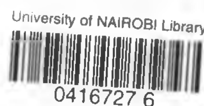
REGENERATING CALLUS, ROOTS AND SHOOTS OF *Jatropha curcas (L)* IN-VITRO AND OPTIMIZING SEED GERMINATION

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF A MASTER OF SCIENCE DEGREE IN PLANT BREEDING

BY

HENGA.S.AMONDI

A56/8109/06


University of NAIROBI Library

0416727 6

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

UNIVERSITY OF NAIROBI

2010

DECLARATION

I Henga Sylvia Amondi declare that this thesis is my original work and has not been presented for a degree in any other university,

HENGA S. AMONDI

Sign..Date.08-09-10

This thesis has been submitted with our approval as supervisors:

1. **Dr. E.C.K. NGUGI**

Department of Plant science and crop protection

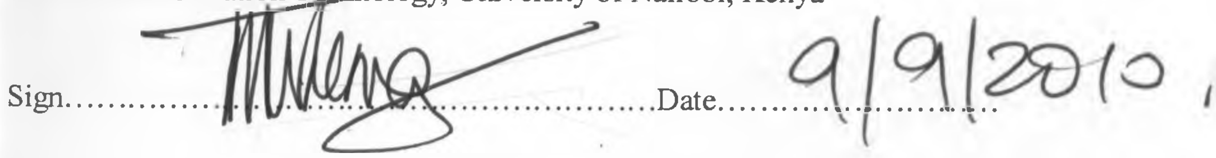
Faculty of Agriculture, University of Nairobi, Kenya

Sign..Date.08/09/2010

2. **PROF.T.WAEMA**

Department of Computer Science

School of Information Technology, University of Nairobi, Kenya



Sign..Date.9/9/2010

Kab Afr QK 495.5 .E9H4 C.2

DEDICATION

I dedicate this work to my dear husband and friend Lawrence, without whose love and support this work would not have been accomplished. You are a priceless jewel and we will match on together to the highest of heights. May God bless you richly and fulfill all the desires of your heart.

To My son Reagan, you are such a precious gift, a motivation that gives me every reason to press on against all odds. May you live to see your children's' children and to declare the goodness of the Lord in the land of the living.

To my Dear mum and Dad Thomas and Joyce Henga, who sacrificed to take me through school, denying yourselves comforts and pleasure, to give me a life. May your name and legacy be echoed across generations to come.

The Nairobi Miracle land Worship Church, Friends, Colleagues, Thanks for your prayers and encouragement. For sure, your love is more than just a word.

To God be all the Glory. This is the doing of the Lord and it's marvelous in our eyes. Amen

AKNOWLEDGMENT

The success of this thesis was made possible through the input of several individuals and institutions. I sincerely express my gratitude to the University of Nairobi for awarding me a scholarship that saw me through this level of study. I also appreciate the London University that facilitated my research project through funding and technical input.

I herein acknowledge the invaluable assistance from my supervisor, Dr. E.C.K. Ngugi who was the key guide in this research and Prof.T.Waema whose input was unmatched. Their corrections, guidance and positive criticism were key in the general success of this venture.

The Village E Science for Life project members, Mr. Orwa, Ammon, Anne, and Joseph for their support and encouragement during the study. Through you I was able to acquire special skills on Information technology and Agriculture as concerns rural communities.

Special appreciation to The Laboratory technician Ms.Cecelia, who gave such sure directions that enabled the smooth running of the project. Her perseverance and encouragement was immeasurable.

The KARI team, Dr. Musymi, Justina and Quinata were of great assistance during my time in the biotechnology laboratory in KARI. This project is sponsored by Village e-Science for Life (VeSeL)

Table of Contents

DECLARATION i
DEDICATION ii
AKNOWLEDGMENT iii
TABLE OF CONTENTS iv
LIST OF TABLES vii
LIST OF PLATES xi
LIST OF APPENDICES xii
ABBREVIATIONS xiii
ABSTRACT xiv
CHAPTER 1: Introduction 1
1.1Origin and distribution 1
1.2Uses 2
1.3 Problem statement and justification 4
1.4 Objectives 6
1.5 Hypotheses 6
CHAPTER 2: Literature Review 7
2.1 Botanical Description 7
2.2 Ecological requirements 7
2.3 Jatropha Production and Productivity 8
2.5 Jatropha oil 8
2.6 Tissue culture 10
2.6.1 Growth regulators/hormones 11
2.6.2 Media components 12
2.6.3 Application of tissue culture 13
2.6.4 Effects of hormones on plant regeneration 13
2.7 Jatropha seed propagation 14
2.7.1 Effect of environment on seed germination 15
2.7.2 Other factors affecting germination 15
2.8 Effect of planting material on growth and regeneration 16
2.9 Effect of seed source on germination 17
2.9.1 REFERENCES(Chapter 1 & 2) 18
CHAPTER THREE: Effect of hormones and explants on callus formation, rooting and shooting of *Jatropha curcas L.* cultured in-vitro 24
3.1 Abstract 24
3.2 Introduction 24
3.3 Materials and methods 25
3.3.2 Chemicals 25
3.3.3.Media Preparation 26
3.3.4. Explants Sterilization 26
3.3.5 Experimental design 27
3.4 RESULTS 29
3.4.0 Experiment 1 28
3.4.1 Days to Callus formation 29

_3.4.2 Callus amount 29
_3.4.3 Days to shooting........ 30
_3.4.4 Shoot length 31
_3.5 Experiment 2 31
_3.5.1 Days to callus formation 31
_3.5.2 Callus amount 32
_3.5.3 Days to shooting........ 33
_3.6 Experiment 3 33
_3.6.1 Amount of callus produced........ 33
_3.6.2 Days to shooting........ 34
_3.6.3 Number of shoot buds per explants 35
_3.7 Experiment 4 35
_3.7.1 Days to shooting........ 35
_3.7.2 No of shoot buds 36
_3.8.3 Shoot length 36
_3.7.4 Days to root........ 37
_3.8 Experiment 5 38
_3.8.1 Days to shooting........ 38
_3.8.2 Number of shoot buds 38
3.8.3 Shoot length........ 39
_3.8.4 Days to root........ 40
_3.9 DISCUSSION........ 42
_3.9.6 Conclusion 45
_3.9.7 REFERENCES(Chapter three) 47
CHAPTER FOUR: Assessment of the effects of temperature, base medium and nursery container on the germination of *Jatropha curcas* seed varieties........ 50
4.1 Abstract........ 50
4.2 Introduction 50
4.3Materials and methods........ 51
__4.4 Experimental design........ 51
4.5 Greenhouse seed germination 51
__4.5.1 Greenhous Experiment A 51
__4.5.2Measurements 52
4.5.3RESULTS 52
4.5.4 Greenhouse Experiment 1 52
__4.5.5 Height at 14 days........ 52
__4.5.6 Percent Germination........ 53
__4.5.7 Leaves per plant at 14 days........ 53
__4.5.8 Days to germination 54
__4.5.9 Height at 21 days........ 54
__4.5.9.1Noumber of leaves per plant (21 days)........ 55
__4.5.9.2 DISCUSSION........ 55
4.7 GREENHOUSE EXPERIMENT B........ 58
__4.7.1 Days to emergence........ 58

__4.7.2 Stem length 59
__4.7.3 Number of leaves 59
__4.7.4 Percent germination... 60
__4.7.5 Discussion.. 61
4.8 TEMPERATURE EXERIMENTS 62
4.8.0 Growth conditions 62
__4.8.1 Days to sprouting 63
__4.8.2 Percentage germination... 64
__4.8.3 Stem Length... 64
__4.8.4 Number of Leaves.. 64
__4.8.5Days to rooting 64
__4.8.6 DISCUSSION... 65
4.9 LAB DESKTOP SEED GERMINATION 66
4.9.0 Growth conditions 66
__4.9.1 Days to sprouting 66
__4.9.2 Percent germination... 67
__4.9.3 Root length 68
__4.9.4 Shoot length 69
__4.9.5 Discussion.. 70
REFERENCES...72
CHAPTER FIVE :General conclusion and recommendations 73
5.1Conclusion.. 73
5.2 Recommendations... 74
LIST OF APPENDICES...................... 75

LIST OF TABLES

Table 1: Physiochemical parameters of Jatropha seed oil....10

Table 2: Physical and chemical properties of Jatropha curcas. L oil....10

Table 3: Components of Murashige & Skoog basic media.... 14

Table 4: Mean effect of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA2mg/l, NAA4mg/l+IBA2mg/l and Murashigr&Skoog on days to callus formation of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....29

Table 5: Mean effect of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA2mg/l, NAA4mg'l+IBA2mg/l and MS on amount of callusing of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media 30

Table 6: Mean effects of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA2mg/l, NAA4mg'l+IBA2mg/l and MS on days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media 30

Table 7: Mean effects of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA2mg/l, NAA4mg'l+IBA2mg/l and MS on shoot length of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....31

Table 8: Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on days to callus formation of Jatropha curcas Shoot tip,leaf and stem explants cultured in MS media....32

Table 9: Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on amount of callus produced from Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....32

Table 10: Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Table 11: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on amount of callus obtained from Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....33

Table 12: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....34

Table 13: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of shoot buds per explant of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media....35

Table 14: Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on days to Shooting of Jatropha curcas varieties cultured in MS media....36

Table 15: Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on number of shoot buds produced by Jatropha curcas varieties cultured in MS media....36

Table 16: Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on shoot length of Jatropha curcas varieties cultured in MS media....37

Table 17: Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on number of days to rooting of Jatropha curcas varieties cultured in MS media....37

Table 18: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to shooting Jatropha curcas seed varieties cultured in MS media...38.

Table 19: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of shoot buds produced by Jatropha curcas seed varieties cultured in MS media....39

Table 20: Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on length of shoots produced by Jatropha curcas seed varieties cultured in MS media..40

Table 21: Mean effects of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to rooting of Jatropha curcas seed varieties cultured in MS media..40

Table 22: Mean heights of Jatropha curcas varieties at at 14 days after planting as affected by use of polythene container, polythene cover, and sand/soil medium treatments.....53

Table 23: Effects of use of polythene container, polythene cover, sand/soil medium on the percentage germination of three Jatropha curcas varieties under nursery production....53.

Table 24: Effects of use of polythene container, polythene cover, sand/soil medium on the

number of leaves per plant of three Jatropha curcas varieties under nursery production.....54

Table 25: Effects of use of polythene container, polythene cover, sand/soil medium on the number of days to germination of three Jatropha curcas varieties under nursery production.....54

Table 26: Mean heights of three Jatropha curcas varieties at 21days of nursery planting as affected by use of polythene container, polythene cover, and sand/soil medium treatments.....55

Table 27: Mean leaf count of three Jatropha curcas varieties at 21days of nursery planting as affected by use of polythene container, polythene cover, and sand/soil medium treatments.....55

Table 28: Mean number of days to emergence of seven Jatropha curcas seed varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.....58

Table 29: Mean stem length of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.....59

Table 30: Mean number of leaves of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.....60

Table 31: Mean %germination of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, and sand/soil mixture and manure treatments.....61

Table 32: Mean number of days to sprouting of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.....63

Table 33: Mean percent germination of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.....63

Table 34: Mean stem length of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.....64

Table 35: Mean number of leaves of two Jatropha curcas varieties as affected by various temperature treatments in an incubator..64
Table 36: Mean no. of days to rooting of two Jatropha curcas varieties as affected by various temperature treatments in an incubator..65
Table 37: Mean number of days to sprouting of Jatropha curcas seed varieties germinated in Petri dishes as affected by use of filter paper base, cotton base and pre/soaking treatments..67
Table 38: Mean percentage germination of Jatropha curcas seed varieties germinated in petri dishes as affected by use of filter paper base, cotton base and pre/soaking treatments..68
Table 39: Mean length of roots produced by Jatropha curcas seed varieties germinated in Petri dishes as affected by use of filter paper base, cotton base and pre/soaking treatments..69
Table 40: Mean length of shoots produced by Jatropha curcas seed varieties germinated in Petri dishes as affected by use of filter paper base, cotton base and –pre/soaking treatments..70

LIST OF PLATES

Plate 1. A mass of callus obtained from in-vitro cultured leaf explants on MS+BAP (8ml/l) +(IBA4ml/l)..41

Plate 2. Shooting of shoot buds in-vitro on MS + BAP (1mg/l) +kn (0.5mg/l) +As (0.5mg/l)....41

Plate 3. A Jatropha curcas seed germinating in-vitro under MS+BAP (1mg/l) +Zn (0.5mg/l)....41

Plate 4. Plantlet obtained from in-vitro cultured seeds on MS+ BAP (1mg/l) +kn (0.5mg/l) +As (0.5mg/l)..41

LIST OF APPENDICES

APPENDIX 1: Analysis of variance (ANOVA) of (1a)number of days to callusing, (1b) amount of callus formation,(1c) days to shooting(1d) shoot length of *Jatropha curcas* varieties subjected to BAP,IBA and NAA treatments..75

APPENDIX 2: Analysis of variance (ANOVA) of (2a)number of days to callusing, (2b) amount of callus formation,(2c) days to shooting of *Jatropha curcas* shoot tip, leaf and stem explants subjected to BAP,IBA,NAA and BAP/NAA treatments...............................76

APPENDIX 3: Analysis of variance (ANOVA) of (3a)amount of callusing,(3b) days to shooting(3c)No of shoot buds of Jatropha curcas shoot tip, leaf and stem explants subjected to BAP/IAA,BAP/Kn, BAP/IBA, BAP/Zeatin, BAP/Kn/As treatments.......77

APPENDIX 4: Analysis of variance (ANOVA) of (4a)number of days to shooting, (4b) no. of shoot buds,(4c) shoot length(4d)days to rooting of *Jatropha curcas* varieties subjected to BAP, NAA,IBA,Zeatin, IAA and Kinetin treatments..78

APPENDIX 5: Analysis of variance (ANOVA) of (5a)number of days to shooting, (5b) no. of shoot buds,(5c) shoot length(5d)Days to rooting of *Jatropha curcas* varieties subjected to BAP/IAA, BAP/Kn ,BAP/ IBA, BAP/Zeatin, NAA/Kn BAP /Kn/As treatment..79

APPENDIX 6: Analysis of variance (ANOVA) of (6a) height at 14 days, (6b) percent germination, and (6c) no. of leaves (6d) Days to germination (6e) height at 21 days (6f) No of leaves at 21 days of *Jatropha curcas* varieties subjected to polythene container, polythene cover, and sand/soil medium treatments..80

APPENDIX 7: Analysis of variance (ANOVA) of (7a) days to germination (7b) stem length, (7c) no. of leaves, (7d) percent germination of seven *Jatropha curcas* varieties subjected to polythene container, polythene cover, and sand/soil medium treatments..82

APPENDIX 8: Analysis of variance (ANOVA) of (8a) days to sprouting, (5b) shoot length, and (8c) percent germination (8d) no. of leaves (8e) days to rooting of *Jatropha curcas* varieties subjected to temperature treatments..83

APPENDIX 9: Analysis of variance (ANOVA) of (9a) days to sprouting, (9b) percent germination, (9c) root length (9d) stem length of *Jatropha curcas* varieties....85

ABBREVIATIONS OF HORMONES USED

BAP: 6-benzylaminopurine,

IAA: Indole-3-acetic acid,

IBA Indole-3-butyric acid,

NAA: 1-naphthylacetic acid,

Zn: 4-hydroxy-3-methyl-trans-2-butenylaminopurine,

Kn: 6-furfurylaminopurine

ANOVA: analysis of variance;

ABSTRACT

Jatropha curcas is among the important tree crops in the world with a potential for biofuel production. The crop is drought resistant and thrives well in warm tropical climates. However, propagation material is a major production constraint. Currently seed and stems are the main materials used for propagation. Reports have shown that the seed has several aspects that constrain production efforts such as low viability, poor germination rates and varying cultivation information. Laboratory and greenhouse experiments were conducted to test for the best micro propagation and seed germination methods in the plant.

Tissue culture trials using MS media with supplements of various hormonal concentrations including BAP, IBA, IAA, NAA, Kinetin, Zeatin and Adenine sulphate. From experiment 1, callus formation using explants (shoot tip, leaf and stem), the media containing BAP4mg/l+IBA2mg/l gave the earliest callus formation date of mean of 8.3 on stems compared to BAP8mg/l+IBA4mg/l, NAA4mg/l+IBA2mg/l and control(No treatment) and earliest days to shooting, mean 15 on shoot tips. The BAP4mg/l+1BA2mg/l combination gave the highest callus amount of mean 2.3, and the longest shoots, mean O.26 on shoot tips.

In experiment 2, BAP8mg/l, IBA4mg/l, NAA2mg/l and BAP8mg/l+NAA2mg/l were used to culture stem, leaf and shoot tip explants. The BAP8mg/l+NAA2mg/l combination gave the earliest callus formation dates of mean 8.3 on shoot tips, BAP gave highest amount of callus of 2.2 on shoot tip while shooting was only observed on shoot tip under BAP culture with a mean of 9.2 days to shooting.

Experiment three involved using BAP1mg/l+IAA0.2mg/l,BAP1mg/l+Kn0.5mg/l, BAP1mg/l+IBA1mg/l, BAP1mg/l+Zeatin0.5mg/l, BAP1mg/l+Kn0.5mg/l+As2mg/l applied on shoot tip, stem and leaf explants in *Jatropha curcas*. Only the shoot tips produced shoots with the earliest mean days to shooting of 7.47 and mean number of shoots of 3.4 in the BAP1mg/l+Kn0.5mg/l+As2mg/l combination.

The fourth involved trials on four seed variety levels TS Morogoro, TZ Arusha, Pemba and Zanzibar cultured in BAP(1mg/l), IAA(0.2mg/l),NAA(0.2mg/l), IBA(1mg/l) ,Zeatin(0.5mg/l), Kinetin(0.5mg/l). BAP 1mg/l gave the earliest mean days to shooting of 21 and highest mean

number of shoot buds of 1.2, the longest shoots with mean 0.32cm and earliest days to rooting with a mean of 22

Experiment 5, four seed varieties, TS Morogoro, TZ Arusha, Pemba and Zanzibar cultured in BAP1mg/l+IAA0.2mg/l, BAP1mg/l+Kn0.5mg/l, BAP1mg/l+IBA1mg/l, BAP1mg/l+Zeatin0.5mg/l, NAA0.2mg/l+Kn0.5mg/l and BAP1mg/l+Kn0.5mg/l+As2mg/l. Cultures Morogoro variety in BAP1mg/l+IBA1mg/l combination gave the earliest mean number of days to shooting of 17.2 and the highest mean shoot length of 0.32cm. The highest number of shoot buds with mean of 1.2 was obtained from the same variety under BAP1mg/l+IBA1mg/l and BAP1mg/l+Kn0.5mg/l+As2mg/l combinations. The earliest mean of days to rooting, mean 20.6 was attained form the BAP1mg/l+IBA1mg/l combinations with the Morogoro variety.

