SUMMARY AND CONCLUSION

Introduction:

Energy Independence is one of the vital areas to make India a developed nation. Among different types of energy sources bio-energy through plant/animal route has to play a great role as the end of fossil fuel age has already began. Plants yielding oil are considered suitable for production of bio-fuel particularly biodiesel. *Jatropha curcas* is a plant, which has come in prominence in India in past couple of years. It was mainly known in India among herbalists for its several medicinal properties.

The genus Jatropha of Euphorbiaceae family is one of the prospective biodiesel yielding tree crops. It is morphologically a diverse genus comprising 160-175 species of shrubs, rhizomatous shrubs, herbs and small trees. About nine species of Jatropha have been recorded in India. Out of these nine species *Jatropha curcas* is one of the most important biodiesel yielding crop. *Jatropha curcas* commonly called as ratanjyot, chandrajyot, Jamal gota, Jangli arandi, Kala aranda and physic nut etc, is multipurpose tree of significant economic importance. It is native of Mexico and tropical South America (Jubera, et. al., 2009). The plant is reported to have been introduced in Asia and Africa by Portuguese as an oil yielding plant. Now it is occurring throughout India including Andaman Island in semi wild condition. It is found throughout most tropics and is known nearly by 200 different names indicating its significance and various possible uses. The *specific epithet*, "curcas", was first used by Portuguese doctor Garcia de Orta more than 400 years ago and is of uncertain origin. Common names include Barbados Nut, Purging Nut, Physic Nut, or JCL (abbreviation of *Jatropha curcas* Linnaeus).

*Jatropha curcas* L. grows as a large shrub or small tree. Its leaves are large green to pale-green leaves with male and female flowers are produced on the same inflorescence, averaging 20 male flowers to each female flower, or 10 male flowers to each female flower. Fruits are produced in winter, or there may be several crops during the year if soil moisture is good and temperatures are sufficiently high and the seeds are mature when the capsule changes from green to yellow.

The seed oil ‘*Jatropha* oil’ can be easily processed to partially or fully replace petroleum based diesel fuel (Forson, 2004). Thus, the use of this plant for large-scale
bio-diesel production is of great interest with regards to solving the energy shortage, reducing carbon emission and increasing the income of farmers (Keith, 2000; Zhou et al., 2006).

*Jatropha* seeds contain 46–58% of oil on kernel weight and 30–40% on seed weight (Subramanian et al., 2005). It shows promise for use as an oil crop for biodiesel (Foidl & Elder, 1997; Henning, 1998). The main advantages of using biodiesel are its renewability, better quality exhaust gas emission and biodegradability. It does not contribute to a rise in the level of carbon dioxide in the atmosphere (Korbitz, 1999; Beet et al., 2002; Sims, 2001). The oil is also a rich source of hydrocarbon (27.0–48.5% of seed oil) and in the recent past, *Jatropha curcas* has evoked much interest all over the world as potential petro crop (Martin & Mayeux, 1985). Such a multiple utility biofuel crop needs genetic improvement in order to alter its status of wild perennial form to a cultivable crop with higher yield and oil content.

The oil is non-edible due to the presence of a toxic substance, ‘curcascine’; it is renewable resource a safe source of energy and a viable alternative to diesel, kerosene, LPG, furnace oil, coal and fuel wood (Martin and Mayeux, 1985). All parts of *Jatropha* (seeds, leaves and bark) have been used in traditional medicine and for veterinary purposes for a long time (Duke, 1988). Some compounds (Curcacyclline A) with antitumor activities were found in this plant. The seed oil can be applied to treat eczema and skin diseases and to soothe rheumatic pain (Heller, 1996). The 36% linoleic acid (C18:2) content in *Jatropha* kernel oil is of possible interest for skincare. The oil is used as a cathartic purgative (Jamalgota) and for the treatment of skin ailments (Duke, 1988). The latex itself has been found to be strong inhibitors to watermelon mosaic virus (Tewari and Shukla, 1982). The leaves and latex are used in healing of wounds, refractory ulcers, and septic gums and as a styptic in cuts and bruises.

6.2 Material and Method

6.2 Explant collection:

The seedling and mature plants were used for study the in vitro propagation of *J. curcas*. Seeds of *Jatropha curcas* were collected from five different places located in Chhattisgarh. Hundred seeds of each provenance namely Bilaspur, Jagdalpur, Mahasamund, Pendra and Raipur were planted in separate nursery beds in the
Botanical Garden of School of Life Sciences of Pandit Ravishankar Shukla University, Raipur to study the growth in plants of different provenance. The plant height and number of nodes were recorded individually, after 90 days. Nodal segments and apical buds from seedlings of each provenance were used as explants, for in vitro propagation studies. Twigs of mature trees (more than 2 years) were collected from the premises of School of Life Science. Nodal segments and apical buds were used as explants for in vitro propagation studies. Seeds were collected from mature tree and zygotic embryos were used as explants for calls initiation and differentiation studied.

**Establishment of Explants**

The plan of work used in this study was as follows:

**6.2.3 Sterilization of explants:**

**(I) Sterilization of seedling explants:**

Explants from the seedling origin of different provenance were taken from plants of four to twelve months-old plants. Nodal and apical explants, 1-2 cm in length were cut and used as explants. All the explants were washed in tap water and then with labolene (0.2%). Following a 5 minute sterilization treatment in 0.2% (w/v) aqueous solution of mercuric chloride (HgCl₂) the explants were washed 4-5 times in sterile double distilled water under aseptic condition. The explants were inoculated in culture jars containing 35.0 ml of media in aseptic conditions.

**(II) Sterilization tree explants:**

Explants were taken from mature tree of *J. curcas* grown at the botanical garden of Pt. Ravishankar University, Raipur. Nodal segment and apical bud were cut into 1 to 2 cm length along with node. All the explants were washed in tap water & then with labolene (0.2%). Following a 5 min. Sterilized treatment in 0.5% (w/v) aqueous solution of mercuric chloride (HgCl₂) & then washed 4-5 times in sterilized double distilled water under aseptic condition. The explants were inoculated in culture jars containing 35.0 ml media in aseptic conditions.

**3.1.5 Explants establishment:**

**3.1.5.1 Establishment of seedling explants:**

**Effect of BAP on explants establishment:**

The nodal segment and apical bud were selected from seedling plant and inoculated in MS medium with different concentration of BAP (0.0, 0.25, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the bud break response in vitro.
Effect of TDZ on explants establishment:
The nodal segment and apical bud were selected from seedling plants and inoculated in MS medium with different concentration of TDZ (0.0, 0.25, 0.5, 1.0 and 2.0, 4.0 mg l⁻¹) to study the bud break response in vitro.

Effect of different media on explants establishment:
The nodal segment and apical bud were selected from seedling plants and inoculated on different basal media (MS, WPM, SH, B₅) containing 1.0 mg l⁻¹ BAP to study the bud break response in vitro.

Effect of different provenances on explants establishment:
The nodal segment and apical bud from Bilaspur, Jagdalpur Pendra, Mahasamund and Raipur plants were placed on MS medium supplemented with 1.0 mg l⁻¹ BAP to study the bud break response in vitro.

3.1.5.2 Establishment of mature explants:

Effect of PVP on browning of medium and explants during establishment:
The nodal segment were selected from mature tree and inoculated in MS medium supplemented with different concentration of PVP (50 mg l⁻¹, 100 mg l⁻¹ and 200 mg l⁻¹) to study the browning of medium in mature tree.

Effect of BAP on explants establishment:
The nodal segment and apical bud were selected from mature tree and inoculated in MS medium supplemented with different concentration of BAP (0.0, 0.25, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the bud break response in vitro in mature tree.

Effect of TDZ on explants establishment:
The nodal segment and apical bud were selected from mature tree and inoculated in MS medium supplemented with different concentration of TDZ (0.0, 0.25, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the in vitro responses.

Effect of different media on explants establishment:
The nodal segment and apical bud were selected from mature tree and inoculated on different basal media (MS, SH, WPM and B₅) supplemented with 2.0 mg l⁻¹ BAP for explants establishment.
3.1.6 In vitro shoot proliferation:

3.1.6.1 In vitro shoot proliferation in cultures of seedling origin:

After 8 weeks, the micro-nodes were placed on shoot multiplication medium to study the following:

**Effect of BAP and NAA in MS medium on shoot proliferation:**

The micro-nodes were inoculated on MS medium supplemented with different concentration in combination of BAP + NAA to study the shoot proliferation response in seedling origin.

**Effect of BAP and NAA in MS medium on shoot proliferation:**

The apical buds were inoculated on MS medium supplemented with different concentration in combination of BAP + NAA to study the shoot proliferation response in seedling origin.

**Effect of different media with BAP and NAA on shoot proliferation:**

The micro-nodes were inoculated on different media (MS and WPM) supplemented with BAP + NAA to study the shoot proliferation in seedling origin.

**In vitro shoot proliferation in cultures of different provenances grown on MS medium with BAP + NAA:**

The micro-nodes from different provenance were inoculated on MS medium supplemented with BAP + NAA to study the shoot proliferation response.

**Effect of different additives on shoot proliferation:**

Individual micro-nodes were placed on MS medium supplemented with BAP + NAA and adjuvant (s). The following adjutants were used:

(a) Adenine sulphate (0.0, 10.0, 20.0 & 40.0 mg l⁻¹)
(b) Activated charcoal (0.0, 25.0, 50.0 & 100.0 mg l⁻¹)
(c) Citric Acid (0.0, 25.0, 50.0 & 100.0 mg l⁻¹)
(d) PVP (0.0, 200.0, 400 & 600 mg l⁻¹)

**Effect of liquid medium in Growtek on shoot proliferation:**

The nodes of micro nodes were inoculated on MS medium supplemented with 0.0, 0.25, 0.5, 1.0, and 2.0 mg l⁻¹ BAP and without agar to compare liquid media on shoot proliferation in seedling origin.
Effect of sub culture:

Micro nodes were sub cultured on MS medium supplemented with one time low concentration of relevant PGRs to study shoot multiplication potential of micro-nodes during sub culture.

3.1.6.2 In vitro shoot proliferation in cultures of mature origin:

After 8 weeks, the micro nodes of micro shoots developed from explants of seedling origin were placed on shoot multiplication medium to study the following:

Effect of BAP and NAA in MS medium on shoot proliferation:

The micro-nodes were inoculated on MS medium supplemented with different concentration in combination of BAP + NAA to study the shoot proliferation in mature origin.

Effect of BAP and NAA in MS medium on shoot proliferation:

The apical buds were inoculated on MS medium supplemented with different concentration in combination of BAP + NAA to study the shoot proliferation in mature origin.

Effect of different media with BAP on shoot proliferation:

The micro-nodes were inoculated on different media (MS, and WPM) supplemented with 1.0 mg l⁻¹ BAP + 0.5 mg l⁻¹ NAA to study the shoot proliferation in mature origin.

