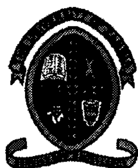

THE UNIVERSITY OF ZAMBIA

Micropropagation of Lusala (*Dioscorea hirtiflora*)

Dissertation submitted in partial fulfilment of requirements for the award of the BACHELOR'S OF SCIENCE DEGREE OF AGRICULTURAL SCIENCES.

Gerald Serenje

SCHOOL OF AGRICULTURAL SCIENCES

DEPARTMENT OF CROP SCIENCES

November 2002

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DEDICATION

To my mother, Ms. Susan Phiri, my brother and sisters, and to my daughter Rachel.

ACKNOWLEDGEMENT

Sincere gratitude is extended to those who assisted in the study, M. Mataa (Ph.D.), as Project Supervisor, K. Munyinda (PhD.) who helped with the analysis of the data, Mr. B. Mwenebanda (Msc. Thesis)- unpublished that helped me in developing the study. Lastly, to Ms. O. Kamanga and Mr. J. Phiri whose presence and advice in the Tissue Culture Laboratory made my work possible.

ABSTRACT

The research study was carried out in the Tissue Culture Laboratory of the Crop Science Department at the School of Agricultural Sciences of the University of Zambia. The objective of the study was to evaluate micropropagation as a method of propagating Lusala (*Dioscorea hirtiflora).* The experiment was designed as a split plot with landrace as main plot and media as the subplot. Four landraces, Mongu, Chongwe, Choma and Namwala – were evaluated in five different media namely; (a) Plain MS, (b) MS + 2.5 ppm BAP (BAP (c) MS + 1.5 ppm BAP (d) MS + 1.5 ppm BAP + 1.5 ppm NAA (e) MS + 1.5 ppm NAA. The explants used in the study were axillary buds. The experiment was conducted from March 2002 to September 2002, the following parameters were measured: shoot proliferation, regeneration and contamination.

The results showed that landraces and media gave different responses to the measured parameters. Namwala had high response at 0.88 shoots per explant, 1.24 shoot proliferation per explant, 0.18 explant responded to rooting and 0.12 explants got contaminated. The least response on landraces was Chongwe at 0.4 shoots per explant, 0.42 shoot proliferation per explant, no rotting and 0.24 explant got contaminated.

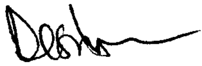
Media MS + 1.5 ppm BAP + 1.5 ppm NAA and MS + 1.5 ppm NAA had high response at 0.725 shoots per explant, 1.025 shoot proliferation per explant, 0.2 roots per explant and 0.1 explant got contaminated. Hormone free media had the lowest response at 0.475

shoots per explant, 0.275 shoot proliferation, no rooting and a high contamination rate at 0.35 per explant.

APPROVAL

The undersigned herewith certify that this dissertation entitled 'Micropropagation of Lusala (*Dioscorea hirtiflora*)' was not previously presented for the award of a degree, and the work contained herein were carried out by GERALD I. SERENJE under my supervision. The dissertation hereby submitted to the School of Agricultural Sciences in partial fulfilment of requirements for the award of the Bachelor's degree of Agricultural Sciences in Crop Science is accepted.

Approved by



M. Mataa (PhD)

Chapter 1

1.0 Introduction

Yams *(Dioscorea spp.)* are perennial climbing plants with edible underground tubers and alternate leaves. Yams have a fibrous root system and are slightly more drought resistant than cassava. Yams are obtained from several species of the genus Dioscorea, a very large genus of climbing plants widely distributed throughout the tropics. The genus is the largest and most important of the ten genera which are included in the family Dioscoreaceae, a monocotyledonous family derived from the Liliaceae, comprising a group of broad-leaved climbing plant with tuberous roots and small, often unisexual flowers Flowering and seed production is very irregular (Cobley, 1963).

More than 95% of the world's yams are currently grown in sub-Saharan Africa, with the remainder grown in the West Indies and parts of South and Central America. There are over 600 yam species grown throughout the world. Production of yams in Africa is largely confined to the 'Yam Zone', comprising Cameroon, Nigeria, Benin, Togo, Ghana and Cote'd'voire where approximately 90% of the world's production takes place (John- Libbey, 2002). According to FAO (2002) statistics, 37.5 million tonnes of yams were produced world wide in 2000, 96% of this in Africa. The leading producer was Nigeria with 26 million tonnes, followed by Ghana with 2-9 million tonnes (John-libbey, 2002). Tropical areas that have long rainy season are best for this crop. Temperatures of 25-30°C or more are optimum for growing yams. Below 20°C the growth is stunted and yams cannot tolerate frost. Yams require a loose, fertile soil. Traditionally, the crop is grown on mounds as well as ridges (Soenarjo, 1995). The tuber is rich in

starch. They may also contain varying amounts of a poisonous alkaloid, dioscorine that is present in considerable quantities in some species. In many of the edible types the small amounts present are easily destroyed by boiling (Cobley, 1963).

Yams are used locally as a vegetable and are always cooked in some form or another since they leave an unpleasant effect in the month and throat if eaten raw (Cobley, 1963). John-Libbey (2002) reported that the major constraints to yam production were weeds, lack of planting and staking materials, costs of labour, storage problems and pest and diseases. During the first 4 months of growth, weeds are a serious problem. Weed competition during this period may reduce yields by as much as 43% (John-Libbey, 2002). Low availability and the high cost of planting material is a major constraint for yam production in Africa. Planting material may account for up to 50% of the cost of the production. About 10 000 seed yams are needed to plant a one hectare field. Lack of staking material can reduce the production of large yam setts that produce plants with extensive shoot systems. Many aspects of yam production – planting, weeding, staking, and harvesting – require considerable amounts of labour. Studies indicate that the cost of labour account for over 40% of yam production costs.

The storage life of yams is linked to their dormancy. After harvesting yams enter into dormancy after which they begin to sprout and quickly lose their dietary value. The high moisture content of yams (70-80%) makes them especially susceptible to attacks by microorganisms while in storage. Current theories envisage dormancy as either from an excess of growth inhibiting over stimulating

substances. There could be some endogenous inhibitors. Gibberellins are often credited with being the promotive agents that break dormancy. The buds of vegetative prop gules, such as tubers require long periods of exposure to low temperatures (below 3-4°) before they will resume growth (Milthorpe and Moorby, 1981).

