PREREQUISITES FOR BIOCROPS UP-SCALING II: AN ASSESSMENT OF THE VEGETATIVE METHOD OF PROPAGATION FOR OILFEROUS PLANT SPECIES WITH POTENTIAL FOR BIODIESEL PRODUCTION

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ABSTRACT

The rooting ability of hardwood cuttings from six selected non- edible oilferous plant species with potential for biodiesel production namely Telfairia pedata, Jatropha curcas, Excoecaria bussei, Croton macrostachyus, Croton megalocarpus and Ricinus communis was assessed on 4 different potting media i.e. forest top soil, sandy, clay and loamy soil. The proportion of cuttings that rooted were evaluated for the root numbers, root length, root dry weight, sprouting and callus formation. Complete randomized design (CRD) was adopted for the experiments and each of the species was replicated 4 times using 5 hardwood cuttings per plot (5 L capacity pots). An ANOVA was computed to test for the significance of variations between all treatments while Tukey-Kramer Multiple Comparisons test was used to test for the differences between treatment means. Jatropha curcas rooted well both in a non-mist propagator and open nursery pots, Telfairia pedata rooted only in the nursery pots, Excoecaria bussei sprouted in both non-mist propagator and open nursery pots but did not root throughout the entire experimental period of 3 months. Croton macrostachyus, Croton megalocarpus and Ricinus communis neither sprouted nor rooted calling for propagation methods other than using hardwood cuttings. Possibly all Excoecaria bussei cuttings which callused would have eventually differentiated into roots had the experiment been allowed to run for more than 3 months.

Key Words: Propagation, rootability, non-mist propagators, *Excoecaria bussei*, *Telfairia pedata*

INTRODUCTION

Provision of fuel energy sustainably is posing global threat and Tanzania is not an exception to this threat. Promotion of oil producing plants species with potential of producing biodiesel has been given a research priority by University of Dar es Salaam (UDSM) under PISCES project since 2005 from which plant species whose oil quality more or less parallels that of biodiesel is in progress. Among these are *Telfairia pedata, Croton macrostachyus, Croton megalocarpus, Ricinus communis*, *Legenaria cenerea* and *Excoecaria bussei. Telfairia pedata* is currently grown in solitary stands around homesteads for local ethnic culinary diets mostly in Kilimanjaro region, *Croton species* grown in Tanzania are found mostly in ornamental settings, *Ricinus communis* in Tanzania grows as a succession plant in the open wild or disturbed and abandoned fields while *Excoecaria bussei* is still in the wild in rocky areas of the miombo woodlands (Moshi *et al*. 2010).

If oil from the above mentioned plant species has to be sustainably harvested for biodiesel production, these plant species must be cultivated on a large scale. For this to happen, they have to be produced *en mass* to satisfy propagule requirement by potential growers. Unfortunately, there is limited information on their most appropriate and rapid methods of propagation. This gap justified conduction of this study whereby vegetative method of propagating these species using cuttings was experimented.

A cutting is any detached plant part which when given favourable conditions can regenerate new roots and consequently a new plant identical to the parent plant. Cuttings can either be leaf bud cutting, soft wood, semi-hardwood, hardwood or root cutting depending on where on the parent plant a cutting is severed (Hartman *et al*. 2002). The type of cutting selected and rooting success normally depend on the environment, season and the type of plant species in question and may be promoted by treatments done to the cuttings including wounding and hormones (Nyomora and Mnzava 1982; Amri *et al*. 2008; 2009; 2010) as well as cutting length, number of nodes and number of leaves (Leakey *et al*. 1990), use of mist propagators (Magingo *et al*. 2001) or use of mycorrhizae (Washa *et al*. 2012).

Planting media on the other hand, play an important role in enhancing soil physical and chemical properties and thereby increasing the penetrating capacity of roots in the planting media. When suitable environment with proper aeration, sufficient water and nutrient availability was offered by the medium, excellent roots system developed which in turn resulted in the luxurious growth of plants (Neelam and Ishtiaq 2001). Henley (1974) found root penetration of two *Pilea* species grown in light soil, timely grounded sphagnum peat moss were four times greater than that of plants grown in compact peat moss. Similarly shoot lengths were 50% greater in non-compact treatments.
The objective of this study was to assess the performance of the identified oil producing plant species with potential of producing biodiesel under vegetative method of propagation and whether using cuttings could be a viable method for their large scale production.