Effect of different additives on shoot proliferation:

Individual nodes were placed on MS supplemented with 1.0 mg l⁻¹ BAP + 0.5 mg l⁻¹ NAA and adjuvant (s). The following adjuvants were used:

(a) Adenine sulphate (0.0, 10.0, 20.0 & 40.0 mg l⁻¹)
(a) Activated charcoal (0.0, 50.0, 100.0 & 200.0 mg l⁻¹)
(c) Citric Acid (0.0, 25.0, 50.0 & 100.0 mg l⁻¹)
(d) PVP (0.0, 200, 400 & 600 mg l⁻¹)

Effect of sub culture:

Micro nodes were sub cultured on MS medium supplemented with one time low concentration of relevant PGRs to study shoot multiplication potential of micro-nodes during sub culture.
3.1.9 Callus Initiation and Shoot Development of *J. curcas*:

Fruits of *J. curcas* of the family Euphorbiaceae were collected from a mature tree growing in SOS in Life science in Pt. Ravishankar Shukla University Raipur. Testa of seeds was removed mechanically, and zygotic embryos were excised. Embryos were disinfected with 0.1% (w/v) mercuric chloride for 3-4 min and rinsed 5-6 times with sterile distilled water. Sterilized zygotic embryos were used as explants for somatic embryogenesis.

3.1.9.1 Callus initiation:

**Effect of 2, 4 D in MS medium on callusing:**

The zygotic embryos were inoculated on MS medium supplemented with different concentration of 2, 4 D (0.0, 0.5, 1.0, 2.0 and 4.0 mgl⁻¹) to study the callusing.

3.1.9.2 Effect of 2, 4 D and BAP on callus and shoot Initiation:

Effect of different concentration of 2, 4 D and BAP on callus and shoot initiation: After eight weeks, the callus grown on MS+ 2, 4-D were sub cultured on MS medium supplemented with combination of 2, 4 D and BAP (0.25+0.25, 0.25+0.5, 0.25+1.0, 0.25+2.0, 0.5+0.25, 0.5+0.5, 0.5+1.0, 0.5+2.0, 1.0+0.25 and 1.0+0.5 mgl⁻¹) to study the effect on shoot initiation and development.

3.1.7 Rooting:

3.1.7.1 Rooting in micro shoots of seedling origin:

The micro shoots were placed on rooting medium for following studies

**Effect of different concentration of IBA on rooting:**

The micro-shoots were inoculated on ½ MS medium supplemented with different concentration of IBA (0.0, 0.5, 1.0, and 2.0, 4.0 mgl⁻¹) to study the in vitro rooting in seedling origin.

**Effect of different concentration of NAA on rooting:**

The micro-shoots were inoculated on ½ MS medium supplemented with different concentration of NAA (0.0, 0.5, 1.0, and 2.0, 4.0 mgl⁻¹) to study the in vitro rooting in seedling origin.
Effect of combination of IAA on rooting:

The micro-shoots were inoculated on ½ MS medium supplemented with different concentration of IAA (0.0, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the in vitro rooting.

Effect of different medium on rooting:

The micro-shoot were inoculated on different media (½ MS and ½ WPM) supplemented with 4.0 mg l⁻¹ IBA to study the in vitro rooting in seedling origin.

Effect of different concentration activated charcoal on rooting:

The micro-shoots were inoculated on ½ MS medium supplemented with 4.0 mg l⁻¹ IBA and different concentration of activated charcoal (25.0, 50.0, 100.0 mg l⁻¹) to study the in vitro rooting in seedling origin.

3.1.7.2 Rooting in micro shoots of mature origin:
The shoots were placed on rooting medium for following studies:

Effect of different concentration of IBA on rooting:

The micro shoots were inoculated on ½ MS medium supplemented with different concentration of IBA (0.0, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the in vitro rooting in mature origin.

Effect of different concentration of NAA on rooting:

The micro-shoots were inoculated on ½ MS medium supplemented with different concentration of NAA (0.0, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the in vitro rooting in mature origin.

Effect of combination of IAA on rooting:

concentration of IAA (0.0, 0.5, 1.0, and 2.0, 4.0 mg l⁻¹) to study the in vitro rooting in mature origin.

Effect of different medium on rooting:

The micro-shoot were inoculated on different media ((½ MS and ½ WPM,) supplemented with 4.0 mg l⁻¹ IBA to study the in vitro rooting.

Effect of different concentration of activated charcoal on rooting:

The micro-shoots were inoculated on ½ MS medium supplemented with 4.0 mg l⁻¹ IBA and different concentration of activated charcoal (25.0, 50.0, 100.0 mg l⁻¹) to study the in vitro rooting.

3.1.8 Hardening:
3.1.8.1 Hardening of plantlets of seedling origin:

**Primary Hardening:**

The in vitro regenerated plantlets of *J. curcas* were removed from the culture tube and washed thoroughly to remove the agar. Plantlets were dipped in fungicide (0.1 % Bavistin) for 10 minutes and transplanted into net pots containing coco peat. The plantlets were reared under semi controlled temperature (25-32 °C) light (2000 lux) and covered with polyethylene to provide humidity (70-80%).

**Secondary hardening:**

After 4 week, plantlets were transplanted into nursery begs containing sand: soil (1:1) for gradual acclimatization to outdoor condition. After one month plants were shifted to Net House and kept there until plantation in the field.

3.1.8.2 Hardening of plantlets of mature tree origin:

This stage involves transfer of plantlets from aseptic condition to the environment of the green house and ultimately to the final location. The shoots were rooted and plants were transferred to soil in polybags.

**Primary Hardening:**

The in vitro regenerated plantlets of *J. curcas* were removed from the culture tube and washed thoroughly to remove the agar. Plantlets were dipped in fungicide (0.1 % Bavistin) for 10 minutes and transplanted into net pots containing Soil-rite. The plantlets were reared under semi controlled temperature (25-32 °C) light (2000 lux) and covered with polyethylene to provide humidity (70-80%).

**Secondary hardening:**

After 4 week, plantlets were transplanted into nursery begs containing sand: soil (1:1) for gradual acclimatization to outdoor condition. After one month plants were shifted to Net House and kept there until plantation in the field.

3.1.10 Study parameter and data analysis:

The parameters studies during explants establishment stage, proliferation stage, rooting, hardening stages, callus initiation stage and shoot development stage were as follows:
3.1.10.1 Explants establishment stage:
During this stage the data were recorded for the following:
(1) Shoot bud initiation,
(2) Shoot no per explants,
(3) Length per shoot,
(4) Node number per shoot.

3.1.10.2 Shoot proliferation stage:
Number of shoots produced from each node, length of each micro-shoot and number of nodes on each proliferated shoot were recorded.
The data were expressed as the following:
(1) Shoot number per node,
(2) Length per shoot,
(3) Node number per shoot.

3.1.10.5 Callus initiation stage:
During this stage the data were recorded for the following:
(1) Callus initiation percentage,
(2) Callus area.

3.1.10.6 Shoot initiation and proliferation stage:
During this stage the data were recorded for the following:
(1) Mean number of shoot on callus,
(2) Mean length per shoot,
(3) Mean number of nodes.

3.1.10.3 Rooting Stage:
During rooting stage, the data were recorded for following:
Number of rooted micro-shoots, length of root. The data were expressed as the following:
- Rooting percentage,
- Root number per micro shoot,
- Root length per explants.
3.1.10.4 Hardening:

Survival of hardened plants was recorded with respect to particular substratum used during the primary and secondary hardening stage.

**Statistical Procedure:**

Statistical methods were used for comparison of treatment means for the different parameters measured during optimizing the protocol for micro-propagation. Completely randomized Designs were used. Each experiment consisted of 10 replicates and experiment was repeated 3 times. The data was analysed by using one or two-way analysis of variance (ANOVA), standard error (SE) was plotted after means. Mean comparisons were made by least significant difference at the 5% probability level.

**RAPD analysis:**

Random Amplified polymorphic DNA (RAPD) marker were used to measure genetic diversity of *J.curcas* a important medicinal plant, collected from five different provenance of Chhattisgarh i.e. Raipur, Bilaspur, Pendra, Mahasamund and Jagdalpur.

3.2.2 Procedure:

3.2.3. DNA Extraction:

3.2.3.1. Leaf samples:

The seeds of different provenances were grown in a nursery bed. Total DNA was extracted from leaf samples collected from individual plants in nursery. The sample size was 3 plants per provenance. Total 15 plants from five provenances were included in the study. The distance between plants collected was at least 70 km to increase the possibility of detecting the variation potential within each population.

3.2.4.2 DNA extraction methods:

Khanuja et al. (1999) DNA extraction methods were tested with samples. Young leaves of *J.curcas* was taken from the seedling of different provenance and DNA was extracted from per gram of fresh leaves ground to a fine powder in a mortar in liquid nitrogen.

3.2.4.2.1 Extraction of genomic DNA from 15 samples was performed according to Khanuja et al. (1999) using CTAB:
3.2.5 RAPD PCR:

3.2.4.1 PCR for DNA amplification

Amplification of genomic DNA was made on a DNA Engine thermal cycler (MJ Research, USA), using the arbitrary decamers. The 15 primers were selected from the different provenances.

6 Optimization of RAPD Protocol:

Because the RAPD-PCR technology is sensitive to change in experimental parameters, a total 15 primers were initially screened against 15 plants selected from all five populations. The effect of magnesium, template DNA concentration, pH values, and length of the denaturation stage of the amplification were all examined. When trying to optimize annealing temperatures, we ran the test reaction at 35 °C, 39 °C and 42 °C. The decamer primer can be clearly amplified at 35 °C. Subsets of 20 primers for further analysis were based on the following criteria: (i) consistent, strong amplification products, and (ii) production of uniform, reproducible fragments between replicate PCRs.

3.2.4.8 Construction of dendrogram:

Calculate the SI between each sample and use UPGMA algorithm to construct the dendrogram.

Results:

1 Shoot bud culture:

Studies were carried out on micro propagation of the J. curcas a medicinally important tree. The experiments were planned to study the influence of explants source, medium, plant growth regulators, adjuvant, etc., on growth performance at one or the other developmental stages of in vitro propagation. The observations were recorded at the end of each experiment and data were analyzed using statistical methods.

. The results of the studies on in vitro propagation from explants of seedling and established trees of J. curcas were as follows:

4.1.1 Explants of Jatropha curcas:

4.1.1.1 Seedlings of different provenances:

Hundred seeds of each provenance namely Bilaspur, Jagdalpur, Mahasamund, Pendra and Raipur were planted in separate nursery beds to study the growth in plants
of different provenance. The plant height and number of nodes were recorded individually, after 90 days. The plants of Pendra provenance were $6.63 \pm 0.23$ and $4.83 \pm 0.16$. Analysis of variance showed significant difference in plant height and number of nodes per plant. Pendra provenance produced maximum nodes per plant followed by Bilaspur, Raipur, Mahasamund and Jagdalpur provenances. Pendra > Bilaspur > Raipur > Mahasamund > Jagdalpur

4.1.2 Sterilization of explants:

Seeds of \textit{J. curcas} were collected from five different places of Chhattisgarh state viz. Bilaspur, Mahasamund, Jagdalpur, Pendra and Raipur. Seeds of each place were planted in Pt. Ravishankar University, garden. The nodal segment and apical bud explants were used to initiate cultures from garden grown six to twelve month old seedlings. Apical buds and nodal segments of established trees more than 2 years old plant of \textit{J. curcas} were used as explants for micro propagation.

4.1.2.1 Sterilization of seedling explants:

The nodal segments from 6 - 12 month old plants of \textit{J. curcas} were cut, washed in running tap water, sterilized with 0.2\% HgCl$_2$ for 5 to 15 minutes duration, rinsed 3-4 times in sterilized distilled water and inoculated on MS medium supplemented with (1.0 mg$^{-1}$) BAP. The contamination and bud break responses were recorded after 15 days.