Rainfed, Irrigated and Kitui varieties of Jatropha were subjected to four treatment levels; plastic pots covered with polythene paper, polythene bag container, the third plastic pots containing soil sand mixture and the fourth plastic pots only. The irrigated variety under polythene cover gave the best heights of mean 16.50cm at 14 days and 17.23cm at 21 days after planting. The rainfed variety under polythene bag container and the irrigated variety under polythene covered container gave the highest percent germination of mean 81.25%. Under polythene cover, the irrigated variety gave highest mean number of 3 leaves per plant at 14 days after planting and mean 3.75 leaves at 21days after planting. The earliest mean of germination days of 6.5 was obtained from Kitui variety under polythene bag containers.

Seven seed varieties were tested for germination under five sets of treatment in the first experiment. Plastic pots covered with polythene paper, polythene bag container, the third plastic pots containing soil sand mixture, the fourth plastic pots only and the fifth had soil manure mixture in plastic pots. The earliest mean germination date of 10.25 were obtained from both polythene(TS Morogoro) and covered containers(TS Morogoro, Arusha and T3 Tanga) while the best mean height of 17.9cm was obtained from the covered plants(Pemba). Both the polythene container and covered trials had an equal mean number of 3 leaves per plant. The highest mean percentage germination was 100% and obtained from seeds germinated in plastic containers(TZ Arusha).

In the temperature experiment, two seed types were subjected to 34,36,38 and 40 degree temperature treatments. At 40 degrees, the earliest mean of days to sprouting, the highest germination percentage of 88% (irrigated variety), the highest mean stem length of 4.2cm and the highest number of leaves, 3(all varieties) were attained.

On the laboratory desktop, seven seed varieties, were placed in two sets of base medium: petridishes aligned with filter paper and petridishes aligned with cotton wool. Each of the treatments attained a mean early sprouting date of 3 except the filter paper. Medium containing cotton wool base gave highest mean percent germination of 100% on the TZ Arusha variety. The highest mean root length of 1.7 cm was attained by presoaked Arusha variety while the cotton base gave the highest shoot length of 10.5cm.

The experiments used the completely randomized design(CRD). Data was analyzed by ANOVA to detect significant differences between means. Means differing significantly were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level.

CHAPTER 1: Introduction

1.1 Origin and distribution

Jatropha curcas is a multipurpose tree growing naturally in countries of the Equatorial Americans, mainly Mexico (Vikram, 2008) and Central America, from where it spread to other tropical countries. It's now cultivated worldwide although it's highly adapted to arid and semi-arid conditions (Hikwa, 1995). The crop is found in almost all tropical and sub-tropical countries. *Jatropha curcas*, a perennial shrub, belongs to the family Euphorbiaceae. The center of origin of jatropha is uncertain, but it is believed to have originated in Mexico and Central America. It is being cultivated all over the world now.

The genus Jatropha is derived from the Greek words, jatrós (doctor) and trophé (food), which alludes to its uses in medicine. It is being primarily cultivated due to its usefulness as a biofuel. The crop is more popular as an alternative fuel option in the developing countries compared to the United States. Though little is known in the United States, jatropha is widely viewed as a potential wonder plant in many parts of the world where longtime fuel shortages have led to development of alternative energy sources to power automobiles and factories (Vikram, 2008).It is listed, e.g., as a weed in Brazil, Fiji, Honduras, India, Jamaica, Panama, Puerto Rico, and Salvador (Holm et al, 1979).

Jatropha is a promising biofuel feedstock in Kenya, where its mainly found in Western, Nyanza, Rift Valley, Coast and Eastern provinces along rivers and in bush lands (Maundu & Tengnas, 2005,Reinhard,). *Jatropha curcas* grows well in the entire East Africa region. In Kenya, This makes it a reliable species for marginal lands, reducing competition for space with food-crops (Greco et.al 2007). A conservation effort by some stakeholders has led to establishment of demonstration plots in areas such as Nyanza, Makueni, Kitui, Thika, Namanga, Kajiado and Marsabit.

1.2Uses

The species is widely grown in the tropics as living fences because it is easily propagated by cuttings and not browsed by cattle. There are several ways the crop has been used which differs with the country in question. The seeds contain 30-35% oil which is used as an insecticide, for soap production and numerous other purposes (Sharma et.al., 1995). The seed oil can also be used as a substitute for diesel oil in engines and in recent years special interest has been shown in the cultivation of physic nut in energy plantations (Breitenstein, 2002). The oil has high saponification value and is being extensively used in some countries to make soap. It also burns without emitting smoke and is therefore useful as an illuminant (Gubitz, 1997). Press cake made from the plant is valuable as organic manure. It has nitrogen content similar to chicken manure, approximately 3.2-3.8 %(Reinhard,2008).

The seeds are not edible mainly due to a high content of toxic proteins but all parts of the plant are used in traditional medicine (Jones& Miller, 1992) However, some provenances have been reported to produce edible seed and in Mexico the seeds from a non-toxic variety are eaten after roasting. Being drought tolerant, it can be used to reclaim eroded areas (Henning, 1996). Unfortunately it is host for the cassava virus that can be transmitted to the crops and it should never be used for fences around cassava fields. Currently no exact figures for the yields are available from the producing countries but trials from India, for instance gave a medium yield of 4 kilograms per tree per year and about 10,000 kilograms of seeds per hectare plus about 3,400 liters of oil per hectare (Suma, Gupt& Khabiruddin, 1997). The oil obtained from *Jatropha curcas* seeds has several uses as raw material for many industrial products. For instance, due to its high saponification value, it's being extensively used in making soap in some countries. It can also be used as an illuminant as it burns without emitting smoke (Breitenstein, 2002).In India, the oil is used for making turkey red oil, adulteration of olive oil and the nuts can be strung on grass and burned like candlenuts (Watt & Breyer-Brandwijk, 1962). Besides the oil, it has been discovered that the back of Jatropha curcas yields a dark blue dye which is a useful coloration for clothes, fishing nets and

lines (Gubitz, 1997). Jatropha has a number of medicinal values. The latex of the shrub contains an alkaloid commonly called jatrophine which is believed to have anti-cancerous properties. In countries such as India, the plant has been used to fight cancer and related illnesses (Reinhard,2008). A probe into preliminary research has indicated that Jatropha may display some Anti-Tumor properties, Anti-Malarial properties. There is also a probe into issues related to HIV/AID's and immune system response enhancement (List, 1969). The crop is also a potential source of pesticide in plants as depicted in the latex that was strongly inhibitory to watermelon mosaic virus in a study (Tewari & Shukla, 1982).

According to Hartwell, the extracts are used in folk remedies for cancer and are reported to be abortifacient, anodyne, antiseptic, cicatrizant, depurative, diuretic, emetic, hemostat, lactagogue, narcotic, purgative, rubefacient, styptic, vermifuge, and vulnerary. Physic nut is a folk remedy for alopecia, anasorca, ascites, burns, carbuncles, convulsions, cough, dermatitis, diarrhea, dropsy, dysentery, dyspepsia, eczema, erysipelas, fever, gonorrhea, hernia, incontinence, inflammation, jaundice, neuralgia, paralysis, parturition, pleurisy, pneumonia, rash, rheumatism, scabies, sciatica, sores, stomachache, syphilis, tetanus, thrush, tumors, ulcers, uterosis, whitlows, yaws, and yellow fever (Duke and Wain, 1981; List and Horhammer, 1979). The oil cake from Jatropha seeds is rich in Nitrogen, Phosphorous, Potassium and other plant essential nutrients and can be a good source of organic manure. The leaves can be used in feeding tusser silkworms. Direct fermentation of seedcake and pulp produces an organic fertilizer that has high potential for export to developed countries (Gubitz, 1997). Pounded leaves is applied near horses' eyes to repel flies in some countries (Watt and Breyer-Brandwijk, 1962).

1.3 Problem statement and justification

Jatropha is a drought resistant, inedible oilseed bearing tree which does not compete with food crops for good agricultural land or adversely impact the rainforest. It grows in tropical and sub-tropical regions and produces high yields of inedible vegetable oil that can be used to produce high-quality biodiesel(James, 1983). Jatropha can grow

on a wide range of land types, including non-arable, marginal and waste land(. In regard to fuel consumption, developing countries are the worst hit by rising prices as they are net importers of oil, besides consuming twice as much oil per dollar of GDP as the United States (Karen, 2007). The rising costs and inaccessibility of fossil fuel leaves about 2 billion people without reliable energy sources worldwide. Since the two-thirds of the people in the developing world who derive their incomes from agriculture and Jatropha based biodiesel has enormous potential to change their situation for the better and poverty can be broken by Jatropha cultivation as this dedicated crop has a huge potential for replication world -wide, improving the livelihood of many more. At the community level, farmers that produce dedicated energy crops can grow their incomes and grow their own supply of affordable and reliable energy while at the national level, producing more biofuels will generate new industries, new technologies, new jobs and new markets(Breitenstern,2002). At the same time, producing more biofuels will reduce energy expenditures and allow developing countries to put more of their resources into health, education and other services for their neediest citizens.

In recent years studies have shown that Jatropha oil burns with one fifth the carbon emission of fossil fuels. Scientists estimate that if even a quarter of the continent's arable land were plowed into Jatropha plantations, output would surpass 20 million barrels a day (Reinhard, 1996). With the combination of oil production and erosion control and the ability to grow in marginal areas with poor soil and low rainfall, this species has great potential in rural development as a source of household income and at the same time creating environmental benefits. Since Jatropha is produced as a low input crop, it's a potentially useful crop in rural agro-industrial development (Moyo, 1998)

It has been established that one of the major challenges facing the Jatropha is production and availability of planting material. The seed that is commonly used in most of today's cultivation, has varying levels of dormancy that may be difficult to break and plants regenerated from seeds seem to take very long period to maturity (Jukarin,

Yamada & Sakaguchi, 1998).The first yield from a crop propagated through seeds is obtained after 2-4 years after planting (Dierk, 2001)

Some farmers purchase seedlings but these are costly for the small scale producer and accessing the possibilities of farmer's nursery production may go a long way in availing the seeds to such farmers and possible commercialization of the seedlings is one way of generating income that could be adopted by the local communities in arid areas such as Kambu division (VeSeL, 2007)

The present study may thus contribute to addressing this problem by assessing alternative methods of propagation of this crop. Although this crop may contribute to rural development by income generation and increasing the efficiency of rural and agricultural processes, production varies greatly and profitable claims are made without well-founded truths. Being an uncultivated wild species, it's not known what procedures for tissue cultures are best followed to regenerate the crop although a few attempts have been made in other countries. This could form a major breakthrough in the Jatropha world since planting materials are a major drawback to current and future production schemes.

The question of germination and viability requires urgent attention if farmers are to benefit from the crop. This is because the information available is limited and unreliable, giving varying ideas on productivity, viability, germination potential, varieties that are questionable.

Good nursery work is the basis of successful afforestation activities and a probe into possible improvement techniques such as use of Sand, polythene bags and other practices may improve productivity. Good management of the nursery could possibly result in seedling production at the end of the first year itself. Nurseries supply seedlings to the farmers in their villages thus providing an alternative to the expensive seeds. Yet in order to guarantee good quality seedlings and survival in the field after planting, good nursery practices should be followed. To raise sufficient nursery stock, information on choice of site, soil medium and variety needs to be considered carefully, areas that the present study seeks to address.

1.4 Objectives

Major Objectives

Efficient plant regeneration via explants and media and optimizing germination of *Jatropha curcas*.

Specific Objectives

To test the effect of hormones and explants on Jatropha callus formation, rooting and shooting in vitro.

To test the effect of temperature, variety, germination container and sand on germination.

1.5 Hypotheses

Various explants and hormones are not effectively useful in obtaining callus roots and shoots under tissue culture propagation of *Jatropha curcas.*

The application of different temperature, soil type, planting container and covering does not affect germination of *Jatropha curcas*.

CHAPTER 2: Literature Review

2.1 Botanical Description

Jatropha curcas or Physic Nut belongs to the family Euphorbiae and is closely related to castor and cassava. It's a drought tolerant perennial growing well in marginal or poor soil (Heller, 1996). Other varieties of Jatropha apart from *Jatropha curcas* include *Jatropha curcas, Jatropha integrerrima, Jatropha gossypifolia, Jatropha glandulifera, Jatropha tanjorensis, Jatropha multifida, Jatropha podagrica, and Jatropha integrerrima* (Deghan&Webster, 1979)

The plant is a small tree or shrub with smooth gray bark, which exudes whitish colored watery latex when cut. It normally grows between 3-5m in height but can attain 8-10m high under favorable conditions (Morton, 1977). It has large pale green leaves and diameter up to 20 cm. The trunk is straight, branching low above the ground; bark is thin and yellowish. Leaves are 6 x 15 cm and lobed (Shmook, 1996).

Flowers small and greenish, unisexual with male and female flowers at the same tree (Hikwa, 1995) although individually and female flowers are larger than the male ones(Deven, 2007). The trees are deciduous, shedding the leaves in the dry season. In permanently humid regions, flowering occurs throughout the year (Felger, 1985).The flowers are pollinated by insects especially honey bees (Morton, 1977)

Fruit is a gray-brown capsule while seeds are black, about 2 cm long and 1 cm thick (James, 1983). An inflorescence approximately yields a bunch of 10 or more fruits which are ovoid and when seeds mature, a three bi-valved cocci forms (Patric, 2007)

2.2 Ecological requirements

Jatropha curcas is drought tolerant and grows in semi-arid regions with mean annual rainfall of between 500mm-1, 000mm (Dierk, 2001). It thrives well in altitudes between 0-500m with mean annual temperature of between 20-28 degrees. The crop is majorly found in the tropics and sub-tropics and thrives in hot areas. It does not require heavy fertilization or irrigation (James, 1983). Although it does not require heavy fertilization or irrigation, fertile soils results in improved yields (Hikwa, 1995). It grows in a very wide variety of soils, including gravelly, sandy and saline soils. Jatropha sheds its

leaves in winter, thus improving soil fertility around the area and providing nutrients to the crop (Perry, 1980).

2.3 Jatropha Production and Productivity

The plant can either be grown from seeds, stem cuttings, grafting budding or air layering. The seeds are sown in a seedbed either through broadcasting or in rows (Centre for Jatropha Promotion,2009). The first shoots are expected after 4 to 7 days of sowing (Wegmeshaus, 1993). Seedlings are usually transplanted into either 6 x 9cm or bigger sized potting polythene papers (bags) when the third leaf starts to develop or after a period of two weeks. Transplanting to the field is usually determined by the on-set of rainy season (Shmook, 1996). While *Jatropha* curcas may start yielding from 9–12 months time, the effective yield in most crops is obtained only after 2 - 3 years time (Deven, 2007). If planted in hedges, the reported productivity of *Jatropha* is from 0.8 kg. to 1.0 kg. of seed per meter of live fence. The seed production is around 3.5 tons / hectare. Seed production ranges from about 0.4 tons per hectare in first year to over 5 tons per hectare after 3 years (Wegmershaus, 1993).

2.5 Jatropha oil

The non-edible vegetable oil of *Jatropha curcas* is a viable alternative to diesel oil as it has comparable physiochemical and performance characteristics. Car engine can be run with *Jatropha curcas* without much change in design (Gaydou & Menet, 1982). The major advantages of Jatropha oil as a diesel oil substitute lies on its higher flash point and cetane number. It has been proven on 4-stroke Mitsubishi engine in Japan (Kandpal& Mira, 1994). Research indicates the quality of jatropha oil is better than most other crop oils for making jet fuel. Jatropha-based fuel also produces about half the harmful carbon emissions of fossil fuel (ARS, USDA, 2002). Researchers in the United States have also found that an acre of jatropha plants can yield 5 to 7 times more oil than other potential feed stocks such as soybeans (D1 oils, 2005). Jatropha oil is significantly cheaper than crude oil. Jatropha could be used to produce a barrel of fuel for around $43, less than the cost of sugar-cane-based ethanol ($45 per barrel) or corn-based ethanol ($83 per barrel) currently favoured in the United States (Peter, 2008,

Patric, 2007)). There is no controversy on growing jatropha as feedstock for fuel because it is a non-food crop (Centre for Jatropha Promotion,2009). Research says that farmers trying to recover from citrus canker or greening may use jatropha. Because it fares well in bad soil, the crop might be helpful for lands unsuitable for traditional agriculture (FAO, 1994). The oil has similar properties to palm oil. It can be used in place of kerosene and diesel and as a substitute for fuel wood (Deven, 2007).

Table 1: Physiochemical parameters of Jatropha seed oil

PARAMETER	QUANTITY
1. Density at 15degrees Celsius (g/cm3)	0.920
2. Viscosity at 30 degrees Celsius	17.152
3. Flash point (Degrees)	240
4. Sulphur content (ppm)	Not measured
5. Iodine value (mg iodine/g)	105.2+/- 0.7
6. Acid value (Mg KOH/g)	3.5+/- 0.1
7. Calorific value (MJ/Kg)	40.7

Literature (Akintayo, 1993)

Table 2: Physical and chemical properties of JCL oil

Oil (%)	48.5
Specific Gravity (kg/m3)	929.7
Refractive index	1.4650
Iodine Number	97.5
Saponification value	102.9
Calorific Vale (MJ/kg)	41.77
Kinematic Viscosity (cSt)	49.93
Fatty Acid Distribution of Jatropha oil	
Fatty Acid	**% by weight**
Palmetic Acid	
Stearic Acid	
Oleic Acid	
Linoliec Acid	

Linolenic Acid	

Source: Federation of Indian Chambers of Commerce and Industry (FICCI) website

2.6 Tissue Culture

The technique of tissue culture consists of taking a piece of a plant such as a stem tip, node, meristem, embryo, or even a seed and placing it in a sterile, (usually gel-based) nutrient medium where it multiplies (Ajay, 2001). The formulation of the growth medium is changed depending upon whether you are trying to get the plant to produce undifferentiated callus tissue, multiply the number of plantlets, grow roots, or multiply embryos for "artificial seed"(Kyte&Lydiane, 1996). Micropropagation is the art and science of plant multiplication *in vitro*. The process includes many steps--stock plant care, explant selection and sterilization, media manipulation to obtain proliferation, rooting, acclimation, and growing on of liners (Dan L, 1991). The basis of tissue culture revolves around two characteristics of plants; totipotency and plasticity(Dodd, 1998)The production of plant through tissue culture operates on the fact that a plant cell has the ability to regenerate a whole plant, an aspect called totipotency(Collin,1998).

