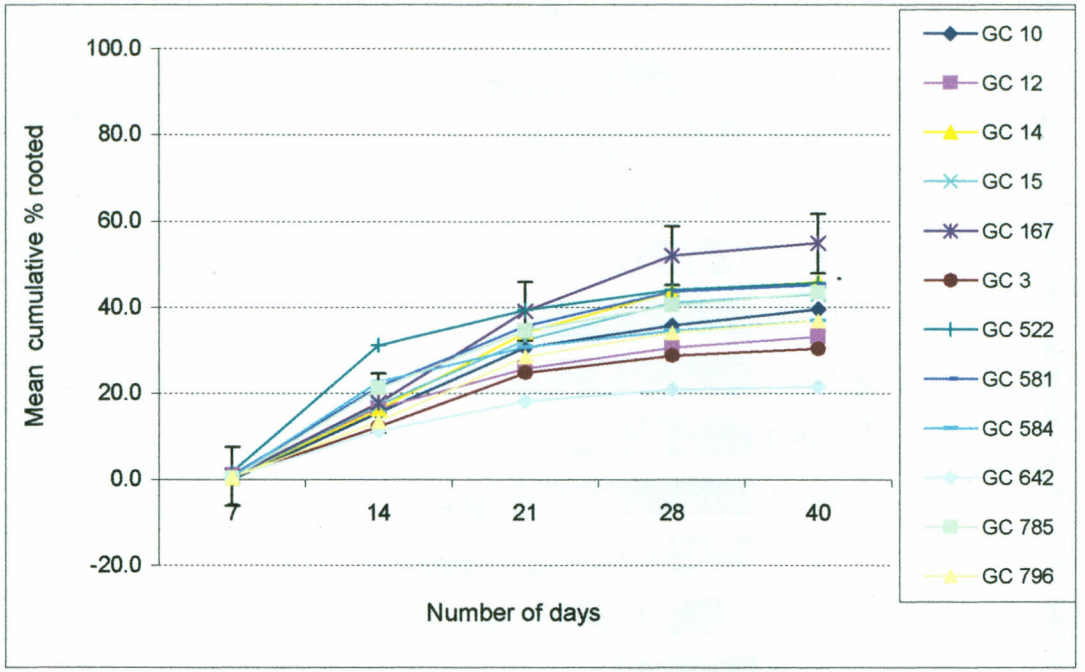

Figure 4.1: Trend of mean cumulative rooting percentage of EGCs under different rooting media and IBA levels.

Statistical analyses at the 40th day, showed a highly significant difference ($p<0.01$, ANOVA) in rooting and shooting among different EGCs under different rooting media and IBA concentrations, where GC 167 had the highest mean rooting percentage (54.8%) followed by GC 14 (45.8%), and GC 642 having the least (21.5%) mean rooting percentage. GC 15 had the highest shooting percentage (78.1%) followed by GC 584 (73.8%) and GC 3 had the least shooting percentage (43.3%) (Figure 4.2).

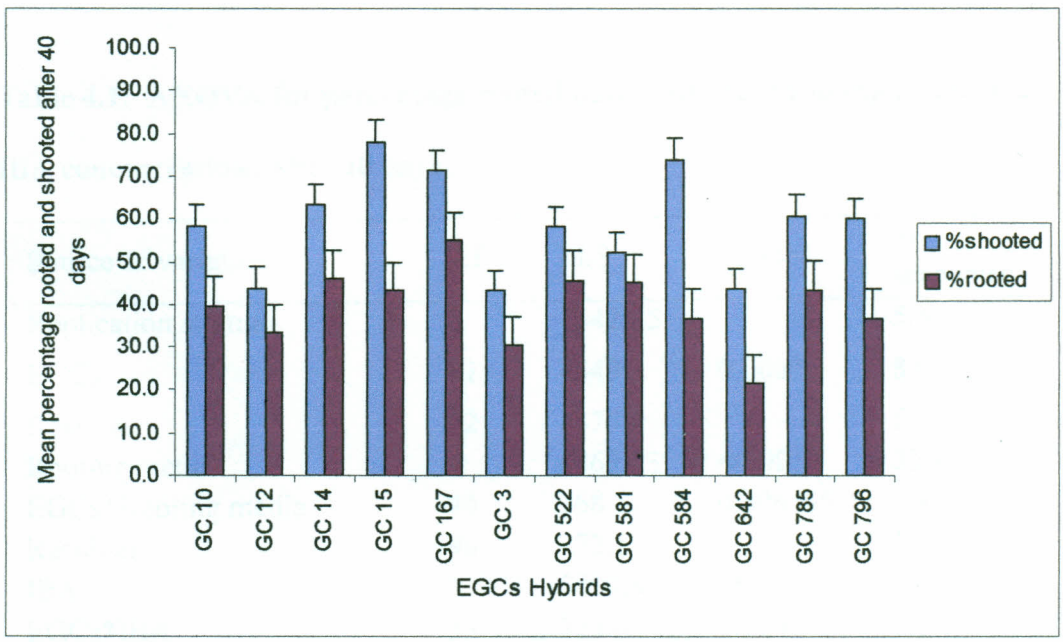

Figure 4.2: Overall mean cumulative rooting and shooting percentage of different EGCs under different rooting media and IBA concentrations after 40 days.

Among the treatments experimented, EGCs accounted for 8.6% and 14.4% of the total variability in rooting and shooting, respectively (Table 4.1 and 4.2). The rest were accounted for by rooting media, IBA concentrations and their respective interactions which were highly significant ($p<0.01$). Interaction effect between EGCs and rooting media, EGCs and IBA concentrations as well as rooting media and IBA concentrations were not significant ($p<0.01$).

Table 4.1: ANOVA for percentage rooted under different rooting media and IBA concentrations after 40 days

Source of variation	d.f	M.S	F.prob.	% accounted
Replication stratum	2	15425.5		5.3
EGCs	11	4549.2	0.008*	8.6
Residual	22	1375.9		5.2
Rooting media	4	48697.3	<0.001*	33.4
EGCs*Rooting media	44	768	0.496 NS	5.8
Residual	96	772.1		12.7
IBA	3	5019.5	<0.001*	2.6
EGCs*IBA	33	320.6	0.103 NS	1.8
Rooting media*IBA	12	197	0.624 NS	0.4
EGCs*rooting media*IBA	132	418.8	<0.001*	9.5
Residual	360	238.6		14.7
Total	719			100.0

* Significant at $p<0.01$ and NS not significant at 5%

Table 4.2: ANOVA for percentage shooting under different rooting media and IBA concentrations after 40 days

Source of variation	d.f	M.S	F.prob.	% accounted
Replication stratum	2	914.5		0.3
EGCs	11	8301.1	<0.001*	14.4
Residual	22	697.8	<0.001*	2.4
Rooting media	4	73193.2	<0.001*	46.1
EGCs*Rooting media	44	1525.7	<0.001*	10.6
Residual	96	291.6		4.4
IBA concentrations	3	1216.1	<0.001*	0.6
EGCs*IBA	33	748.2	<0.001*	3.9
Rooting media*IBA	12	488.3	<0.001*	0.9
EGCs*Rooting media*IBA	132	441.3	<0.001*	9.2
Residual	360	129.5		7.3
Total	719			100.0

Significant at $p<0.01$

This implied that rooting media and IBA concentrations had different effect on rooting and shooting ability of EGCs as shown in Figure 4.3 where sand soil had

the least mean rooting among different EGCs as compared to clay sub soil that had the highest mean rooting percentages.

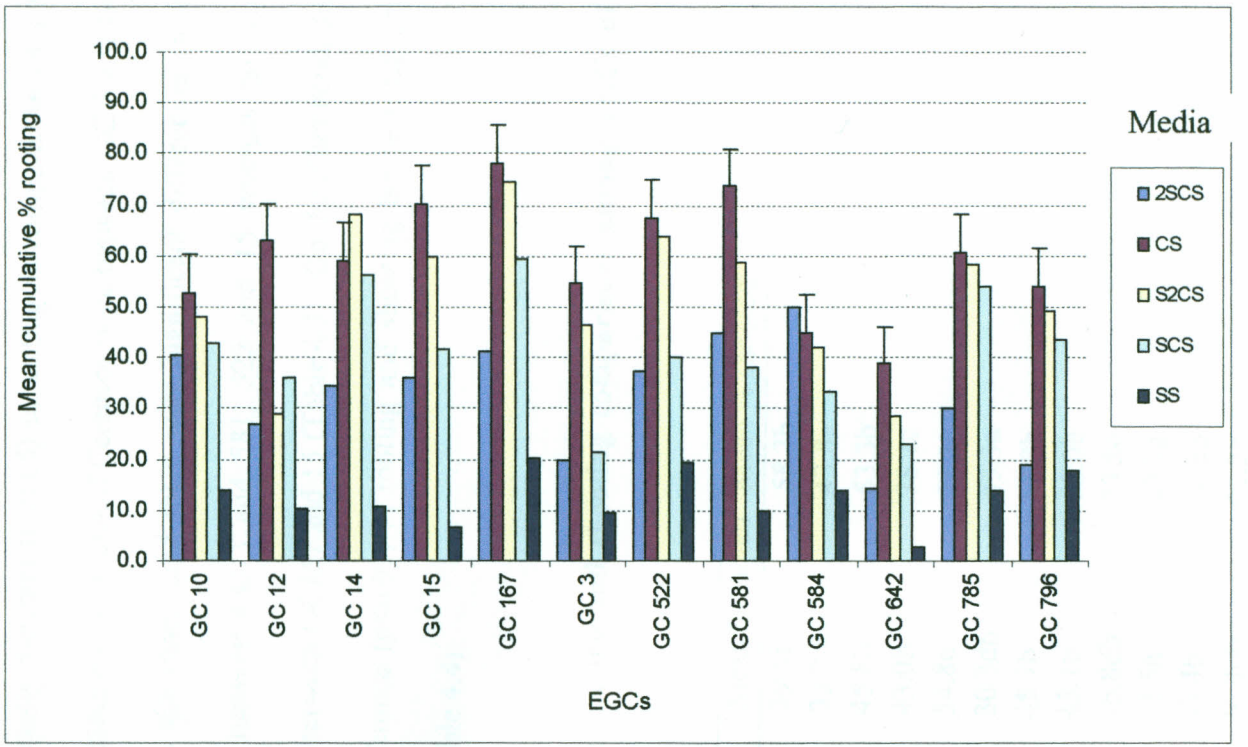

Figure 4.3: Mean cumulative rooting ability for EGCs at different levels of rooting media after 40 days

Orthogonal comparisons on the basis of (S.e.d) among EGCs mean rooting percentages under different rooting media and IBA concentrations showed that GC 642 was significantly different ($p<0.05$) in rooting as compared to GCs 10, 581, 167 and 584 whereas no significant differences were between GC 3 and GCs 581, 522 and 15. On the other hand there were significant differences ($p<0.05$) in shooting ability between GC 3 and 581, 522 and 15 whereas no significant differences were between GC167 and 15 (Table 4.3). On the other hand, there was a significant difference ($p<0.01$) in rooting and shooting between CS and SCS, 2SCS and SS (Table 4.4).

Table 4.3: Mean rooting and shooting comparisons among EGCs after 40 days

EGCs	Mean % rooted	Mean % shooted
GC 10	39.6b	58.3b
GC 12	33.2db	43.8a
GC 14	45.8b	63.3b
GC 15	43.0b	78.1c
GC 167	54.8c	71.3c
GC 3	30.3db	43.3a
GC 522	45.7b	58.3b
GC 581	45.1b	52.0b
GC 584	36.8db	73.8c
GC 642	21.5a	43.7a
GC 785	43.4b	60.8b
GC 796	36.8db	60.2b
S.e.d	6.772	4.823

Key: a, b, c , db = Values with the same letters are not significantly different ($p<0.05$).