The explants exposed for 10 minutes to mercuric chloride showed 100\% bud break response without contamination. Nodal segments exposed for 13 minutes duration reduced contamination as well as bud break response considerably.

4.1.2.2 Sterilization of established tree explants:

Apical buds and nodal segments of established trees more than 2 years old plant of \textit{J. curcas} were used as explants for micro propagation. The twigs were collected from botanical garden of SOS in Life science, Pt. Ravishankar University, Raipur.

The apical and nodal segment from fresh shoot of garden grown plant of \textit{J. curcas} were cut, washed in running tap water, sterilized with 0.5\% HgCl$_2$ for different durations, 5 to 15 minutes, rinsed 3-4 times in sterilized distilled water and inoculated on MS medium supplemented with 2.0 mg$^{-1}$ of BAP. The contamination and bud break responses were recorded after 10 days.
The explants exposed for 10 minutes to mercuric chloride showed 100 % bud break response without contamination. Nodal segments exposed for 17 minutes duration reduced contamination as well as bud break response considerably.

4.1.4 Establishment of explants:

4.1.3.1 Establishment of seedling explants:

The nodal segments and apical buds from 6 - 12 months old seedlings of *J. curcas* were used as explants for in vitro propagation studies:

4.1.3.1.1 Effect of MS with BAP on establishment of seedling explants

A. Nodal explants

The nodal segments were inoculated on MS medium supplemented with BAP (0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/l). The shoot bud initiation occurred maximum 100% of with mean shoot no./explant ± SE was 2.13 ± 0.03 & mean shoot length was 2.75 cm ± 0.06 and mean node no. was 2.98 ± 0.07 in this medium However, the explants on MS medium with low and high concentrations of BAP showed reduced average shoot length and node number.

The ANOVA showed significant effect of different levels of BAP in MS medium on shoots per explants at \( p \leq 0.001 \), mean shoot length at \( p \leq 0.010 \) and mean node number at \( p \leq 0.010 \). These results suggested that 2.0 mg/l BAP in MS medium was suitable medium for initiating shoot bud culture from nodal segments of seedlings.

B. Apical bud explants:

Apical buds were inoculated on MS medium supplemented with BAP (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg/l). The shoot bud initiation occurred maximum 100% of apical bud explants placed on MS medium supplemented with 1.0 mg/l BAP. In 1.0 mg/l BAP medium, the explants produced 2.19 ± 0.03 shoots per explants with 2.79 ± 0.05 shoot length (cm) per shoot and 3.15 ± 0.07 nodes per shoot.

The ANOVA showed significant effect of different levels of BAP in MS on mean shoot length at \( p \leq 0.001 \) and mean node number at \( p \leq 0.010 \) but there was no difference in shoots per explants. These results suggest that 2.0 mg/l BAP in MS medium was suitable medium for establishment in *J. curcas* from apical bud in seedling plant.
4.1.3.1.2  Effect of MS medium with TDZ on establishment of seedling explants:

4.1.3.1.3  Nodal explants:

Nodal segments were inoculated on MS medium supplemented with different concentration of TDZ (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg l⁻¹). The nodal segment placed on MS medium supplemented with 0.5 mg l⁻¹ TDZ showed 80% shoot bud initiation, maximum number of shoots per explants 1.88 ± 0.10, mean length (cm) per shoot 2.31 ± 0.16 and mean node number per shoot 2.47 ± 0.17.

The ANOVA showed significant effect of MS with different levels of TDZ on mean shoot number per explants at p ≤ 0.0, mean shoot length (cm) at p ≤ 0.001 and mean node number at p ≤ 0.010. These results suggested that MS medium supplemented with 0.5 mg l⁻¹ TDZ was a suitable medium for establishment of nodal explants of seedlings.

Apical bud explants:

Apical buds were inoculated on MS medium supplemented with different concentration of TDZ (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg l⁻¹). The apical bud explants placed on MS medium supplemented with 0.5 mg l⁻¹ TDZ showed 85% shoot initiation responses, maximum number of shoots per explants 2.07 ± 0.10, maximum shoot length (cm) per shoot 2.46 ± 0.14 and maximum nodes per shoot 2.55 ± 0.15.

The ANOVA showed significant effect of MS medium with different levels of TDZ mean shoot length at p ≤ 0.001 and mean node number at p ≤ 0.010 but there is no difference in number of shoot per explants. These results suggested that MS medium supplemented with 1.0 mg l⁻¹ TDZ was a suitable for establishment of apical bud explants of seedlings.

4.1.3.1.4  Effect of different medium with BAP on establishment of seedling explants:

4.1.3.1.5  Nodal explants:

Nodal segments were inoculated on MS medium, SH medium, WPM medium and B₅ medium supplemented with 1.0 mg l⁻¹ BAP. The nodal segment placed on MS, SH, WPM and B₅ medium with 1.0 mg l⁻¹ BAP showed 100%, 0%, 46% and 0% shoot bud initiation responses respectively. The maximum shoot number per explant 2.02 ± 0.01,
maximum shoot length per shoot 2.56 ± 0.03 and maximum node number per shoot 2.80 ± 0.03 produced in MS medium with 1.0 mg l⁻¹ BAP.

The ANOVA showed significant effect on mean shoot number at p ≤ 0.001, mean shoot length at p ≤ 0.001 and mean node number at p ≤ 0.010. These results suggested that MS medium supplemented with (1.0 mg l⁻¹) BAP was a suitable medium for establishment of nodal segment of seedling plants.

**Apical bud explants:**

The apical buds were inoculated on MS medium, SH medium, WPM medium and B₅ medium supplemented with 1.0 mg l⁻¹ BAP. The apical bud placed on MS, SH, WPM and B₅ medium with 1.0 mg l⁻¹ BAP showed 100%, 0%, 53% and 0% shoot bud initiation responses, respectively. The mean shoot number per explants 2.16 ± 0.03, mean length per shoot 2.56 ± 0.03 and mean node number per shoot 2.80 ± 0.03 produced in MS medium supplemented with 1.0 mg l⁻¹ BAP.

The ANOVA showed significant effect of mean shoot length and mean node number at p ≤ 0.001. These results suggested that MS medium supplemented with 2.0 mg l⁻¹ BAP was a suitable medium for establishment of apical bud explants of seedlings.

**4.1.3.1.4 Effect of provenance on in vitro establishment of seedling explants:**

**A. Nodal explants:**

The nodal segment were excised from the seedlings of Pendra, Raipur, Bilaspur, Mahasamund and Jagdalpur provenances and inoculated on MS medium supplemented with (1.0 mg l⁻¹) BAP, to study the effect of different provenances on the establishment of explants.

The explants derived from five different provenances showed no variation in percent shoot bud initiation response. Shoot bud initiation occurred in 100% in all five different provenances. The number of shoots per explants, mean shoot length (cm) per shoot, and mean number of node per shoot for Pendra provenance were 2.20 ± 0.05, 2.83 ± 0.08, 2.72 ± 0.06, respectively.

The ANOVA showed significant effects of provenances on shoots per explants at p ≤ 0.010, mean shoot length (cm) at p ≤ 0.001 and mean node number a p ≤ 0.010. In general, the nodal explants from Pendra provenance showed better explants.
establishment response than the explants from Raipur, Bilaspur, Mahasamund and Jagdalpur provenances.

B. **Apical bud explants:**

Apical bud were excised from the seedlings of Pendra, Raipur, Bilaspur, Mahasamund and Jagdalpur provenances and inoculated on MS medium supplemented with (1.0 mg l$^{-1}$) BAP, to study the effect of different provenances on the establishment of explants.

The explants derived from five different provenances showed no variation in percent shoot bud initiation response. Shoot bud initiation occurred in 100% in all five different provenances. The number of shoots per explants, mean shoot length (cm) per shoot, and mean number of node per shoot for Pendra provenance were $2.34 \pm 0.06$, $2.93 \pm 0.08$, $2.82 \pm 0.07$, respectively.

The ANOVA showed significant effects of provenances on shoots per explants at $p \leq 0.010$, mean shoot length (cm) at $p \leq 0.001$ and mean node number at $p \leq 0.010$. In general, the nodal explants from Pendra provenance showed better explants establishment response than the explants from Raipur, Bilaspur, Mahasamund and Jagdalpur provenances.

### 4.1.3.2 Establishment of mature tree explants:

The nodal segments and apical buds of established trees (more than 2 years old) were used as explants for *in vitro* propagation studies.

#### 4.1.3.2.1 Effect different levels of PVP in MS medium with BAP on leaching of cultures during establishment of nodal segments of mature trees:

Nodal explants of mature trees were inoculated on MS medium supplemented with 2.0 mg l$^{-1}$ BAP and different levels of PVP (50, 100, 200 mg l$^{-1}$). The nodal pieces placed on MS medium supplemented with 2.0 mg l$^{-1}$ BAP and 100 mg l$^{-1}$ PVP showed slight leaching only 6% cultures, shoot bud initiation in 100 % explants, $3.0 \pm 0.40$ shoot per explants, $4.0 \pm 0.55$ cm length per shoot and $4.0 \pm 0.52$ nodes per shoot.

The ANOVA showed significant effect of MS with different levels of PVP on shoot per explants at $p \leq 0.001$, mean shoot length at $p \leq 0.010$ and mean node number at $p \leq 0.010$. These results suggest that 2.0 g l$^{-1}$ PVP in MS medium was suitable adsorbent to control browning without adverse effect on establishment of explants of mature trees.
4.1.3.2.2 Effect of different levels of BAP in MS medium on establishment of mature tree explants:

4.1.3.2.3 Nodal explants:

Nodal segments of mature trees were inoculated on MS medium supplemented with different concentrations (0.0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg l\(^{-1}\)) of BAP and 100 mg l\(^{-1}\) PVP. The nodal pieces placed on medium with BAP (2.0 mg l\(^{-1}\)) showed 96% shoot bud initiation, 2.32 ± 0.03 shoot per explants, 3.24 ± 0.06 cm length per shoot and 3.29 ± 0.07 nodes per shoot.

The ANOVA showed significant effect of MS with different levels of BAP on shoot per explants at \(p \leq 0.010\), mean shoot length at \(p \leq 0.001\) and mean node number at \(p \leq 0.010\). These results suggest that 2.0 mg l\(^{-1}\) BAP in MS medium was suitable medium for explants establishment of mature trees.

A. Apical bud:

Apical buds were inoculated on MS medium supplemented with different concentration (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg l\(^{-1}\)) of BAP and 100 mg l\(^{-1}\) PVP. MS medium supplemented with 2.0 mg l\(^{-1}\) BAP showed maximum percentage of shoot bud initiation 100%, the explants produced 2.27 ± 0.04 shoots per explants with 2.99 ± 0.08 length (cm) per shoot and 3.33 ± 0.09 nodes per shoot.

The ANOVA showed significant effect of MS with different levels of BAP on mean shoot length at \(p \leq 0.001\) and mean node number at \(p \leq 0.010\). These results suggest that 2.0 mg l\(^{-1}\) BAP in MS medium was suitable medium for explants establishment of mature trees.