MATERIALS AND METHODS

Experimental Site and Source of Planting Material

Cuttings from four of the recommended oil producing plant species, *i.e.*, *Ricinus communis*, *Telfairia pedata*, *Jatropha curcas* and *Excoecaria bussei* were harvested from Manyara, Moshi, Arusha and Iringa regions in August, 2009. *Teilfairia pedata* cuttings were from solitary vines found around homesteads in Moshi. *Ricinus communis and Excoecaria bussei* were reaped from plants growing in the wild in Arusha, Manyara and Iringa regions in August 2009. Respective stem cuttings were excised from mature shoots of donor plants and packed into wet old newspapers which were then enclosed in cool boxes (10 °C), and transported to UDSM nursery site where the experiment was conducted.

Experimental Design and Treatments for Assessment of Potting Media

Complete randomized design (CRD) was adopted for the vegetative propagation experiments. Each species was replicated 4 times and 5 cuttings per species measuring 30 cm long were inserted into the media to the depth of at least one node in 5 L capacity pots. Each type of media was also replicated. The potting media tested were: Forest top soil which was mined from a nearby natural forest, clay soil from a nearby anthill, sand soil from a nearby dry rain water stream and loam soil which was composited from sand, silt, and clay soils in a ratio of 2:2:l by volume. Both non-mist propagator and open nursery were used for each set of treatments. The cuttings were sprayed with a fine jet of water whenever the no-mist propagator was opened to maintain high humidity. A cutting was considered rooted if it had at least one primary root ≥ 1 mm long.

After three months, the proportion of cuttings that had rooted was evaluated for the root numbers, root length, root dry weight and callus formation. Destructive sampling was done by over irrigating the pots and shaking the soil loose off the plant roots. Measurements of root dry weight were made after thoroughly loosening the soil clinging to the roots using a pressurized spray of irrigation water. All cut root material were collected and thoroughly dried at 85 °C until attainment of constant weight and then weighed.

Data Analysis

ANOVA test was used to test the significance of the variations between all treatments, and Tukey-Kramer test was used to test the differences between treatment means. Tables and figures were used to summarize the data. The significance level for all tests was 5% (α = 0.05).

RESULTS

Subsequent results are from the non-mist propagator because rooting in the open nursery setting was very poor.

Number of Roots Formed 3 Months after the Experimental Setting-up

Telfairia pedata had significantly more roots than other species in all soil types varying from 84, 81, 52 and 50 in forest top soil, loam soil, clay soil and sand soil respectively while *Ricinus communis* had significantly fewer number of roots in all soil types ranging from 33, 26, 25, and 15 in loam soil, clay soil, forest top soil, and sand soil respectively. *Excoecaria bussei* and *Jatropha curcas* root numbers were not statistically different in sandy and clay soils but the two species were significantly different in forest and loamy soils (Figure 1 a, b, c, d).

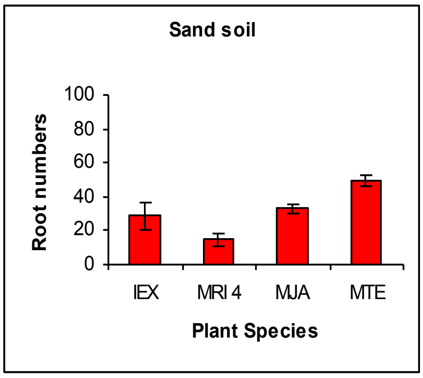
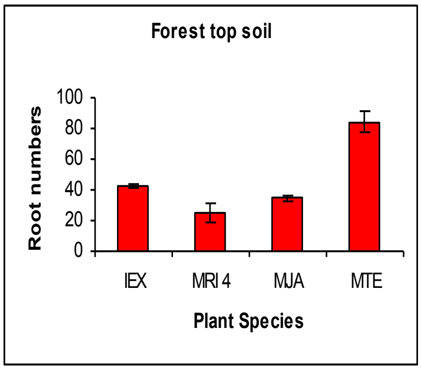