Table 4.4: Overall mean rooting and shooting percentages of rooting media after 40 days

Rooting media	% rooted	% shooted
CS	59.9c	81.9d
S2CS	52.3c	69.8c
SCS	40.8b	66.8c
2SCS	33.0b	53.0b
SS	12.5a	23.0a
S.e.d	3.275	2.012

.**Key**: a, b, c,d = Values with the same letter are not significantly different ($p<0.05$). Clay sub soil (CS) and sand soil (SS) mixed in the following ratios: 1:0 (SS), 1:1 (SCS), 1:2 (S2CS), 0:1 (CS) and 2:1 (2SCS).

This showed that effect of rooting media was consistent in rooting and shooting among different EGCs and IBA concentrations as illustrated by figure 4.4.

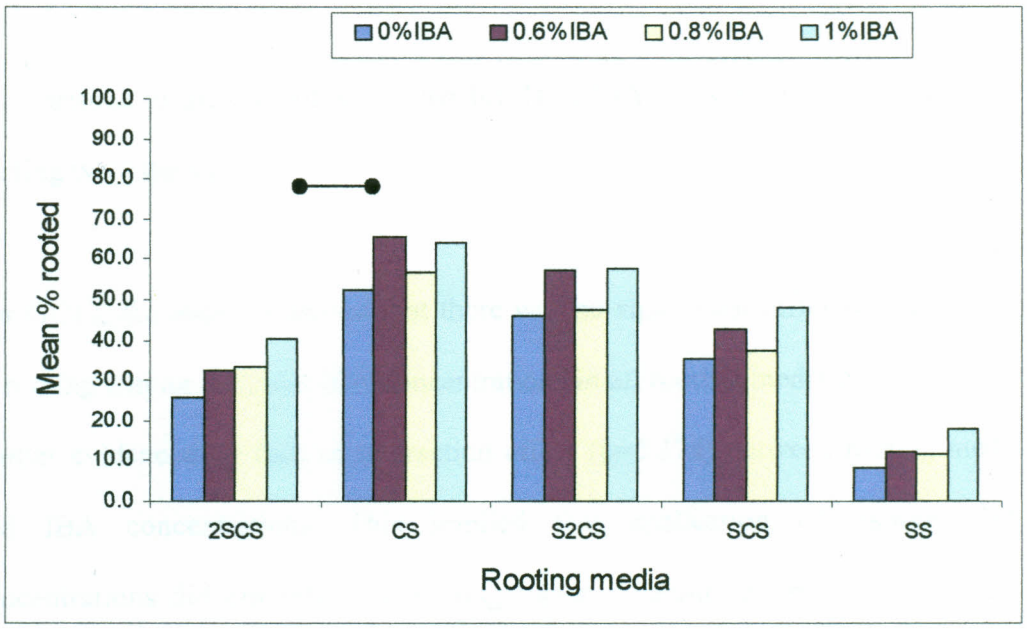

Figure 4.4: Mean rooting percentage under different IBA concentrations and rooting media after 40 days

Clay sub soil had the highest rooting percentage in all different IBA concentrations, while SS had the lowest rooting percentage in all IBA concentrations tested.

Examining the effect of rooting media and IBA concentrations on each EGCs using logistic regression modeling on the basis of number of cuttings rooted, the results showed that for GC 10, under clay sub soil, there was no significant effect of IBA concentrations, even though the chances of the cuttings rooting under 1% of IBA were about 3.6 times higher than those of the control (0%) IBA. The

comparisons of the control with other levels of IBA showed that their chances of rooting were the same.

For GC12, the results revealed that there was no significant difference ($p=0.936$) in rooting among different IBA concentrations in all rooting media used. This was further evidenced by lack of interaction effect ($p=0.478$) between rooting media and IBA concentrations. This implied that application of various IBA concentrations did not influence rooting under different rooting media. On the other hand the model revealed that there was a significant difference ($p<0.01$) in rooting under different rooting media where chances of cutting rooting under clay sub soil (CS) were about 27.8 times higher than 2SCS (Table 4.5).

Table 4.5: Logistic regression results on rooting of GC12 under different rooting media and IBA

Parameter	s.e of the estimate	t.prob	Odds ratio
Constant	1.18	0.049	
CS	1.38	0.021	27.8
S2CS	1.39	0.325	4.0
SCS	1.35	0.149	7.3
SS	2.17	0.657	0.38
0.6%	1.49	0.659	1.9
0.8%	1.36	0.164	6.9
1.0%	1.35	0.101	9.6

Reference levels: Media, 2SCS, IBA 0%

This high level of significance showed that GC12 roots better under CS in any IBA concentrations, even though 1% IBA showed higher rooting than other IBA concentrations tested (figure 4.5).

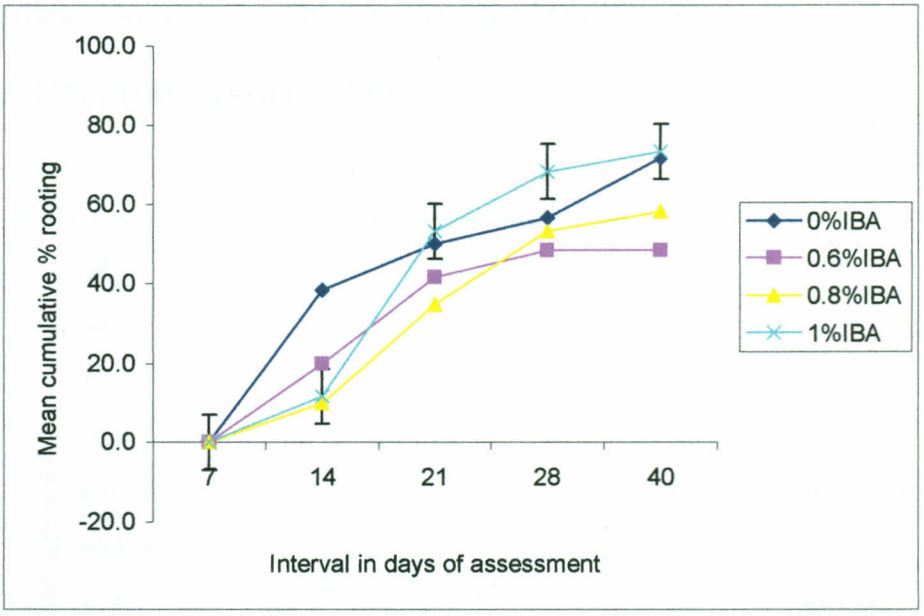

Figure 4.5: Trend of % rooting for GC12 under CS under different IBA concentrations

One percent (1%) IBA gave the highest rooting percentage, while 0% had the lowest rooting percentage. Results showed that for GC 14, there was no significant difference ($p>0.05$) in rooting between 2SCS and SS, SCS, CS and S2CS. Similarly, there was no significant difference among IBA concentrations in rooting under different rooting media. This implied that GC14 can root equally in all the rooting media tested and IBA concentrations. On the other hand, the chances of GC14 shooting under CS were about 62 times higher than in 2SCS implying that CS played a significant role in influencing shooting of the cuttings. Equally, shooting was about 6, 5 and 4.9% times higher in 0.6%, 0.8% and 1% IBA

concebtration, respectively. This implied that IBA to some extent influenced shooting of GC14 cuttings (figure 4.6).

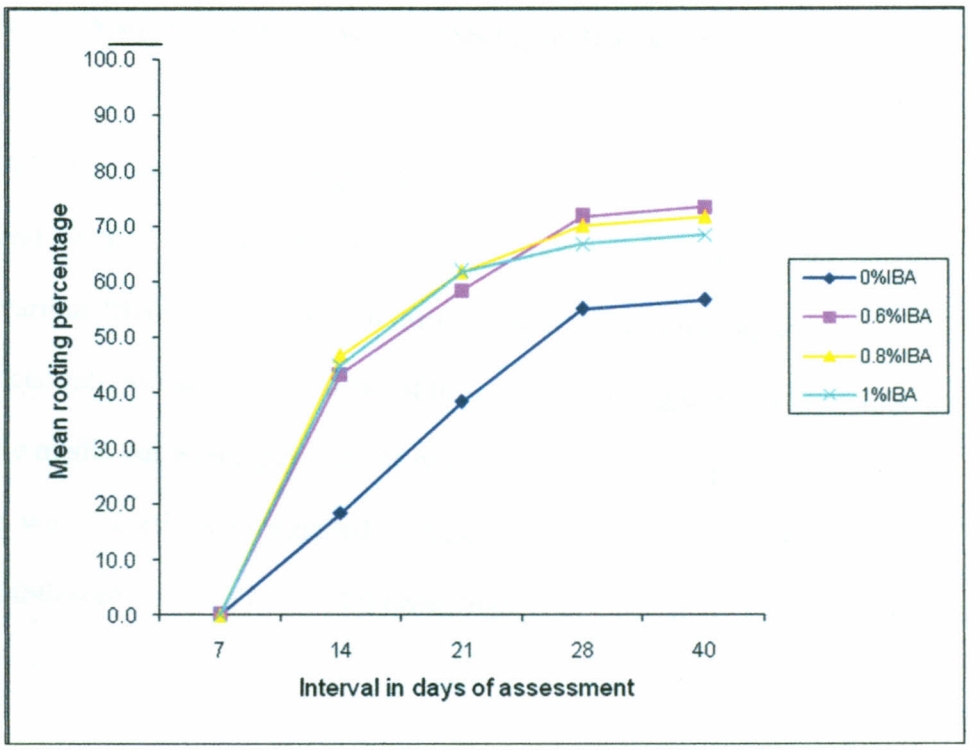

Figure 4.6: Trend of % shooting for GC14 under CS with different IBA concentrations

In GC 15, there was no significant difference ($p>0.05$) in rooting among different rooting media and IBA concentrations. The mean number of roots varied from 12.7 to 17 as compared to the mean number of shoots which varied from 27-34. On the other hand there was a significant difference ($p<0.05$) in shooting between

On the other hand there was a significant difference ($p<0.05$) in shooting between 2SCS and CS, S2CS and SCS where the chances of cuttings shooting under CS, S2CS and SCS were 13.4, 13.4 and 8.8 times higher than in 2SCS.

In GC167, there were also no significant differences ($p>0.05$) in rooting between 2SCS and SCS, CS, S2CS Equally, no significant differences were obtained among various IBA concentrations in influencing rooting, implying that any of the IBA concentrations had equal chances of influencing rooting of GC 167 cuttings in any of the media but would perform fairly better in S2CS. This was also shown in shooting where cuttings propagated in S2CS had 7.3 times higher chances of shooting followed by those in CS (5.0 times higher) as compared to cuttings under 2SCS.

There was a significant difference ($p<0.05$) in rooting of GC 3 cuttings under CS as compared to 2SCS where the chances of the cuttings rooting were 10.6 times higher as compared to 2SCS. Also, there was no significant difference ($p>0.05$) in rooting between the different IBA concentrations. In addition, the chances of cuttings shooting under S2CS and CS were about 12.2 and 7.2 times higher than in 2SCS, respectively. This still stressed a significant role of rooting media in rooting and shooting of the cuttings.

In GC 522, the chances of rooting and shooting under CS and S2CS were about 3.6 and 3.4 times higher as compared to 2SCS, respectively. This showed that the addition of sand to clay sub soil did not significantly influence or increase the chances of the cuttings rooting. On the other hand the application of IBA to GC 522 cuttings did not significantly ($p>0.05$) in influence rooting. This was also the case in the interaction effect implying that rooting of the cuttings in either rooting media was independent from one IBA level to the other.

For GC 581, there was no interaction effect ($p=0.890$) between rooting media and IBA. Similarly, there was no significant difference ($p=0.124$) in rooting and shooting among the IBA treatments but there was high significant difference ($p<0.01$) between the rooting media where the chances of the cuttings rooting under CS, S2CS were 5.1 and 2.6 times higher than in 2SCS, respectively. Sand soil showed dismal performance in rooting across all the EGCs and IBA concentrations. The average number of roots and shoots under clay sub soil ranged from 11.7-17 and 22-30, respectively.