Many pests affect yams; such as insects, nematodes, fungal and pathogens; bacterial diseases and viruses, which either single, or in combination reduce yields as well as deterioration of the quality of the tuber in storage. The major insect and nematodes pests are yam beetles *(Prionorycytes caniculus)*, yam crickets (*Gymnogryllus lucens*), mealy bugs (*Planococcus citri*), yams scale insects *(Aspidiella hartii)*, yam weevil *(Palaeopus dioscorae)* yam tuber beetle (*Heterligus meles*), yam nematode *(Scutellonema bradys)*, root-knot nematode *(Meloidogyne spp.)* and lesion nematode *(Pratylenchus spp.)*. Yam anthracnose *(Glomerella cingulata)* (Waller and Hill, 1988), leaf spot caused by *Cercospora* spp., leaf blight, crown gall disease, tuber rot, bacterial rot, yam mosaic virus, and water yam virus are the most important diseases. Pierik (1987) defines *in vitro culture* as the culture or media under sterile conditions of plants, cells and protoplasts of plants, seeds, embryos, organs, tissues and explants. Since yams are commonly vegetatively propagated, micro propagation may be possible *in vitro.* To conduct micro propagation, nutrient media all of different components can be evaluated. Media is generally composed of inorganic salts, plant growth hormones, vitamins, amino acids, supplementary carbon sources, water and the medium matrix. However, the choice of a particular medium depends mainly on

the species of the plant, the tissue organ to be cultured and the purpose of the experiment (Pierik, 1987).

In vitro propagation has been used in agriculture, for instance with potatoes (*solanum tuberosum*), Apples (*Malus domestica*), Pears (*Pyrus communis*), Tea (*Camellia sinensis*), sugarcane (*Saccharum officinarum*), many ornamental bulbs and tuberous plants. There are a number of advantages of micro propagation. It allows the rapid multiplication of planting materials. Almost any part of the plant can be used and small pieces of plants can be used to produce a large number of plants in a small space within a relatively short time. It is possible to produce plantlets that are normally free of fungi and bacteria and in some cases also viruses. Micro propagation has an advantage over conventional breeding methods. Especially with unpredictable weather conditions, there is perfect conditioning (nutrient media and physical conditions enabling precise timing of cutting production, the effect of seasons can be eliminated and year round production achieved seeing that conventional methods are highly season dependent. Micro propagation is cheaper in terms of labour, planting material and space and also reduces disease incidence, consequently the cost on pesticides since the propagules are disease free.

Lusala *(Dioscorea hirtiflora)* is an important traditional relish used alone or in combination with other relish preparations in Zambia, especially Central and Southern Provinces. The mode of crop harvesting entails uprooting the whole plant. Over exploitation, land clearing and cultivating has reduced *Dioscorea hirtiflora* in the wild. Therefore, there may be need to start cultivating the species to prevent it from getting extinct and to explore its commercial value (Mingochi,

2001). *In vitro* culture may be one approach to relieve pressure on the wild gene pool and ensure sustainable use.

1.2 Objectives

The overall objective was to evaluate rapid and cost effective methods of propagating Lusala. Specifically the study evaluated different media for propagating (shoot develop and rooting) Lusala and response of different ecotypes of Lusala to these media.

Chapter 2

LITERATURE REVIEW

Yams are economically important food staple crop, grown mainly in the humid and sub humid tropics. They constitute a multi species, polyploid crop, which is vegetatively, propagated from whole tubers or tuber pieces (Degras, 1993). The basis of the popularity of yams is their eating quality (Dorosh, 1988; Nweke et al, 1992). Yams are important not only for farm household food security, but also as a source of cash income (Mingochi, 2001). Unfortunately, in spite of their relative importance, yams have suffered from research neglect with the result that the problems which presently require research attention are wide ranging, and the knowledge base for the crop, from which to tackle those problems, is veal and frustratingly inadequate (FAO, 1997). This is especially the case with respect to their genetic improvement where such characteristics of the yam plant such as recalcitrance in flowering, tuber dormancy, and an extended juvenile growth phase, put limitations on the efficiency and potential success of a breeding programme (Nweke et al, 1992). It has been assumed that conventional breeding was impossible because of poor flowering and fertility (IITA, 1994). Unlike in other crops where the male and female flowers are on the same plant, yam flowers are borne on separate plants (Dioeciousness). Therefore flowering is difficult to synchronise when the female flowers are ready, the male flowers are not ready and vice versa (IITA, 1994).

The development of free flowering, fertile populations and the ease of production of countless seedlings suggest that for the first time one important species can be improved by modern techniques (Okereke, 1975). In recent years a few scientists

have devoted study to the African yam (IITA, 1987). The application of in vitro techniques to rapid propagation of yam was stimulated and enhanced by the discovery of useful extractable secondary metabolites (e.g. diosgenin) from certain yam species for the pharmaceutical industry. (Lakshni, 1986). The use of apical meristem and nodal segment culture systems for the production of virus-free plants and for the international exchange of disease-free clonal material and conserving germplasm provides enormous incentive for yam tissue culture research and plant production for in vitro materials (Lakshni, 1986).

The hypothesis has been established that plant growth regulators are required for yam microtuber induction in Dioscorea alata and that certain types of growth regulators are more effective than others are enhancing induction frequencies. (Amirato, 1982). Specific types of cytokinins and auxins may be very important for multiplication of certain yam species and for inducing certain types of yam development (Uduebo, 1971). Early research effort in yam micro propagation concentrated on the manipulation of media supplements such as plant growth regulators to promote shoot formation in order to allow for more effective levels of propagation. In particular, the addition of kinetin, either alone or with other specific auxin, to MS media (Murashige and Skoog, 1962) has resulted in higher shoot bulking with responses being species – dependent (Cortes – Moullor and Liu, 1982).

Media Composition

The composition of the nutrient medium is an important media parameter, which must be optimised in order to obtain successful plant regeneration. Multitudes of plant tissue culture media are suitable for plant regeneration. The formulation

developed by Murashige and Skoog (1962) is the most common medium employed with great success (Appendix 2).

