EVALUATION OF SEED SOURCE VARIATION AND CLONAL PROPAGATION TECHNIQUES IN *Jatropha curcas* LINN.

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Forestry

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VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2007

DECLARATION

I hereby declare that this thesis entitled "**Evaluation of seed source variation and clonal propagation techniques in *Jatropha curcas* Linn.**" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any University or Society.

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ACKNOWLEDGEMENT

It is with utmost respect and great devotion, I place on record my deep sense of gratitude and indebtness to my major advisor Dr. N. K. Vijayakumar, Associate Dean, College of Forestry, whose pragmatic suggestions, erudite guidance, unstinted mental support, friendly cooperation and parental concern throughout the study period made my thesis work an easy task. I express my heartfelt and sincere thanks to him.

My deep sense of gratitude goes to my advisory committee member, Dr. P. K. Ashokan, Professor and Head, Department of Tree Physiology & Breeding, College of Forestry, for his constructive suggestion, timely cooperation and encouragement and valuable guidance throughout the conduct of my study work.

I take this opportunity to place on record my sincere gratitude to Dr. K. Gopikumar, Professor and Head, Department of Forest Management & Utilization, College of Forestry, for his timely advice and constant help in a way of extending the facilities available in the department for conducting the present study.

I owe my sincere thanks to Dr. K. Vidyasagaran, Associate Professor, Department of Forest Management & Utilization, College of Forestry, for his valuable suggestion, constant encouragement, timely help in availing me to use the nursery and engaging the labour facilities.

I extend my unreserved thanks Mr. A. V. Santhoshkumar, Asst. Professor, Department of Tree Physiology & Breeding, College of Forestry, for his cooperation and availing the facilities for the conduct of experiment.

I am whole heartedly obliged to Dr. K. Sudhakara, Professor, Department of Silviculture & Agroforestry, College of Forestry; Mr. S. Gopakumar, Assistant Professor, Department of Forest Management & Utilization, College of Forestry; Dr. T. K. Kunhamu, Assistant Professor, Department of Silviculture & Agroforestry, College of Forestry; Dr. P. O. Nameer, Associate Professor, Department of Wildlife Sciences, College of Forestry and Dr. E. V. Anoop, Associate Professor, Department of Wood Science, College of Forestry, for kindly providing me valuable advice and various facilities for the conduct of the study.

Let me place on record my heartiest thanks to Mr. Rohini Ballave Saikhiya, Mr. Kumar Divayam, Mr. Harsha T. Hegde and Tippeshi L. Chauhan, Mrs. Sharada, Mr. Arun Visa, Mr. Abhilash G, Ms. Nataliya, for their friendly help during the course of my work.

The constant support and help provided by Mr. Ravindra, P.C and Ms. Jinsy M. Joseph will always be remembered.

Words cannot describe the cooperation extended by my friends Mr.Chikkanna, Mr.Samom Khelen, Mr.Shankar, Mr.Srikanth, Mr. Naveen, Mr.Praveen, Mr.Kishore, Mr.Manikandan, Mr.Rathish, Mr. Deviprasad, Mr.Puttuswamy, Mr.Jaba, Mr.Madhu, Mr.Gupta, Mr.Sahu, Mr.Imrose, Mr.Srini, Mr.Anto, Ms.Deepa Kuriakose, Mr.Money, Mr. K.B.Sridhar and Mr.Vinod.

The help rendered by Mr.Prasad, Mrs.Prema, Mrs.Rashmi, Mrs.Mini, Mrs.Deepa, Mrs.Seena, Mrs.Shantha, Ms.Preethi, Mrs.Sali and Mrs.Subindu, Ms.Sweety and Mrs.Eeshwari is also remembered with gratitude. My special thanks to Mrs.Jini and Mrs.Jyothi for their patience and complimentary gesture during my computer usage.

I express my deep sense of gratitude to Kerala Agricultural University for extending financial and technical support for pursuance of my study and research.

My inspiration, my loving parents Mr. and Mrs. M. P. Kalkoor and my loving sister Mrs. Shwetha Prasanna and my brother Mr. Shreesha Kalkoor were always with me with there uninhibited moral support, bonded affection. I behold to them forever for all I am today and hope to be in the future.

Anisha Kalkoor,M

Dedicated to

My parents

and

My teacher

Dr.N.K.Vijayakumar

CONTENTS

ABBREVIATIONS

Avg.	Average
BA	Benzyl adenine
cm	Centimeter
cv.	Cultivar
^0C	Degree celsius
dia.	Diameter
DAS	Days after sowing
DAP	Days after planting
Fig.	Figure
hrs.	Hours
et al.	Coworkers
GA_3	Gibberlic acid
g /gm	Grams
$HgCl_2$	Mercuric chloride
IAA	Indole 3- acetic acid
IBA	Indole 3-butyric acid
KAU	Kerala Agricultural University
K / kin	Kinetin
MAT	Mean annual temperature
MAR	Mean annual rainfall
m	Meter
Max	Maximum
mg	Milligram
min.	Minutes
Min	Minimum
mM	Milli molar
mm	Millimeter
µm	Micro meter
MS	Murashige and Skoog (1962) medium
NAA	Naphthalene acetic acid
No.	Number
ppm	Parts per million
Rh	Relative humidity
spp.	Species
TDZ	Thidiazuron
WPM	Woody Plant Medium of Lloyd and McCown (1980)
wt.	Weight
2ip	N-6 - (2 –isopentyl) adenine
2, 4-D	2, 4-Dichlorophenoxy acetic acid

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Introduction

INTRODUCTION

'Energy Independence' is one of the vital areas to make India a developed nation. Among different types of energy sources bioenergy through plant/animal route has to play a great role as the end of fossil fuel age has already began. Among various bioenergy sources plant route is considered very promising because of its renewable nature. Plants yielding oil are considered suitable for production of biofuel particularly biodiesel. Because of heavy requirement of edible oil, tree borne nonedible seeds are considered ideal for Indian condition for production of biodiesel. Tree borne oil seeds are best and potential alternative to mitigate the current and future oil crisis and also to transform the vast stretch of wasteland to green oil fields (Singh, 2006). The potential species identified so far include neem (*Azadirachta indica*), karanj (*Pongamia pinnata*), mahua (*Madhuca latifolia*), undi (*Callophyllum inophyllum*) and ratanjyot (*Jatropha curcas*). Among them *Jatropha curcas* is identified as the most potential biodiesel source and comparing with other sources this has added advantages as rapid growth, higher seed productivity, suitability for tropical and sub tropical regions of the world etc.

Jatropha curcas Linn. belonging to family Euphorbiaceae, is commonly known as ratanjyot, safed arand, physic nut, purging nut and chanderjyot. It is a large shrub or tree native to the American tropics, but commonly found and utilized throughout most of the tropical and subtropical regions of the world. Jatropha exhibits a wide environmental tolerance; it is found in seasonally dry tropics as well as equatorial regions and is well adapted for cultivation within the vast areas of marginal and degraded lands in the semi-arid and arid tropics. The plant is a multipurpose tree of significant importance because of its several industrial and medicinal uses. In addition to production of oil, promotion of *Jatropha curcas* plantation will generate tremendous job opportunities among rural and tribal masses. The byproducts

obtained during the production of biodiesel give rise to bio-fertilizers and glycerol, which add the value of the crop.

Such a multiple utility biofuel crop needs improvement in order to screen better genotypes with higher seed yield, higher oil content, fast growth, resistance against pest and disease and other performance characteristics. The improvement work in this species is very limited. Among trees, considering the different levels of variation present in a species, between seed sources and between trees within population variation are two categories that may account for over 90 per cent of the total variation observed in most of the species (Zobel and Talbert, 1984). Genetic differences associated with the place of origin have been several times as great as that among individual trees within a stand. Hence, it becomes necessary to conduct seed source testing prior to a more intensive breeding work. The selection of good seed source through extensive survey and assessment of genetic variation is the prerequisite for the success of any large scale plantation programme.

In plantations of tropical species use of traditional method of planting by seeds has a disadvantage of segregation and slower rate of growth in the progeny. This problem can be overcome by asexual methods of reproduction or clonal propagation. Planting materials produced by this method are true to type in nature. Though many methods of macro propagation like grafting, layering, budding, rooting of cuttings, etc. can be used, many tree species of economic importance are not amenable to these methods and even where it is possible, the number of propagules produced from limited quantities of available germplasm will be quite insufficient to meet the demands of extensive planting programmes. This problem can be overcome through micro propagation. Micro propagation is a technique of clonal propagation through various tissue culture techniques. The main objective of micro propagation

through tissue culture is to produce a large number of uniform propagules of known and desirable genotypes in the shortest possible time.

Against this backdrop, the current study was carried out with the following objectives:

1. To estimate the variability among different seed sources of *Jatropha curcas* Linn.
2. To standardize clonal propagation methods in *Jatropha curcas* Linn. through macro and micro propagation techniques.

Review of Literature

REVIEW OF LITERATURE

The literature pertaining to *Jatropha curcas* and other important tree borne oilseeds relevant to the present study are reviewed here under.

2.1 Seed source variation

2.1.1 Seed source variability for seed parameters

Seed is one of the most important inputs for forest nursery production and plantation. A good quality seed should possess uniform size, weight, color and freedom from pest and diseases to produce vigorous seedlings. Several provenance trials indicated the presence of considerable variations in seed size and weight (Anon, 1973 and Murthy, 1973a, b). It was also evident from several studies that viability increases with increase in seed size (Hedegart, 1974; Bhumibhamonm *et al.*, 1980 and Kaosaard, 1981). Variation in drupe size, fillingness, germination, etc. had also been documented differently by different authors (Gupta and Pattanath, 1975; Gupta and Kumar, 1976; Prasad and Parvez, 1986; Masilamani and Dharmalingam, 1999). The seed sources of *Jatropha curcas* exhibits considerable amount of variation in seed parameters and germination characteristics (Kumar *et al.*, 2004b).

Seed size and weight are important characteristics of plant species, which depend on a variety of factors like seed source, genetic make up and the environment where it is growing (Cavers and Steel, 1984). A close relationship between seed size, seed weight and seed quality has been documented for many tropical hardwoods (Halos, 1983). Higher germination and seedling development of *Jatropha curcas* were observed in heavier seeds than in the lighter seeds.

Heavy and large seeds contain more food than smaller seeds, which are attributed to higher seedling growth parameters (Kandya, 1979 and Stanton, 1985).

Kumar *et al.* (2003) studied seed source variation of *Jatropha curcas* seeds collected from five different locations in Tamil Nadu. They found that large seeds contained more moisture compared to medium and small seeds that tend to result in higher germination. Number of seeds per kilogram was highest in Paripati seed source and lowest in Walayar seed source. The seed moisture showed significant variation among the collections from different locations of Tamil Nadu and Kerala that ranged from 6.1 per cent in Walayar seed source to 4.2 per cent in Paripatti seed source.

Geetanjali *et al.* (2003) assessed the germination potential of *Jatropha curcas* seeds based on seed size. They found that 100 seed weight of three categories varied from 70.82 gm in large, 51.08 gm in medium and 34.85 gm in small seeds, respectively. They concluded that there was a significant increase in germination percent and seedling vigor with increasing seed weight. Similarly, Kumar (2003) reported that for all the seed traits studied from five locations of *Jatropha curcas* in Tamil Nadu, 1000 seed weight, seed length, breadth and thickness were higher in Coimbatore seed source followed by Madurai seed source and he confirmed that germination was higher in larger seeds and it gradually declined with reducing seed size. In another study it was found that the average fruit weight was highest in ripe fruits than unripe fruit and 100 seed weight was highest in ripe fruit than the dry opened fruits. The maximum seed length of 7.33 mm was recorded in Darpur seed source and minimum of 6.45 mm was recorded in Saran seed source. The maximum seed width of 6.16 mm was recorded in Mahendrawal seed source and minimum (5.25 mm) was recorded in Sathyal seed source. Kumar *et al.* (2003) noticed significant

difference in size and weight of seeds and the ratio of large, medium and small seeds were found to be 4:2:1 in Annur seed lot and 3:2:1 on seeds from Coimbatore, Madurai, Mettupalyam and Palani seed sources.

Kumar *et al.* (2003) reported variation in seed traits of *Acacia nilotica* collected from different locations. They reported significant correlation of seed weight with seed length and germination percent. They confirmed that seed weight could be considered as an important trait in early selection of seed sources. The seed sources with higher seed weight are expected to give higher germination percent. The highest seed weight of 39.97 mg was recorded in Rampur seed source and the lowest seed weight was recorded in Basoli seed source (25.99 mg). The maximum number of seeds per gram was recorded in Basoli seed source (40.16/g), while, the minimum was recorded in Rampur seed source (25.13/g). In a similar study in *Pongamia pinnata* it has been reported that out of six locations in Tamil Nadu, seed weight was highest in Salem seed lot followed by Pillur and Erode, while the lowest was in seeds of Karur (Kumar *et al.*, 2003). The number of seeds per kilogram was highest in seed of Karur followed by Coimbatore and lowest in Salem. It was also found that moisture content varied significantly among the seed lots, which varied from 6.3 per cent in Salem to 5.3 percent in Karur seed source.

Kumar *et al.* (2003) noticed significant variation for seed weight, length and breadth in *Prosopis cineraria*. The average seed weight varied from 39.6 mg per seed to 72.0 mg per seed in different seed sources and most of the seed sources had significantly higher seed weights than the general mean. They concluded that variation in seed characteristics of *P. cineraria* have been attributed to mother tree difference and as a result of large variation in climatic conditions in areas of its natural distribution.

Among the various seed sources of *Acacia catechu* the seed length varied from 7.79 mm to 10.41 mm and seed width from 6.06 to 8.84 mm (Bhat and Chavan, 2003). The Rajapura seed source excelled all other seed sources for seed length and width. They also observed that highest and lowest average 100 seed weight was in Nauni and Chakla seed sources, respectively, and that seed source with higher seed length and width also possessed highest seed weight. It was confirmed that seedling of *Acacia catechu* from large sized seeds showed significant increase in seedling parameters (Kumar, *et al.*, 2003).

2.1.2 Seed source variability for germination attributes

Germination potential of *Jatropha curcas* seeds from in and around Tamil Nadu and Kerala was assessed by Kumar and Swarnkar (2003). The germination varied from 83 per cent in Walayar seed source to 57 percent in Paripati seed source. They confirmed that the higher germination and seedling development of *Jatropha curcas* were observed in the heavier seed than in the lighter seeds. It was found that germination and vigor index was highest in Walayar seed source and least in Paripati seed source. Seed germination of *J. curcas* was 82, 73, and 49 per cent in Madurai seed source and 65, 58 and 37 per cent in Palani seed sources for large, medium and small seeds, respectively (Kumar, 2003). In a similar study, the germination varied from 99, 60 and 20 per cent in large, medium and small seeds respectively (Geetanjali, *et al.*, 2003). They also found that germination was highest in dry opened fruit, which was not significantly different from that of ripe fruits while unopened fruits had significantly lower germination per cent. Variation in seed parameters and germination of *Jatropha curcas* were noticed in the seeds collected from ten different seed sources, the germination and vigor index was reported to be highest in seeds of Walayar and while Paripati showed least (Kumar *et al.*, 2003). Similarly, Kumar *et al.* (2003)

reported that seed weight varied significantly from Coimbatore seed lot recording the largest seed weight and Madurai the least.

Dagar *et al.* (2004) studied the effect of seed weight on germination potential using different categories of seeds (100 to 800 milligrams per seed) in *J. curcas*. In the first three categories there was no germination and the other categories exhibited 20, 30, 60, 85, and 90 percent germination, respectively. Hence it was concluded that the seed weight has direct relation with the germination of seeds.

Balakrishnan and Singh (1995) reported that large seeds in *Acacia nilotica* (32.2%), medium seeds in *Erythrina indica* (44.2%) and small seeds in *Albizia lebbeck* (26.6%), *Cassia siamea* (38.5%) and *Populus dulce* (52.0%) exhibited the maximum germination. Rajiv (1996) has reported significant difference in seed germination between seed source of neem from 18 geographical sources of India. He also noticed that neem seeds sown in plastic trays in a glasshouse gave significantly higher germination (65-94%) than seeds sown in poly bags (23-51%).

Kumar and Toky (1996) observed variation in germination among twelve seed sources of *Albizzia lebbeck* from India. They reported that the germination in different seed sources of *Albizzia lebbeck* varied from 16.7 per cent to 38.05 per cent. The Nalgarh seed source exhibited highest germination per cent (38.07 %), which was significantly different from the rest of the seed sources, which suggested that seed sources with higher seed weight showed maximum germination percent than that of smaller and lighter seeds, probably, because of more food reserves in the endosperm. The seed germination in *Acacia catechu*

varied from 5 – 94 per cent in incubator and 8-50 per cent in nursery (Bhat and Chavan, 2000).

Germination test was conducted to study the performance of *Pongamia pinnata* seeds collected from different locations and found that there was average germination per cent in all the locations (Kumar *et al.*, 2003). Lavania and Singh (2004) reported that the germination of *Populus ciliata* from different seed sources differed significantly with each other at 1.0 per cent level and ranged from 41.75 per cent in Kathpudia seed source to 71. 55 per cent in Nainital seed source.

2.1.3 Seed source variability for seedling parameters

In *Jatropha curcas* Kaushik *et al.* (2003) reported that the height of seedling was significantly influenced by the rooting medium and size of seeds. Large sized seeds gave rise to 32 per cent increase in germination in comparison to small sized seeds. The maximum height of seedling (46.77 cm) was recorded in large sized seeds sown in rooting media containing sand, soil and FYM in the ratio of 1:1:1. The size of seeds and rooting media also significantly influenced the root length. The root length was 17.60 cm and 12.80 cm in large and small sized seeds, respectively. The collar diameter varied significantly in all the treatments. In both the rooting media collar diameter of seedlings increased with increasing seed size and weight. The large sized seeds also produced seedlings with greater number of leaves; their increase being 17 percent in large sized seeds compared to smaller ones. They observed that dry matter production of seedlings also varied significantly by seed size and growing media.

The seedling vigor of *Jatropha curcas* as determined by leaf, shoot and root weight was significantly higher in seeds from dry opened fruits. The seeds from both ripe and dry opened fruits produced vigorous seedlings (Geetanjali, *et al.*, 2003).

Kumar *et al.* (2003) studied the effect of seed size on seedling growth of *Jatropha curcas*. They found higher germination and seedling vigor with heavier seeds. In comparison of different seed sources for seedling parameters they found that root length, shoot length, vigor index and biomass production were highest in the seeds collected from Coimbatore and lowest in the Madurai collection. They also found that shoot growth was faster than root growth. In another study they found that seedlings from Walayar seed source exhibit highest root length, shoot length and biomass accumulation after 50 days of sowing.

In a comparison of the effect of seed size and seedling vigor, Balakrishnan and Singh (1995) reported that large seeds recorded maximum seedling height in *Albizzia lebbeck* (3.07 cm) and *Erythrina indica* (3.86 cm), medium seeds in *Acacia nilotica* (4.98 cm) and small seeds in *Cassia siamea* (3.37 cm) and *Pithecellobium dulce* (4.03 cm).

Munendrappa *et al.* (1997) reported that the vigor of *Tectona grandis* seedlings varied among the seed sources with those from Dharwad and Mettupalyam expressing better growth than those from Shimoga and Bangalore. Similarly, Kumar *et al.* (2003) reported that root length, shoot length and biomass of *Pongamia pinnata* were higher in seedlings raised from Salem seeds compared to other seed sources in the study.

Lavania and Singh (2004) reported that total seedling length varied significantly from 3.15 to 4.99 cm in the seedlings of different seed sources in *Populus ciliata.* The maximum seedling length of 4.99 cm was observed in Bhowali seed source followed by 4.57 cm in Ranichuari and minimum 3.15 cm in Kathpudia seed source. The seedling vigor varied from 131.51 to 335.50 showing a significant difference in different seed sources. The maximum leaves per seedling were found in the Bhowali (5.8) and minimum in Kathpudia (4.1).

In a study conducted by Nandeshwar *et al.* (2005) it was found that large sized seeds gave slightly higher shoot biomass production than small sized and medium sized seeds of *Buchanania lanzan.* For root length, root biomass and total biomass, seedlings from medium sized seeds were superior to the rest.

2.1.4 Seed source variation for oil content and yield

In *Jatropha curcas* oil and seed yields are influenced by climate, intensity of management, site quality, size of the plant and genetic potential. Highest yield can be expected only under the most favorable growing conditions. Seed oil content varied from 29-55 per cent in the states of Chattisgrh, Andhra Pradesh, Orissa and Tamil Nadu. The plants growing in Pendra Road (Chattisgarh) recorded seeds having the highest oil content. In general, the oil yield of *Jatropha curcas* was 35-40 per cent of the seed yield (Puri and Swamy, 2003).

Keremane *et al.* (2003) studied growth and yield traits of three genotypes of *Jatropha curcas* at three population densities. They found the highest seed yield of 2766.2 kg per hectare in medium density of 3333 plants per hectare and

confirmed that this variation might be due to greater number of fruits per tree in this spacing.

According to Puri and Swamy (2003) age of the plant did not have any effect on the oil content. However, seeds of moist localities had higher oil content than drier localities. Among the dry localities oil content varied significantly i.e. the seeds from Vertisol (Red soil) were having higher oil content than those from Entisols and Altisol. During storage of seeds, irrespective of storage temperature, the oil content decreases about 2.0 per cent with time.

Kumar *et al.* (2003) reported that among the seeds from ten different locations in Tamil Nadu, the Walayar seed contained the highest per cent of oil (60.08 %) followed by Attapady (59.9 %) and the lowest was noticed in Paripati seed source (47.4 %). The oil content varied from 30-48 percent in the states of Chattisgarh, Andhra Pradesh, Orissa and Tamil Nadu. It was found to be highest in Chattisgarh (48.25 %) and lowest in Tamil Nadu (30.5%). Oil yield from seeds collected in the plains was 15 per cent and that of the hills about 19 per cent of seed weight (Kannan, 2003).

Variation in seed oil content between different seed sources has been reported in other oil yielding tree species also. Nadagoudar and Nataraja (1997) observed variability in total oil content in seeds of neem from 20 locations, which ranged from 19.03 to 28.03 per cent. They noticed four per cent higher oil content from seeds of old trees than in irrigated areas. They also reported wide variation for azadirachtin content of seeds between the trees at Dharwad (5.48 %) and Bijapur (6.8%).

Significant variation among the six climatic zones for oil content in *Pongamia pinnata* was reported by Kumaran *et al.* (2003). It was concluded that Cauvery delta recorded the maximum mean oil content of 37.49 per cent followed by western zone (33. 26 %) and northwestern zone recorded the lowest value of 29.87 per cent. They finally concluded that the locations such as Cauvery delta zone might be focused upon for future exploration of genetic material because of high oil content. They also reported that oil content of karanj kernel was highly influenced by soil type and the range was from 32.79 per cent to 39.57 per cent. The oil content was highest in coastal alluvium (39. 57%) while in red soil it was least (34.59%). Parthiban, *et al.* (2003) reported that among the 30 seed sources, 11 recorded significantly higher oil content than the general mean. The oil content ranged from 51.50 per cent to 26.48 per cent.

2.2 Clonal propagation

Clonal propagation technique will be useful in capturing maximum genetic gains in quickest time (Swamy *et al.*, 2002) and it has an added advantage in the establishment of clonal seed orchard, clonal banks, propagation of exceptional hybrids and of selected plants on large scale (Gera *et al.*, 2000).

2.2.1 Macro propagation

There are several ways of vegetative propagation. The three main types in forest tree propagation are grafting, air layering and the use of cuttings. Propagation by cuttings is the most convenient and cheapest method and usually preferred when possible.

Though uniform and identical true to type planting stock can be developed through clonal propagation techniques, scientific studies and literature regarding appropriate vegetative propagation techniques in jatropha are quite scanty.

Swamy and Singh (2006) reported that *Jatropha curcas* can be propagated more cheaply and quickly by means of vegetative propagation. Studies conducted on the rooting of stem cuttings revealed that the rooting potential mainly depends on season, age and size of cuttings. The shoots should be healthy and smooth. Cuttings of *J. curcas* taken from the base of the stem gave better response as compared to the cuttings taken from the tip and middle portion of the stem (Kuashik and Kumar, 2005). In another study it was observed that one year old shoots preferably from the middle of the branch measuring 20-25 cm in length with 4-5 buds are the best suited material for propagation of jatropha as they give nearly 80-90 per cent rooting (Singh *et al.*, 2006).

Clavo *et al.* (2000) reported the practice of vegetative propagation of *Jatropha curcas* for raising live fences in pastures. They also reported great variability in the rooting of the cuttings when grown in different localities. Zahavi (2005) studied the establishment ability and cover development of vegetatively propagated *Jatropha curcas* plants. The study revealed that *Jatropha curcas* has comparatively high establishment success than the other species. Kureel (2006) has listed a number of ongoing experiments to develop quality-planting material for mass propagation and the standardization of efficient propagation techniques in *Jatropha curcas*.

Gupta and Dutta (1999) studied the influence of plant growth regulators on the rooting and sprouting performance in stem cuttings of *Jatropha*

pandurifolia 'rosea'. They used 15 cm long stem cuttings and various concentrations of IBA and NAA. They observed that higher levels of IBA and NAA have significantly reduced the growth of the cuttings.

In a study conducted by Kumar and Swarnkar (2003) to evaluate the rooting performance of *Jatropha gossypifolia* stem cuttings comprised of two sets of treatments. The first set comprised 24 hr dipping in different concentration of IBA and the other set comprised of 3-4 min dip in different concentration of IBA. It was observed that IBA concentration of 50 and 75 ppm for the 24 hr dip promoted higher rooting, survival and vigor. Similarly, IBA concentration of 1000 and 1500 ppm in the 3-4 min dip were superior over the other treatments in relation to rooting, survival and vigor. They also reported that higher concentrations of IBA (2000 ppm, 2500 ppm) show negative rooting response. Lack of well developed rooting system in higher concentrations of IBA through exogenous application seems that it has raised the IBA content to supra-optimum level causing considerable reduction in rooting (Nanda *et al.*, 1968).

Singh *et al.* (2003) studied the effects of NAA and IBA on rooting of stem cuttings in *Bougainvillea*. They treated the semi-hard wood cuttings with various concentrations of NAA and IBA for five seconds by dipping the basal ends in the growth regulator solution. They concluded that NAA at 1500 ppm + IBA at 1000 ppm combination was the best treatment with respect to rooting percentage, root length, root fresh weight and root dry weight.

Similar study was conducted by Stancato *et al.* (2003) in *Rhipsalis grandiflora*. Influence of origin of cuttings and different levels of IBA on the vegetative propagation of *Parinari curatellifolia* was examined by Mwang-ingo *et al.* (2003). Cuttings from the basal portion and treatment with 375 ppm of IBA

was found to be the best treatment with respect to rooting percentage, number of roots and root length.

In *Anogeissus pendula* the treatment with IBA was found to be less effective for vegetative propagation through rooting of cuttings (Rai *et al.*, 2002). Effect of IBA and age of the cuttings on rooting and survivability of *Murayya keonigi* stem cuttings was reported by Ranganathappa *et al.* (2002). All the treatments have shown positive response compared to the control.

Clonal propagation through stem cuttings in *Ceiba pentandra* (Linn.) Gaertn. was studied by Rajendran *et al.* (2002). They used IAA, IBA and NAA in different concentrations to induce rooting and found that among all the treatments IBA 3000 ppm was the best with respect to sprouting percentage, percentage of rooting, average number of roots and root length. Similar work in *Alnus nitida* was done by Thakur and Pant (2002). Maximum root- shoot ratio was observed in 800 ppm IAA and 1600 ppm IBA treatments. Maximum shoot height was found in 1600ppm IBA treatment.

Standardization of vegetative propagation techniques in some medicinal plants was attempted by Philip *et al.* (1991). They studied the effect of different rooting hormones, age and length of cuttings on rooting of cuttings. In this study IBA was found to be the best rooting hormone and rooting response increased with increasing maturity and length of cuttings. Similar studies were done in *Ficus glomorata* L. (Bhatt and Badoni, 1993), *Pinus nigra* var *maritime* (Spanos *et al.*, 1993), *Terminalia bellerica* Roxb., *Terminalia chebula* Retz. (Bhardwaj *et al.*, 1993), *Ulmus villosa* (Bhardwaj and Mishra, 2005), *Vitex nigundo* (Tewary *et al.*, 2004) and *Albizzia amara* (Handa *et al.*, 2003). All these studies indicated the positive effect of hormones on rooting of cuttings.

2.2.2 Micro propagation

Mass production of selected clones through *in vitro* techniques – 'micro propagation' is of great importance in clonal forestry to overcome the constraints like scarce seed supplies, germination problems, long regeneration time, etc. (Leaky, 1987). It has long been apparent that genetic gains can be captured by clonal propagation (Durzan, 1998) and under such circumstances *in vitro* propagation is known to be a possible method in many tree species especially where conventional methods are not economically feasible (Bonga, 1982).

Plant tissue culture is defined as the technique of cultivating cells, tissues or organs of plants on artificial media under aseptic conditions. During the last few decades tissue culture has made considerable amount of progress in the fields of fundamental botany and applied science like, agriculture, horticulture, forestry etc.

2.2.2.1 Plant tissue culture: a historic perspective

The concept of developing whole plant from a single cell came from the cell theory proposed by Schwann (1839). The theory states that each living cell of an organism if provided with proper environment would be capable of independent development. Cell theory gave birth to the concept of totipotency which forms the basic principle behind tissue culture. The principle states that it should be possible to produce an organism from any of its nucleated cells since all information needed to specify an organism is contained in its DNA.

It was Haberlandt (1902) who for the first time cultured cells on artificial medium.He observed obvious growth of cells on the media. But the cultures lacked cell division. Later, White (1931) found out that the material used by Haberlandt were all mature differentiated cells lacking any meristematic activity. Research during the later period (1940-1950) was mainly concentrated on determining the nutritional requirements for obtaining sustained growth of tissues in cultures. The discovery of plant hormones and vitamins contributed tremendously to the development of tissue culture. The most classical demonstration of hormonal control of organogenesis in callus cultures came from Skoog and Miller (1957). They demonstrated that shoot and root initiation could be regulated by a subtle ratio of auxin and cytokinin.

Muir and Hildebrandt (1954) reported the growth of cell cultures in liquid medium for the first time. Later Jones *et al.* (1960) were successful in inducing growth in single isolated cells. The field of protoplast culture was thrown open by Cocking (1960) when he isolated protoplast for the first time. Over the years protoplasts have been isolated from different plant parts like roots, leaves, coleoptiles, fruit tissues, pollen mother cells etc. Isolation and culture of protoplasts lead to the discovery of somatic hybridization, which is a very powerful tool for research in somatic cell genetics.

Morel (1960) employed meristem culture for the first time for clonal propagation. This technique is also used to produce virus free plants (Walkey, 1980). Guha and Maheswari (1964) cultured anthers for the first time to develop haploid cultures. This technique is also used to double the

chromosome number of plants developed from anther to produce homozygous diploids (Maheswari and Rangaswami, 1963).

One of the applications of tissue culture in plant breeding is embryo culture. Various hybrid embryos have been successfully cultured and germinated *in vitro* (Maheswari and Rangaswami, 1963). This can help in producing distant hybrids. This area is now on limelight since it leads to the isolation of plants which are tolerant to pesticides, alkalinity, pathogens etc. Nickell (1964) was the first to demonstrate that variants could be isolated from sugarcane cultures. Later many investigators worked in this field (Nabors *et al.*, 1975; Smith and McComb, 1981). One of the major advantages is that the induction and screening of mutants can be done at cellular level. This technique is used for the conservation of rapidly depleting biodiversity. This has been employed in preserving the tissues of many species like chrysanthemum (Bannier and Steponkus, 1972), datura (Bajaj, 1976), nicotiana (Maddox *et al.*, 1983), etc.

Secondary metabolites of plants which are having industrial and medicinal importance can be extracted from the cell cultures. This technique has been tried successfully in many plants like *Dioscorea deltoidea* (Kaul *et al.*, 1969), *Morinda citrifolia* (Zenk *et al.*, 1975), *Nicotiana tubacum* (Tabata and Hiraoka, 1976), etc.