Figure 1: a



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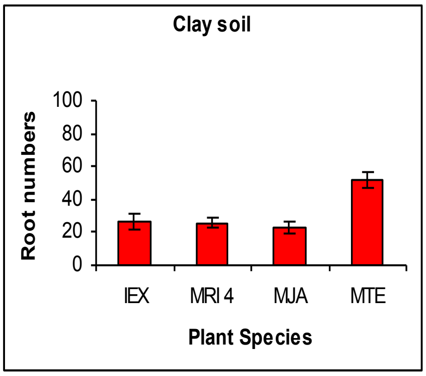

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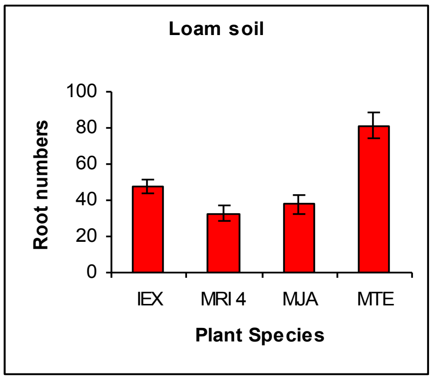


Figure 1: d

Figure 1: a, b, c, d: Number of Roots Formed in Different Soil Types 180 Days after planting the cuttings. IEX= *Excoecaria bussei,* MRI 4=*Ricinus communis*, MJA= *Jatropha curcas*, MTE= *Telfairia pedata.*

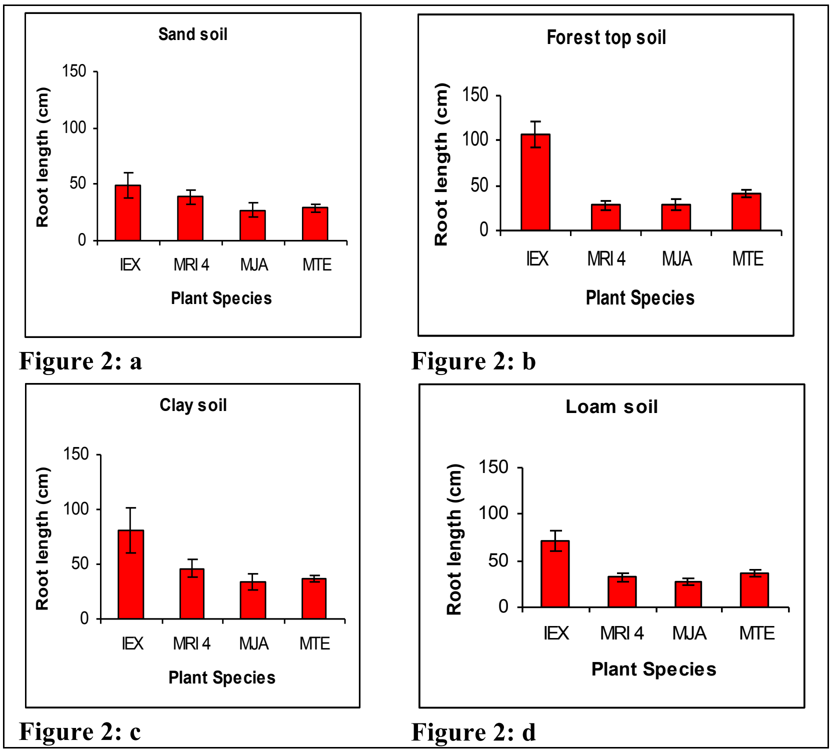


Figure 2 a, b. c. d: Root Length of Roots Formed in Different Soil Types 180 Days after planting. IEX=*Excoecaria bussei,* MRI 4= *Ricinus communis*, MJA= *Jatropha curcas*, MTE= *Telfairia pedata.*