4.1.3.2.4 Effect of different levels of TDZ MS medium on establishment of mature tree explants:

4.1.3.2.5 Nodal explants:

Nodal segments were inoculated on MS medium supplemented with different concentration (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg l\(^{-1}\)) of TDZ and 100 mg l\(^{-1}\) PVP. The nodal pieces placed on medium with 1.0 mg l\(^{-1}\) TDZ showed 90% shoot bud initiation, 2.04 ± 0.06 shoots per explants with 2.58 ± 0.09 length (cm) per shoot and 2.83 ± 0.10 nodes per shoot.
The ANOVA showed significant effect of MS with different levels of TDZ on shoot per explants, mean shoot length and mean node number at $p \leq 0.010$. These results suggest that 1.0 mg l$^{-1}$ TDZ in MS medium was suitable medium for explants establishment of mature trees.

**Apical buds:**

Apical buds were inoculated on MS medium supplemented with different concentration (0.0, 0.25, 0.5, 1.0, 2.0 & 4.0 mg l$^{-1}$) of TDZ and 100 mg l$^{-1}$ PVP. The apical buds placed on medium with 1.0 mg l$^{-1}$ TDZ showed best percentage of shoot bud initiation responses 90%, the explants produced $2.10 \pm 0.05$ shoot per explants with $2.72 \pm 0.08$ length (cm) per shoot and $2.94 \pm 0.10$ nodes per shoot.

The ANOVA showed significant effect of MS with different levels of TDZ on mean shoot length at $p \leq 0.001$ and mean node number at $p \leq 0.010$. These results suggest that 1.0 mg l$^{-1}$ TDZ in MS medium was suitable medium for explants establishment of mature trees.

**4.1.3.2.4 Effect of different medium on establishment of mature trees:**

**A. Nodal segments:**

Nodal segment were inoculated on MS medium, SH medium, WPM medium and B$_5$ medium supplemented with 2.0 mg l$^{-1}$ BAP and 100 mg l$^{-1}$ PVP. The maximum percentage of shoot bud initiation 96%, the maximum shoot number per explants 2.04 ± 0.04, length per shoot $2.59 \pm 0.07$ and node number per shoot $2.81 \pm 0.08$ produced in MS medium with 2.0 mg l$^{-1}$ BAP.

The ANOVA showed significant effect of mean shoot number at $p \leq 0.010$, mean shoot length at $p \leq 0.001$ and mean node number at $p \leq 0.001$.These results suggested that MS medium supplemented with (2.0 mg l$^{-1}$) BAP was a suitable for establishment of mature trees from nodal segments.

**B. Apical buds:**

The apical buds were inoculated on MS medium, SH medium, WPM medium and B$_5$ medium supplemented with 2.0 mg l$^{-1}$ BAP and 100 mg l$^{-1}$ PVP. The apical bud placed on MS medium with 2.0 mg l$^{-1}$ BAP showed maximum shoot bud initiation 100%, the maximum shoot number per explant $2.13 \pm 0.03$, length per shoot $2.72 \pm 0.05$ and node number per shoot $2.98 \pm 0.07$.

The ANOVA showed significant effect of mean shoot length at $p \leq 0.001$ and mean node number at $p \leq 0.010$.These results suggested that MS medium
supplemented with BAP (2.0 mg/l) was a suitable for establishment of apical bud of mature trees.

4.1.4 Shoot proliferation:

4.1.4.1 In vitro shoot proliferation in cultures of seedling origin:

Experiments were performed to enhance shoot proliferation. The cultures established on MS medium with (1.0 mg/l) BAP were used for shoot proliferation. In each experiment 10 replicates were used and each experiment was repeated three times. The observations were recorded after 8 weeks.

4.1.4.1.1 Effect of different levels of BAP and NAA in MS medium on shoot proliferation:

A. Shoot proliferation from nodal segments:

The nodal segments developed on establishment medium MS medium with 1.0 mg/l BAP (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The micro nodes placed on MS medium with 0.5 mg/l BAP and 0.5 mg/l NAA showed best shoot proliferation response. They produced maximum number of shoots per micro node 4.09 ± 0.01, length per shoot 4.14 ± 0.03 and 4.20 ± 0.07 nodes per shoot.

The ANOVA showed significant difference in maximum shoot number, shoot length and node number at p ≤ 0.001. The results suggested that MS medium supplemented with 0.5 mg/l BAP and 0.5 mg/l NAA was suitable medium for in vitro shoot proliferation from micro nodes of seedling origin.

B. Shoot proliferation from apical buds

The apical buds of micro shoots on MS medium with 1.0 mg/l BAP (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The apical buds placed on MS medium with 0.5 mg/l BAP and 0.5 mg/l NAA showed better response than other concentration in combination of BAP and NAA. They produced maximum 4.15 ± 0.01 shoots per apical bud, 4.23 ± 0.02 length per shoot, 4.30 ± 0.06 nodes per shoot.

The ANOVA showed significant difference in maximum shoot number, shoot length and node number at p ≤ 0.001. The results suggested that MS medium
supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA was suitable medium for shoot proliferation from apical buds of seedling origin.

Apical segments showed better response than nodal bud, so apical segment used for further studies.

**4.1.4.1.2 Effect of different media with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA on in vitro shoot proliferation:****

The apical segments of shoots developed on establishment medium MS medium with 1.0 mg\textsuperscript{l}\textsuperscript{1} BAP (medium I) were inoculated on medium II with different formulations (MS, SH, WPM or B\textsubscript{5}) and supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA for shoot proliferation. The micro nodes on MS medium with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP + 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA showed better response compared to SH, WPM and B\textsubscript{5} media. The micro nodes placed on MS medium supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA showed mean number of shoots per micro node 4.48 ± 0.18, mean length (cm) per micro shoot 3.51 ± 0.19 and nodes per micro shoot 3.07 ± 0.07.

The ANOVA showed significant difference in maximum shoot number at \( p \leq 0.001 \), shoot length at \( p \leq 0.01 \) and node number at \( p \leq 0.01 \). The results suggested that MS medium supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA (medium II) was suitable for in vitro shoot proliferation from nodal segments of seedling origin.

**4.1.4.1.3 Effect of provenance on shoot proliferation***

The apical segments of shoots developed from explants of different provenances of *J. curcas* on explants establishment medium MS medium with 1.0 mg\textsuperscript{l}\textsuperscript{1} (medium I) were inoculated on shoot proliferation medium MS supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA (medium II). The micro nodes of Pendra provenance placed on MS medium supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP and 0.5 mg\textsuperscript{l}\textsuperscript{1} NAA showed maximum shoot number per micro node 4.55 ± 0.02, maximum shoot length (cm) per micro shoot 3.72 ± 0.10 and number of nodes per micro shoot 3.33 ± 0.09.

The ANOVA showed significant difference in shoot length at \( p \leq 0.01 \) and node number at \( p \leq 0.01 \), and shoot number at \( p \leq 0.001 \). In general, the micro nodes from Pendra provenance placed on MS medium supplemented with 0.5 mg\textsuperscript{l}\textsuperscript{1} BAP
and 0.5 mg l\(^{-1}\) NAA showed better shoot proliferation response than those of Bilaspur, Jagdalpur, Mahasamund and Raipur provenances.

4.1.4.1.4 Effect of adjuvant on in vitro shoot proliferation from nodes of seedling origin:

The micro nodes were inoculated on MS medium supplemented with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA and different concentrations of adenine sulphate (AS) or polyvinylpyrrolidone (PVP) or citric acid (CA) or activated charcoal (AC) for shoot proliferation.

(e) Effect of adenine sulphate on shoot proliferation

The micro nodes were inoculated on MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA and different concentrations (0.0, 10.0, 20.0 or 40.0 mg l\(^{-1}\)) of adenine sulphate. The maximum shoot number per micro node, mean shoot length (cm) per micro shoot and number of nodes per micro shoot on MS with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA and 40.0 mg l\(^{-1}\) adenine sulphate were 5.0 ± 0.04, 4.62 ± 0.08 and 4.60 ± 0.05. The ANOVA showed significant difference in mean shoot number at \(p \leq 0.001\), mean shoot length at \(p \leq 0.010\) and mean node number at \(p \leq 0.010\). The adenine sulphate at 10.0 mg l\(^{-1}\) concentration caused increase in mean shoot number, shoot length and node number. The results suggested that MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA supplemented with 40.0 mg l\(^{-1}\) adenine sulphate was suitable medium for in vitro shoot proliferation from micro nodes of seedling origin.

(f) Effect of PVP on shoot proliferation

The micro nodes were inoculated on MS with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA 40.0 mg l\(^{-1}\) adenine sulphate and different concentrations (0.0, 200.0, 400.0 or 600.0 mg l\(^{-1}\)) of PVP. The micro nodes placed on MS medium supplemented with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA+ 40.0 mg l\(^{-1}\) adenine sulphate and 200.0 mg l\(^{-1}\) PVP showed mean shoot number per micro node 44 ± 0.05, mean shoot length (cm) per micro shoot 4.28 ± 0.08 and number of nodes per micro shoot 4.36 ± 0.05.

The ANOVA showed significant difference in shoot length at \(p \leq 0.0\) and node number at \(p \leq 0.010\), and shoot number at \(p \leq 0.001\). The results suggested that MS
medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate supplemented with 200 mg l\(^{-1}\) PVP was suitable medium for in vitro shoot proliferation from micro nodes of seedling origin.

**Effect of activated charcoal on shoot proliferation:**

The micro nodes were inoculated on MS with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate and different concentrations (0.0, 25.0, 50.0 or 100.0 mg l\(^{-1}\)) of activated charcoal. The micro nodes placed on MS medium supplemented with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate without activated charcoal and showed maximum shoot number per micro node 4.18 ± 0.01, mean shoot length (cm) per micro shoot 4.28 ± 0.01 and mean number of nodes per micro shoot 4.39 ± 0.07.

The ANOVA showed significant difference on mean shoot number at \(p \leq 0.001\), mean shoot length at \(p \leq 0.001\) and mean node number at \(p \leq 0.010\). The results suggested that activated charcoal in MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate inhibited shoot proliferation from micro node of seedling origin.

**Effect of citric acid on shoot proliferation:**

The micro nodes were inoculated on MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate and different concentrations (0.0, 25.0, 50.0 or 100.0 mg l\(^{-1}\)) of citric acid. The micro nodes placed on MS medium supplemented with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate and 25.0 mg l\(^{-1}\) citric acid were 4.72 ± 0.08, 4.51 ± 0.06 and 4.45 ± 0.07 respectively.

The ANOVA showed significant difference in mean shoot length and node number, and shoot number at \(p \leq 0.001\). The results suggested that MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA + 40.0 mg l\(^{-1}\) adenine sulphate supplemented with 25.0 mg l\(^{-1}\) citric acid was suitable medium for shoot proliferation from micro node of seedling origin.

**4.1.4.1.5 Sub culture of nodal segments from micro shoots of seedling origin:**

Micro nodes of seedling origin were inoculated on MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA and 10.0 mg l\(^{-1}\) adenine sulfate. After every 45 days, the micro nodes were excised inoculated on fresh medium for subculture. The sub
culturing was carried out up to 5 cycles. The micro nodes at 3rd subculture showed mean number per micro node 5.24 ± 0.01, mean shoot length per micro shoot 4.91 ± 0.02 and node number per micro shoot 4.69 ± 0.03. In general, the shoot length, node number and node number gradual increase from first to third subculture and then declined in 4th and 5th sub culture.

The ANOVA showed significant effect of sub culturing on shoots per explants at p ≤ 0.001, mean shoot length at p ≤ 0.001 and mean node number at p ≤ 0.001. It is concluded that sub culturing up to 3rd cycles was beneficial for shoot proliferation.