For the case of GC 584 and GC 642, modeling of rooting did not provide dependable results due to effect of collinearity between rooting media and IBA concentrations. However there was a significant difference in shooting between rooting media where the chances of the cuttings shooting under CS were about five and 2.2 times higher in GC584 and 642 than those of 2SCS, respectively. This

was very different from GC785 where the chances of the cuttings rooting in S2CS were 6.4 times higher than in SCS. This was followed by CS at 2.2 times higher than 2SCS. This showed that unlike most of other EGCs, GC 785 was very prolific in rooting under clay sub soil fortified with sand. This was also evidenced in shooting. On the other hand GC 796 had significant rooting and shooting in CS as compared to other rooting media where the chances of cuttings rooting under it were about 3.8 times higher than 2SCS and 4.9 times higher in shooting. S2CS and SCS had also high rooting and shooting than 2SCS.

4.2 Effect of different rooting media and leaf surface area on rooting of EGC clones

The results showed that at day seven the cuttings of different leaf surface area under different rooting media had started rooting where cuttings with leaf surface area of $100cm^2$ had the highest rooting and shooting percentage. As rooting progressed, it was observed that rooting was directly proportional to leaf surface area and duration (days), where rooting percentage increased with increasing leaf surface area and duration. After 40 days, most cuttings across all rooting media had shooted better when their leaf surface area was at least 80 cm^2 with 0 cm^2 (no leaves) least performing in shooting (figure 4.7).

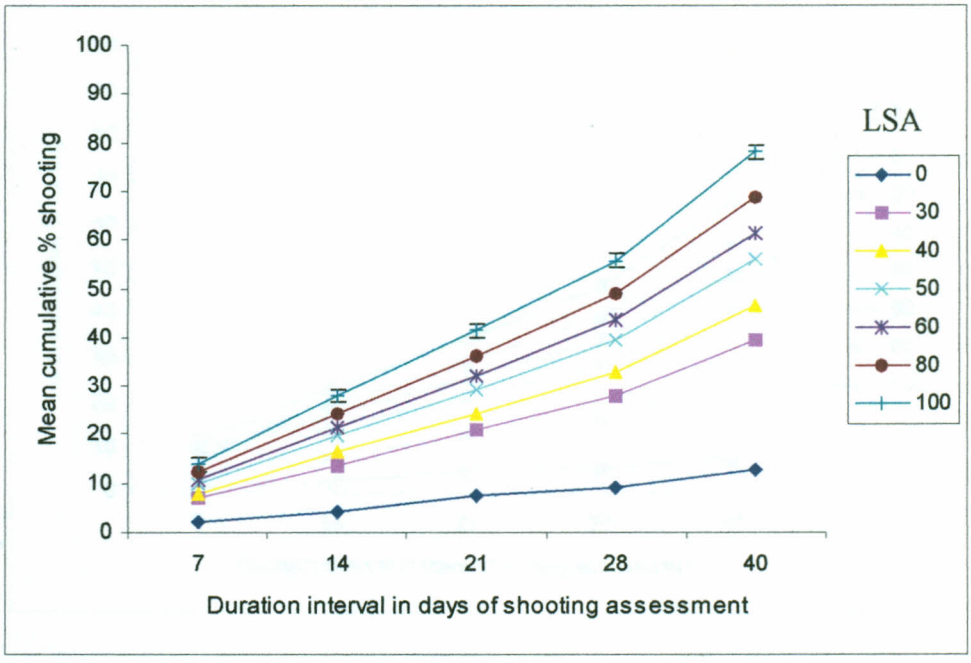

Figure 4.7: Overall mean shooting percentage of leaf surface area at different days, in clay sub soil 1

Leaf surface area of 100cm^2 had the highest shooting percentage, while 0 cm^2 had the lowest shooting percentage (figure 4.7). Most of the cuttings across all the rooting media had rooted better with 100 cm^2 followed by 80 cm^2 whereas 0 cm^2 had least overall rooting (figure 4.8). This showed that the larger the leaf surface area of the leaf, the better the rooting of cutting for all the 12 EGCs experimented.

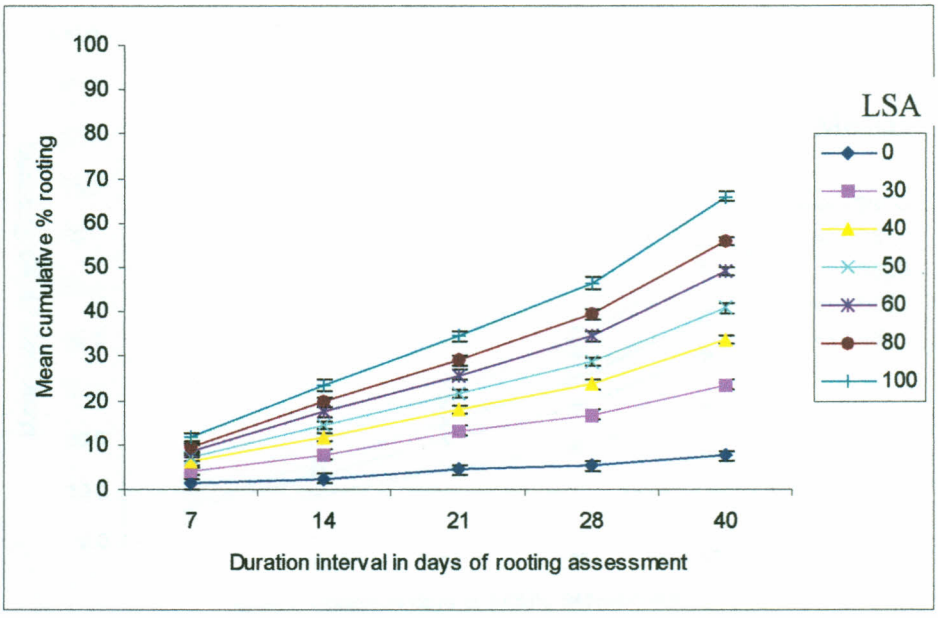

Figure 4.8: Mean rooting percentage of cuttings with different leaf surface area in different days, in clay sub soil

As indicated in figure 4.8, rooting followed the same trend as shooting with the highest rooting being recorded in cuttings with a leaf area of $100cm^2$, while the lowest rooting in cuttings with a leaf area of $0cm^2$. Looking at media, more rooting was observed under CS followed by S2CS with SS having the least rooting (figure 4.9a).

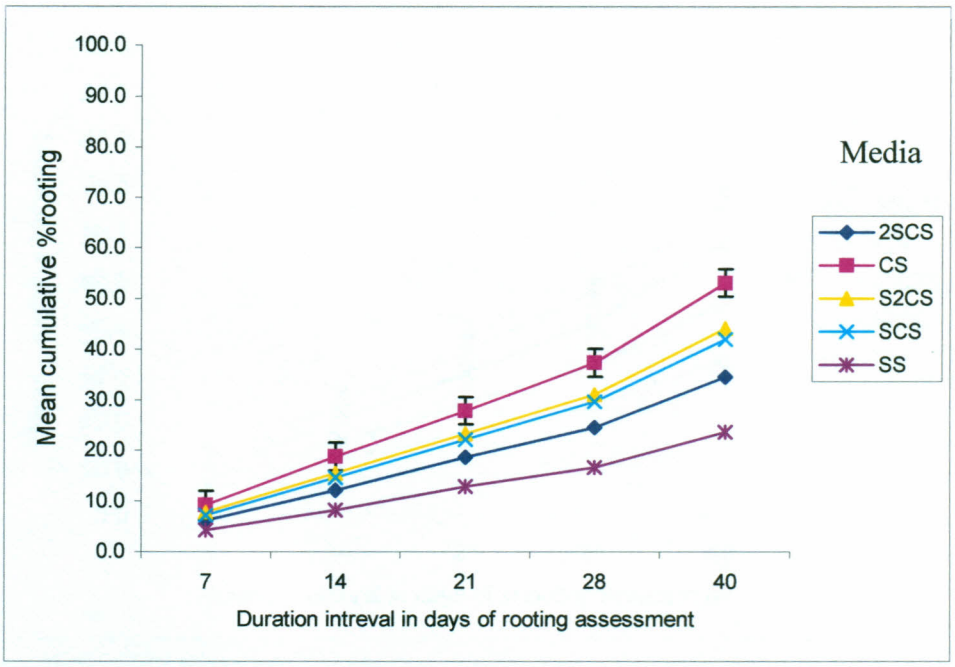

Figure 4.9a: Mean rooting percentage of cuttings in different rooting media at different days of assessment, in clay sub soil

Clay sub soil had the highest mean shooting percentage followed by SCS whereas SS alone had the least shooting as in the case of rooting. This indicated that most of the EGCs cuttings performed poorly in rooting and shooting under sand soil (figure 4.9b).

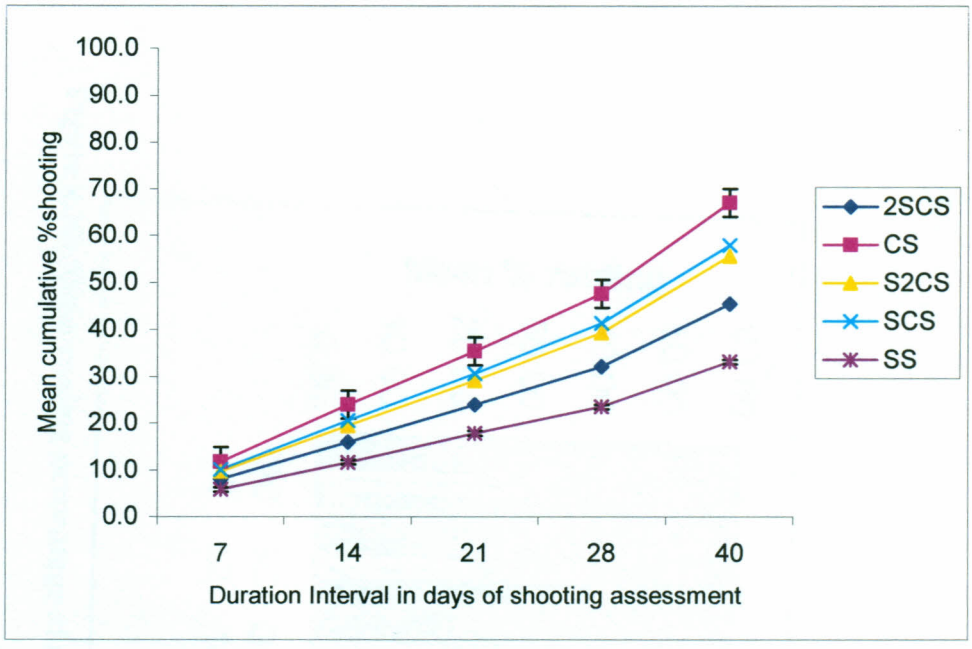

Figure 4.9b: Mean shooting percentage of rooting media at different days of assessment

Clay sub soil had the highest shooting percentage, followed by SCS, SS had the lowest shooting percentage (figure 4.9b).

The cuttings of GC 15 had the highest mean percentage rooting and shooting whereas GC 642 and GC 796 had the least mean percentage rooting and shooting, respectively across all media tested (figure 4.10).