2.2.2.2 Micro propagation through tissue culture in tree species

The big boom in plantation forestry during the past two decades has created an ever increasing demand for quality planting materials. Micro propagation because of its inherent advantage is regarded as the best way to

meet this demand. With this view, the technique has been tried in many economically important trees and various other woody species including jatropha. A very brief account of some of the salient works carried out in jatropha and other important species are reviewed below.

Jatropha species

Techniques were developed for regeneration from various explants of *Jatropha curcas* by Sujatha and Muktha (1996). Regeneration from hypocotyls, petiole, and leaf explants were evaluated on a range of concentrations of zeatin, kinetin, and BA either singly or in combination with IBA. Higher regeneration from hypocotyls and petiole explants were obtained on BA with IBA than zeatin or kinetin supplemented media. Leaf discs from the third expanding leaf exhibited higher regeneration potential than those from fourth leaf. Independent of the explant type, direct adventitious shoot bud induction was highest on MS medium with 2.22 μM BA and 4.9 μM IBA. Regenerated shoots could be rooted on growth regulator free full strength MS medium.

A simple, rapid, efficient and reproducible protocol for direct regeneration of plantlets from shoot tips of *Jatropha curcas* was developed by Sardana *et al.* (1998). Regeneration was observed from shoot tips on a combination of GA3 (3.0 mg l^{-1}) and IAA (3.0 mg l^{-1}) on MS medium. Plantlets were acclimatized and successfully transferred to pots and finally to the field.

An efficient 2-stage method for plant regeneration from leaf explant derived embryogenic callus of *Jatropha curcas* was developed and standardized by Sardana *et al.* (2000). Stage 1 was induction of embryogenic callus and formation of globular embryos which required MS medium, Gamborgs medium

containing MS basal salts and Gamborgs vitamins supplemented with 6- benzyl adenine (3.0 mg l^{-1}) and IAA (3.0 mg l^{-1}). Stage 2 was induction of plantlets, which was on MS medium containing GA (3.0 mg l^{-1}) and IAA (1.0 mg l^{-1}). Somatic embryos developed into normal plantlets on full MS medium containing 3.0 per cent sucrose.

A method for production of clonal plants from *Jatropha curcas* by *in vitro* production of multiple shoots from nodal segments was developed by Rajore *et al.* (2002). The nodal segments were cultured on MS medium supplemented with cytokinins viz., kinetin, BA and auxins viz., IAA and NAA. Cultures were maintained at 25°C with less than 16 hrs photo periods. Multiple shoot production was obtained on MS medium fortified with kinetin at 2.0 mg l^{-1} and 1.5 mg l^{-1} IBA. However, addition of various additives viz., ascorbic acid (10.0 mg l^{-1}), citric acid (50.0 mg l^{-1}), adenine sulphate (25.0 mg l^{-1}), and glutamine (100.0 mg l^{-1}) showed synergistic effect in shoot proliferation and its development. *In vitro* produced shoots were transferred on rooting medium which comprised of full and half strength MS medium incorporated with auxins *viz.*, IAA, IBA, NAA and 2, 4-D (1.0-6.0 mg l^{-1}). Best rooting was obtained on 1/2 strength MS medium containing NAA (5.0 mg l^{-1}). Plantlets were transferred for primary hardening in a sterilized mixture of sand and vermiculite (3:1) and were established in soil with survival rate of 60-80 per cent.

The growth and development of jatropha zygotic embryos *in vitro* were studied by Jesus *et al.* (2003) and found that embryos were not affected by medium and GA3 concentration. The growth and development were most pronounced in half strength MS medium without GA3.

Protocol for direct shoot regeneration from different explants of *Jatropha integerrima* was developed by Sujatha and Dhingra (1993). Prolific adventitious shoot bud initiation was obtained using a combination of 2.2 or 4.4 μM BA and 4.9 μM IBA. Reduction of IBA concentration (2.5μM) promoted further development of shoots. Regenerated shoots readily rooted on MS medium lacking growth regulators.

The effect of explants (hypocotyls, stem, peduncle, and leaf) on shoot induction was investigated by Sujatha *et al.* (2000) *on Jatropha integerrima* using MS medium containing BA, kinetin or zeatin (0.1- 2.0 mg l^{-1}) in combination with IBA (1.0 mg l^{-1}). BA was most effective in promoting shoot induction. Highest shoot regeneration potential was observed on stem and leaf explants. However, leaf segments failed to regenerate on medium supplemented with kinetin and zeatin. Differences due to explant, cytokinin type, its concentration and their interactions were significant.

Acacia species

Nodal segments from the seedlings of *Acacia mangium* could give rise to plantlets when cultured on MS media containing 0.5 mg/l BA (Ahmed, 1990). Multiple shoots were developed from axillary buds excised from *in vitro* grown seedlings of *Acacia auriculiformis* on Gamborg's (B5) basal medium supplemented with coconut milk and benzyl adenine (BA) 10^{-6} M concentration. These shoots, transferred individually to B5 medium, containing indole-3-acetic acid (IAA) 10^{-7} M or naphthalene acetic acid (NAA) 10^{-6} or 10^{-7} M produced roots (Mittal, *et al.*, 1989). Cotyledonary nodal explants of *Acacia nilotica* differentiated multiple shoots on B5 medium supplemented with BA (1.5 mg l^{-1}). Individual shoots, when

transferred to B5 medium containing IAA (2.0 mg l^{-1}) produced healthy roots in 100 per cent cultures (Dewan *et al.*, 1992). Xie and Hong (1992) reported somatic embryogenesis and whole plant regeneration in callus cultures derived from immature zygotic embryos of *Acacia mangium*. Embryogenic callus was induced on Murashige and Skoog (MS) medium containing combinations of thidiazuron (TDZ) 1.0-2.0 mg/l, IAA (0.25-2.0 mg/l) and a mixture of amino acids.

Aegle marmelos

Rapid clonal multiplication of *Aegle marmelos* (L.) was achieved by enhanced axillary bud proliferation on MS nutrient medium (Ajithkumar and Seeni, 1998). Bud break was dependent on cytokinin supply, but the synergistic combination of BA (2.5 mg l^{-1}) and IAA (1.0 mg l^{-1}) induced the formation of shoots. Shoot cuttings were best rooted in half-strength MS medium with IAA (0.5 mg l^{-1}) or IBA (10.0 mg l^{-1}).

Ailanthus triphysa

Nodal explants were used and maximum bud initiation was obtained on MS medium supplemented with 2.0 mg l^{-1} BA and 2.0 mg l^{-1} kinetin. Rooting was obtained on half strength MS medium containing 4.0 mg l^{-1} IAA and 0.4 mg l^{-1} IBA (Natesha, 1999).

Albizzia lebeck

Multiple shoots were produced from the hypocotyl, root, cotyledon and leaflet explants of *Albizzia lebeck*, both directly and indirectly. Rooting was

achieved on transfer of the shoots to MS medium containing 2.0 mg l^{-1} IAA (Paramjith and Maheshwari, 1982).

Anogeissus pendula

Joshi *et al.* (1991) were successful in obtaining multiple shoots from cotyledonary and epicotyledonary nodes. Cotyledonary nodes, which produced 15-20 shoots, were found better than epicotyledonary nodes, which produced 4-5 shoots when cultured on MS medium containing 1.0 mg/l BA and 0.1 mg/l IAA. Shoots were rooted on half strength MS medium containing 15 mg /l of IBA and 0.1 mg/l of kinetin.

Artocarpus heterophylus

Roy *et al.* (1996) reported multiple shoot production from shoot tip and nodal segments of *Artocarpus heterophylus* cultured on MS medium supplemented with BA (2.5 mg l^{-1}) and IAA (0.5 mg l^{-1}). Excised shoots were rooted on half strength MS medium containing NAA and IBA each at 1.02 mg l^{-1} concentration.

Azadirachta indica

Somatic embryogenesis was obtained in neem (*Azadirachta indica*) using mature seeds, which were cultured on MS medium supplemented with TDZ. TDZ was very effective and induced somatic embryogenesis across a wide range of concentrations (1-50 μm). However, somatic embryogenesis was accompanied by callus formation at concentrations of 20 μm and above. Plants were regenerated from both directly formed somatic embryos and somatic embryos

derived from cell suspensions placed on semisolid medium devoid of growth regulators. Regenerated plantlets continued to grow after transfer to greenhouse environment and were similar phenotypically to zygotic seedlings (Murthy and Saxena, 1998). Evaluation of azadirachtin production in micro propagated plantlets was attempted by Roshni (2003). It was reported that the Murashige and Skoog (MS) medium is better than Woody Plant Medium (WPM). Murashige and Skoog medium (MS) when supplemented with kinetin showed positive effects in terms of establishment and growth of the culture.

Caesalpinia pulcherima

Nodal explants from the trunk sprouts were used for callusing on MS medium containing NAA alone and 2, 4-D in any combination, except BA. The greatest rooting was obtained in medium containing IAA and cytokinin (Rao *et al.*, 1998).

Cleistanthus collinus

Cleistanthus collinus was micro propagated using nodal explants on MS medium supplemented with BA (2.2 μM). Shoot proliferation was enhanced when the BA concentration was lowered to 1.1μM. Rooting was achieved on half strength MS medium with 22.8 μM IAA (Quraishi *et al.*, 1994).

Dalbergia species

Direct regeneration of somatic embryos is reported from immature zygotic embryos of *Dalbergia latifolia* (Rao and Sita, 1994). Immature embryos were used as explants. Pre-culture on high 2, 4-D medium for 4 weeks induced

direct somatic embryogenesis and that was expressed during the second culture phase in the presence of low 2, 4-D along with a high sucrose concentration. Embryos were separated and transferred to the MS medium containing BA (0.5–1.0 mg l^{-1}) where they developed into plantlets.

Mahato (1992) was successful in obtaining multiple shoots from the nodal segments of Indian rosewood. Woody Plant Medium (WPM) as well as MS medium were found to be suitable for primary culture establishment. Kannan (1995) found that WPM with kinetin at 1.0 mg l^{-1} and IAA at 0.1 mg l^{-1} was best for getting enhanced release of axillary buds in *Dalbergia latifolia*. Multiple shoots (3.5 shoots / explant) were induced on MS medium by adding 2.0 mg l^{-1} BA. *In vitro* rooting was obtained in half-strength basal medium after giving a pulse treatment with 1000 mg l^{-1} IBA solution to base of the shoots produced from buds of young trees. Direct shoot and root formation was noticed from nodal explants of young trees when cultured on WPM supplemented with1.0 or 2.0 mg l^{-1} IAA. Rooting could not be obtained from shoots produced from nodal explants of mature elite rosewood trees.

Excocaeria agallocha

Nodal segments were cultured on MS medium containing BA, zeatin and IBA in concentrations of 13.3 µM, 4.65 µM and 1.23 µM, respectively. Multiple shoot induction was complemented with efficient shoot elongation, and repeated subculture of binodal segments from axillary shoots resulted in 10-12 shoots per explant in 3 months. Rooting was achieved by growing shoots in the new medium with 0.23 µM IBA (Rao *et al.*, 1998).

Emblica officianalis

The *in vitro* multiplication of *Emblica officinalis* (*Phyllanthus emblica*) plantlets from nodal shoot explants was attempted by Maneesh *et al.* (2001). Dipping of explants in 1.0 per cent bavistin (carbendazim) for 60-90 minutes followed by 0.1 per cent $HgCl_2$ treatment for 8 minutes significantly reduced explant contamination. Multiple shoot production was obtained in MS medium containing kinetin at 0.2, 0.4, and 0.6 mg l^{-1}. Supplementation with 1.0 mg l^{-1} GA_3 (gibberllic acid) and 0.4 mg l^{-1} kinetin favoured the internodal elongation of shoots. Ying *et al.* (2002) attempted adventitious induction of shoots using tender buds of *Phyllanthus emblica* as explants on MS medium. Results showed that MS medium supplemented with BA (0.5 mg l^{-1}) and NAA (0.1 mg l^{-1}) is suitable for adventitious bud inducement and the rate of differentiation reached more than 90 per cent. MS medium with BA (0.1 mg l^{-1}) and NAA (0.1 mg l^{-1}) is suitable for the growth of shoots. 2-3 adventitious buds were regenerated in the base. Rooting was achieved on half strength MS with NAA (0.25 mg l^{-1}) or with NAA (0.25 mg l^{-1}) + IBA (0.25 mg l^{-1}).

Eucalyptus species

Pattanaic and Vijaykumar (1997) induced multiple shoots in *Eucalyptus globulus* using nodal segments cultured on MS medium containing IBA (0.5 mg l^{-1}). Rooting was obtained on half strength MS medium containing IAA (0.5 mg l^{-1}) and IBA (0.5 mg l^{-1}). Nodal explants of *Eucalyptus tereticornis* were cultured on MS medium (Prabha *et al.*, 2000). Cultures established on medium containing BA (1.0 mg l^{-1}) + NAA (0.1 mg l^{-1}) and then transferred to medium containing 1.0 mg l^{-1} BA + 1.0 mg l^{-1} NAA gave maximum multiplication of shoots. Best rooting was observed on half strength MS medium with 0.5 mg l^{-1} IBA.

Gmelina arborea

Thirunavoukkarasu and Debata (1998) successfully induced shoots of *Gmelina arborea* by culturing axillary buds on McCowns medium for woody plants supplemented with different concentrations of BA (0.25 - 0.5 mg l^{-1}). Clonal micro propagation of *Gmelina arborea* using axillary buds from 1 to 2 year old plants as explants was tried by Melendez and Contreras (2000). These buds were cultured on a half strength MS medium containing myoinositol (100 mg l^{-1}), thiamine (0.10 mg l^{-1}), nicotinic acid (0.5 mg l^{-1}), pyiridoxin (0.1 mg l^{-1}) and glycine (2.0 mg l^{-1}). Multiple shoots were obtained after 6 weeks of culture. Rooting was induced on medium containing NAA (0.1 mg l^{-1}).

Hevea brasiliensis

Multiple shoots from axillary buds of *Hevea brasiliensis* was obtained on MS medium supplemented with kinetin (1.0 mg l^{-1}), 2, 4-D (1.0 mg l^{-1}), sucrose (20.0 g l^{-1}) and Difco agar (4.0 g l^{-1}). Rooting was obtained on MS medium containing NAA (5.0 mg l^{-1}), IBA (3.0 mg l^{-1}), sucrose (50 g l^{-1}) and 4.0 g l^{-1} Difco agar (Mendanha *et al.*, 1998).

Pterocarpus santalinus

Efficient protocols were established for *in vitro* shoot multiplication of *Pterocarpus santalinus*. The highest shoot bud regeneration was achieved by culturing mesocotyl explants on B5 medium fortified with 3.0 mg l^{-1} BA and 1.0 mg l^{-1} NAA. Shoots treated with IAA, NAA and IBA (1.0 mg l^{-1} each) prior to transferring them to the rooting medium exhibited better rooting than those with no prior treatment (Anuradha and Pullaiah, 1999).

Pterocarpus marsupium

In vitro propagation of *Pterocarpus marsupium* was reported by Tiwari, *et al.* (2004). Nodal segments were used as explants. The maximum number of shoot induction was obtained on MS medium containing 3.0 mg l^{-1} BA and 0.5 mg l^{-1} NAA. Shoot length was maximum in MS medium supplemented with 0.2 mg l^{-1} IBA. Regenerated plants were acclimatized and successfully transferred under field conditions.

Santalum album

Direct organogenesis from shoot tips of sandal has been reported by Sanjay *et al.* (1998). Adventitious buds were initiated on MS medium containing various combinations of cytokinins. Radhakrishnan *et al.* (2001) reported micro propagation in sandal using nodal segments as explants. Callus initiation and organogenesis was highest on MS medium supplemented with BA at 4.0 mg l^{-1}. Rooting was observed in MS medium containing 3.0 mg l^{-1} IBA.

Tectona grandis

Tiwari *et al.* (2002) reported an improved protocol for micro propagation of teak. Maximum average number of shoots was obtained on MS medium supplemented with 22.2 μM BA and 0.57 μM IAA. *In vitro* raised shoots were dipped in IBA 99.8 μM for two minutes to obtain rooting. An efficient protocol for micro propagation of teak through tissue culture using seedling explants has been standardized by Sharma (2000).

Tamarindus indicus

Regeneration of plants *via.* adventitious bud formation from mature zygotic embryo axis of tamarind has been reported by Mehta *et al.* (2004). Explants consisting of longitudinal section of the embryo axis with attached cotyledon were cultured on MS medium with various combinations and concentrations of NAA, BA and sucrose. Induction of adventitious shoot buds was achieved on the cut surface of the axis when cultured in a medium containing NAA (2.69 μM), BA (44.39 μM) and 4 per cent sucrose. A medium consisting of zeatin (0.91 μM), BA (2.22 μM), calcium pantothenate (0.41 μM) and biotin (0.40 μM) supported differentiation of the buds to form elongated shoots. The shoots developed roots in a half strength MS medium with 2.0 per cent sucrose following a 72-hour treatment with auxin mixture in the dark. On transfer to soil 24 per cent plants survived.

Vateria indica

Organogenesis and embryogenesis in *Vateria indica* were attempted by Divatar (1994). Moderate callusing was obtained from leaf and internodal segments cultured on MS medium and half strength MS medium supplemented with growth regulators like 2iP, 2,4-D and IBA. However, further response from callus was not observed.

Materials and Methods

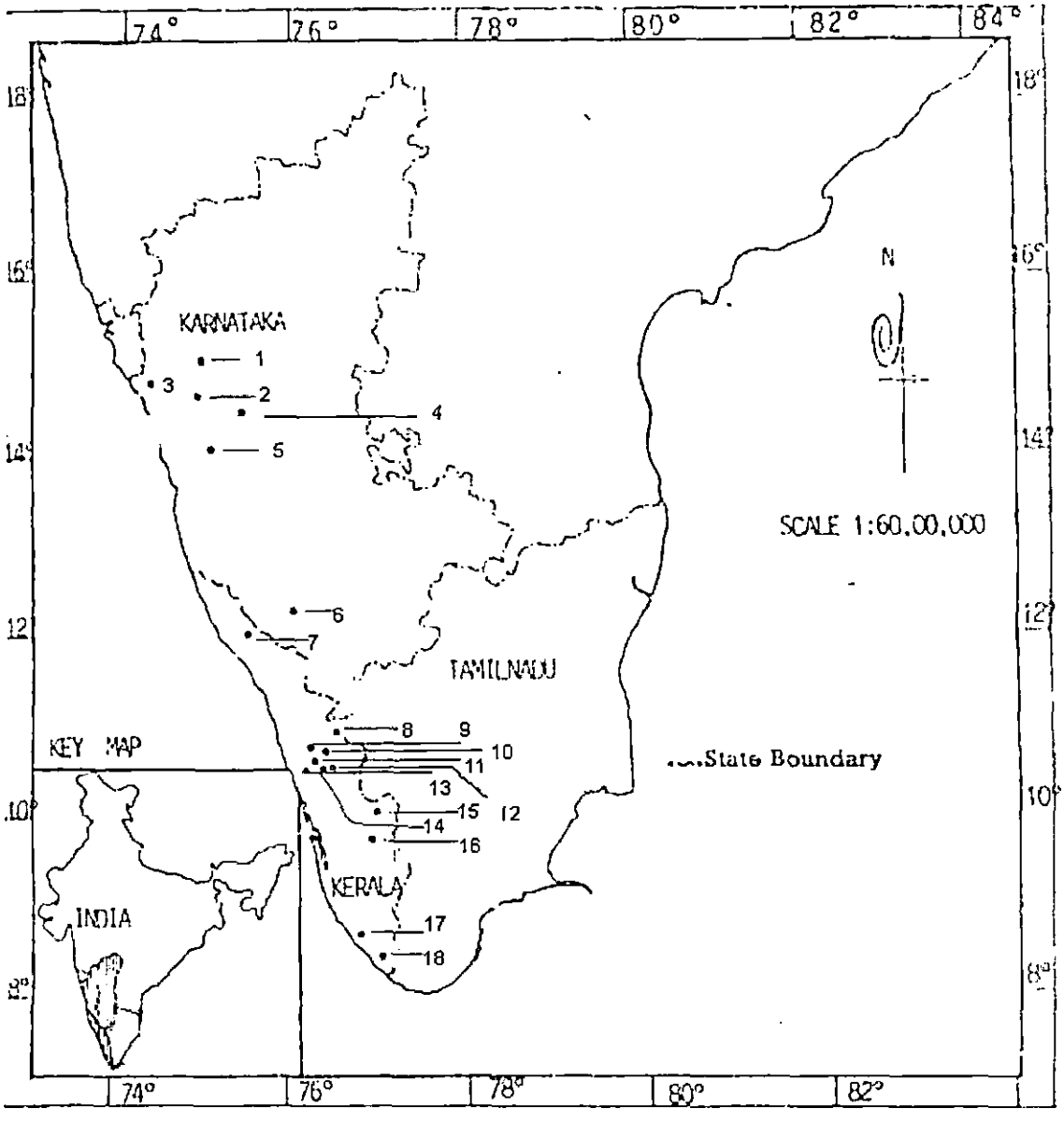
MATERIALS AND METHODS

The present investigation titled "Evaluation of seed source variation and standardization of clonal propagation in *Jatropha curcas* Linn." was undertaken during the year 2005-2007 in the nursery, the research field and tissue culture laboratory of the College of Forestry, Vellanikkara, Trichur District, Kerala, India which is situated at 10^0 32' N latitude and 76^0 10' E longitude at an altitude of 22.25 m above MSL. The location experiences a humid tropical climate with a mean annual rain fall of 2668.6 mm most of which is received between June to September. The minimum temperature varies from 22.2^{0}C (December) to 24.7^0 C (May) and maximum from 28.6^0 C (July) to 36.2^0 C (March). The details of the materials used and techniques / methodology employed in the different experiments during the course of investigation are described in this chapter.

3.1 Seed source variation studies

3.1.1 Materials

The experimental material for this study consisted of 18 seed sources of *Jatropha curcas* collected from different parts of Kerala and Karnataka (Fig.1).While field evaluation was carried out for all the 18 seed sources, seed parameters were studied for 17 seed sources. While collecting the material regulations for seed source sampling concerning minimum number of trees and distance between parent trees were followed (Lauridsen and Olesen, 1990). Seeds from individual trees were mixed and used as seed source in the present investigation.



1. Dharwad
2. Banavasi
3. Sirsi
4. Bhadravathi
5. Shimoga
6. Kushalnagar
7. Kasargod
8. Palakkad
9. Guruvayur
10. Madakathara
11. Athani
12. Chirakakod
13. Chavakkad
14. KAU
15. Marayur
16. Pala
17. Trivandrum
18. Neyantingara

Fig. 1 Map showing the locations of different seed sources

3.1.2 Methods

3.1.2.1 Survey and collection of seed material

The survey was done across various geographical localities of different agro climatic zones spread over different districts. During the survey, it was noticed that the fruiting season varies in different agro climatic zones. Based on seed availability and fruiting season at different time, seeds were collected from 17 different seed sources while seedlings were collected from one seed source to carry out the present investigation. The meteorological data of these locations are presented in Appendix I.

For the collection of seeds, five to ten plants approximately of same age and girth were identified in each seed source during January 2006 to May 2006. Trees located at minimum 100 m apart were selected to avoid narrowing down of the genetic variation within a seed source. Some times, trees on farm lands, roadsides and fences were used for collection of seeds. Mature fruits were picked directly from the plant and some times fruits fallen on the ground were also collected. The fruits were broke open and seeds were extracted and kept open for drying separately under sunlight. The seeds collected from each seed source were labeled and kept separately in cloth bags.

3.1.2.2 Recording observations on seed traits

Data on various quantitative traits of the seeds were recorded for all the 17 seed sources. The seed parameter studies were carried out with a random sample of 25 seeds from each source with five replications. The characters studied and techniques adopted to record the observations are given below.

Seed length

The length of seed from the base to tip was measured using vernier caliper and expressed in cm.

Seed breadth

The breadth at the middle of seed was measured using vernier caliper and expressed in cm.

Seed length to breadth ratio

It is the ratio between seed length and breadth.

Estimation of oil content

This was done at The Energy Resource Institute (TERI), New Delhi. Oil content was expressed as percentage of seed weight.

Hundred seed weight

Determination of hundred seed weight was done as per ISTA (1993). For this, 5x100 seeds were counted at random from the seed sample, weighed and recorded in gram (g).

3.1.2.3 Nursery experiment

Seedling evaluation of 18 seed sources was conducted during the year 2006-2007 in the nursery, College of Forestry, Vellanikkara. The weather parameters during the experimental period during March to July are presented in Appendix II.

3.1.2.3.1 Seed treatment

Seeds from each accession were bulked separately. Equal numbers of seeds were immersed in tap water for overnight prior to the sowing.

3.1.2.3.2 Sowing and lay out of the experiment

Seeds were sown in plastic trays containing river sand. The trays were arranged in CRD with three replications during the month of March. Known numbers of seeds from each seed source were sown randomly in the plastic trays. The seeds were dibbled at 1.0 cm depth with a spacing of 3x3 cm^2 in the sand. The trays were watered regularly and germination counts were recorded every day.

3.1.2.3.3 Recording seed germination attributes

Number of seeds germinated was monitored everyday till no further germination is noticed. Emergence of the shoot above the sand level as well as opening of the cotyledon was considered as germination. Based on the germination counts, the following parameters were recorded.

Germination per cent

$$\text{Germination per cent} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Mean daily germination

$$\text{Mean daily germination} = \frac{\text{Total germination}}{\text{Total number of days}}$$

Peak value of germination

$$\text{Peak value of germination} = \frac{\text{Final germination percent}}{\text{The number of days that took to reach peak germination}}$$

Germination value

Germination value was estimated according to the Method prescribed by Czabtor (1962).

$$\text{Germination Value (GV)} = \text{PV X MDG}$$

Where,

PV = Peak value of germination

MDG = Mean daily germination

3.1.2.3.4 Recording seedling attributes

After completion of germination, the seedlings were transplanted to polybags. Polybags measuring 7"x5" (250 gauge) were filled with potting mixture containing soil, sand and farm yard manure in the ratio of 1:2:1 and used for the purpose. Seedling characters were studied for 14 seed sources. The polybags containing seedlings were arranged in the nursery in CRD with three replications (Fig. 2). Each treatment comprised of 25 seedlings for the respective seed sources. Five seedlings from each replication were randomly selected for taking biometrical observations. The following observations were recorded at specific periodic intervals.

R1	R2	R3
T1	T6	T14
T2	T13	T13
T3	T2	T12
T4	T12	T11
T5	T10	T10
T6	T3	T9
T7	T8	T8
T8	T14	T7
T9	T7	T6
T10	T5	T5
T11	T11	T4
T12	T4	T3
T13	T1	T2
T14	T9	T1

Fig. 2 Nursery lay out of 14 seed sources

Shoot length:

The height of the shoot was measured from the base of the seedling to the growing tip using meter scale at an interval of fifteen days and expressed in cm.

No. of leaves per seedling:

The total number of leaves was counted on the selected plants from each replication and expressed as average number of leaves per seedling. The observation was taken at an interval of fifteen days.

Collar diameter:

The collar diameter was measured slightly above the soil surface at the bulged portion of the seedling at an interval of fifteen days using a digital caliper and expressed in millimeters.

3.1.2.4 Field experiment

A seed source evaluation trial has been laid out in Block 10 of the College of Forestry farm at Vellanikkara. Two month old seedlings of the 18 seed sources were planted in a randomized block design (RBD) with three replications. The spacing adapted was 2 x 2 m with 10 seedlings per treatment per replication. The layout of the field experiment is given in Fig. 3.

The survival percentage and establishment percentage were recorded in the first two months after planting. The other observations were recorded on five seedlings in each replication from three months after planting (MAP) at two month interval as described below:

12	17	1	9	2	16
4	7	15	10	13	14
3	8	18	11	5	6

Replication I

7	10	9	11	8	18
14	13	15	17	16	12
6	3	4	2	5	1

Replication II

13	14	15	16	17	18
7	8	9	10	11	12
1	2	3	4	5	6

Replication III

Fig. 3 Field lay out of 18 Seed sources

Plant height

The plant height was measured from the ground level to the tip of the stem using a meter scale and the mean was expressed in centimeters (cm).

Basal diameter

The basal diameter was measured at the base of the stem (near the ground at the collar position) using a digital caliper and the mean was expressed in centimeters (cm).

Number of branches

All the branches were counted and recorded in whole number. The data was expressed as the number of branches other than the main stem.

Number of leaves

The total number of leaves in the selected plants was counted from each replication and the average was reported as number of leaves per seedling.

3.1.2.5 Statistical analysis

Data recorded from the various experiments were tabulated and subjected to statistical analysis separately. Analysis of variance was carried by using MSTAT-C package. Mean values were compared with Duncan's Multiple Range Test (DMRT) values, which were calculated separately for each parameter.

3.2 Clonal propagation techniques in jatropha by rooting of cuttings

3.2.1 Materials

The stem cuttings used for this study were collected from a two year old plantation of *Jatropha curcas* maintained in the farm of the College of Forestry, Vellanikkara. The different types of cuttings used in this experiment are shown in Plate 1 and described below:

Sl. No	Type of cutting	Length of cutting (cm)
1.	Softwood cuttings Collected from the top succulent portions of the stem	10
		20
2.	Semi-hardwood cuttings Collected from the medium matured middle portion of the stem	10
		20

3.2.2 Methods

3.2.2.1 Growth regulator treatment

Three different auxins, namely, indole acetic acid (IAA), indole 3- butyric acid (IBA) and naphthalene acetic acid (NAA) were used for treating the cuttings. Each of these hormones was used in three concentrations, viz., 100 ppm, 250 ppm and 500 ppm. In addition to this, each type of cutting had a control treated with distilled water and an absolute control with no treatment at all. The different treatment combinations are presented in Table 1.

Semi-hardwood 20 cm cuttings

Softwood 20 cm cuttings

Semi-hardwood 10 cm cuttings

Softwood 10 cm cuttings

Plate 1 Different types of cuttings used for the rooting in *Jatropha curcas*

Table 1 Details of treatments for rooting in stem cuttings of *Jatropha curcas*

Treatment No.	Treatments
1	IAA-100-S0FT-10
2	IAA-100-S0FT-20
3	IAA-100-S.HARD-10
4	IAA-100- S.HARD -20
5	IAA-250-S0FT-10
6	IAA-250-S0FT-20
7	IAA-250- S.HARD -10
8	IAA-250- S.HARD -20
9	IAA-500-S0FT-10
10	10. IAA-500-S0FT-20
11	IAA-500-S.HARD-10
12	IAA-500-S.HARD-20
13	IBA-100-S0FT-10
14	IBA-100-S0FT-20
15	IBA-100-S.HARD-10
16	IBA-100- S.HARD -20
17	IBA-250-S0FT-10
18	IBA-250-S0FT-20
19	IBA-250- S.HARD -10
20	IBA-250- S.HARD -20
21	IBA-500-S0FT-10
22	IBA-500-S0FT-20
23	IBA-500-S.HARD-10
24	IBA-500-S.HARD-20
25	NAA-100-S0FT-10
26	NAA-100-S0FT-20
27	NAA-100-S.HARD-10
28	NAA-100-S.HARD-20
29	NAA-250-S0FT-10
30	NAA-250-S0FT-20
31	NAA-250-S.HARD-10
32	NAA-250-S.HARD-20
33	NAA-500-S0FT-10
34	NAA-500-S0FT-20
35	NAA-500-S.HARD-10
36	NAA-500-S.HARD-20
37	CONTROL WITH WATER–SOFT-10
38	CONTROL WITH WATER–SOFT-20
39	CONTROL WITH WATER - S.HARD-10
40	CONTROL WITH WATER - S.HARD-20
41	CONTROL without water - SOFT-10
42	CONTROL without water - SOFT -20
43	CONTROL without water - S.HARD-10
44	CONTROL without water - S.HARD -20

3.2.2.2 Lay out of the experiment

The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Treatments included two age, two length cuttings and five levels of hormonal treatment (including two controls). Thus, there were forty-four treatment combinations each having 20 cuttings. The nursery lay out of the experiment is presented in Appendix III.