Environmental factors

The intensity of light, type and duration of the light play a very important role in the morph genesis of the culture. In many species, the explants tend to establish well under 500-1000 lux illumination using 16 hours photoperiod (Pierik, 1987). Fluorescent tubes mounted outside the growth rack usually supply light. Pierik (1987) found that a dark period is necessary for the formation of shoot, the length of the dark period will depend on the species.

Culture temperature is usually maintained at 25°C. Some species may require varying temperatures for optimum growth. It has been found that the optimum temperature for *in vitro* growth and development is generally 3-4°C higher than *in vivo* (Pierik, 1987). The temperature ranges from 20 – 28°C. When the incubation temperature is raised above 28°C, water tends to condense on the plants and container walls, which may restrict growth (Hu and Wang, 1983). Bhojwane (183) reports that Seabrook and Cummings in 1982, tested a range of temperature regimes for the shoot and bulbils formation in leaf base tissue cultures of Narcissus and found that more shoot were formed at a constant temperature of 25°C. But Tisserate (1987) says that some species may require varying temperature treatments for optimum growth.

Explant

Two sorts of plant material can be used for isolation of explants in vitro culture: Plants grown under controlled environment in a green house or those grown in the

open field. In practice, if an explant is isolated from a plant that has grown outside there is much greater chance of infections taking place, (Pierik, 1987).Tisserate (1987) noted that practically any part of the plant can be successfully cultured in vitro and regenerate plantlet provided the explant is obtained at the proper physiological stage of development. Immature tissue and organs are invariably more morphologically plastic in vitro than mature tissues and organs. Morph genesis potential is used to describe the ability of the explant to regenerate plants in vitro. Evans et al (1983) has shown that leaves can be responsive to culture. Other studies such as those of Lineburge and Wanstreet (1995) have shown that buds, leaves and roots of various species can be amenable to tissue culture.

MATERIALS and METHODS

Four Lusala (*Dioscorea hirtiflora*) ecotypes namely; Mongu, Chongwe, Choma and Namwala were used in the experiment respectively. These were obtained from the National Irrigation Research Station – Nanga, Mazabuka in March, 2002 and brought to the Green House. These were maintained in the green house by weeding and watering and then transferred to the tissue culture lab of the Crop Science Department. The media were (1) Plain MS (2) Ms + 2.5 ppm BAP, (3) Ms + 1.5 ppm BAP (4) MS + 1.5 ppm BAP + 1.5 ppm NAA, (5) MS + 1.5 ppm NAA.

Axillary buds were used as explants, these were about 2mm in size. These explants were rinsed in 79% ethanol for 30 seconds to remove dust particles and get rid of air bubbles. The explants were then disinfected in 1% sodium hypochlorite for 3 minutes. This was followed by a thorough rinsing of explants in sterile distilled water 3 to 4 times. The instruments were sterilized each time after handling tissue by dipping them in 95% ethanol, flaming them and allowing them to cool. The work surface was regularly scrubbed with 95% ethanol as well. The Lusala explants were inoculated in the test tubes containing the media covered with caps. The caps of the culture test tube containing MS media were removed, the inoculum transferred on the medium, the neck of the test tube flamed and cap replaced in quick succession. The process of sterilization of explants took place in a laminar flow chamber hood.

The experimental design used was a split plot with ten replications. The main plot was ecotype and subplot was the media. The parameters measured were shoot regeneration, number of shoots, number of roots and contamination rate. All measurements were taken

after two weeks of culture. M Stat (University of Michigan) computer program was used to analyse the data. Differences were considered significant at $P \leq 0.05$.

Chapter 4

RESULTS

Shoot regeneration responses of different Lusala ecotypes

The ecotypes responded differently to treatments (Fig.1). Choma and Namwala readily produced shoots (85%), Mongu at 60% was intermediate. Chongwe showed the least ability to produce shoots, only 40% of the inoculated materials produced shoots. Generally the rate of shoot proliferation was low. Namwala produced about 1.2 shoots per explant (Fig. 2). The other ecotypes produced less shoots, the lowest was Chongwe where most of cultures failed to produce shoots giving an average of 50%.

The influence of media type on culture regeneration

The hormone free media produced the least shoot regeneration (Fig. 3), only 45% of the cultures produced shoots. The other media significantly encouraged shoot regeneration averaging 70%. The data on the effect of media on shoot proliferation showed that MS+1.5ppm NAA media had the highest response at 102.5%. Hormone free media showed the lowest response to shoot proliferation at 27.5%. The MS+2.5ppm BAP and MS+1.5ppm BAP + 1.5ppm NAA media were second response at 100% respectively and MS+1.5ppm BAP was the third at 72.5% (Fig.4).

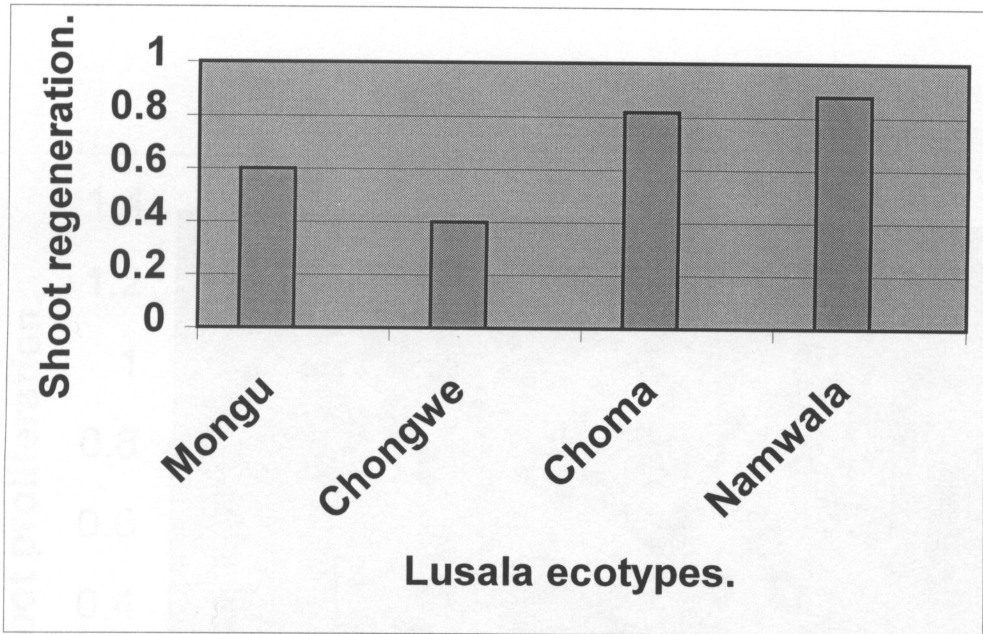


Figure. 1: Shoot regeneration responses of different Lusala (*Dioscorea hirtiflora*) ecotypes.