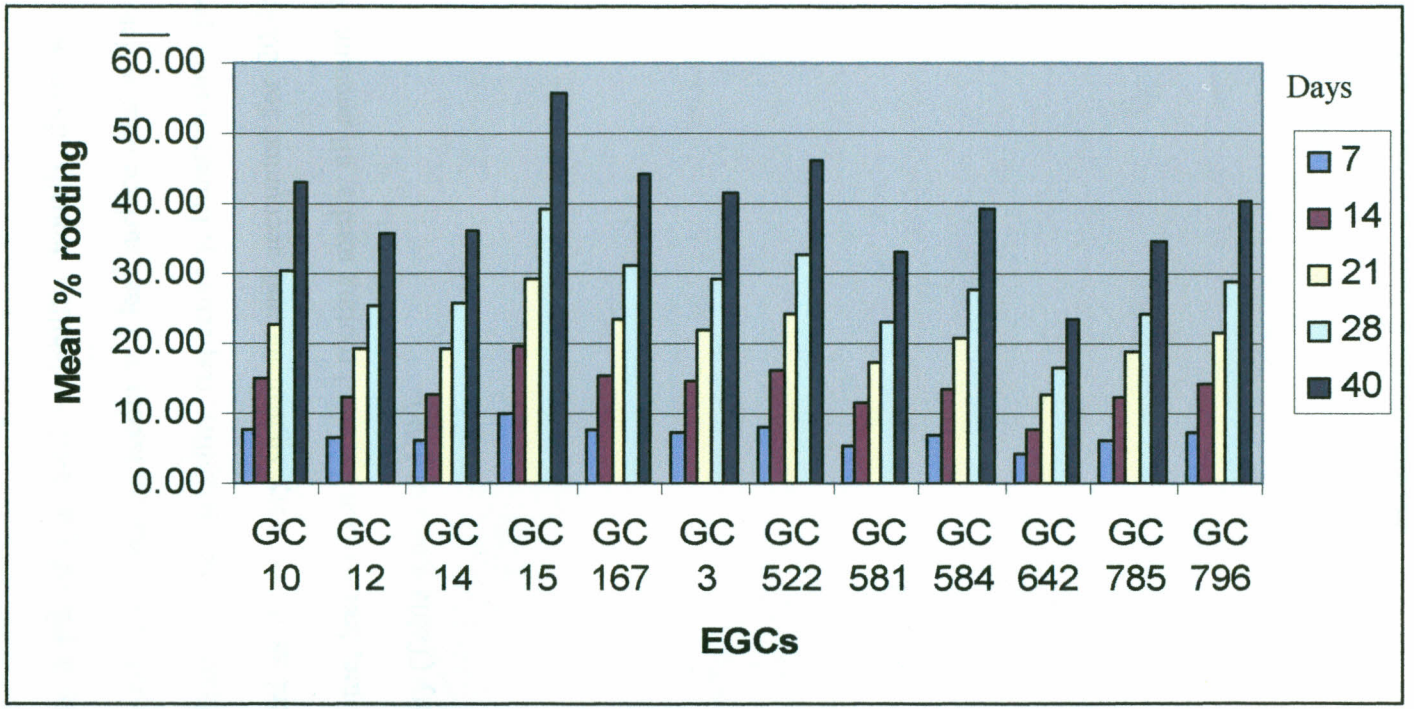

Figure 4.10: Mean rooting percentage of cuttings for duration at different levels of EGCs, in clay sub soil

Analysis of variance showed that there was a highly significant difference ($p<0.01$) in rooting and shooting among different EGCs (Table 4.6). This accounted for 5.4% of the total variability. Equally there was a significant difference ($p<0.01$) for rooting media and leaf surface area which accounted for 9.1 and 31.9% of the total variability, respectively. Duration since propagation was equally critical as it was very significant and accounted for 20.4% of the total variability. Time, leaf surface area and rooting media, all accounted for 61.4% of total variability (Table 4.6a and 4.6b).

Table 4.6a: ANOVA for duration, EGCs, rooting media and leaf surface area in rooting

Source of variation	d.f.	M.S.	F prob.	% accounted
Replication	2	53222.87		4.8
Duration	3	150838.72	<.001	20.4
EGCs	11	10992.07	<.001	5.4
Rooting media (Rmedia)	4	50632.13	<.001	9.1
Leaf_Surface area(leaf SA)	6	117917.54	<.001	31.9
Duration.EGCs	33	505	<.001	0.8
Duration*Rmedia	12	2361.18	<.001	1.3
EGCs*Rmedia	44	1335.11	<.001	2.6
Duration*leaf_SA	18	5418.19	<.001	4.4
EGCs*leaf_SA	66	327.85	<.001	1.0
Rmedia*leaf_SA	24	620.86	<.001	0.7
Duration*EGCs.Rmedia	132	62.89	0.999	0.4
Duration*EGCs.leaf_SA	198	16.64	1	0.1
Duration*Rmedia*leaf_SA	72	33.25	1	0.1
EGCs*Rmedia.leaf_SA	264	189.08	<.001	2.2
Duration*EGCs.Rmedia*leaf_SA	792	10.4	1	0.4
Residual	3358	95.28		14.4
Total	5039			100

Table 4.6b: ANOVA for duration, EGCs, rooting media and leaf surface area in shooting

Source of variation	d.f.	M.s.	F pr.	% accounted
Replication	2	120.73		0.0
Duration	3	258687.04	<.001	27.3
EGCs	11	29915.76	<.001	11.6
Rmedia	4	71443.34	<.001	10.0
leaf_SA	6	140737.2	<.001	29.7
Duration*EGCs	33	1403.94	<.001	1.6
Duration*Rmedia	12	3232.33	<.001	1.4
EGCs*Rmedia	44	2591.41	<.001	4.0
Duration*leaf_SA	18	6382.16	<.001	4.0
EGCs*leaf_SA	66	731.54	<.001	1.7
Rmedia*leaf_SA	24	982.05	<.001	0.8
Duration*EGCs.Rmedia	132	118.53	<.001	0.6
Duration*EGCs*leaf_SA	198	36.54	0.062	0.3
Duration*Rmedia*leaf_SA	72	54.14	<.001	0.1
EGCs*Rmedia*leaf_SA	264	298.38	<.001	2.8
Duration*EGCs.Rmedia*leaf_SA	792	14.74	1	0.4
Residual	3358	31.37		
Total	5039			100

It is also worthy to note that there was significant ($P<0.01$) interaction effect between duration, EGCs, rooting media and leaf surface area implying that the occurrence of rooting was independent on the day of rooting. However, the significant interaction effect between EGCs, rooting media and leaf surface area showed that the variation in rooting was strongly influenced by different levels of each factor. This means that for a given EGC and rooting media, different levels of leaf surface area would respond differently in rooting.

Orthogonal comparisons between EGCs showed that showed that there was a significant difference in rooting and shooting between GC 642 and GCs 581, 796, 167 and 15 (Table 4.7).

Table 4.7: Orthogonal comparisons in rooting and shooting of EGCs after 40 days

EGCs	% Rooted	% Shooting
GC 10	43.1c	58.6c
GC 12	35.8b	40.5b
GC 14	36.3b	44.0b
GC 15	55.7d	75.9e
GC 167	44.4cd	51.8bc
GC 3	41.6c	65.0d
GC 522	46.2c	64.6cd
GC 581	33.0b	58.5c
GC 584	39.0b	50.1b
GC 642	23.5a	45.7b
GC 785	34.8b	35.8a
GC 796	40.5b	31.6a
s.e.d	0.554	0.3141

Values with the same letters are not significantly different ($p<0.01$). On the other hand there was a significant difference ($p<0.01$) between sand soil and 2SCS, S2CS, SCS and CS whereas there was no significant difference ($p>0.05$) between 2SCS, S2CS and SCS (Table 4.8).

Table 4.8: Mean rooting percentage of EGC hybrids in different rooting media after 40 days

Rooting media	Mean % rooted
CS	53.1
S2CS	44.1
SCS	42.0
2SCS	34.6
SS	23.7
s.e.d	2.177

This implied that addition of sand soil in any ratio to clay sub soil did not have a big influence in rooting. In addition, clay sub soil was highly significantly different ($p<0.01$) in rooting and shooting from the others. Similarly there was highly significant difference ($p<0.01$) in rooting and shooting between 0 cm^2 and the rest of the levels of leaf surface area (Table 4.9).

Table 4.9: Orthogonal comparisons for rooting and shooting of cuttings with different leaf surface areas after 40 days

leaf surface area (cm^2)	% rooted	% shooted
0	7.5a	12.7a
30	23.6b	39.4b
40	33.7c	46.6b
50	40.8cd	55.8c
60	49.1cd	61.4c
80	55.9d	68.8cd
100	65.9e	78.1e
s.e.d	0.423	0.240

Values with the same letters are not significantly different ($p<0.01$). Treatments with $0cm^2$ had all leaves cut off

On the basis of the hypothesis of the study where the question of concern was to find out what appropriate rooting media and leaf surface area that can be used for further vegetative propagation of the commercially available EGCs, the logistic regression of each EGC hybrid under each media and different leaf surface area results are as described in each of the following sub heading for every EGC hybrid.

GC 10

The results showed that the chances of the cuttings rooting under 2SCS whose leaf surface area is 30, 40, 50, 60 and 80 cm^2 were about 18, 23, 34, 48 and 63 times higher than for those with a leaf surface area of 0 cm^2 (Table 4.10). This stressed the importance of the leaf area in vegetative cuttings as demonstrated by the 100cm^2 leaf area whose chances of rooting were very high (127 times higher).

Table 4.10: Logistic regression results on rooting for LSA under 2SCS after 40 days

Parameter	s.e.	t prob.	Odds ratio
Constant	1.46	0.014	0.01
leaf_SA 30	1.53	0.079	18.0
leaf_SA 40	1.52	0.057	23.3
leaf_SA 50	1.51	0.035	34.2
leaf_SA 60	1.51	0.022	48.3
leaf_SA 80	1.51	0.016	63.1
leaf_SA 100	1.51	0.006	127.3

Similar trends were also found in other rooting media (SS, SCS, CS and 2SCS) as shown in figures (4.11a-4.11e) where higher mean number of roots was observed in clay sub soil. Overall, the results of this study show that the increase of leaf

surface area in any rooting media, resulted in an increase in number of roots for GC 10 cuttings.

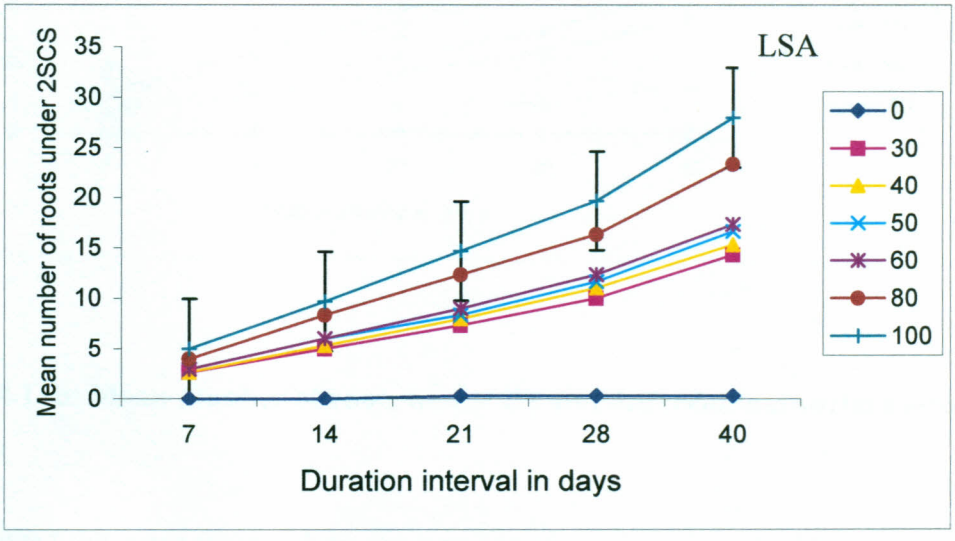

Figure 4.11a: Mean number of roots under 2SCS and different leaf surface area

Figure 4.11a shows that the higher the leaf surface area, the better the rooting, with the highest number of roots being recorded in $100cm^2$, and the lowest being recorded in $0cm^2$ in 2SCS.