3.2.2.3 Recording of observations

Days to sprout and sprouting percentage

Days taken for sprouting were noted separately for each treatment. The cuttings which produced visible sprouts were counted separately for each treatment and their percentage was worked out. Five successfully rooted cuttings were selected randomly from each treatment for taking the biometric observations. The following observations were recorded at monthly interval.

Number of shoots

The number of new shoots produced was recorded and expressed as average for each treatment.

Height

The total length of all shoots produced from the cuttings was measured from the base to the tip of shoot using a tailor's tape and expressed in centimeters.

Number of leaves

Number of leaves produced by individual rooted cuttings was recorded.

Collar diameter of the new shoots

The collar diameter of the shoots was measured at the base of the shoot with a vernier caliper and expressed in centimeters.

Recording root parameters

At the end of the study five seedlings were selected randomly from each treatment for taking observations on root growth. This was replicated three times. The study involved recording observations on following parameters

Main root length

The length of the longest root of each of the seedlings was measured and expressed in centimeters.

Total length of root

Total length of all primary and secondary roots measured and expressed in centimeters.

Number of primary roots

The number of primary roots produced per seedling was counted and expressed.

Number of secondary roots

The number of secondary roots produced per seedling was counted and expressed.

Fresh weight of roots

Roots were removed carefully from the soil, washed thoroughly, weighed in an electric balance and weights were expressed in grams.

Dry weight of roots

The dry weight was found out after oven-drying the roots at 60 to 80^0 C and expressed in gm.

Collar diameter

The diameter of the root collar measured using vernier caliper and expressed in mm.

3.2.2.4 Statistical analysis

All the biometrical observations were analyzed statistically using the analysis of variance technique applied to CRD with the help of MSTAT-C package.

3.3 Clonal propagation in jatropha through micro propagation

Work on micro propagation of *Jatropha curcas* was initiated by employing bud culture technique using nodal segments as explants. Existing facilities in the tissue culture laboratory of Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara was used for this study. Various materials used and methods employed in the study are explained below.

3.3.1 Materials

3.3.1.1 Culture media

For culturing the explants MS medium (Murashige and Skoog, 1962), Woody Plant Medium (WPM) (Lloyd and McCown, 1980) and B5 medium (Gamboorg, *et al.*, 1976) were used in the present study. The composition of the different media

used is presented in Appendix IV. The media was fortified with different concentrations of growth hormones including two cytokinins (kinetin and BA) and an auxin (IBA) either singly or in combination. The chemicals used for preparing media were of analytical grade from Sisco Research Lab, Merck or Sigma.

3.3.1.2 Explants

The explants used in the present experiment are nodal segments collected from mature plants of *Jatropha curcas.*

3.3.2 Methods

3.3.2.1 Preparation of stock solution

For the easiness in media preparation, stock solutions were made. Each stock was prepared separately. For this required quantities of the chemicals were weighed accurately. About 20 ml of distilled water was taken in a beaker and the chemicals were added one after the other and dissolved by constant stirring. Care was taken while the preparation of iron stock since it precipitates readily. To avoid this Na_2EDTA and $FeSO_4.7H_2O$ were dissolved in separate beakers with approximately 200 ml distilled water each. Both beakers were placed on hot plates and brought to the point of almost boiling. Then $FeSO_4.7H_2O$ solution was added slowly to Na_2EDTA over a 15 minute period with constant stirring. Then the volume was made up to one liter in a volumetric flask by adding distilled water. The mixture was allowed to cool in room temperature. The stock solutions were labeled indicating the stock number and date of preparation. They are stored in amber coloured bottles under refrigerated condition.

3.3.2.2 Preparation of the media

The quantity of stock solutions required to prepare one liter media were pipetted out into a 1000 ml beaker. Then inositol (100 mg) and sucrose (30 g) was added. Sufficient volume of water was added to dissolve the mixture. The volume was made up to one liter and the pH was adjusted to 6.5 using pH paper. To this medium 8 g of agar was added and dissolved by boiling. About 15 ml of media was then distributed into cleaned culture tubes of 150 x 25mm size. The culture tubes were then plugged with cotton.

3.3.2.3 Sterilization of culture medium

The media was sterilized using a pressure cooker at pressure of 1.06 kg cm^{-2} for 20 minutes at 121 ° C. The sterilized media was stored in culture room.

3.3.2.4 Sterilization of equipments

All metal and glass instruments and other accessories were wrapped in paper and sterilized in an autoclave at 1.06 kg cm^{-2} pressure for 20 minutes at 121^0C temperature. They were then stored in hot air oven. Forceps, scissors etc. were again dipped in alcohol and flamed at the time of use.

3.3.2.5 Collection and preparation of explants

Stem segments of approximately 30 to 40 cm were cut from mature plants of *Jatropha curcas* using a sharp secature and brought to the laboratory as soon as possible to avoid desiccation. The leaves were removed close to the stem leaving small part of the petiole. Stem segments were first washed in tap water and then they

were made into single node cuttings which were dipped in a mixture of the systemic fungicide, Bavistin 0.2 per cent and the contact fungicide Indofil M- 45 0.2 per cent each for about 30 minutes. Then they were taken out, washed under running water and then washed with soap solution. The soap is removed by washing again in tap water.

3.3.2.6 Surface sterilization of explants

Surface sterilization is done under perfect aseptic condition in a laminar air flow chamber. It was done using 0.1 per cent mercuric chloride for varying durations. For this the explants were immersed in the sterilant for stipulated period with occasional stirrings by swirling movements. After that, they were washed three times with sterilized distilled water so as to remove the traces of sterilant.

3.3.2.7 Inoculation of explants

Culturing is done under perfect aseptic condition in a laminar air flow cabinet. For this cotton plug of the test tubes containing the media was opened near a flame and one nodal segment each was transferred to the medium using a forceps. The cotton plug was immediately replaced. Then cultures were properly labeled and were kept in the culture room maintained at 25±2^{0}C and with a light intensity of 2000 lux for 16 hours light period.

3.3.2.8 Induction of bud break and shoot development

For induction of bud break and shoot development various treatment combinations of growth regulators were tried as given in Table 2. For each treatment a minimum of fifteen tubes were used and was replicated three times. Observations

Table 2 Growth regulator combinations used for induction of bud break and shoot development

Media	Cytokinins		Auxin –IBA
	BA(mgl^{-1})	Kinetin (mgl^{-1})	
MS	0.5		
MS	1.0		
MS	2.0		
MS	3.0		
MS		0.5	
MS		1.0	
MS		2.0	
MS		3.0	
MS			0.5
MS			1.0
MS			2.0
MS	0.5	0.5	
MS		1.0	
MS		2.0	
MS		3.0	
MS	1.0	0.5	
MS		1.0	
MS		2.0	
MS		3.0	
MS	2.0	0.5	
MS		1.0	
MS		2.0	
MS		3.0	
MS	3.0	0.5	
MS		1.0	
MS		2.0	
MS		3.0	
WPM	0.5		
WPM	1.0		
WPM	2.0		
WPM	3.0		
WPM		0.5	
WPM		1.0	
WPM		2.0	
WPM		3.0	

were taken for a period of four weeks with an interval of seven days. The data collected are presented on the basis of cultures that remained uncontaminated. The observations which are recorded in each treatment are the following:

Number of cultures contaminated

Number of cultures showing contamination were counted and expressed as percentage of total number of cultures.

Number of cultures showing bud break

Number of cultures showing bud initiations were expressed as percentages of total number of surviving cultures.

Time taken for bud initiation

Time taken for bud initiation was recorded and expressed in days.

Average number of leaves

Average number of leaves was worked out as mean of the total number of leaves from the number of cultures showing leaf production.

Maximum number of leaves

Maximum number of leaves was expressed as maximum number of leaves produced per explant in a particular treatment.

Average number of shoots per culture

The average number of shoots per culture was expressed as mean of the total number of shoots produced in different cultures of a particular treatment.

Average length of shoots per culture

The average length of shoots per culture was expressed as a mean of total length of shoots from the number of cultures showing shoot development.

Maximum shoot length

Maximum shoot length was expressed as the maximum length of shoots produced per explant in a particular treatment.

Number of cultures rooted *in vitro*

Number of culture rooted *in vitro* was counted and expressed as percentage total cultures in a particular treatment.

Number of cultures showing callus production

Number of cultures showing callusing were counted and expressed as percentage of total cultures.

Number of cultures showing browning

Number of cultures showing browning were counted and expressed as percentage of total number of cultures.

3.3.2.9 Statistical analysis

The data generated were statistically analyzed using the statistical package MSTAT-C.

Results

RESULTS

The present study on the evaluation of seed source variation and standardization of clonal propagation techniques in *Jatropha curcas* was carried out during 2005-07 in the College of Forestry. Results of the different experiments under this study are presented below.

4.1 Evaluation of seed source variation in *Jatropha curcas*

Evaluation of *Jatropha curcas* collected from a total of 18 seed sources from two states of the peninsular India, viz., Kerala and Karnataka was carried out. While field evaluation was carried out for all the 18 seed sources, seed parameters and seedling characters were studied for 17 and 14 seed sources, respectively. The results obtained from the study are presented here under.

4.1.1 Seed source variation in seed parameters

A general view of the variation in seed and kernel of some of the seed sources are given in Plate 2 and 3 and the data pertaining to the various seed parameters recorded in seeds collected from different seed sources are furnished in Tables 3 and 4.

4.1.1.1 Seed length

A statistically significant variation was found between the seed sources with respect the seed length (Table 3). The maximum value for seed length was recorded by Kasargod seed source (1.90 cm). However, the length of seeds collected from Palakkad (1.85 cm), Kasargod (1.90 cm), Guruvayur (1.85 cm), Athani (1.85),

Table 3 Seed parameters and germination characteristics of seeds from different seed sources of *Jatropha curcas.*

Sl. No	Seed source	Seed length (cm)	Seed width (cm)	Length breadth ratio (%)	100 seed weight (g)	Germination percentage (%)	MDG	PVG	GV
1.	Athani	1.85^{ABC}	1.12^{A}	60.54 ^{BC}	63.44^{F}	25.33^{F}	1.41^{E}	5.07^{G}	7.15^{G}
2.	Banavasi	1.73^{FG}	1.11^{A}	64.16 ^{B}	71.39^{CDE}	32.00^{E}	0.97^{G}	6.40^{E}	6.21^{G}
3.	Bhadravathi	1.71^{G}	1.10^{A}	64.31 ^{B}	63.78^{F}	21.66^{G}	0.72^{H}	4.33^{H}	3.12^{H}
4.	Chavakkad	1.60^{H}	1.12^{A}	70.07^{A}	65.70^{F}	31.48^{E}	1.01^{G}	6.30^{E}	6.36^{G}
5.	Chirakakod	1.89^{A}	1.02^{B}	54.15 ^{D}	54.44^{G}	6.07^{J}	0.48^{K}	0.76^{N}	0.14^{K}
6.	Dharwad	1.65^{H}	1.02^{B}	61.85 ^{BC}	63.65^{F}	17.77^{H}	0.99^{G}	2.22^{K}	2.20^{HI}
7.	Guruvayur	1.85^{ABC}	1.14^{A}	61.62 ^{BC}	64.83^{F}	16.88^{H}	0.54^{I}4	3.38^{I}	1.83^{I}
8.	Kasargod	1.90^{A}	1.14^{A}	60.03 ^{BC}	71.57^{CDE}	47.35^{C}	1.43^{E}	9.47^{C}	13.54^{D}
9.	KAU	1.72^{G}	1.23^{A}	65.33 ^{B}	72.52^{BCD}	48.15^{C}	3.44^{B}	9.63^{C}	33.13^{B}
10.	Kushalnagar	1.86^{AB}	1.17^{A}	62.91 ^{BC}	46.64^{H}	32.56^{E}	1.92^{D}	5.43^{F}	10.43^{E}
11.	Madakathara	1.75^{EFG}	1.01^{B}	57.72^{CD}	54.31^{G}	13.57^{I}	0. 4^{J}	2.71^{J}	1.19^{IJ}
12.	Neyantingara	1.75^{EFG}	1.13^{A}	64.77^{B}	74.53^{AB}	92.61^{A}	4.87^{A}	15.44^{A}	75.19^{A}
13.	Pala	1.75^{EFG}	1.13^{A}	64.77^{B}	69.02^{E}	35.8^{AD}	1.19^{F}	7.16^{D}	8.52^{F}
14.	Palakkad	1.85^{ABC}	1.13^{A}	61.12^{BC}	73.65^{ABC}	71.90^{B}	2.18^{C}	14.38^{C}	31.35^{C}
15.	Shimoga	1.80^{CDE}	1.12^{A}	62.47^{BC}	70.41^{DE}	18.21^{H}	0.59^{I}	3.64^{I}	2.15^{HI}
16.	Sirsi	1.78^{DEF}	1.15^{A}	64.61^{B}	71^{CDE}	13.70^{I}	0.44^{J}	1.71^{L}	0.75^{JK}
17.	Trivandrum	1.81^{BCD}	1.16^{A}	64.10^{B}	75.72^{A}	6.66^{J}	0.37^{J}	1.33^{J}	0.49^{JK}
SEm +/-		0.02	0.02	1.64	0.85	0.69	0.33	0.12	0.33

MDG= Mean daily germination; PVG= Peak value of germination; GV=Germination value
Figures with the same alphabet do not differ significantly

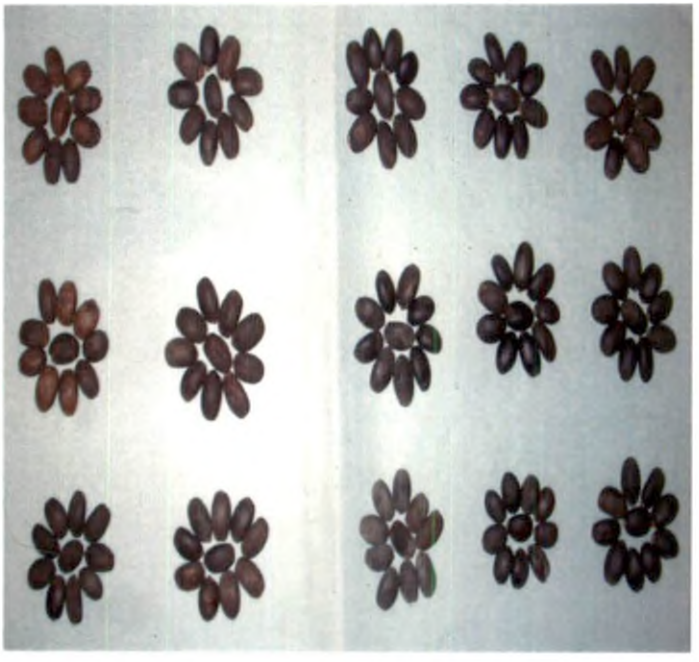


Plate 2 Seeds of *Jatropha curcas* from some seed sources

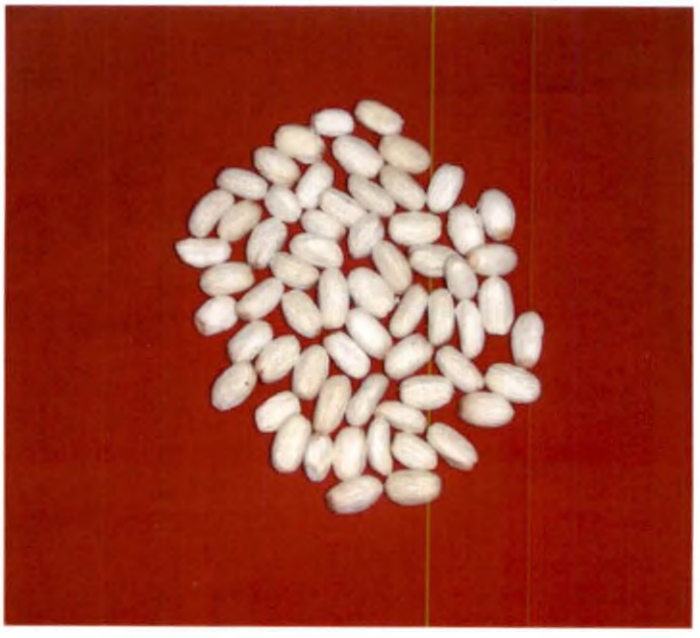


Plate 3 Kernel of *Jatropha curcas* seeds

Chirakakod (1.89 cm) and Kushalnagar (1.86 cm) were found on par with each other. Seed length was least in Dharwad (1.65 cm) and Chavakkad seed sources (1.60 cm), which were found on par with each other.

4.1.1.2 Seed width

Seed collected from different seed sources exhibited significant differences in their seed width as presented in Table 3. However, 14 seed sources were found on par with each other for this parameter, which in turn were superior to Dharwad (1.02 cm), Chirakakod (1.02 cm) and Madakathara (1.01cm) seed sources. Seed width was highest (1.23 cm) in KAU seed source and was least (1.01 cm) in Madakathara seed source.

4.1.1.3 Seed length breadth ratio

Significant difference was observed for this character across different seed sources. Seed length breadth ratio ranged between 70.07 and 54.15 per cent and the highest was for Chavakkad (70.07 %) seed source and the least (54.15 %) was for Chirakakod. However, all the other seed sources were found on par with each other.

4.1.1.4 Hundred seed weight

Hundred seed weight of the different seed sources showed statistically significant variation ranging from 46.44 g to 75.72 g (Table 3). The maximum value (75.72 g) for hundred seed weight was recorded by Trivandrum seed source followed by Neyantingara (74.53 g) and Palakkad (73.65 g) seed sources, which were found on par with each other. The least value (46.44 g) was recorded by Kushalnagar seed source.

4.1.1.5 Germination percentage

The germination percentage showed a significant difference among the seeds collected from different seed sources (Table 3). The germination percentage varied from 6.07 per cent to 92.61 per cent. Maximum germination percentage (92.61 %) was observed in Neyantingara seed source, which was followed by Palakkad, KAU and Kasargod seed sources. Germination percentage was least in Trivandrum (6.66 %) and Chirakakod (6.07 %) seed sources.

4.1.1.6 Mean daily germination

The value of the mean daily germination (MDG) varied significantly across different seed sources as presented in Table 3. Wide variations were observed in the MDG between the seed sources ranging from 0.19 to 4.87. Significantly higher (4.87) MDG was recorded in Neyantingara seed source followed by KAU (3.44) and Palakkad (2.18) while, the minimum (0.18) MDG was recorded for Chirakakod seed source. However, three other seed sources, namely, Athani, Kasargod and Kushalnagar recorded higher values of MDG over the general mean.

4.1.1.7 Peak value of germination

The data pertaining to the peak value of germination (PVG) is furnished in Table 3. A significant difference was observed in the peak value of germination among the different seed sources. The maximum value (15.44) was recorded for Neyantingara seed source and the minimum (0.76) for Chirakakod seed source. However, six seed sources, viz., Chavakkad (6.30), Banavasi (6.40), Pala (7.16), Kasargod (9.47), KAU (9.63) and Palakkad (14.38) in addition to Neyantingara, recorded higher values of PVG over the general mean.

4.1.1.8 Germination value

Variation in the germination value (GV) was significant between the different seed sources (Table 3). The germination value varied as high as 75.23 to as low as 0.15 in different seed sources. Neyantingara seed source exhibited the highest value (75.23) followed by Palakkad (31.35), Kasargod (13.54) and KAU (33.13). The least (0.15) was recorded for Chirakakod seed source.

4.1.1.9 Kernel to seed weight ratio

Kernel to seed weight ratio was maximum in Palakkad seed source (67.46 %) and minimum (50.90 %) in Kushalnagar seed source (Table 4). Eight seed sources, namely, Sirsi, Shimoga, Bhadravathi, Dharwad, Chavakkad, KAU, Chirakakod, Trivandrum in addition to Palakkad seed source, recorded higher values of kernel to seed weight ratio over general mean.

4.1.1.10 Seed oil content percentage

Seed oil content was estimated for fourteen seed sources. The oil content varied considerably among different seed sources, ranging from 24.34 per cent to 41.00 per cent. The highest oil content was recorded in Dharwad seed source (41.00 %) followed by Shimoga (39.45 %) and Kasargod (39.28 %) seed sources. Apart from these, five other seed sources, namely, Guruvayur, Chavakkad, KAU, Neyantingara and Trivandram recorded higher values for seed oil content over general mean (Table 4).

Table 4 Kernel to seed weight ratio and seed oil content (%) in different seed sources of *Jatropha curcas.*

Sl. No.	Seed source	Kernel to seed weight ratio (%)	Seed oil content (%)
1.	Athani	61.65	NA*
2.	Banavasi	61.31	NA*
3.	Bhadravathi	65.90	NA*
4.	Chavakkad	62.90	35.88
5.	Chirakakod	65.42	30.99
6.	Dharwad	66.00	41.00
7.	Guruvayur	58.80	34.42
8.	Kasargod	61.89	39.28
9.	KAU	62.50	33.82
10.	Kushalnagar	50.90	32.29
11.	Madakathara	57.14	30.28
12.	Neyantingara	61.00	34.75
13.	Pala	59.29	24.34
14.	Palakkad	67.46	26.64
15.	Shimoga	64.80	39.45
16.	Sirsi	64.64	32.26
17.	Trivandrum	64.33	33.98
Mean		62.11	33.53

Note: * - Seed sources not analyzed for their seed oil content due to the limited quantities of seeds and fungal contamination of seeds

4.1.2 Seed source variation in seedling characters in nursery

Biometric observations like seedling height, number of leaves and collar diameter of *Jatropha curcas* seedlings of 14 seed sources recorded at 15, 30, 45 and 60 days after sowing (DAS) are presented in Table 5 to 7.

4.1.2.1 Seedling height

The data pertaining to seedling height recorded at different growth stages are presented in Table 5. The variations were not significant in all the growth phases. At 15 DAS, the maximum (0.24 m) height was shared by Kasargod and Shimoga seed sources while, the minimum (0.19 m) was recorded for Kushalnagar and Chirakakod seed sources. The same trend of height increment was observed at 30 and 45 DAS. At 60 DAS, the maximum height (0.29 m) was observed in Palakkad and Shimoga seed source while the lowest height (0.24 m) was registered for Kushalnagar seed source. For the entire growth period of 60 days in nursery, the minimum growth was observed in Kushalnagar seed source.

4.1.2.2 Number of leaves

Data on number of leaves per seedling at different growth phases (15, 30, 45 and 60 DAS) are presented in Table 6. There were no significant differences among the seed sources with respect to this character throughout the observation period in the nursery. At 15 DAS, the maximum leaf number (6.0) was observed in Guruvayur seed source and the minimum (4.86) was in Dharwad seed source. At 60 DAS, relatively higher (7.0) values for leaf number were recorded for Sirsi and Chirakakod while, the lowest (6.0) for Kushalnagar, Athani, Dharwad and Bhadravathi seed source.

Table 5 Seedling height (m) of different seed sources of *Jatropha curcas*

Sl. No.	Seed source	DAS 15	DAS 30	DAS 45	DAS 60
1.	Athani	0.23	0.25	0.27	0.28
2.	Banavasi	0.21	0.23	0.25	0.27
3.	Bhadravathi	0.21	0.23	0.26	0.28
4.	Chavakkad	0.23	0.25	0.26	0.28
5.	Chirakakod	0.19	0.22	0.23	0.25
6.	Dharwad	0.21	0.24	0.26	0.28
7.	Guruvayur	0.22	0.24	0.26	0.28
8.	Kasargod	0.24	0.25	0.26	0.28
9.	KAU	0.22	0.23	0.25	0.27
10.	Kushalnagar	0.19	0.20	0.22	0.24
11.	Madakathara	0.22	0.24	0.26	0.28
12.	Palakkad	0.23	0.25	0.27	0.29
13.	Shimoga	0.24	0.26	0.28	0.29
14.	Sirsi	0.20	0.22	0.23	0.24
SEm (+/-)		0.02	0.02	0.02	0.02

DAS: Days after sowing

Table 6 Number of leaves in seedlings of different seed sources of *Jatropha curcas* seedlings

Sl. No.	Seed source	DAS 15	DAS 30	DAS 45	DAS 60
1.	Athani	5.46	5.67	5.40	6.00
2.	Banavasi	5.46	5.73	5.67	6.33
3.	Bhadravathi	5.34	5.67	5.33	6.00
4.	Chavakkad	5.33	6.00	5.27	6.33
5.	Chirakakod	5.27	5.53	5.80	7.00
6.	Dharwad	4.86	5.93	4.67	6.00
7.	Guruvayur	6.00	5.47	5.67	6.33
8.	Kasargod	5.00	5.27	5.00	6.33
9.	KAU	5.27	5.47	5.47	6.67
10.	Kushalnagar	5.00	5.53	5.20	6.00
11.	Madakathara	5.53	6.27	5.73	6.33
12.	Palakkad	5.33	5.29	4.80	6.33
13.	Shimoga	5.93	5.80	5.27	6.67
14.	Sirsi	5.00	5.80	5.60	7.00
SEm (+/-)		0.31	0.17	0.33	0.48

DAS: Days after sowing

4.1.2.3 Collar diameter

Collar diameter of seedlings recorded at different growth intervals (15, 30, 45 and 60 DAS) did not differ significantly except at 45 DAS (Table 7). At this stage the highest value (12.83 mm) of collar diameter was observed in Athani seed source followed by Chirakakod, Shimoga, KAU and Guruvayur with the collar diameter12.61 mm, 12.55 mm, 12.53 mm and 12.50 mm, respectively, which were on par with each other. Least value (8.49mm) for collar diameter was attained by Sirsi seed source. At 60 DAS also the trend remained more or less the same though the values were not significantly different.

4.1.3 Seed source variation in field performance

On the onset of monsoon, 60 days old seedlings of eighteen seed sources were planted in the field at an espacement of 2m x 2m.

Survival and establishment of the seedlings in various seed sources was recorded for the first three months after planting. Seedlings of all the seed sources exhibited 100 per cent survival and establishment. No seedling mortality was noticed in any of the seed sources.

Various growth parameters at 90, 150, 210, 270 and 330 days after planting (DAP) were recorded (Plate 4 and 5). The data are presented in Table 8 to 11.

Table 7 Collar diameter (mm) in seedlings of different seed sources of *Jatropha curcas*

Sl. No.	Seed source	DAS 15	DAS 30	DAS 45	DAS 60
1.	Athani	9.45	10.96	12.83^{A}	12.96
2.	Banavasi	9.08	11.01	12.37^{AB}	12.52
3.	Bhadravathi	8.02	9.96	11.16^{AB}	11.4
4.	Chavakkad	8.97	11.13	12.39^{AB}	12.58
5.	Chirakakod	8.16	10.28	12.61^{A}	12.77
6.	Dharwad	8.16	10.00	11.93^{AB}	12.14
7.	Guruvayur	9.09	10.87	12.50^{A}	12.62
8.	Kasargod	9.52	10.84	11.84^{AB}	12.06
9..	KAU	9.63	11.01	12.53^{A}	12.65
10.	Kushalnagar	7.62	9.07	10.07^{BC}	10.14
11.	Madakathara	8.12	10.38	11.69^{AB}	11.79
12.	Palakkad	9.09	10.63	12.06^{AB}	12.27
13.	Shimoga	9.39	10.85	12.55^{A}	12.79
14.	Sirsi	8.10	10.10	8.49^{C}	11.49
SEm (+/-)		0.62	0.53	0.71	0.58

DAS: Days after sowing
Figures with the same alphabets do not differ significantly

Plate 4 Field Photo of seed source evaluation (180 DAP)

Plate 5 Field Photo of seed source evaluation (300 DAP)

4.1.3.1 Plant height

Highly significant variations were observed at all the growth intervals (90, 150, 210, 270 and 330 DAP) between the seed sources with respect to plant height (Table 8).

At 90 DAP, the maximum height was observed for KAU (0.90 m) followed by 0.79 m for Kasargod, which were found on par with each other and the minimum for Kushalnagar (0.36 mm). Kasargod later replaced the maximum height growth from 150 days (1.03 m) to 330 days (1.37m) after planting. For the entire growth period of 330 days in the field, the minimum growth rate was observed in Kushalnagar seed source. The second maximum height growth was observed for the KAU seed source from 150 to 330 DAPs. It was seen that Sirsi, Shimoga, Banavasi, Bhadravathi, Madakathara, Marayur, Athani and Neyantingara seed sources are almost on par with each other throughout the growth stages with respect to the seedling height.

4.1.3.2 Number of branches

Data on number of branches per plant recorded at different growth intervals are presented in Table 9. No significant difference in number of branches was observed for the first two growth phase. But, after 210 DAP, statistically significant difference was observed between seed sources. At 210 DAP the maximum (1.17) number of branches was observed in Palakkad seed source followed by Kasargod, KAU and Chavakkad seed sources, which were on par with each other, while, the minimum was recorded for Kushalnagar seed source. At 270 DAP also the same trend of growth was exhibited by all seed sources with Palakkad (3.53) on the top position and Kushalnagar (0.29) seed source at the bottom. It was seen that Palakkad,

Table 8 Plant height (m) of different seed sources of *Jatropha curcas*

Sl. No.	Seed source	90 DAP	150 DAP	210 DAP	270 DAP	330 DAP
1.	Athani	0.58^{BCD}	0.69^{CDE}	0.72^{BCDE}	0.75^{CDE}	0.83^{EF}
2.	Banavasi	0.52^{CD}	0.76^{BCD}	0.78^{BC}	0.80^{BCD}	0.90^{DE}
3.	Bhadravathi	0.53^{CD}	0.64^{CDEF}	0.69^{BCDE}	0.76^{BCD}	0.86^{EF}
4.	Chavakkad	0.67^{BC}	0.83^{ABC}	0.91^{AB}	1.01^{AB}	1.14^{BC}
5.	Chirakakod	0.56^{BCD}	0.79^{ABC}	0.83^{BC}	0.89^{BC}	1.06^{C}
6.	Dharwad	0.60^{BC}	0.70^{CDE}	0.77^{BCD}	0.84^{BC}	1.04^{CD}
7.	Guruvayur	0.63^{BC}	0.82^{ABC}	0.85^{BC}	0.91^{BC}	1.02^{CD}
8.	Kasargod	0.79^{AB}	1.03^{A}	1.08^{A}	1.16^{A}	1.37^{A}
9.	KAU	0.90^{A}	0.99^{AB}	1.08^{A}	1.15^{A}	1.22^{B}
10.	Kushalnagar	0.36^{D}	0.42^{F}	0.49^{E}	0.51^{E}	0.56^{H}
11.	Madakathara	0.59^{BCD}	0.65^{CDEF}	0.68^{BCDE}	0.75^{CDE}	0.83^{EF}
12.	Marayur	0.57^{BCD}	0.65^{CDEF}	0.69^{BCDE}	0.74^{CDE}	0.83^{EF}
13.	Neyantingara	0.65^{BC}	0.69^{CDE}	0.73^{BCD}	0.76^{BCD}	0.82^{EF}
14.	Pala	0.45^{CD}	0.49^{EF}	0.53^{DE}	0.58^{DE}	0.63^{GH}
15.	Palakkad	0.56^{BCD}	0.75^{BCDE}	0.84^{BC}	0.91^{BC}	1.05^{CD}
16.	Shimoga	0.60^{BC}	0.63^{CDEF}	0.69^{BCDE}	0.78^{BCD}	0.85^{EF}
17.	Sirsi	0.51^{CD}	0.62^{CDEF}	0.65^{CDE}	0.68^{CDE}	0.75^{EFG}
18.	Trivandrum	0.52^{CD}	0.52^{DEF}	0.53^{DE}	0.66^{CDE}	0.73^{FG}
	SEm(+/-)	0.07	0.08	0.07	0.08	0.05

DAP: Days after planting

Figures with the same alphabets do not differ significantly

Table 9 Number of branches per plant of different seed sources *Jatropha curcas*

Sl. No.	Seed source	90 DAP	150 DAP	210 DAP	270 DAP	330 DAP
1.	Athani	0.07	0.33	0.23^{DE}	0.67^{DE}	2.68^{H}
2.	Banavasi	0.13	0.60	0.54^{ABCDE}	1.73^{ABCDE}	4.27^{E}
3.	Bhadravathi	0.00	0.07	0.33^{DE}	0.93^{DE}	3.27^{G}
4.	Chavakkad	0.00	0.20	0.97^{ABC}	2.93^{ABC}	5.47^{B}
5.	Chirakakod	0.12	0.28	0.60^{ABCDE}	1.82^{ABCDE}	4.33^{DE}
6.	Dharwad	0.60	0.60	0.80^{ABCD}	2.33^{ABCD}	4.80^{C}
7.	Guruvayur	0.27	0.27	57^{ABCDE}	1.73^{ABCDE}	4.14^{E}
8.	Kasargod	0.07	0.20	1.10^{AB}	3.33^{AB}	6.35^{A}
9.	KAU	0.13	0.33	1.10^{AB}	3.33^{AB}	$4.4C^{DE}$
10.	Kushalnagar	0.00	0.00	0.10^{E}	0.29^{E}	2.33^{H}
11.	Madakathara	0.00	0.33	0.43^{CDE}	1.33^{CDE}	3.62^{FG}
12.	Marayur	0.00	0.27	0.77^{ABCD}	2.20^{ABCD}	4.77^{C}
13.	Neyantingara	0.30	0.62	0.53^{BCDE}	1.58^{ABCDE}	4.02^{EF}
14.	Pala	0.00	0.20	0.33^{DE}	1.00^{DE}	3.39^{G}
15.	Palakkad	0.07	0.22	1.17^{A}	3.53^{A}	6.43^{A}
16.	Shimoga	0.73	0.73	0.43^{CDE}	1.33^{CDE}	3.68^{FG}
17.	Sirsi	0.00	0.29	0.70^{ABCDE}	2.07^{ABCDE}	4.70^{CD}
18.	Trivandrum	0.08	0.53	0.62^{ABCDE}	1.87^{ABCDE}	4.25^{E}
	SEm+/-	0.16	0.23	0.19	0.56	0.14

DAP: Days after planting
Figures with the same alphabets do not differ significantly

Kasargod, Guruvayur, Sirsi, Banavasi, Dharwad, Chavakkad, Marayur, KAU, Chirakakod and Trivandrum seed sources were on par with each other at this stage of growth. At 330 DAP, highly significant variation was observed between seed sources in respect of number of branches produced. The maximum number (6.43) of branches was observed in Palakkad seed source followed by 6.35 in Kasargod and 5.47 in Chavakkad seed source, while the least (2.33) number of branch was recorded for Kushalnagar seed source.

