2.2 REVIEW OF LITERATURE

The idea of totipotency of plant cells was put forward by G. Haberlandt, the great German physiologist who (1902) suggested that “one could successfully cultivate artificial embryos from vegetative cells”.

Steward et al., (1958) demonstrated the totipotency of higher plant cells in unambiguous terms with their success in forming somatic embryos from cultured root phloem cells.

The combined use of IAA and kinetin by Skoog and Miller (1958) illustrated the synergism association with these compounds regarding the development of synchronized shoot-root plant regeneration.

Rao and Narayanaswamy (1972) reported that the young stem derived callus is highly viable, whereas the callus derived from the leaf bits was soft and could not be maintained beyond a second or third subcultures found in Tylophora indica.

Murashige (1962) stated that the development of shoots on basal media may be due to stimulation by endogenous hormones or some signals related to wounding, which play a vital role during the induction of regeneration, or the ratio of ions present in the medium. The difference in the number of shoots formed in leaf and internode explants may be a result of differences in the regeneration potential of different explants, which is attributed by the physiological state, age and cellular differentiation among the constituent cells.
Staba, (1980) reported that the microbes multiply and compete with growing explant for nutrients, while releasing chemicals which can alter culture environments e.g. pH and inhibit explants growth or cause death.

Beck and Componetti (1983) reported the necessity of the cytokinin for shoot initiation.

According to George & Sherrington (1984) the combination of auxins and cytokinins promote cellular differentiation and also organogenesis.

Stafford, (1986) reported plant derived compounds include many terpenes, polyphenols, steroids, alkaloids and glycosides.

Arora and Bhojwani (1989) reported the in vitro propagation of *Saussurea lappa* Clarke. In their work they have induced high frequency of shoots from leaf explants on MS media supplemented with NAA (0.5 µM).

Fowler (1993) stated that callus formation occurs from revered process of cell differentiation, known as dedifferentiation or redifferentiation.

Hsu *et al.*, (1993) developed a simple and effective method for plant propagation of *Bupleurum. falcatum* using terminal and lateral buds. Among the two explants tested for regeneration potential, terminal bud explants were most responsive. The induction of shoots was achieved in the one-fourth MS basal medium (containing full-strength organic constituent) supplemented with 1 mg/l BA, 0.2 mg/l NAA, and 1% sucrose. The maximum number of adventitious buds (13 adventitious buds per responding explant) developed when the terminal buds
were cultured in the liquid MS basal medium supplemented with 1 mg/l BA for one month. Addition of amino acids [proline (100 mg/l), tryptophan (100 mg/l), glutamine (100 mg/l) and asparagine (100 mg/l)] or coconut milk (10%) to the culture medium facilitated shoot growth and reduced browning of tissue.

According to Roja (1994) since production of secondary metabolites is generally higher in differentiated tissues, there are attempts to cultivate shoot cultures and root cultures for the production of medicinally important compounds, these organ cultures are relatively more stable.

George (1996) reported that 2,4-D shows effect on the RNA metabolism by inducing the transcription of messenger RNA capable of coding proteins required for the growth and hence, promoting a chaotic cell proliferation, i.e., callus formation.

According to Jager and Van Staden (1996). 2,4-D is said to show unorganized growth regulation and is supportive for the organogenesis together with the cytokinins.

Natarajan et al., (1999) developed a protocol for callus-mediated shoot regeneration from stem explants of *Hybanthus enneaspermus*. Green compact nodular calli are obtained on MS medium containing 2.0 mg/l 2,4-D and 0.5 mg/l BAP. Shoot buds were induced and elongated on MS basal medium with 5.0 mg/l BAP. The shoots were rooted in MS medium with 2.0 mg/l IBA, and then were successfully hardened and transferred to the field.
Rout (2000). Explants in cultures release phenol compounds, which are oxidised by enzymes known as polyphenol oxidase, and cause the media to turn brown by transferring explants into new culture media on regular intervals.

Matkowski (2000) stated that browning can be minimized by adding antioxidants or phenol absorbents for e.g. ascorbic acid, glutathione, activated charcoal and polyvinylpyrrolidone.

In Adenophora triphylla (Chen et al., 2001) standardized an efficient plant regeneration system from stem internode explants. Adventitious shoots were induced by culturing the explants on MS basal medium supplemented with 2.22 - 35.51 µM BA in combination with 0.54 µM NAA. The regeneration potential varied with the developmental stage of the stem explant and growth regulator combinations. The stem explants obtained from the uppermost region of the shoot, near to the shoot tip, were the most competent for the regeneration, and the competence decreased as the stem matured.

Begum et al., (2002) reported in vitro rapid clonal propagation of Ocimum basilicum L. Subculture of in vitro shoots produced a highest frequency of rooting on MS media containing 1.0 mg/l NAA. Plantlets were successfully established under ex vitro condition.

Rao and Ravishankar (2002) reported that in medicinal and aromatic plants the metabolites of medicinal importance biosynthesized and accumulate in different organs such as roots, leaves and shoots.
Bais et al., (2002) reported the in vitro propagation of *Spilanthes mauritiana* DC. Maximum shooting was achieved on MS media with BA (1.0 mM) and NAA (0.1 mM) with minimal callusing from axillary bud explants. Shoots rooted best in MS media supplemented with IAA (0.2 mM) and the plants were well established in the soil.

Wala (2003) in *Curculigo orchioides* Gaertn. multiple shoots from the meristem tip culture on MS medium supplemented with BA (2.21 µM). The shoots were rooted either on half strength of MS basal medium or on the one supplemented with NAA (0.53 µM). In vitro plantlets were transferred to pots containing a mixture of vermiculite and soil (1:1) for acclimation for a period of two-three weeks.