The regeneration of whole organisms depends upon the concept that all plant cells can, given the correct stimuli, express the total genetic potential of the parent plant. This maintenance of genetic potential is called 'totipotency'. In practical terms though, identifying the culture conditions and stimuli required to manifest this totipotency can be extremely difficult and it is still a largely empirical process (Fowler, 2000). The plasticity allows plants to alter their metabolism; growth and development to best suit their environment (Ramage, 2002). Particularly important aspects of this adaptation, as far as plant tissue culture and regeneration are concerned, are the abilities to initiate cell division from almost any tissue of the plant and to regenerate lost

organs or undergo different developmental pathways in response to particular stimuli (Gamburg, 2002).

One can use protoplasts, leaf pieces or roots to generate a new plant using media and hormones (Dodd 1998). The formulation of the growth medium is changed depending upon whether you are trying to get the plant to produce undifferentiated callus tissue, multiply the number of plantlets, grow roots, or multiply embryos for "artificial seed"(Debergh,1991). Tissue culture cells have a small vacuole, lack chloroplasts and photosynthetic pathways and the structural or chemical features that distinguish so many cell types within the intact plant are absent (Collin, 1998).Tissue cultured cells can also be induced to re-differentiate into whole plants by alterations to the growth media (Ramage, 2002).

2.6.1 Growth regulators/hormones

Plant growth regulators are chemicals that when used in small amounts promote and influence the growth, development, and differentiation of cells and tissues. Although they naturally occur within plants, several related compounds can be produced artificially by man (Thomas, 1979). The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. For example, an excess of auxin will often result in a proliferation of roots, while an excess of cytokinin may yield shoots (Huibo & Zihui, 2006) A balance of both auxin and cytokinin will often produce an unorganized growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as well as the medium composition (Else, 2001). There are five classes of plant hormones including Auxin, Cytokinins, Ethylene, Abscisic acid and Gibberellins (Dodd, 1995). Auxins are found in embryo of seed, young leaves, and meristems of apical buds and stimulates cell elongation. They take part in phototropism, gravitropism, apical dominance, and vascular differentiation, inhibit abscission prior to formation of abscission layer, stimulate ethylene synthesis and fruit development and induce adventitious roots on cuttings (Donelly, 1988).

Cytokinins are synthesized in roots and transported to other organs. They stimulate cell division, reverse apical dominance, are involved in shoot growth and delay leaf sequence (Smith, 1992). Ethylene is found occurring in tissues of ripening fruits, nodes of stems, senescent leaves and flowers. Abscisic Acid is found in leaves, stems, and green fruit and stimulates stomatal closure. Gibberellins occurs in meristems of apical buds and roots, young leaves and embryo. It stimulates shoot elongation, bolting and flowering in biennials and regulates production of hydrolytic enzymes in grains (Trigiano, 1996).

2.6.2 Media components

One of the most complete media is in the Murashige and Skoog shoot multiplication medium B (MSMB) (Sigma Catalog No. M7149).

Table 3:Components of Murashige & Skoog basic media

Components	Quantity (g/l)
Macronutrient salt solution	
Ammonium nitrate	1.650
Calcium Chloride anhydrous	0.3320
Magnesium Sulphate	0.1807
Potassium Nitrate	0.1900
Potassium Phosphate monobasic	0.170
Ammonium Sulphate	N/A
Micronutrient salt solution	
Boric acid	6.2
Cobalt Chloride	0.025
Cupric Sulphate	0.025
Na2-EDTA-2H2O	37.3
Manganese Sulphate-H2O	16.9
Molybdic acid	0.25
Potassium Iodide	0.83
Zinc Sulphate-7H2O	8.6
Ferrous Sulphate-H2O	0.0278
Thiamine-Hcl	0.000100

M & S Complete Medium	Quantity
Sucrose	30
Agar	8
Vitamins	
Glycerine	0.00200
Myo-inositol	0.1000
Nicotinic Acid	0.005
Pyridoxine-Hcl	0.0005
Hormones	
Indole-3-butyric acid (IBA)	
6-Benylaminopurin (BAP)	
Ph 10.5 at room temperature	

2.6.3 Applications of tissue culture

There are several areas that tissue culture techniques are useful, including; micro propagation using meristem(Fowler,2000) and shoot culture to produce large numbers of identical individuals ,screening programmes of cells, rather than plants for advantageous characters(Rost & Thomas, 1979), large-scale growth of plant cells in liquid culture as a source of secondary products, crossing distantly related species by protoplast fusion and regeneration of the novel hybrid(Collin, 1998), production of dihaploid plants from haploid cultures to achieve homozygous lines more rapidly in breeding programmes and as a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants and removal of viruses by propagation from meristematic tissues(Roca & Davide, 2004)

2.6.4 Effects of hormones on plant regeneration

In one trial on Jatropha, shoot bud proliferation from axillaries was assessed on an initial basal Murashige and Skoog (MS) salt medium supplemented with different concentrations of benzyl adenine (BA), kinetin and thidiazuron (TDZ) followed by subculture to medium with 4.4-8.9 μM BA. Regardless of the concentration of BA in the

subculture medium, shoot multiplication rate was optimum (10–12.3) with primary culture on medium supplemented with 2.3–4.5 µM TDZ (Shujantha et.al, 2005)

Elsewhere in Jatropha, efficient adventitious shoot regeneration from leaf tissues was achieved with culture on medium with 8.9–44.4 µM BA + 4.9 µM indole-3-butyric acid (IBA) followed by transfer to medium supplemented with 8.9 µM BA + 2.5 µM IBA.

In another study of jatropha tissue culture, adventitious shoot buds were induced from very young leaf explants of in vitro germinated seedlings as well as mature field-grown plants cultured on Murashige and Skoog's (MS) medium supplemented with thidiazuron (TDZ) (2.27 µM), 6-benzylaminopurine (BA) (2.22 µM) and indole-3-butyric acid (IBA) (0.49 µM). The presence of TDZ in the induction medium has greater influence on the induction of adventitious shoot buds, whereas BA in the absence of TDZ promoted callus induction rather than shoot buds. Induced shoot buds were multiplied and elongated into shoots following transfer to the MS medium supplemented with BA (4.44 µM), kinetin (Kn) (2.33 µM), indole-3-acetic acid (IAA) (1.43 µM), and gibberellic acid (GA_3) (0.72 µM). Well-developed shoots were rooted on MS medium supplemented with IBA (0.5 µM) after 30 days. Regenerated plants after 2 months of acclimatization were successfully transferred to the field without visible morphological variation (Ajay & Deore, 2007)

2.7 Jatropha seed propagation

Jatropha can be established from seed, seedlings and cuttings. Plants from seeds develop a taproot and four lateral roots, while cuttings do not develop a taproot but only lateral roots. Two seeds resembling peanuts are present in the nuts. Seeds are non-edible and are toxic. The toxicity symptoms include acute abdominal pain and nausea about half hour after ingestion (Degharn, 1979). Some suggestions have been made to claim that the best result is saplings from seeds raised in a nursery in polythene bags, (about 3 - 4 months) which are planted just before the rainy season (Deven, 2007). Plants from cuttings are not so resistant to drought, because they don't develop a taproot, which reaches far into the depth of the soil. If the seeds are put directly into

the soil, they might be attacked by cattle which step on them and eat them, because the repellant is not yet developed since grazing cows sometimes dig into the soil with the hoofs.

2.7.1 Effect of environment on seed germination

A seed contains an embryonic plant in a resting condition, and germination is its resumption of growth. Seeds will begin to germinate when the soil temperature is in the appropriate range and when water and oxygen are available (Siegal, 1962) One of the most usual causes of failures with seed is watering. Seeds need a supply of moisture and air in the soil around them. The Polythene bag method is highly recommended as it's believed to be very useful in helping to overcome rotting problem. Watering of containers of very small seeds if done from below, allows the water to creep up until the surface glistens (Coupland et.al,). Fortunately most seeds are tolerant of a wide range of temperatures but it is wise to try to maintain a steady, not fluctuating temperature (Martin, 1972).

Ventilation and light are other important factors of germination (Pfaler, 1981). The depth of sowing also affects germination of plants. Sowing too deeply results in a seed that has only enough food within itself for a limited period of growth and a tiny seed sown too deeply soon expends that energy and dies before it can reach the surface (Rocal & Arlt, 2005)

2.7.2 Other factors affecting germination

Some perennials and tree and shrub seeds can be very slow and erratic in germination. This may sometimes be due to seed dormancy *(*Berlin, 2000*)*; a condition which prevents the seed from germinating even when it is perfectly healthy and all conditions for germination are at optimum (Takayama, 2005). The natural method is to sow the seeds out of doors somewhere where they will be sheltered from extremes of climate, predators, etc. and leave them until they emerge, which may be two or three seasons later. Dormancy, however, can be broken through artificial means (Huibo, 2001).

Some seeds, e.g. Sweet peas, ipomoea, have hard seed coats which prevent moisture being absorbed by the seed. Scratching or abraiding can be achieved by chipping the seed with a sharp knife at a part furthest away from the 'eye', by rubbing lightly with sandpaper or with very small seed pricking carefully once with a needle. (Smith, 1992). Soaking is beneficial in some ways, for instance it can soften a hard seed coat and also leach out any chemical inhibitors in the seed which may prevent germination. As each seed swells it should be removed and sown before it has time to dry out (Shrivastava, 2002).

Some seeds need a period of moisture and cold after harvest before they will germinate-usually this is necessary to either allow the embryo to mature or to break dormancy (Takanyama, 2005). The seeds must be moist whilst being pre-chilled, but it doesn't usually benefit them to be actually in water or at temperatures below freezing (Huibo et.al 2006).
Light also seems to be beneficial after prechilling and so pre-chilled seeds should have only the lightest covering of compost over them, if any is required, and the seed trays etc. should be in the light and not covered with brown paper.
Some seeds have a combination of dormancy's and each one has to be broken in turn and in the right sequence before germination can take place (Rost & Thomas,2004).

2.8 Effect of planting material on growth and regeneration

Seeds are widely used as planting material by many farmers who cultivate *Jatropha curcas*. However, sources reveal that plants from seeds produce after 2-3 years from planting date (Hikwa et.al, 1997). Other observations show that they may yield after 2-4 years of planting depending on environmental conditions (Singh, 2008). Seeds have limited viability and loose almost 50% viability within 15 months of harvest. Propagation from seeds also leads to genetic variability and makes the crop prone to diseases as the seeds may acquire the diseases during early stages of growth from environment or inherit from the parent plant. (Ginwal & Shrivastava.2004). Vegetative

propagation offers the advantage in developing true to type disease free varieties of economic and commercially important plants for clonal multiplication. (Shujantha, 2005)

2.9 Effect of seed source on germination

Wide range of variation has been observed for each character studies. A first attempt experiment in India that collected and investigated seeds from various sources in the country showed that seeds from different sources gave varying results when rated for germination and growth rates (Norman,1993). They suggested that species from one region might vary in their growth rates possibly due to altitude, temperature, soil conditions and rainfall (Ginwal &Shrivastava 2004)

2.9.1 REFERENCES(Chapter 1 & 2)

Ajay C. Deore and T. Sudhakar Johnson.,2008: High-frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L.: an important biodiesel plant.

Akintayo, E.T, 2004: characteristics and composition of *Parkia and* Jatropha *curcas* oils and cakes.Bioresource technology, 1992, 307-210

ARS, USDA. 2002: Evaluation and bioinduction of energy components of Jatropha curcas.

Becker et al., 1999: Studies on Propagation of non-toxic variety of Jatropha curcas, Stuttgart, Germany.

Berlin / Heidelberg., 2004 : Dormancy termination of western white pine (Pinus monticola Dougl. Ex D. Don) seeds is associated with changes in abscisic acid metabolism Journal Planta Publisher Springer ISSN 0032-0935 (Print) 1432-2048 (Online) Issue Volume 218, Number 4 / February, 2004 Category Original Article DOI 10.1007/s00425-003-1139-8 Pages 630-639.

Berlin. C Al, H. W., and H. M ORISHIMA, 2000 Genomic regions affecting seed shattering and seed **dormancy** in rice.

Black M, Butler J, Hughes M., 1987: Control and development of dormancy in cereals. In: Mares DJ, ed. Fourth International Symposium on Pre-Harvest Sprouting in Cereals, Boulder, Co. USA: Westview Press, 379-92

Breitenstein/Shila.,2002: Enterprise of Trust – economic welfare in rural areas through the use of renewable energies.

Centre for Jatropha promotion and Biodiesel. 2009: Non food Biodiesel.

Collin, H.A. and S. Edwards., 1998: Plant Cell. Culture

Dan Lineberger. Plant Tissue Culture INFORMATION EXCHANGE, Texas A & M University

Debergh, P.C. and R.H. Zimmerman., 1991: Micropropagation, Technology and Application. Kluwer Ac Henning, Reinhard K., Jatropha Development Project to the Sudan – Report of a Mission to 'UNIDO, Khartoum, 2001, unpublished; ademic Publishers. Lab design, info on labs worldwide, in depth discussions of problems.

Deghan, B. and G.L. Webster.,1979: Morphology and infrageneric relationships of the genus Jatropha (Euphorbiciae). University of California Publications in Botany, Vol.74.

Deven Leigh., 2007: Jatropha Biomass: Energy for the Future Deven Leigh 6/2/2007

Dierk Hesselbach., 2001: Guide n the cultivation and use of Jatropha curcas. For farmers and field extension staff.

Dodds, J. H. and Roberts, L. W.,1995: *Experiments in plant tissue culture*. Cambridge University Press, Cambridge.

Donnelly, D.J., and Vidaver, D.W., 1988: Glossary of Plant Tissue Culture, Portland, Timber Press. Good definitions of tissue culture terms.

Duke, J.A. and Atchley, A.A., 1984: Proximate analysis. In: Christie, B.R. (ed.), The handbook of plant science in agriculture. CRC Press, Inc., Boca Raton, FL.

Duke, J.A. and Ayensu, E.S.,1985: Medicinal plants of China. Reference Publications, Inc. Algonac, MI.

Duke, J.A. and Wain, K.K., 1981: Medicinal plants of the world. 3 vols.

D1 Oils:2005 csr best practice, Article 13, London, United Kingdom, 2005.

Else M.A.1; Coupland D.2; Dutton L.3; Jackson M.B.,2001: *Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (Ricinus communis) despite diminished delivery of ABA from the roots to shoots in xylem sap.* Physiologia Plantarum, Volume 111, Number 1, January 2001, pp. 46-54(9) Blackwell Publishing

Felger, Richard, Mary, Moser.1985: People of the Desert Sea Ethinobotany of the Seri-Indians; Tucson University of Arizona Press.

Food and Agricultural Organization. 1994: Jatropha curcas: *a multipurpose species for problematic sites.* World Bank Land Resources Series No. 1: *MFP News,* Vol. 4, No. 3, January-March 1994.)

Fowler, M. R., 2000: Plant cell culture, laboratory techniques. In *Encyclopedia of cell technology* (ed. R. E. Spier), pp. 994–1004. Wiley, New York.

Gamborg, O. L., 2002: Plant tissue culture. Biotechnology. Milestones. *In vitro Cellular and Developmental Biology—Plant*, **38**, 84–92.

Gaydou, A.M.L.Menet, G. Ravelojaona and P. Geneste., 1982: Vegetable energy sources in Madagaskar; ethyl alcohol and oil seeds (French). Oleagineux 37(3): 135-141.

Gübitz et al.,1997: Biofuels and Industrial Products from *Jatropha curcas*, developed from the Symposium "Jatropha 97", Managua, Nicaragua, Febr. 1997.

H.S Ginwal, P.S Rawat & R.L Shrivasta., 2004: Seed source variation in growth, performance and oil yield of Jatropha curcas in Central India.

Huibo Ren, Zhihui Gao, Lin Chen , Kaifa Wei, Jing Liu, Yijuan Fan, William J. Davies, Wensuo Jia,{dagger} and Jianhua Zhang,{dagger} JXB.,2006: *Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit.* Advance Access originally published online on September 18, 2006. Journal of Experimental Botany 2007 58(2):211-219.

Hikwa (1995), Henning (1996), Makkar, Becker& Schmook (1997) Agronomy Research Institute, Department of research and Specialist services, Harare, Zimbabwe.

Heller J.,1996: Physic nut Jatropha curcas-Promoting the conservation and use of underutilized and neglected crops.

Henning R., 1996: Combating Desertification, The Jatropha Project of Mali, West. Africa.

Henning R., 2000b: The Jatropha Manual. A guide to the intergrated exploitation of Jatropha Plant in Zambia

Henning, Reinhard K., 1999: The Jatropha System in Zambia – Evaluation of the existing Jatropha activities and proposals for an implementation strategy in Southern Province of Zambia, 1999 (non published feasibility study).

Henning, Reinhard K., 2001: Jatropha Development Project to the Sudan – Report of a Mission to UNIDO, Khartoum, 2001, unpublished.

Henning, Reinhard K., 2004: The Jatropha website http://www.jatropha.org, 1997 – 2004:

Henning, Reinhard K., 1997: Combating Desertification by integrated Utilization of the Jatropha plant – Experiences of the Jatropha Project in Mali, West Africa, 1997;

Holm, L.G., Pancho, J.V., Herberger, J.P., and Plucknett, D.L., 1979: A geographical atlas of world weeds. John Wiley & Sons, New York.

Jacobs DI, van der Heijden R, Verpoorte R (2000) Proteomics in plant biotechnology and secondary metabolism research. Phytochem Anal **11:** 277-287

Jonnes N & MillerJ.H.,1992: Jatropha curcas, a multipurpose species for problematic sites, Land resource series.

Kandpal, J.B and Mira Madan.,1994: Jatropha curcas: a renewable energy source for meetind future energy needs.

Karen Palmer., 2007: Newsweek International, Feb. 19, 2007 issue. Jatropha, Europe's answer for biofuels?

Kyte, Lydiane and J. Kleyn.,1996: Plants from Test Tubes: An Introduction to Micropropagation, 3rd ed., Timber Press, 1996. Good basics for the beginning amateur or grower.

List, P.H. and Horhammer, L., 1969–1979: Hager's handbuch der pharmazeutischen praxis. vols 2–6. Springer-Verlag, Berlin.

Maundu, P. and B.O.Tengnas (eds)., 2005: Useful trees and shrubs for Kenya.

Martin FW., 1972: "In Vitro Measurement of Pollen Tube Growth Inhibition". *Plant Physiol* **49** (6): 924–925. PMID 16658085.

Morton, J.F., 1977: Major medicinal plants. C.C. Thomas, Springfield, IL.

Morton, J.F., 1981: Atlas of medicinal plants of middle America. Bahamas to Yucatan. C.C. Thomas, Springfield, IL.