4.1.3.3 Number of leaves

The number of leaves per plant showed significant difference among seed sources studied at different growth intervals (Table 10). At 90 DAP, significantly highest (28.00) number of leaves per plant was recorded in KAU seed source followed by 26.53 in Kasargod seed source and 25.27 in Guruvayur seed source, which were found on par with each other. The lowest number of leaves (11.30) per plant was recorded for Kushalnagar seed source.

At 150 DAP, the maximum (23.33) number leaves per plant was recorded for Kasargod and the minimum (6.64) for Kushalnagar. However, at 210 DAP KAU seed source exhibited the maximum (13.17) value, while the minimum (1.80) was by Kushalnagar seed source. Even at 270 DAP, the same trend of growth was continued in almost all the seed sources. At 330 DAP also a highly significant difference among different seed sources were observed in respect of number of leaves. KAU seed source exhibited maximum (81.00) value followed by 71.22 for Kasargod and 68.22 for Chavakkad seed source, while the minimum (10.60) was recorded for Kushalnagar seed source. The KAU seed source was consistently showing significantly higher leaf production through out its growth phases. For the entire

Table 10 Number of leaves per plant of different seed sources of *Jatropha curcas*

Sl. No.	Seed source	90 DAP	150 DAP	210 DAP	270 DAP	330 DAP
1.	Athani	20.00^{ABCDE}	9.20^{D}	4.57^{CD}	18.33^{CD}	24.70^{J}
2.	Banavasi	19.27^{ABCDE}	21.20^{AB}	6.73^{ABCD}	26.73^{BCD}	38.73^{H}
3.	Bhadravathi	16.60^{CDE}	12.87^{ABCD}	7.27^{ABCD}	29.07^{ABCD}	43.04^{FG}
4.	Chavakkad	21.27^{ABCD}	13.80^{ABCD}	11.00^{ABC}	43.87^{ABC}	68.22^{C}
5.	Chirakakod	21.83^{ABCD}	20.08^{AB}C	8.60^{ABC}	34.67^{ABC}	52.15^{E}
6.	Dharwad	24.93^{ABC}	15.47^{ABCD}	11.33^{AB}	45.60^{AB}	67.27^{C}
7.	Guruvayur	25.27^{ABC}	15.40^{ABCD}	6.93^{ABCD}	27.73^{ABCD}	41.00^{G}
8.	Kasargod	26.53^{AB}	23.33^{A}	11.27^{AB}	45.13^{AB}	71.22^{B}
9.	KAU	28.00^{A}	21.13^{AB}	13.17^{A}	52.60^{A}	81.00^{A}
10.	Kushalnagar	11.30^{E}	6.64^{D}	1.80^{D}	7.22^{D}	10.60^{K}
11.	Madakathara	17.40^{CDE}	11.53^{BCD}	6.50^{BCD}	26.07^{BCD}	38.55^{H}
12.	Marayur	19.80^{ABCDE}	12.40^{BCDD}	7.13^{ABCD}	28.53^{ABCD}	41.64^{FG}
13.	Neyantingara	20.80^{ABCD}	9.22^{D}	5.80^{BCD}	23.24^{BCD}	33.29^{I}
14.	Pala	15.37^{DE}	$9.40C^{D}$	5.80^{BCD}	23.42^{BCD}	33.65^{I}
15.	Palakkad	17.93^{BCDE}	15.57^{ABCD}	8.93^{ABC}	35.73^{ABC}	55.24^{D}
16.	Shimoga	24.27^{ABC}	8.47^{D}	7.37^{ABCD}	29.47^{ABCD}	43.60^{F}
17.	Sirsi	16.73^{CDE}	10.61^{BCD}	5.90^{BCD}	23.53^{BCD}	33.74^{I}
18.	Trivandrum	15.33^{DE}	7.07^{D}	7.10^{ABCD}	28.40^{ABCD}	41.60^{FG}
	SEm+/-	2.59	3.19	1.9	7.61	0.78

DAP: Days after planting
Figures with the same alphabets do not differ significantly

growth period of 330 days, the minimum growth rate was observed for Kushalnagar seed source.

4.1.3.4 Collar diameter

Data on collar diameter at different growth phases (90, 150, 210, 270 and 330 DAP) are presented in Table 11. Statistically significant difference was observed between seed sources in all growth stages except at 90 DAP.

At 150 DAP the collar diameter was highest (44.96 mm) in KAU seed source followed by 44.77 mm in Kasargod and 43.06 in Chavakkad seed sources while, the least (29.95 mm) was recorded in Kushalnagar seed source. The maximum collar diameter was later replaced by Kasargod seed source from 210 days to 330 days after planting. For the entire growth period of 330 days, the maximum collar diameter was observed in Kasargod seed source and minimum in Kushalnagar seed source. At 330 DAP, the maximum collar diameter (49.04 mm) was recorded for Kasargod seed source followed by 47.80 mm for KAU and 47.76 mm for Chavakkad seed source. The least (31.80 mm) collar diameter was recorded for Kushalnagar seed source.

4.2 Clonal propagation of *Jatropha curcas* through rooting of cutting

Results of experiments conducted on clonal propagation through rooting of cuttings in the nursery, College of Forestry, Vellanikkara during 2006-2007 are presented below.

4.2.1 Shoot parameters

The data pertaining to the shoot parameters recorded at 180 DAP in different treatments are furnished in Table 12 to 21.

Table 11 Plant Collar diameter (mm) of different seed sources of *Jatropha curcas*

Sl. No.	Seed source	90 DAP	150 DAP	210 DAP	270 DAP	330 DAP
1.	Athani	33.75	37.74^{DEFG}	38.45^{BCDEFGH}	39.13^{CDE}	41.36^{FG}
2.	Banavasi	31.25	34.58^{FGH}	37.54^{CDEFG}	39.10^{CDE}	41.53^{FG}
3.	Bhadravathi	32.05	36.23^{EFGH}	37.82^{CDEFG}	39.21^{CDE}	39.05^{HI}
4.	Chavakkad	35.9	43.06^{AB}	45.13^{ABC}	46.27^{ABC}	47.76^{AB}
5.	Chirakakod	34.86	42.11^{ABC}	44.25^{ABCD}	45.57^{ABCD}	44.84^{CD}
6.	Dharwad	34.87	40.4B^{CD}	42.83^{ABCDE}	44.14^{ABCDF}	43.19^{DEF}
7.	Guruvayur	33.39	38.34^{CDEF}	38.27^{BCDEFGH}	39.98^{BCDE}	44.51^{CD}
8.	Kasargod	37.44	44.70^{A}	47.34^{A}	48.68^{A}	49.04^{A}
9.	KAU	39.41	44.96^{A}	46.7^{AB}	47.89^{AB}	47.8^{AB}
10.	Kushalnagar	26.15	29.95^{I}	30.34^{G}	30.97^{F}	31.80^{J}
11.	Madakathara	32.18	35.58^{EFGH}	36.52^{DEFG}	37.82^{DE}F	46.40^{BC}
12.	Marayur	32.34	37.28^{DEFG}	39.3^{ABCDEF}	40.39^{BCDE}	42.10^{EFG}
13.	Neyantingara	33.63	36.77^{DEFG}	39.83^{ABCDEF}	41.23^{ABCDE}	43.00^{DEF}
14.	Pala	27.34	32.56^{HI}	33.78^{FG}	35.03^{EF}	37.64^{I}
15.	Palakkad	32.16	39.62^{BCDE}	42.07^{ABCDE}	42.59^{ABCDE}	44.06^{DE}
16.	Shimoga	33.21	35.66^{EFGH}	38.4^{BCDEFG}	39.35^{CDE}	40.5^{GH}
17.	Sirsi	29.56	33.54^{GHI}	34.29^{FG}	35.82^{EF}	37.88^{I}
18.	Trivandrum	31.80	34.48^{FGH}	34.81^{EFG}	35.88^{EF}	37.03^{I}
	SEm+/-	2.48	1.26	2.51	2.44	0.66

DAP: Days after planting
Figures with the same alphabets do not differ significantly

4.2.1.1 Per cent sprouting

A statistically significant variation was observed between the treatments with respect to this character (Table 12 and 13). In softwood cuttings, no significant difference was observed between two length categories. Most of them were found on par with each other. The sprouting was highest (79.17 %) in cuttings treated with IAA 250 ppm, irrespective of their length, which was followed by 78.34 per cent in IAA 100 ppm, while the least (44.17 %) was in treatment consisting of NAA 500 ppm.

Same trend was noticed in semi-hardwood cuttings for this character. Irrespective of the length of cuttings, the highest (88.34 %) was exhibited by one of the controls having no treatment. This was followed by 85.84 per cent sprouting in IBA100 ppm and IBA 250 ppm as against the least (69.17 %) in NAA 500 ppm.

In 10 cm cuttings, irrespective of their age, the highest (73.34 %) sprouting was observed in IBA 100 ppm and IBA 250 ppm. The least (47.50 %) sprouting was exhibited by NAA 500 ppm. In 20 cm cuttings, highest (90.84 %) sprouting was noticed in IAA100 ppm and the least (65.83 %) was in NAA 500 ppm. In all the treatments, the 20 cm length cuttings found superior to that of 10 cm, irrespective of their age.

In general, a significant difference was observed between the treatments. The highest (80.00 %) rate of sprouting was exhibited in treatment involving IAA100 ppm followed by 79.58 per cent on IBA 100 ppm. However, all these were on par with each other and with the two controls. The least (56.67 %) sprouting was observed in treatment consisting NAA 500 ppm.

Table 12 Effect of growth regulators and age of cuttings on sprouting (%) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean (%)
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	66.67 ABCDEFGH	90.00 ABC	78.34	71.67 ABCDEF	91.67 AB	81.67	80.00 A
2.		250	73.33 ABDEF	85.00 ABCD	79.17	56.67 DEFGHI	90.00 ABC	73.34	76.25 A
3.		500	65.00 BCDEFGH	43.33 GHI	54.17	66.67 ABCDEFGH	93.33 AB	80.00	67.08 AB
4.	IBA	100	66.67 ABDEFGH	80.00 ABCDE	73.34	80.00 ABCDE	91.67 AB	85.84	79.58 A
5.		250	60.00 DEFGHI	85.00 ABCD	72.50	86.67 ABCD	85.00 ABCD	85.84	79.17 A
6.		500	65.0B CDEFGHI	80.00 ABCDE	72.50	76.67 ABCDE	75.00 ABCDEF	75.84	74.17 A
7.	NAA	100	41.6 HIJ	75.00 ABCDEF	58.34	73.00 ABCDEF	83.33 ABCDE	78.17	68.33 AB
8.		250	45.00 FGHI	60.00 CDEFGHI	52.5	76.67 ABCDE	81.68 ABCDE	79.18	65.83 AB
9.		500	35.00 I	53.33 EFGHI	44.17	60.00 CDEFGHI	78.33 ABCDE	69.17	56.67 B
10.	Control (water)		75.00 ABCDEF	78.33 ABCDE	76.17	70.00 ABCDEFGH	81.67 ABCDE	75.84	76.25 A
11.	Control		56.67 DEFGHI	70.00 ABCDEFGH	63.34	80.00 ABCDE	96.67 A	88.34	75.83 A
Mean			59.09	72.73	65.91	72.55	86.21	79.38	72.65

The figures with the same alphabets do not differ significantly

Table 13 Effect of growth regulators and length of cuttings on sprouting (%) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	66.67 ABCDEFGH	71.67 ABCDEF	69.17	90.00 ABC	91.67 AB	90.84	80.00 A
2.		250	73.33 ABDEF	56.67 DEFGHI	65.00	85.00 ABCD	90.00 ABC	87.50	76.25 A
3.		500	65.00 BCDEFGH	66.67 ABCDEFGH	65.84	43.33 GHI	93.33 AB	68.33	67.08 AB
4.	IBA	100	66.67 ABDEFGH	80.00 ABCDE	73.34	80.00 ABCDE	91.67 AB	85.84	79.58 A
5.		250	60.00 DEFGHI	86.67 ABCD	73.34	85.00 ABCD	85.00 ABCD	85.00	79.17 A
6.		500	65.0B CDEFGHI	76.67 ABCDE	70.84	80.00 ABCDE	75.00 ABCDEF	77.50	74.17 A
7.	NAA	100	41.6 HIJ	73.00 ABCDEF	57.34	75.00 ABCDEF	83.33 ABCDE	79.17	68.33 AB
8.		250	45.00 FGHI	76.67 ABCDE	60.84	60.00 CDEFGHI	81.68 ABCDE	70.84	65.83 AB
9.		500	35.00 I	60.00 CDEFGHI	47.50	53.33 EFGHI	78.33 ABCDE	65.83	56.67 B
10.	Control (water)		75.00 ABCDEF	70.00 ABCDEFGH	72.50	78.33 ABCDE	81.67 ABCDE	80.00	76.25 A
11.	Control		56.67 DEFGHI	80.00 ABCDE	68.34	70.00 ABCDEFGH	96.67 A	83.34	75.83 A
Mean			59.09	72.55	65.82	72.73	86.21	79.47	72.65

The figures with the same alphabets do not differ significantly

Table 14 Effect of growth regulators and age of cuttings on shoot length (m) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	0.08 S	0.19 EFGHIJK	0.130	0.16 GHIJKLMN	0.26 ABCD	0.214	0.172 A
2.		250	0.09 QRS	0.17 FGHIJKLM	0.128	0.15 HIJKLMNOPQ	0.27 ABC	0.209	0.169 AB
3.		500	0.09 RS	0.17 FGHILKLM	0.125	0.09 QRS	0.28 AB	0.186	0.156 ABC
4.	IBA	100	0.08 S	0.19 EFGHIJK	0.135	0.20 EFGHI	0.23 BCDEF	0.212	0.176 A
5.		250	0.14 IJKLMNOPQRS	0.18 EFGHIJKL	0.158	0.14 IJKLMNOPQRS	0.23 BCDE	0.183	0.171 AB
6.		500	0.10 OPQRS	0.13 KLMNOPQRS	0.117	0.19 EFGHIJ	0.21 CDEFG	0.196	0.157 ABC
7.	NAA	100	0.10 PQRS	0.16 GHIJKLMNOP	0.127	0.10 NOPQRS	0.21 DEFGH	0.154	0.141 BC
8.		250	0.09QRS	0.16 GHIJKLMNO	0.125	0.13 LMNOPQRS	0.19 EFGHIJK	0.159	0.142 BC
9.		500	0.10 NOPQRS	0.15 IJKLMNOPQR	0.125	0.11 MNOPQRS	0.19 EFGHIJK	0.151	0.138 C
10.	Control (water)		0.11 MNOPQRS	0.18 EFGHIJKL	0.147	0.13 JKLMNOPQRS	0.24 BCDE	0.184	0.166 ABC
11.	Control		0.10 NOPQRS	0.15 IJKLMNOPQR	0.125	0.16 GHIJKLMN	0.32 A	0.244	0.185 A
Mean			0.10	0.17	0.131	0.14	0.24	0.190	0.161

The figures with the same alphabets do not differ significantly

Table 15 Effect of growth regulators and length of cuttings on shoot length (m) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	0.08 S	0.16 GHIJKLMN	0.12	0.19 EFGHIJK	0.26 ABCD	0.23	0.172 A
2.		250	0.09 QRS	0.15 HIJKLMNOPQ	0.12	0.17 FGHIJKLM	0.27 ABC	0.22	0.169 AB
3.		500	0.09 RS	0.09 QRS	0.09	0.17 FGHILKLM	0.28 AB	0.22	0.156 ABC
4.	IBA	100	0.08 S	0.20 EFGHI	0.14	0.19 EFGHIJK	0.23 BCDEF	0.22	0.176 A
5.		250	0.14 IJKLMNOPQRS	0.14 IJKLMNOPQRS	0.14	0.18 EFGHIJKL	0.23 BCDE	0.20	0.171 AB
6.		500	0.10 OPQRS	0.19 EFGHIJ	0.14	0.13 KLMNOPQRS	0.21 CDEFG	0.17	0.157 ABC
7.	NAA	100	0.10 PQRS	0.10 NOPQRS	0.10	0.16 GHIJKLMNOP	0.21 DEFGH	0.18	0.141 BC
8.		250	0.09QRS	0.13 LMNOPQRS	0.11	0.16 GHIJKLMNO	0.19 EFGHIJK	0.17	0.142 BC
9.		500	0.10 NOPQRS	0.11 MNOPQRS	0.11	0.15 IJKLMNOPQR	0.19 EFGHIJK	0.17	0.138 C
10.	Control (water)		0.11 MNOPQRS	0.13 JKLMNOPQRS	0.12	0.18 EFGHIJKL	0.24 BCDE	0.21	0.166 ABC
11.	Control		0.10 NOPQRS	0.16 GHIJKLMN	0.13	0.15 IJKLMNOPQR	0.32 A	0.24	0.185 A
Mean			0.10	0.14	0.12	0.17	0.24	0.20	0.1610.21

The figures with the same alphabets do not differ significantly

4.2.1.2 Shoot length

Shoot length has shown significant difference between various treatments (Table 14 and 15). Maximum (0.19 m) height was noticed in the control followed by 0.18 m in the cuttings treated with IBA 100 ppm, while the least (0.14 m) height was exhibited by treatment involving NAA 500 ppm irrespective of their age and length. In both softwood and semi-hardwood cuttings, two categories of length showed a significant difference in the height and the 20 cm cuttings were found superior to 10 cm cuttings. In all the treatments the performance of semi-hardwood cuttings for this character was superior to that of softwood cuttings. In softwood cuttings a significant difference was noticed between two age categories for this character. The maximum (0.19 m) height was obtained in treatments IAA100 ppm and IBA 100 ppm. The least (0.13) was obtained in IBA 500 ppm. In semi-hardwood cuttings also same trend was continued with a maximum of 0.24 m in control (water) and the minimum of 0.15 m in NAA 100 ppm.

A significant difference in the height was observed between two (10 cm and 20 cm) length cuttings. The cuttings treated with IAA 100, 250 and 500 ppm exhibited the maximum (0.14 m) height. The least (0.09 m) was exhibited by IAA 500 ppm, irrespective of their age. In case of 20 cm cuttings, the maximum height (0.24 m) was noticed in control against the least (0.17 m) in cuttings treated with IBA 500, NAA 250 and NAA 500 ppm. In all the above experiments the 10 cm long cuttings performed better over the 20 cm cuttings with respect to the height.

4.2.1.3 Number of shoots

The data pertaining to the effect of various growth hormones on the number of shoots are presented in Table 16 and 17. A significant difference among the

Table 16 Effect of growth regulators and age of cuttings on number of shoots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	1.33 EFGHI	1.93 BCDEF	1.63	2.33 AB	1.60 CDEFGHI	1.93	1.79 AB
2.		250	1.13 HI	1.80 BCDEFGH	1.47	2.80 A	2.13 BCD	2.45	1.96 A
3.		500	1.60 CDEFGHI	1.80 BCDEFGH	1.70	1.67 CDEFGHI	1.53 CDEFGHI	1.55	1.63 BCD
4.	IBA	100	1.20 GHI	1.73 BCDEFGHI	1.47	1.80 BCDEFGH	1.80 BCDEFGHI	1.80	1.64 BCD
5.		250	1.87 BCDEFGH	1.53 CDEFGHI	1.68	1.67 BCDEFGHI	2.00 BCDE	1.83	1.76 ABC
6.		500	1.07 I	1.40 EFGHI	1.25	2.20 BC	1.60 CDEFGHI	1.90	1.58 BCD
7.	NAA	100	1.20 GHI	1.20 GHI	1.2	2.20 BC	1.53 CDEFGHI	1.85	1.53 BCD
8.		250	1.23 GHI	1.67 BCDEFGHI	1.43	1.33 EFGHI	2.00 BCDE	1.67	1.55 BCD
9.		500	1.31 FGHI	1.40 EFGHI	1.36	1.30 FGHI	1.33 EFGHI	1.30	1.33 D
10.	Control (water)		1.20 GHI	1.47 DEFGHI	1.35	1.33 EFGHI	1.87 BCDEFG	1.59	1.47 CD
11.	Control		1.27 FGHI	1.20 GHI	1.25	2.00 BCDE	1.80 BCDEFGH	1.90	1.57 BCD
Mean			1.31	1.56	1.44	1.88	1.74	1.80	1.62

The figures with the same alphabets do not differ significantly

Table 17 Effect of growth regulators and length of cuttings on number of shoots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	1.33 EFGHI	2.33 AB	1.83	1.93 BCDEF	1.60 CDEFGHI	1.77	1.79 AB
2.		250	1.13 HI	2.80 A	1.97	1.80 BCDEFGH	2.13 BCD	1.95	1.96 A
3.		500	1.60 CDEFGHI	1.67 CDEFGHI	1.64	1.80 BCDEFGH	1.53 CDEFGHI	1.65	1.63 BCD
4.	IBA	100	1.20 GHI	1.80 BCDEFGH	1.50	1.73 BCDEFGHI	1.80 BCDEFGHI	1.77	1.64 BCD
5.		250	1.87 BCDEFGH	1.67 BCDEFGHI	1.76	1.53 CDEFGHI	2.00 BCDE	1.75	1.76 ABC
6.		500	1.07 I	2.20 BC	1.65	1.40 EFGHI	1.60 CDEFGHI	1.50	1.58 BCD
7.	NAA	100	1.20 GHI	2.20 BC	1.70	1.20 GHI	1.53 CDEFGHI	1.35	1.53 BCD
8.		250	1.23 GHI	1.33 EFGHI	1.27	1.67 BCDEFGHI	2.00 BCDE	1.83	1.55 BCD
9.		500	1.31 FGHI	1.30 FGHI	1.31	1.40 EFGHI	1.33 EFGHI	1.35	1.33 D
10.	Control (water)		1.20 GHI	1.33 EFGHI	1.27	1.47 DEFGHI	1.87 BCDEFG	1.67	1.47 CD
11.	Control		1.27 FGHI	2.00 BCDE	1.65	1.20 GHI	1.80 BCDEFGH	1.50	1.57 BCD
Mean			1.31	1.88	1.59	1.56	1.74	1.65	1.62

The figures with the same alphabets do not differ significantly

Table 19 Effect of growth regulators and length of cuttings on number of leaves in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	4.00 CDEFG	5.13 ABCDEFGH	4.57	6.53 ABCDE	7.13 ABCD	6.83	5.61
2.		250	2.40 H	6.93 ABCD	4.67	6.13 ABCDEFG	5.73 ABCDEFGH	5.93	5.30
3.		500	2.93 FGH	3.00 FGH	2.83	6.27 ABCDEF	7.30 ABC	6.79	4.81
4.	IBA	100	2.93 FGH	6.33 ABCDEF	4.63	6.47 ABCDE	6.73 ABCD	6.60	5.82
5.		250	5.20 ABCDEF	4.40 CDEFGH	4.80	6.33 ABCDEF	4.20 CDEFGH	5.27	5.04
6.		500	3.20 EFGH	5.73 ABCDEFGGH	4.47	4.60 CDEFGH	5.53 ABCDEFGH	5.07	4.77
7.	NAA	100	3.13 EFGH	2.80 GH	2.97	4.53 CDEFGH	6.20 ABCDEFGH	5.37	4.17
8.		250	3.73 DEFGH	4.18 CDEFGH	3.97	6.13 ABCDEFG	8.20 A	7.17	5.56
9.		500	4.27 CDEFGH	4.40 CDEFGH	4.35	5.33 ABCDEFGH	6.80 ABCD	6.07	4.96
10.	Control (water)		4.13 CDEFGH	4.47 CDEFGH	4.30	5.13 ABCDEFGH	5.67 ABCDEFGH	5.40	5.24
11.	Control		4.80 BCDEFGH	3.87 DEFGH	4.34	3.87 DEFGH	8.07 AB	5.97	5.18
Mean			3.70	4.66	4.17	5.57	6.51	6.04	5.13

The figures with the same alphabets do not differ significantly

treatments was noticed for this character. Irrespective of their age and length, the cuttings exhibited maximum (1.96) number of shoots in IAA 250 ppm followed by 1.79 in IAA 100 ppm, which were on par with each other and superior to one of the controls treated with water. The number of shoots was least (1.33) in the treatment involving NAA 500 ppm.

In softwood cuttings significant differences were observed between two length categories in cuttings treated with IAA 100, 250, 500 and IBA 100. In all these treatments 20 cm cuttings were found superior to 10 cm cuttings. In case of semi-hardwood cuttings a significant difference was observed in almost all the treatments except those cuttings which were treated with IBA 100 and IBA 500 ppm. No significant difference was observed between 10 cm and 20 cm cuttings in both the age categories. Semi-hardwood cuttings were found superior to softwood cuttings in all the treatments, as it is evident from their mean values.

4.4.1.4 Number of leaves

No significant difference was observed between the treatments for this character (Table 18 and 19). Semi-hardwood cuttings performed relatively superior to softwood cuttings in all the treatments. Irrespective of their age and length, the highest (6.53) number of leaves was found in semi-hardwood cuttings treated with IBA 100 pm followed by 6.33 in semi- hardwood cuttings treated with IAA 250 ppm. In softwood cuttings, the maximum (5.77) number of leaves was obtained in treatment involving IBA 250 and the least (3.83) in NAA 100 ppm.

No significant difference was noticed between the two length categories. However, it was evident from their mean values that the 20 cm cuttings were superior to 10 cm cuttings with respect to this character.

Table 18 Effect of growth regulators and age of cuttings on number of leaves in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	4.00 CDEFG	6.53 ABCDE	5.25	5.13 ABCDEFGH	7.13 ABCD	5.96	5.61
2.		250	2.40 H	6.13 ABCDEFG	4.27	6.93 ABCD	5.73 ABCDEFGH	6.33	5.30
3.		500	2.93 FGH	6.27 ABCDEF	4.46	3.00 FGH	7.30 ABC	5.15	4.81
4.	IBA	100	2.93 FGH	6.47 ABCDE	4.70	6.33 ABCDEF	6.73 ABCD	6.53	5.82
5.		250	5.20 ABCDEF	6.33 ABCDEF	5.77	4.40 CDEFGH	4.20 CDEFGH	4.30	5.04
6.		500	3.20 EFGH	4.60 CDEFGH	3.90	5.73 ABCDEFGGH	5.53 ABCDEFGH	5.63	4.77
7.	NAA	100	3.13 EFGH	4.53 CDEFGH	3.83	2.80 GH	6.20 ABCDEFGH	4.50	4.17
8.		250	3.73 DEFGH	6.13 ABCDEFG	4.92	4.18 CDEFGH	8.20 A	6.20	5.56
9.		500	4.27 CDEFGH	5.33 ABCDEFGH	4.82	4.40 CDEFGH	6.80 ABCD	5.10	4.96
10.	Control (water)		4.13 CDEFGH	5.13 ABCDEFGH	5.47	4.47 CDEFGH	5.67 ABCDEFGH	5.00	5.24
11.	Control		4.80 BCDEFGH	3.87 DEFGH	4.35	3.87 DEFGH	8.07 AB	6.00	5.18
Mean			3.70	5.57	4.70	4.66	6.51	5.56	5.13

The figures with the same alphabets do not differ significantly

4.2.1.5 Collar diameter

The data on the effect of growth hormones on collar diameter were found significant as shown in Table 20 and 21. However, except for NAA 250 ppm, that performed least (0.12 mm) all the other treatments were found on par with each other. No significant differences were observed between two age categories. However, semi- hardwood cuttings exhibited comparatively higher mean values for collar diameter in all the treatments over the softwood cuttings.

In 10 cm cuttings almost all the treatments were on par with each other except for IBA 100, IBA 250 and control, which has shown significant difference between the two age categories. The maximum collar diameter (15.35 mm) was observed in control as against the least (11.84 mm) in IAA 500. In 20 cm cuttings the maximum (14.40 mm) collar diameter was noticed in IAA 250 treated cuttings and the least (11.59 mm) was observed in NAA 250 ppm. However, it was found that the 20 cm cuttings were better than the 10 cm cuttings with respect to the collar diameter.