Root Length of the Formed Roots

Results on root length showed that, *Excoecaria bussei* had significantly the longest roots in all soil types ranging from 106.8 cm, 80.3 cm, 70.8 cm and 48.8 cm in forest top soil, clay soil, loam soil and sandy soil respectively while *Jatropha curcas* had shortest roots in all soil types varying from 34.3 cm, 28 cm, 27.3 cm, and 27 cm, in clay soil, forest top soil, sand soil and loam soil, respectively as shown in figure2. Root lengths for *Ricinus communis*, *Jatropha curcas* and *Telfairia pedata* were not significantly different in all soil types (Figure 2).

Roots Dry Weight

Jatropha curcas had highest root dry weight in all soil types. *Ricinus communis* had lowest root dry weight in all soil types 5.5 g, 2.4 g, and 2 g, in forest top soil, clay soil and sand soil respectively except in sandy soil where *Telfairia pedata* had lowest root dry weight of 5.7 g. (Figure 3 a, b, c and d). Clay and sandy soil did not confer much seedling growth as evidenced by relatively lower root dry weight (Figure 3) and total plant biomass (Figure 4) than forest top soil and loam soil.

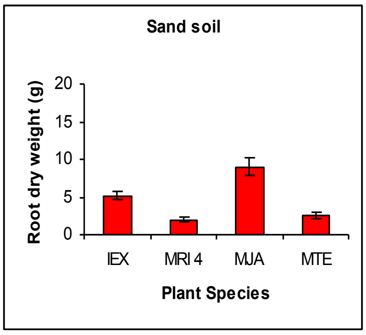
Total plant biomass was highest for *Jatropha curcas* in loam and forest soil i.e. 70 to 75 respectively followed by *Telfairia pedata* in the same media. Total plant biomass was lowest for *Ricinus communis* and *Excoecaria bussei* mostly in sandy and clay soil.

Seedlings Shoot/Root Ratio

All tested species had different shoot/root ratio biomass in different soil types where by *Telfairia pedata* had highest shoot/root ratio of 7.8, 6.8, 6.6, and 5.1 in sandy, forest, clay and loam soil respectively. *Excoecaria bussei* had the lowest shoot/root ratio in almost all soil types (Table 1).

Correlation of Different Parameters of Cuttings

As shown in Table 2, root numbers significantly and positively correlated with root length (α = 0.01) and callusing (α = 0.05), while root length significantly and positively correlated with callus formation at α = 0.01. Insignificant correlation was revealed between root characteristics with sprouting or between sprouting and callus formation (Table 2).



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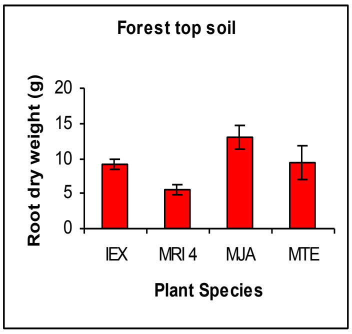

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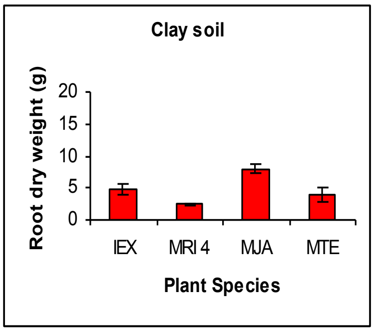

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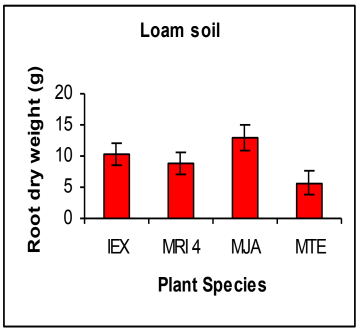
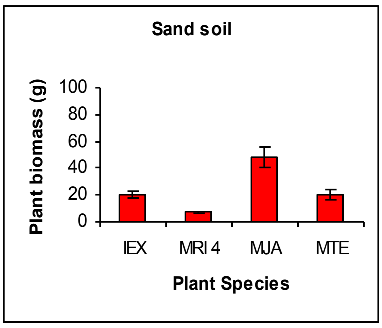