**4.1.4.1.6 Effect of liquid medium MS with BAP in Growtek culture vessels on in vitro shoot proliferation:**

The micro nodes were inoculated on liquid MS medium supplemented with different concentrations (0.0, 0.25, 0.5, 1.0, 2.0 mg l⁻¹) of BAP. The micro nodes placed on MS medium supplemented with 1.0 mg l⁻¹ BAP showed mean shoot number per micro node 4.38 ± 0.08, mean shoot length (cm) per micro shoot 3.63 ± 0.11 and mean nodes per micro shoot 3.14 ± 0.11.

The ANOVA showed significant difference in mean shoot number, mean shoot length and mean node number (at p ≤ 0.001) in liquid MS medium supplemented with different concentration of BAP. The results suggested that MS medium supplemented with 1.0 mg l⁻¹ BAP was suitable medium for shoot proliferation from seedling origin nodes in growtek culture vessels.

**4.1.4.2 In vitro shoot proliferation in cultures of from mature tree origin:**

Experiments were performed to enhance shoot proliferation. The cultures established on MS medium with (2.0 mg l⁻¹) BAP, were used for in vitro shoot proliferation. Nodes and apical buds were separated and inoculation on shoot proliferation medium. In each experiment 10 replicates were used and each experiment was repeated three times. The observations were recorded after 8 weeks.

**4.1.4.2.1 Effect of different levels of BAP and NAA in MS medium on in vitro shoot proliferation:**

**C. In vitro shoot proliferation from nodal segments:**

The micro nodes of shoots elongated from explants of mature tree on explants establishment medium MS with 2.0 mg l⁻¹ (medium I) were inoculated on shoot
proliferation medium MS with different concentrations of BAP or combination of BAP and NAA (medium II). The micro nodes placed with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA showed better response than other concentrations of BAP and combinations of BAP and NAA. The micro node placed on MS medium supplemented with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA showed maximum number of shoots per micro node 4.08 ± 0.01, length per shoot 4.12 ± 0.03, nodes per shoot 4.19 ± 0.07.

The ANOVA showed significant difference in shoot number at \(p \leq 0.001\), shoot length at \(p \leq 0.010\) and node number at \(p \leq 0.010\). The results suggested that MS medium supplemented with the combination of 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA was suitable medium for in vitro shoot proliferation in cultures of mature tree origin.

**In vitro shoot proliferation from apical buds:**

The apical buds of micro shoots elongated from explants of mature tree on explants establishment medium MS medium with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} (medium I) were inoculated on shoot proliferation medium MS medium with different concentrations of BAP and combinations of BAP and NAA (medium II). The apical buds placed on MS medium with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA showed better response than other concentration of BAP and combination of BAP and NAA. The apical buds placed on MS medium supplemented with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA showed maximum number of shoots per apical buds 4.11 ± 0.01, 4.12 ± 0.02 length per shoot, 4.09±0.09 node per shoot.

The ANOVA showed significant difference in maximum shoot number, shoot length and node number at \(p \leq 0.001\). The results suggested that MS medium supplemented with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA was suitable medium for in vitro shoot proliferation from apical buds of mature tree origin.

Apical segments shows better shoot proliferation response

**4.1.4.2.2 Effect of different media with 0.25 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.25 mg\textsuperscript{1}l\textsuperscript{-1}NAA on in vitro shoot proliferation:**

The micro nodes of shoots developed from explants of mature tree on explants establishment medium MS with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} (medium I) were inoculated on medium II with different formulations (MS, SH, WPM or B\textsubscript{5}) supplemented with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA for shoot proliferation. The nodal segments on MS medium with 1.0 mg\textsuperscript{1}l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1}l\textsuperscript{-1} NAA showed better response compared to SH, WPM and B\textsubscript{5}
media. The micro nodes placed on MS medium supplemented with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA showed maximum number of shoots per micro node 4.53 ± 0.09, mean length (cm) per micro shoot 3.21 ± 0.12 and nodes per micro shoot 3.51 ± 0.12.

The ANOVA showed significant effect on mean shoot number at p ≤ 0.001, mean shoot length at p ≤ 0.010 and mean node number at p ≤ 0.010. These results suggested that MS supplemented with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA was a suitable medium for shoot proliferation from micro nodes of mature tree origin.

4.1.4.2.3 Effect of adjuvant on shoot proliferation from cultures of mature tree origin:

The micro nodes of *Jatropha curcas* were inoculated on MS medium supplemented with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA and different adjuvant adenine sulphate (AS) or polyvinylpyrrolidone (PVP) or activated charcoal (AC) or citric acid (CA) on different concentration.

(a) Effect of adenine sulphate on shoot proliferation:

The micro nodes were inoculated on MS medium with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA and different concentrations (0.0, 10.0, 20.0 or 40.0 mg\text{\textsuperscript{l}}) of adenine sulphate. The mean shoot number per micro node, mean length (cm) per micro shoot and nodes per micro shoot placed on MS medium supplemented with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA and 40.0 mg\text{\textsuperscript{l}} adenine sulphate were 4.91 ± 0.06, 4.61 ± 0.08 and 4.58 ± 0.05, respectively.

The ANOVA showed significant difference in mean shoot number at p ≤ 0.010, mean shoot length at p ≤ 0.010 and mean node number at p ≤ 0.001. The adenine sulphate increased shoot length, mean shoot number and mean node number. The results suggested that MS with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA supplemented with 40.0 mg\text{\textsuperscript{l}} adenine sulphate was suitable medium for shoot proliferation from micro node of mature tree origin.

(b) Effect of PVP on shoot proliferation:

The micro nodes were inoculated on MS medium with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA and different concentrations (200.0, 400.0 or 600.0 mg\text{\textsuperscript{l}}) of PVP. The micro nodes placed on MS media with 1.0 mg\text{\textsuperscript{l}} BAP + 0.5 mg\text{\textsuperscript{l}} NAA and 200.0 mg\text{\textsuperscript{l}} PVP showed mean shoot number per micro node 4.44 ± 0.07, mean length (cm) per micro shoot 4.46 ± 0.07 and nodes per micro shoot 4.34 ± 0.07.
The ANOVA showed significant difference in shoot number at \( p \leq 0.010 \), shoot length at \( p \leq 0.010 \), and node number at \( p \leq 0.001 \). The results suggested that 200.0 mg\( \text{l}^{-1} \) PVP in MS medium with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA was suitable medium for shoot proliferation from micro node of mature tree origin.

### (c) Effect of activated charcoal on shoot proliferation:

The micro nodes were inoculated on MS with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA and different concentrations (0.0, 25.0, 50.0 or 100.0 mg\( \text{l}^{-1} \)) of activated charcoal. The micro nodes placed on MS medium supplemented with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA and without activated charcoal showed maximum shoot number 4.15 ± 0.01, mean shoot length (cm) 4.16 ± 0.02 and mean nodes per shoot 4.21 ± 0.09.

The ANOVA showed significant effect of MS with 0.25 mg\( \text{l}^{-1} \) BAP + 0.25 mg\( \text{l}^{-1} \) NAA and different concentrations of activated charcoal on shoots per micro node at \( p \leq 0.010 \), mean shoot length at \( p \leq 0.001 \) and mean node number at \( p \leq 0.010 \). The results suggested that MS supplemented with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA and 25 mg\( \text{l}^{-1} \) activated charcoal was suitable medium for shoot proliferation from micro node of mature tree origin.

### Effect of citric acid on shoot proliferation:

The micro nodes were inoculated on MS medium with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA and different concentrations (0.0, 25.0, 50.0 or 100.0 mg\( \text{l}^{-1} \)) of citric acid. The micro nodes placed on MS medium supplemented with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA and 25.0 mg\( \text{l}^{-1} \) citric acid were 4.72 ± 0.06, 4.55 ± 0.07 and 4.50 ± 0.06.

The ANOVA showed significant difference in shoot number at \( p \leq 0.001 \) mean shoot length at \( p \leq 0.010 \) and node number at \( p \leq 0.010 \). The results suggested that MS medium with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA supplemented with 25.0 mg\( \text{l}^{-1} \) citric acid was suitable medium for shoot proliferation from micro nodes of mature tree origin.

### 4.1.4.2.4 Effect of sub culture on shoot proliferation from nodal segments of mature tree origin:

Micro nodes were inoculated on MS medium with 1.0 mg\( \text{l}^{-1} \) BAP + 0.5 mg\( \text{l}^{-1} \) NAA, 40 mg\( \text{l}^{-1} \) adenine sulphate. After every 45 days, the micro nodes were excised and inoculated on fresh medium for subculture. The sub culturing was carried out up to 5 cycles. The micro nodes produced mean shoot number per micro nodes 5.22 ±
0.01 and mean shoot length (cm) per micro shoot 4.90 ± 0.02 and mean node number per micro shoot 4.68 ± 0.02 at the end of 3rd subculture. In general, the shoot number, the shoot length, and node number gradually increased from first to third subculture. After third cycle the shoot number, the shoot length and node number declined.

The ANOVA showed significant effect on sub culturing on mean shoot number at p ≤ 0.001, mean shoot length at p ≤ 0.010 and mean node number at p ≤ 0.010. It is concluded that sub culturing up to 3rd cycles was beneficial for shoot proliferation from nodal segments of mature tree origin.

### 4.2 Callusing and Shoots Initiation:

#### 4.2.1 Effect of 2, 4 D on callus initiation:

Zygotic embryos of *Jatropha curcas* were inoculated on MS medium supplemented with different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l⁻¹) of 2, 4 D (Table 52, Plate 75).

MS medium supplemented with 2, 4 D (2.0 mg l⁻¹) showed maximum 95% callus induction response and maximum callus area 10.14 ± 0.10 cm² per explants.

The ANOVA showed significant effect of MS with different levels of 2, 4 D on percent of callus induction response and callus area at p ≤ 0.001. These results suggested that 2.0 mg l⁻¹ 2, 4 D in MS medium was suitable medium for callus initiation in *Jatropha curcas*.

#### 4.2.2 Effect of different combinations of 2,4 D and BAP on Shoot Differentiation

Zygotic embryo derived callus grown on MS with 2.0 mg l⁻¹ 2,4 D were inoculated on MS medium supplemented with different concentrations in combinations of 2,4 D and BAP.

MS medium supplemented with 0.5 mg l⁻¹ 2,4 D and 1 mg l⁻¹ BAP showed maximum 95% frequency of shoot with maximum callus area 10.14 ± 0.10 cm²

The ANOVA showed significant effect of MS with different levels of combination of 2, 4 D and BAP on mean number of shoot and callus area at p ≤ 0.001. These results suggest that 1.0 mg l⁻¹ 2, 4 D and 0.25 mg l⁻¹ BAP in MS medium was suitable medium for initiation and development of callus area and shoots induction from Zygotic embryo derived callus grown on MS with 2.0 mg l⁻¹ 2,4 D.
Summary and Conclusion

**Effect of different concentrations in combinations of 2,4 D and BAP on shoots from Zygotic embryo derived callus**

Zygotic embryo derived callus grown on MS with 2.0 mg l\(^{-1}\) 2,4 D were inoculated on MS medium supplemented with different concentrations in combinations of 2,4 D and BAP.

