Summary and Conclusion
Man is familiar with edible roots and tubers since the dawn of civilization. These species grew over extended period of time and were available as secure food to the primitive societies. Roots and tubers contain a large group of plant species yielding starchy roots, tubers, rhizomes, corms and stems. About 400 vegetable crops are commercially cultivated worldwide that include approximately 38 root, 23 tuber, 14 rhizome, 11 corm and 10 bulb crops. India holds a rich diversity of tropical root and tuber crops. While some of them are cultivated, others grow as wild plants. Tribal, inhabiting near forest, gather wild roots and tubers for their personal use, and as a salable commodity. Considering their importance for food, medicine and industry, these under utilized species can be grown at commercial scale. The wild species Curcuma angustifolia yields edible rhizome, whereas Dioscorea hispida and Pueraria tuberosa produce edible tubers. Besides having food value, these rhizomes and tubers are endowed with medicinal properties.

Curcuma angustifolia Roxb belongs to family - Zingiberaceae. It is commonly known as East Indian arrowroot and travancore starch. The vernacular names of Curcuma angustifolia are - tayakshira (Sanskrit), tikhur (Hindi), tavakhira (Marathi), ararutkizhangu (Tamil) and ararut gaddalu and palagunda (Telugu). World wide edible species of the Genus Curcuma are - C. aeruginosa, C. alismatifolia, C. amada, C. angustifolia, C. aromatic, C. caulina, C. heyneana, C. leucorrhiza, C. longa, C. mangga, C. Montana, C. pierrana, C. pseudo-montana, C. purpurascens, C. rotundata, C. rubescens, C. xanthorrhiza and C. zedoaria.
Tikhur is a perennial herb with short, globular rootstock, which emits long ellipsoid tuber-bearing fleshy roots. The tuber is processed to obtain tikhur, which is sold in the market. Tikhur flour is cooked and consumed in many parts of India. It grows wild in its natural habitat and usually perpetuates through vegetative reproduction. Overexploitation has made tikhur scarce in natural habitat and costly in the market. Cultivation of sufficiently prolific ones would greatly enhance its production and quality. Its rhizome contains starch, glucose, protein, and fat. Rhizome oil contains 11-hydroxyisomer, methyl eugenol, palm acid and camphor. Tubers yield starch, resembling arrowroot, which is easily digestible. It is recommended for invalids and children. Starch recovery is 1% from the tuber, and is in high demand. Tuber powder, 10-20 gm, with porridge is useful in reducing intestinal inflammation. It's unctuous, carminative and astringent properties help in diarrhea, dysentery, and flatulence. It is also aphrodisiac, diuretic and a cardio-tonic.

Dioscorea hispida Dennst (Synonyms: D. daemona Roxb., D. hirsuta Dennst, D. triphylla), an important member of family – Dioscoreaceae, is commonly known as baichandi. The vernacular names of Dioscorea hispida are – karukandu (Hindi), podavakilangu (Malayalam), baicandi (Marathi), peiperenda (Tamil) and tella-ginigeddalu and pulidumpa (Telugu). It is a left-twining climber with alternate leaves of three leaflets. It bears subterranean tubers that can produce industrial starch and edible flour. In many parts of India, people consume sweet baichandi chips of the processed tubers containing 88.34% carbohydrates. Forest dwellers collect and process tubers to prepare baichandi chips, which is now-a-days scarce and costly commodity. The tubers contain toxic alkaloid called dioscorine, which runs throughout the whole plant. Dioscorine is bitter and poisonous; it produces paralysis of the central nervous system, and in general behaves like picrotoxin. Tubers are eaten after complete elimination of toxic properties, cooked, steamed, eaten with coconut milk, as porridge after steaming by adding palm sugar, or dried and powdered to make gadoong cake. There is a possibility of utilizing the tubers for production of industrial starch and edible flour.
Members of *Dioscorea* are generally called yam and they are the staple food-stuff for many millions. They are also a standby food for many populations in the time of famine. In addition, a number of wild species have found widespread use as a source of the steroidal sapogenin, diosgenin, which is the precursor in the commercial synthesis of sex hormones and corticosteroids. Genus *Dioscorea* includes several economical and edible species such as *Dioscorea alata*, *D. altissima*, *D. puber*, *D. antaly*, *D. arachnida*, *D. atropurpurea*, *D. opposita*, *D. belophylla*, *D. hemandroid*, *D. brasiliensis*, *D. brevipetiolata*, *D. bulbifera*, *D. cayennensis*, *D. cirrhosa*, *D. colocasiifolia*, *D. hispida*, *D. decaisneana*, *D. tectorum*, *D. depamperata*, *D. divaricata*, *D. dodecandura*, *D. dumetorum*, *D. esculenta*, *D. javiera*, *D. fargesii*, *D. gibbiflora*, *D. glandulosa*, *D. globosa*, *D. gracillima*, *D. hamiltonii*, *D. hastata*, *D. hastatifolia*, *D. heptaneura*, *D. illustrata*, *D. pentaphylla*, *D. japonica*, *D. koarao*, *D. kratica*, *D. latifolia*, *D. lusititia*, *D. laxiflora*, *D. luzonensis*, *D. maciba*, *D. myriantha*, *D. oppositifolia*, *D. ovinala*, *D. papuana*, *D. p. rei*, *D. pyrifolia*, *D. quinqueloba*, *D. rotundata*, *D. septemloba*, *D. spicata*, *D. tomentosa*, *D. tricopoda*, *D. trifolia* and *D. villosa*.