4.2.2 Root parameters

4.2.2.1 Main root length

No significant difference between treatments was noticed in the effect of growth hormones on the main root length (Table 22 and 23). The two age categories did not differ significantly with respect to their main root length. However, the semi-hardwood cuttings were found better than softwood cuttings as evident from their mean values. Difference between the two length categories were also non significant. However, in cuttings treated with IAA 250, IAA 500, IBA 100 and IBA 250, the 20 cm cuttings exhibited their superiority over the 10 cm cuttings with respect to this character. However, irrespective of their age and length, the cuttings treated with IAA

Table 20 Effect of growth regulators and age of cuttings on collar diameter (mm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	14.01 ABCDEFGHIJ	12.08 EFGHIJKLM	13.06	15.35 ABCD	14.45 ABCDEFGH	14.90	13.95 A
2.		250	12.15 DEFGHIJKLM	12.11 DEFGHIJKLM	12.12	15.26 ABCDE	14.31 ABCDEFGHI	14.75	13.45 AB
3.		500	12.84 CDEFGHIKLM	11.68 GHIJKLM	12.26	10.83 KLM	16.09 AB	13.46	12.86 AB
4.	IBA	100	10.96 JKLM	12.27 DEFGHIJKLM	11.62	15.25 ABCDE	13.17 BCDEFGHIJKLM	14.23	12.93 AB
5.		250	10.74 LM	13.71 BCDEFGHIJKLM	12.23	15.98 ABC	15.08 ABCDEF	15.53	13.88 A
6.		500	13.13 BCDEFGHHIJKLM	11.97 GHIJKLM	12.45	14.41 ABCDEFGH	13.38 BCDEFGHIJKLM	13.89	13.17 AB
7.	NAA	100	11.82 GHIJKLM	12.98 BCDEFGHIJ	12.40	14.05 ABCDEFGHIJK	13.68 BCDEFGHIJKL	13.87	13.14 AB
8.		250	11.36 GHIJKLM	11.97 FGHIJKLM	11.67	13.91 ABCDEFGHIJKL	11.21 HIJKLM	12.56	12.12 B
9.		500	11.07 IJKLM	12.71 DEFGHIJKLM	11.83	14.35 ABCDEFGH	13.85 ABCDEFGHIJKL	14.10	12.97 AB
10.	Control (water)		13.73 BCDEFGHIJKL	10.37 M	12.58	16.96 A	14.15 ABCDEFGH	15.55	13.80 A
11.	Control		12.12 DEFGHIJKLM	13.06 BCDEFGHIJKLM	12.58	14.53 ABCDEFGH	15.17 ABCDE	14.85	13.72 A
Mean			12.18	12.26	12.51	14.63	14.05	14.33	13.27

The figures with the same alphabets do not differ significantly

Table 21 Effect of growth regulators and length of cuttings on collar diameter (mm) of shoots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	14.01 ABCDEFGHIJ	15.35 ABCD	14.68	12.08 EFGHIJKLM	14.45 ABCDEFGH	13.27	13.95 A
2.		250	12.15 DEFGHIJKLM	15.26 ABCDE	13.71	12.11 DEFGHIJKLM	14.31 ABCDEFGHI	13.21	13.45 AB
3.		500	12.84 CDEFGHIJKLM	10.83 KLM	11.84	11.68 GHIJKLM	16.09 AB	13.89	12.86 AB
4.	IBA	100	10.96 JKLM	15.25 ABCDE	13.11	12.27 DEFGHIJKLM	13.17 BCDEFGHIJKLM	12.72	12.93 AB
5.		250	10.74 LM	15.98 ABC	13.36	13.71 BCDEFGHIJKLM	15.08 ABCDEF	14.40	13.88 A
6.		500	13.13 BCDEFGHHIJKLM	14.41 ABCDEFGH	13.77	11.97 GHIJKLM	13.38 BCDEFGHIJKLM	12.68	13.17 AB
7.	NAA	100	11.82 GHIJKLM	14.05 ABCDEFGHIJK	12.94	12.98 BCDEFGHIJ	13.68 BCDEFGHIJKL	13.33	13.14 AB
8.		250	11.36 GHIJKLM	13.91 ABCDEFGHIJKL	12.64	11.97 FGHIJKLM	11.21 HIJKLM	11.59	12.12 B
9.		500	11.07 IJKLM	14.35 ABCDEFGH	12.71	12.71 DEFGHIJKLM	13.85 ABCDEFGHIJKL	13.28	12.97 AB
10.	Control (water)		13.73 BCDEFGHIJKL	16.96 A	15.35	10.37 M	14.15 ABCDEFGH	12.26	13.80 A
11.	Control		12.12 DEFGHIJKLM	14.53 ABCDEFGH	13.33	13.06 BCDEFGHIJKLM	15.17 ABCDE	14.12	13.72 A
Mean			12.18	14.63	13.41	12.26	14.05	13.16	13.27

The figures with the same alphabets do not differ significantly

Table 22 Effect of growth regulators and age of cuttings on main root length (cm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	20.72^{ABC}	22.68 ^{ABC}	21.7	22.89 ^{ABC}	19.47 ^{ABC}	21.18	21.44
2.		250	24.47 ^{ABC}	21.67 ^{ABC}	23.07	27.11 ^{A}	22.05 ^{ABC}	24.58	23.83
3.		500	22.80 ^{ABC}	20.25 ^{ABC}	21.56	17.55 ^{BC}	25.89 ^{AB}	21.72	21.62
4.	IBA	100	20.00 ^{ABC}	21.55 ^{ABC}	20.78	26.00 ^{AB}	21.33 ^{ABC}	23.67	22.22
5.		250	24.50 ^{ABC}	21.77 ^{ABC}	23.14	27.33 ^{A}	20.22 ^{ABC}	23.78	23.46
6.		500	23.89 ^{ABC}	18.47 ^{ABC}	21.18	23.00 ^{ABC}	23.00 ^{ABC}	23.00	22.09
7.	NAA	100	19.67 ^{ABC}	21.11 ^{ABC}	20.39	21.78 ^{ABC}	19.56 ^{ABC}	20.67	20.53
8.		250	19.44 ^{ABC}	20.90 ^{ABC}	20.17	18.44 ^{ABC}	25.50 ^{AB}	21.97	21.07
9.		500	22.61 ^{ABC}	25.17 ^{AB}	23.89	22.58 ^{ABC}	18.78 ^{ABC}	20.68	22.28
10.	Control (water)		23.91 ^{ABC}	23.44 ^{ABC}	23.68	24.22 ^{ABC}	21.18 ^{ABC}	22.70	23.19
11.	Control		21.33 ^{ABC}	23.83 ^{ABC}	22.58	18.67 ^{ABC}	15.67 ^{C}	17.17	19.87
Mean			22.12	21.89	22.01	22.69	21.15	21.92	21.96

The figures with the same alphabets do not differ significantly

Table 23 Effect of growth regulators and length of cuttings on the main root length (cm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	20.72^{ABC}	22.89^{ABC}	21.81	22.68^{ABC}	19.47^{ABC}	21.18	21.44
2.		250	24.47^{ABC}	27.11^{A}	25.89	21.67^{ABC}	22.05^{ABC}	21.86	23.83
3.		500	22.80^{ABC}	17.55^{BC}	18.90	20.25^{ABC}	25.89^{AB}	23.07	21.62
4.	IBA	100	20.00^{ABC}	26.00^{AB}	23.00	21.55^{ABC}	21.33^{ABC}	21.44	22.22
5.		250	24.50^{ABC}	27.33^{A}	25.92	21.77^{ABC}	20.22^{ABC}	21.00	23.46
6.		500	23.89^{ABC}	23.00^{ABC}	23.45	18.47^{ABC}	23.00^{ABC}	20.74	22.09
7.	NAA	100	19.67^{ABC}	21.78^{ABC}	20.73	21.11^{ABC}	19.56^{ABC}	20.34	20.53
8.		250	19.44^{ABC}	18.44^{ABC}	18.94	20.90^{ABC}	25.50^{AB}	23.20	21.07
9.		500	22.61^{ABC}	22.58^{ABC}	22.54	25.17^{AB}	18.78^{ABC}	21.96	22.28
10.	Control (water)		23.91^{ABC}	24.22^{ABC}	24.07	23.44^{ABC}	21.18^{ABC}	22.31	23.19
11.	Control		21.33^{ABC}	18.67^{ABC}	20.00	23.83^{ABC}	15.67^{C}	19.75	19.87
Mean			22.12	22.69	22.29	21.89	21.15	21.53	21.96

The figures with the same alphabets do not differ significantly

250 ppm exhibited the highest (23.83 cm) value for main root length and the least was in the control (19.87 cm).

4.2.2.2 Total length of roots

A significant difference was noticed among the cuttings treated with different growth hormones and is given in Table 24 and 25. Irrespective of their age and length, the cuttings produced maximum (98.71 cm) root length in IAA 250 followed by IBA 250 (97.56 cm), which were on par with each other. The least (69.32 cm) root length was exhibited by control that was treated with water. No significant difference was noticed between the two age categories. But the semi-hardwood cuttings were found superior over softwood cuttings. The length of cuttings also had no significant effect on the total length of roots. However, the 20 cm cuttings exhibited comparatively higher values than 10 cm cuttings as is evident from their means (Plate 6).

4.2.2.3 Root collar diameter

Data pertaining to the root collar diameter is presented in Table 26 and 27. Collar diameter of the roots differed significantly between treatments. Irrespective of their age and length the cuttings treated with IAA 100 ppm exhibited highest (31.57 mm) collar diameter and the least was in control that was treated with water.

A significant difference was also noticed between two age categories as it is evident from their individual values in all the treatments the semi-hardwood cuttings showing comparatively higher values than softwood cuttings. No significant difference was found in between the two length categories. However, the higher mean

Table 24 Effect of growth regulators and age of cuttings on total length of roots (cm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	87.71 BCDEFGHI	89.50 BCDEFGH	88.61	86.22 BCDEFGHIJ	115.97 ABC	102.10	94.85 AB
2.		250	83.25 BCDEFGHI	86.42 BCDEFGHIJ	84.84	117.97 AB	107.19 ABCDE	112.58	98.71 A
3.		500	86.39 BCDEFGHI	80.97 CDEFGHIJ	83.68	104.00 ABCDEF	91.00 ABCDEFGH	97.50	90.59 AB
4.	IBA	100	73.55 DEFGHI	96.55 ABCDEFGH	85.05	94.99 ABCDEFGH	103.00 ABCDEF	99.00	92.02 AB
5.		250	83.78 BCDEFGHI	73.89 DEFGHIJ	78.84	124.78 A	107.78 ABCD	116.28	97.56 A
6.		500	75.44 DEFGHIJ	85.00 BCDEFGHI	80.22	67.00 GHIJ	94.58 ABCDEFGH	80.79	80.51 BC
7.	NAA	100	81.22 CDEFGHIJ	79.65 DEFGHIJ	80.44	74.56 DEFGHIJ	100.00 ABCDEFG	87.28	83.56 ABC
8.		250	62.00 HIJ	71.90 EFGHIJ	66.95	65.33 GHIJ	86.67 BCDEFGH	76.00	72.35 C
9.		500	76.94 DEFGHIJ	68.94 FGHIJ	72.94	81.38 CDEFGHIJ	53.44 IJ	67.41	70.18 C
10.	Control (water)		48.17 J	66.10 GHIJ	57.14	83.44 BCDEFGHIJ	79.55 DEFGHIJ	81.50	69.32 C
11.	Control		66.33 GHIJ	71.11 FGHIJ	68.22	77.11 DEFGHIJ	76.87 DEFGHIJ	76.99	72.54 C
Mean			74.98	81.50	78.23	88.80	89.96	89.38	83.84

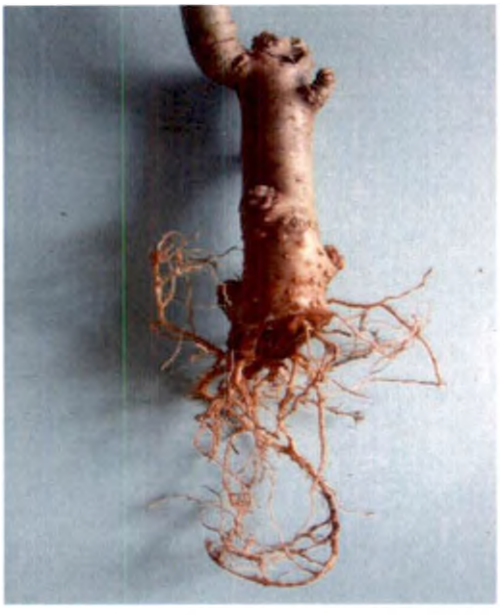
The figures with the same alphabets do not differ significantly

Table 25 Effect of growth regulators and length of cuttings on total length of roots (cm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	87.71 BCDEFGHI	86.22 BCDEFGHIJ	86.97	115.97 ABC	89.50 BCDEFGH	102.74	94.85 AB
2.		250	83.25 BCDEFGHI	117.97 AB	100.61	86.42 BCDEFGHIJ	107.19 ABCDE	96.81	98.71 A
3.		500	86.39 BCDEFGHI	104.00 ABCDEF	95.20	80.97 CDEFGHIJ	91.00 ABCDEFGH	85.99	90.59 AB
4.	IBA	100	73.55 DEFGHI	94.99 ABCDEFGH	84.27	96.55 ABCDEFGH	103.00 ABCDEF	99.78	92.02 AB
5.		250	83.78 BCDEFGHI	124.78 A	104.28	73.89 DEFGHIJ	107.78 ABCD	90.84	97.56 A
6.		500	75.44 DEFGHIJ	67.00 GHIJ	71.22	85.00 BCDEFGHI	94.58 ABCDEFGH	89.79	80.51 BC
7.	NAA	100	81.22 CDEFGHIJ	74.56 DEFGHIJ	77.89	79.65 DEFGHIJ	100.00 ABCDEFG	89.83	83.56 ABC
8.		250	62.00 HIJ	65.33 GHIJ	63.67	71.90 EFGHIJ	86.67 BCDEFGH	79.29	72.35 C
9.		500	76.94 DEFGHIJ	81.38 CDEFGHIJ	79.16	68.94 FGHIJ	53.44 IJ	61.19	70.18 C
10.	Control (water)		48.17 J	83.44 BCDEFGHIJ	65.81	66.10 GHIJ	79.55 DEFGHIJ	72.83	69.32 C
11.	Control		66.33 GHIJ	77.11 DEFGHIJ	71.72	71.11 FGHIJ	76.87 DEFGHIJ	73.99	72.54 C
Mean			74.98	88.80	81.89	81.50	89.96	85.73	83.84

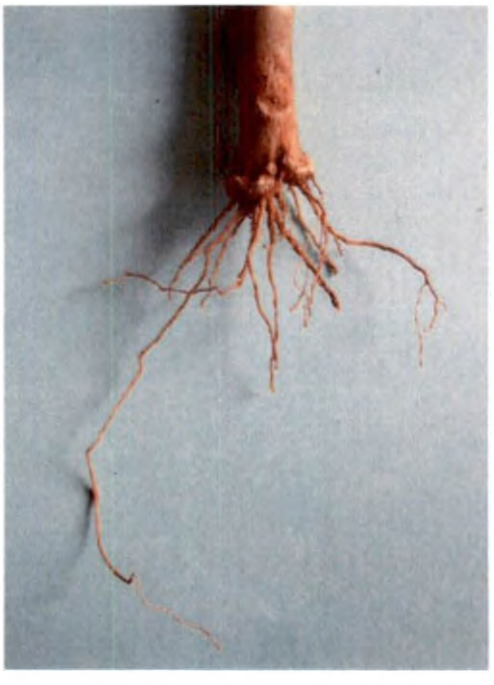
The figures with the same alphabets do not differ significantly

Rooting in semi-hardwood cutting of 20cm



Rooting in semi-hardwood cutting of 10cm

Rooting in softwood cutting of 20cm



Rooting in softwood cutting of 10cm

Plate 6 Root formation in different types of cuttings

Table 26 Effect of growth regulators and age of cuttings on root collar diameter (mm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	26.35 FGHIJKLMNO	31.55 BCDEFGHI	28.95	36.76 ABC	31.60 BCDEFGHI	34.18	31.57 A
2.		250	22.50 MNOP	28.42 DEFGHIJKLM	25.46	37.48 AB	32.07 BCDEFG	34.78	30.14 AB
3.		500	25.54 HIJKLMNOP	24.24 KLMNOP	24.89	26.41 FGHIJKLMNO	27.09 EFGHIJKLMN	26.75	25.82 DE
4.	IBA	100	25.32 IJKLMNOP	27.03 EFGHIJKLMN	26.18	34.44 ABCD	33.10 ABCDE	33.77	29.97 AB
5.		250	20.37 P	27.11 EFGHIJKLMN	23.74	38.44 A	31.95 BCDEFGH	35.20	29.46 ABC
6.		500	20.67 OP	25.15 IJKLMNOP	22.91	38.44 A	29.24 DEFGHIJKL	33.84	28.39 BCDE
7.	NAA	100	23.41 LMNOP	27.20 EFGHIJKLMN	25.31	32.73 ABCDEF	31.53 BCDEFGHI	32.13	28.72 ABCD
8.		250	23.15 LMNOP	25.72 GHIJKLMNOP	24.24	32.97 ABCDE	30.15 DEFGHIJK	31.56	28.00 BCDE
9.		500	22.37 NOP	28.05 DEFGHIJKLM	25.21	30.84 CDEFGHIJ	30.06 DEFGHIJK	30.45	27.83 BCDE
10.	Control (water)		20.32 P	25.42 IJKLMNOP	22.87	29.03 DEFGHIJKLM	27.46 EFGHIJKLMN	28.25	25.56 E
11.	Control		24.41 JKLMNOP	24.01 KLMNOP	24.21	29.38 DEFGHIJKL	28.60 DEFGHIJKLM	28.99	26.60 CDE
Mean			23.13	26.72	24.93	33.36	30.26	31.81	28.37

The figures with the same alphabets do not differ significantly

Table 27 Effect of growth regulators and length of cuttings on root collar diameter (mm) in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	26.35 FGHIJKLMNO	36.76 ABC	31.56	31.55 BCDEFGHI	31.60 BCDEFGHI	31.58	31.57 A
2.		250	22.50 MNOP	37.48 AB	29.99	28.42 DEFGHIJKLMN	32.07 BCDEFG	30.25	30.14 AB
3.		500	25.54 HIJKLMNOP	26.41 FGHIJKLMNO	25.98	24.24 KLMNOP	27.09 EFGHIJKLMN	25.67	25.82 DE
4.	IBA	100	25.32 IJKLMNOP	34.44 ABCD	29.88	27.03 EFGHIJKLMNO	33.10 ABCDE	30.07	29.97 AB
5.		250	20.37 P	38.44 A	29.41	27.11 EFGHIJKLMNO	31.95 BCDEFGH	29.53	29.46 ABC
6.		500	20.67 OP	38.44 A	29.56	25.15 IJKLMNOP	29.24 DEFGHIJKL	27.20	28.39 BCDE
7.	NAA	100	23.41 LMNOP	32.73 ABCDEF	28.07	27.20 EFGHIJKLMNOP	31.53 BCDEFGHI	29.37	28.72 ABCD
8.		250	23.15 LMNOP	32.97 ABCDE	28.06	25.72 GHIJKLMNOP	30.15 DEFGHIJK	27.94	28.00 BCDE
9.		500	22.37 NOP	30.84 CDEFGHIJ	26.61	28.05 DEFGHIJKLMN	30.06 DEFGHIJK	29.06	27.83 BCDE
10.	Control (water)		20.32 P	29.03 DEFGHIJKLM	24.68	25.42 IJKLMNOP	27.46 EFGHIJKLMN	26.44	25.56 E
11.	Control		24.41 JKLMNOP	29.38 DEFGHIJKL	26.90	24.01 KLMNOP	28.60 DEFGHIJKLM	26.31	26.60 CDE
Mean			23.13	33.36	28.25	26.72	30.26	28.49	28.37

The figures with the same alphabets do not differ significantly

values of semi-hardwood cuttings made it comparatively superior over the softwood cuttings.

4.2.2.4 Fresh weight of roots

No significant difference was noticed among treatments with respect to the fresh weight of roots (Table 28 and 29). Irrespective of their age and length the cuttings treated with IAA 250 ppm exhibited highest (3.86 g) and the least (2.84 g) was exhibited by control that was treated with none. In both softwood and semi-hardwood cuttings two categories of length showed no significant difference in the fresh weight of roots. In all the treatments, semi-hardwood cuttings performed superior to that of softwood cuttings. The length of cutting had no effect on the performance with respect to this character. Both 10 and 20 cm long cuttings were on par with each other. However, the 20 cm cuttings were marginally superior to 10 cm cutting as it is evident from their mean value.

4.2.2.5 Dry weight of roots

A highly significant difference was noticed between the treatments with respect to dry weight of roots as presented in Table 30 and 31. Irrespective of their age and length the cuttings treated with NAA 500 ppm exhibited the highest (1.34 g) followed by 1.24 g in IAA 250, which were on par with each other while, the least (0.81g) was in the control.

No significant difference was observed between the two age categories except in cuttings treated with NAA 500 ppm, in which semi-hardwood cuttings were superior to softwood cuttings. However, all the other treatments were on par with each other with respect to the dry weight of roots. The effect of length of cuttings on

Table 28 Effect of growth regulators and age of cuttings on fresh weight (g) of roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	3.04^{ABCD}	4.30^{ABCD}	3.67	3.88^{ABCD}	3.29^{ABCD}	3.59	3.63
2.		250	3.11^{ABCD}	3.39^{ABCD}	3.25	4.82^{A}	4.12^{ABCD}	4.47	3.86
3.		500	3.04^{ABCD}	3.16^{ABCD}	3.10	2.38^{BCD}	4.16^{ABCD}	3.27	3.19
4.	IBA	100	1.97^{D}	2.86^{ABCD}	2.42	3.76^{ABCD}	3.55^{ABCD}	3.66	3.04
5.		250	2.43^{ABCD}	3.15^{ABCD}	2.79	4.74^{AB}	4.36^{ABCD}	4.55	3.67
6.		500	2.15^{CD}	3.14^{ABCD}	2.65	3.48^{ABCD}	3.60^{ABCD}	3.54	3.09
7.	NAA	100	2.99^{ABCD}	2.82^{ABCD}	2.81	3.70^{ABCD}	3.58^{ABCD}	3.64	3.27
8.		250	1.92^{D}	3.95^{ABCD}	2.94	3.07^{ABCD}	4.72^{AB}	3.90	3.42
9.		500	3.37^{ABCD}	3.82^{ABCD}	3.60	4.52^{ABC}	3.30^{ABCD}	3.91	3.76
10.	Control (water)		2.65^{ABCD}	3.84^{ABCD}	3.25	4.65^{AB}	4.03^{ABCD}	4.34	3.79
11.	Control		2.38^{BCD}	3.16^{ABCD}	2.77	3.18^{ABCD}	2.65^{ABCD}	2.92	2.84
Mean			2.64	3.42	3.03	3.83	3.76	3.80	3.41

The figures with the same alphabets do not differ significantly

Table 29 Effect of growth regulators and length of cuttings on fresh weight (g) of roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	3.04^{ABCD}	3.88^{ABCD}	3.46	4.30^{ABCD}	3.29^{ABCD}	3.80	3.63
2.		250	3.11^{ABCD}	4.82^{A}	3.97	3.39^{ABCD}	4.12^{ABCD}	3.76	3.86
3.		500	3.04^{ABCD}	2.38^{BCD}	2.71	3.16^{ABCD}	4.16^{ABCD}	3.66	3.19
4.	IBA	100	1.97^{D}	3.76^{ABCD}	2.87	2.86^{ABCD}	3.55^{ABCD}	3.21	3.04
5.		250	2.43^{ABCD}	4.74^{AB}	3.59	3.15^{ABCD}	4.36^{ABCD}	3.76	3.67
6.		500	2.15^{CD}	3.48^{ABCD}	2.82	3.14^{ABCD}	3.60^{ABCD}	3.37	3.09
7.	NAA	100	2.99^{ABCD}	3.70^{ABCD}	3.35	2.82^{ABCD}	3.58^{ABCD}	3.20	3.27
8.		250	1.92^{D}	3.07^{ABCD}	2.50	3.95^{ABCD}	4.72^{AB}	4.34	3.42
9.		500	3.37^{ABCD}	4.52^{ABC}	3.92	3.82^{ABCD}	3.30^{ABCD}	3.56	3.76
10.	Control (water)		2.65^{ABCD}	4.65^{AB}	3.65	3.84^{ABCD}	4.03^{ABCD}	3.94	3.79
11.	Control		2.38^{BCD}	3.18^{ABCD}	2.78	3.16^{ABCD}	2.65^{ABCD}	2.91	2.84
Mean			2.64	3.83	3.24	3.42	3.76	3.59	3.41

The figures with the same alphabets do not differ significantly

Table 30 Effect of growth regulators and age of cuttings on dry weight (g) of roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	1.07 BCDEFG	1.28 BCDE	1.18	1.19 BCDE	0.90 BCDEFG	1.05	1.11 ABCD
2.		250	0.95 BCDEFG	1.08 BCDEFG	1.02	1.58 AB	1.34 ABCDE	1.46	1.24 AB
3.		500	0.95 BCDEFG	0.96 BCDEFG	0.96	0.88 CDEFG	1.22 BCDE	1.05	1.00 BCD
4.	IBA	100	0.48 FG	1.10 BCDEFG	0.79	1.14 BCDEF	1.03 BCDEFG	1.09	0.94 BCD
5.		250	0.78 DEFG	0.95 BCDEFG	0.87	1.44 ABCD	1.30 BCDE	1.37	1.12 ABCD
6.		500	0.65 EFG	0.69 EFG	0.67	1.18 BCDE	0.73 EFG	0.96	0.82 D
7.	NAA	100	1.15 BCDEF	0.81 DEFG	0.98	1.11 BCDEFG	0.43 G	0.770	0.88 CD
8.		250	1.27 BCDE	0.88 CDEFG	1.08	1.34 BCDE	1.01 BCDEFG	1.18	1.13 ABCD
9.		500	0.94 BCDEFG	1.97 A	1.46	1.50 ABC	0.96 BCDEFG	1.23	1.34 A
10.	Control (water)		0.82 EFG	1.15 BCDEFG	0.99	1.34 BCDE	1.28 BCDE	1.31	1.15 ABC
11.	Control		0.66 EFG	0.87 CDEFG	0.77	0.93 BCDEFG	0.78 DEFG	0.86	0.81 D
Mean			0.88	1.07	0.98	1.24	0.99	1.11	1.05

The figures with the same alphabets do not differ significantly

Table 31 Effect of growth regulators and length of cuttings on dry weight (g) of roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	1.07 BCDEFG	1.19 BCDE	1.13	1.28 BCDE	0.90 BCDEFG	1.09	1.11 ABCD
2.		250	0.95 BCDEFG	1.58 AB	1.27	1.08 BCDEFG	1.34 ABCDE	1.21	1.24 AB
3.		500	0.95 BCDEFG	0.88 CDEFG	0.92	0.96 BCDEFG	1.22 BCDE	1.09	1.00 BCD
4.	IBA	100	0.48 FG	1.14 BCDEF	0.81	1.10 BCDEFG	1.03 BCDEFG	1.07	0.94 BCD
5.		250	0.78 DEFG	1.44 ABCD	1.11	0.95 BCDEFG	1.30 BCDE	1.13	1.12 ABCD
6.		500	0.65 EFG	1.18 BCDE	0.92	0.69 EFG	0.73 EFG	0.71	0.82 D
7.	NAA	100	1.15 BCDEF	1.11 BCDEFG	1.13	0.81 DEFG	0.43 G	0.62	0.88 CD
8.		250	1.27 BCDE	1.34 BCDE	1.31	0.88 CDEFG	1.01 BCDEFG	0.95	1.13 ABCD
9.		500	0.94 BCDEFG	1.50 ABC	1.22	1.97 A	0.96 BCDEFG	1.47	1.34 A
10.	Control (water)		0.82 EFG	1.34 BCDE	1.08	1.15 BCDEFG	1.28 BCDE	1.22	1.15 ABC
11.	Control		0.66 EFG	0.93 BCDEFG	0.80	0.87 CDEFG	0.78 DEFG	0.83	0.81 D
Mean			0.88	1.24	1.06	1.07	0.99	1.03	1.05

The figures with the same alphabets do not differ significantly

the dry weight of roots was found non significant. All the treatments were on par with each other.

4.2.2.6 Number of primary roots

A significant difference was noticed between treatments for the number of primary roots (Table 32 and 33). Irrespective of their age and length, the maximum (12.42) number of roots was recorded in IAA 250 ppm and NAA 250 ppm while, the least (8.58) was exhibited by control that was treated with none.

All the treatments were on par with each other between two age categories but, a significant difference was observed between the two length categories. In treatment IAA 100 ppm, 20 cm long cuttings were found superior over 10 cm long cuttings.

4.2.2.7 Number of secondary roots

The effect of growth hormones on the number of secondary roots was found significant as shown in the Table 34 and 35. Irrespective of their age and length, the maximum (14.58) number of secondary roots was observed in cuttings treated with IAA 100 ppm followed by 12.75 in IAA 250 and IBA 250 ppm, which were on par with each other. A significant difference was observed between the age categories in treatment consisting NAA 250 ppm and control (water), in which, the semi-hardwood cuttings were found superior over softwood cuttings. However, all the other treatments were found on par with each other with respect to the number of secondary roots.

Table 32 Effect of growth regulators and age of cuttings on number of primary roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	8.67 CDEFG	14.67 AB	11.67	12.33 ABCDEFG	9.67 BCDEFG	11.00	11.33 AB
2.		250	9.67 BCDEFG	14.00 ABCD	11.84	12.33 ABCDEFG	13.67 ABCDE	13.0	12.42 A
3.		500	10.00 BCDEFG	12.67 ABCDEF	11.34	9.67 BCDEFG	10.67 ABCDEFG	10.17	10.75 ABC
4.	IBA	100	8.33 DEFG	11.33 ABCDEFG	9.83	11.33 ABCDEFG	11.67 ABCDEFGH	11.50	10.67 ABC
5.		250	9.67 BCDEFG	10.33 ABCDEFG	10.00	10.33 ABCDEFG	16.00 A	13.17	11.58 AB
6.		500	8.00 EFG	12.33 ABCDEFG	10.17	11.33 ABCDEFG	11.00 ABCDEFG	11.17	10.67 ABC
7.	NAA	100	11.67 ABCDEFG	10.00 BCDEFG	10.84	9.67 BCDEFG	14.33 ABC	12.00	11.42 AB
8.		250	8.33 DEFG	14.00 ABCD	11.17	13.67 ABCDE	13.67 ABCDE	13.17	12.42 A
9.		500	11.00 ABCDEFG	11.67 ABCDEFG	11.34	9.67 CDEFG	12.33 ABCDEFG	11.00	11.17 AB
10.	Control (water)		6.67 G	9.67 BCDEFG	8.17	10.33 ABCDEFG	10.67 ABCDEFG	10.50	9.33 BC
11.	Control		7.00 FG	7.33 FG	7.17	9.33 BCDEFG	10.67 ABCDEFG	10.00	8.58 C
Mean			9.00	11.64	10.32	10.91	12.21	11.56	10.94

The figures with the same alphabets do not differ significantly

Table 33 Effect of growth regulators and length of cuttings on number of primary roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	8.67 CDEFG	12.33 ABCDEFG	10.50	14.67 AB	9.67 BCDEFG	12.17	11.33 AB
2.		250	9.67 BCDEFG	12.33 ABCDEFG	11.00	14.00 ABCD	13.67 ABCDE	13.84	12.42 A
3.		500	10.00 BCDEFG	9.67 BCDEFG	9.84	12.67 ABCDEF	10.67 ABCDEFG	11.67	10.75 ABC
4.	IBA	100	8.33 DEFG	11.33 ABCDEFG	9.83	11.33 ABCDEFG	11.67 ABCDEFGH	11.50	10.67 ABC
5.		250	9.67 BCDEFG	10.33 ABCDEFG	10.00	10.33 ABCDEFG	16.00 A	13.17	11.58 AB
6.		500	8.00 EFG	11.33 ABCDEFG	9.67	12.33 ABCDEFG	11.00 ABCDEFG	11.67	10.67 ABC
7.	NAA	100	11.67 ABCDEFG	9.67 BCDEFG	10.67	10.00 BCDEFG	14.33 ABC	12.17	11.42 AB
8.		250	8.33 DEFG	13.67 ABCDE	11.00	14.00 ABCD	13.67 ABCDE	13.84	12.42 A
9.		500	11.00 ABCDEFG	9.67 CDEFG	10.34	11.67 ABCDEFG	12.33 ABCDEFG	12.00	11.17 AB
10.	Control (water)		6.67 G	10.33 ABCDEFG	8.50	9.67 BCDEFG	10.67 ABCDEFG	10.17	9.33 BC
11.	Control		7.00 FG	9.33 BCDEFG	8.17	7.33 FG	10.67 ABCDEFG	9.00	8.58 C
Mean			9.00	10.91	9.96	11.64	12.21	11.93	10.94

The figures with the same alphabets do not differ significantly

Table 34 Effect of growth regulators and age of cuttings on number of secondary roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Age of cuttings						Grand mean
			Softwood			Semi-hardwood			
			10 cm long	20 cm long	Mean	10 cm long	20 cm long	Mean	
1.	IAA	100	14.33^{AB}	14.33^{AB}	14.33	14.67^{AB}	15.00^{A}	14.84	14.58^{A}
2.		250	10.33^{BCDE}	13.00^{ABCD}	11.67	13.67^{ABC}	14.00^{AB}	13.84	12.75^{AB}
3.		500	12.33^{ABCDE}	12.00^{ABCDE}	12.17	13.33^{ABC}	13.67^{ABC}	13.50	12.83^{B}
4.	IBA	100	11.00^{ABCDE}	14.33^{AB}	12.67	12.67^{ABCD}	13.00^{ABCD}	12.84	12.75^{AB}
5.		250	11.33^{ABCDE}	11.67^{ABCDE}	11.50	14.00^{AB}	13.00^{ABCD}	13.50	12.50^{B}
6.		500	11.67^{ABCDE}	12.67^{ABCD}	12.17	11.67^{ABCDE}	14.00^{AB}	12.84	12.50^{B}
7.	NAA	100	13.33^{ABC}	11.00^{ABCDE}	12.17	13.33^{ABC}	12.67^{ABCD}	13.00	12.58^{B}
8.		250	8.67^{DE}	8.33^{E}	8.50	13.33^{ABC}	13.00^{ABCD}	13.17	10.83^{B}
9.		500	9.33^{CDE}	10.67^{ABCDE}	10.00	10.67^{ABCDE}	12.67^{ABCD}	11.67	10.83^{B}
10.	Control (water)		8.33^{E}	12.33^{ABCDE}	10.33	14.00^{AB}	12.33^{ABCDE}	13.17	11.75^{B}
11.	Control		10.33^{BCDE}	13.33^{ABC}	11.83	12.00^{ABCDE}	13.00^{ABCD}	12.50	12.17^{B}
Mean			11.00	12.15	11.58	13.03	13.30	13.17	12.37

The figures with the same alphabets do not differ significantly

Table 35 Effect of growth regulators and length of cuttings on number of secondary roots in cuttings of *Jatropha curcas*

Sl. No	Growth regulator	Concentration (ppm)	Length of cuttings						Grand mean
			10 cm			20 cm			
			Softwood	Semi-hard wood	Mean	Softwood	Semi-hard wood	Mean	
1.	IAA	100	14.33^{AB}	14.67^{AB}	14.50	14.33^{AB}	15.00^{A}	14.67	14.58^{A}
2.		250	10.33^{BCDE}	13.67^{ABC}	12.00	13.00^{ABCD}	14.00^{AB}	13.50	12.75^{AB}
3.		500	12.33^{ABCDE}	13.33^{ABC}	12.83	12.00^{ABCDE}	13.67^{ABC}	12.84	12.83^{B}
4.	IBA	100	11.00^{ABCDE}	12.67^{ABCD}	11.84	14.33^{AB}	13.00^{ABCD}	13.67	12.75^{AB}
5.		250	11.33^{ABCDE}	14.00^{AB}	18.58	11.67^{ABCDE}	13.00^{ABCD}	12.34	12.50^{B}
6.		500	11.67^{ABCDE}	11.67^{ABCDE}	11.67	12.67^{ABCD}	14.00^{AB}	13.34	12.50^{B}
7.	NAA	100	13.33^{ABC}	13.33^{ABC}	13.33	11.00^{ABCDE}	12.67^{ABCD}	11.84	12.58^{B}
8.		250	8.67^{DE}	13.33^{ABC}	11.00	8.33^{E}	13.00^{ABCD}	10.67	10.83^{B}
9.		500	9.33^{CDE}	10.67^{ABCDE}	10.00	10.67^{ABCDE}	12.67^{ABCD}	11.67	10.83^{B}
10.	Control (water)		8.33^{E}	14.00^{AB}	11.17	12.33^{ABCDE}	12.33^{ABCDE}	12.33	11.75^{B}
11.	Control		10.33^{BCDE}	12.00^{ABCDE}	11.17	13.33^{ABC}	13.00^{ABCD}	13.17	12.17^{B}
Mean			11.00	13.03	12.02	12.15	13.30	12.73	12.37

The figures with the same alphabets do not differ significantly

The effect of length of cuttings on the number of secondary roots were found non significant. All the treatments were at par with each other. However, the 20 cm cuttings, by virtue of their higher mean values were found superior to 10 cm cuttings.