M.Shujantha, H.P.S Makkar & K.Becker .,2005: Shoot buds proliferation from axillary nodes and leaf nodes.

Moyo, S.K.,1998: exploring the potential of Jatropha curcas L. in Rural Development and Environmental Protection.

Norman C. Deno, 139 Lenor Dr., 1993: State College PA 16801, USA. *Seed Germination: Theory and Practice*, An extensive study of the germination rates of a huge variety of seeds under different experimental conditions, including temperature variation and chemical environment.

Openshaw K., 2000: A review of Jatropha curcas: An oil plant of unfulfilled promise. Biomass and Energy 19:1-5

Patrick Barta., 2007: The Wall Street Journal, 2007

Perry, L.M., 1980: Medicinal plants of east and southeast Asia. MIT Press, Cambridge.

Pfahler PL., 1981: "In vitro germination characteristics of maize pollen to detect biological activity of environmental pollutants". *Environ. Health Perspect.* **37**: 125–32. doi:10.2307/3429260. PMID 7460877.

Ramage, C. M. and Williams, R. R., 2002: Mineral nutrition and plant morphogenesis. *Invitro Cellular and Developmental Biology—Plant*, **38**, 116–24.

Reinhard K. Henning, bagani, Rothkreuz 11, D-88138., 2008: Jatropha curcas in Africa, an evaluation. Weissensberg, Germany.

R.N.Singh, D.K. Vyas, N.S, L. Srivastava, Madhuri Narra., 2008: Holistic approach to utilize all parts of Jatropha fruit for energy. Renewable Energy, Volume 33, Issue 8, August 2008, Pages 1868-1873

Roca, M.G.; Arlt, J., Jeffree, C.E.; Read, N.D., 2005: Cell biology of conidial anastomosis tubes in Neurospora crassa. Eukaryotic Cell 4: 911-919.

Roca M., M.G.;2004: Davide, L.C.; Davide, L.M.; Mendes-Costa, M.C.; Schwan, R.F.;

Rost, Thomas L., and T. Elliot Weier., 1979: *Botany: a brief introduction to plant biology.* New York: Wiley. Pages 155-170. ISBN 0-471-02114-8

Schmook, Birgit., 1996: Jatropha curcas L. Gensammlung Halbinsel Yucatan und Veracruz/Mexico, 1996.

Sharma et.al. 1995: Jatropha oil and diversity of use.

Smith, Roberta H., 1992: Plant Tissue Culture-Techniques and Experiments. Academic Press. $35.00. Good introduction and broad base for college course.

S. M. Siegel, L. A. Rosen., 1962: *Effects of Reduced Oxygen Tension on Germination and Seedling Growth* Physiologia Plantarum 15 (3), 437–444.

Srivastava, L. M., 2002: *Plant growth and development hormones and environment.* Amsterdam:AcademicPress.Page143.

Staubmann R, Ncube I, Gubitz GM, Steiner W, Read JS.,1999: Department of Biotechnology, Graz University of Technology, Graz, Austria

Sukarin, W., Yamada,Y. &Sakaguchi,S.,1987: Characteristic of physic nut. Jatropha curcas L. as a new biomass crop in the tropics. Jpn. Agric.Res. Quart. Japan 20(4):302-303

Surma G.D, Gupta S.N &Khabiruddin, M., 1997: Cultivation of Jatropha curcas as a future source of hydrocarbons and other Industrial products.

Symposium "Jatropha 97", Managua, Nicaragua, Febr.

Takayama S, Isogai A ., 2005: "Self-incompatibility in plants". *Annu Rev Plant Biol* **56**: 467–89. doi:10.1146/annurev.arplant.56.032604.144249. PMID 15862104

Tewari, J.P. and Shukla, I.K.,2008: Inhibition of infectivity of 2 strains of watermelon mosaic virus by latex of some angiosperms. Geobios. Jodhpur, India. 9(3):124–126.

Trigiano, Robert N, and Dennis J. Gray, eds.,1996: Plant Tissue Culture Concepts and Laboratory Exercises. CRC Press. $65.00. For the advanced student.

Takayama S, Isogai A (2005). "Self-incompatibility in plants". *Annu Rev Plant Biol* **56**: 467–89.

Vikram Koundinya .,2008: Jatropha. Iowa State University

Watt, J.M. and Breyer-Brandwijk, M.G.,1962: The medicinal and poisonous plants of southern and eastern Africa. 2nd ed. E. &S. Livingstone, Ltd., Edinburgh and London.

Wegmershaus, R. Oliver, G.,1993: Jatropha curcas in Zimbabwe, Growers Handbook; Plant Oil & Engine Development Group Ptv. Ltd., Harare, 1993 (non published copy);

Wheals, A., 2004: Conidial anastomoses fusions between Colletotrichum species. Mycological Research. 108, 11: 1320-1326.

Wiemer, Hans-Jürgen.,1996: Financial and Economic Analysis of the Jatropha System, Report for GTZ, 1996;

CHAPTER THREE: Effect of hormones and explants on rooting and shooting of *Jatropha curcas L.* cultured in-vitro.

3.1 Abstract

Jatropha curcas is among the most important tree crops in the world yet sufficient good quality propagation material is a major production constraint. The use of hormones and different parts of a plant in tissue culture has been reported to affect the productivity of most plants including *Jatropha curcas*. Being a recalcitrant plant, there are difficulties in the reproduction process. The present study tried to regenerate a protocol that would assist in producing the crop through in-vitro culture.

Laboratory and greenhouse experiments were conducted to investigate the best micro propagation and seed germination methods in Tissue culture Laboratory at Kabete campus and the Kenya Agricultural Research Institute, National Agricultural Laboratories(KARI, NAL) in Nairobi district during 2007 to 2009. A possible regenerative protocol for induction of adventitious shoots and roots from seed and shoot tip cultures of Jatropha curcas was developed. Callus was easily developed from MS media with supplements of BAP, IBA and or NAA from all the explants (shoot tip, leaf and stem) with BAP8ml and IBA4ml combination performing best. Adventitious shoots were induced from in vitro germinated shoot tip explants of nursery grown seedlings cultured on Murashige and Skoogs (MS) medium supplemented with BAP and indole/3/butyric acid IBA . In cultured seeds, the best shooting was obtained from MS medium supplemented with BAP, Kn and Adenine Sulphate combination.

The presence of Kinetin and Adenine sulphate in the induction medium has greater influence in the induction of adventitious shoots. Rooting was obtained from seeds cultured on MS supplemented with Kinetin and Adenine sulphate.

3.2 Introduction

Jatropha curcas commonly known as physic nut is a perennial shrub that has oily seeds with a potential for producing biodiesel oil(Swarup 2004). Jatropha curcas

production may not affect production of important food crops because it thrives in non productive lands. Tissue culture presents one of the best methods of obtaining true to type plants. This will help in meeting existing demand for planting material and enable future development of the crop. A technique for large scale regeneration of shoots and roots in vitro produced from shoot tip, stem and leaf explants was developed from the present study. Shoots and roots were successfully obtained from seed embryo culture. The first yield from a crop propagated through seeds is obtained after 2-4 years after planting (Dierk, 2001)

Some farmers purchase seedlings but these are costly for the small scale producer and accessing the possibilities of farmer's nursery production may go a long way in availing the seeds to such farmers and possible commercialization of the seedlings is one way of generating income that could be adopted by the local communities in arid areas such as Kambu division in Machakos district (VeSeL, 2007)

The present study may thus contribute to addressing this problem by assessing alternative methods of propagation of this crop. Although this crop may contribute to rural development by income generation and increasing the efficiency of rural and agricultural processes, production varies greatly and profitable claims are made without well-founded truths. Being a wild species that is being domesticated, there are not yet clear procedures for tissue cultures are best followed to regenerate the crop. This could form a major breakthrough in Jatropha since planting materials are a major drawback to current and future production schemes.

3.3 Materials and methods

3.3.2 Chemicals

All chemicals were of analytical grade and were purchased from either Kobian (K Ltd. The chemicals included hormones such as BAP, IBA, IAA,NAA, Kn, Zn and As. Others were agar, sucroce, macro and micronutrients.

3.3.3 Media Preparation

The basic media involved preparing the Murashige and Skoog, 1962 Standard media which included Macronutrients, Micronutrients, agar and sucrose. The media was supplemented with BAP, IBA, IAA, NAA, Kinetin, Zeatin, Adenine Sulphate, either alone or in combinations.

EXPERIMENT	CHEMICALS USED	Page
Experiment 1	BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA2mg/l, NAA4mg/l/l+IBA2mg/l, Control (Ms only). Basic media used is Murashige & Skoog (MS) basic media.	
Experiment 2	BAP4mg/l, IBA2mg/l, NAA4mg/l and Control(MS only)	
Experiment 3	BAP1mg/l+0.2mg/lIAA, BAP1mg/l+0.5mg/lKn, BAP1mg/l+1mg/lIBA, BAP1mg/l+0.5mg/lZeatin, BAP1mg/l +0.5mg/l Kn+2mg/l As,	
Experiment 4	BAP1mg/l, NAA4mg/l,IBA1mg/l, Zeatin0.5mg/l, IAA0.2mg/l and Kinetin0.5mg/l	
Experiment 5	BAP1mg/l+0.2mg/lIAA, BAP1mg/l+0.5mg/lKn, BAP1mg/l+1mg/lIBA, BAP1mg/l+0.5mg/lZeatin, NAA4mg/l+Kn0.5mg/l and BAP1mg/l +Kn0.5mg/l +As2mg/l	

3.3.4 Explants Sterilization

Eight month old plants of Jatropha curcas growing in polythene pots from Kambu, Machakos were obtained and kept in the green house. The youngest leaves vere extracted using a sterilized scapel and placed in sterile distilled water in a jar. Similarly, stems were cut and the tip of shoots were cut and excised from the parent plants. The explants were first rinsed in running tap water.Two to three drops of liquid washing detergent(2.0 Teepol) was put into sterile distilled water in a jar. The tops were placed in the jar and the screw lids replaced after which the jar was shaken for 5 minutes. The water was poured off and rinsing was done 3 times using sterile distilled water. The explants were then dipped in 70% alcohol for a few seconds and rinsed in sterile distilled

water. Into 30ml of household bleach 2 drops of detergent were added in a jar then the explants were added and shaken intermittently for 10 minutes. The mixture was drained and water added, covered and shaken then taken to the transfer chamber.

The transfer chamber was sprayed with isopropyl alcohol. The instruments, work surface and gloved hands were sterilized time and again using the alcohol spray and solution.

3.3.4 Culturing

Forceps and knives were immersed for 30seconds in 10%bleach diluted in distilled water and these were used to cut and transfer the explants into media. The stems and leaves were cut into small pieces before culturing. The cultured explants were then placed on the growth chamber. Sub-culturing into fresh media was done after every three weeks.

Embryos from seeds of three varieties were used. The seeds were sterilized and then pre-germinated before being cultured into the growth medium.

All tissue culture experiments were carried out in culture tubes (150times25) mm containing 20ml of culture medium ph of media was adjusted to 7.0+ or-0.1 as recommended for tissue culture experiments.

3.3.5 Experimental design

The experimental design used was completely randomized design (CRD). Data was analyzed by ANOVA to detect significant differences between means. Means separation was done using Duncan's Multiple Range Test (DMRT) at 5% probability level. Variability of data was expressed as mean standard deviation.

In experiment 1, the plant part levels were three and were subjected to four levels of treatment. Each of the treatment was replicated ten times. The total number of observations was 120. Different combinations including BAP8mg/l+IBA4mg/l, BAP4/l+IBA2mg/l, NAA4mg/l/l+IBA2mg/l, Control (Ms only) was supplemented to the MS media at specified concentrations. All the levels of hormonal treatment were able to cause callus formation in at least one of the set ups. However not all the levels produced shoots? Contamination took place in some set ups.

In experiment 2, the plant part levels were three and were subjected to four levels of treatment. Each of the treatment was replicated ten times. The total number of observations was 120. Different concentrations of the hormones BAP8mg/l, IBA4mg/l, NAA4mg/l and Control (MS only) were supplemented to the MS media at specified concentrations.

Experiment 3 the plant part levels were three and were subjected to four levels of treatment. Each of the treatment was replicated ten times. The total number of observations was 120. It involved trials of various hormonal combinations with BAP including BAP/IAA, BAP/Kn, BAP/IBA, BAP/Zeatin, and BAP/Kn/As. It was majorly design to test the effect of hormonal combinations on shooting of shoot tip, stem and leaf explants in Jatropha curcas. The concentrations were BAP (1mg/l), IAA (0.2mg/l), IBA (1mg/l) Zeatin (0.5mg/l), Kinetin (0.5mg/l), Adenine Sulphate (2mg/l)

The fourth involved trials on four seed variety levels TS Morogoro, TZ Arusha, Pemba and Zanzibar subjected to six hormonal treatment levels, BAP, NAA,IBA, Zeatin, IAA and Kinetin. With each treatment replicated 5 times, the number of observations was 120. The concentrations used were BAP (1mg/l), IAA (0.2mg/l), NAA (0.2mg/l), IBA (1mg/l) Zeatin (0.5mg/l), Kinetin (0.5mg/l).

In experiment 5, four seed varieties,TS Morogoro, TZ Arusha, Pemba and Zanzibar were subjected to 6 hormonal combinations BAP/IAA, BAP/Kn, BAP/IBA, BAP/Zeatin, NAA/Kn and BAP/Kn/As. Each was replicated 5 times giving a total observation of 120. Shooting was rare and was only observed in a few of the cultures containing BAP/IBA, BAP/Zeatin and BAP/Kn/As. The concentrations used included BAP (1mg/l), IAA (0.2mg/l), IBA (1mg/l) Zeatin (0.5mg/l), Kinetin (0.5mg/l), Adenine Sulphate (2mg/l)

Data collection

A 30 cm meter rule was used to measure the lengths of shoots while the number of days taken to callus formation, shooting and rooting were simply counted and recorded. The callus was classified into three groups; large=3, medium=2, small=1 and the records were taken through physical observation.

3.4 RESULTS

3.4.0 Experiment 1: Results

3.4.1 Days to Callus formation

The general callus formation was observed within 2 to 4 weeks after culturing of the shoot tip, leaf and stem explants grown on MS medium supplemented by various concentrations of plant growth regulators.

In this trial BAP8ml in combination with IBA gave the earliest callus formation days. The effect of plant parts on the number of days to callus formation was not significant ($p \geq 0.05$), while the treatment type also did not significantly ($p \geq 0.05$) affect the number of days to callus formation. The interaction between plant part and treatment also did not affect the days to callusing significantly ($p \geq 0.05$) as indicated in the table 4.

Table 4 Mean effect of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA8mg/l, NAA4mg/l/l+IBA2mg/l, Control(MS) on days to callus formation of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones				
	BAP8mg/l+IBA4mg/l	BAP4mg/l+IBA2mg/l	NAA4mg/l+IBA2mg/l	NO TREATMENT	Mean
shoot tip	10.6	11.9	10.9	12.6	11.5
Leaf	8.8	12.6	13.5	18.6	13.7
Stem	8.3	14.7	15.9	14.8	13.4
Mean	**9.23**	13.7	13.43	13.33	
LSD ($p \leq 05$)	5.35				
				LSD	4.64

3.4.2 Callus amount

The callus amount was grouped into three, where 1 represent small, 2 represent medium and 3 represent large. In this trial BAP8ml in combination with IBA4ml, and BAP4ml in combination with IBA2ml had set ups with the highest amount of callus.

The plant parts affected the amount of callus formed significantly, while the treatment type did not significantly affect the amount of callus formed. The interaction

between plant part and treatment however affected the amount of callus formed significantly as indicated in the table 5 below.

Table 5 Mean effect of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA8mg/l, NAA4mg/l/l+IBA2mg/l and MSon amount of callus formed of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones				
	BAP8mg/l+IBA4mg/l	BAP4mg/l+IBA2mg/l	NAA4mg/l+IBA2mg/l	NO TREATMENT	Mean
shoot tip	2.3	2.3	2	1	1.9
Leaf	0.6	0.6	0.5	1.1	0.7
Stem	0.8	1.4	1.1	0.7	1
Mean	1.23	1.43	1.2	0.93	
LSD (p≤05)	0.48				

LSD (P≤05) 0.42

3.4.3 Days to shooting

None of the cultures of the leaf and stem explants under this set of produced any shoots i.e. shoots were observed only in the shoot tip explants. Only the first two treatment levels, BAP8ml+IBA4ml and BAP4ml+IBA2ml combinations produced some level of shooting. In general shooting was quite minimal with most cultures producing no shoots at all. The plant parts affected the number of days to shooting significantly. The treatment type had a significant effect on the number of days to shooting. The interaction between plant part and treatment also affected the number of days to shooting significantly as indicated in the table 6.

Table 6 Mean effects of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA8mg/l, NAA4mg/l/l+IBA2mg/l and MS on days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones				
	BAP8mg/l+IBA4mg/l	BAP4mg/l+IBA2mg/l	NAA4mg/l+IBA2mg/l	NO TREATMENT	Mean
shoot tip	19.7	15	0	0	8.68
Leaf	0	0	0	0	0
Stem	0	0	0	0	0
Mean	5.65	5	0	0	
LSD (p≤05)	3.84				

LSD (P≤05) 3.23

3.4.4 Shoot length

The plant parts affected the length of shoots formed significantly. The treatment type had a significant effect on the length of shoots formed. The interaction between plant part and treatment also affected the length of shoots formed significantly as indicated in the table 7.

Table 7 Mean effects of BAP8mg/l+IBA4mg/l, BAP4mg/l+IBA8mg/l, NAA4mg/l/l+IBA2mg/l and MS on shoot length (cm) of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones					
	BAP8mg+l/IBA4mg/l	BAP4mg/l+IBA2mg/l	NAA4mg/l+IBA2mg/l	NO TREATMENT	Mean	LS (p
shoot tip	0.35	0.26	0	0	0.15	0.05
Leaf	0	0	0	0	0	
Stem	0	0	0	0	0	
Mean	0.12	0.09	0	0		
LSD ($p \leq 05$)	0.065					

3.5 Experiment 2

All the levels of hormonal treatment were able to cause callus formation in at least one of the set ups. However not all the levels produced shoots. Contamination took place in quite a number of the set ups thus affection the results.

3.5.1 Days to callus formation

The treatment containing BAP+NAA had the earliest callus formation dates with a mean of 8.3 days observed on the cultured shoot tip explants. The effect of plant parts on number of days taken to callus formation was not significant. The treatment type also did not have a significant effect on the number of days to callus formation. The interaction between plant part and treatment also did not significantly affect the number of days to callus formation as indicated in the table 8.