*Pueraria tuberosa* (family- Papilionaceae/Fabaceae) is commonly known as Indian Kudzu and Patal Kumda. The vernacular names of *Pueraria tuberosa* are bankumra, sural, bilaikand, bharda and tirra (Hindi), shimia, batraji (Bengali), vidarikand, phagvelo and khakarvel (Gujarati), gumadigida (Kannad), sirala and bisalu (Kumaun), ghorbel (Marathi), siali (Punjabi) and darigummadi (Telugu). It is usually propagated through seeds and tuber. Widespread destruction of natural habitat and indiscriminate use of tubers has restricted reproduction and regeneration of this plant. Plant species is now considered threatened. This plant possesses many isoflavonones among these most abundant is puerarin. Genus *Pueraria* includes several edible tuber-bearing species such as *Pueraria edulis*, *P. hirsuta*, *P. lobata*, *P. montana*, *P. phaseoloides*, *P. pseudo-hirsuta*, *P. thomsonii* and *P. tuberosa*.

*Patal Kumda* tubers are eaten raw or boiled; may also be used for extraction of starch. Leaves are used as fodder for horses and cattle. Roots are demulcent and
refrigerant; tubers are also used as cataplasm on swollen joints and as a lactagogue. Tubers are large, 25-30 cm broad and 30-60 cm long weighing up to 35 kg. It is unctuous, anabolic, nutritive, cholagogue, carminative, cardio tonic, hemostatic, aphrodisiac, galactogogue, diuretic, complexion enhancer and rejuvenator. It reduces intestinal dryness in hepato-splenomegaly, cardiac debility, bleeding disorder and hoarseness of voice, cough, dysuria and phthisis. Tuber mainly contains starch, sugar and resin. Tuber also contains beta-sitosterol, stigmasterol, daidzein, puerarin and diacetylpuerarin. It contains 64.6% total carbohydrate on dry matter basis.

There are three main routes of in vitro propagation namely axillary shoot proliferation, organogenesis and somatic embryogenesis. Plant tissue culture techniques have been successfully employed for propagation of several plant species. However, every plant species and sometimes, different varieties of the same species require specific chemical(s) and culture condition(s) for their development in vitro. Besides, there are problems like contamination, browning of explants, juvenility, hyperhydricity, low rate of multiplication, lack of differentiation, rooting and acclimatization, etc. Thus, thorough investigation is required to establish in vitro propagation procedure for each species. The present research work was planned to study in vitro propagation in Curcuma angustofolia, Dioscorea hispida and Pueraria tuberosa.

6.1 Objectives:
The objectives of the present study were as follows:

A. Curcuma angustofolia

- To study the effect of BAP and provenance on establishment of rhizome bud explants.
- To study the influence of different media (MS, SH and WPM), nature of media (liquid vs semisolid), provenance, PGR and adjuvant(s) on shoot proliferation of Curcuma angustifolia.
- Subculture of micro shoots and determination of shoot multiplication rate
• To attempt for micro rhizome formation in vitro
• Acclimatization of in vitro raised plantlets
• Field trial of in vitro regenerated plants

B. Dioscorea hispida
• To study the effect of BAP and provenance on establishment of nodal explants.
• To study the influence of different media (MS, SH and WPM), nature of media (liquid vs semisolid), PGR and adjuvant(s) on shoot proliferation of Dioscorea hispida.
• Subculture of micro shoots and determination of shoot multiplication rate
• To attempt for micro tuberization in vitro
• To find out the effects of PGR, etc. on rooting of micro shoots
• Acclimatization of in vitro raised plants
• Field trial of in vitro regenerated plants

C. Pueraria tuberosa
• To study the effect of different media, PGR and adjuvant(s) on establishment of nodal explants from field grown plants.
• To study the effect of BAP on in vitro germination of the seeds of Pueraria tuberosa
• To study the effect of node position on establishment of shoot bud cultures
• To find out the effect of BAP on shoot proliferation
• To find out the effect of PGRs on rooting of micro shoots
• To establish callus culture from immature leaf and petiole sections and attempt differentiation
• Acclimatization of in vitro raised plants

6.2 MATERIALS AND METHODS:
The materials and methods used in the present investigation were as follows:

A. Curcuma angustifolia
Rhizomes of *C. angustifolia* were collected from four places of Chhattisgarh state namely Bilaspur, Dhamtari, Pithora and Raipur. These rhizomes were planted separately in the flowerbeds of Botanical Garden of School of Life Sciences. The rhizomes were also planted in sand bed in side the greenhouse for sprouting.