4.3 Clonal propagation of *Jatropha curcas* through micro propagation

Results of various experiments conducted on micro propagation of *Jatropha curcus* Linn. in the tissue culture laboratory of Department of Tree Physiology and Breeding, College of Forestry, Vellanikkara during 2006-2007 are presented below.

4.3.1 Surface sterilization of explants

Results of various surface sterilization treatments on culture contamination are presented in Table 36. It was found that the treatment effects differed significantly. Among the various surface sterilization treatments tried, 15 minute dip of explants in 0.1 per cent mercuric chloride ($HgCl_2$) as well as treatment involving a one-hour dip in 0.2 per cent fungicidal solution (Bavistin and Indofil M 45) followed by 15 minute dip in 0.1 per cent $HgCl_2$ were found to be most effective in controlling contamination of explants. These treatments were significantly superior to all the other treatments. The surface sterilization with 0.1 per cent $HgCl_2$ dip for 12 minutes and fungicidal solution mixture for one hour followed by 0.1 per cent $HgCl_2$ for 10 minutes were found to be at par with a contamination rate of 39.0 per cent and 37.31 per cent, respectively. Ethyl alcohol (70 %) dip for 1-2 minute alone was found to be totally ineffective in controlling culture contamination.

4.3.2 Seasonal variation in the rate of culture contamination

The seasonal influence on culture contamination of nodal explant cultured is evident from the results presented in Table 37. The rate of fungal and bacterial infection varied greatly with respect to the season of collection of explants. All the

Table 36 Effects of various surface sterilants on culture establishment of *Jatropha curcas*

Treatment no.	Treatment Details	Contamination (%)		
		1st week	2nd week	3rd week
1.	$HgCl_2$ (0.1 %) for 10 min.	12.81 D	88.3 B	100.00 A
2.	$HgCl_2$ (0.1 %) for 12 min.	14.90 C	29.17 D	39.00 C
3.	$HgCl_2$ (0.1 %) for 15 min.	3.47 E	15.40 E	25.00 D
4.	Ethyl alcohol (70 %) for 1-2 min.	73.33 A	100.00 A	100.00 A
5.	$HgCl_2$ (0.1 %) for 15 min+ Ethyl alcohol (70 %) for 1-2 min.	0.00 F	100.00 A	100.00 A
6.	Fungicidal solution (0.2 % Bavistin + 0.2 % indofil) for 30 min.+$HgCl_2$ (0.1 %) for 15 min.+	0.00 F	34.57 D	100.00 A
7.	Fungicidal solution (0.2 % Bavistin+ 0.2 % indofil) for 1 hr. + $HgCl_2$ (0.1 %) for 15 min.	0.00 F	14.40 E	24.19 D
8.	Fungicidal solution (0.2% Bavistin + 0.2% indofil) for 30 min.+$HgCl_2$ (0.1 %) for 10 min.	0.00 F	72.21 C	100.00 A
9.	Fungicidal solution (0.2% Bavistin + 0.2% indofil) for 1 hr.+ $HgCl_2$ (0.1 %) for 10 min.	18.05 B	29.72 D	37.31 C
10.	Fungicidal solution (0.2% Bavistin+ 0.2% indofil) for 1 hr.+ Ethyl alcohol (70 %) for 1-2 min.+ $HgCl_2$ (0.1 %) for 15 min.	0.00 F	38.03 D	89.91 B
Sem +/-		0.58	2.91	2.65

Figures with the same alphabet do not differ significantly

Table 37 Seasonal influence on contamination and culture establishment in axillary bud cultures of *Jatropha curcas*

Month	Contamination (%)		Survival of cultures (%)	Culture establishment (%)
	F.C	B.C		
January	68.7	1.6	29.71	20.40
February	40.5	3.5	56.00	41.70
March	19.0	0.2	80.80	60.53
April	11.7	NIL	88.30	79.60
May	9.2	NIL	90.80	83.60
June	100.0	NIL	NIL	NIL
July	98.2	NIL	1.80	NIL
August	97.5	NIL	2.50	NIL
September	98.2	NIL	1.80	NIL
October	96.5	2.5	1.00	NIL
November	86.4	2.7	10.88	NIL
December	79.1	3.1	17.85	4.59

F.C. - Fungal contamination
B.C. - Bacterial contamination

cultures initiated during rainy season got contaminated while they showed better survival during March to May. Infection was as high as 40-100 per cent during other months of the year. Fungal infection was identified as the main source of contamination during culture establishment. Culture contamination was highest during the months of June to October.

4.3.3 Standardization of basal medium for culture establishment

The data on culture establishment and growth in various media tried are presented in Table 38. A significant difference was observed between three media tried with respect to percent leaf initiation, shoot initiation and callusing. Among all the media tried, MS medium has shown relatively higher bud initiation (32.40 %). Significantly high percentage (27.58 %) of leaf initiation, shoot initiation (8.88 per cent), average shoot length (1.06 cm) and maximum shoot length (1.67 cm) was observed in MS medium, which was found to be significantly superior over the other two basal media.

4.3.4 Effect of plant growth regulators on bud initiation and growth

Based on screening trials MS medium and WPM were selected and supplemented with various concentrations of cytokinins (BA and kinetin) and auxin (IBA) singly and in combination to study their effect on axillary bud cultures (Plate 7 & 8). The results of these treatments are detailed below.

4.3.4.1 Effect of BA on bud break and shoot development in MS medium

The data on the effect of various concentrations of BA (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) on different growth parameters are presented in the Table 39. Highest

Table 38 Culture establishment from nodal explants of *Jatropha curcas* in different basal media

Media	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %	Browning %
	% culture	Days	% culture	Days	Avg.	Max	% culture	Days	Avg.	Max.		
MS BASAL	32.40	8.33^{B}	27.58 ^{A}	13.00	1.19	1.67	8.88 ^{A}	15.33 ^{A}	1.08 ^{A}	1.67 ^{A}	19.99 ^{A}	42.39 ^{A}
WPM BASAL	30.07	7.00 ^{B}	5.55 ^{B}	9.67	1.33	1.67	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	15.55 ^{A}	0.00 ^{B}
B5 BASAL	18.50	12.00^{A}	4.17 ^{B}	6.00	0.50	0.67	0.00 ^{B}	0.00 ^{B}	0.00^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}
SEm+/-	4.13	0.69	2.99	4.49	0.59	0.67	1.28	0.19	0.13	0.19	2.57	3.49

Figures with the same alphabet do not differ significantly

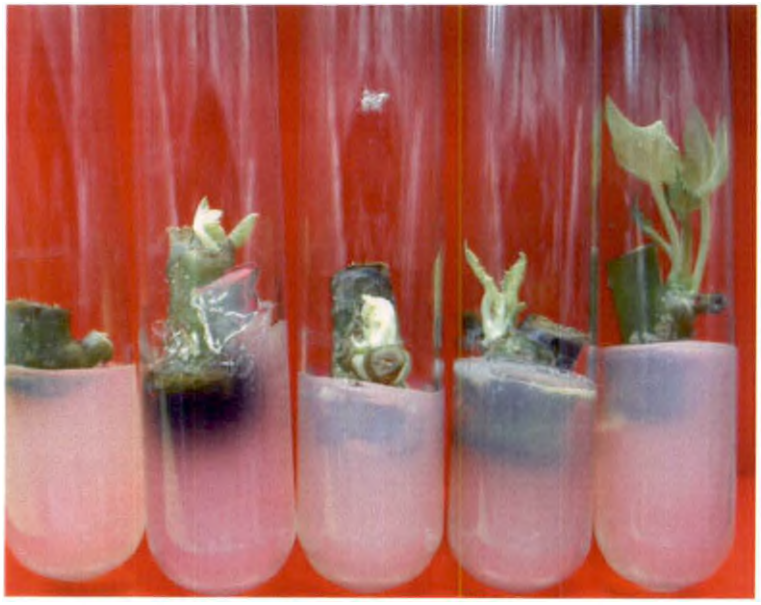


Plate 7 Stages of development and leaf morphologies of *Jatropha curcas* in MS

Plate 8 Stages of development and leaf morphologies of *Jatropha curcas* in WPM

Table 39 Effect of BA on bud break and shoot development in axillary bud cultures of *Jatropha curcas* in MS medium

Concentration of BA (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %	Browning %
	% culture	Days	% culture	Days	Avg.	Max.	% culture	Days	Avg.	Max.		
0.5	31.16 ^{B}	7.33	22.14	10.00	2.83	3.33	19.31 ^{B}	14.00	2.53	3.20	16.65	29.05^{A}
1.0	52.48 ^{A}	7.00	24.52	10.67	1.97	2.67	37.07 ^{A}	16.00	2.33	3.17	21.03	28.81^{A}
2.0	55.55 ^{A}	6.67	28.88	10.33	1.85	3.00	34.88 ^{A}	15.67	2.63	3.67	23.89	13.33^{B}
3.0	29.91 ^{B}	7.50	24.04	12.00	1.25	2.75	20.71 ^{B}	19.00	1.75	2.25	2.97	38.10^{A}
SEm+/-	6.16	0.28	2.34	0.84	0.40	0.56	2.90	1.20	0.34	0.46	2.97	3.52

Figures with same alphabet do not differ significantly

percentage (55.55 %) of bud initiation was observed in MS medium containing 2.0 mg l^{-1} BA followed by MS + 1.0 mg l^{-1} BA (52.58 %), which were found to be on par with each other and significantly superior to other two concentrations of BA attempted. No significant difference was observed in number of days taken by the cultures for bud break. Highest shoot initiation (37.07 %) was obtained in MS medium added with 1.0 mg l^{-1} BA followed by 34.88 per cent in the media containing 2.0 mg l^{-1} BA. These treatments were on par with each other and were significantly superior to medium containing 0.5 and 3.0 mg l^{-1} BA. All the cultures took almost same time for shoot development and there was no significant difference in the number of days taken for shoot development. Relatively higher (28.88 %) leaf initiation percentage was obtained in medium containing 2.0 mg l^{-1} BA (Plate 9). However, there was no significant difference between the media with respect to leaf initiation percentage, average number of leaves, maximum number of shoots, average shoot length, maximum shoot length and callusing percentage.

Callusing was highest (23.89 %) in medium containing 2.0 mg l^{-1} BA followed by 1.0 mg l^{-1} BA (21.03 %). Callusing was least (2.97 %) when medium was supplemented with 3.0 mg l^{-1} BA. Browning of the medium was noticed in some of the treatments. It was highest (38.10 %) in medium containing 3.0 mg l^{-1} BA followed by 29.05 per cent in media added with 0.5 and 1.0 mg l^{-1} BA, which were found to be on par with each other. Least browning (13.33 %) was recorded in the medium containing 2.0 mg l^{-1} BA.

4.3.4.2 Effect of BA on bud break and shoot development in WPM medium

Effect of different concentrations (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) of BA in WPM on axillary bud cultures of *Jatropha curcas* was studied and the data presented in Table 40. Highest bud initiation (71.57 %), which was significantly superior over the

Table 38 Culture establishment from nodal explants of *Jatropha curcas* in different basal media

Media	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %	Browning %
	% culture	Days	% culture	Days	Avg.	Max	% culture	Days	Avg.	Max.		
MS BASAL	32.40	8.33^{B}	27.58 ^{A}	13.00	1.19	1.67	8.88 ^{A}	15.33 ^{A}	1.08 ^{A}	1.67 ^{A}	19.99 ^{A}	42.39 ^{A}
WPM BASAL	30.07	7.00 ^{B}	5.55 ^{B}	9.67	1.33	1.67	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	15.55 ^{A}	0.00 ^{B}
B5 BASAL	18.50	12.00^{A}	4.17 ^{B}	6.00	0.50	0.67	0.00 ^{B}	0.00 ^{B}	0.00^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}
SEm+/-	4.13	0.69	2.99	4.49	0.59	0.67	1.28	0.19	0.13	0.19	2.57	3.49

Figures with the same alphabet do not differ significantly

Plate 9 Leaf initiation in MS+ BA 2.0 mg l^{-1}

Plate 12 Shoot development in MS+ 2.0 mg l^{-1}BA + 1.0 mgl^{-1} kin

Table 40 Effect of BA on bud break and shoot development in axilary bud cultures of *Jatropha curcas* in WPM medium

Concentration of BA (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %
	% culture	Days	% culture	Days	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5	71.57 ^{A}	9.00	42.78	14.33	2.47 ^{A}	4.00	36.67 ^{A}	20.33	2.55 ^{A}	3.50 ^{A}	32.22^{A}
1.0	42.96 ^{B}	8.33	33.46	16.33	1.31 ^{B}	2.33	21.28 ^{B}	18.00	1.00 ^{C}	2.33 ^{B}	18.83^{B}
2.0	38.51 ^{B}	8.67	21.80	16.67	1.39 ^{B}	2.00	5.55 ^{C}	21.00	3.00 ^{A}	3.00 ^{AB}	5.5^{C}
3.0	44.44 ^{B}	8.33	36.67	15.00	1.17 ^{B}	1.13	26.67 ^{AB}	20.00	2.00 ^{B}	2.50 ^{B}	3.63^{BC}
Sem+/-	6.20	1.22	6.91	0.87	0.25	0.75	4.31	0.83	0.15	0.22	3.63

Figures with same alphabets do not differ significantly

other treatments, was found when 0.5 mg l^{-1} BA was supplemented in the medium, which in turn were found to be on par with each other. There was no, significant difference in number of days taken by the cultures for bud break, leaf initiation and maximum number of leaves. However, in all these parameters the medium containing 0.5 mg l^{-1} BA was found relatively superior over the others. Average number of leaves was significantly higher (2.47) in the medium added with 0.5 mg l^{-1} over all the other media. Highest shoot production (36.67%) also was obtained in WPM containing 0.5 mg l^{-1} BA followed by the medium containing 1.0 mg l^{-1} BA (21.28 %). The least rate of shoot initiation (5.55 %) was recorded when WPM was supplemented with 2.0 mg l^{-1} BA. There was no significant difference in the number of days taken for shoot development. Average shoot length obtained was highest (3.0 cm) in WPM containing 2.0 mg l^{-1} BA followed by WPM containing 0.5 mg l^{-1} BA with the shoot length of 2.55 cm, which were on par with each other. Maximum shoot length (3.5 cm) was obtained in WPM containing 0.5 mg l^{-1} BA and the least (2.33 cm) obtained in WPM containing 1.0 mg l^{-1} BA. Highest percent of callusing (32.33%) was obtained in WPM containing 0.5 mg l^{-1} BA and the least (3.63%) was in medium containing 3.0 mg l^{-1} BA.

4.3.4.3 Effect of kinetin on bud break and shoot development in MS medium

For induction of bud break and shoot development four concentrations of kinetin (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) were supplemented to MS medium. The results of these treatments are presented in Table 41. Among the different concentrations of kinetin, bud break was highest (66.18%) in medium containing 1.0 mg l^{-1} kin and minimum (26.67%) in medium containing 3.0 mg l^{-1} kin. Maximum leaf initiation (52.09%) was observed in the medium containing 1.0 mg l^{-1} kin followed by the medium supplied with 0.5 mg l^{-1} kin (33.98 %) and the least (25.55%) was observed in medium containing 3.0 mg l^{-1} kin. Treatment effect on the time taken for leaf

Table 41 Effect of kinetin on bud break and shoot development in axilary bud cultures of *Jatropha curcas* in MS medium

Concentration of kinetin (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %	Browning %
	% culture	Days	% culture	Days	Avg.	Max..	% culture	Days	Avg.	Max.		
0.5	37.75 ^{B}	7.00	33.98 ^{B}	12.00 ^{A}	2.94 ^{A}	5.00 ^{B}	28.89 ^{B}	14.33	1.50 ^{AB}	2.67 ^{B}	27.34 ^{B}	22.20 ^{C}
1.0	66.18 ^{A}	6.00	52.09 ^{A}	7.67 ^{B}	3.47 ^{A}	8.67 ^{A}	52.08 ^{A}	14.33	2.17 ^{A}	4.00 ^{A}	47.64 ^{A}	13.05 ^{D}
2.0	37.73 ^{B}	6.67	26.64 ^{C}	10.33 ^{A}	1.25 ^{B}	2.00 ^{C}	22.20 ^{B}	14.33	0.92 ^{B}	2.00 ^{B}	17.77 ^{C}	28.85 ^{B}
3.0	26.67 ^{B}	6.50	25.55 ^{C}	10.83 ^{A}	1.13 ^{B}	2.00 ^{C}	26.66 ^{B}	14.50	1.50 ^{AB}	2.25 ^{B}	23.33 ^{BC}	40.00 ^{A}
Sem +/-	3.65	0.46	2.10	0.63	0.32	0.67	2.06	0.313	0.22	0.34	2.80	1.25

Figures with the same alphabets do not differ significantly

initiation was found significant and it was as high as 10.83 days when medium supplemented with 3.0 mg l^{-1} kin and as low as 7.67 days at the levels of 1.0 mg l^{-1} kin. However, the medium containing 0.5, 2.0 and 3.0 mg l^{-1} kin were at par with each other with respect to time taken for leaf initiation. Average number of leaves obtained was maximum (3.47) in MS medium containing 1.0 mg l^{-1} kin and was minimum (1.13) in 3.0 mg l^{-1} kin. Treatment of 0.5 mg l^{-1} kin and 1.0 mg l^{-1} kin did not differ significantly with respect to average number of leaves. Maximum number of leaves obtained was highest (8.67) in medium containing 1.0 mg l^{-1} kin and was minimum (2.0) in medium containing 2.0 kin and 3.0 mg l^{-1} kin. Shoot initiation was highest (52.08 %) in medium containing 1.0 mg l^{-1} kin, which was significantly superior to all the other media and minimum (22.20 %) was obtained in medium supplemented with 2.0 mg l^{-1} kin. There was no significant difference between the treatments of 0.5, 2.0 and 3.0 mg l^{-1} kin with respect to their effect on percent shoot development. Average as well as maximum shoot length was highest in MS medium containing 1.0 mg l^{-1} kin and was least (0.92 cm) with 2.0 mg l^{-1} kin. All the other treatments were at par for these two parameters. Callusing was highest (47.64 %) in MS medium containing 1.0 mg l^{-1} kin and it was least (17.77 %) in MS + 2.0 mg l^{-1} kin (Plate 10). A significantly higher number of cultures exhibited browning of the medium when supplemented with 3.0 mg l^{-1} kin.

4.3.4.4 Effect of kinetin on bud break and shoot development in WPM

The WPM was supplemented with four different concentrations (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) of kinetin to study their effect on axillary bud cultures of jatropha. The results obtained are presented in Table 42. Maximum bud break (66.51%) was obtained in medium containing 0.5 mg l^{-1} kin, which was followed 50.98 per cent in WPM +1.0 mg l^{-1} kin. Minimum was obtained in WPM containing 2.0 mg l^{-1} kin. Leaf initiation was highest (48.23 %) in WPM + 1.0 mg l^{-1} kin followed by WPM+

Table 42 Effect of kinetin on bud break and shoot development in axillary bud cultures of *Jatropha curcas* in WPM medium

Concentration of kinetin (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %
	% culture	Days	% culture	Days	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5	66.51 ^{A}	8.67	42.89 ^{A}	18.00 ^{A}	2.76 ^{A}	4.33 ^{A}	28.21 ^{A}	22.00 ^{A}	2.28 ^{A}	3.0 ^{A}	15.86^{AB}
1.0	50.98 ^{B}	9.00	48.23 ^{A}	18.00 ^{A}	2.23 ^{A}	4.00 ^{A}	22.40 ^{A}	20.67 ^{A}	2.17 ^{A}	2.67 ^{A}	10.58^{BC}
2.0	15.43 ^{C}	10.00	0.00 ^{C}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	6.98^{C}
3.0	39.70 ^{B}	10.00	18.67 ^{B}	17.00 ^{A}	2.14 ^{A}	3.33 ^{A}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	0.00 ^{B}	18.53^{A}
SEm+/-	3.97	0.83	4.07	1.12	0.31	0.69	2.50	0.53	0.08	0.17	2.09

Figures with the same alphabet do not differ significantly

Plate 10 Callusing in MS

Plate 11 Callusing in WPM

0.5 mg l^{-1} kin (42.89 %) which were found on par with each other and was least (0%) in WPM+ 2.0 mg l^{-1} kin. None of the cultures showing bud initiation could produce leaves in medium supplemented with 2.0 mg l^{-1} kin. Shoot initiation was highest (28.21%) in medium containing 0.5 mg l^{-1} kin followed by 2.0 mg l^{-1} kin showing 22.4 per cent shoot initiation. However, these treatments were found on par with each other with respect to per cent shoot initiation. The medium containing 2.0 and 3.0 mg l^{-1} kin failed to produce shoots. There was no significant difference in the time taken for shoot initiation between the treatments of 0.5 and 1.0 mg l^{-1} kin. Average shoot length obtained was maximum (2.28 cm) in medium containing 0.5 mg l^{-1} kin followed by 2.17 cm in medium containing 1.0 mg l^{-1} kin. Maximum shoot length obtained was highest (3.0 cm) in medium containing 0.5 mg l^{-1} kin followed by 2.67 cm in medium containing 1.0 mg l^{-1} kin.

Almost all the combinations of kin in WPM induced callusing in explants (Plate 11). Highest rate of callusing (18.53 %) was noticed in WPM + 3.0 mg l^{-1} kin and the least (6.98 %) was in WPM+2.0 mg l^{-1} kin, which were differed significantly with each other.

4.3.4.5 Effect of IBA on culture establishment and growth in MS medium

The data on the effect of different concentrations viz., 0.5, 1.0 and 2.0 mg l^{-1} of IBA supplemented to MS medium is presented as mean values of respective treatment (Table 43). Highest bud initiation (61.57 %) was observed in MS + 2.0 mg l^{-1}IBA and least (43.85 %) in MS +0.5 mg l^{-1}IBA. Highest leaf initiation (44.44 %) and maximum number of leaves (3.33) and average number of leaves (2.57) were observed in MS+1.0 mg l^{-1} IBA and were least in MS + 0.5 mg l^{-1}IBA. Rooting was observed in MS medium supplemented with 1.0 mg l^{-1} BA. Callusing was highest (23.39 %) in MS medium containing 1.0 mg l^{-1}IBA followed by 17.78 per cent in

Table 43 Effect of IBA on bud break and shoot development in axillary bud cultures of *Jatropha curcas* in MS medium

Concentration of IBA (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %
	% culture	Days	% culture	Days	Avg.	Max.	% culture	Days	Avg.	Max.	
0.5	43.85	7.00	32.87	15.67	1.17	2.33	0.00	0.00	0.00	0.00	17.78
1.0	57.25	7.33	44.44	15.33	2.57	3.33	0.00	0.00	0.00	0.00	23.39
2.0	61.57	7.33	38.15	15.00	1.83	2.67	0.00	0.00	0.00	0.00	0.00

media containing 0.5 mg l^{-1}IBA. Callusing was absent in MS medium containing 2.0 mg l^{-1}IBA.

4.3.4.6 Effect of BA and kinetin on culture establishment and growth in MS medium

Sixteen combinations of BA and kinetin were tried in order to exploit the synergistic effect of two cytokinins for inducing bud break and shoot development (Plate 12). Results of these experiments are presented in the Table 44.

The interaction effect of all BA and kinetin levels was found significant. Highest bud initiation (71.06 %) was obtained in MS supplemented with 0.5 mg l^{-1} BA+0.5 mg l^{-1}kin. At 3.0 +2.0 mg l^{-1} of BA and kin, respectively, no bud initiation was noticed. MS+ 2.0 mg l^{-1} BA+2.0 mg l^{-1} kin took least (6.67 days) number of days for bud initiation compared to the time taken by other levels of BA and kinetin. Other treatment combinations did not differ significantly from this in their effects on number of days taken for bud break. Highest leaf initiation (60 %) was observed in MS + 0.5 mg l^{-1}BA +1.0 mg l^{-1} kin followed by 59.18 per cent in MS +0.5 mg l^{-1} BA +0.5 mg l^{-1} kin. MS + 2.0 mg l^{-1} BA+0.5 mg l^{-1} kin took least (13.33 days) and MS + 2.0 mg l^{-1} BA+2.0 mg l^{-1} kin took maximum (17.0 days) number of days for leaf initiation. Average (3.5) and maximum (5.33) number of leaves obtained was highest in medium containing 2.0 mg l^{-1}BA+0.5 mg l^{-1} kin. However, media containing 3.0 +0.5, 3.0 +1.0, 3.0 +2.0, 3.0 +3.0 mg l^{-1} BA and kin, respectively could not produce leaves.

Maximum shoot development (57.78 %) was observed in MS + 0.5 mg l^{-1}BA+1.0 mg l^{-1} kin followed by 53.20 per cent in MS+0.5 mg l^{-1}BA+0.5 mg l^{-1} kin and 50.98 per cent in medium containing 0.5 mg l^{-1}BA+2.0 mg l^{-1} kin. The buds

Table 44 Effect of combination of BA and kinetin on bud break and shoot development in axillary bud cultures of *Jatropha curcas* in MS medium

Concentration of BA+ kinetin (mg l^{-1})	Bud initiation		Leaf initiation		Number of leaves		Shoot initiation		Shoot length (cm)		Callusing %	Browning %
	% culture	Days	% culture	Days	Avg.	Max.	% culture	Days	Avg.	Max.		
0.5+0.5	71.06^{A}	7.33^{BC}	59.18^{A}	14.67^{BC}	2.50^{AB}	3.33^{ABC}	53.20^{A}	18.67^{BC}	3.33^{A}	3.67^{ABC}	0.00^{D}	14.05^{C}
0.5+1.0	68.14^{A}	7.33^{BC}	60.00^{A}	14.67^{BC}	2.67^{AB}	4.00^{AB}	57.78^{A}	19.33^{AB}	2.33^{B}	4.33^{A}	11.30^{B}	4.00^{D}
0.5+2.0	68.37^{A}	7.33^{BC}	45.84^{B}	14.33^{CD}	1.83^{ABC}	3.00^{ABC}	50.98^{A}	20.33^{A}	2.00^{BC}	3.50^{ABC}	5.66^{C}	12.69^{C}
0.5+3.0	49.60^{B}	7.33^{BC}	38.26^{C}	14.33^{CD}	1.83^{ABC}	2.00^{ABC}	41.11^{B}	17.33^{C}	2.50^{B}	2.50^{C}	6.14^{C}	19.20^{B}
1.0+0.5	34.90^{C}	10.33^{A}	19.65^{DE}	15.33^{BC}	1.83^{ABC}	2.33^{ABC}	16.89^{C}	18.67^{BC}	2.17^{BC}	4.00^{AB}	6.18^{C}	24.44^{A}
1.0+1.0	69.51^{A}	7.00^{BC}	49.76^{B}	14.33^{CD}	1.67^{ABC}	2.00^{ABC}	42.21^{B}	19.33^{AB}	2.37^{B}	3.00^{BC}	0.00^{D}	19.60^{B}
1.0+2.0	56.82^{B}	7.67^{BC}	44.44^{BC}	14.67^{BC}	1.50^{ABC}	2.33^{ABC}	40.00^{B}	20.3^{A}	2.17^{BC}	4.00^{AB}	6.66^{C}	23.00^{A}
1.0+3.0	50.98^{B}	7.33^{BC}	38.56^{C}	15.33^{BC}	1.50^{ABC}	2.67^{ABC}	40.00^{B}	17.33^{C}	1.83^{BC}	3.00^{BC}	0.00^{D}	24.44^{A}
2.0+0.5	28.01^{CD}	7.33^{BC}	14.01^{E}	13.33^{D}	3.50^{A}	5.33^{A}	14.01^{C}	18.67^{BC}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{E}
2.0+1.0	49.02^{B}	7.33^{BC}	23.14^{D}	15.67^{B}	1.67^{ABC}	2.67^{ABC}	19.99^{C}	18.67^{BC}	1.50^{C}	3.00^{BC}	15.55^{A}	0.00^{E}
2.0+2.0	22.22^{DE}	6.67^{C}	13.33^{EF}	17.00^{A}	1.00^{BC}	1.00^{BC}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	6.66^{C}	0.00^{E}
2.0+3.0	15.55^{E}	8.33^{B}	6.66^{FG}	15.00^{BC}	2.00^{ABC}	2.00^{ABC}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{E}
3.0+0.5	16.67^{E}	7.50^{BC}	0.00^{G}	0.00^{E}	0.00^{C}	0.00^{C}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	6.66^{C}	0.00^{E}
3.0+1.0	26.66^{CD}	8.00^{BC}	0.00^{G}	0.00^{E}	0.00^{C}	0.00^{C}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{E}
3.0+2.0	0.00^{F}	0.00^{D}	0.00^{G}	0.00^{E}	0.00^{C}	0.00^{C}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{E}
3.0+3.0	26.66^{D}	8.00^{BC}	0.00^{G}	0.00^{E}	0.00^{C}	0.00^{C}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{D}	0.00^{E}
SEm+/-	2.70	0.45	2.36	0.36	0.60	1.01	2.86	0.49	0.22	0.39	0.67	0.85

Figures with the same alphabet do not differ significantly

initiated in other treatments failed to produce shoots.