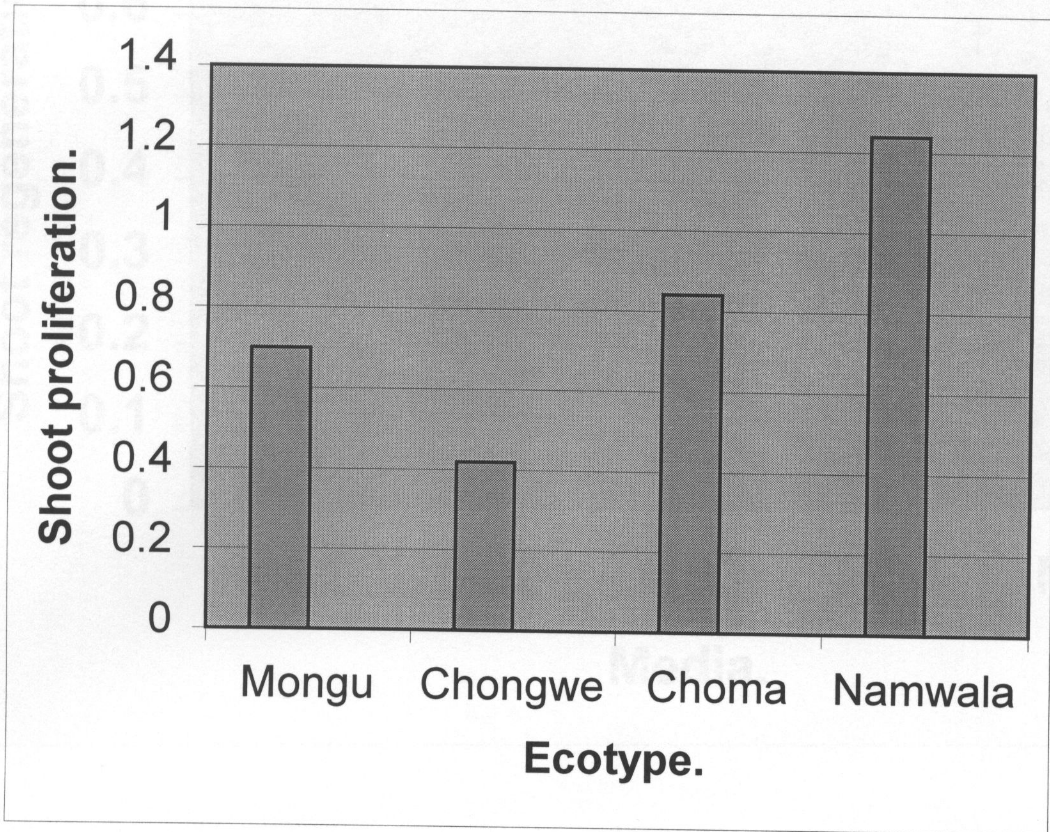


Figure. 2: Effect of Lusala (*Dioscorea hirtiflora)* ecotype on shoot proliferation. Data was pooled across different culture media.