The rhizome buds, 2-3 cm long, were excised from rhizome under aseptic condition, sterilized in 0.2% mercuric chloride for different durations (5 to 20 minutes).

The rhizome buds were inoculated on MS medium supplemented with different concentrations of BAP (0.0, 1.0, 3.0 or 5.0 mg/l).

Rhizome buds obtained from the plants of Bilaspur, Dhamtari, Pithora and Raipur provenances were inoculated on the culture establishment medium (MS supplemented with 3.0 mg/l BAP) to study the in vitro response of different provenances.

Elongated shoots were placed on different media (MS, SH and WPM) and BAP concentrations (0.0, 1.5, 3.0 or 6.0 mg/l) to study their effect on shoot proliferation.

Shoot proliferation response of different provenances on Woody Plant Medium (WPM) supplemented with 3.0 mg/l BAP was also investigated.

Effects of sucrose (15.0, 30.0, 60, 90 g/l), sub culturing (up to 8th passages), different adjuvants (adenine sulfate at 0.0, 25.0, 50.0 or 100.0 mg/l; casein hydrolysate at 0.0, 25.0, 50.0 or 100.0 mg/l and combination of adenine sulfate and casein hydrolysate at 12.5:12.5, 25.0:25.0, 50.0: 50.0, 100.0 :100.0), BAP in combination with Kinetin (3.0:0.0, 2.0:1.0, 1.5:1.5 or 3.0: 0.0), IAA (0.25, 0.5 or 1.0 mg/l), NAA (0.25, 0.5 or 1.0 mg/l) and liquid medium on shoot proliferation were investigated.

Micro shoots were inoculated on WPM medium supplemented with different concentration of BAP (0.0, 1.5, 3.0 or 6.0 mg/l) and sucrose (30.0, 60.0 or 90.0 g/l) and placed under different light regime to induce micro rhizome
In vitro regenerated plantlets were acclimatized in greenhouse condition prior to transfer to the field. The hardened plants of *C. angustifolia* were transplanted into the field along with the rhizome at different plant-to-plant spacing (15 cm, 30 cm and 45 cm) to compare the performance of tissue culture raised plants and rhizome grown plants.

**B. Dioscorea hispida**

Tubers of *D. hispida* were collected from four different places of Chhattisgarh state namely Bilaspur, Dhamtari, Pithora and Raipur. Tubers of each place were collected and planted separately in the flowerbeds of Botanical Garden of the School of Life Sciences. Tubers were also planted on the sand beds inside the greenhouse for sprouting.

The greenhouse-grown plants were suitable to initiate cultures. The Nodal segments of *D. hispida* were used as explants. Nodal segments from new sprouts were cut, washed in running tap water and sterilized with 0.2% HgCl₂ for different durations (3 to 20 minutes).

Surface sterilized nodal segments were inoculated on MS medium supplemented with different concentrations of BAP (0.0, 1.0, 3.0 or 5.0 mg/l) to establish axenic shoot cultures.

Nodal segments obtained from Bilaspur, Dhamtari, Pithora and Raipur provenances were inoculated on establishment medium (MS supplemented with 1.0 mg/l BAP) to study the in vitro response of different provenances.

Micro shoots were excised and individual node was placed on different media to study the effect of different media formulations viz. MS (Murashige and Skoog), SH (Schenk and Hildebrandt) and WPM (Lloyd and McCown) on shoot proliferation. All media were supplemented with 1.0 mg/l BAP.

Individual micro nodes were placed on WPM medium containing 1.0 mg/l BAP (control), 1.0 mg/l Kinetin and their combination (0.5:0.5 mg/l) to study the effect of Kinetin on shoot proliferation.
Effects of different additives namely PVP (0.0, 250.0, 500.0 or 1000.0 mg/l); Citric acid (0.0, 25.0, 50.0 or 100.0 mg/l); Adenine sulfate (0.0, 10.0, 20.0 or 40.0 mg/l) and Activated charcoal (100.0, 200.0 or 400.0 mg/l) on shoot-proliferation were investigated.

Effects of IAA (0.0, 0.1, 0.25 or 0.5 mg/l) and NAA (0.0, 0.1, 0.25 or 0.5 mg/l) on in vitro shoot proliferation was investigated.

The micro shoots were inoculated on WPM containing 1.0 mg/l BAP + 20.0 mg/l AS + 0.25 mg/l IAA and with or without agar to compare semisolid and liquid media.

Micro nodes were sub cultured on the fresh medium every six weeks, for six consecutive sub cultures on WPM supplemented with 1.0 mg/l BAP to study shoot multiplication potential of micro nodes.

Micro shoots were inoculated on WPM supplemented with 1.0 mg/l Kinetin or, 1.0 mg/l IAA or 1.0 mg/l NAA or 1.0 mg/l IBA or without PGR and different concentrations of sucrose (15.0, 30.0, 60.0 or 90.0 gm/l) to induce micro tuber formation and incubated for 90 days under three different photoperiods (16h light: 8h dark, 8h light: 16h dark or 0h light: 24h dark).