Table 8 Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on days to callus formation of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones					
	BAP8mg/l	IBA4mg/l	NAA2mg/l	BAP8mg/l+ NAA2mg/l	Mean	LSD (p≤05)
shoot tip	13.1	10.6	13.3	8.3	11.34	4.04
Leaf	10.4	9.6	10.3	11.1	10.35	
Stem	10.6	12.4	8.7	10.4	10.53	
Mean	11.37	10.87	10.77	9.93		
LSD (p≤05)	4.66					

3.5.2 Callus amount

Callus formation was generally low. The effect of plant parts on amount of callus formed was not significant. The treatment type however had a significant effect on the amount of callus formed. The interaction between plant part and treatment also did not significantly affect the amount of callus formation as indicated in the table 9. The cultures containing IBA had the largest amount of callus being observed while BAP+NAA combination had the lowest.

Table 9 Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on amount of callus produced from Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones					
	BAP8mg/l	IBA4mg/l	NAA2mg/l	BAP8mg/l+ NAA2mg/l	Mean	LSD (p≤05)
shoot tip	0.8	2.2	1.4	0.4	1.2	0.41
Leaf	0.7	2.0	1.1	0.5	1.8	
Stem	0.6	1.9	1.1	0.5	1.3	
Mean	0.7	2.03	1.2	0.47		
LSD (p≤05)	0.47					

3.5.3 Days to shooting

Shooting of cultures was generally low and negligible in most of the treatments and explants. Actually shooting was only observed in the shoot tip explants cultures. Only the cultures containing BAP treatment had some level of shooting with the rest giving none. The use of different plant parts significantly affected the number of days taken to shooting.

The treatment type had a significant effect on the days to shooting. The interaction between plant part and treatment also had a significant effect on the days to shooting as indicated in the table 10.

Table 10 Mean effect of BAP8mg/l, IBA4mg/l, NAA2mg/l, BAP8mg/l+NAA2mg/l on days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones					
	BAP8mg/l	IBA4mg/l	NAA2mg/l	BAP8mg/l+ NAA2mg/l	Mean	LSD (p≤05)
shoot tip	9	0	0	0	2.3	1.9
Leaf	0	0	0	0	0	
Stem	0	0	0	0	0	
Mean	3.07	0	0	0		
LSD(p≤05)	2.19					

3.6 Experiment 3

3.6.1 Amount of callus produced

Callus formation was quite substantial in most of the observations. The effect of plant parts on amount of callus formed was significant. The treatment type however had a significant effect on the amount of callus formed. The interaction between plant part and treatment significantly affect the amount of callus formed as indicated in the table 11. The cultures containing IBA had the largest amount of callus being observed while BAP+NAA combination had the lowest.

Table 11 Mean effect BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on amount of callus obtained from Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP/Kn/AS	Mean	LSD (p≤05)
shoot tip	0.35	0.26	0	0	0.15	0.0	0.8	0
Leaf	1.4	0.8	0.6	1.0	1.0	2.4	2.3	
Stem	2.8	1.8	1.6	2.6	0.8	2.6	2.5	
Mean	2.3	1.6	1.7	1.9	1.5	1.7		
LSD (p≤05)	0.51							

3.6.2 Days to shooting

Although shooting was observed, it was generally low and negligible in most of the treatments and explants. Only the shoot tip explant cultures gave significant observations of shoots being produced with the stem explants producing shoots only when cultured in the BAP+Kn+As combination medium. All the hormonal treatments used in the shoot tip explants cultures produced some level of shooting except the BAP/IAA combination. The use of different plant parts significantly affected the number of days taken to shooting . The treatment type had a significant effect on the days to shooting. The interaction between plant part and treatment also had a significant effect on the days to shooting as indicated in the table 12.

Table 12 Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to shooting of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP/Kn/AS	Mean	LSD (p≤05)
shoot tip	0.0	29.8	22.4	30.8	23.2	31.6	23.0	4.9
Leaf	0.0	0.0	0.0	0.0	0.0	0.0	2.3	
Stem	0.0	0.0	0.0	0.0	0.0	24.0	4.1	
Mean	0.0	9.9	7.5	1.9	10.2	7.7		
LSD(p≤05)6.97								

3.6.3 Number of shoot buds per explants

The use of different plant parts significantly affected the number of shoot buds observed. The treatment type had a significant effect on the number of shoot buds produced per explant culture. The interaction between plant part and treatment also had a significant effect on the number of shoot buds observed as indicated in the table.

Table 13 Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of shoot buds per explant of Jatropha curcas shoot tip, leaf and stem explants cultured in MS media

Explant	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP+Kn+AS	Mean	LSD (p≤05)
shoot tip	0.0	2.2	1.6	2.0	0.6	3.4	1.6	0.3
Leaf	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Stem	0.0	0.0	0.0	0.0	0.0	1.0	1.2	
Mean	0.0	0.7	0.5	0.7	0.2	1.5		
LSD (p≤05)	0.4							

3.7 Experiment 4

3.7.1 Days to shooting

Shooting was observed only in the BAP, Zeatin and Kinetin treatments. The cultures containing NAA, IBA and IAA did not produce any shoots. Shooting occurred within three to six weeks after culturing. The variety level did not significantly affect the number of days taken to shooting. The treatment type had a significant effect on the days to shooting. The interaction between plant part and treatment also had a significant effect on the days to shooting as indicated in the table 14.

Table 14 Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on days to shooting of Jatropha curcas varieties cultured in MS media

Variety	Hormones							
	BAP	NAA	IBA	Zeatin	IAA	Kinetin	Mean	LSD (p≤05)
TS MOROGORO	21.0	0.0	0.0	26.6	0.0	23.0	11.8	5
TZ ARUSHA	23.6	0.0	0.0	26.4	0.0	27.2	12.9	
PEMBA	22.2	0.0	0.0	24.2	0.0	23.0	11.6	
ZANZIBAR	22.8	0.0	0.0	27.2	0.0	32.4	13.7	
Mean	5.3	0.0	0.0	26.1	0.0	26.4		
LSD (p≤05)	7.3							

3.7.2 No of shoot buds

Generally, the number of shoot buds observed was between 1 and 2.The use of different varieties did not significantly affect the number of shoot buds observed in the experiment. The treatment type had a significant effect on the number of shoot buds produced per explant culture. The interaction between variety and treatment also had a significant effect on the number of shoot buds observed as indicated in the table 15.

Table 15 Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on number of shoot buds produced by Jatropha curcas varieties cultured in MS media

Variety	Hormones							
	BAP	NAA	IBA	Zeatin	IAA	Kinetin	Mean	LSD (p≤05)
TS MOROGORO	1.2	0	0	0.8	0	1.2	0.5	0
TZ ARUSHA	1	0	0	0.8	0	1	0.5	
PEMBA	0.6	0	0	0.6	0	0.6	0.3	
ZANZIBAR	0.8	0	0	0.8	0	0.8	0.4	
Mean	0.3	0.0	0.0	0.8	0.0	0.9		
LSD (p≤05)	0.3							

3.7.3 Shoot length

The highest mean length was observed in the cultures containing Kinetin treatments. The variety level did not affect the length of shoots formed significantly. The treatment type had a significant effect on the length of shoots formed. The

interaction between variety and treatment also affected the length of shoots formed significantly as indicated in the table 16 below.

Table 16 Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on shoot length (cm) of Jatropha curcas varieties cultured in MS media

Variety	Hormones							
	BAP	NAA	IBA	Zeatin	IAA	Kinetin	Mean	LSD (p≤05)
TS MOROGORO	0.32	0	0	0.14	0	0.26	0.1	0
TZ ARUSHA	0.08	0	0	0.1	0	0.22	0.1	
PEMBA	0.14	0	0	0.14	0	0.24	0.1	
ZANZIBAR	0.1	0	0	0.18	0	0.18	0.1	
Mean	0.1	0.0	0.0	0.1	0.0	0.2		
LSD (p≤05)	0.1							

3.7.4 Days to root

Roots were only produced from cultures containing BAP, Zeatin and Kinetin hormonal treatments. Rooting occurred within three to six weeks after culturing.

The variety level did not significantly affect the number of days taken to rooting. The treatment type had a significant effect on the days to rooting. The interaction between variety and treatment also had a significant effect on the days to rooting as indicated in the below.

Table 17 Mean effect of BAP, NAA, IBA, Zeatin, IAA, Kinetin on number of days to rooting of Jatropha curcas varieties cultured in MS media

Variety	Hormones							
	BAP	NAA	IBA	Zeatin	IAA	Kinetin	Mean	LSD (p≤05)
TS MOROGORO	22.0	0.0	0.0	28.0	0.0	25.0	12.5	6.5
TZ ARUSHA	27.0	0.0	0.0	29.4	0.0	30.0	14.4	
PEMBA	24.0	0.0	0.0	25.2	0.0	24.8	12.3	
ZANZIBAR	32.6	0.0	0.0	30.8	0.0	33.4	16.1	
Mean	5.9	0.0	0.0	28.4	0.0	28.3		
LSD (p≤05)	8.0							

3.8 Experiment 5

3.8.1 Days to shooting

The earliest shooting was observed on day 20 after culturing while the latest was on day 33.The variety level significantly affected the number of days taken to shooting. The treatment type had a significant effect on the days to shooting. The interaction between variety and treatment also had a significant effect on the days to shooting as indicated in the table 18 below

Table 18 Mean effect of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to shooting *Jatropha curcas* seed varieties cultured in MS media.

Variety	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP+Kn+AS	Mean	LSD (p≤05)
TS MOROGORO	0.0	0.0	17.2	25.2	0.0	20.2	10.4	4.4
TZ ARUSHA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
PEMBA	0.0	0.0	20.6	22.4	0.0	0.0	7.2	
ZANZIBAR	0.0	0.0	29.2	29.8	0.0	0.0	9.8	
Mean	0.0	0.0	16.8	19.4	0.0	5.1		
LSD (p≤05)	5.4							

3.8.2 Number of shoot buds

The number of shoot buds observed in this experiment was between 1 and 2.The use of different varieties did significantly affect the number of shoot buds observed in the experiment. The treatment type had a significant effect on the number of shoot buds produced per explant culture. The interaction between variety and treatment also had a significant effect on the number of shoot buds observed as indicated in the table 19.

Table 19 Mean effects of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of shoot buds produced by Jatropha curcas seed varieties cultured in MS media

Variety	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP+Kn+AS	Mean	LSD (p≤05)
TS MOROGORO	0.0	0.0	1.2	0.8	0.0	1.2	0.5	0.2
TZ ARUSHA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
PEMBA	0.0	0.0	0.6	0.6	0.0	0.0	0.2	
ZANZIBAR	0.0	0.0	0.8	1.0	0.0	0.0	0.3	
Mean	0.0	0.0	0.7	0.6	0.0	0.3		
LSD (p≤05)	5.4							

3.8.3 Shoot length

The highest mean length was observed in the cultures containing BAP/IBA treatment combination. The variety level affected the length of shoots formed significantly. The treatment type had a significant effect on the length of shoots formed. The interaction between variety and treatment also affected the length of shoots formed significantly as indicated in the table 20.

Table 20 Mean effects of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on length of shoots produced by Jatropha curcas seed varieties cultured in MS media

Variety	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP+Kn+AS	Mean	LSD (p≤05)
TS MOROGORO	0.0	0.0	0.3	0.1	0.0	0.2	0.1	0.04
TZ ARUSHA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
PEMBA	0.0	0.0	0.1	0.1	0.0	0.0	0.5	
ZANZIBAR	0.0	0.0	0.2	0.2	0.0	0.0	0.6	
Mean	0.0	0.0	0.7	0.6	0.0	0.3		
LSD (p≤05)	0.04							

3.8.4 Days to root

Roots were only produced from cultures containing BAP+IBA, BAP+ Zeatin and BAP+Kn+As hormonal treatments. Rooting occurred within 4 to 7 weeks after culturing. The variety level significantly affects the number of days taken to rooting. The treatment type had a significant effect on the days to rooting. The interaction between variety and treatment also had a significant effect on the days to rooting as indicated in the table 21 below.

Table 21 Mean effects of BAP+IAA, BAP+Kn, BAP+IBA, BAP+Zeatin, NAA+Kn, BAP+Kn+AS on number of days to rooting of Jatropha curcas seed varieties cultured in MS media.

Variety	Hormones							
	BAP+IAA	BAP+kn	BAP+IBA	BAP+Zeatin	NAA+Kn	BAP+Kn+AS	Mean	LSD (p≤05)
TS MOROGORO	0.0	0.0	23.2	29.8	0.0	29.4	10.4	4.40
TZ ARUSHA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
PEMBA	0.0	0.0	24.8	25.4	0.0	0.0	7.2	
ZANZIBAR	0.0	0.0	34.4	35.2	0.0	0.0	9.8	
Mean	0.0	0.0	20.6	22.6	0.0	7.4		
LSD (p≤05)	6.45							

1. A mass of callus obtained from in-vitro cultured leaf explants on MS+BAP (8ml/l) + (IBA4ml/l)

2. Shooting of shoot buds in-vitro on MS + BAP (1mg/l) +Kn (0.5mg/l) +As (0.5mg/l)

A Jatropha curcas seed germinating in-vitro under MS+BAP (1mg/l) +Zn (0.5mg/l)

Plantlet obtained from in-vitro cultured seeds on MS+ BAP (1mg/l) +kn (0.5mg/l) +As (0.5mg/l)

3.9 DISCUSSION

Jatropha curcas aspect of being a latex producing plant has posed a major challenge in its micro propagation trials. Seeds that are the most commonly used for propagation does not give a plant population that is true to the parent stock due to cross pollination. This is why the present study tries to use hormonal combinations and additives which have shown increased potential for a breakthrough in obtaining a protocol. From experiment 1, callus formation using explants (shoot tip, leaf and stem), the media containing BAP4mg/l+IBA2mg/l gave the earliest callus formation date of mean of 8.3 on stems compared to BAP8mg/l+IBA4mg/l, NAA4mg/l+IBA2mg/l and control(No treatment) and earliest days to shooting, mean 15 on shoot tips. The BAP4mg/l+1BA2mg/l combination gave the highest callus amount of mean 2.3, and the longest shoots, mean 0.26 on shoot tips.

In experiment 2, the BAP8mg/l+NAA2mg/l combination gave the earliest callus formation dates of mean 8.3 on shoot tips, BAP gave highest amount of callus of 2.2 on shoot tip while shooting was only observed on shoot tip under BAP culture with a mean of 9.2 days to shooting.

Experiment 3 results showed that only the shoot tips produced shoots with the earliest mean days to shooting of 7.47 and mean number of shoots of 3.4 in the BAP1mg/l+Kn0.5mg/l+As2mg/l combination.

In the fourth experiment BAP 1mg/l gave the earliest mean days to shooting of 21 and highest mean number of shoot buds of 1.2, the longest shoots with mean 0.32cm and earliest days to rooting with a mean of 22

Experiment 5 indicated that cultures of Morogoro variety in BAP1mg/l+IBA1mg/l combination gave the earliest mean number of days to shooting of 17.2 and the highest mean shoot length of 0.32cm. The highest number of shoot buds with mean of 1.2 was obtained from the same variety under BAP1mg/l+IBA1mg/l and BAP1mg/l+Kn0.5mg/l+As2mg/l combinations. The earliest mean of days to rooting, mean 20.6 was attained form the BAP1mg/l+IBA1mg/l combinations with the Morogoro variety.

Callus production response was quite easy when most of the treatments were used. There were major problems encountered during initiation of shoots and roots from explants. Most of the growth regulator formulations showed very slow response to explants and were unable to support growth of explants and necrosis occurred. There was generally a differential response of the explants to the treatments which could be attributed to the age and physiological condition of the donor plant. Shooting response was favored by the presence of BAP, Zeatin and Kinetin alone or in combination.

IAA, IBA, NAA are plant growth regulators called auxins (plant physiology journal online). According to Taiz L(1998) auxins are known to promote cell growth and cell division and depending on a specific tissue they may either promote lateral expansion(roots) or axial elongation(they participate in geotrophism, phototropism, hydrotropism and other developmental changes and also induce sugar and mineral accumulation at the site of application(Janipher L, 2000).

Cytokinins are involved mainly in cell growth (Kieber, 2002) and differentiation and promote cell division and include Kinetin and Zeatin among others (Chen et al 1985 and (Mok, 2001). They also function in shoot and root morphogenesis among other things (Sakakibara, 2006).

Reduced nitrates such as amino acids and amides have been reported to improve cell proliferation and regeneration in specific genotypes (Vasudevan et.al, 2004). In the present study, adenine sulphate significantly affects rooting and shooting and supports report by Montague et.al, 1979 that amino acids serve as a precursor in polyamide synthesis.

Callus formation

A callus is a mass of differentiated cells. The present experiment indicates that Jatropha curcas easily produces callus when cultured in vitro. All the explants shoot tip, leaf and stem parts easily produced callus in this case and there was no major problem here. Although most of the growth hormones tried BAP, NAA, IBA, IAA were able to produce a certain amount of callus, IBA appeared to be the best in gaining a good quantity of callus. The ability of all the present hormones to produce calli is supported

by Nannapat et. al, 2000 who performed a study using various concentrations of BA and IBA and reported similar results in all his trials. However, the study also shows that BAP+NAA combination is shown to produce callus at the earliest time possible. This supports results by Shujantha and Mukta, 1996. One major problem experienced, apart from contamination was the fact that most calli turned brown and ceased growth after some time. This darkening of calli could probably be due to production and oxidation of phenolic compounds released by explants (Monacilli et al, 1995).

Shooting and hormones

Shooting response was favored by the presence of BAP, Zeatin and Kinetin alone or in combination. The present experiment depicts the use of BAP as an important auxin in the induction of shoot buds and proliferation of shoot buds of Jatropha curcas. This agrees with the findings of Arocikasamy et al 2000. The best shooting medium contained BAP1mg/l+ Kn0.5mg/l+As0.2mg/l, a result which is similar to the findings of Mukul et al 2007. In the present study the presence of BAP in cultures is shown to enhance shooting. This confirms reports that BA plays an important role in the production of shoots in many members of the Euphobiae Family (Ripley and Preece, 1986). In addition, Nitish et.al 2007 in an experiment, defined the role of BAP as key in shoot proliferation. Results also show that the presence of adenine sulphate also encourages shoot induction. The use of Kinetin and Zeatin in successful shoot production as communicated in this study in Jatropha curcas is also reported by Sujantha & Mukta (1996).Kinetin is also useful in shoot proliferation (Nitish et.al, 2007) The use of additives such as Adenine Sulphate as seen in the study, is a key factor in the production of shoots from Jatropha curcas, a concept that supports reports by Sarika & Meenakshi, 2008.