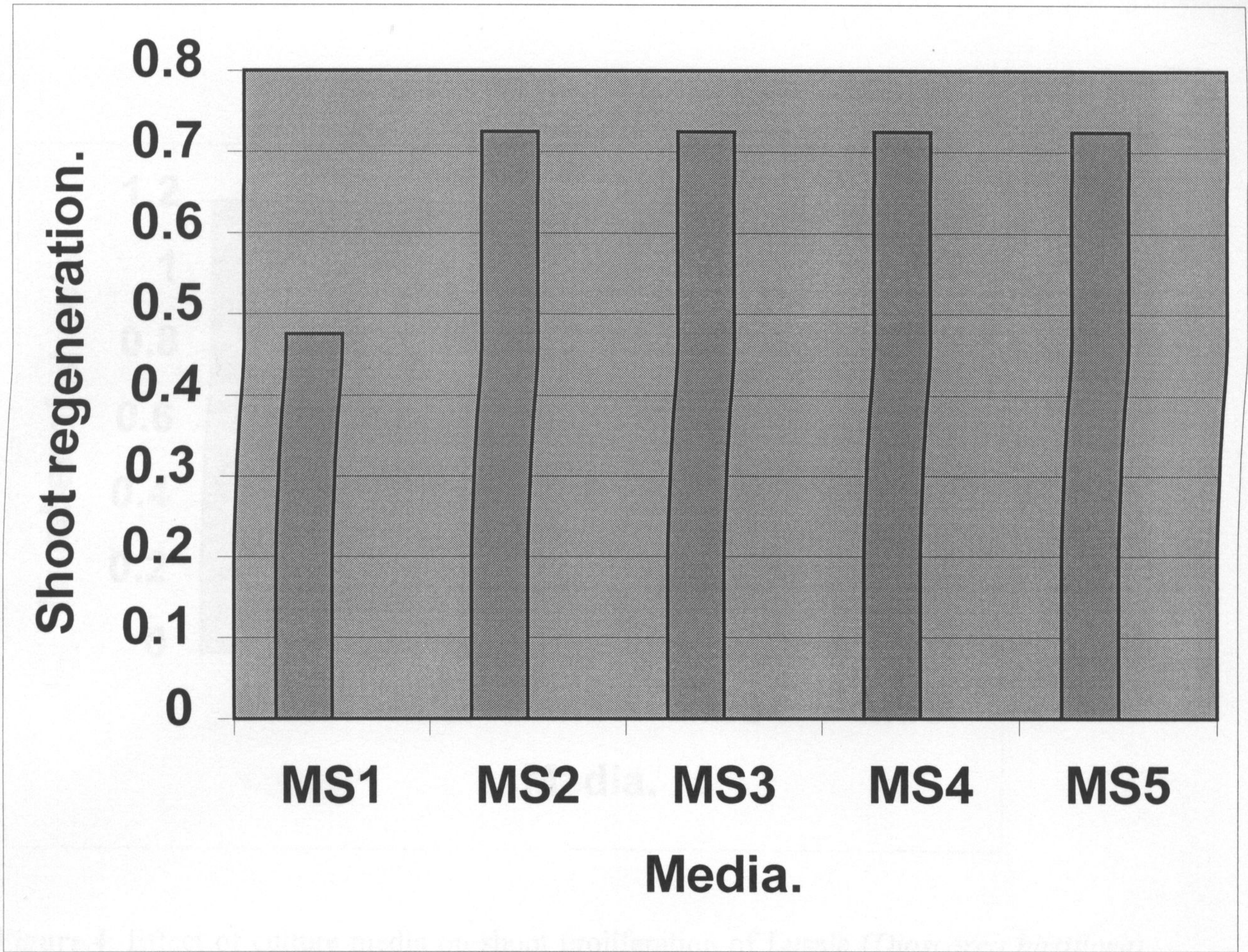


Figure 3. Effect of culture media on shoot regeneration of Lusala (*Dioscorea hirtiflora* on 5 different culture media (MS1) Plain MS (MS2) Ms + 2.5 ppm BAP, (MS3) Ms + 1.5 ppm BAP (MS4) MS + 1.5 ppm BAP + 1.5 ppm NAA, (MS5) MS + 1.5 ppm NAA.

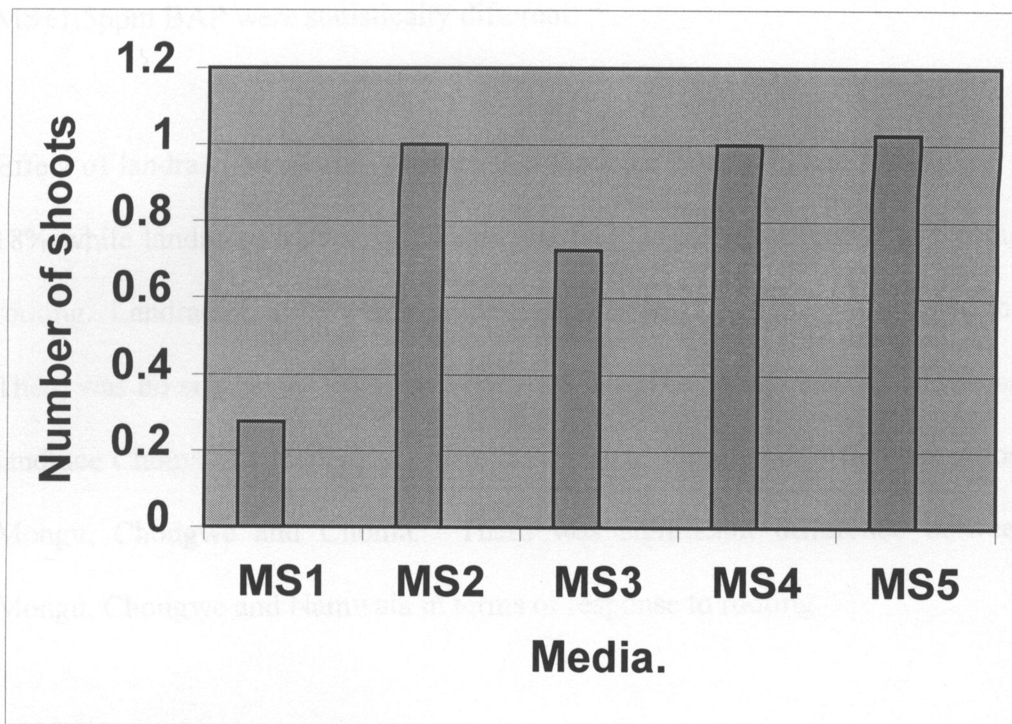


Figure.4: Effect of culture media on shoot proliferation of Lusala (*Dioscorea hirtiflora)* on 5 different culture media (MS1) Plain MS (MS2) Ms + 2.5 ppm BAP, (MS3) Ms + 1.5 ppm BAP (MS4) MS + 1.5 ppm BAP + 1.5 ppm NAA, (MS5) MS + 1.5 ppm NAA.

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MS+2.5ppm BAP, MS+1.5ppmBAP+1.5ppm NAA and MS+1.5ppm NAA media showed no significant difference in terms of response to shoot number. Hormone free media and MS+1.5ppm BAP were statistically different.

Effect of landrace on rooting showed that landrace Namwala had the highest response at 18% while landraces Mongu and Chongwe had the lowest response to rooting with zero rooting. Landrace Choma was second in response at 12%. This is illustrated in figure 5. There was no significant difference between landrace Mongu and Chongwe and between landrace Choma and Namwala. Also there was no significant difference among landrace Mongu, Chongwe and Choma. There was significant difference between landrace Mongu, Chongwe and Namwala in terms of response to rooting.

The response of different landraces to rooting treatments

The effect of media on rooting was found that MS+1.5ppm NAA media had the highest response to rooting at 20% while hormone free media; MS+1.5ppmBAP and MS+2.5ppm BAP 1, 2 and 3 were the lowest and did not show any rooting response. Media MS+1.5ppm BAP + MS+1.5ppm NAA was the second in terms of response to rooting at 17.5% (Fig. 6). Statistically, there is no significant difference among hormone free media, MS+1.5ppm BAP and MS+2.5ppmBAP and also between media MS+1.5ppm BAP +MS+1.5ppm NAA. There is statistical difference between hormone free media, MS+1.5ppm BAP, MS+2.5ppm BAP and media MS+1.5ppm BAP+ 1.5ppm NAA and MS+1.5ppm NAA.