Figure 3: d

Figure 3: Root Dry Weight 180 Days after germination. (a) Loam soil, (b) Forest top soil, (c) Sand soil and (d) Clay soil. IEX= *Excoecaria bussei,* MRI 4= *Ricinus communis*, MJA=*Jatropha curcas*, MTE= *Telfairia pedata.*



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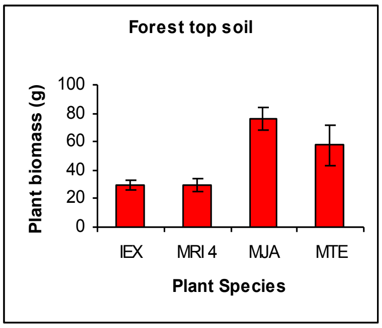

Figure 4: b

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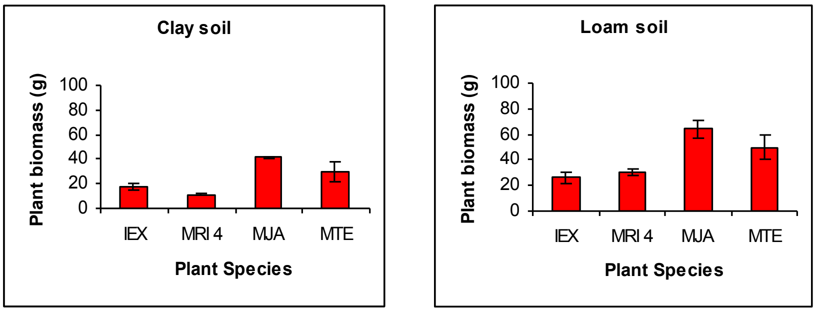

Figure 4: c **Figure 4: d**

Figure 4: Seedling Total Plant Biomass 180 Days after planting the cuttings. (a) Sand oil, (b) Clay soil, (c) Loam soil and (d) Forest top soil IEX= *Excoecaria bussei,* MRI 4= *Ricinus communis*, MJA= *Jatropha curcas*, MTE= *Telfairia pedata.*

Table 1: Shoot/Root Ratio of Seedlings Biomass of the Tested Plant Species in Different Media

	Shoot dry wt. (g)	**Root dry wt. (g)**	**Shoot/Root ratio**
Sand soil			
Excoecaria bussei	15.2	5.2	2.9
Ricinus communis	5.1	2	2.6
Jatropha curcas	38.8	9	4.3
Telfairia pedata	17.6	2.6	6.8
Clay soil			
Excoecaria bussei	12.7	4.8	2.6
Ricinus communis	8.6	2.4	3.6
Jatropha curcas	33.6	8	4.2
Telfairia pedata	25.7	3.9	6.6
Loam soil			
Excoecaria bussei	15.8	10.3	1.5
Ricinus communis	21.7	8.8	2.5
Jatropha curcas	51.2	12.9	4.0
Telfairia pedata	44.4	5.7	7.8
Forest soil			
Excoecaria bussei	20.7	9.2	2.3
Ricinus communis	24.2	5.5	4.4
Jatropha curcas	63.3	13	4.9
Telfairia pedata	48	9.4	5.1

Table 2: Correlation Coefficients of Propagules Rooting and Sprouting Parameters

Parameters	Root numbers	Root length (cm)	Sprouting %	Callusing %
Root numbers	-	0.991**	0.476	0.870*
Root length (cm)	0.991**	-	0.399	0.928**
Sprouting %	0.476	0.399	-	0.145
Callus formation %	0.870*	0.928**	0.145	-

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The need to determine root length and numbers in this study was meant to assess for potential capacity for nutrient and water uptake from the surrounding medium. Roots also anchor the plant in place to keep it from being washed away or toppling over, so a health root system is required for optimal plant growth. Rooting by stem cutting is one of the most cost effective vegetative propagation methods for mass multiplication of species. Stem cuttings have the desired genetic constitution of the plant species and also since cutting are taken from an adult plant, propagation using cuttings would induce flowering and fruiting much earlier than seedlings. In addition, it is also most useful for plant species whose seeds are difficult to germinate.