MS medium supplemented with 0.5 mg l\(^{-1}\) 2,4 D and 1 mg l\(^{-1}\) BAP showed maximum mean shoot number 3.06 ± 0.07, mean shoot length 3.61 ± 0.11 and mean node number 3.59 ± 0.11.

The ANOVA showed significant effect of MS with different levels of combination of 2, 4 D and BAP on mean number of shoot, mean shoot length, and mean node number at p ≤ 0.001. These results suggest that 0.5 mg l\(^{-1}\) 2, 4 D and 1.0 mg l\(^{-1}\) BAP in MS medium was suitable medium for initiation and shoots induction from Zygotic embryo derived callus grown on MS with 2.0 mg l\(^{-1}\) 2,4 D. The apical micro shoots were transferred to the medium supplemented with NAA and BAP.

**Effect of different levels of BAP and NAA in MS medium on shoot proliferation of shoots from Zygotic embryo derived callus:**

The apical segments developed on establishment 0.5 mg l\(^{-1}\) 2, 4 D and 1.0 mg l\(^{-1}\) BAP in MS medium (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The micro nodes placed on MS medium with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA showed best shoot proliferation response. They produced maximum number of shoots per micro node 3.06 ± 0.07, length per shoot 3.61 ± 0.11, nodes per shoot 3.49 ± 0.11. The micro nodes on other concentrations in combinations of BAP and NAA produced less number of shoots.

The ANOVA showed significant difference in maximum shoot number, shoot length and node number at p ≤ 0.001. The results suggested that MS medium supplemented with 0.5 mg l\(^{-1}\) BAP and 0.5 mg l\(^{-1}\) NAA was suitable medium for in vitro shoot proliferation from micro nodes of Zygotic embryo derived callus origin.
In vitro rooting from micro shoot:

4.1.5.1 Effect of auxins on in vitro rooting in micro shoots of seedling origin:

4.1.5.1.1 Effect of IAA:

Micro shoots of seedling origin were inoculated on ½ MS medium with different concentration (0.0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹) of IAA. The micro shoots placed on ½ MS medium with 4.0 mg l⁻¹ IBA were 66%, 1.85 ± 0.11, 2.06 ± 0.15 with 44% callusing.

The ANOVA showed significant difference in percent response at $p \leq 0.010$, root length at $p \leq 0.001$ and root number at $p \leq 0.001$. The results revealed that ½ MS supplemented with 4.0 mg l⁻¹ IBA showed maximum root length and maximum root induction responses from micro shoots of seedling origin.

4.1.5.1.2 Effect of NAA:

Micro shoots were inoculated on ½ MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l⁻¹) of NAA. The micro shoots placed on ½ MS medium with all the above concentration showed no initiation response on root number, root length per micro shoot. There were 100% callusing.

The ANOVA showed significant difference in percentage of rooting, mean root number at $p \leq 0.010$ and mean root length at $p \leq 0.001$. The results revealed that ½ MS supplemented with NAA, is not suitable for rooting of micro shoots of *Jatropha curcas*.

4.1.4.1.3 Effect of IBA on in vitro rooting of micro shoots

Micro shoots were inoculated on ½ MS medium with (0.0, 0.5, 1.0, 2.0 and 4.0, mg l⁻¹) IBA. In all these concentration maximum percentage of root initiation responses 60%, maximum root number per micro shoot 2.76 ± 0.05 and maximum root length per micro shoot 2.93 ± 0.05 recorded in 4.0 mg l⁻¹ IBA.

The ANOVA showed significant effect of MS medium with different level of IBA + NAA on percentage of root initiation responses at $p \leq 0.010$, root number at $p \leq 0.010$ and mean root length at $p \leq 0.001$. These results suggest that MS medium supplemented with 4.0 mg l⁻¹ IBA is suitable medium for rooting from micro shoot of seedling origin.
4.1.5.1.4 Effect of different media with 4.0 mg/l IBA on rooting of micro shoots:

Micro shoots were inoculated on different medium ½ MS and ½ WPM medium with 4.0 mg/l of IBA. The micro shoots placed on ½ MS medium with 4.0 mg/l IBA showed root initiation responses 50%, mean shoot number per micro shoot 1.57 ± 0.12 and mean root length per micro shoot 1.23 ± 0.09.

The ANOVA showed significant difference in percent rooting response, root length at p ≤ 0.010 and root length at p ≤ 0.001. The results revealed that ½ MS supplemented with 4.0 mg/l IBA, showed maximum root length and maximum root induction responses from micro shoot of seedling origin.

e4.1.5.1.5 Effect of activated charcoal on rooting of micro shoots:

The micro shoots were inoculated on half - MS medium containing 25.0, 50.0 and 100.0 mg/l activated charcoal and 4.0 mg/l IBA and then were transferred to medium without IBA. Micro shoots on 50.0 mg/l charcoal showed maximum rooting 80%, with 3.03 ± 0.06 mean root numbers per micro shoot and 3.18 ± 0.04 mean root lengths (cm) per micro shoot.

ANOVA revealed significant difference in root induction response, mean root number at p ≤ 0.010 and root length at p ≤ 0.001 obtained on half MS medium supplemented with 4.0 mg/l IBA and different concentrations of activated charcoal. The micro shoots, placed on half - MS medium supplemented with 4.0 mg/l IBA 50 mg/l activated charcoal showed best root induction and root length

4.1.5.1 Effect of auxins on in vitro rooting of micro shoot from mature tree origin:

4.1.5.1.1 Effect of IAA:

Micro shoots were inoculated on ½ MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 or 4.0 mg/l) of IAA. The micro shoots placed on ½ MS medium with 4.0 mg/l IAA showed maximum root initiation 60%, mean root number per micro shoot 1.62 ± 0.09 and mean root length per micro shoot 1.87 ± 0.13.

The ANOVA showed significant difference in percent of root initiation response, root length and root length at p ≤ 0.010. The results revealed that ½ MS supplemented with 4.0 mg/l IAA, induced maximum rooting, maximum root number and maximum root length from micro shoot of mature tree origin.
4.1.5.2.2 Effect of NAA:

Micro shoots were inoculated on ½ MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l⁻¹) of NAA. The micro shoots placed on ½ MS medium with all the above concentration showed no initiation response on root number, root length per micro shoot. There were 100% callusing.

The ANOVA showed significant difference in percent of root response, root number at p ≤ 0.010 and root length at p ≤ 0.001. The results revealed that ½ MS medium supplemented NAA is not suitable for rooting of micro shoots of *Jatropha curcas* of mature tree origin.

4.1.4.2.3 Effect of IBA:

Micro shoots were inoculated on ½ MS medium with different concentration (0.0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹) of IBA. In all these concentration maximum percentage of root initiation responses 60% maximum root number 2.73 ± 0.05 and maximum root length 2.88 ± 0.05 recorded in 4.0 mg/l IBA.

The ANOVA showed significant effect of MS medium with different level of IBA + NAA on percentage of rooting responses, root number and mean root length at p ≤ 0.010. These results suggest that MS medium supplemented with 4.0 mg l⁻¹ IBA is suitable medium for in vitro rooting from micro shoot of mature tree origin.

4.1.5.2.3 Effect of different media with 4.0 mg l⁻¹ IBA on rooting:

Micro shoot were inoculated on ½ MS medium, ½ WPM medium supplemented with 4.0 mg l⁻¹ IBA. The micro shoots placed on ½ MS and ½ WPM medium with IBA (4.0 mg l⁻¹) showed 50% and 30%, root initiation responses, respectively. The mean root number per micro shoot 1.39 ± 0.10, mean root length per micro shoot 1.55 ± 0.14 produced in MS medium with 4.0 mg l⁻¹ IBA.

The ANOVA showed significant effect on root initiation responses at p ≤ 0.010, mean root number at p ≤ 0.10 and mean root length at p ≤ 0.010. These results suggested that ½ MS medium supplemented with 4.0 mg l⁻¹ IBA was a suitable for rooting in micro shoot of mature tree origin.
4.1.5.2.5 Effect of activated charcoal on rooting in micro shoots of mature tree origin:

The micro shoots were inoculated on half - MS medium containing different concentrations (25.0, 50.0 and 100.0 mg l\(^{-1}\)) of activated charcoal and 4.0 mg l\(^{-1}\) IBA and then transferred to medium without IBA. Micro shoots on 50.0 mg l\(^{-1}\) charcoal showed maximum percentage of root induction 90%, with 3.11 ± 0.09 root numbers per micro shoot and 3.30 ± 0.09 root length (cm) per micro shoot.

ANOVA revealed significant difference in root initiation, root number and root length obtained with half MS medium supplemented with 4.0 mg l\(^{-1}\) IBA and 50 mg l\(^{-1}\) activated charcoal, at p ≤ 0.001. These result suggested that half - MS medium supplemented with 4.0 mg l\(^{-1}\) IBA + 50 mg l\(^{-1}\) charcoal was best medium for rooting from micro shoots of mature origin.

4.1.6 Hardening of plantlets of seedling origin:

(a) Primary hardening:

In vitro regenerated plantlets were washed thoroughly with running water to remove agar, dipped for few seconds in 0.1 % bavistin and then transferred to root trainer containing different types of substratum. The root trainers were filled with either soil rite or mixture of soil and sand (ratio 1:1). The root trainers were placed in greenhouse for primary hardening. The plantlets transferred to root trainer filled with cocopeat showed 90% survival of the micro plants. The survival was 70% of plantlets that were transferred to root trainer containing mixed soil and sand. These results suggest that cocopeat was better for primary hardening of micro shoots as compared to soil and sand mixture.

(b) Secondary hardening:

After primary hardening, the plants were transferred to polyethylene bags and placed in a net house. The plantlets transferred to root trainer filled with soil rite showed 80% survival of the micro plants. The survival was 60% of plantlets that were transferred to root trainer containing mixed soil and sand. After 60 days of acclimatization, the height of the plantlets was 6.14 cm.

These results suggest that cocopeat was better for secondary hardening of *Jatropha curcas* micro shoots compare to soil and sand mixture.
4.1.7 Hardening of plantlets of mature tree origin

(a) Primary hardening:

In vitro regenerated plantlets were washed thoroughly to remove agar, washed with running water dipped for few seconds in 0.1 % bavistin and then transferred to root trainer containing different types of substratum. The net pots were filled with either coco peat or mixture of soil and sand (ratio 1:1). The root trainers were placed in green- house for primary hardening. The plantlets transferred to root trainer filled with soil rite showed 80% survival of the micro plants. The survival was 60% of plantlets that were transferred to root trainer containing mixed soil and sand.

These results suggest that coco peat was better for primary hardening of mature origin micro shoots compare to soil and sand mixture.

(c) Secondary hardening

After primary hardening, the plants were transferred to polyethylene bags and placed in a net house, the plantlets transferred to root trainer filled with coco peat showed 70% survival of the micro plants .The survival was 60% of plantlets that were transferred to root trainer containing mixed soil and sand (Table 50). After 60 days of acclimatization, the height of the plantlets was 5.65 cm.

These results suggested that coco peat was better for secondary hardening of micro shoots as compared to soil and sand mixture.

Conclusion:

Micropropagation:

Explant source:

The seedling and mature plants were used for study the in vitro propagation of J. curcas. Seeds of Jatropha curcas were collected from five different places located in Chhattisgarh. Hundred seeds of each provenance namely Bilaspur, Jagdalpur, Mahasamund, Pendra and Raipur were successfully germinated in separate nursery beds in the Botanical Garden of School of Life Sciences of Pandit Ravishankar Shukla University, Raipur to study the growth in plants of different provenance. Nodal segments and apical buds from seedlings of each provenance were used as explants, for in vitro propagation studies.
Twigs of mature trees (more than 2 years) were collected from the premises of School of Life Science. Nodal segments and apical buds were used as explants for in vitro propagation studies.