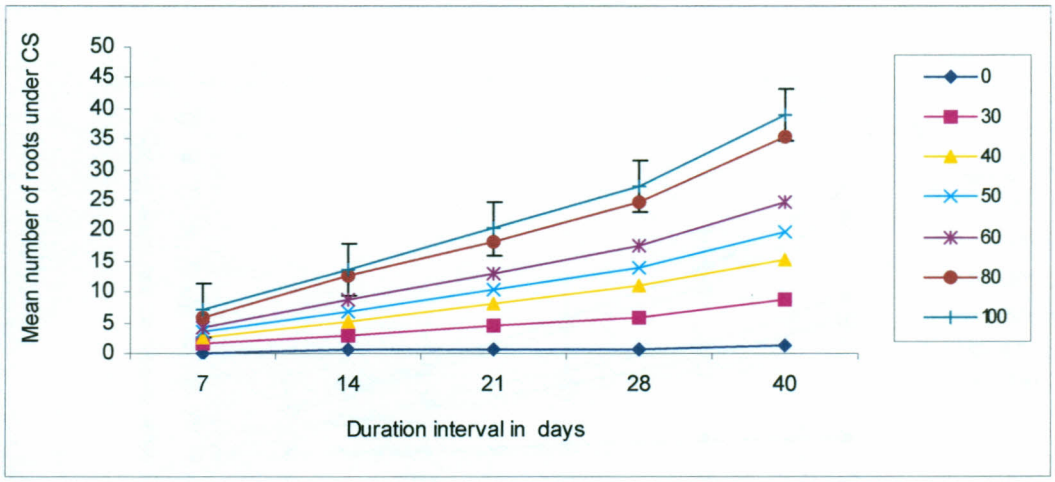

Figure 4.11b: Mean number of roots under CS and different leaf surface area

CS followed the same trend, where the number of roots increased with increasing leaf surface area (Figure 4.11b). The mean number of roots increased with increasing leaf area for S2CS, as shown in figure 4.11c

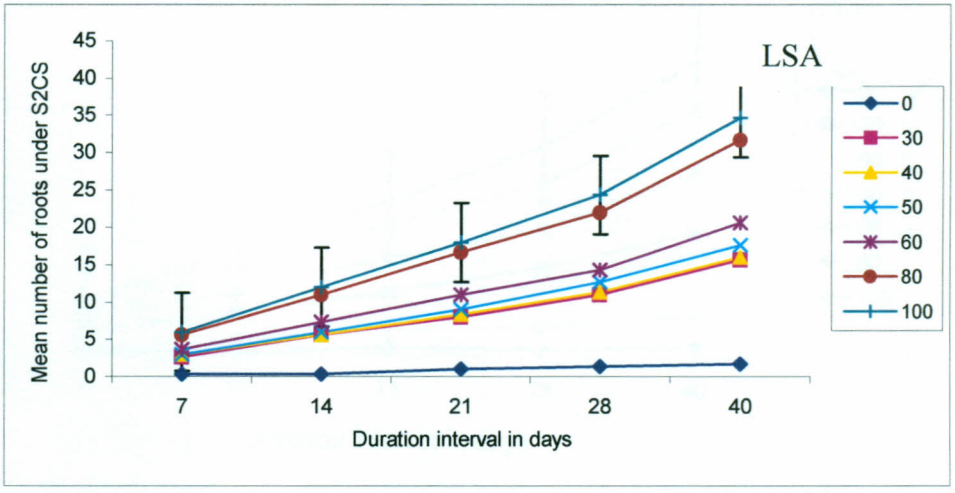

Figure 4.11c: Mean number of roots under S2CS and different leaf surface area

For SCS, a similar trend was observed where rooting was directly proportional to increase in leaf area as indicated in figure 4.11d

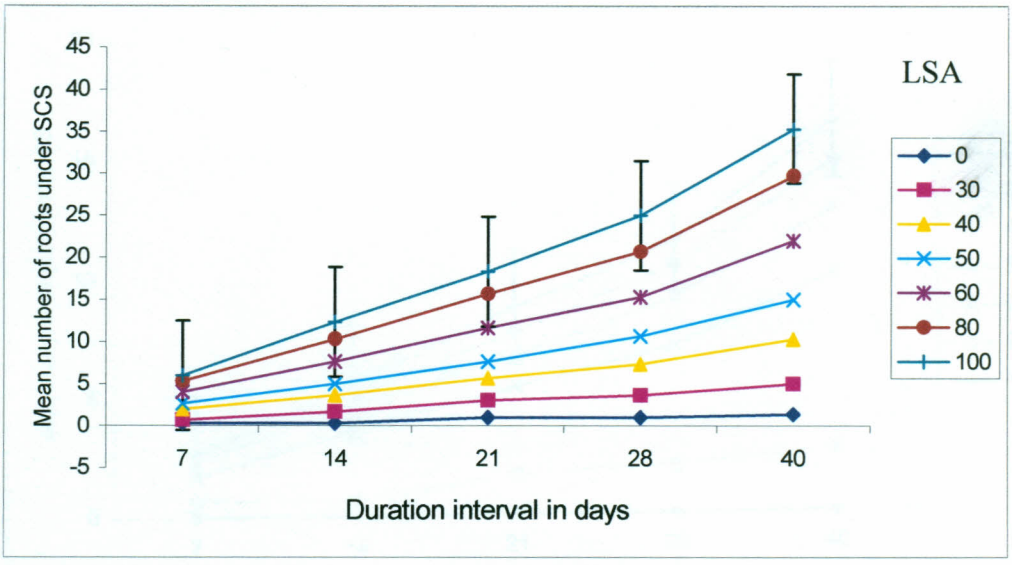

Figure 4.11d: Mean number of roots under SCS and different leaf surface area

The mean number of roots increased with increasing leaf area as in figure 4.11e for SS.

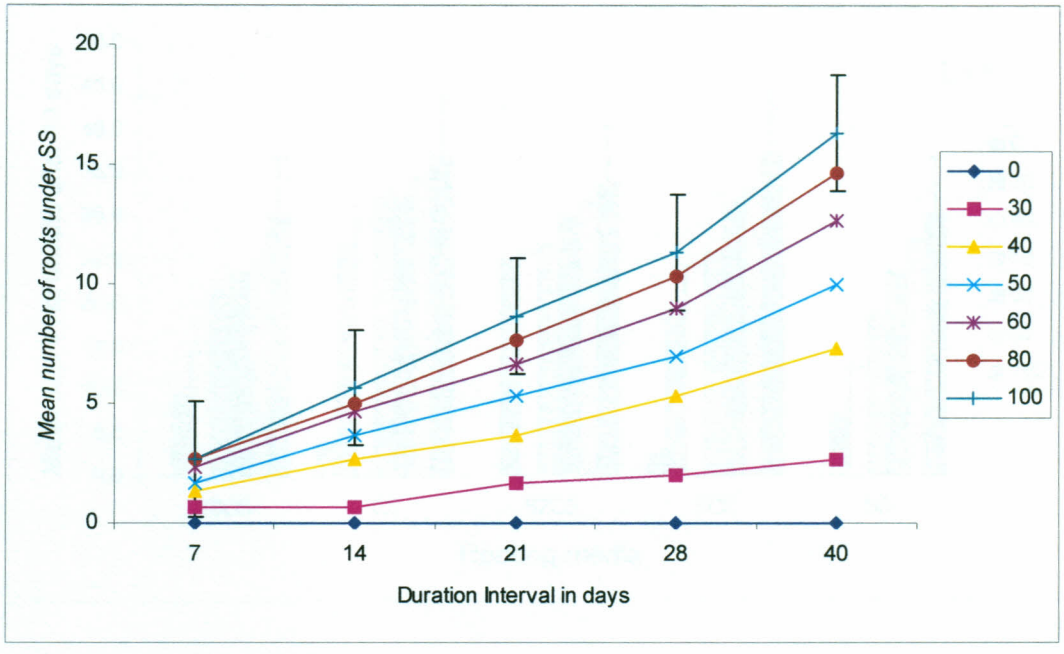

Figure 4.11e: Mean number of roots under SS and different leaf surface area

Equally, the number of shoots followed the same trend where the minimum and maximum mean number was 2.7 and 33.7, after 40 days, respectively (Figure 4.12).

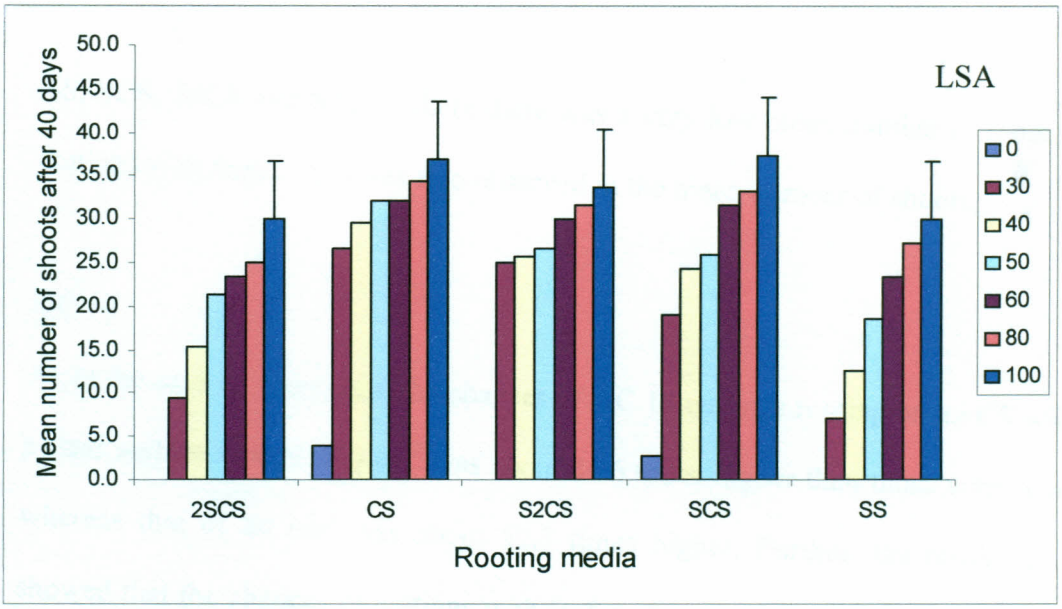

Figure 4.12: Mean number of shoots under different rooting media and leaf surface area after 40 days

Figure 4.12 shows for each given media, the number of shoots increased with increasing leaf area.

GC 12.

The results revealed that the chances of cuttings rooting under CS with a leaf surface area of 100 cm^2 was about 99 times higher than those with $0cm^2$ whereas that of 80 cm^2 was about 27 times higher. This further demonstrated that the leaf surface area and the rooting media were very critical in influencing the rooting ability of the cuttings. Almost similar results were found in other rooting media

SS, SCS, 2SCS and S2CS, where there was a very low mean number of roots for cuttings with 0cm^2. This was also observed in the mean number of shoots.

GC 14

As in the case of other GCs, the chances of GC 14 cuttings rooting under CS with a leaf surface area of 100 cm^2 was about 84.6 times higher than those with 0cm^2 whereas that of 80 cm^2 was about 52.3 times higher. Further, the results also showed that the chances of cuttings with leaf surface area 40 and 50 rooting were close to one another. Similar results were also observed for shooting where the chances of cuttings shooting with leaf surface area of 100 cm^{2}was about 841 times higher as compared to 0cm^2. The other rooting media tested showed a similar trend in shooting and rooting under different leaf surface areas.

GC 15

Unlike in other EGCs where CS has been dominantly having higher rooting than other media, in this case the results showed that the cuttings of GC15 had rooted better in SCS where the chances of cuttings with a leaf surface area of 100 cm^2 rooting, was about 8.3 times higher than cuttings with a leaf surface area of 0 cm^2. This was followed by cuttings with a leaf surface area of 80 cm^2. On the other hand the chances of cuttings with a leaf surface area was 100cm^2 shooting, were about 32.8 times higher than 0 cm^2. Similar trend was observed on other rooting

media where the cuttings with higher leaf surface area had high number of roots and shoots, respectively.