Micro shoots were excised and placed vertically on half strength WPM supplemented with different concentration of NAA (1.0, 2.0 or 4.0 mg/l), IBA (1.0, 2.0 or 4.0 mg/l) and activated charcoal (25.0, 50.0 or 100.0 mg/l) to study their effect on root induction.

The in vitro regenerated plantlets of Dioscorea hispida were removed from the culture bottles and washed thoroughly and transplanted into net pots containing different substrates namely Sand: Soil: Farm Yard Manure (1:1:1 by volume) and Coco peat. After 4 weeks, plants were transplanted to nursery bags containing sand: soil: FYM (1:1:1) and gradually acclimatized to outdoor conditions. After one month, plants were shifted to Net House and kept there until plantation in the field. Hardened plants of D. hispida were planted in the field along with the natural tubers to evaluate their field performance.
**C. Pueraria tuberosa**

The tubers of *P. tuberosa* were collected from Forest of Raigarh district of Chhattisgarh state, and planted in flowerbed of Botanical Garden of the School of Life Sciences.

Seeds were collected and aseptically inoculated on MS medium supplemented with 0.5 mg/l BAP or without BAP. Seedlings obtained via *in vitro* seed germination were used for further studies.

Effect of node position on explants establishment was investigated. Four nodes were numbered from base to apex. The nodal segments were cut and inoculated on the MS medium supplemented with 0.5 mg/l BAP.

Micro shoots were excised and micro nodes were inoculated on the MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0 and 2.0 mg/l) to determine the effect of BAP on shoot proliferation.

Effects of IBA (0.0, 0.5, 1.0 and 2.0 mg/l), IAA (0.0, 0.5, 1.0 and 2.0 mg/l) and NAA (0.0, 0.5, 1.0 and 2.0 mg/l) was studied on *in vitro* rooting of micro shoots of *P. tuberosa*.

*In vitro* rooted plantlets were removed carefully from the culture medium and washed under the running tap water and transferred into the net pots containing coco peat and sand, soil and manure. After 4 weeks plants were shifted into the nursery bags containing mixture of sand, soil and manure (1:1:1) for the secondary hardening.

Young leaf and petiole segments of the field grown plants were used as explants for callus induction. Explants were treated with 0.2 % HgCl₂ for 10 minutes and washed in sterilized distilled water for 3 – 4 times and aseptically placed on the MS medium supplemented with different concentration of growth regulators 2, 4-D (1.0 mg/l or 2.0 mg/l), NAA (0.5 mg/l or 1.0 mg/l), BAP (1.0 mg/l or 2.0 mg/l), combination of 2,4-D and BAP (1.0:1.0, 1.0:2.0, 2.0:1.0 or 2.0:2.0 mg/l, respectively) and combination of NAA and BAP (0.5:1.0, 0.0.5:0:2.0, 1.0:1.0 or 1.0:2.0 mg/l, respectively) to study their effects on callusing. Callus induced from
explants on the first media were transferred to the second media to study their effects on differentiation.

Nodal segments of new sprouts of field-grown *Pueraria tuberosa* were aseptically inoculated on different media (MS, SH and WPM) supplemented with BAP, Kinetin or 2-ip (each 0.01, 0.1 or 1.0 mg/l), MS medium supplemented with 2.0 mg/l BAP and adenine sulfate (25.0, 50.0 or 100.0 mg/l) and MS medium supplemented with 2.0 mg/l BAP and PVP (250.0, 500.0 or 1000.0 mg/l) to study shoot bud elongation.

6.3 Results

A. *Curcuma angustifolia*

Explants establishment

- Pithora provenance produced maximum rhizome fresh weight per plant (245.5 ± 15.93 g) followed by Raipur, Bilaspur and Dhamtari provenances.
- Treatment of explants with 0.2% mercuric chloride for 15 minutes duration reduced contamination to 10%, and showed 80% shoot bud elongation.
- Best (80%) rhizome buds explants establishment occurred on MS medium with 3.0 mg/l BAP.
- Rhizome bud explants from Pithora provenance showed highest shoot bud induction 80%, shoots per explants 1.9 ± 0.18, shoot length per plant 49.10 ± 1.77mm, leaf number per plant 2.7 ±0.30 and maximum leaf width 18.0 ± 0.68 mm at explants establishment stage, which were higher than those of Bilaspur, Dhamtari, and Raipur provenances.

Shoot Proliferation

- WPM supplemented with 3.0 mg/l BAP was better than MS and SH media for in vitro shoot proliferation. It induced maximum 4.1± 0.41 shoots per micro shoot, mean shoot length (mm) 42.5 ± 2.18, mean leaf number 9.2± 0.87, mean leaf width (mm) 15.3 ± 0.52 and mean root number 8.4 ± 0.79.
At shoot proliferation stage also Pithora provenances showed maximum number of shoots per micro shoot 3.9 ± 0.23, mean shoot length (mm) 41.0 ± 2.61, mean number of leaves 7.6 ± 0.64, mean leaf width (mm) 16.2 ± 0.66 and mean root number 8.9 ± 0.82. The *in vitro* shoot proliferation of Pithora provenance was better than Bilaspur Dhamtari and Raipur provenances. Pithora provenance was used for further experiments.