Seeds were collected from mature tree and zygotic embryos were used as explants for calls initiation and differentiation studied.

**Sterilization of explants:**

Sterilization of seedling origin explant with 0.2% (w/v) aqueous solution of mercuric chloride (HgCl₂) for 5 min was suitable to achieve maximum establishment and minimum level if contamination for nodal and apical explant.

Sterilization of mature origin explant with 0.5% (w/v) aqueous solution of mercuric chloride (HgCl₂) for 5 min was suitable to achieve maximum establishment and minimum level if contamination for nodal and apical explant.

**Establishment of Explants:**

**Establishment of seedling Explants:**

**Nodal segment:**

The nodal explant placed on MS media with 1.0 mg l⁻¹ BAP showed 100% frequency of shoot bud induction. The mean shoot no./explant ± SE was 2.13 ± 0.03 & mean shoot length was 2.75 cm ± 0.06 and mean node no. was 2.98 ± 0.07 in this medium. The nodal segment placed on WPM, SH and B₅ medium containing 1.0 mg l⁻¹ BAP showed less % frequency of shoot bud induction. Similarly, nodes on MS medium with TDZ showed comparatively less shoot bud initiation than MS with BAP.

The nodal segment from seedling of Pendra provenance exhibits 100% shoot bud initiation with maximum shoot number, shoot length and node number per node 2.20 ± 0.05, 2.83 ± 0.08, 2.72 ± 0.06 respectively whereas nodes derived from seedling of Bilaspur, Jagdalpur, Mahasamund and Raipur showed 100% shoot bud initiation but produces less shoot number, shoot length and node number per node. The provenances significantly affect the shoot number, shoot length, node number.

**Apical bud explant:**

The apical explant placed on MS media with 1.0 mg l⁻¹ BAP showed 100% frequency of shoot bud induction. The mean shoot no./explant ± SE was 2.13 ± 0.03
& mean shoot length was 2.75 cm ± 0.06 and mean node no. was 2.98 ± 0.07 in this medium. The apical segment placed on WPM, SH and B5 medium containing 1.0 mg/l BAP showed less % frequency of shoot bud induction. Similarly, nodes on MS medium with TDZ showed comparatively less shoot bud initiation then MS with BAP.

The apical segment from seedling of Pendra provenance exhibits 100% shoot bud initiation with maximum shoot number, shoot length and node number per node 2.20 ± 0.05, 2.83 ± 0.08, 2.72 ± 0.06 respectively whereas nodes derived from seedling of Bilaspur, Jagdalpur, Mahasamund and Raipur showed 100% shoot bud initiation but produces less shoot number, shoot length and node number per node. The provenances significantly affect the shoot number, shoot length, node number.

Establishment of Explants:

Establishment of mature tree Explants:

Apical explants of mature trees were inoculated on MS medium supplemented with 2.0 mg/l BAP and different levels of PVP (0, 50, 100, 200 mg/l). The nodal pieces placed on MS medium supplemented with 2.0 mg/l BAP and 100 mg/l PVP showed slight leaching only 6% cultures, shoot bud initiation in 100% explants, 2.06 ± 0.01 shoot per explants, 2.62 ± 0.23 cm length per shoot and 2.83 ± 0.26 nodes per shoot. However, PVP at higher or lower concentration reduced shoot bud initiation.

Nodal segment:

The nodal explant placed on MS media with 1.0 mg/l BAP showed 100% frequency of shoot bud induction. The mean shoot no./explant ± SE was 2.13 ± 0.03 & mean shoot length was 2.75 cm ± 0.06 and mean node no. was 2.98 ± 0.07 in this medium. The nodal segment placed on WPM, SH and B5 medium containing 1.0 mg/l BAP showed less % frequency of shoot bud induction. Similarly, nodes on MS medium with TDZ showed comparatively less shoot bud initiation then MS with BAP.

Nodal segment were inoculated on MS medium, SH medium, WPM medium and B5 medium supplemented with 2.0 mg/l BAP and 100 mg/l PVP showed maximum 96% percentage of shoot bud initiation, the maximum shoot number per explants 2.04 ± 0.04, length per shoot 2.59 ± 0.07 and node number per shoot 2.81 ±
0.08. The % of shoot initiation reduces with following media WPM, SH and B5. The media significantly affect the shoot number, shoot length, node number.

**Apical bud explant:**

The apical explant placed on MS media with 1.0 mg\textsuperscript{l} BAP showed 100% frequency of shoot bud induction. The mean shoot no./explant ± SE was 2.13 ± 0.03 & mean shoot length was 2.75 cm ± 0.06 and mean node no. was 2.98 ± 0.07 in this medium. The apical segment placed on WPM, SH and B\textsubscript{5} medium containing 1.0 mg\textsuperscript{l} BAP showed less % frequency of shoot bud induction. Similarly, nodes on MS medium with TDZ showed comparatively less shoot bud initiation then MS with BAP.

The apical buds were inoculated on MS medium, SH medium, WPM medium and B\textsubscript{5} medium supplemented with 2.0 mg\textsuperscript{l} BAP and 100 mg\textsuperscript{l} PVP showed maximum shoot bud initiation 100% with maximum shoot number per explant 2.13 ± 0.03, length per shoot 2.72 ± 0.05 and node number per shoot 2.98 ± 0.07. The % of shoot initiation reduces with following media WPM, SH and B5. The media significantly affect the shoot number, shoot length, node number.

**Shoot proliferation:**

*In vitro shoot proliferation in cultures of seedling origin:*

**Shoot proliferation from nodal segments:**

The nodal segments developed on establishment medium MS medium with 1.0 mg\textsuperscript{l} BAP (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The micro nodes placed on MS medium with 0.5 mg\textsuperscript{l} BAP and 0.5 mg\textsuperscript{l} NAA showed best shoot proliferation response. They produced maximum number of shoots per micro node 4.09 ± 0.01, length per shoot 4.14 ± 0.03, nodes per shoot 4.20 ± 0.07. The micro nodes on other concentrations in combinations of BAP and NAA produced less number of shoots.

**Shoot proliferation from apical buds**

The apical buds of micro shoots on MS medium with 1.0 mg\textsuperscript{l} BAP (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The apical buds placed on MS medium with 0.5 mg\textsuperscript{l} BAP and 0.5 mg\textsuperscript{l} NAA showed better response than
other concentration in combination of BAP and NAA. They produced maximum 4.15 ± 0.01 shoots per apical bud, 4.23 ± 0.02 lengths per shoot, 4.30 ± 0.06 nodes per shoot. Apical segments showed better response than nodal bud, so apical segment used for further studies.

The apical segments of shoots developed on establishment medium MS medium with 1.0 mg l⁻¹ BAP (medium I) were inoculated on medium II with different formulations (MS, SH, WPM or B₅) and supplemented with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA for shoot proliferation. The micro nodes on MS medium with 0.5 mg l⁻¹ BAP + 0.5 mg l⁻¹ NAA showed mean number of shoots per micro node 4.48 ± 0.18, mean length (cm) per micro shoot 3.51 ± 0.19 and nodes per micro shoot 3.07 ± 0.07 which was better response as compared to SH, WPM and B₅ media.

**Effect of provenance on shoot proliferation**

The apical segments of shoots developed from explants of different provenances of *J. curcas* on explants establishment medium MS medium with 1.0 mg l⁻¹ (medium I) were inoculated on shoot proliferation medium MS supplemented with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA (medium II). The micro nodes of Pendra provenance placed on MS medium supplemented with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA showed maximum shoot number per micro node 4.55 ± 0.02, maximum shoot length (cm) per micro shoot 3.72 ± 0.10 and number of nodes per micro shoot 3.33 ± 0.09. In general, the micro nodes from Pendra provenance showed better shoot proliferation response than those of Bilaspur, Jagdalpur, Mahasamund and Raipur provenances.

**Effect of adjuvant on in vitro shoot proliferation from nodes of seedling origin**

The micro nodes were inoculated on MS medium with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA and different concentrations (0.0, 10.0, 20.0 or 40.0 mg l⁻¹) of adenine sulphate. The shoot number per micro node, mean shoot length (cm) per micro shoot and number of nodes per micro shoot on MS with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA and40.0 mg l⁻¹ adenine sulphate were 5.00 ± 0.04, 4.62 ± 0.08 and 4.60 ± 0.05. The shoot proliferation response was less with other adjuvant (PVP, AS and AC.) The results suggested that MS medium with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA supplemented with 40.0 mg l⁻¹ adenine sulphate was suitable medium for in vitro shoot proliferation from micro nodes of seedling origin in compare to other adjuvant.
Micro nodes of seedling origin were inoculated on MS medium with 0.5 mg/l BAP and 0.5 mg/l NAA and 10.0 mg/l adenine sulphate. After every 45 days, the micro nodes were excised inoculated on fresh medium for subculture. The subculturong was carried out up to 5 cycles. The micro nodes at 3rd subculture showed maximum mean number per micro node 5.24 ± 0.01, mean shoot length per micro shoot 4.91 ± 0.02 and node number per micro shoot 4.69 ± 0.03 followed by 4th & 5th subculture. In general, the shoot number, the shoot length, and node number gradually increased from first to third subculture. After third cycle the shoot number, the shoot length and node number declined. It is concluded that sub culturing up to 3rd cycles was beneficial for shoot proliferation.

The micro nodes were inoculated on liquid MS medium supplemented with different concentrations (0.0, 0.25, 0.5, 1.0, 2.0 mg/l) of BAP. The micro nodes placed on MS medium supplemented with 1.0 mg/l BAP showed mean shoot number per micro node 4.38 ± 0.08, mean shoot length (cm) per micro shoot 3.63 ± 0.11 and mean nodes per micro shoot 3.14 ± 0.11. The results suggested that MS medium supplemented with 1.0 mg/l BAP was suitable medium for shoot proliferation from seedling origin nodes in Growtek culture vessels.

**In vitro shoot proliferation in cultures of from mature tree origin:**

**Effect of different levels of BAP and NAA in MS medium on in vitro shoot proliferation:**

**In vitro shoot proliferation from nodal segments:**

The micro nodes of shoots elongated from explants of mature tree on explants establishment medium MS with 2.0 mg/l (medium I) were inoculated on shoot proliferation medium MS with different concentrations of BAP or combination of BAP and NAA (medium II). The micro nodes placed with 1.0 mg/l BAP + 0.5 mg/l NAA showed better response than other concentrations of BAP and combinations of BAP and NAA. The micro node placed on MS medium supplemented with 1.0 mg/l BAP + 0.5 mg/l NAA showed maximum number of shoots per micro node 4.08 ± 0.01, length per shoot 4.12 ± 0.03, nodes per shoot 4.19 ± 0.07 which is suitable for shoot proliferation.

The apical buds of micro shoots elongated from explants of mature tree on explants establishment medium MS medium with 1.0 mg/l (medium I) were inoculated on shoot proliferation medium MS medium with different concentrations.
of BAP and combinations of BAP and NAA (medium II). It showed better response than other concentration of BAP and combination of BAP and NAA. The apical buds placed on MS medium supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA showed maximum number of shoots per apical buds 4.11 ± 0.01, 4.12 ± 0.02 length per shoot, 4.09±0.09 node per shoot. The results suggested that MS medium supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA was suitable medium for in vitro shoot proliferation from apical buds of mature tree origin. Apical segments shows better shoot proliferation response.