GC 167

The results showed that the chances of cuttings with a leaf surface area of 100 cm^2 rooting under CS were about 2.9 times higher as compared to 0cm^2 whereas that of 30, 40, 50, 60 and 80 cm^2 were 1.7, 2.1, 2.2, 2.7 and 2.8 times higher, respectively. For the case of shooting, the chances were 319, 188, 129, 87, 50 and 37.9 times higher for 100, 80, 60, 50, 40 and 30 cm^2 than 0cm^2, respectively. This was also observed in other rooting media.

GC 3

Cuttings with a leaf surface area of 100cm^2 had the highest mean number of roots under clay sub soil. CS had about 45 times higher chances of rooting than 0cm^2 whereas 80 cm^2 had about 20 times higher chances as compared to 7 times higher for 60cm^2 with 50cm^2 having about 5.7 times higher while 30 and 40 cm^2 had about 2.7 and 4.7 times higher chances of rooting respectively as compared to 0 cm^2. On the other hand cuttings with 100cm^2 leaf surface area had about 59 times higher chances of shooting than 0cm^2 whereas 30, 40, 50, 60 and 80cm^2 had 2, 3.2, 4, 7.6 and 14 times higher chances than the control respectively. This trend was also found with the other rooting media.

GC 522

In this case 100cm^2 had more rooting in S2CS where the chances of rooting were about 17.8 times higher than in 0cm^2. 30, 40, 50, 60 and 80 cm^2 had about 3.4, 5.1, 9.6 and 10.4 chances times higher in rooting as compared to control. Shooting followed a similar trend where the cuttings with a leaf surface area of 100cm^2 had about 11.8 times higher chances of shooting than the control. A similar trend was observed for 80 cm^2. 30, 40, 50 and 60 cm^2 had about 2.5, 5.3, 6.1 and 8.1 times higher chances in rooting as compared to the control respectively. Overall, this is the trend that was found in other rooting media.

GC 581

In this case, SCS had the highest mean number of roots and shoots with 100cm^2 leading in both cases. The logistic regression failed to compute reliable estimates due to collinearity.

GC 584

CS had the highest mean number of roots with 100cm^2 as compared to the other leaf areas. The chances of rooting and shooting of the cuttings in all the leaf surface area were at least 10 times higher as compared to the control. This trend was similar with other rooting media.

GC 642

The results showed that 100cm^2 cuttings had about 3.8 times chances higher in rooting under CS a compared to 0cm2 (Table 13). Similarly it had about 4.2 times higher chances in shooting as compared to the control. CS and 100cm^2 were critical in enhancing the rooting ability of the cuttings across most of the EGCs. This trend was also found in other rooting media.

GC 785

More rooting occurred under clay sub soil where 100cm2 cuttings had higher mean number of roots and had about 20.4 times chances in rooting as compared with the control. It was followed by 80cm2 with 17.7 times chances higher in rooting as compared with 60cm2 of 15.4 times higher and 50cm2 with 11 whereas 30 and 40cm2 had about 4.3 and 9.6 times higher than the control. Similar trend was observed with other rooting media. However shooting analysis had a problem of collinearity.

GC 796

The results revealed that 100 cm^2 cuttings had about 41 times chances of rooting as compared to control. This was followed by 80, 60, 50, 40 and 30 cm^2 at 21.7, 20.3, 19, 14.5 and 10.2 times higher as compared with control, respectively. Equally in shooting 100cm2 had the leading whose chances were about 45 times higher as compared with the control. It was equally followed by 80, 60, 50, 40 and

$30cm^2$ with 29.5, 23.3, 18, 14.8 and 9.1 times higher than the control, respectively. Similar trend was observed among other rooting media.

4.3 Effect of different leaf surface area and IBA concentrations on the rooting of EGCs hybrids

The results showed that there was minimal rooting at day seven but most of the cuttings had started rooting at day 14. This progressed till at day 40 when the study was terminated. GC 15 had the highest mean rooting percentage (57.3%) among the EGCs experimented and was followed by GC 785 (56.6%) (Table 4.11).

Table 4.11: Mean cumulative rooting percentage for EGCs

	Mean % rooting across days of assessment				
EGCs	7	14	21	28	40
GC 10	0.0	18.7	27.6	37.1	52.9
GC 12	0.0	13.3	20.1	27.1	38.2
GC 14	0.1	13.5	20.6	27.1	38.6
GC 15	0.1	20.2	30.1	40.2	57.3
GC 167	0.2	18.8	27.9	37.6	53.0
GC 3	0.1	12.1	18.7	24.8	35.1
GC 522	0.0	11.8	18.2	24.2	34.2
GC 581	0.1	15.2	22.4	29.7	42.2
GC 584	0.0	14.3	21.4	28.2	40.3
GC 642	0.1	14.4	22.4	29.6	41.8
GC 785	0.1	19.9	29.6	39.8	56.6
GC 796	0.0	16.1	24.0	32.0	45.4
s.e.d=2.132					

GC 15 had the highest shooting percentage (73.7%), closely followed by GC 642 (70.2%). GC 14 had the lowest shooting percentage (56.4%) as indicated in figure 4.13.

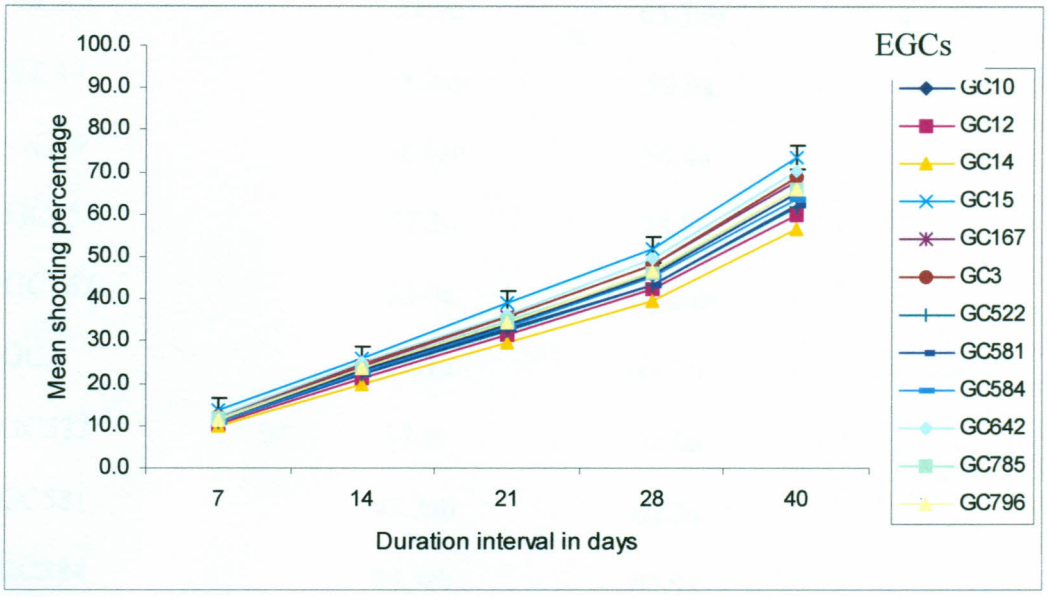

Figure 4.13: Mean number of shoots for different EGCs

ANOVA and logistic regression analysis (analysis of deviance) showed a significant difference ($p<0.01$) in rooting and shooting among the different EGCs at the end of the experimental period (Table 4.12).

Table 4.12: Mean comparisons in rooting/shooting of EGCs

EGCs	% rooted	% shooted
GC10	52.9c	65.3ab
GC12	38.2ab	59.9a
GC14	38.6ab	56.4a
GC15	57.3c	73.7c
GC167	53.0c	68.2ab
GC3	35.1a	68.7ab
GC522	34.2a	62.0a
GC581	42.2ab	62.3a
GC584	40.3ab	63.9a
GC642	41.8ab	70.2c
GC785	56.6c	66.1a
GC796	45.4ab	66.2a
s.e.d	3.350	2.479

Values with the same letter are not significantly different ($p<0.01$)

This shows that different EGCs had different abilities of rooting. They accounted for very negligible percentage (2.8%) of the total variability as compared to duration (37.3%) leaving others explained by, duration, leaf surface area, IBA and interaction effect among various factors in question. This showed that the number of days the cuttings took before rooting were very critical. Overall the mean

number of roots ranged from 11 to 18 for all EGCs with different leaf surface areas and IBA concentrations. However at each EGCs level with same leaf surface areas under different IBA concentration the mean number of roots ranged between zero and 26.

On the other hand there was also highly significant difference ($p<0.01$) in shooting and rooting among different levels of leaf surface area of the cuttings where $100cm^2$ had the highest mean cumulative percentage rooting and shooting at the end of the experiment (Table 4.13). The leaf surface area accounted 21% of the total variability. This was in line with objective of the study examining the appropriate LSA and IBA concentration that would be suitable in influencing rooting of the cuttings.

Table 4.13: Mean cumulative rooting/shooting percentages at day 40

leaf Surface area cm^2	% rooted	% shooting
0	5.1	11.3
30	27.2	52.4
40	38.7	62.8
50	47.4	71.8
60	55.5	79.1
80	63.8	86.1
100	74.9	93.1
s.e.d	2.559	1.894

Further to this, the results showed no significance difference ($p=0.755$) in rooting among different levels of IBA concentrations. It accounted for less than one percent of the total variability in all its interaction effect and itself. This implied almost all EGCs cuttings of different leaf surface area rooted equally under different IBA concentrations (Figure 4.14a and 4.14b)

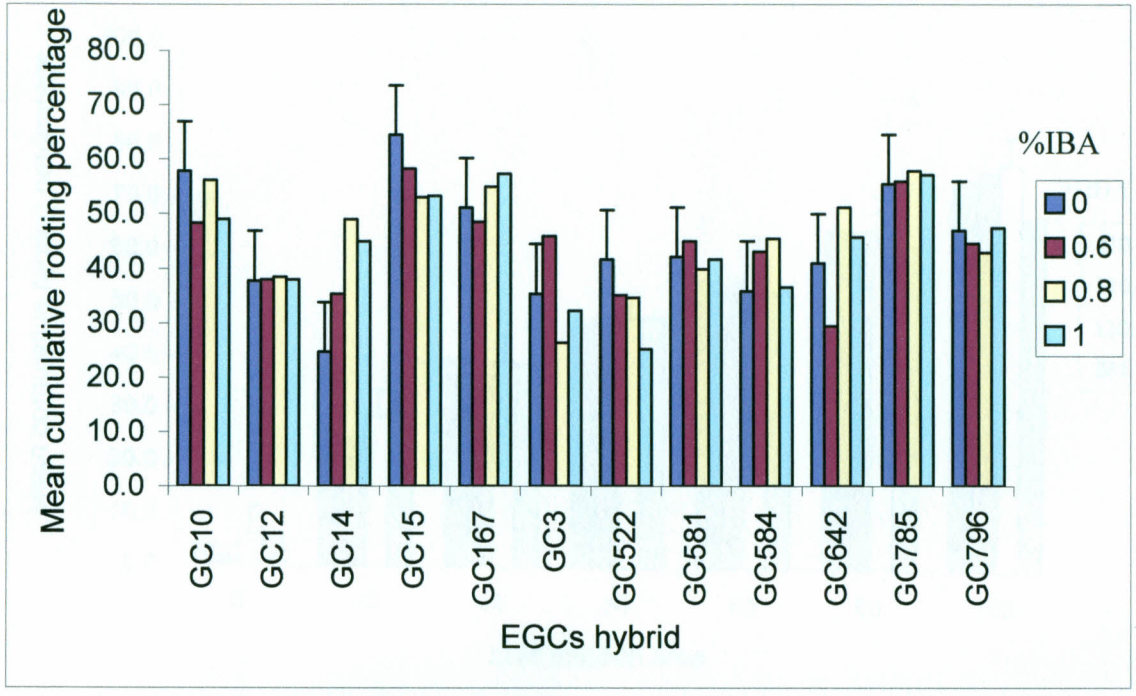

Figure 4.14a: Mean cumulative rooting % of EGCs under different IBA concentrations