The micro shoots placed on WPM containing 3.0 mg/l BAP and 30.0 g/l sucrose produced maximum number of shoots per micro shoot 4.5 ± 0.43, mean shoot length (mm) 43.2 ± 1.82, mean leaf number 8.6 ± 0.57, mean leaf width (mm) 14.2 ± 0.59, and mean root number 8.9 ± 0.71.

Micro shoots of *Curcuma angustifolia* were inoculated on WPM with 3.0 mg/l BAP, 30.0 g/l sucrose and 0.8% agar (semi solid medium). After every 45 days, the micro shoots were excised and a single shoot was inoculated on fresh medium of same composition for subculture. The subculture was carried out up to 8th cycle. The shoot number increased gradually up to 6th subculture and then stabilized. Shoot length exhibited gradual increase from first to eighth subculture. At 6th subculture number of shoots per micro shoot and mean shoot length (mm) were 9.3 ± 0.38 and 71.5 ± 0.94, respectively.

Semisolid WPM containing 3.0 mg/l BAP, 1.0 mg/l IAA, 25.0 mg/l adenine sulfate and 30.0 g/l sucrose induced maximum number of shoots per micro shoot 6.9 ± 0.32, mean shoot length (mm) 62.8 ± 1.71, mean number of leaves 15.6 ± 0.53, mean leaf width (mm) 9.6 ± 0.57.

The micro shoots placed in liquid medium showed significantly better response than those on semisolid medium. The micro shoots inoculated in liquid WPM containing 3.0 mg/l BAP, 1.0 mg/l IAA, 25.0 mg/l adenine sulfate and 30.0 g/l sucrose produced shoot number per micro shoot 8.16 ± 0.34, mean shoot length (mm) 69.33 ± 0.69, mean leaf number 14.75 ± 0.72, mean leaf width (mm) 15.83 ± 0.38 and mean root number 11.25 ± 0.45.
• Different concentrations of Kinetin, NAA or casein hydrolysate did not show any improvement in shoot proliferation.

Micro rhizome formation:
• Best micro rhizome formation occurred in the micro shoots placed on WPM with 3.0 mg/l BAP and 60.0 g/l sucrose under 16h Light: 8h Dark photoperiod. On this medium the micro shoots exhibited maximum micro rhizome number 5.0 ± 0.36, micro rhizome length (mm) 35.0 and micro rhizome weight (mg) 375.0 mg.

Root initiation:
• In micro shoots of *C. angustifolia*, rooting occurred during shoot multiplication stage. The rooted plants were separated and used for hardening.

Hardening of plantlets:
• The rooted plants transferred to net pots containing coco peat showed 100.0 percent survival. Secondary hardening was carried out in the nursery bags containing mixed soil (in ratio of 1 sand: 1 soil: 1 FYM). In this stage, plants showed 92.0 % survival.

Field performance of tissue culture (TC) plants
• TC plants and rhizome grown plants were planted at the spacing of 15.0, 30.0 and 45.0 cm to assess field performance. Comparatively, the tissue culture plants showed better rhizome formation per plant at all the three spacing. However, best rhizome growth of tissue culture plants occurred at 45 cm spacing. The tissue culture plants showed fresh weight 303.93 ± 11.83 g per plant, which was significantly superior to fresh weight 227.3 ± 11.13 g per plant of rhizome grown plants.
B. *Dioscorea hispida*:

Explants establishment:

- Treatment of nodal segments with 0.2% mercuric chloride for 10 minutes duration reduced contamination to 0.0%, and showed 100% shoot bud elongation.

- Best (100%) nodal bud explants establishment occurred on MS medium with 1.0 mg/l BAP. On this medium, the explants produced $2.2 \pm 0.29$ shoots per explants with $30.8 \pm 1.89$ mm maximum shoot length and $2.58 \pm 0.38$ nodes per shoot.

- Effect of provenance was investigated at explants establishment stage. Tubers were collected from Bilaspur, Dhamtari, Pithora and Raipur, and planted separately in green house for sprouting. Newly sprouted buds were excised, sterilized and inoculated on medium to study the effect of provenance on the explants establishment. The cultures initiated from different provenances did not differ significantly at establishment stage.

Shoot Proliferation

- The micro nodes of *D. hispida* were inoculated on MS, SH and WPM medium each supplemented with 1.0 mg/l BAP. The micro nodes inoculated on WPM with 1.0 mg/l BAP exhibited maximum in vitro shoot proliferation. On this medium, the shoot number, maximum shoot length (mm) and number of node per shoot were $3.1 \pm 0.23$, $36.1 \pm 2.33$, $2.56 \pm 0.15$, respectively.