Contamination rate

The effect of landrace on contamination rate showed that landrace Mongu had the lowest contamination at 3.9% while landrace Chongwe had the highest rate of contamination. Landrace Choma was the second in terms of contamination at 5.2% and landrace Namwala was the third at 4.6% (Figure 7).

The effect of media on contamination showed that hormone free media had the highest contamination at 35% whilst media MS+1.5ppm BAP was the lowest with no contamination. Media MS+1.5ppm BAP+1.5ppm NAA was second at 20% while media MS+2.5ppm BAP and MS+1.5ppm NAA were third at 10% respectively (Figure. 8). Media MS+2.5ppm BAP and MS+1.5ppm NAA had no statistical difference in terms of response to contamination. Hormone free media, MS+1.5ppm BAP and MS+1.5ppm NAA were statistically different. Media MS+1.5ppmBAP and MS+1.5ppmNAA had no significant difference and between media MS+2.5ppm BAP and MS+1.5ppm NAA, there was no significant difference.

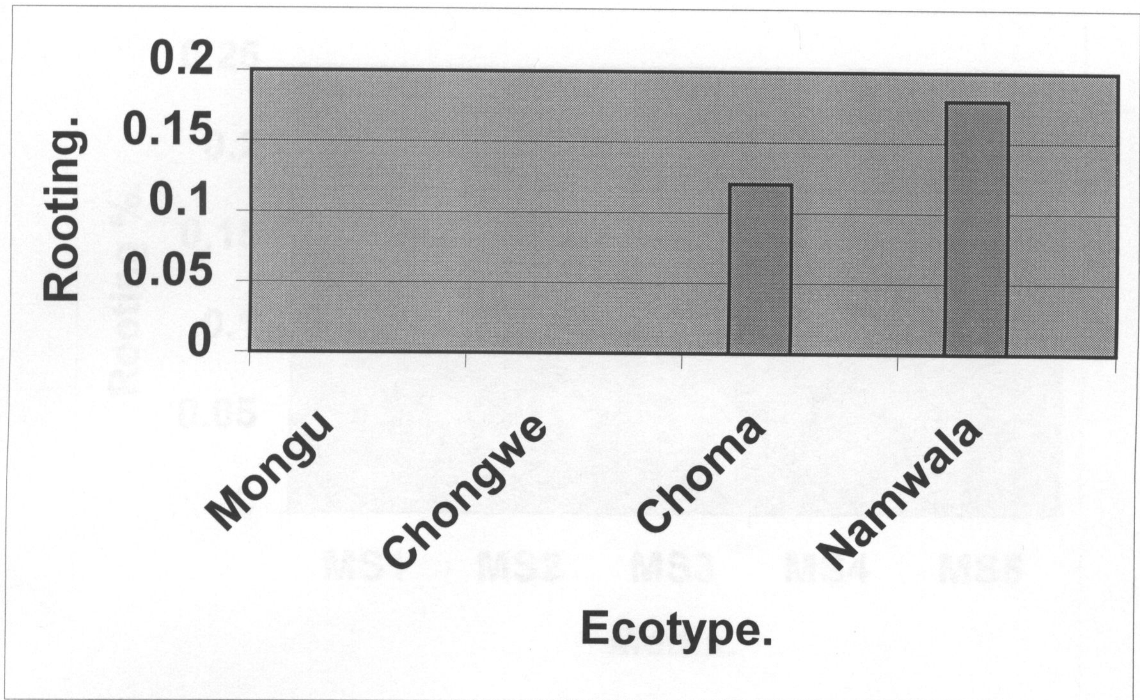

Figure. 5: Response of five Lusala ecotypes to root inducing treatments.

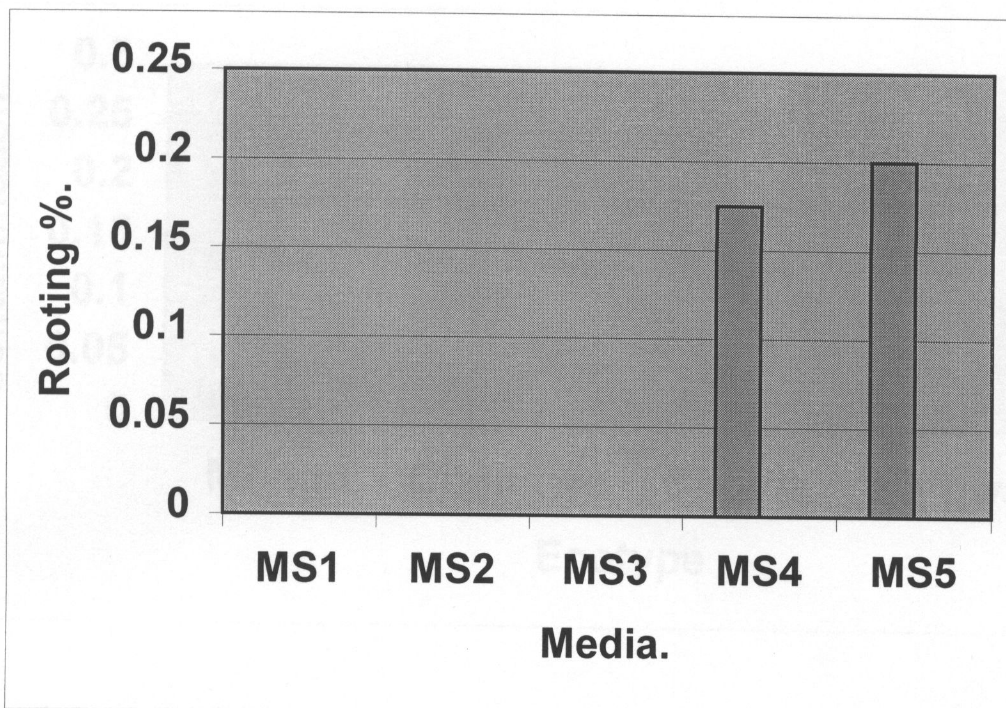

Figure.6: Root development in Lusala (*Dioscorea hirtiflora*) cultured on 5 different media (MS1) Plain MS (MS2) Ms + 2.5 ppm BAP, (MS3) Ms + 1.5 ppm BAP (MS4) MS + 1.5 ppm BAP + 1.5 ppm NAA, (MS5) MS + 1.5 ppm NAA.