The micro nodes of shoots developed from explants of mature tree on explants establishment medium MS with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} (medium I) were inoculated on medium II with different formulations (MS, SH, WPM or B\textsubscript{5}) supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA for shoot proliferation. The nodal segments on MS medium with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA showed better response compared to SH, WPM and B\textsubscript{5} media. The micro nodes placed on MS medium supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA showed maximum number of shoots per micro node 4.53 ± 0.09, mean length (cm) per micro shoot 3.21 ± 0.12 and nodes per micro shoot 3.51 ± 0.12. The apical segments on MS medium with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA showed better response compared to SH, WPM and B\textsubscript{5} media. These results suggested that MS supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA was a suitable medium for shoot proliferation from micro nodes of mature tree origin.

Effect of adjuvant on shoot proliferation from cultures of mature tree origin:

The micro nodes were inoculated on MS medium with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA and different concentrations (0.0, 10.0, 20.0 or 40.0 mg\textsuperscript{1} l\textsuperscript{-1}) of adenine sulphate. The micro nodes placed on MS medium supplemented with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA and 40.0 mg\textsuperscript{1} l\textsuperscript{-1} adenine sulphate were number of shoots per micro node 4.91 ± 0.06, mean length (cm) per micro shoot 4.61 ± 0.08 and nodes per micro shoot 4.58 ± 0.05. The shoot proliferation response was less with other adjuvant (PVP, AS and AC.) The results suggested that MS medium with 0.5 mg\textsuperscript{1} l\textsuperscript{-1} BAP and 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA supplemented with 40.0 mg\textsuperscript{1} l\textsuperscript{-1} adenine sulphate was suitable medium for in vitro shoot proliferation from micro nodes of mature origin in compare to other adjuvant.

Micro nodes were inoculated on MS medium with 1.0 mg\textsuperscript{1} l\textsuperscript{-1} BAP + 0.5 mg\textsuperscript{1} l\textsuperscript{-1} NAA, 40 mg\textsuperscript{1} l\textsuperscript{-1} adenine sulphate (Table 37). After every 45 days, the micro nodes
were excised and inoculated on fresh medium for subculture. The sub culturing was carried out up to 5 cycles. The micro nodes produced mean shoot number per micro nodes 5.22 ± 0.01 and mean shoot length (cm) per micro shoot 4.90 ± 0.02 and mean node number per micro shoot 4.68 ± 0.02 at the end of 3rd subculture. In general, the shoot number, the shoot length, and node number gradually increased from first to third subculture. After third cycle the shoot number, the shoot length and node number declined. It is concluded that sub culturing up to 3rd cycles was beneficial for shoot proliferation.

**Callusing and Shoots Initiation:**

Zygotic embryos of *Jatropha curcas* were inoculated on MS medium supplemented with different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l⁻¹). MS medium supplemented with 2, 4 D (2.0 mg l⁻¹) of 2, 4 D showed maximum 95% callus induction response and maximum callus area 10.14 ± 0.10 cm² per explants.

Zygotic embryo derived callus grown on MS with 2.0 mg l⁻¹ 2,4 D were inoculated on MS medium supplemented with different concentrations in combinations of 2,4 D and BAP. MS medium supplemented with 0.5 mg l⁻¹ 2,4 D and 1 mg l⁻¹ BAP showed maximum 95% frequency of shoot with maximum callus area 10.14 ± 0.10 cm², with mean shoot number 3.06 ± 0.07, mean shoot length 3.61 ± 0.11 and mean node number 3.59 ± 0.11 per callus area.

Instead of somatic embryo there was shoot developed from the callus which was further used as explant for shoot proliferation in the combination of BAP and NAA. These results suggest that 1.0 mg l⁻¹ 2, 4 D and 0.25 mg l⁻¹ BAP in MS medium was suitable medium for initiation and development of callus area and shoots induction from Zygotic embryo derived callus grown on MS with 2.0 mg l⁻¹ 2,4 D. The apical micro shoots were transferred to the medium supplemented with NAA and BAP.

The apical segments developed on establishment 0.5 mg l⁻¹ 2, 4 D and 1.0 mg l⁻¹ BAP in MS medium (medium I) were inoculated on shoot proliferation medium MS medium with different concentration in combination of BAP and NAA (medium II). The micro nodes placed on MS medium with 0.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ NAA showed best shoot proliferation response. They produced maximum number of shoots per micro node 3.06 ± 0.07, length per shoot 3.61 ± 0.11, nodes per shoot 3.49 ± 0.11. The results suggested that MS medium supplemented with 0.5 mg l⁻¹ BAP and 0.5
mg⁻¹ NAA was suitable medium for in vitro shoot proliferation from micro nodes of Zygotic embryo derived callus origin.

**In vitro rooting from micro shoot:**

**Effect of auxins on in vitro rooting in micro shoots of seedling origin:**

Micro shoots of seedling origin were inoculated on ½ MS medium with different concentration (0.0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹) of IAA. The micro shoots placed on ½ MS medium with 4.0 mg l⁻¹ IAA were 66%, mean shoot number per micro shoot 1.85 ± 0.11 and mean root length per micro shoot 2.06 ± 0.15 with 44% callusing.

Micro shoots were inoculated on ½ MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 mg l⁻¹) of NAA. The micro shoots placed on ½ MS medium with all the above concentration showed no initiation response on root number, root length per micro shoot. There were 100% callusing.

Micro shoots were inoculated on ½ MS medium with (0.0, 0.5, 1.0, 2.0 and 4.0, mg l⁻¹) IBA. In all these concentration maximum percentage of root initiation responses 60%, maximum root number per micro shoot 2.76 ± 0.05 and maximum root length per micro shoot 2.93 ± 0.05 recorded in 4.0 mg l⁻¹ IBA. These results suggest that MS medium supplemented with 4.0 mg l⁻¹ IBA is suitable medium for rooting from micro shoot of seedling origin.

Micro shoots were inoculated on different medium ½ MS and ½ WPM medium with 4.0 mg l⁻¹ of IBA. The micro shoots placed on ½ MS medium with 4.0 mg l⁻¹ IBA showed root initiation responses 50%, mean shoot number per micro shoot 1.57 ± 0.12 and mean root length per micro shoot 1.23 ± 0.09 (Table 41). The percentage of root initiation responses, mean root number and mean root length in ½ WPM medium with 4.0 mg l⁻¹ IBA was low.

The micro shoots were inoculated on half - MS medium containing 25.0, 50.0 and 100.0 mg l⁻¹ activated charcoal and 4.0 mg l⁻¹ IBA and then were transferred to medium without IBA. Micro shoots on 50.0 mg l⁻¹ charcoal showed maximum rooting 80%, with 3.03 ± 0.06 mean root numbers per micro shoot and 3.18 ± 0.04 mean root lengths (cm) per micro shoot. The micro shoots, placed on half - MS medium supplemented with 4.0 mg l⁻¹ IBA 50 mg l⁻¹ activated charcoal showed best root induction and root length.
Effect of auxins on in vitro rooting of micro shoot from mature tree origin:

Micro shoots were inoculated on ½ MS medium with different concentration (0.0, 0.5, 1.0, 2.0 or 4.0 mg l\(^{-1}\)) of IBA. In all these concentration maximum percentage of root initiation responses 60% maximum root number 2.73 ± 0.05 and maximum root length 2.88 ± 0.05 recorded in 4.0 mg/l IBA in camparisison to IAA and NAA. IAA shows less root initiation response and NAA had not responded for rooting. These results suggest that MS medium supplemented with 4.0 mg l\(^{-1}\) IBA is suitable medium for in vitro rooting from micro shoot of mature tree origin.

Hardening of plantlets:

The plantlets transferred to root trainer filled with coco peat showed 90% survival of the micro plants. The survival was 70% of plantlets that were transferred to root trainer containing mixed soil and sand. After primary hardening, the plants were transferred to polyethylene bags and placed in a net house. The plantlets transferred to root trainer filled with coco peat showed 80% survival of the micro plants. The survival was 60% of plantlets that were transferred to root trainer containing mixed soil and sand. These results suggest that coco peat was better for secondary hardening of *Jatropha curcas* micro shoots compare to soil and sand mixture for both seedling and mature origin micro shoot.

RAPD analysis:

Genomic DNA was isolated from fresh tender leaf tissues of 15 plants (B1-B3, J1-J3, P1-P3, M1-M3 and R1-R3 15) of *Jatropha curcas* collected from different locations (Table 54). DNA was extracted using Khanuja et al., (1999) methods and the yield was noted down on the basis of visual intensity of bands in 0.8% agarose gel under trans-illuminator. Maximum yield (up to 500 ng per gm of leaf tissue) was obtained with method described by Khanuja et al., (1999).

The OPA-08, OPB-08 primers produced distinct, highly reproducible amplification profile for all the screened samples. These 2 primers were selected for RAPD analysis of *J.curcas*.

The OPA-08 primers produced total 27 bands; the number of bands ranged from 04 to 13 bands, with an average of 8.4 bands per primer; 10 bands (37.0%) of these were polymorphic.
Polymorphism in Bilaspur provenance: Primer OPA-08 amplified 3 bands from 3 DNA samples of Bilaspur provenance, out of which 3 bands were polymorphic, with number of bands ranging from 6 to 9. The average percentage of polymorphic bands was 100 %.

Polymorphism in Jagdalpur provenance: Primer OPA-08 amplified 10 bands from 3 DNA samples of Jagdalpur provenance, out of which 3 bands were polymorphic, with number of bands ranging from 1 to 10. The average percentage of polymorphic bands was 30 %.

Polymorphism in Mahasamund provenance: Primer OPA-08 amplified 7 bands from 3 DNA samples of Mahasamund provenance, out of which 3 bands were polymorphic, with number of bands ranging from 1 to 8. The average percentage of polymorphic bands was 42 %.

Polymorphism in Pendra provenance: Primer OPA-08 amplified 4 bands from 3 DNA samples of Pendra provenance, out of which 2 bands were polymorphic, with number of bands ranging from 6 to 9. The average percentage of polymorphic bands was 50 %.

Polymorphism in Raipur provenance: Primers OPA-08 amplified 3 bands from 3 DNA samples of Raipur provenance, out of which only 1 bands were polymorphic, with number of bands ranging from 5 to 8. The average percent of polymorphic bands was 33%.

Cluster analysis and dendrogram: Leaf samples were obtained from seedlings of Bilaspur (B1 to B3), Jagdalpur (J1 to J3), Mahasamund (M1 to M3), Pendra (P1 to P3), Raipur (R1 to R3) provenances (Table 54). Based on the data of RAPD amplified products, dendrogram for 15 samples of J. curcas were constructed using UPGMA algorithm. A perusal of dendrogram indicates that all the accessions initially fall under two major clusters I & II. Cluster I contain P2 from Pendra which form single group. While cluster II is further divided into two major group A & B at (25% similarity coefficient). The groups A contain J3 and M3 while group B is divided into two sub cluster I and II. Sub cluster I contain B1, B2, B3, P3, M2, R1, R2 and R3. Sub cluster II contains J1, J2, P1 and M1.