- Addition of kinetin in WPM did not enhance in vitro shoot proliferation in *D. hispida*.

- Addition of 1000.0 mg/l PVP in WPM with 1.0 mg/l BAP the culture medium was completely controlled blackening/ browning of the medium. The micro nodes placed on WPM supplemented with 1.0 mg/l BAP and 1000.0 mg/l PVP showed shoot number $3.4 \pm 0.27$, maximum shoot length (mm) $38.0 \pm 1.40$ and number of nodes per micro shoot $3.66 \pm 0.82$. Citric
acid, casein hydrolysate and adenine sulfate could neither improve *in vitro* shoot proliferation nor could control the leaching. Addition of the activated charcoal in the WPM inhibited *in vitro* shoot proliferation of *D. hispida*.

- Addition of IAA in WPM with 1.0 mg/l BAP significantly enhanced shoot proliferation. The micro nodes placed on WPM supplemented with 1.0 mg/l BAP and 0.25 mg/l IAA showed maximum shoot number 3.4 ± 0.32, maximum shoot length (mm) 51.4 ± 2.06 and number of nodes per micro shoot 3.3 ± 0.20. In NAA supplemented medium, no significant increase in shoot proliferation was observed.

- The micro nodes of *D. hispida* were inoculated on WPM supplemented with 1.0 mg/l BAP, 20 mg/l AS, 0.25 mg/l IAA and 0.7% agar (semisolid medium) as well as in WPM supplemented with 1.0 mg/l BAP, 20 mg/l AS and 0.25 mg/l IAA without agar (liquid medium). In liquid medium, the shoot number, shoot length and number of nodes per micro shoot were reduced and the shoots were hyperhydrated.

- Effect of subculture on *in vitro* shoot proliferation in *D. hispida* was investigated for 6 cycles on WPM medium supplemented with 1.0 mg/l BAP. Shoot number, maximum shoot length (mm) and number of nodes per micro shoot showed gradual increase up to 5th sub culture. At 5th sub culture shoot number, maximum shoot length (mm) and number of nodes per micro shoot were 6.08 ± 0.23, 53.5 ± 0.68 and 3.41 ± 0.26, respectively.

**Micro tuber formation:**

- Best tuber formation occurred in micro shoots inoculated on WPM containing 60.0 g/l sucrose, 1.0 mg/l NAA and placed under 8h light: 16h dark regime. All the micro shoots placed on WPM with 1.0 mg/l NAA and 60.0 g/l sucrose showed tuber formation with average tuber weight 520.0 mg.

- WPM medium supplemented with Kinetin as well as IAA in combination with sucrose and placed under 8h light: 16h dark regime failed to induce
tuber formation in micro shoots of *D. hispida*. Micro tubers were stored in dry condition at room temperature. The micro tubers sprouted in the month of June, which is the natural growing season for them.

- Cultures in WPM medium supplemented with IBA or NAA (1.0 mg/l) in combination with sucrose (60.0 or 90.0 g/l) were also incubated in the complete darkness. Under total dark condition 80% micro shoots dried within 8 weeks. The micro shoots that survived were hyper hydrated and etiolated, and did not produce micro tuber.

Root initiation:

- Excised micro shoots, approximately 3.0 cm long, were placed on half-WPM medium supplemented with IAA, NAA or IBA (each 0.0, 1.0, 2.0 or 4.0 mg/l), or activated charcoal (25.0, 50.0 or 100.0 mg/l). Best root induction 90% with root number 2.67 ± 0.41 and root length 12.56 ± 1.25 mm occurred in micro shoots placed on half-WPM with 2.0 mg/l IAA. The micro shoots on medium containing 25.0 mg/l activated charcoal, the root induction, root number and root length were 70%, 2.5 ± 0.50 mm and 11.75 ± 4.37, respectively. In other media, either percent root induction or number root and root length were not satisfactory. In some cases, the roots originated from callus at the base of micro shoots.

Hardening of plantlets

- *In vitro* regenerated plantlets were transferred to net pots containing different substrates for primary hardening. The net pots were filled with either coco peat or mixture of soil, sand and farmyard manure (ratio 1:1:1). Best (86%) survival of plantlets was observed on net pots filled with coco peat. Secondary hardening was done in the nursery bags filled with soil mixture (sand, soil and farmyard manure in 1:1:1 ratio), where they showed 90% survival.
Field performance of tissue culture (TC) plants

- Hardened TC plants of *D. hispida* (approximate height 30 cm) with were transplanted into the field along with the sprouting natural tubers as control. The plants developed from tissue culture raised plants showed shoot number 1.0 ± 0.0, plant height (cm) 38.3 ± 2.64, tuber weight (g) 59.5 ± 4.11 before drying. The plants developed from natural tubers showed shoot number 1.0 ± 0.0, plant height (cm) 171.8 ± 3.28 and tuber weight (g) 493.0 ± 36.75. Single shoot was obtained from both TC plants and natural tubers. The plants developed from natural tubers were superior to those developed from TC plants.