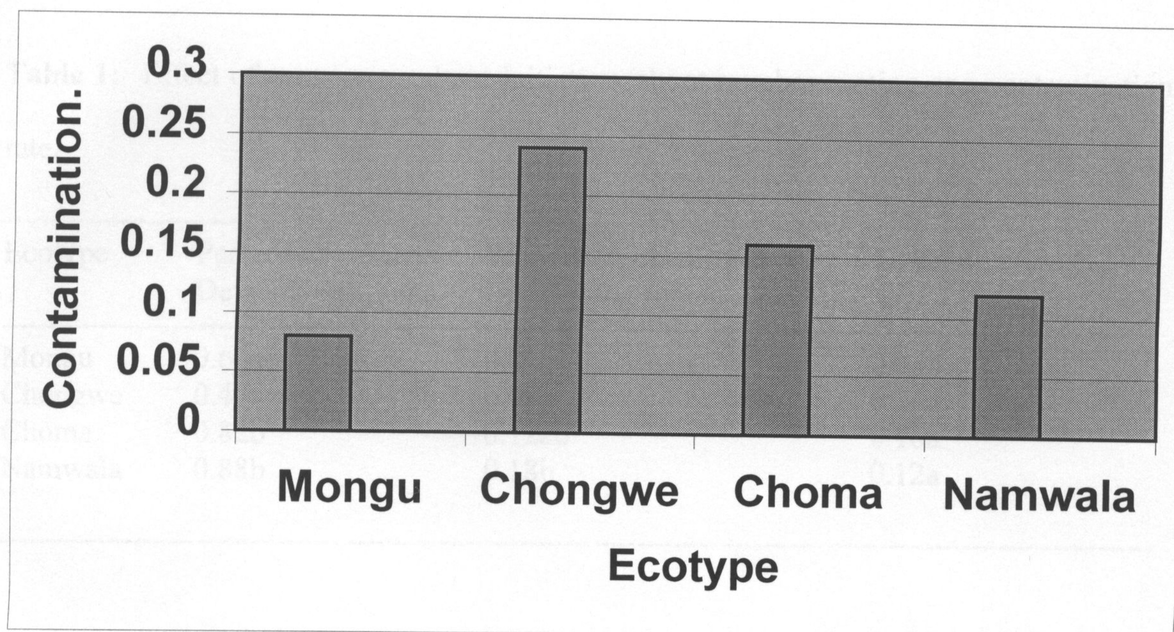

Figure. 7: Proportion of contaminated cultures in different Lusala (*Dioscorea hirtiflora*) ecotypes.

Table 1: Effect of ecotype on shoot initiation, shoot number rooting and contamination rate.

Ecotype	Percent of cultures Developing shoots	Proportion of cultures Developing root	Contamination %
Mongu	0.60ab	0.00a	0.08a
Chongwe	0.40a	0.00a	0.24a
Choma	0.82b	0.12ab	0.16a
Namwala	0.88b	0.18b	0.12a

Table 2. Effect of media on shoot development, shoot, number, rooting and contamination rate

Culture media	Percent of cultures Developing shoots	Number of shoots	Proportion of cultures Developing root	Contamination %
Plain MS	0.48a	0.23a	0.00a	0.35c
MS + 1.5 ppm BAP	0.73b	0.73b	0.00a	0.00a
MS + 2.5 ppm BAP	0.73b	1.00c	0.00a	0.10ab
MS + 1.5 ppm BAP + 1.5 ppm NAA	0.73b	1.00c	0.18b	0.20b
MS + 1.5 ppm NAA	0.73b	1.03c	0.20b	0.10ab

Chapter 5

DISCUSSION

Landrace Mongu and Chongwe had low shoot regeneration and low shoot proliferation. This could have been due to some dormancy effects on the landraces i.e. the age and position of buds affect shoot regeneration. Pierik (1987) reported that juvenile explants remain juvenile in tissue culture and regenerate very easily while adult explants remain adult and regenerate with repeated sub culturing to fresh media. Buds that are positioned on the top part of the plant (new buds) will regenerate very easily compared to the old buds situated on the lower positions of the plant. Also the greater the size of the bud at inoculation the lower the shoot regeneration and the higher the contamination rate, as the microorganisms will have a large area to develop.

Mongu and Chongwe ecotypes did not respond to rooting. This could have been attributed to the tough texture of these explants. In the hormone free media, there was low shoot regeneration, indicating the low levels of endogenous cytokinins. Hormone containing media resulted in shoot regeneration but BAP at 2.5 ppm reduced shoot development. The media that did not contain an auxin (NAA) did not respond to rooting. Only the media MS + 1.5 ppm BAP + 1.5 ppm NAA and MS + 1.5 ppm NAA produced roots.

Choma and Namwala ecotypes showed some similar characteristics in the response to the parameters measured. This similarity of the landraces in most characteristics could make one believe that these two landraces were closely related, or could have been the same ecotypes given the proximity of Choma to Namwala.

Chapter 6

CONCLUSION

Although the potential of propagating local Lusala ecotypes was demonstrated but there is need to optimize propagation media to induce shoot and root proliferation. Conditioning methods to harden propagules and make them tolerant to non-controlled conditions have to evaluated together with molecular characterization of the different ecotypes to avoid unnecessary duplication.

Chapter 7

7.0 References

Amirato, P.V. **1982**. Growth and Morphogenesis in culture of the Monocot yam, Dioscorea. Pg. 169 – 179. In Plant Tissue Culture, Japan.

Bhojwane, S.S. and Razdan, M.K. **1983**. Plant Tissue Culture. Elsevier Science Publishers B.V. New York.

Cobbley, L.S. **1963**. An Introduction to the Botany of Tropical Cops. Pg. 178. Western Printing Services, Bristol.

Cortes – Monllor, A and L. J. Liu. **1982**. Tissue Culture Propagation of yam in Puerto Rico, J. Agric. Univ. Puerto Rico 25: 419-428.