Rooting

The development of roots posed a serious problem in most of the trials in the present study, and was worse when plant parts were used in the cultures. The response from seed embryo culture is shown to have a greater potential in root regeneration.

Here again the use of BAP,IBA, Kinetin, Zeatin combinations proves fruitful. Aneesha& Muppla 2009, reports the successful use of IBA in induction of roots.

Explants

The findings from this study shows that the use of shoot tips has an advantage over the Leaf and stem explants in invitro propagation of Jatropha curcas. This confirms earlier report by Sarika & Menakshi(2008) who successfully used shoot tips to produce plantlets. Also shoot tips are most widely used and most efficient in micro propagation because it involves exploitation of already present buds in the stock plant(Pierik,1991). The leaf and stem explants only produce callus efficiently and there's almost negligible response to shooting and rooting hormones used in this study. Pierik 1991, reports that nodal meristems or the shoot tips are an important source tissue of micro propagation and plants raised from them are comparatively more resistant to genetic variation.

Seed embryo culture

Using seed embryos in culture as opposed to plant parts is depicted to produce better response with respect to shooting and rooting from the present study. This was specially enhanced by the presence of BAP,IBA, Kinetin and Zeatin hormones. Combination of hormones is also shown to enhance plantlet formation as seen in the present study where BAP+IBA and BAP+Kn+As combinations had the best shooting and rooting results in the comparisons. When the hormones were used in combination as in experiment five, the shooting occurred earlier, with a mean of 17.2 compared to 22 days obtained from the single hormones in experiment four.

3.9.1 Conclusion

The present study has developed a protocol with the potential of reproducing Jatropha curcas through tissue culture. The basic media is Murashige and Skoog(MS) basic media and the best plant parts are seed embryos. Seeds are soaked for a few hours in a jar and then the seed coat is removed. The embryos are then sterilized and placed in MS media containing BAP, 1mg/l combined with Kinetin and Adenine sulphate, to generate shoots. Subculturing using the same media is done after every two weeks to

obtain roots. The plantlet formed is then taken to the greenhouse for hardening before transplanting.

As an alternative, one could use shoot tip explants containing a shoot bud. This is sterilized and cultured in the same media to obtain shoot and roots. Shoot tips are good because they are less dependent on genotypes and contain the shoot apical meristem.

3.9.2 REFERENCES(Chapter three)

Aneesha Singh, Muppla Parandhami Reddy, Jitendra Chikara and Sweta Singh.,2009: A simple regeneration protocol from stem explants of *Jatropha curcas*—A biodiesel plant. Discipline of Wasteland Research, Central Salt & Marine Chemicals Research Institute (Council of Scientific and Industrial Research), Bhavnagar 364002, Gujarat, India Available online 14 November 2009

Anonymous.,1959: *wealth of India/Raw materials*, Vol 5.(Publication and Information Directorate CSIR New Delhi) 1959 293-297.

Arocikasamy S, Varghese G and Ignacimuthu S,2000: High Frequency Regeneration of Chickpea(*Cicer arietinum L.)*Plantlets from leaf callus, Phytomorphology, 50(2000) 297-302)

Bhasabutra R and Sutiponpeibum S.,1982: Jatropha curcas oil as a substitute for diesel engine oil. *Renew energy Rev J, 4*,(1982) 56=

Chen, C.et al. 1985. Localization of Cytokinin Biosynthetic Sites in Pea Plants and Carrot Roots. Plant Physiology 78:510-513.

Dierk Hesselbach.,2001: Guide n the cultivation and use of Jatropha curcas. For farmers and field extension staff.

E.F. George, M.A. Hall, and G-J. De Klerk (eds).,2008: *Plant propagation by tissue culture. Vol 1. The background. 3rd edition* Springer, Dordrecht, 501pp. ISBN-13=978-1-4020-5004-6

Grim, C.,1996: The Jatropha project in Nicaragua,Bagani Julu(Mali) 1,pp. 10-14.Gupta.

Kieber. J.J., 2002: Tribute to Folke Skoog: Recent advances in our understanding of cytokinin biology. Journal of Plant growth regulation 21, 1-2.

Jennifer L. Nemhauser, Lewis J. Feldman and Patricia C. Zambryski.,2000: Auxin and ETTIN in Arabidopsis gynoecium morphogenesis. Development 127, 3877-3888 (2000).

Mok.DWS and Mok. MC.2001. Cytokinin metabolism and action. *Annual Review of Plant Physiology and Plant Molecular Biology 52:449*

Monacelli, B.,G,Pascua,A.Cuteri, A.Verusio,B.Botta and G.D Monache.,1995: Histological study of callus formation and optimization of cell growth in *Taxus baccata.159,cytobios*, 81:159-170

Montague D. K. (ed), Philadelphia, W. B. Saunders Co., 1989: Polyamide metabolism in embryotic cells of *Daucus carota L.* Changes in arginine decarboxylase activity. Plant Physiol

Mukul Manjari Datta, Priyana Muherjee, Biswajit Ghosh and Timir Baran Jha., 2007: In Vitro Propagation of Biodiesel plant Jatropha curcas L. Department of Botany, Presidency college, Kolkata 700 073, India. Current science, Vol.93, No.10, 25 November 2007

Murashige, T. and Skoog F., 1962: A revised medium for Rapid growth of Bioassays in tissue culture with tobacco tissues. Physiol.Plant. 1962, 15,473-479

Nannapat Thepsamran, Chockpisit Thepsithar and Aree Thongpukdee., 2000: Multiple shoot induction of Jatropha curcas. Department of Biology, Faculty of science. Silpakorn University, Nakhon Pathorm 73,000, Thailand

Nitish Kumar, K. G. Vijay Anand and Muppala P. Reddy., 2010: Shoot regeneration from cotyledonary leaf explants of Jatropha curcas: a biodiesel plant. **Published online:** 7 March 2010

Pierik RLM., 1991: Commercial aspects of micro propagation in: Horticulture-New techniques and applications. Kluwer, Doldretch.The Netherlands. Pp 141-153

Tiedman, J. and Hawker, J.S. In vitro propagation of Latex producing plants. Ann, Bott. 1982, 49, 273-279.

Rajore S, Batra A., 2005: Efficient plant regeneration via shoot tip explants in Jatropha curcas L.J.Plant Biochem. Biotech 14:73-75

Sakakibara, H.2006. Cytokinins: Activity, Biosynthesis, and Translocation. *Annual Review of Plant Physiology and Plant Molecular Biology 431-449*

Sardana J, Batra A, Ali DJ., 2000: An Expedicious method for regeneration of somatic embryos of Jatropha curcas L. Phytomorphology 5(3/4):239-242

Sardana J, Batra A, Ali DJ.,1998: In vitro plantlet formation and micro propagation

of *Jatropha curcas L. Adv. Plant Sci. 11 (2):1679169*

Sarjana J, Batra A & Sharma R.,1998: In vito plantlet formation and micro propagation of *Jatropha curcas L.* Adv. Plant Sci.11 (1998) 167-169

Sarika S, & Meenakshi B.,2008: In vito propagation of Physic nut Jatropha curcas L: Influence of Additives. Laboratory of Agal biotechnology, Department of Bioscience, Barkatullah University. Bhopal, India. International Journal of Intergrative biology, IJIB, 2008. Vol.3. No. 1, 73

Singh.,2009: A simple regeneration protocol from stem explants of *Jatropha curcas*—A biodiesel plant. Discipline of Wasteland Research, Central Salt & Marine Chemicals Research Institute (Council of Scientific and Industrial Research), Bhavnagar 364002, Gujarat, India Available online 14 November 2009

Sujantha M and Mukta N.,1996: Morphogenesis and plant receneration from tissue culture of *Jatropha curcas*. Plant cell tissue organ cult, 44 (1996) 135-141

Taiz, L. & Zeiger, E.,1998: Plant Physiology. 2nd edition. Massachusetts: Sinauer Associates, Inc. 792p.

Vasudevan,Abu R. Huaizhu Wu, Antonios M. Xydakis, Peter H. Jones, E. O'Brian Smith, John F. Sweeney, David B. Corry and Christie M. Ballantyne.,2004: Eotaxin and Obesity

Village E Science for Life.,1997: Intergrating Information technology Agriculture in rural communities.

CHAPTER FOUR: Assessment of the effects of Temperature, base medium and nursery container on the germination of *Jatropha curcas* seed varieties

4.1 Abstract

Two experiments were carried out in the greenhouse to assess the effect of plastic container and sand soil mixture, polythene cover and polythene container on germination of Jatropha curcas seed varieties. In the green house, the use of polythene cover and polythene container gave highest germination rates of mean 100%. The highest height of 17.9cm was also obtained from seeds planted in polythene paper over plastic container and sand soil mixture treatments on 7 Jatropha curcas varieties.

A trial on the effect of temperature on 2(Rainfed/Irrigated variety)Jatropha curcas seed regeneration indicated that increasing temperature increases the germination rate with an optimum of about 40 degrees being the best for maximum germination. Desktop germination on 7(Ts Morogoro,T3 Tanga,Zanzibar,T6 Dar,TZ Arusha,Pemba,Arusha) seed varieties indicated the medium containing cottonwool base gave higher germination rates over the use of filter paper,presoaking and plain petridishes to germinate the seeds.

4.2 Introduction

Jatropha curcas is normally propagated by seeds and cuttings, and commercial propagation used seeds as the main planting material. Some of the problems experienced in the production include low germination, poor seed viability, scanty and delayed rooting of seedlings and vegetative cuttings (Heller, 1996 & Openshaw, 2000). Hot and humid weather is preferred for successful germination of seeds. Seeds can either be taken through nursery production or sown directly in the field. Germination may be done on polythene bags or plastic papers. The medium for nursery growth may be soil, mixed with sand or manure. The question of germination and viability requires urgent attention if farmers are to benefit from the crop. This is because the information

available is limited and unreliable, giving varying ideas on productivity, viability, germination potential, varieties that are questionable.

Good nursery work is the basis of successful afforestation activities and a probe into possible improvement techniques such as use of Sand, polythene bags and other practices may improve productivity. Good management of the nursery could possibly result in seedling production at the end of the first year itself. Nurseries supply seedlings to the farmers in their villages thus providing an alternative to the expensive seeds. Yet in order to guarantee good quality seedlings and survival in the field after planting, good nursery practices should be followed. To raise sufficient nursery stock, information on choice of site, soil medium and variety needs to be considered carefully, areas that the present study seeks to address.

4.3 Materials and methods

Temperature trial involved two seed varieties, rain fed variety A and irrigated variety B which were germinated in an incubator under 32,34,36 and 38degrees.Desktop seed germination used petridishes, cotton wool, filter paper, water and seeds.

Greenhouse seed trials involved use of pots, soil, sand, polythene bags which were purchased from supermarkets in Nairobi and were of size 8x12 inches.

4.4 Experimental design

The exp used the completely randomized design(CRD). Data was analyzed by ANOVA to detect significant differences between means. Means differing significantly were compared using Duncan's Multiple Range Test (DMRT) at 5% probability level. Variability of data was expressed as mean+standard deviation.

4.5 Greenhouse seed germination

4.5.1 Greenhouse Experiment A

Seed varieties, Rainfed, Irrigated and Kitui were subjected to four treatment levels. The first involved planting seeds in a plastic pot and containing soil and covered with plastic paper. The second had seeds planted in polythene bags, the third plastic pots containing soil sand mixture and the fourth plastic pots only.

There were three variety levels subjected to four levels of treatment. Each of the treatment was replicated four times. The total number of observations was 48.

4.5.2Measurements

Measurements of heights, number of days to germination, percentage germination, number of leaves and was made. A 30cm ruler was used to take the measurements for height while the number of days germination were measured by observing the time of sprouting of seeds or emergence of explants. The number of leaves produced were observed and counted. All the records were taken and kept in a notebook.

4.5.3 RESULTS

4.5.4 Greenhouse Experiment

In general, all the levels of treatment were able to cause germination of at least a plant per batch in each of the set ups. The measurements of height taken later, days of planting were higher than the measures taken on the earlier date.

4.5.5 Height at 14 days

The variety type significantly ($p \leq 0.05$) affected the heights measured after 14 days of planting such that there were differences across the varieties.

The application of different treatments had significant effect on the height as shown in table 22 below. There was significant interaction between variety and treatment levels.

Table 22 Mean heights of Jatropha curcas varieties at 14 days after planting as affected by use of polythene container, polythene cover, sand/soil medium treatments.

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	11.5	1.0	0.0	4.3	4.2	2.9
Irrigated	4.3	16.5	7.8	7.5	8.5	
Kitui	24.0	0.0	0.0	25.2	8.2	
Mean	8.3	3.9	9.6	6.1		
LSD(p≤05)	3.4					

4.5.6 Percent Germination

There was a general variation in percentage germination of seeds planted in different treatment conditions, but the effect of the treatments was not significant ($p \geq 0.05$) as shown below. The type of variety also did not significantly affect the percentage germination. The interaction between the variety and treatment was not significant.

Table 23 Effects of use of polythene container, polythene cover, sand/soil medium on the percentage germination of Jatropha curcas varieties under nursery production.

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	81.3	31.3	37.5	31.3	45.3	20.7
Irrigated	50.0	37.5	81.3	57.5	56.6	
Kitui	31.3	37.5	43.8	25.0	34.4	
Mean	54.2	35.4	54.2	37.9		
LSD (p≤05)	24.0					

4.6.7 Leaves per plant at 14 days

The effect of different treatments on the number of leaves per plant counted 14 days after planting was not significant. On average, almost all the plants had the same number of leaves during this stage of growth as shown in table. The variety type had a significant effect on the number of leaves produced per plant at 14 days of planting. The interaction between variety and treatment was significant. However, for the rain fed variety planted in sand/soil treatment, there were no leaves produced at this time of

growth. The Kitui variety had averagely higher number of leaves compared to the rain fed and irrigated varieties.

Table 24 Effects of use of polythene container, polythene cover, sand/soil medium on the number of leaves per plant after14 of Jatropha curcas varieties under nursery production.

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	2.0	0.5	0.0	1.0	0.9	0.7
Irrigated	1.5	0.8	3.0	1.5	1.7	
Kitui	1.8	1.5	2.3	1.5	1.5	
Mean	1.8	0.9	54.2	1.8	1.3	
LSD (p≤05)	1.8					

4.5.8 Days to germination

The variety type significantly ($p \leq 0.05$) affected the days to germination such that there were differences across the varieties.

The application of different treatments had no significant effect on the days to germination as shown in table 25. There was significant interaction between variety and treatment levels.

Table 25 Effects of use of polythene container, polythene cover, sand/soil medium on the number of days to germination of Jatropha curcas varieties under nursery production

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	10.8	16.3	19.8	9.5	14.1	3.1
Irrigated	9.8	9.5	8.5	12.0	9.9	
Kitui	7.8	12.5	6.5	8.5	8.8	
Mean	9.4	12.8	54.2	11.6		
LSD (p≤05)	3.5					

4.5.9 Height at 21 days

The effect of different treatments on the height of plants measured 21 days after planting was not significant. The variety type also had no significant effect on the

heights at this stage of growth. However, the interaction between variety and treatment was significant.

Table 26 Mean heights of Jatropha curcas varieties at 21days of nursery planting as affected by use of polythene container, polythene cover, and sand/soil medium treatments.

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	14.6	4.4	2.3	5.9	6.8	3.5
Irrigated	6.8	6.6	17.2	10.3	10.2	
Kitui	8.8	10.8	12.7	7.6	9.9	
Mean	10.0	7.3	10.7	7.9		
LSD (p≤05)	4.0					

4.5.9.1No. of leaves per plant (21 days)

The variety type did not significantly (p ≥0.05) affected the days to germination. The application of different treatments had no significant (p ≥0.05) effect on the days to germination as shown in table 27. There was significant interaction between variety and treatment levels.

Table 27 Mean leaf count of Jatropha curcas varieties at 21days of nursery planting as affected by use of polythene container, polythene cover, sand/soil medium treatments

Variety	Treatment					
	Polythene	Control	Covered	Sand/soil	Mean	LSD(p≤05)
Rainfed	3.0	2.3	1.8	2.0	2.3	0.8
Irrigated	2.3	2.0	3.8	2.8	2.7	
Kitui	3.0	2.8	2.5	2.0	2.6	
Mean	2.8	2.3	2.7	2.3		
LSD (p≤05)	0.9					

4.5.9.2 DISCUSSION

The irrigated variety under polythene cover gave the best heights of mean 16.50cm at 14 days and 17.23cm at 21 days after planting. The rainfed variety under polythene bag container and the irrigated variety under polythene covered container gave the highest percent germination of mean 81.25%. Under polythene cover, the irrigated variety gave highest mean number of 3 leaves per plant at 14 days after

planting and mean 3.75 leaves at 21days after planting. The earliest mean of germination days of 6.5 was obtained from Kitui variety under polythene bag containers.

The maximum height was obtained in plants grown under polythene cover treatment where a mean height of 16.5cm was attained by the irrigated variety. There are several factors that affect seed growth and they include moisture content in soil and atmosphere. This observation could have resulted from the fact that when plants are covered, there's reduced moisture loss through evapotranspiration. The water vapor in the atmosphere is higher, limiting excess release from the plants. Covering preserves soil moisture. The moisture is thus well used for the plant growth.

The maximum height could also have resulted from increased temperature within the covered area, which is reported to favor increased growth rate in most plants. In fact the rate of seed growth has been reported to be directly proportionate to the rise in temperature. A study carried out on germination also gave a general report that increased soil moisture and temperature have a positive influence on growth (Jacob, 2000).

On the other hand the maximum height of these plants could be an effect of the variety. The irrigated seed variety, having been obtained from seeds planted under irrigation requires good soil moisture, and thus performed well when covered. There was low germination in the rain fed seed varieties grown under cover. This could have resulted from a problem with the seed variety or dormancy of the seed.

The polythene cover treatment and polythene paper container exhibited the highest percentage of germinated seeds. Dormancy and viability are important aspects of seed germination. In this case it's possible that a rise in the soil temperature might have resulted in an increased breakage of dormancy since one way of breaking dormancy is by heating. Although some seeds require a chilling to break dormancy, others require heat to do so (Black , 1987)

The polythene containers used were black in colour and so the high temperature could have been blamed on increased heat absorption and retention by the black

polythene papers, which in turn increased the possibility of sprouting of the seed, giving a higher percentage compared to others. Our observation is in line with Henning who recommends that Jatropha seeds should be germinated in plastic bags filled with soil and manure (Henning, 2000b).

The highest number of leaves was obtained in the plants that were grown under cover. This could be due to the factor of moisture and temperature that favored increased growth. With the polythene cover, there was increased retention of water in the soil and moisture is useful for the transfer of mineral nutrients from soil to plant for usage in the manufacture of food.