*C. Pueraria tuberosa*

Explants establishment:

- The tubers were collected from Raigarh forest of Chhattisgarh state. Some of these tubers sprouted and formed shoots. These plants showed flowering and fruiting in fourth year of plantation. Seeds of *P. tuberosa* were collected, sterilized and inoculated on MS medium supplemented with 0.5 mg/l BAP or without BAP. *In vitro* germination occurred in 40 % seeds placed on MS medium without BAP and in 60% of seeds on MS supplemented with 0.5 mg/l BAP. The seedlings on MS medium without BAP showed height (mm) 87.6 ± 1.12 and number of nodes per plant 2.0 ± 0.32. The seeds on MS medium with 0.5 mg/l BAP showed height (mm) 70 ± 1.70 and nodes per plant 4.0 ± 0.32. These *in vitro* grown seedlings served as source of nodal explants for micro propagation.

- Starting from first (cotyledon-node), second, third and fourth nodal segments were excised and inoculated on MS medium supplemented with 0.5 mg/l BAP. The first node produced maximum number of shoots per micro node.
2.8 ± 0.37, maximum shoot length (mm) 28.6 ± 1.36 and nodes per micro shoot 2.4 ± 0.25.

- The nodal segments were collected from field grown plants of *P. tuberosa*, sterilized under aseptic condition and inoculated on various (MS, SH and WPM) basal medium supplemented with PGR or PGR + adjuvant to initiate axillary shoot bud culture, but the nodal segments did not respond to any of the medium tested.

**Shoot proliferation**

- The micro nodes were inoculated on the MS medium supplemented with BAP (0.0, 0.5, 1.0 or 2.0 mg/l). The micro nodes exhibited best shoot proliferation on MS medium supplemented with 1.0 mg/l BAP. It showed shoots per micro node 3.4 ± 0.39, shoot length (mm) 35.0 ± 1.87, and nodes per shoot 2.7 ± 0.29.

**Root initiation**

- The micro shoots of *P. tuberosa* were inoculated on half MS medium supplemented with IBA, IAA or NAA (each 0.0, 0.5, 1.0 or 2.0 mg/l). The micro shoots showed 100% root initiation when placed on half MS medium supplemented with 0.5 or 1.0 mg/l IBA. However, maximum number of root per micro shoot 13.2 ± 1.38 and maximum root length (mm) 37.6 ± 1.51 were recorded in cultures rooted on half MS with 1.0 mg/l IBA.

**Hardening of plantlets**

*In vitro* regenerated plantlets were transferred to net pots containing coco peat or mixed soil (soil: sand: FYM in 1:1:1 ratio) to study the effect of substratum on primary hardening. The percent survival of tissue culture plants on coco peat and mixed soil were 86.67 and 66.67, respectively. For secondary hardening the plants were transferred to the nursery bags containing mixture of sand, soil and manure (1:1:1) and placed in the greenhouse where they showed 100% survival.

**Callus Induction**
Effect of plant growth regulators on callusing in petiole segments and leaf explants of *P. tuberosa*

**Petiole segments**

Petiole segments, 1.0 cm long, were inoculated on MS medium supplemented with 2,4-D or BAP (each 1.0 and 2.0 mg/l) for callus induction. Callus induction occurred in 100% petiole explants placed on the first medium MS with 1.0 or 2.0 mg/l 2,4-D. The callus areas (mm²) on 1.0 and 2.0 mg/l 2,4-D were 81.0 ± 9.98 and 68.5 ± 5.10, respectively. However, the callus transferred to second medium dried after 4 weeks and failed to differentiate.

**Leaf explants:**

Leaf segments, 1.0 cm², were inoculated on MS medium supplemented with 2, 4-D or BAP (each 1.0 and 2.0 mg/l) for callus induction. The leaf segments placed on MS medium supplemented with 1.0 mg/l 2,4-D showed percent callus induction 80% and callus area (mm²) 137.2 ± 13.40, and those on MS medium with 2.0 mg/l 2,4-D exhibited callus induction percent 100.0 and callus area (mm²) 189.9 ± 12.45. However, the callus transferred to second medium did not differentiate.

**6.4 Conclusion**

- *Curcuma angustifolia*

  1. The Bilaspur, Dhamtari, Pthora and Raipr provenances of *C. angustifolia* show variation in per plant rhizome production during field plantation. The order of rhizome production per plant of these provenances was – Pthora (245.5 ± 15.93) > Raipur (205 ± 12.76) > Bilaspur (203 ± 10.96) > Dhamtari (150.5 ± 21.15).

  2. Treatment of explants with 0.2 % mercuric chloride for 15 minutes is essential to sterilize subterranean explants, rhizome buds.