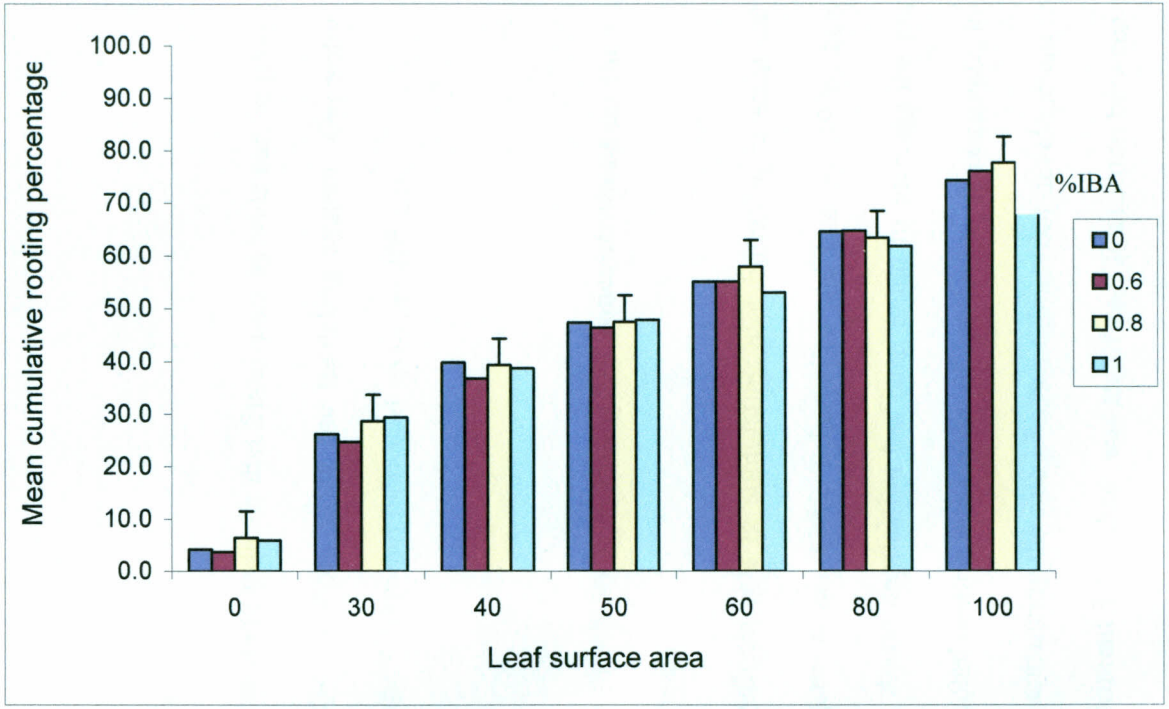

Figure 4.14b: Mean cumulative rooting % of LSA under different IBA concentrations

CHAPTER FIVE

5.0 DISCUSSION

Results of this experiment indicate that Eucalyptus hybrids can be propagated in a non mist propagation system, using the tunneling system. Generally, minimal rooting started on the 7th day and increased up to the 40th day.

Effect of different indole butyric acid (IBA) concentrations on the rooting of different EGC hybrids

Auxins play a significant role in stimulating root initiation in stem cuttings of woody plants (Poupard *et al.*, 1994; Tchoundjeu and Leakey, 1996; Tchoundjeu and Leakey, 2000). Auxin application has been found to increase the number of roots initiated per rooted cutting in a variety of species, as, for example, in *Shorea leprosula* as well as enhancing the rate of adventitious root development (Aminah *et al.*, 1995). In experiment 3.3.1, there was significant interaction between rooting media, EGCs and IBA on percentage rooted, with highest rooting being observed with 0.6% IBA concentration, in clay sub soil. However, results from logistic analysis carried out individually for all EGCs showed no significant interaction effect of IBA and media on the rooting of the 12 EGCs tested. This indicates that the EGC hybrids did not require the addition of exogenous IBA for rooting. It is

possible that EGCs have sufficient endogenous auxins and do not require exogenous IBA. Exclusion of IBA would reduce the cost of vegetative propagation of EGC hybrids.

Rooting media and IBA concentration had a different effect on rooting and shooting ability of EGCs. The effects of combining IBA and rooting media could not be identified by ANOVA implying that the rooting ability of the 12 different EGCs were independent in each rooting media and IBA concentration.

Many authors have reported differences in rooting frequency depending on the exogenous auxin or combination of auxins used (Felker and Clarke, 1981; Klass *et al.*, 1987), with IBA often giving the best results (Teklehaimanot *et al.*, 1996). However, in experiment 3.3.1 and 3.3.3, the cuttings rooted without any application of IBA, with the control having higher rooting percentages in some cases than the test. This is not unusual as the same findings were recorded by Shiembo *et al.*, (1996a) where the overall effect of IBA on rooting of *Irvingia gabonensis* was relatively slight. According to Ofori *et al.*, (1996) experiments with a range of tree species have indicated highly contrasting responses to IBA addition. *Milicia exelsa* recorded a relatively high rooting percentage without applied IBA, implying that it is well supplied with endogenous auxins. This could be the same case for EGCs. Similar results have been reported in a number of tropical tree species such as *Shorea macrophylla* (Lo, 1985) *Nauclea diderrichii* (Leakey, 1990) and *Vochysia hondurensis* (Leakey *et al.*, 1990).

Effect of rooting media on the rooting of EGC hybrids.

One of the most important criteria for the successful rooting of cuttings is suitable rooting medium (Macdonald, 1986; Ercis *et al.* 2001). Generally, there was variability in rooting and shooting of different EGCs under various rooting media with sand having the lowest mean rooting and shooting percentages and clay sub soil the highest mean rooting and shooting percentages. This contradicts the results of Leakey *et al.*, 1990; Nyansi, 2004 and Atangana *et al.*, 2006, where high rooting percentages were recorded in sand media in *Cordia alliodora*, *Garcinia. kola* and *Acacia floribunda* leafy stem cuttings, respctively.

Species tend to have an optimal substrate for rooting which is linked to their hydromorphic or xeromorphic status (Loach, 1985) and the effects of this on the water relations of the cuttings (Mese´n *et al.*, 1997a). *C. alliodora*, *G. Kola* and *A florbunda*) are xeromorphic and this explains why they recorded high rooting percentages in sand soil. However, the findings of this study indicate that EGCs are hydromorphic and this would explain why they rooted better in clay sub soil. These findings are supported by Indieka (2007) with *Melia volkensii* which is a hyrdromorphic tree species, where cuttings rooted best in clay soils.

In experiment 3.3.1 and 3.3.2, different hybrid clones rooted differently in different rooting media. This could be brought about by clonal differences between EGC clones. Clonal differences between the 12 different EGC hybrid varieties came about through a selection and breeding process carried out in South Africa for *E grandis* and *E camadulensis* species. The selection and breeding process involved establishment of provenance trials. A provenance trial includes trees of the same species but of different origin and if the difference is more obvious they are called varieties (Olensen, 1976). The *E grandis* and *E camadulensis* were selected from different regions (origins) in South Africa. Hybridization of *E grandis* and *E camadulensis* from different origins, gave rise to the 12 different EGC hybrid varieties. These varieties although belonging to the same species, do not have the same genetic makeup because their origins before selection and hybridization are different.

Differences between clones have also been observed by Atangana *et al.,* (2006) with rooting ability of *A. floribunda*. Such differences in rooting ability have also been reported for some forest tree species such as *Platanus occidentalis*, (Cunningham, 1986), *Albizia guachapele* (Mese´n *et al.*, 2001) et cetera. Other causes for differences in rooting ability between clones could be due to genetic differences in cutting morphology and/or physiology (Leakey, 2004). Genetic potential, as well as propagation environment, postseverance treatment, cutting

origin and environment, stockplant physiology and management, have been reported to influence rooting (Hartmann *et al.*, 2002; Leakey, 2004).

The effect of media on rooting could be projected to growing field conditions with different soil types bringing out the aspect of geographic mapping of EGCs based on their rooting performance in different media. Results from regression analysis showed that GC 12, 15, 3, 522 and 581 had higher chances of rooting in clay sub soil compared to any other media while GC 785 and 167 had higher chances of rooting in S2CS. This could possibly be translated to mean that GC 12, 15, 3, 522, 581 could grow well in areas with clay soils, while GC 785 and 167 could perform well in areas with slightly sandy soils. Since all the EGCs performed poorly in sand soil, this could be translated to mean that the EGCs would not perform well in areas with predominantly sandy soils.

The preference of different EGCs to specific media could also be attributed to hybridisation process of *E. grandis* and *E. camadulensis*. *E. grandis* performs well in high rainfall areas (rainfall not less than 900mm), while *E. camadulensis* in dry areas (rainfall of 450-900mm). EGCs that have a preference to clay soil could be having more of the *E. grandis* characteristics and less of the *E. camadulensis* characteristics, because clay is more water retentive which is characteristic of moist areas where *E. grandis* grows. The opposite is true for EGC varieties that have a preference for sand in clay sub soil (GC 167 and 785).

The roooting and shooting success was highest in CS and lowest in SS. Soil texture affects the retention of water and the effect of water infiltration. According to an experiment conducted by Ercis *et al.,* (2002) on Kiwi fruits, both rooting ratio and root number increased because of increasing water retention capacity of media at low tensions (< 0.33 atm). Clay sub soil has a high water holding capacity because it is very finely textured, meaning there was more water available for the cuttings. In contrast, sand soil permits rapid infiltration and percolation of water and is incapable of retaining large quantities of water. This is supported by Janick (1986), where clay soils were classified as having a water capacity of between 133-208 mm per meter of soil, compared to sandy soil with water capacity of 63-104 mm per meter of soil.

According to Ellyard and Ollerenshaw, (1984) interaction between the rooting medium and auxin treatment could effect rooting response, since increased water uptake could be expected to increase auxin uptake and consequently to stomatal conductance and photosynthesis (Mesen *et al.*, 1997b).

The lowest rooting and shooting percentages were observed with increasing amounts of sand to clay. Aeration and water holding capacity of the media are often negatively correlated (Loach 1985). This is because larger the air spaces between the soil particles meaning more aeration, the lower the water holding

capacity of the media. Ofori *et al.*, (1996) has identified sand as having a relatively low water holding capacity and cuttings inserted in this media had displayed lower values of foliar Relative water Content (RWC) and lower rooting percentages and root mass. These factors could explain why higher rooting percentages were recorded with clay than sand. Higher rooting percentages were observed with CS followed by S2CS, then SCS, 2SCS and lastly SS. Water uptake is particularly important for overcoming the initial physiological shock when cuttings are taken, which can lead to high moisture deficits, leaf abscission and cutting death in drought sensitive species (Newton and Jones, 1993). As suggested for *I. gabonensis* (Shiembo *et al.*, 1996a), the relatively higher rooting percentage recorded in sawdust for *Pausinystali. johimbe* cuttings, may be attributed to its relatively high air/water ratio and water content. This could be the same case with clay sub soil.

Effect of leaf surface area on rooting of EGC hybrids

Rooting of leafy cuttings seems to be affected by a combination of factors influencing root formation (Dick *et al.*, 2004). In experiment 3.3.2, there was significant interaction effect between EGCs, rooting media and leaf surface area, showing that the variation in rooting was strongly influenced by different levels of each factor. For a given EGC and rooting media, different levels of leaf surface area created a different response to rooting. GCs 12, 14, 167, 3, 584, 642 and 796

rooted best in CS, while 15 and 522 rooted best in SCS and S2CS respectively. This trend was also observed with *A. floribunda* (Atangana *et al.*, 2006) where increase in rooting percentage of leafy stem cuttings was observed by combining the factors of clone and rooting media.