The seeds grown under cover exhibited the earliest number of days to germination. This could be due to the fact that there was enough moisture during germination since the covering helped in preserving the moisture. Jacob concluded in a study that a moderate watering intensity increases the germination rare, showing that the crop requires adequate moisture at this stage of growth (Jacob 2000)
The effects of both treatment and variety on plant height were not significant at this level. This could be explained by the fact that the plant has fast growth rate after germination, irrespective of the treatment that was made initially. This result confirms that obtained by a study that also observed that when germination is initiated, the growth rate is very fast (Jacob, 2000)

The overall effect of the various treatments and varieties on the number of leaves was not significant at this level. Most of the plants had obtained about 3 leaves by this level of growth. However the highest number of plants with 3 leaves was observed in the covered treatment, which could be explained by the fact that these plants, having germinated earlier had access to the external growth factors such as light which fastens growth.

4.7 GREENHOUSE EXPERIMENT B

Seven seed varieties were tested for germination under four sets of treatment in the first experiment. Treatment one involved planting seeds in plastic container filled with soil and covered with polythene bag. Treatment two involved planting seeds in plastic container filled with half soil and half sand, treatment three had seeds planted in polythene bags filled with soil and the final treatment which acted as control had seeds planted in plastic containers filled with soil. Seeds were planted at the same depth, watered at equal intervals. Each of the treatments was replicated four times.

4.7.1 Days to emergence

In this set up, the variety type significantly ($p \leq 0.05$) affected the days to emergence such that there were differences across the varieties. The application of different treatments had a significant ($p \leq 0.05$) effect on the days to emergence as shown in table 28 below. There was no significant interaction between variety and treatment levels ($p \geq 0.05$). Seeds germinated in the polythene bags and those covered using plastic bags emerged earliest.

Table 28 Mean number of days to emergence of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.

Variety	Treatment						
	Polythene	Control	Covered	Sand/soil	Manure	Mean	LSD (p≤05)
TS morogoro	10.3	15.3	10.3	12.5	15.3	12.7	2.8
T3 Tanga	11.0	15.5	10.3	13.5	24.0	14.9	
Zanzibar	13.0	16.8	12.8	16.5	20.8	16.0	
T6 Dar	11.3	16.3	14.0	17.5	18.5	15.5	
TZ Arusha	11.0	13.0	12.0	18.5	25.0	15.9	
Pemba	11.8	17.3	13.8	20.0	19.5	16.5	
Arusha	12.0	18.3	9.5	21.0	24.3	17.0	
Mean	11.5	16.0	11.8	17.1	21.4		
LSD (p≤05)	2.4						

4.7.2 Stem length

The highest mean height of 17.9cm was obtained from seeds that were germinated in the covered containers while the lowest mean of4.3cm was obtained from seeds planted in soil/manure mixture. The variety type significantly ($p \leq 0.05$) affected the length of stems such that there were differences across the varieties. The application of different treatments also had a significant ($p \leq 0.05$) effect on the length of stems as shown in table 29 below. However, there was no significant interaction between variety and treatment levels ($p \geq 0.05$).

Table 29 Mean stem length of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.

Variety	Treatment						
	Polythene	Control	Covered	Sand/soil	Manure	Mean	LSD (p≤05)
TS morogoro	12.6	7.9	12.6	4.8	4.3	8.4	1.4
T3 Tanga	13.0	8.2	14.0	6.1	5.1	9.3	
Zanzibar	13.9	8.3	13.8	6.7	5.8	9.7	
T6 Dar	16.2	9.1	17.9	9.2	5.7	11.6	
TZ Arusha	17.6	7.1	16.3	8.9	7.1	11.4	
Pemba	17.2	9.6	17.2	8.6	5.9	11.7	
Arusha	15.6	9.0	13.6	8.9	7.3	10.9	
Mean	15.2	8.5	15.0	7.6	5.9		
LSD (p≤05)	1.1						

4.7.3 Number of leaves

The variety type did significantly affected the number of leaves produced($p \leq 0.05$). The application of different treatments had a significant($p \leq 0.05$) effect on the number of leaves as shown in table below. There was no significant interaction between variety and treatment levels.

Table 30 Mean number of leaves of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments.

Variety	Treatment						
	Polythene	Control	Covered	Sand/soil	Manure	Mean	LSD (p≤05)
TS morogoro	3	2	3	2	2	2.2	0.3
T3 Tanga	3	2	3	2	2	2.4	
Zanzibar	3	2	3	2	2	2.3	
T6 Dar	3	2	3	2	2	2.3	
TZ Arusha	3	3	3	2	2	2.6	
Pemba	3	2	3	2	2	2.4	
Arusha	3	3	2	3	2	2.6	
Mean	2.9	2.4	2.8	2.0	1.9		
LSD (p≤05)	0.3						

4.7.4 Percent germination

The effect of the treatments on the percentage germination was significant as shown in figure 28 below. The type of variety also did significantly affect the percentage germination($p \leq 0.05$). The interaction between the variety and treatment was also significant($p \leq 0.05$). The highest mean percentage germination was 100% and obtained from seeds germinated in plastic containers while the lowest percentage(mean=25%) was obtained from seeds germinated in polythene bags and manure/soil mixture.

Table 31 Mean %germination of seven Jatropha curcas varieties germinated in the greenhouse as affected by use of polythene container, plastic container, covering, sand/soil mixture and manure treatments

Variety	Treatment						
	Polythene	Control	Covered	Sand/soil	Manure	Mean	LSD (p≤05)
TS morogoro	68.8	37.5	68.8	43.8	25.0	48.8	11.4
T3 Tanga	43.8	56.3	37.5	31.3	43.8	42.5	
Zanzibar	87.5	75.0	62.5	75.0	37.5	67.5	
T6 Dar	62.5	62.5	50.0	68.8	25.0	53.8	
TZ Arusha	93.8	100.0	93.8	93.8	56.3	87.5	
Pemba	25.0	37.5	37.5	37.5	25.0	32.5	
Arusha	68.8	37.5	75.0	43.8	50.0	55.0	
Mean	64.3	58.0	60.7	56.3	37.5		
LSD (p≤05)	9.6						

4.7.5 Discussion

The use of nurseries to germinate seeds before transplanting to the field is very important and an essential part in producing plants such as Jatropha curcas. Apart from environmental aspects, other factors such as soil type and containers/pots used may affect the both germination and growth rates. Seven seed varieties were tested for germination under five sets of treatment in the first experiment. Plastic pots covered with polythene paper, polythene bag container, the third plastic pots containing soil sand mixture, the fourth plastic pots only and the fifth had soil manure mixture in plastic pots. The earliest mean germination date of 10.25 were obtained from both polythene(TS Morogoro) and covered containers(TS Morogoro, Arusha and T3 Tanga) while the best mean height of 17.9cm was obtained from the covered plants(Pemba). Both the polythene container and covered trials had an equal mean number of 3 leaves per plant. The highest mean percentage germination was 100% and obtained from seeds germinated in plastic containers(TZ Arusha).

The present study confirms that using polythene bags as container and covering plants with a plastic paper has a positive effect and gives the earliest days to emergence

compared to the plastic container, sand/soil mix and manure treatments. This could be explained by the fact that plastic bags conduct a lot of heat which increases the soil temperatures. The high temperatures, then increase the rate of physiological reactions in the plants and this is translated into increased germination and growth(Demosthenis,2000). In addition Jatropha is a heat loving plant that thrives well in high temperature conditions(Grim,1996).

The covered containers also resulted in seedlings with` the highest stem lengths, a result which confirms that temperature proportionately affects growth, through an influence on the rates of photosynthesis and translocation.

Although the use of cover on containers resulted in increased emergence and growth, the percentage of plants that germinated under this treatment was lowest, with a mean of 25%. There was probably so much heat in the covered containers that could have destroyed the seeds and reduced their viability. Interestingly, the seeds planted normally in plastic containers(control) gave the highest mean percentage of 100% with respect to the TZ Arusha variety.

Communications from the present experiment shows that different varieties give different results when Jatropha curcas is germinated in the greenhouse or nursery. The effect of variety was significant in all the experiments in the set up. The result obtained from nursery germination of seeds will differ with respect to the variety used.

4.8 TEMPERATURE EXPERIMENTS

4.8.0 Growth conditions

The two seed types, rain fed and irrigated variety were cleaned under running tap water. The seeds were placed in petridishes aligned with cottonwool, labeled and placed in an incubator under 34,36,38 and 40 degree temperature treatments. Germination rates were assessed daily for two weeks. Data on the best temperature rates were recorded. Also days to emergence, heights of shoots, number of leaves per seedling were recorded on a daily basis.

4.8.1 Days to sprouting

The mean effect of temperature treatments on the number of days to sprouting of Jatropha seeds germinated under incubation was not significantly different as shown in table 32 below. The mean effect of variety on days to sprouting was not significant ($p \geq 0.05$) and the mean interaction between variety and treatment was also not significant($p \geq 0.05$).

Table32 Mean number of days to sprouting of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.

Variety	Treatment					
	34D	36D	38D	32D	Mean	LSD(p≤05)
Rainfed	3.8	3.8	3.2	3.0	3.5	1.0
Irrigated	2.6	3.8	3.8	3.0	3.3	
Mean	3.2	3.8	3.5	3.0		
LSD (p≤05)	1.4					

4.8.2 Percentage germination

The treatment type significantly affected the mean percentage germination such that the percentage germination of plants at 40 degrees was higher than the mean percentage germination at 43 degrees Celsius($p \leq 0.05$). The variety type did not affect the percentage germination significantly($p \geq 0.05$). The interaction between variety and treatment was not significant($p \geq 0.05$).

Table 33 Mean percent germination of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.

Variety	Treatment					
	34D	36D	38D	32D	Mean	LSD(p≤05)
Rainfed	42.7	37.5	44.0	75.0	49.8	14.5
Irrigated	24.0	24.0	59.0	88.0	48.8	
Mean	33.3	30.8	51.5	81.5		
LSD (p≤05)	20.5					

4.8.3 Stem Length

There was a significant effect of various treatments on the length of stems of plants($p \leq 0.05$). The plant height increased with the increase in temperature. The effect of variety on length was however not significant($p \geq 0.05$) and the interaction between variety and treatment was also insignificant($p \geq 0.05$).

Table 34 Mean stem length of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.

Variety	Treatment					
	34D	36D	38D	32D	Mean	LSD(p≤05)
Rainfed	1.5	1.8	2.6	4.2	2.5	0.6
Irrigated	1.5	1.5	2.4	4.1	2.4	
Mean	1.5	1.6	2.5	4.2		
LSD (p≤05)	0.8					

4.8.4 No. of Leaves

The variety type did not significantly ($p \geq 0.05$) affect the number of leaves produced by the plants. The application of different treatments had no significant effect on the number of leaves produced as shown in table 35 ($p \geq 0.05$).

There was also no significant interaction between variety and treatment levels($p \geq 0.05$).

Table 35 Mean number of leaves of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.

Variety	Treatment					
	34D	36D	38D	32D	Mean	LSD(p≤05)
Rainfed	2.2	2.2	2.2	3.0	2.4	0.6
Irrigated	1.8	1.8	2.2	2.8	2.2	
Mean	2.0	2.0	2.2	2.9		
LSD (p≤05)	0.8					

4.8.5Days to rooting

The variety type did not significantly ($p \geq 0.05$) affect the days to rooting of Jatropha curcas varieties germinated under controlled temperature. The application of

different treatments had no significant effect on the days to rooting as shown in table 36 below. There was no significant interaction between variety and treatment levels.

Table 36 Mean no. of days to rooting of two Jatropha curcas varieties as affected by various temperature treatments in an incubator.

Variety	Treatment					
	34D	36D	38D	32D	Mean	LSD(p≤05)
Rainfed	4.4	3.6	3.8	4.6	4.1	0.8
Irrigated	3.2	4.2	2.8	4.0	3.6	
Mean	3.8	3.9	3.3	4.3		
LSD (p≤05)	1.2					

4.8.6 DISCUSSION

Growth is promoted when temperature rises and is inhibited when temperature falls since temperature affects translocation of materials, respiration and building of new cells(Dennis,2009). The present study confirms temperature as an important factor in germination of plants varieties as documented by Demosthenis 2000. In the temperature experiment, two seed types were subjected to 34,36,38 and 40 degree temperature treatments. At 40 degrees, the earliest mean of days to sprouting, the highest germination percentage of 88% (irrigated variety), the highest mean stem length of 4.2cm and the highest number of leaves, 3(all varieties) were attained.

However, the present temperatures did not affect time of sprouting significantly possibly because they were generally very high. Jatropha curcas is a heat loving plant and has been reported to thrive well in relatively high temperatures(Surma,1997). This is supported by the fast sprouting observed, where the earliest occurs on day 3 and the latest on day 6. This result is supported by claims from the green Africa foundation that Jatropha curcas germination takes about 4 to 10 days.

The present study has shown that an increase in temperature increases growth rate as depicted in the response of heights to temperature(Demosthenis,2000). The highest mean length of the stems(4.2cm) is obtained at the highest temperature,

42degrees Celsius. This result is supported by literature that temperature differences affect the rates of photosynthesis, transpiration and respiration in plants(Galtier et.al,1993)This occurs in most seeds and is explained by the fact that heat treatment helps in breaking seed dormancy at a faster rate thus increases rate of germination The present study shows that a higher percentage of germination is obtained at higher temperatures compared to lower ones. In fact an average of 88% germination is obtained at 40 degrees, compared to the 24% mean obtained at 36degrees in the irrigated seed variety. Although not significant, the number of leaves produced at the high temperature is more that those produced at lower temperatures in the range used. This could be explained by the fact that temperature increases photosynthetic rates (Lafta&Lorenzen,1995). The present study however, does not show any positive influence of temperature on rooting of the explants especially with reference to the ranges used.

4.9 LAB DESKTOP SEED GERMINATION

4.9.0 Growth conditions

Seven seed varieties, were placed in two sets of treatment. The first one included petridishes aligned with filter paper while the second involved petridishes aligned with cottonwool. Water was admitted into the petridishes as need arose.

4.9.1 Days to sprouting

Sprouting of seeds took place within one week. The earliest sprouting was observed on day 3 after setting while the latest was on day 7.The variety level significantly affected the number of days taken to sprouting ($p \geq 0.05$). The treatment type had no significant effect on the days to sprouting ($p \leq 0.05$). The interaction between variety and treatment also had a significant effect($p \geq 0.05$) on the days to sprouting as indicated in the table 37.

Table 37 Mean number of days to sprouting of Jatropha curcas seed varieties germinated in petridishes as affected by use of filter paper base, cotton base and pre/soaking treatments

Variety	Treatment					
	Filter paper	Cotton base	presoaked	control	Mean	LSD(p≤05)
TS morogoro	2.3	3.5	2.3	2.3	2.6	0.8
T3 Tanga	4.0	4.8	4.8	3.5	4.3	
Zanzibar	5.3	5.0	5.8	5.0	5.3	
T6 Dar	4.5	6.5	3.8	5.5	5.1	
TZ Arusha	2.8	3.0	3.8	3.0	3.1	
Pemba	4.0	4.5	5.5	6.0	5.0	
Arusha	1.8	1.8	3.0	3.5	2.5	
Mean	3.5	4.1	4.1	4.1		
LSD(p≤05)	0.6					

4.9.2 Percent germination

The highest mean percent germination of 100% was attained when seeds from the TZ arusha variety were germinated on cotton wool base The treatment type did not significantly affect the mean percentage germination($p \geq 0.05$). The variety type affected the percent germination significantly such that there were differences across the varieties($p \leq 0.05$). The interaction between variety and treatment was not significant ($p \geq 0.05$).

Table 38 Mean percentage germination of Jatropha curcas seed varieties germinated in petridishes as affected by use of filter paper base, cotton base and pre/soaking treatments.

Variety	Treatment					
	Filter paper	Cotton base	presoaked	control	Mean	LSD(p≤05)
TS morogoro	56.3	62.5	56.3	43.8	54.6	15.3
T3 Tanga	43.8	56.3	37.5	50.0	46.9	
Zanzibar	87.5	75.0	62.5	75.0	75.0	
T6 Dar	62.5	62.5	50.0	68.8	61.0	
TZ Arusha	93.8	100.0	93.8	93.8	95.3	
Pemba	25.0	37.5	37.5	37.5	34.4	
Arusha	50.0	37.5	75.0	43.8	51.6	
Mean	59.8	61.6	59.9	58.9		
LSD (p≤05)	11.6					

4.9.3 Root length

The highest mean root length obtained under the desktop seed germination was 1.7cm while the lowest mean was 0.1cm. The variety type significantly ($p \leq 0.05$) affected the length of stems such that there were differences across the varieties. However, the application of different treatments had no significant($p \geq 0.05$) effect on the length of roots as shown in table 39 and figure 36 below. However, there was no significant interaction between variety and treatment levels($p \geq 0.05$).

Table 39 Mean length of roots produced by Jatropha curcas seed varieties germinated in petridishes as affected by use of filter paper base, cotton base and pre/soaking treatments.

Variety	Treatment					
	Filter paper	Cotton base	presoaked	control	Mean	LSD(p≤05)
TS morogoro	0.9	1.1	0.7	0.7	0.8	0.2
T3 Tanga	0.3	0.3	0.3	0.2	0.3	
Zanzibar	0.5	0.5	0.5	0.4	0.5	
T6 Dar	0.5	0.4	0.4	0.3	0.4	
TZ Arusha	0.9	0.8	0.7	0.6	0.8	
Pemba	0.1	0.2	0.3	0.3	0.2	
Arusha	1.2	0.6	1.7	1.1	1.1	
Mean	0.6	0.6	0.6	0.5		
LSD (p≤05)	0.2					

4.9.4 Shoot length

The treatment involving the cotton base gave the highest mean shoot length of 10.5cm while that containing the presoaked seeds gave the lowest shoot length of 3.5cm. The variety type significantly ($p \le 0.05$) affected the length of shoots such that there were differences across the varieties. However, the application of different treatments had no significant($p \ge 0.05$) effect on the length of shoots as shown in table 40 and figure 37 below. There was also no significant interaction between variety and treatment levels ($p \ge 0.05$).

Table40 Mean length of shoots produced by Jatropha curcas seed varieties germinated in petridishes as affected by use of filter paper base, cotton base and –pre/soaking treatments.