  3. MS medium supplemented with 3.0 mg/l BAP was optimum for the establishment of explants.
4. WPM supplemented with 3.0 mg/l BAP was better than MS and SH medium for *in vitro* shoot proliferation.
5. Sub culturing up to 6th cycles was beneficial for *in vitro* shoot proliferation, as gradual increase in shoot number occurs during these subcultures. The results reveal that approximately 1, 15,855 plantlets can be produced from a single rhizome bud explants within 36 weeks, after establishment of culture.
6. WPM medium with 3.0 mg/l BAP, 1.6 mg/l A, 30g/l sucrose and 25.0 mg/l adenine sulfate was suitable medium for shoot proliferation. Using this medium for sub culture, number of units can be increased further.
7. The semisolid medium may be replaced with liquid medium for further increase in shoot proliferation. However, the technique may be costly as liquid cultures need shaking.
8. Micro rhizomes formation is possible from the micro shoots grown on WPM supplemented with 3.0 mg/l BAP and 60 g/l sucrose at 16h light: 8h dark regime.
9. Rooting of micro shoots occurred during shoot multiplication stage and even at explants establishment stage.
10. Coco peat and FYM are suitable substrate for primary hardening and secondary hardening, respectively.
11. It is advantageous to use tissue culture plants of *Curcuma angustifolia* as planting material rather than natural rhizomes for cultivation. The plant-to-plant spacing of 45.0 cm is beneficial than other spacing. The tissue culture plants performed better than the rhizome grown plants during field trial.

- **Dioscorea hispida**
  1. Genotypic variation was not observed in *D. hispida* during explants establishment.
  2. MS medium supplemented with 1.0 mg/l BAP was suitable medium for explants establishment. It induced shoot elongation in 100% nodal explants.
3. WPM was found more suitable basal medium than MS and SH. WPM medium supplemented with 1.0 mg/l BAP induced maximum 3.1 ± 0.23 shoots per micro node, maximum shoot length (mm) 36.1 ± 2.33 and maximum nodes per micro shoot 2.56 ± 0.15 at shoot proliferation stage.

4. Kinetin or combination of BAP and Kinetin in WPM with 1.0 mg/l did not improve shoot proliferation in D. hispida.

5. Addition of 1000 mg/l PVP completely checked blackening/browning of the medium. WPM with 1.0 mg/l BAP and 1000.0 mg/l PVP induced maximum 3.4 ± 0.27 shoots per micro node, 38.0 ± 1.40mm maximum shoot length and 3.66 ± 0.82 nodes per micro shoot.

6. Addition of adenine sulfate, casein hydrolysate, citric acid and activated charcoal failed to enhance shoot proliferation in D. hispida.

7. WPM supplemented with 1.0 mg/l BAP and 0.25 mg/l IAA enhanced in vitro shoot proliferation in D. hispida. It induced 3.4 ± 0.32 shoots per micro node with maximum shoot length 51.4 ± 2.06mm and nodes per micro shoot 3.3 ± 0.2.

8. Addition of NAA in WPM with 1.0 mg/l BAP gradually decreased shoot proliferation.

9. The cultures hyper hydricity within 15 days of inoculation, whereas on semisolid medium the cultures grew normally.

10. Shoot number, maximum shoot length (mm) and number of nodes per micro shoot showed gradual increase up to 5th cycle. It is estimated that approximately 3,61,035 propagules can be obtained from a single node in 30 weeks after explants establishment.

11. Best (100%) micro tube formation and healthy micro tubers (520 mg/micro tuber) can be achieved on WPM supplemented with 1.0 mg/l NAA and 60.0g/l sucrose under 8h light:16h dark.

12. Root induction is a problem in micro shoots D. hispida Best (90%) root induction can be obtained in micro shoots placed on half - WPM with 2.0 mg/l IAA.
13. Coco peat and FYM are suitable substrate for primary hardening and secondary hardening, respectively.

14. Plants developed from old tubers were superior to those developed from TC plants.

15. The micro tubers cannot be directly used for cultivation. These micro tubers should be subjected to more cycles ex vitro. However, the number of cycles should be standardized.

*Pueraria tuberosa*

1. Complete in vitro plantlet regeneration can be achieved through seedling nodes.

2. *In vitro* raised seedlings are suitable explants to initiate bud culture.

3. Field grown plants are not amenable to tissue culture.

4. The first, second, third and fourth nodes of *P. tuberosa* seedlings show a significant difference during explants establishment. The first nodes (cotyledon node) of the *in vitro* raised seedling are best explants for *in vitro* shoot culture establishment in *P. tuberosa*.

5. MS medium supplemented with 1.0 mg/l BAP is best medium for shoot proliferation from nodal explants seedling origin.

6. Half MS supplemented with 1.0 mg/l IBA was suitable for root induction. The micro shoots placed on half-MS with 0.5 or 1.0 mg/l IBA.

7. Coco peat and FYM are suitable substrate for primary hardening and secondary hardening, respectively.

8. Dedifferentiation of leaf and petiole tissues of *P. tuberosa* can be achieved on variety of media. However, redifferentiation is difficult. A variety of media were tested for differentiation but none of them could induce caulogenesis or somatic embryogenesis.