Leaf surface area was directly proportional to rooting ability, with $100cm^2$ having the highest rooting and shooting percentages and 0 cm^2 having the least rooting and shooting percentages from the 7^{th} to 40^{th} day across all EGCs. This showed that the higher the leaf area, the better the rooting and shooting across all the rooting media. This demand for a leaf has been reported by Tchoundjeu *et al.*, (2002) for *Prunus africana*, Shiembo *et al.*, (1996a) for *I. gabonensis*, Newton and Jones (1993a) for *Terminalia spinosa*, Aminah, (1991) for *Hopea odorata* and *Leakey et al.*, (1982) for *Triplochiton scleroxylon*. The importance of leaf areas on the rooting ability of cuttings of tropical species is well known for many species, and has been reported to influence the balance between assimilate gain by photosynthesis and water loss by transpiration (Leakey and Coutts, 1989) in the tropical hardwood *T. scleroxylon*. Many studies have suggested that the supply of current assimilates produced by active photosynthesis during propagation is essential for adventitious roots production (Leakey and Coutts, 1989; Newton and Jones, 1993a). This could explain the findings reported here.

Recent measurements of photosynthesis in *E. grandis* cuttings during the rooting process have demonstrated a strong relationship between rooting success and net photosynthetic rate and stomatal conductance (Hoad and Leakey, 1996), which is also affected by adaptations to pre-severance stockplant environment (Hoad and Leakey, 1994). It can be hypothesised that the differences between species in optimum leaf area reflect adaptations to light environments (quantum flux efficiency, chlorophyll density, stomatal density, leaf size and specific leaf area), perhaps depending on the species position in ecological succession (Tchoundjeu and Leakey, 1996).

Some studies have reported the inability of leafless cuttings to root. Studies conducted by Tchoundjeu *et al.*, (2002) show that leafless cuttings of *P. africana*, did not root and rapidly become moribund. This was also the case for *P. johimbe* (Tchoundjeu *et al.*, 2004), and many other temperate or tropical species, e.g. *T. scleroxylon* (Leakey *et al.*, 1982), *Khaya ivorensis* (Tchoundjeu and Leakey, 1996), where rooting ability is affected by leaf area and leafless cuttings did not root. In this study, EGC cuttings with no leaves rooted. The ability for leafless cuttings of EGCs to root indicates that they are a resilient species that are easy to root.

Rooting was observed with leafless cuttings of EGC hybrids (although very low) probably due to both current assimilates and carbohydrates produced by the

coppice before harvesting was stored in the stem. This may have influenced rooting as what was observed with *M. excelsa* (Ofori *et al.*, 1996). In *T. scleroxylon*, the inability of leafless cuttings to root was associated with rapid depletion of carbohydrates in the stem (Leakey *et al.*, 1982).

In this study, variation in leaf area had significant effects on the final rooting percentage of leafy cuttings, contrasting with the results obtained from *T. spinosa* (Newton and Jones, 1993a) and *N. diderrichii* (Leakey, 1990). Increase in leaf area also led to an increase in number of shoots and roots. This is in agreement with results obtained for *K. ivorensis* (Tchoundjeu and Leakey, 1996) and *Gnetum africanum* (Shiembo *et al.*, 1996b) but in contrast with results obtained from *I. gabonensis* (Shiembo *et al.*, 1996a) and *P. johimbe* (Tchoundjeu *et al.*, 2004) where rooting of these tree species has been shown to be sensitive to variation in leaf area, but no beneficial effects on mean number of roots per cutting was found following leaf reduction.

Previous research with *T. scleroxylon* under controlled environment conditions has identified an optimum leaf area of around 50^2cm (Leakey *et al.*, 1982; Leakey and Coutts, 1989). According to Nketiah *et al.*, (1998), significantly higher rooting percentages were obtained with a leaf area of 100 cm^2 than the other areas tested in the same *T. scleroxylon*. The propagation protocol transferred from South Africa had set a leaf area of 50 cm^2 as the ideal leaf area under mist propagation

system. However, in the non mist propagation system (tunneling) $100cm^2$ gave the best results. It would be interesting to investigate the leaf area that would give maximum rooting potential above $100cm^2$.

These conflicting results may be attributed to the different propagation environments employed in these studies. The non-mist propagation system used in the investigation with Nketiah *et al.*, (1998), appears to be at least as effective at maintaining high relative humidity around the cuttings, as the more conventional mist system used by Leakey *et al.*, (1982). As a result, larger-leaved cuttings in the non-mist system may be less often subjected to the severe water deficits which can arise during propagation Newton and Jones, (1993b). The same can also be said for the EGCs where larger leaved cuttings in the tunnels were subjected to less water deficits due to the high relative humidity.

Reduction in production costs of EGC hybrids through the use of the tunneling system

The technology adopted from South Africa for propagating the Eucalyptus hybrid clones is very advanced for the rural farmer to adopt. This system requires the use of electricity and a reliable source of water. The misting of cuttings is computerized and the watering is programmed to mist the cuttings after every few minutes, to keep the cuttings moist preventing desication. A stand by generator is

therefore necessary in case of a power blackout. The water used requires filtering so as to prevent the misters from getting blocked with particles. This necessitates the control of water quality. Skilled labour is therefore required to manage this system.

The media used for rooting the cuttings in the mist propagation system is synthetic (peat and vermiculite) raising production costs, and is not readily available for farmers in the rural areas. In addition to this rooting of some varieties of the EGC hybrids in this system is low, with an average of 50% further raising production costs.

On the other hand, the tunneling system is a simple and cheaper method of propagating Eucalyptus hybrids. The infrastructure used is cheap and the inputs such as clay sub soil and polyethene sheets are readily available. Since it is a non mist propagation system, the use of electricity is eliminated. Watering is done fortnightly, greatly reducing the quantity of water used compared to the mist propagation system.

The rooting success of EGC hybrids in this system is higher than that of mist propagation system. In addition to this, results from this study show that the application of IBA was not necessary for the rooting of EGC cuttings. This will further reduce production costs. The cost of production of a cutting propagated under the tunneling system is Ksh 6 while that of cutting propagated under the

mist propagation system is Ksh 10. The difference in cost is due to the use of cheaper inputs and higher rooting percentages in the tunneling system.

5.1 CONCLUSION

EGCs are amenable to vegetative propagation under the tunneling system. Overall, the best media that would give high number of shoots and roots as well as rooting percentages is clay sub soil. This is due to its high water holding capacity as opposed to sand soil which recorded very low rooting percentages due to its low water retention capacity.

Results from this experiment show that leaf area of $100cm^2$ gave the best results. It is not clear whether these results could be improved by increasing leaf areas as this was not investigated.

EGC hybrids are well supplied with endogenous auxins, hence their ability to root without the application of auxin. The application of IBA in propagating the clonal hybrids would appear to be unnecessary either in terms of root number or rooting percentage. This will further reduce the cost for propagation of EGC hybrids. It would appear that varying the clay sub soil and sand in the rooting media could be a simple way of carrying out geographic mapping for the different EGC hybrid clones

Results from this study provide a useful tool in reducing production costs as the current green house mist propagation technology adopted from South Africa is too expensive for small scale farmers to adopt. The tunneling system which is a non mist propagation system, eliminates the need for electricity, reduces the amount of watering to once in two weeks, eliminates the use of IBA and has higher rooting success compared to the mist propagation system. The use of locally available material coupled with the high rooting and shooting percentages recorded in some EGC varieties makes the tunneling system an economical farmer friendly method of propagation of EGC hybrid clones.

5.2 Recommendations

Results from this experiment indicate that commercial propagation of EGCs under the tunneling system is realistic, especially in the rural areas, given the high rooting percentages achieved. This will eventually lead to increased forest cover.

Further work based on research questions that arose form this study is required to:

- Investigate the effect of increasing leaf surface area beyond $100cm^2$ on rooting and shooting of EGCs
- Investigate the effect of increasing the rooting duration beyond 40 days
- Investigate further, the aspect and possibility of geographic mapping of the different EGC hybrid clones by using different ratios of clay subsoil and sand soil

- Investigate post rooting performance of the different EGC hybrid clones in the different media used in the study
- Investigate the effect of nutrient status and pH of the media on the rooting of EGC hybrid clones before the cuttings are planted
- Optimise the use of tunnels instead of mist propagation system to reduce costs and increase number of cuttings produced, on a large scale, in Karura and other geographical areas

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APPENDIX I: Logistic regression model

The logistic regression model is applicable when we have a binomial observations of the form y_i/n_i, for i=1,2….n where y_i is number of successes out of n_i Bernoulli trials. The expected value of the random variable associated with the observations y_i is given by $E(Y_i) = n_i p_i$ where p_i is the corresponding response probability and Y_i is the random variable of the y_i success. It then follows that the linear logistic model for the dependence of a p_i on the values $x_1, x_2, \ldots x_k$ of k explanatory variable $X_1, X_2 \ldots X_K$ is such that

$$Logit\ (P_i) = \log\ (p_i\ /1-p_i) = \beta_0 + \beta_1 x_{1i} + \ldots\ldots + \beta_k x_{ki} \ldots\ldots (1)$$

where β_0 is the constant parameter $\beta_1, \beta_2, \ldots\ldots \beta_k$ are the parameter coefficient of the corresponding explanatory variables (Collet, 2003, pp 58).

On some re-arrangement

$$p_i = Exp\ (\beta_0 + \beta_1 x_{1i} + \ldots\ldots + \beta_k x_{ki})\ /\ [1 + exp\ (\beta_0 + \beta_1 x_{1i} + \ldots\ldots + \beta_k x_{ki})] \ldots\ldots (2)$$

On fitting the model, the unknown parameter coefficients of the explanatory variable have first to be estimated using the methods of maximum likelihood whose function is given by

$$L\ (\beta) = \log\ L\ (\beta) = \prod_{i=1}^{n} (n_i C y_i + p_i^{yi}\ (1-p^i)^{ni-yi} \ldots\ldots (3)$$

Where $\prod$=Product and C= combination

This likelihood depends on the unknown success probability p_i, which in turn depends on the parameter coefficient of the explanatory variables. Maximizing the likelihood function and taking the derivatives with respect to β results to a set of k+1 unknown equations equated to zero, which can be solved numerically providing the estimated β coefficients of the explanatory variables. At this stage the estimated coefficient do not directly indicate the effect of change in the corresponding explanatory variable on probability (p_i) of the outcome occurring, rather the coefficients reflects the effect of individual explanatory variable on its log of odds which can be either a positive or negative value. The positive coefficient means that the log of odds increases as the corresponding independent variable increases and vice versa. Consequently if the log of odds is positively (negatively) related to an independent variable both odds and probability p_i of the outcome are also positively (negatively) related to that variable). The only difference is that this relationship is linear for the log of odds and non linear for odds and probability of the outcome.

Following the general overview of the logistic regression model development, data generated from each experiment will be modeled independently. For instance the effect of rooting media on rooting will be analyzed by letting p_{ijk} be the probability of a cutting rooting under i^{th} *EGCs*, j^{th} propagation media and k^{th} IBA concentration, for i = 1,2,3…12 ; j = 1, 2,..5 and k=1, 2….4. Then the logistic regression model characterizing the rooting of *EGCs* shoot cutting and correctly predicting the category of outcome for individual cases is given by.

$$Logit\ (p_{iik}) = log\ (p_{ijk}/1\text{-}p_{ijk}) = \beta_0 + \beta_{1i}\ EGcs + \beta_{2j} Rom.2_j + \beta_{3k} IBA_{3k} \quad \ldots\ldots\ldots (4)$$

where β_0= constant parameter

β_{1i} = Coefficient parameters associated with various EGCs

β_{2j}= parameter coefficient associated with the rooting media

Rom. = rooting media

β_{3kj}= Coefficient parameters associated with various levels of IBA

IBA= IBA concentrations.

It then follows that all models fitted for hormonal concentration (IBA. leaf area and number of buds would apply the same procedure as in model 4.

Wald test statistic for testing the statistical significance of each coefficient (β) in the model would be used. However, Menard (1995) warns that for large coefficient the standard errors are inflated lowering the Wald statistics (chi- square) value. Consequently, R- squared (R^2) would be used to measure percentage of deviance explained or accounted by the explanatory variables in the model.