Highest average (3.33 cm) as well as maximum (4.33 cm) length of shoot was noticed in MS + 0.5 mg l^{-1}BA+0.5 mg l^{-1} kin and lowest (0 cm) for both was observed in MS +2.0 mg l^{-1}BA+0.5 mg l^{-1} kin. Highest rate of callusing (15.55%) was noticed in MS + 2.0 mg l^{-1}BA+1.0 mg l^{-1} kin followed by 11.30 per cent callusing in MS + 0.5 mg l^{-1}BA+1.0 mg l^{-1} kin. Callusing was absent in other treatment combinations. Media browning was observed in some cultures, which was highest (24.44 %) in MS + 1.0 mg l^{-1}BA+3.0 mg l^{-1} kin.

Discussion

DISCUSSION

Availability of energy at reasonable cost is of vital importance for the economic development of any nation considering the import burden of petroleum products and pollution caused by the use of these products. Government of India as well as various research institutions and automobile industries in our country are taking keen interest in biodiesel. Mainly, biodiesel is being produced by the crops like sunflower, soybean, mustard oil etc. in many parts of the world. As the nation is facing a shortage of edible oils, it would not be feasible to produce biodiesel from edible oils. However, the country has enormous potential to produce tree borne oil seeds for biodiesel production. Among the potential tree borne oil seeds jatropha is one of the best alternatives (Kureel, 2006).

Jatropha curcas, a wild oil seed crop, has been identified as a potential source of producing biodiesel. Compared with other sources this has added advantages as rapid growth, higher seed productivity as well as suitability for tropical and sub tropical regions of the world (Katwal and Soni, 2003). The nonedible oil of *Jatropha curcas* has requisite potential of providing a promising and commercially viable alternative to diesel as it has the desired physico-chemical and performance characteristics comparable to petro-diesel (Kureel, 2006). There is a wide awakening to use jatropha oil as biofuel particularly in countries like India, where the resources to import fossil fuel is poor (Venkatachalam, 2003).

Notwithstanding the reported versatility of *Jatropha curcas* owing to its short gestation period, excellent regeneration capability, long productive life, adaptability to fragile and harsh environments etc., any such plans for large scale commercialization are unlikely to succeed without back up of appropriate and relevant region specific and situation specific planting materials and agro production

techniques. In the light of these limitations and the limitations associated with the available non descript genetic materials and half assembled technologies, any indiscriminate and hasty expansion of *Jatropha curcas* into all conceivable agro ecological and crop growing situations may cause irreparable damage to an otherwise 'wonder fuel' and harm the immense opportunities it otherwise offers in the long run.

In this context, the present study was undertaken to evaluate different seed sources for their genetic variation, to standardize efficient method of clonal propagation techniques and to develop quality planting material for mass propagation. The results of various studies conducted are discussed in this chapter.

5.1 Evaluation of seed source variation in *Jatropha curcas*

Among trees, considering the various levels of variation present in a species, between seed sources and between trees within population variation are two categories that may account for over 90.00 per cent of the total variation observed in most of the species (Zobel and Talbert, 1984). Genetic differences associated with the place of origin have been several times as great as that among individual trees within a stand. Hence, it becomes necessary to exploit such genetic variation by conducting seed source testing prior to a more intensive breeding work.

Such an approach will be of great use in a biofuel crop like *Jatropha curcas* in order to screen better genotypes with higher growth, yield and oil content. Crop improvement work in this species is very scanty. In India seed source research is focused on few major species like *Tectona grandis, Azadirachta indica, Acacia nilotica, Dalbergia latifolia* and *Pinus roxburghi,* to screen the naturally available genetic variation to select the best planting material for higher productivity and for future breeding work. Limited amount of genetic variation studies were attempted

through province trials in *Jatropha curcas* (Heller, 1996 and Ginwal *et al.*, 2004). For successful promotion of large scale plantations, there is a need for carefully planned and well directed seed source research. The most successful tree improvement programme is that where proper seed and seed sources were used. The loss from using wrong sources can be great and even disastrous (Zobel and Talbert, 1984).

Evaluation of *Jatropha curcas* collected from a total of 18 seed sources of Kerala and Karnataka was carried out at Vellanikkara, Trichur, during 2005-2007. While field evaluation was carried out for all the 18 seed sources, seed and seedling characteristics were studied for 17 and 14 seed sources, respectively.

Seed being one of the most important inputs for forest nursery production and plantation establishment its quality should be assured in any selection programme (Lauridsen and Olesen, 1990). A good quality seed should possess uniform size, weight, color and freedom from pest and diseases to produce vigorous seedlings. Several provenance trials indicated the presence of considerable variations in seed size and weight (Anon, 1973 and Murthy, 1973 a,b). In the present study, significant variations were observed among the seed sources for their seed characters, viz., seed length, seed width, seed length:breadth ratio, 100 seed weight, kernel: seed weight ratio, seed oil content, germination percentage, mean daily germination, peak value of germination and germination value. The highest value for seed length was recorded by Kasargod seed source and the lower values were by Dharwad and Chavakkad seed sources. With respect to the seed width, seeds of KAU seed source exhibited the maximum value while, the minimum was by Madakathara. Veerendra *et al.* (1996) reported such variations in seed length and width between neem seed sources.

Toon *et al.* (1990) in a study found that seed size is an indication of the quality of seeds and their genetic potentialities. Vinod (1997) referred that larger

sized seeds have greater advantages over smaller seeds with reference to germination and further performance of the seedlings of neem.

Higher values for 100 seed weight were recorded by Trivandrum, Neyantingara and Palakkad seed sources. A direct relation between seed length and seed width was observed with the 100 seed weight. This may be due to the fact that the weight is contributed by the fillingness of the seeds, kernel weight, etc. Such relationships between seed size, weight and seed quality has been documented for many hardwood species of the tropics (Halos, 1983). However, seeds from Trivandrum seed source in spite of their heaviness, failed to exhibit higher value for germination percentage. Hughes (1987) assumed such low germination to the defective seed collection and handling such as using unripe seeds.

The seed sources of *Jatropha curcas* exhibits considerable amount of variation in seed parameters and germination (Kumar *et al.*, 2004b). Limited work has been done on jatropha seeds which warrant intensive investigation. In the present study, highest germination percentage was recorded for Neyantingara seed source followed by Palakkad. The higher germination was observed in the heavier seeds than in the lighter seeds. These results were in conformity with the work done on teak by Kumar (1979), Prasad (1996) and in neem by Ponnammal *et al.* (1993). Same trend was observed in case of mean daily germination, peak value of germination and germination value. Neyantingara exhibited highest value for all these parameters. Findings of Dwivedi (1993) that the seeds collected from high rainfall regions possessed higher germination compared to that of drier localities supported the results obtained in our study in case of Palakkad and Neyantingara seed sources.

Jatropha curcas being the choicest biofuel crop, oil content is one of the most important criteria for the selection of the crop. Jatropha contains about 46-58 per cent

of oil on kernel weight and 30-40 per cent on seed weight (Subramanian *et al.*, 2005). This variation in oil content may be due to its geography/ecotype or agro climatic conditions of the locality. (Kumaran, 1991; Kumar *et al.*, 2003; Kannan, 2003; Parthiban, *et al.*, 2003 and Puri and Swamy, 2003). This is evident from the variations noticed for this character in the present study. The oil content of seeds ranged from the lowest of 24.34 in Pala to the highest 41.00 per cent in Dharwad seed source. Such a high degree of variation in oil content was observed in neem seeds collected from different locations of Tamil Nadu by Sridharan *et al.* (1998). Similar variations were also reported by Kaura *et al.* (1998). Puri *et al.* (2005) referred the variations in the oil content in jatropha seeds collected from different regions of Chattisgarh. Such a multiple utility biofuel crop needs improvement in order to screen better genotypes with higher yield and oil content.

A large variation was observed in kernel: seed weight ratio. Ginwal *et al.* (2004) also reported a great variation among seed sources from different localities with respect to this character. Kernel: seed weight ratio was maximum in Palakkad seed source and was minimum in Kushalnagar seed source. No direct relationship was observed between kernel: seed weight ratio and oil content. In some cases in spite of high kernel: seed weight ratio, the oil content was low probably due to their genetic traits suggesting genetic variability persisting in between the seeds sources.

From the above observations, it is seen that the magnitude of seed source variations in seeds is significantly high in most of the characters studied. A significant observation was made that none of the seed sources showed highest value for all the characters. Similar reports were made by Chandragupta *et al.*(1991) and Vakshaya *et al.*(1992) in *Dalbergia sissoo*. However, Kasargod recorded highest values for seed length, KAU for seed width, Trivandrum for 100 seed weight, Neyantingara for germination percentage, mean daily germination, peak value of

germination and germination value, Palakkad for high kernel: seed weight ratio and Dharwad for highest seed oil content. As there is a wide range of variations present in the seed sources with respect to their physical and germination characteristics there is a need for further investigation of more promising trait superiority.

Seed source variations in seedling characters in nursery

Seed source variation in the nursery and in the field is genetic in nature (Sniezko and Stewart, 1989). Kjaer and Foster (1996) stated that better growth, quality and adaptability could be achieved through careful selection of the best sources when raising the seedlings for a planting programme. Gupta *et al.* (1991) while discussing juvenile-adult relationship stated that the seed sources which exhibited better performance at their juvenile stage proved to be consistently superior over a periods of years. Burdon and Sweet (1976) and Rai *et al.* (1982) also reported that one which performs well at juvenile stage may perform equally well at adult stage also. The present study was conducted under this backdrop with a view to delineate the variation in seedling characters of the various seed sources in the objective of identifying superior ones. Out of a total of 18 seed sources studied in this study, seedling parameters were evaluated for 14 seed sources.

Variations in biometric traits observed between the 14 seed sources at various phases of growth from 15 DAS to 60 DAS in the nursery was found non significant except for collar diameter at 45 DAS. Such type of discontinuous variations was also noticed in *Dalbergia sissoo* by Vakshaya *et al.* (1992). These non significant variations may be attributed to marginal genetic variation between the seed sources or the controlled growth condition provided in the nursery.

Seed source variations in field performance

The performance of the plant in the field is very important parameter that tells about the ability of the plant to adjust to the new environment both above and below ground, to produce desirable plant habit to capture maximum light and produce high photosynthates in turn higher yield, resistance to various adverse conditions, etc. Parameters like plant height, number of branches, number of leaves, number of bunches of fruit, number of fruits per bunch and collar diameter are having direct relationship with the ultimate yield of the plant (Shankaran, 2003). So studies conducted to get information on all these characters. So field evaluation of all the seed sources was carried out during the present study.

A significant variation in biometric characters was observed between seed sources at various phases of growth from 90 to 330 DAP. Similar observations were made by Ginwal *et al.* (2004) in *Ecualyptus camaldulensis* and by Demirci and Bilir (2001) in *Cedrus libani.* Variations in plant height were observed between the 18 seed sources at all the growth phases from 90 to 330 DAP which were found significant. At 90 DAP the maximum height was observed for KAU while the minimum was for Kushalnagar. The maximum height was later replaced by Kasargod seed source from 150 DAP to 330 DAP. Kushalnagar seed source remained consistently inferior through out the entire growth phases. In *Jatropha curcas* Kaushik *et al.* (2003) reported that the height of the plant was significantly influenced by the size of seeds. Chhillar *et al.* (2002) reported the direct relationship between seed weight and seedling vigor. These reports back up the results obtained in the present study. Seed source studies in *Jatropha curcas* with respect to the plant height growth are scanty. However, such variations have been reported on many other tree species viz., *Eucalyptis tereticornis* (Otegbeye, 1990), *Pinus oocarpa* (Zashimuddin, *et al.*, 1991), *Acacia auriculiformes* (Aini *et al.*, 1994; Awang, 1994), *Acacia nilotica* (Balakrishnan and Toky, 1995; Ginwal *et al.*, 1995) and *Pinus caribaea* (Swain and Patnaik, 1996).

Seed sources exhibiting faster height growth are generally regarded as suitable for areas where weed competition is severe. In this context, Kasargod seed source is more promising with respect to the plant height concerned (Fig. 4).

The results showed marked differences among seed sources with respect to the number of branches at the growth phases from 210DAP to 330DAP (Fig 5). Palakkad performed best through out the growth phases from 210 to 330 DAP. For the entire period of growth of 330DAP in the field the minimum number of branches was recorded for Kushalnagar seed source. Such seed source variations in number of branches were reported in neem by Dillon *et al.* (2003) and Kumar *et al.* (2004 a,b) in jatropha. Number of branches is an important character that determines plant shape which in turn determines total biomass production. This trait is desirable, if the medicinal, fodder and fuel wood uses of the plant is considered.

Variation in number of leaves was reported in jatropha by Geetanjali *et al.* (2004). The performance of different seed sources on leaf number have been reported in *Gliricidia sp.* (Rajaram, 1990), *Bassia latifolia* (Jenner, 1995), *Acacia nilotica* (Balakrishnan and Toky, 1995) and *Acacia catechu* (Kumar *et al.*, 2004). More number of leaves presumably indicate more leaf area in turn more biomass is allocated to leaves in order to increase the photosynthetic efficiency of plants. This also contributes to the production of more seed yield per plant. Results showed that at 330 DAP, the number of leaves are maximum with the KAU seed source followed by Kasargod (Fig. 6). This can be attributed to the increased height growth of these seed sources at 330 DAP. This is in consonance with the results obtained by Jayasankar (1996) in teak.

The results showed marked differences among seed sources in their performance in terms of collar diameter at 150 DAP (Fig 7). Kasargod excelled all

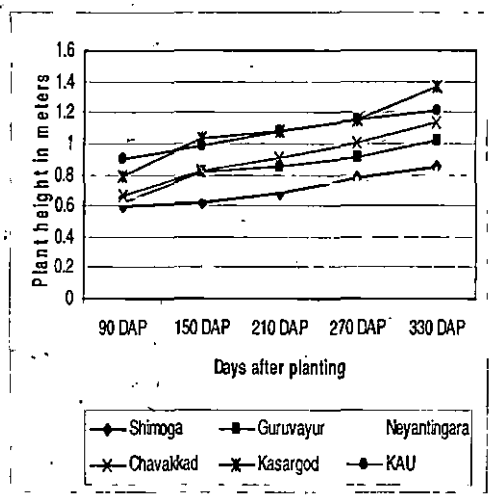


Fig. 4 Seed source variation for plant height (m) in top six seed sources

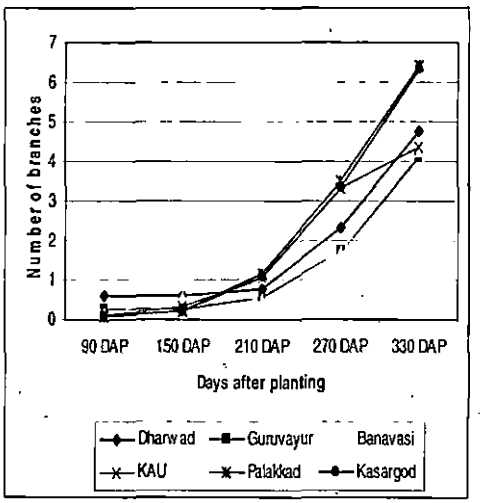


Fig. 5 Seed source variation for number of branches in top six seed sources

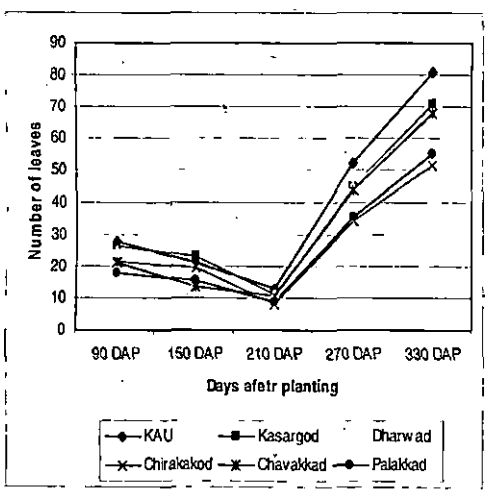


Fig. 6 Seed source variation for number of leaves in top six seed sources

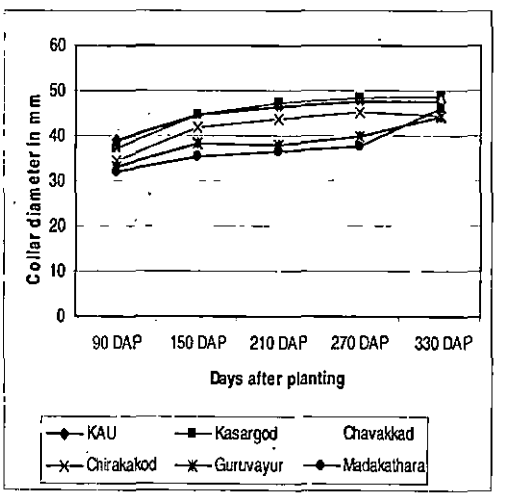


Fig. 7 Seed source variation for collar diameter in top six seed sources

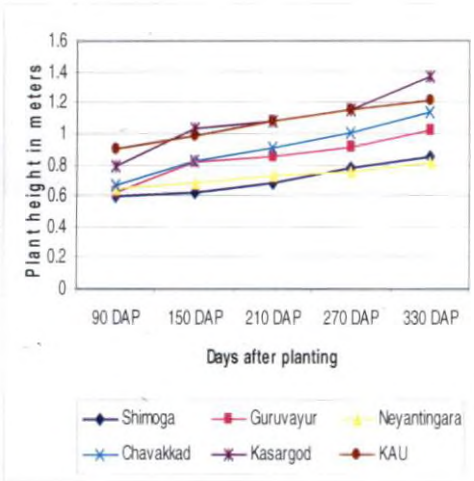


Fig. 4 Seed source variation for plant height (m) in top six seed sources

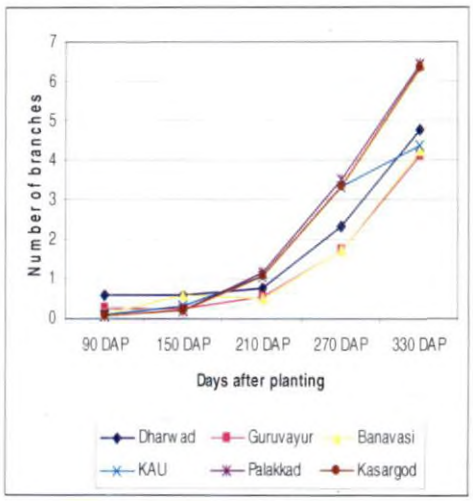


Fig. 5 Seed source variation for number of branches in top six seed sources

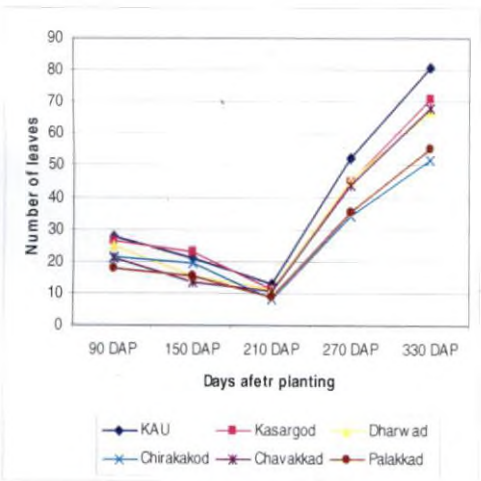


Fig. 6 Seed source variation for number of leaves in top six seed sources

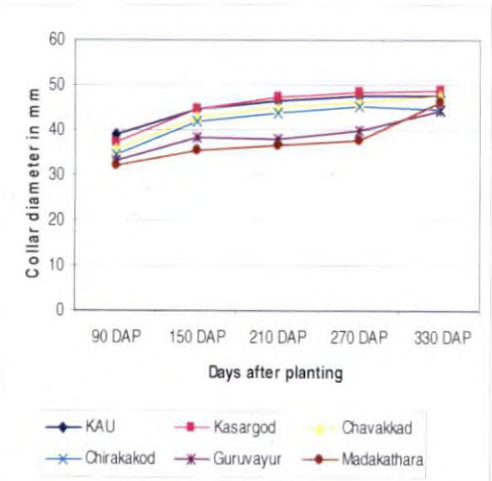


Fig. 7 Seed source variation for collar diameter in top six seed sources

the other seed sources through out the growth phases after 150 DAP, while the worst performance was exhibited by Kushalnagar seed sources. Wide variation among seed sources and between growth phases were reported in *Tectona grandis* (Kedharnath and Matthewa, 1962), *Pinus sp.* (Namkoong and Conkle, 1976), *Leucaena leucocephala* (Palit, 1980) and *Populus sp.* (Jha *et al.*, 1991). The Kasargod seed source excelled all the other seed sources in terms of plant height and collar diameter. These results are in conformity with the findings of Amara (1987) who reported that the seed source which exhibited faster height growth also characterized by quicker radial expansion.

5.2 Clonal propagation through rooting of cuttings

Applied tree improvement programmes consist of not only the development of elite material but the mass production of the improved stock. Unless the improved material is mass multiplied and supplied to the commercial sector the tree improvement programme will be genetic dead end. But the use of traditional method of planting by seeds may not be advisable because of segregation and slower rate of growth. In order to overcome the inherent biological problems connected with the seeds, vegetative propagation could be tried as potential means of production of quality planting stock. Thus, vegetative propagation is generally considered as important part of tree improvement programme in regeneration. The goal is to get the best planting stock with highest genetic quality (Nanda, 1968; Wright, 1975 and Hartmann and Kester, 1983).

Jatropha curcas is a species which is very much amenable to conventional methods of propagation. Swamy and Singh (2006) reported that *Jatropha curcas* can be propagated more cheaply and quickly by means of vegetative propagation through rooting of stem cuttings. Studies conducted on the rooting of stem cuttings revealed that the rooting potential mainly depends on season, age and size of cuttings. Clavo *et*

al. (2000) reported the practice of vegetative propagation of *Jatropha curcas* for raising live fences in pastures. The present experiment was carried out to increase rooting percentage in cuttings and enhance the production rate by propagules from limited number of elite planting stock. This was attempted through various hormone treatments.

A significant difference was observed in the percent sprouting between the treatments. A gradual reduction in the number of cuttings sprouted was noticed from 15 DAP to 60 DAP in all the treatments (Fig. 8). This may be attributed to the death of pre existing axillary buds on the stem due to poor root formation in the cuttings. Maximum sprouting was observed in treatment involving IAA 100 ppm, which was on par with the control. In *Anogeissus pendula* the hormonal treatment was found to be less effective in this respect (Rai *et al.*, 2002). By this it can be presumed that the auxins have no considerable effect on the sprouting of cuttings. Gambhir (2003) also quoted that propagation of *Jatropha curcas* through stem cuttings ensure uniformity and easy establishment without any hormonal treatment. Zahavi (2005) in his study revealed that *Jatropha curcas* has comparatively high establishment success than the other species. May be for this reason the plant is being planted successfully through stem cuttings by farmers as a live fence around their farms. However, the sprouting was significantly higher in the semi-hardwood cuttings than in softwood cuttings. Similar results were also obtained *Platanus acerifolia* (Aiton) Willd. by Dias *et al.* (1999) where the semi-hardwood cuttings increased the sprouting, shooting and rooting process.

As the length of cutting is very important which decides the number of planting stocks that could be prepared from the unit quantity of plant propagules, various length cuttings were used in the study. Lindqust and Ong (2005) stressed the importance of shoot height and diameter while explaining the plant survival in the field. The present study was conducted to know the effect of various growth

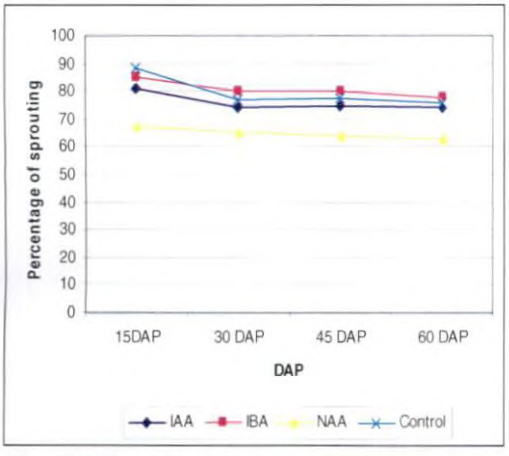


Fig. 8 Effect of auxins on percent sprouting

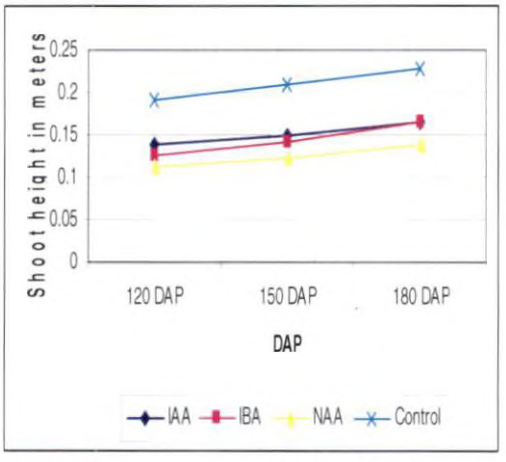


Fig. 9 Effect of auxins on shoot height

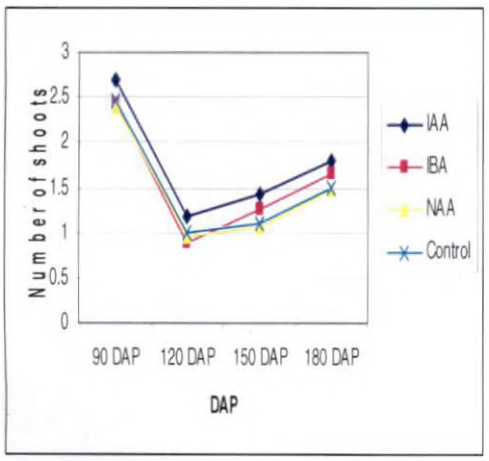


Fig. 10 Effect of auxins on number of shoots

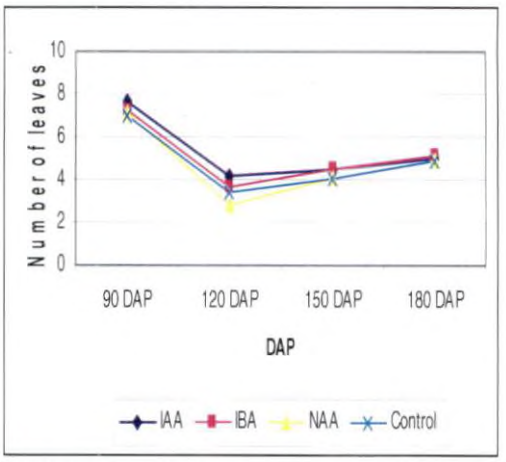


Fig.11 Effect of auxins on number of leaves

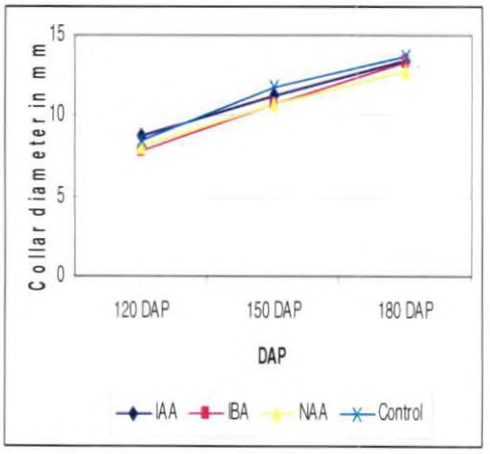


Fig.12 Effect of auxins on collar diameter

hormones on all these characters. A gradual increase in the length of shoots was observed in all the treatments (Fig. 9). In all the treatments, 20 cm length cuttings were found superior to that of 10 cm cuttings with respect to the percentage of sprouting.

There was a significant difference noticed between the treatments with respect of shoot length though the treatments were not superior to the control. Similar results were been reported by Swamy *et al.* (2002). He reported that auxin treatment is not essential and the planting stocks show good growth when planted in good rooting media and irrigated properly. In all the treatment semi-hardwood cuttings were found superior to softwood cuttings. The length of cuttings also showed significant difference in the shoot length. The 20 cm long cuttings performed better in comparison with the 10 cm long cuttings.

Number of shoots is one of the most important characters desired by the grower as it has a direct relation on the total biomass and yield. More the number of fruit bearing branches more will be the seed yield and in turn more oil could be obtained from a unit area. In earlier periods of growth the cuttings showed more number of shoots. But a gradual reduction in the number of shoots was observed in the later stages of growth (Fig.10). These shoots might have originated from the pre existing buds in the stem. As the cuttings lack well developed root system they might have dried. But, as the cuttings start producing new roots an increase in the number of shoots was observed. A significant difference was noticed between the treatments with respect to the number of branches. Irrespective of their age and length, the cuttings exhibited maximum number of shoots in IAA 250 ppm. The results of the experiments conducted by Karoshi and Hegde (2002) in *Pongamia pinnata* and Thakur and Pant (2002) in *Alnus nitida* also support the results of the present study. However, the semi-hardwood cuttings were found superior to softwood cuttings. In all the treatments 20 cm cuttings gave better responses than 10 cm cuttings.

Leaves form an important part in the plant body which acts as a source for the photosynthesis for the production of sink, biomass. So, the study on the number of leaves was done. No significant difference was observed in the number of leaves. The changes in the number of leaves from 90 DAP to 180 DAP are shown in Fig.11 Cuttings in all the hormonal treatments performed in the same manner with respect to this character. However, semi-hardwood and 20 cm cuttings performed better than softwood and 10 cm cuttings, respectively, for this character.

Plant survival in the field greatly depends upon the stem diameter (Lindqust and Ong, 2005). In the present study significant variation in the collar diameter was observed between the two length categories. 20 cm cuttings were found better than 10 cm cuttings with respect to this character. Presence of large number of buds will produce large number of shoots which indirectly might have influenced the collar growth. Singh (2006) also observed that the cuttings of the branch measuring 20-25 cm in length with 4-5 buds are the best suited material for the propagation of jatropha as they give nearly 80-90 per cent rooting. The NAA 250 ppm was inferior to all the other hormonal treatment including control. Fig.12 shows the gradual changes in the collar diameter from 120 DAP to 180 DAP of the shoots

The change of focus from quantity to quality needs to be viewed seriously as recent nursery manuals tend to focus on morphological characteristics for assessing the vitality in the planting stock. Morphological characters are easy and rapid to measure and provide reliable information on seedling / stocking quality. Root size, shape and number, root: shoot ratio, etc. are important as they show the nutrient absorption capacity, anchoring ability and relationship between the transpiration area and water absorption area in the stocking. With this in mind, the present study was designed to estimate some of these root characters also in the nursery.

With respect to the root parameters almost all characters except main root length and fresh weight of roots showed very significant difference between the

treatments. Maximum total length of roots was observed in IAA 250 and IBA 250. Results obtained by Karoshi and Hegde (2002) are also in line with the present study.

Maximum root collar diameter and number of secondary roots were observed in IAA 100, fresh weight of roots and number of primary roots was observed in IAA 250 and NAA 500 exhibited maximum values for dry weight of roots. In all the treatments the least values were recorded for the control. For all the rooting characters studied the semi-hardwood cuttings showed better responses as against the softwood cuttings.

Kaushik and Kumar (2005) also stated about the importance of age of jatropha cuttings on the rooting, sprouting and survival of the stocking. In his study semi-hard wood cuttings which were collected from the base of the stem gave better responses as compared to the softwood cuttings collected from the tip and middle portion of the stem. In the current study also semi-hardwood and 20 cm cuttings showed better performance over the softwood and 10 cm cuttings with respect to the shoot characters (Fig.13 to Fig.17).