Variety	Treatment					
	Filter paper	Cotton base	presoaked	control	Mean	LSD(p≤05)
TS morogoro	7.6	9.2	7.6	6.7	7.8	1.4
T3 Tanga	5.3	4.5	3.5	4.3	4.4	
Zanzibar	8.0	6.4	5.8	7.0	6.8	
T6 Dar	8.6	5.3	5.3	8.0	6.8	
TZ Arusha	10.1	10.5	9.0	9.8	9.8	
Pemba	7.1	7.9	6.4	7.1	7.1	
Arusha	5.5	3.8	5.8	6.2	5.3	
Mean	7.4	6.7	6.2	7.1		
LSD (p≤05)	1.9					

4.9.5 Discussion

In the present study, almost all the seeds sprouted within the same period of time(1week) in all the treatments used, thus implying that germinating seeds in petridishes in the labs result in fast germination irrespective of the base material used.

On the laboratory desktop, seven seed varieties, were placed in two sets of base medium: petridishes aligned with filter paper and petridishes aligned with cotton wool. Each of the treatments attained a mean early sprouting date of 3 except the filter paper. Medium containing cotton wool base gave highest mean percent germination of 100% on the TZ Arusha variety. The highest mean root length of 1.7 cm was attained by presoaked Arusha variety while the cotton base gave the highest shoot length of 10.5cm.

When the number of seeds germinated(%germination) is considered however, the seeds put in petridishes with a cotton base performed better with one of the varieties giving 100% germination. The use of cotton as a base for seed pregermination before planting may be useful in the production of Jatropha curcas.

Laboratory germination of Jatropha curcas seeds is also useful in obtaining seedlings with good roots which may be handy during the latter stages of growth. Roots absorb water and mineral salts and also helps in anchoring the plant into place. They also act as food storage agents.

In addition to the roots, shoots play a vital role in the plant structer, by supporting stems and leaves and are pathways for chanelling raw materials from the roots to the leaves for photosynthesis.

General discussion on germination

Several factors affect the rate of seed germination and seedling growth. These may include seed quality, seed age sowing depth, soil moisture content, soil preparation quality among others(Heller,1996). The present study confirms the fact that using polythene paper enhances germination. Henning 2000c also noticed that pre cultivation of Jatropha seedlings in poly ethylene bags is more appropriate and enables acceleration of the installation of a plantation by at least three months compared to cuttings. The present germination results were generally earlier and ranged between three to ten days. The figures on percentage germination was quite low.

According to FACT foundation, 2006, germination takes 10 days if the moisture conditions are good. The result could also be credited to low viability. Jatropha curcas seeds are oily and so do not store for long and increased storage time results in increased loss of viability. In fact if the period exceeds 15 months, the viability may go to as low as 50%months.(Kobilke 1989)

REFERENCES

A. M. Lafta and J. H. Lorenzen.,1995: Effect of High Temperature on Plant Growth and Carbohydrate Metabolism in Potato. Plant Physiol. 1995 October; 109(2): 637–643. Department of Plant Sciences, North Dakota State University, Fargo, North Dakota 58105.

Dennis Holley.,2009: The role of light intensity and temperature in plant development.

Demosthenis Chachalis and Krishna N. Reddy.,2000: Factors affecting *Campsis radicans* seed germination and seedling emergence.Weed Science 48(2):212-216. 2000 doi: 10.1614/0043-1745(2000)048[0212:FACRSG]2.0.CO;2

Fact foundation.,2006: Handbook on Jatropha curcas First draft March 2006-www.factfuels.org

Galtier N, Foyer CH, Huber J, Voelker TA, Huber SC.,1993: Effects of Elevated Sucrose-Phosphate Synthase Activity on Photosynthesis, Assimilate Partitioning, and Growth in Tomato (Lycopersicon esculentum var UC82B). Plant Physiol. 1993 Feb;101(2):535–543.

Grim, C.,1996: The Jatropha projct in Nicaragua,Bagani Julu(Mali) 1,pp. 10-14.Gupta.

Heller J .,1996: Physic nut Jatropha curcas-Promoting the conservation and use of underutilized and neglected crops.

Henning, R.,2000c: Use of Jatropha curcas oil as raw material and fuei: an intergrated approach to create income and supply energy for rural development. Experiences of the Jatropha project in Mali, West Africa. Presentation at the international meeting "Renewable energy-A vehicle for Local development-11 Folkecenter for renewable energy, Denmark, August, 2000.

Kobilke ,H., 1989: *Untersuchurgen zur bestarndesbegrundung von Purgiernu* (Jatropha curcas L.) Diploma thesis, University of Hohenheim, Stuttgart

Openshaw K.,2000: A review of Jatropha curcas: An oil plant of unfulfilled promise. Biomass and Energy 19:1-5

Surma G.D, Gupta S.N &Khabiruddin, M.,1997: Cultivation of Jatropha curcas as a future source of hydrocarbons and other Industrial products.

CHAPTER FIVE :General conclusion and recommendations

5.1Conclusion

A crop such a *Jatropha curcas* may be regenerated using different protocols, as claims indicate from various sources. It is therefore important that since vegetative propagation may take quite a long time in developing true to type cultivars, the use of tissue culture is a viable option.

The development of appropriate techniques for in vitro culture and micro propagation of *Jatropha curcas* is necessary for germplasm collections, breeding programs and mass propagation. The improvement of the micro propagation of Jatropha curcas is important for Biodiesel production. Micropropagation of *Jatropha curcas* requires more input and time in order to obtain more specific information.

The present study has succeeded in obtaining a protocol for regenerating callus, shoots and roots of Jatropha curcas. The best hormones for callus production is a BAP1mg/l+1BA1mg/l while the best hormones for shooting and rooting are BAP1mg/l+IBA1mg/l and BAP1mg/l+Kn0.5mg/l+As2mg/l combinations. The best plant part for use are the seed embryos, as they are able to produce both roots and shoots at the earliest period possible.

An explant can be a variety of tissues, depending on the particular plant species being cultured. The explant can be used to initiate a variety of culture types, depending on the explant used. Regeneration by either organogenesis or somatic embryogenesis results in the production of whole plants. Different culture types and regeneration methods are amenable to different transformation protocols. Different combinations of culture type and transformation protocol are used depending on the plant species and cultivar being used. In some species a variety of culture types and regeneration methods can be used, which enables a wide variety of transformation protocols to be utilized.

The nursery germination under polythene bag container and the irrigated variety under polythene covered container gave the highest percent germination of mean 81.25% .The use of seed nursery enables healthy control of light, moisture, soil and allows development of saplings. The use of polythene bags as containers and

polythene cover as confirmed by this study gives increased success in germination rates. Seedlings may take about three to six months in the nurseries.

According to this study, the higher the temperature, the higher the germination rates. Here at 40 degrees, the earliest mean of days to sprouting, the highest germination percentage of 88% (irrigated variety), the highest mean stem length of 4.2cm and the highest number of leaves, 3(all varieties) were attained. Germination of seeds can also be carried out in the laboratory desk top using cotton wool. This medium containing cotton wool base gave highest mean percent germination of 100%

5.2 Recommendations

Jatropha curcas is fast becoming an important crop in most parts of the world. Results from the present study indicates that Jatropha curcas plantlets can be regenerated either from seed embryo or shoot tip explants. Not all plant tissue is suited to every plant transformation method, and not all plant species can be regenerated by every method. There is therefore a need to find both a suitable plant tissue culture/regeneration regime and a compatible
plant transformation methodology.

Characterization of seeds and varieties would be useful for breeders with interest on the crop. Multilocational germination trials are required to ascertain the effect of environment on specific genotypes. Research and development to develop quality oil and high yielding genetically improved varieties. Specific tissue culture on commercial lines is also important especially since significant oil production requires large scale planting.

LIST OF APPENDICES

Appendix 1 analysis of variance (ANOVA) of (1a)number of days to callusing, (1b) amount of callusing,(1c) days to shooting(1d) shoot length of Jatropha curcas varieties subjected to BAP,IBA and NAA treatments

1(a)

Source	**DF**	**Anova SS**	**Mean Square**	**F Value**	**P**	**r > F**
Plant part	2	96.3166667	48.15833	0.44		0.645
Treat	3	588.2	196.0667	1.79		0.153
Plant part *treat	6	284.75	47.45833	0.43		0.855

1(b)

Source	DF	Anova SS	Mean Square	F Value	P	r > F
Plant part	2	31.2	15.6	17.66		<0.0
Treat	3	3.8	1.266667	1.43		0.23
Plant part *treat	6	12.8	2.133333	2.42		0.03

1(c)

Source	DF	Anova SS	Mean Square	F Value	P	r > F
Plant part	2	2006.816667	1003.408	17.82	<	0.00
Treat	3	1040.225	346.7417	6.16	0	0.00
plant part *treat	6	2080.45	346.7417	6.16	<	0.00

1(d)

				Mean				
Source		DF	Anova SS	Square	F	Value	P	r > F
plant part		2	0.62016667	0.310083		21.08	<	0.00
Treat		3	0.32358333	0.107861		7.33	0	0.00
plant part	*treat	6	0.64716667	0.107861		7.33	<	0.00

Appendix 2 Analysis of variance (ANOVA) of (2a)number of days to callusing, (2b) amount of callusing,(2c) days to shooting of *Jatropha curcas* shoot tip, leaf and stem explants subjected to BAP,IBA,NAA and BAP/NAA treatments.

2(a)

			Anova	**Mean**			
Source		**DF**	**SS**	**Square**	**F**	**Value**	**Pr > F**
Variety		2	21.61667	10.80833		0.13	0.8781
Treat		3	31.8	10.6		0.13	0.9436
variety*t	Reat	6	215.45	35.90833		0.43	0.856

2(b)

				Mean			
Source		DF	Anova SS	Square	F	Value	Pr > F
Variety		2	0.65	0.325		0.38	0.6843
Treat		3	43.26667	14.42222		16.89	<.0001
variety*t	Reat	6	0.683333	0.113889		0.13	0.9917

2(c)

				Mean			
Source		DF	Anova SS	Square	F	Value	Pr > F
Variety		2	141.0667	70.53333		3.85	0.0242

Treat		3	211.6	70.53333	3.85	0.0116
variety*t	Reat	6	423.2	70.53333	3.85	0.0016

APPENDIX 3 : Analysis of variance (ANOVA) of (3a)amount of callusing,(3b) days to shooting(3c)No of shoot buds of Jatropha curcas shoot tip, leaf and stem explants subjected to BAP/IAA,BAP/Kn, BAP/IBA, BAP/Zeatin, BAP/Kn/As treatments

3(a)

Source		**DF**	**Anova SS**	**Mean Square**	**F**	**Value**	**Pr > F**
plant part		2	47.28889	23.64444		46.77	<.0001
Treat		5	6.088889	1.217778		2.41	0.0446
plant part	*treat	10	15.77778	1.577778		3.12	0.0023

3(b)

Source		DF	Anova SS	Mean Square	F	Value	Pr > F
plant part		2	9012.156	4506.078		49.14	<.0001
Treat		5	2713.122	542.6244		5.92	0.0001
plant part	*treat	10	3319.711	331.9711		3.62	0.0006

3(c)

Source		DF	Anova SS	Mean Square	F	Value	Pr > F
plant		2	48.46667	24.23333		71.51	<.0001

part					
Treat	5	19.46667	3.893333	11.49	<.0001
plant					
part *treat	10	21.26667	2.126667	6.28	<.0001

APPENDIX 4 Analysis of variance (ANOVA) of (4a)number of days to shooting, (4b) no. of shoot buds,(4c) shoot length(4d)days to rooting of *Jatropha curcas* varieties subjected to BAP, NAA,IBA,Zeatin, IAA and Kinetin treatments

4(a)

Source	**DF**	**Anova SS**	**Mean Square**	**F**	**Value**	**Pr**	**> F**
Variety	3	110.625	36.875		0.27	0	0.8450
Treat	5	15493.9	3098.78		22.91		<0.0001
variety*t reat	15	3924.82	261.655		1.93	0	0.0287

4(b)

Source	DF	Anova SS	Mean Square	F	Value	Pr	> F
Variety	3	0.89166	0.29722		1.66	0	0.1810
Treat	5	14.775	2.955		16.49		<0.0001
variety*t reat	15	8.45833	0.56388		3.15	0	0.0003

4(c)

Source	DF	Anova SS	Mean Square	F	Value	Pr	> F
Variety	3	0.041	0.01366		1.46	0	0.2292
Treat	5	0.70266	0.14053		15.06		<0.0001
variety*t reat	15	0.472	0.03146		3.37	0	<0.0001

APPENDIX 5 Analysis of variance (ANOVA) of (5a)number of days to shooting, (5b) no. of shoot buds,(5c) shoot length(5d)Days to rooting of *Jatropha curcas* varieties subjected to BAP/IAA, BAP/Kn ,BAP/ IBA, BAP/Zeatin, NAA/Kn BAP /Kn/As treatments

5(a)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Variety	3	2062.892	687.6306	9.48	<.0001
Treat	5	7965.342	1593.068	21.95	<.0001
variety*t reat	15	4355.958	290.3972	4	<.0001

5(b)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Variety	3	4.425	1.475	12.21	<.0001
Treat	5	9.441667	1.888333	15.63	<.0001
variety*t reat	15	7.525	0.501667	4.15	<.0001

5(c)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Variety	3	0.195667	0.065222	12.94	<.0001
Treat	5	0.484	0.0968	19.2	<.0001
variety*t reat	15	0.333333	0.022222	4.41	<.0001

5(d)

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Variety	3	3277.292	1092.431	10.35	<.0001
Treat	5	11265.18	2253.035	21.34	<.0001
variety*t reat	15	6806.058	453.7372	4.3	<.0001

APPENDIX 6 Analysis of variance (ANOVA) of (6a)height at 14 days, (6b) percent germination,(6c) no.of leaves(6d)Days to germination (6e)height at 21 days (6f) No of leaves at 21 days of *Jatropha curcas* varieties subjected to polythene container, polythene cover, sand/soil medium treatments

6a Dependent variable – Height at 14 days

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	183.8229167	91.9114583	5.46	0.0085
treat	3	219.5572917	73.1857639	4.35	0.0103
variety*treat	6	564.0520833	94.0086806	5.59	0.0004

6b Dependent variable- Percent germination

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	3938.541667	1969.270833	2.35	0.1094
treat	3	3712.500000	1237.500000	1.48	0.2365
variety*treat	6	8128.125000	1354.687500	1.62	0.1701

6c Dependent variable: No. of Leaves at 14 days

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	7.62500000	3.81250000	4.13	0.0243
treat	3	5.72916667	1.90972222	2.07	0.1217
variety*treat	6	15.20833333	2.53472222	2.74	0.0267

6d Dependent variable: Days to germination

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	244.5000000	122.2500000	6.75	0.0032
treat	3	82.7291667	27.5763889	1.52	0.2251
variety*treat	6	299.8333333	49.9722222	2.76	0.0260

6e Dependent variable: Height at 21 days

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	117.0704167	58.5352083	2.51	0.0950
treat	3	98.9883333	32.9961111	1.42	0.2536
variety*treat	6	612.4479167	102.0746528	4.39	0.0020

6f Dependent variable: No. of Leaves at 21 days

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	2	1.62500000	0.81250000	0.70	0.5009
treat	3	2.16666667	0.72222222	0.63	0.6026
variety*treat	6	10.70833333	1.78472222	1.55	0.1908

APPENDIX 7 Analysis of variance (ANOVA) of (7a)days to germination (7b) stem length,(7c) no. of leaves, and (7d)percent germination of seven *Jatropha curcas* varieties subjected to polythene container, polythene cover, sand/soil medium treatments.

7a Dependent variable: Days to germination

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	235.4857	39.24762	1.92	0.0845
Treat	4	1777.471	444.3679	21.72	<.0001
variety*t Reat	24	479.7286	19.98869	0.98	0.502

7b **Dependent variable: Stem length**

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	201.2094	33.53491	7.25	<.0001
Treat	4	2138.93	534.7326	115.66	<.0001
variety*t Reat	24	110.8627	4.61928	1	0.4742

7cDependent variable: No of Leaves

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	2.842857	0.47381	1.93	0.0823
Treat	4	21.78571	5.446429	22.21	<.0001
variety*t Reat	24	7.014286	0.292262	1.19	0.2668

7dDependent variable: Percent germination

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	38294.64	6382.44	19.32	<.0001
Treat	4	12187.5	3046.875	9.22	<.0001
variety*t Reat	24	15812.5	658.8542	1.99	0.009

APPENDIX 8 Analysis of variance (ANOVA) of (8a)days to sprouting, (5b) shoot length,(8c) percent germination(8d)no. of leaves (8e)days to rooting of *Jatropha curcas* varieties subjected to temperature treatments.

8a Dependent variable: Days to sprouting

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	0.225	0.225	0.1	0.759
treat	3	3.675	1.225	0.52	0.6707
variety*treat	3	4.275	1.425	0.61	0.6157

8b Dependent variable: Shoot length

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	0.225	0.225	0.3	0.5859
treat	3	45.054	15.018	20.21	<.0001
variety*treat	3	0.101	0.033667	0.05	0.987

8c **Dependent variable: Percent germination**

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	10.92025	10.92025	0.02	0.8843
treat	3	16405.01	5468.337	10.77	<.0001
variety*treat	3	2302.061	767.3536	1.51	0.2304

8d **Dependent variable: No of Leaves**

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	0.625	0.625	0.78	0.3834
treat	3	5.475	1.825	2.28	0.0981
variety*treat	3	0.275	0.091667	0.11	0.9509

8e **Dependent variable: Days to rooting**

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	1	3.025	3.025	1.83	0.1852
treat	3	5.075	1.691667	1.03	0.3945
variety*treat	3	4.875	1.625	0.98	0.4122

APPENDIX 9 Analysis of variance (ANOVA) of (9a)days to sprouting, (9b) percent germination,(9c) root length(9d)stem length of *Jatropha curcas* varieties subjected to use of filter paper base, cotton base and pre/soaking treatments

9a Dependent variable: Days to sprouting

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	141.2321	23.53869	19.97	<.0001
treat	3	8.071429	2.690476	2.28	0.085
variety*treat	18	41.55357	2.308532	1.96	0.0213

9b Dependent variable: Percentage germination

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	38415.18	6402.53	13.55	<.0001
treat	3	133.9286	44.64286	0.09	0.9629
variety*treat	18	7209.821	400.5456	0.85	0.6403

9c Dependent variable: Root length

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	10.92839	1.821399	17.29	<.0001
treat	3	0.257411	0.085804	0.81	0.4893
variety*treat	18	3.159464	0.175526	1.67	0.0623

9d Dependent variable: Stem length

Source	DF	Anova SS	Mean Square	F Value	Pr > F
variety	6	290.8511	48.47518	6.93	<.0001
treat	3	24.77527	8.258423	1.18	0.3224
variety*treat	18	65.63036	3.646131	0.52	0.9409