Results on the number of roots showed that, *Telfairia pedata* produced many roots in all soil types followed by *Jatropha curcas* and *Excoecaria bussei* while *Ricinus communis* had fewer roots in all soil types except in clay soil. *Excoecaria bussei* had the longest roots in all soil types followed by *Telfairia pedata* and *Ricinus communis* while *Jatropha curcas* had the shortest roots in all soil types. Further analysis indicated that root number and root length were correlated; species with fewer root numbers had longer roots. While this would compensate for root length density, there is still difference in the levels of soil volume to be mined for water and nutrients.

Shoot to root ratio showed that *Telfairia pedata* had the highest shoot/root ratio indicating greater proportion of dry mass allocated to the shoots while *Excoecaria bussei* had the lowest shoot/ root ratio meaning more allocation of carbon to roots than to shoots. *Telfairia pedata* which is a vine would allocate more carbon to shoots in order to reach supporting canopy to get sunlight needed for photosynthesis while the other species would invest more in roots to search for water and nutrients in poor soils where it inherently grows. The effect would be that the proportionately greater shoot mass in *Telfairia pedata* would perhaps make the recruited seedlings less tolerant to drought and/or nutrient deficiency while the opposite would be true for *Excoecaria bussei*.

Jatropha curcas and *Telfairia pedata* rooted well in all soils, *Excoecaria bussei* species sprouted but they did not root at all. This is in agreement with other studies on vegetative propagation which have shown that flushing of buds and shoot growth is not necessarily a good indicator of root development (Puri and Shamet 1988). During vegetative propagation, early differentiation and growth of leaf buds is dependent on food reserves available in the cutting (Duguma 1988). Reports by Davis and Jacobs (2005) revealed that during early establishment of rooted cuttings, energy allocation might be strongly biased towards roots, and therefore shoot growth may reduce root formation because of competition for nutrient reserves. Also Bhatt and Todaria (1990) reported that where root formation lags behind shoot formation, survival of cuttings becomes poor due to assimilation deficiencies and risk of drying due to evapotranspiration. Shoot production therefore may be considered to be an indicator of metabolic activity within the cutting rather than being causally related to root emergence in stem cuttings for vegetative propagation.

Croton macrostachyus, *Ricinus communis*, and *Croton megalocarpus* neither sprouted nor rooted. This is in accordance to other reports (Mbuya *et al*. 1994) that *Croton macrostachyus*, *Ricinus communis*, and *Croton megalocarpus* species are mostly propagated by seeds and wildings and that there are no reports available on vegetative propagation of *Ricinus communis*.

In this study the root length correlated well with root numbers. Greater root length and high number of roots per rooted cuttings suggested well developed root system which is a good indicator of high plant performance in the field. Planting stock with good root system would therefore confer better adaptation in the field and ultimately resulting in better survival and growth. The beneficial effect of long roots is to allow uptake of water and nutrients beyond the initial depletion zone.

Callus percentage correlated positively with root number as well as root length and this as reported elsewhere (Meng *et al*. 2009) arises due to the fact that callus which is an undifferentiated mass of cells is the precursor of adventitious root formation and therefore should be correlated positively with root initiation and growth. The formation and development of callus on cuttings is normally a prerequisite to differentiation of roots on cuttings. Possibly all cuttings which callused would eventually differentiate into roots if more time was given.

In conclusion, for the studied plant species, only *Jatropha curcas* and *Telfairia pedata* can easily be propagated vegetatively using standard method of hardwood cutting while *Excoecaria bussei, Ricinus communis*, and *Croton megalocarpus* cannot be easily propagated using this method for large scale propagule production. This warrants investigation of other means of propagation such as softwood cuttings and as well as propagation using seedmicropropagation.

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