Costas, R.C. Boneta, E. And Silva, S. **1968**. Effects of various cultural practices on yields of yams in Puerto Rico. J. Agric. Univ. P.R. 52: 356 – 361.

Degra, L. **1993**. The yam, a tropical root crop pg. 221 – 226. Macmillan Press Ltd. London, in Co-operation with the Technical Centre for Agricultural and Rural Co-operation, Ede, Netherlands.

Dorosh, P. **1988**. The economics of root and tuber crops in Africa. International Institute of Tropical Agriculture, Ibadan (Research and Crop Management Program, Research monograph No. 1).

Evans, D.A. Sharp, W.R. Amirato, P.V. Yamada, Y. **1983**. Handbook of Plant Cell Culture. Macmillan Publishers Co. New York, pp. 13 – 82.

FAO, **1997**. FAO Yearbook Production Vol. 50. Food and Agricultural Organization of the United Nations, Rome (FAO Statistics Series, No, 135).

Ferguson, T.U. **1972**. The propagation of *Dioscorea spp*. By Vine Cuttings – a critical review. Tropical Root and Tuber Crops News letter 5: 4-7.

Hu, C.Y. and Wang, P.J. **1983** Meristem, Shoot tip and Bud Cultures in Evans, D.A.; sharp, W.R.; Amirato, P.V; Yamada, Y. Handbook of plant cell culture. Pp 177 – 228. Macmillan Publishing Co. New York.

IITA, **1982**. Tuber and Root Crop production manual No. 9. IITA, Ibadan.

IITA, **1994**. Yam breeders break through. Mt. Makulu library CD ROM.

IITA, **1997**. Annual Report for Project 15: Recombinant DNA, Molecular diagnostics and Cellular biotechnology for crop improvement. IITA, Ibadan.

IITA, **2002**. Org/Research/Summaries/Sum Prog. 5 Html. IITA/Crops and Farming Systems – Improving yam based systems.

John – Libbey, **2002**. Eurotext for/articles/agric/7/6/6516/en – resum html. Biotechnological strategies for yams – Promoting food security in Africa.

Lakshni, **1987**. Improved growth of tissue cultures of the onion (*Allium cepa*). Physiology Plant: 41(1977), 70-72

Lineburge, D.R. and Wan Street. **1995**, Micropropagaton of *Ajuga raptans*

Milthorpe and J. Moorby. 1981. An Introduction to Crop Physiology. 2 ed. pp.132,138.

Mingochi, D.S. **2001**. Exploration, collection, characterisation and evaluation of *Dioscorea hirtiflora* (lusala), in vegetable crop Research Report presented at 2001 Annual Planting meeting Ministry of Agriculture Food and Fisheries.

Murashige T and F. Skoog, **1962**. A revised medium growth and Bioessays with tobacco tissue 15: 473 – 497.

Okereke, O.U. **1975**. A new approach to improvement of yam, Nature (Lond.) 254: 134 – 135.

Pierik, R.K.M. **1987**. In vitro culture of Highere Plants Martinus Nijhoff Publishers, Dordrecht.

Soenarjo, R. **1995**. Yam (*Dioscorea* spp.) in Zambia Seed Technology Handbook, Ministry of Agriculture, Food and Fisheries.

Tisserate, B. **1987**. Embryogenesis, orgnogenesis and plant regeneration. Dixon, R.A. Plant Cell Culture, IRL Press, Oxford. Pp. 79 – 144.

Uduebo, A.E. **1971**. Effect of external supply of growth substances on axillary proliferation and development in *D. bulbifera*. Bot. 35: 159 – 163.

Waller, J.M and D.S. Hill.**1988**. Pests and Diseases of Tropical Crops. Field Handbook. pp. 370-371.

Appendix 1: ANOVA Tables of Growth and Development Parameters of four Lusaka (*Dioscorea hirtiflora*) Landraces

ANOVA TABLE FOR SHOOT DEVELOPMENT

Source of Variation	df	Sum of Squares	Mean Square	F. Value	Prob.
Factor A	3	6.940	2.313	12.8519	0.0000
Factor B	4	2.420	0.605	3.3611	0.0111
AB	12	2.460	0.205	1.1389	0.3314
Error	180	32.400	0.180		
CV%		63.32			

ANOVA TABLE FOR SHOOT NUMBER

Source of Variation	df	Sum of Squares	Mean Square	F.Value	Prob.
Factor A	3	18.175	6.058	18.4518	0.0000
Factor B	4	15.020	3.755	11.4365	0.0000
AB	12	5.100	0.425	1.2944	0.2251
Error	180	59.100	0.328		
CV%		71.18			

ANOVA TABLE FOR ROOT DEVELOPMENT

Source of Variation	df	Sum of Squares	Mean Squares	F- Value	Prob.
Factor A	3		0.405	5.5649	
Factor B	4		0.425	5.8397	
AB	12		0.155	2.1298	
Error	180		0.073		
CV%			359.70		

ANOVA TABLE FOR CONTAMINATION RATE

Source of Variation	df	Sum of Squares	Mean Squares	F-Value	Prob.	
Factor A	3	0.700	0.233	3.0882	0.0285	
Factor B	4	2.800	0.700	9.2647	0.0000	
AB	12	8.400	0.700	9.2647	0.0000	
Error	180	13.600	0.076			
CV%		183.25				

NOTE: In the ANOVA Tables Under the source of variation column

A represent Effect of Landrace

B represents Effect of Media

AB represents Landrace/Media Interaction

Appendix 2: Composition of Murashige and Skoog Tissue Culture Media

Source: Plant Tissue Culture (Bhojwana, 1983)

Constituents	**Amounts in mg l^{-1}**
NH_4NO_3	1650
KNO_3	1900
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	3.40
KI	0.83
H_3BO_3	6.2
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
$Na_2MoO_4.2H_2O$	0.25
$CuSO_4.SH_2O$	0.025
$CoCl_2.6H_2O$	0.025
$FeSO_4.7H_2O$	27.8
$Na_2EDTA.2H_2O$	37.3
Organic	
Inositol	100
Nicotinic acid	0.5
Pyridoxine HCL	0.5
Thiamin HCL	0.1
Glycine	2
Sucrose	3%