In the present study, even though there was no much great effect of growth hormones on shooting behaviour, roots flourished well in the presence of root hormone treatment. For almost all the root characters studied the effect of hormone was very prominent as against the control (Fig.18 to Fig.21). The reports of various authors in *Jatropha gossypiifolia* (Kumar and Swarnkar, 2003), *Jatropha curcas* (Zahavi, 2005), *Quercus* spp. (Coggeshall, *et al.*, 2003), Bougainvillea (Singh *et al.*, 2003) and *Rhipsalis* grandiflora (Stancato, *et al.*, 2003) also support the positive effect of growth hormone on the rooting behaviour.

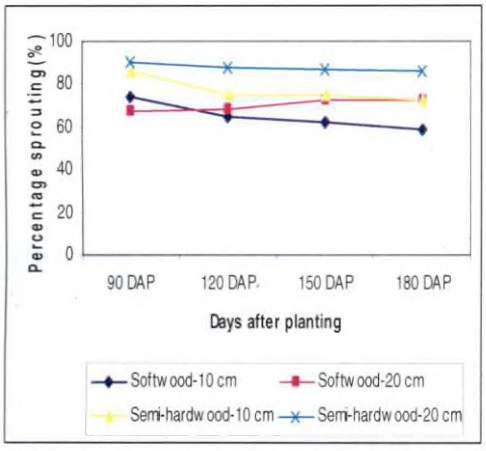


Fig13. Effect of cutting age and length on sprouting

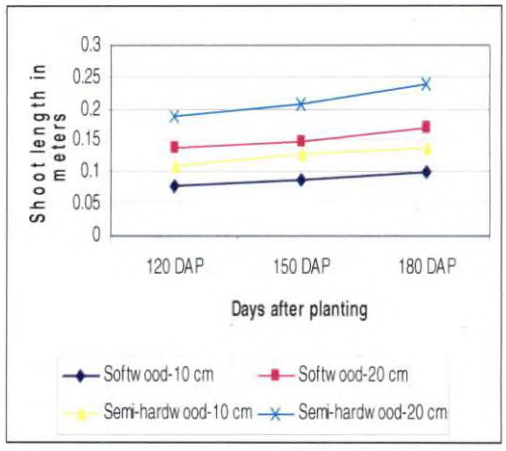


Fig.14 Effect of cutting age and length on shoot length

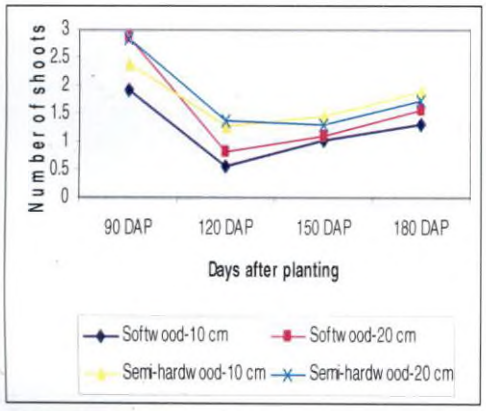


Fig.15 Effect of cutting age and length on number of shoots

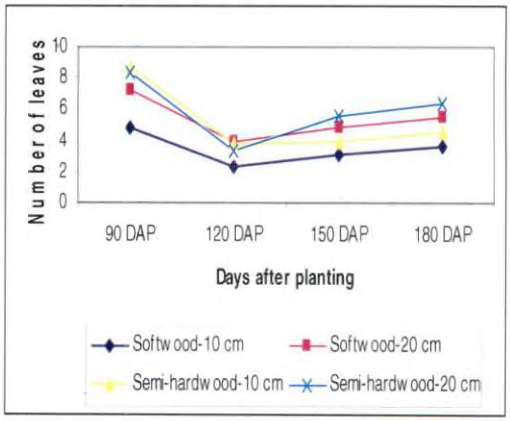


Fig.16 Effect of cutting age and length on number of leaves

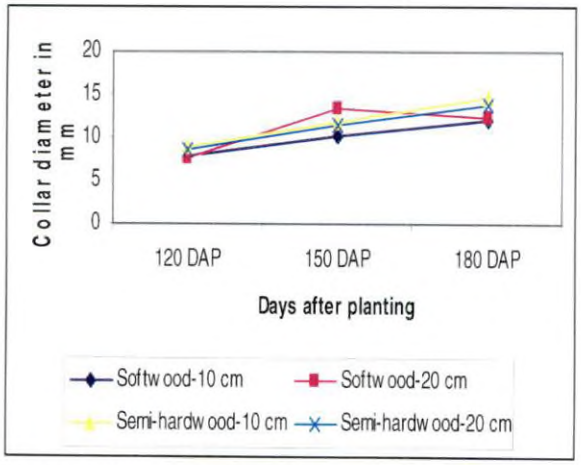


Fig. 17 Effect of cutting age and length on collar diameter (mm)

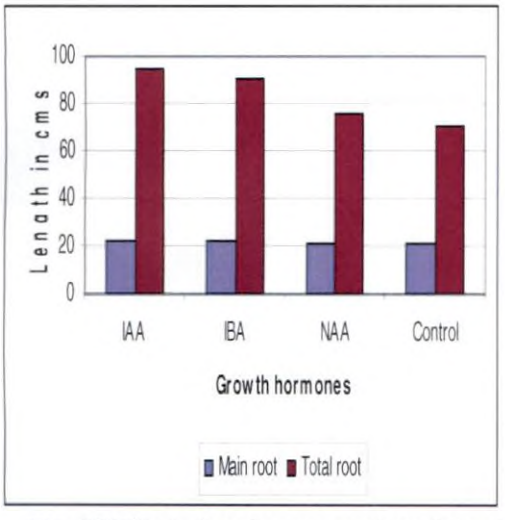


Fig. 18 Effect of auxins on root length (cm)

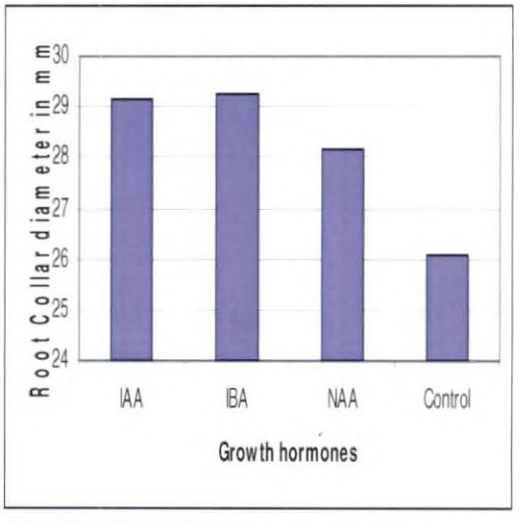


Fig. 19 Effect of auxins on root collar diameter (mm)

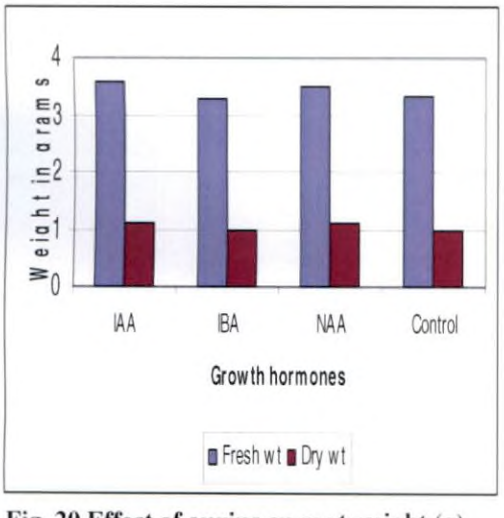


Fig. 20 Effect of auxins on root weight (g)

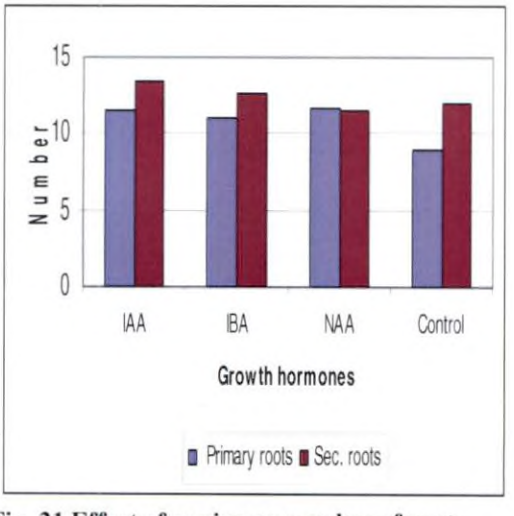


Fig. 21 Effect of auxins on number of roots

5.3 Clonal propagation through micro propagation

Mass production of selected clones through *in vitro* techniques i.e. micro propagation is of great importance in clonal forestry to overcome the constraints like scarce seed supplies, germination problems, long regeneration time, etc. (Leaky, 1987).

Jatropha curcas is a plant where conventional vegetative propagation techniques are economically feasible. However, production of large number of quality propagules from limited amount of elite material necessitates that the micro propagation techniques needs to be standardized. The results of various experiments conducted on micro propagation of *Jatropha curcas* by using nodal explants from mature trees during the present investigation are discussed below.

Surface sterilization and culture contamination

Microbial contamination is the single most important cause of losses in commercial and scientific plant tissue culture laboratories (Leifert and Woodward, 1998). All cultures will end up with contamination if the explants used are not properly disinfected. At times control of contamination is extremely difficult and with many contaminants it is impossible (Leifert and Woodward, 1998). Hence, more emphasis will have to be placed on early detection and prevention of contamination at source itself. In the present study, culture contamination was a serious problem since, the mother plants were growing in the open field. It was observed that there was almost 100 per cent contamination during the rainy season. This may be due to the congenial and conducive weather conditions which favor the rampant proliferation of microbial inoculum in the field.

Among the various measures taken to control culture contamination, a substantial reduction in culture contamination was obtained by immersing the explants in 0.1 per cent $HgCl_2$ for 15 minutes. However, a minimum contamination of cultures was noticed when the explants were dipped in a solution of Bavistin and Indofil M 45 (both at 0.2 %) for 1 hour and finally surface sterilized by using 0.1 per cent $HgCl_2$ for 15 minutes. Use of $HgCl_2$ as a surface sterilant is well documented (George and Sherrington, 1984; Kumar, 1993; Divatar, 1994; Kannan, 1995; Roshni, 2003 and Money, 2006). Pre-soaking of the explants in fungicidal solution for reducing fugal contamination has been suggested by Broome and Zimmerman (1978) in black berry, Money (2006) in *Jatropha curcas*.

It is always desirable for economical feasibility of tissue culture technique to have minimum percentage of contamination. In many cases contamination was found as late as after two weeks of culturing and even after the bud sprout. This may be due to the presence of latent spores of fungus or other microorganisms deep inside the tissues which easily get survived in the surface sterilization treatment and later when metabolic process starts, their growth also is favored and get expressed after a long gap. So, it is advisable to give a treatment to the mother plant growing in the open area by drenching systemic fungicidal solution into the root zone with an interval of 15 days.

Predominant influence of seasonal variation on microbial interference in cultures of *Jatropha curcas* was noticed (Table 37). The peak contamination which was seen during June to October can be directly related to the high ambient relative humidity prevailing during the season, which favors an increased amount of microbial inoculum in the environment. This is further supported by the relatively low contamination rates during the dry seasons of February to May. Such a season dependent culture contamination has been reported earlier by Hu and Wang, 1983

and Divatar, 1994. Seasonal influence on physiological state of plant and its effect on culture establishment has also been reported (Borrod, 1971; Seabrook *et al.*, 1976).

Basal media and effect of various growth regulators on culture establishment and growth

The most extensively used media for micro propagation in trees are MS (Murashige and Skoog, 1962), WPM (Lloyd and Mc Cown, 1980) and B5 medium (Gamboorg *et al.*, 1976). A comparative study of three media on culture establishment and growth of axillary bud was carried out. Through initial screening it was found that MS medium has significantly better performance for the culture characteristics studied in comparison with WPM and B5 medium (Fig. 22). In some earlier studies also Murashige and Skoog medium was reported as the best medium for shoot production in *Jatropha curcas* (Sujatha and Mukta, 1996; Sardana *et al*, 1998; Sujatha *et al.*, 2000; Rajore *et al.*, 2002 and Money, 2006) and in *Jatropha podagrica* (Jesus, *et al.*, 2003).

Phytohormones (auxin and cytokinins) or their synthetic counterparts are required either singly or in combination to initiate and maintain cell division. These hormones are physiologically active in very small quantities. The concentration and ratio of hormone requirement may vary from plant to plant and should be standardized for particular plant tissue. Axillary bud is a predetermined organ with morphogenic potential to develop into a shoot in the absence of apical dominance. The application of cytokinins to the axillary buds can overcome the effects of apical dominance and stimulate lateral buds to grow in the presence of terminal bud (Sachs and Thimman, 1964). Rajore *et al.* (2002) also tried axillary buds for the in *vitro* production of multiple shoots in *Jatropha curcas*.

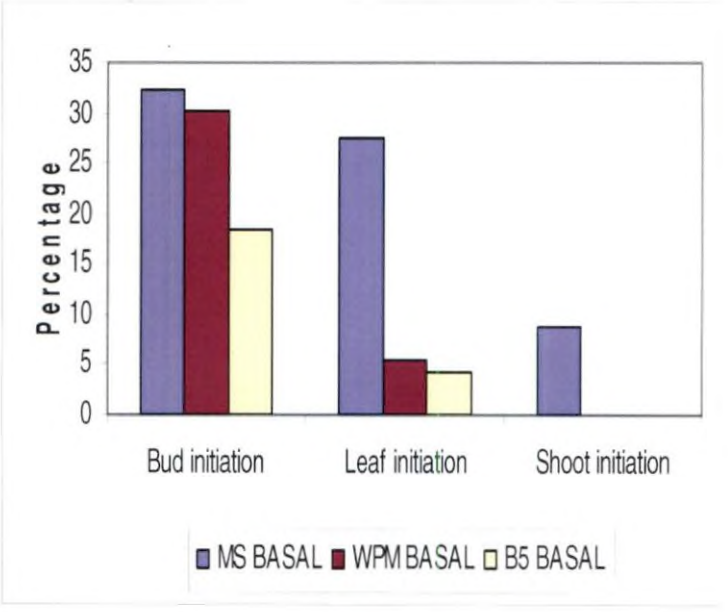


Fig.22 Effect of different basal media on culture response

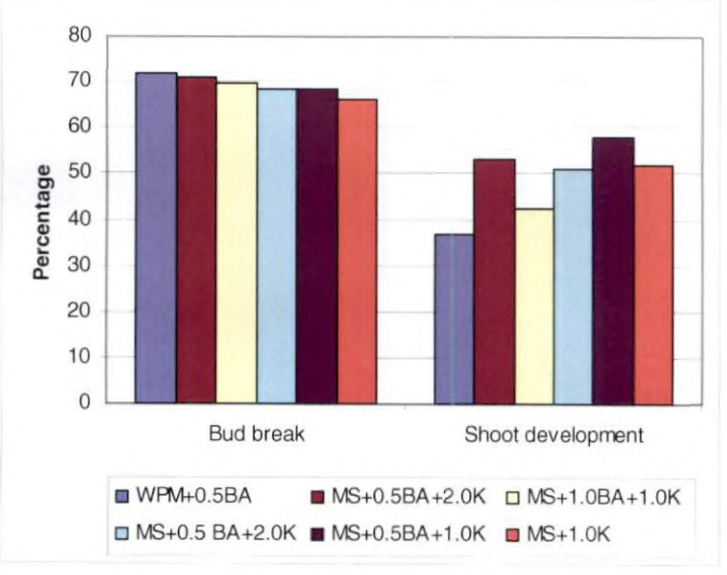


Fig. 23 Top six media combinations and their relative performance

Considering the above observation, more detailed studies were carried out by supplementing the MS medium with various combinations of plant growth regulators for axillary bud cultures of *Jatropha curcas*. Benzyl Adenine (BA) is known to induce better shoot growth and multiplication than any other cytokinins particularly in tree species (Ahmed, 1990). In the present investigation, however, BA was found to be less effective in inducing bud break and leaf initiation in comparison with kinetin when supplemented singly in both MS and WPM media.

Lower concentrations of kin lead to the best growth of shoots and leaves when compared to BA (Table 41 and 42). Similar reports had been documented by many authors in *Jatropha curcas* (Sujatha and Mukta, 1996), *Jatropha integerrima* (Sujatha *et al.*, 2000), *Syzygium cumini* (Roy *et al.*, 1996), *Hevea brassiliensis* Muell. Arg. (Mendanha *et al.*, 1998) and *Azadirachta indica* A. Juss. (Roshni, 2003). Excess callus formation was observed at the tip of the explant and callus production increased with kinetin concentration. Media supplemented with lower concentrations of BA also showed higher callusing.

Results in the present study shows that increase in the BA concentration has lead to a decrease in bud break, leaf initiation, shoot initiation and shoot length (both average and maximum shoot length) obtained. This inverse relationship between concentration of BA and shoot length was earlier reported by Kannan (1995) in *Dalbergia latifolia*. In MS medium the explants performed better when concentration of BA was in between 1.0 to 2.0 mg l^{-1} while, in WPM they performed better at the lower concentrations (0.5 mg l^{-1}) of BA. Sixteen combinations of BA and kinetin were attempted in the present study. Generally there has been an increase in the response and growth performance due to synergistic action of BA and kinetin (Table 44).

Among the auxins, IBA was attempted in the present study. The use of these growth regulators in MS media is reported to be efficient in jatropha by Sujatha and Muktha (1996), Sardana *et al.* (2000), Rajore *et al.* (2002) and others. Three combinations of IBA were tried which produced satisfactory bud break. However, no further shoot development was observed. Rooting was observed in MS media containing 1.0 mg l^{-1} IBA of the combination. Similar result was also obtained by Rajore and Batra (2005) in *Jatropha curcas*.

One of the major problems that was encountered in the present study was the lack of shoot elongation. Certain media combinations have been identified which give over 50.00 per cent shoot induction. Generally these shoots grow to a maximum of about 4.0 cm and get stunted.

Some of the media combinations that have given the best response in terms of bud break and shoot development in axillary bud cultures of *Jatropha curcas* are presented in the Table 45 and Fig. 23.

Table 45 Some of the media combinations showing good bud break and shoot development in axillary bud cultures of *Jatropha curcas*.

Media combination	Bud break (%)	Shoot development (%)
WPM+ 0.5 BA	71.57	36.67
MS+ 0.5 BA+0.5 K	71.06	53.20
MS+1.0 BA+1.0 K	69.51	42.21
MS+0.5 BA+2.0 K	68.14	57.78
MS+0.5 BA+1.0 K	68.37	50.98
WPM+0.5 K	66.51	28.21
MS+1.0K	66.18	52.08
MS+2.0 IBA	61.57	0.00
MS+1.0 IBA	57.25	0.00
MS+1.0 BA+ 2.0 K	56.82	40.00

Summary

SUMMARY

The research progarmme entitled "Evaluation of seed source variation and standardization of clonal propagation techniques in *Jatropha curcas* Linn." was carried out during 2005-2007 in the College of Forestry, Vellanikkara. The salient findings from the study are highlighted below.

Evaluation of seed source variation in *Jatropha curcas*

1. Seed length differed significantly between seed sources. The maximum value for seed length was recorded by Kasargod seed source.
2. Considerable variation was observed in seed width among the seed sources and KAU excelled all the other seed sources in this respect.
3. A significant difference was observed for seed length: breadth ratio across the seed sources. Chavakkad seed source recorded the highest value for seed length: breadth ratio.
4. 100 seed weight varied from 46.44 to 75.72 g. Trivandrum seed source was the best performer in this respect.
5. Germination percentage in the nursery differed greatly between the seed sources. Neyantingara seed source with its highest germination percentage (92.61 %) was found to be superior to all the other seed sources.
6. Considerable variation was observed between the seed sources for the characters like mean daily germination, peak value of germination and germination value. Neyantingara stand first among all the seed sources for these characters.
7. Kernal to seed weight ratio ranged from 50.90 to 67.46 per cent and Palakkad attained maximum value for this character.

8. Oil content of seeds from different seed sources exhibited great variation ranged from 23.34 to 41.00 per cent. Seeds collected from Dharwad with the highest oil content excelled all the other seed sources.
9. Seed source variation for seedling characters viz., seedling height, number of leaves and collar diameter of the seedlings in the nursery were found non significant.
10. Seedling of all the seed sources exhibited 100 per cent survival and establishment. No seedling mortality was observed in any of the seed sources.
11. The best performer in terms of plant height in the field was Kasargod.
12. The maximum number of branches was recorded by Palakkad seed source.
13. The number of leaves per plant showed a significant difference among the seed sources and KAU registered the highest value for number of leaves.
14. For the entire growth period of 330 days, the maximum collar diameter was observed in Kasargod seed source.
15. All the seed sources varied significantly for most of the characters studied. None of the seed sources showed highest value for all the character. Among the eighteen seed sources three seed sources viz., Kasargod, KAU and Palakkad were found to be better in terms of most of the seed and seedling characters studied.

Clonal propagation through rooting of cuttings

1. Significant difference was observed in percent of sprouting of cuttings in different hormone treatment. Highest sprouting was recorded in IAA

100 ppm. The rate of sprouting was significantly influenced by the age of cutting where, the semi-hardwood cuttings gave the best results.

2. Maximum length of shoots was noticed in the control. The length of shoots was greatly influenced by the age and length of cuttings and it attained its maximum value in semi-hardwood, 20 cm cuttings.
3. IAA 250 ppm recorded highest number of shoots. Semi-hardwood cuttings excelled soft wood cuttings and 20 cm cuttings were found superior to 10 cm cuttings with respect to this character.
4. The growth hormones were found to have no significant difference on the number of leaves. However, semi-hardwood and 20 cm cuttings were found to have good results for this character.
5. Semi-hardwood cuttings gave good results than softwood cuttings in respect of collar diameter. Growth hormones had no great influence on the collar diameter, which performed similar to control.
6. Almost all the root characters are found to be greatly influenced by growth hormones.
7. Total length of root exhibited its maximum value in treatment IAA 250.
8. Collar diameter of roots differed significantly between treatments and IAA 100 recorded the maximum value for this character.
9. Growth hormones were found to have no significant effect on the fresh weight of roots.
10. NAA 500 excelled all the other treatments with respect to the dry weight of the roots.
11. Maximum number of primary roots was recorded by IAA 100 while the treatment IAA 250 exhibited maximum number of secondary roots.
12. Semi-hardwood cuttings were found superior to softwood cuttings for all the root characters studied.

13. No significant difference was noticed between two length categories in all the root character studied except number of primary roots where the 20 cm cuttings showed better performance over 10 cm cuttings.

Clonal propagation through micro propagation

1. The culture contamination was noted to be one of the major problems in the jatropha tissue culture.
2. A fungicidal dip in 0.2 per cent each of Bavistin (Carbendazim) and Indofil M-45 (Mancozeb) for 1 hour followed by 15 minutes dip in 0.1 per cent $HgCl_2$ were found to be superior to all the other treatments.
3. The culture contamination was found to be greatly influenced by the season of collection of explants.
4. Out of the three basal media tried, Murashige and Skoog (MS) medium was found to be better than Woody Plant Medium and B5 medium.
5. In MS medium, treatment of 1.0 mg l^{-1} kin was found to be better in terms of bud initiation, leaf initiation and shoot initiation, while in WPM 0.5 mg l^{-1}BA was found to be better for these characters.
6. Murashige and Skoog medium supplemented with 1.0 mg l^{-1}kin was found to be the best medium for shoot production. Highest average number of leaves (3.47), maximum number of leaves (8.67) and maximum shoot length (4.0 cm) was observed in this medium.
7. Rooting was observed in MS medium added with 1.0 mg l^{-1}IBA.
8. The synergistic effect of BA and kinetin in MS medium was found to be better than supplementing them individually to MS medium especially for the enhanced release of axillary buds. The treatment MS+0.5 mg l^{-1} BA+1.0 mg l^{-1}kin has shown as much as 57.78 per cent shoot initiation

and was found to be the best treatment combination to get highest shoot initiation.

9. Increase in the concentration of kinetin reduced the percentage of bud break and leaf initiation.
10. Supplementing MS medium with auxins did not improve the culture response.
11. Callusing was observed in almost all the media combinations. The highest callusing was recorded in MS medium containing 1.0 mg l^{-1}kin.
12. Browning of the medium was noticed in some of the media combinations. It was highest recorded in MS basal medium and MS + 3.0 mg l^{-1}kin.

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Note: * Originals not seen

Appendices

Appendix I

Physical and meteorological data of different geographical seed sources

Seed source	Latitude (N)	Longitude (E)	MAR (mm)	Altitude (m)	MAT (^{0}C)	
					Max.	Min.
Banavasi	$14^0$34'	$75^0$10'	1500-2000	535	35.0	12.0
Bhadravathi	$13^0$50'	$75^0$42'	957	580	-	-
Chavakkad	10^0 53'	$76^0$05'	3129.0	102	23.0	32.0
Dharwad	$15^0$09'-$16^0$34'	$75^0$46'-$77^0$35'	818	397	39.0	16.0
Guruvayur	$10^0$52'	$76^0$21'	2540	25	35.0	20.0
Kasargod	$12^0$50'	$75^0$00'	3548.10	19	31.2	23.6
KAU	$10^0$32'	$76^0$10'	2668.6	22	23.4	28.6-36.2
Kushalnagar	$12^0$47'	$75^0$97'	2800	831	28.0	11.0
Marayur	$10^0$06'	$77^0$09'	1288.3	870	36.2	8.0
Neyantingara	$8^0$23'	$77^0$05'	1600	50	-	-
Pala	$9^0$71'	$76^0$70'	3388	57	-	-
Palakkad	$10^0$47'	$76^0$39'	2075.9	95	20.0	45.0
Shimoga	$14^0$57'	$75^0$35'	833	569	32.6	19.2
Sirsi	$14^0$36'	$75^0$53'	2657	619	35.0	9.5
Trivandrum	$8^0$29'	$76^0$57'	1500	64	34.0	16.0

Source: http:// en.wikipedia.org

Appendix- II

Weather data at Vellanikkara during the study period (2006 July to 2007 June)

Element	Year 2006						Year 2007					
	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Relative humidity (%)	85.55	82.79	84	79	72	57	54	55	63	69	74.1	84.2
Rain fall(mm)	519	550.6	522.2	323.7	79	0	0	0	0	61	86.2	608.6
Rainy days	29	15	17	11	5	0	0	0	0	4	3	17
Sunshine hours	2.09	4.25	3.9	4.8	6.5	7.8	8.7	9.8	8.2	7.7	7.11	3.77
Maximum temperature (^{0}C)	29.49	29.80	29.6	31.0	31.7	31.5	32.5	34.0	36.0	35.7	33.37	29.91
Minimum temperature (^{0}C)	23.26	23.06	23.0	23.0	23.7	23.6	22.0	22.2	24.4	25.0	24.65	23.59

Source: Department of Meteorology, College of Horticulture, KAU, Vellanikkara

Appendix III Nursery lay out for clonal propagation

R 1	R 2	R 3
1	23	44
2	1	43
3	40	42
4	15	41
5	44	40
6	29	39
7	39	38
8	33	37
9	24	36
10	31	35
11	20	34
12	2	33
13	25	32
14	9	31
15	42	30
16	7	29
17	10	28
18	12	27
19	38	26
20	6	25
21	13	24
22	19	23
23	27	22
24	43	21
25	3	20
26	14	19
27	32	18
28	17	17
29	18	16
30	22	15
31	30	14
32	11	13
33	21	12
34	37	11
35	8	10
36	26	9
37	16	8
38	41	7
39	35	6
40	5	5
41	34	4
42	4	3
43	36	2
44	28	1

Appendix IV

Chemical composition (mg/l) of various culture media used *in vitro* propagation of *Jatropha curcas*

Components	MS Medium	WPM medium	B5 Medium
$(NH_4)_2SO_4$	-	-	134
$MgSO_{4\times}7H_2O$	370	370	500
KC1	0.83	-	-
K_2SO_4	-	990	-
$CaC1_{2\times}2H_2O$	440	96	150
KNO_3	1,900	-	3,000
KCl	0.83	-	-
$Ca(NO_3)_{2\times}4H_2O$	-	556.0	-
NH_4NO_3	1,650	400.0	-
$NaH_2PO_{4\times}H_2O$	170.0	170.0	150
KH_2PO_4	170	-	-
$FeSO_{4\times}7H_20$	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3
$MnSO_{4\times}4H_2O$	22.3	22.3	10 (1 H_2O)
$ZnSO_{4\times}7H_2O$	8.6	8.6	2
$CuSO_{4\times}5H_2O$	0.025	0.025	0.025
$CoC1_{2\times}6H_2O$	0.025	-	0.025
K1	0.83	0.83	0.75
H_3BO_3	6.2	6.2	3
$Na_2M_0O_{4\times}2H_2O$	0.25	0.25	0.25
Sucrose	3.0	2.0	2.0
Myo-Inositol	100.0	100.0	100
Nicotinic Acid	0.5	0.5	1.0
Pyridoxine HC1	0.5	0.5	1.0
Thiamine HC1	0.1-1	0.1	10

EVALUATION OF SEED SOURCE VARIATION AND CLONAL PROPAGATION TECHNIQUES IN *Jatropha Curcas* LINN.

By

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Forestry

Faculty of Agriculture
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2007

ABSTRACT

A study was conducted in College of Forestry, Vellanikkara, Trichur, during the period 2005-2007 to evaluate different seed sources *Jatropha curcas*, a potential source of producing biodiesel for their genetic variation and to standardize efficient clonal propagation techniques. The study involved the evaluation of seed sources for their seed and seedling parameters as well as field performance of the plants. Attempts were made to standardize of macro and micro propagation techniques for the multiplication of elite genotypes.

The material used for the evaluation consisted of different seed sources from various parts of Kerala and Karnataka. Although the variation among the seed sources for most of the seedling characters in the nursery was found non significant, a considerable variation was observed among them in their field performance. Considerable variation was also noticed for the seed parameters such as length, width, 100 seed weight, germination percentage, kernel: seed weight ratio and seed oil content. None of the seed sources excelled for all the characters studied. Among the different seed sources three seed sources viz., Kasargod, KAU and Palakkad seed sources were found to be superior in terms of most of the characters studied.

Standardization of rooting of cuttings was attempted with 10 and 20 cm cuttings taken from semi-hardwood and softwood parts of the stem. Three levels each of IAA, IBA and NAA were used for evaluating their efficiency for rooting as well as shoot formation in the stem cuttings. Effect of these hormones on the shoot parameter was found less significant. However, highest sprouting was recorded in IAA 100 ppm where as IAA 250 ppm recorded highest number of shoots. Almost all the root characters were found to be greatly influenced by growth hormones. The semi-hardwood cuttings were found superior to the soft wood cuttings while, the 20 cm

cuttings were found better than 10 cm cuttings with respect to most of the characters studied.

Clonal propagation of *Jatropha curcas* was attempted by micro propagation through tissue culture using nodal segments as explants. Among the three basal media tried viz., MS, WPM and B5 medium, MS was found to be better in terms of bud, leaf and shoot initiation. The culture establishment was greatly influenced by the season of culturing. All explants cultured during the rainy season were got contaminated. A fungicidal dip in 0.2 per cent Bavistin (Carbendazim) and Indofil M- 45 (Mancozeb) for 1 hour followed by 15 minute dip in 0.1 per cent $HgCl_2$ was the most effective surface sterilization procedure. Murashige and Skoog medium supplemented with 1.0 mg l^{-1} kin was found to be the best medium for shoot production. Highest average number of leaves (3.47), maximum number of leaves (8.67) and maximum shoot length was observed in this medium. The synergistic effect of BA and kin in MS medium was found to be better than supplementing them individually especially for the enhanced release of axillary buds. The treatment MS+0.5 mg l^{-1}BA+1.0 mg l^{-1} kin was found to be the best treatment combination to get highest shoot initiation.