Arya et al., (2003) developed a micropropagation protocol for mass multiplication of *Leptadenia reticulata*. They harvested from greenhouse-maintained and field-grown plants were used to establish cultures of *L. reticulata*. The nodal shoot segments were surface-sterilized and cultured on Murashige and Skoog (MS) medium along with additives (25 mg l⁻¹ each of adenine sulfate, arginine, and citric acid, and 50 mg l⁻¹ ascorbic acid) containing 0.6 µM indole-3-acetic acid (IAA) and 9 µM N⁶-benzyladenine (BA).

Datta et al., (2003) reported that *Heliotropium indicum* L. (Boraginaceae) contains the (INO). To study the yield of INO as a function of plant development, plantlets were regenerated in vitro from nodal and hypocotyl explants and also
from hypocotyl callus, on Murashige and Skoog’s (MS) medium supplemented with 1-naphthaleneacetic acid (NAA), 6-benzylaminopurine (BAP), asparagine (Asp) and glutamine (Glu). The regenerated plantlets were rooted on MS supplemented with Glu or gibberellic acid (GA3).

Ghosh and Bannerjee (2003) reported the influence of plant growth regulators on *in vitro* callogenesis and in vitro shoot regeneration from leaf and nodal explants of *Justicia gendurussa* Burm. F.

Oksman *et al.*, (2004) stated that unlike humans and animals, plants are not mobile which makes them very susceptible to attack from pests and predators. To overcome this problem, during metabolism plants produce enormous number of compounds as part of defence mechanism. These compounds are not essential for primary functions like growth, photosynthesis and reproduction and are called secondary metabolites. Secondary metabolites are used as pharmaceutical, agrochemicals, aromatics and food additives.

Vanisree *et al.*, (2004) stated that the endangered status warrants that tissue culture conditions need to be standardized from different parts of the plant so that the cultures can be used for the production of metabolites under laboratory conditions as has been reported in various medicinal plants so as to relieve pressure from its natural habitat.

Shoot tips were excised from in vitro seedlings and established on MS, Nitch and Nitch (NN), or B5 medium. For shoot proliferation, *in vitro* nodal and apical explants were cultured on MS medium containing 0.25–2 µM 6-benzylaminopurine (BA), 6-furfurylaminopurine (kinetin), or thidiazuron (TDZ). Proliferated microshoots were rooted on MS medium supplemented with 2.7–11.4 µM indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), or α-naphthaleneacetic acid (NAA). Results indicated that the shoots established at 100% regardless of media type, however, shoot height, nodes per shoot, and leaf number were highest for explants established on MS medium compared to NN or B5.

Paulsamy *et al.*, (2004) worked on *Berberis tinctoria* Lesch., results of the study exhibited that the basal medium containing BAP and NAA each at 0.5 mg/l was found to be the optimum for callus formation. Shoot proliferation was highly effective in the basal medium supplemented with BAP at 0.5 mg/l. The root initiation was maximum in the basal medium containing the NAA at 1.0 mg/l and the plantlet establishment was successful in the hardening medium composed by vermiculite and soil in the ration of 1:1.

Ali *et al.*, (2005) developed a suitable micropropagation method for *Hypericum perforatum* L. They obtained highest callus on MS media supplemented with kinetin and 2,4-D (0.5 mg/l) in darkness from leaf disc
explants. Highest number of shoots were obtained from leaf callus and easily acclimatized in greenhouse conditions.

Lu (2005) described a protocol for rapid and large-scale in vitro propagation of the valuable medicinal herb *Vitis thunbergii* Sieb. et Zucc.. Culture conditions influencing shoot proliferation and rooting of the two clones (three- and five-lobed) were examined. Three medium formulations, Murashige-Skoog (MS), Woody Plant Medium (WPM) and Nitsch and Nitsch (NN) medium, were tested for growth of shoot tip culture, and WPM was found to have a superior proliferation rate.

Rehman *et al.*, (2005) developed an efficient in vitro protocol for rapid production of plantlets using rhizome tip and lateral bud explants of *Kaempferia galanga* L. on MS medium with auxins and cytokinins and were acclimatized and established in the soil with 85% of success.

Lattanzio V. *et al.*, (2006), after a short reviewed plant phenols and polyphenols as UV sunscreens, signal compounds, pigments, internal physiological regulators or chemical messengers and examines some findings in chemical ecology concerning the role of phenolics in the resistance mechanisms of plants against fungal pathogens and phytophagous insects.

Thomas T. D. and Maseena E.A (2006) established a method for rapid micropropagation of *Cardiospermum helicacabum* through plant regeneration from leaf and nodal explant derived calli. The nodal and leaf segments were
cultured on MS medium supplemented with 2,4-D (0.5–9 µM) for callus induction. Callus production was highest at 5 µM 2,4-D where 96 and 90% of cultured leaf and nodal cuttings produced callus, respectively. The viable calli were maintained at reduced concentration of 2,4-D (2 µM). These calli were transferred to MS medium supplemented with various concentrations of 6-benzyladenine (BA; 2–10 µM) or kinetin (2–10 µM) alone or in combination with indole 3-acetic acid (IAA; 0.2–1.0 µM) for shoot regeneration.

Chandra et al., (2006) reported successful propagation of *Picrorrhiza kurroa* Royle ex Benth. *In vitro* shoot multiplication was achieved using nodal segment on MS media containing 1.0 l M BAP. Rooting was observed in MS media supplemented with IBA. The plantlets raised were well established in the field.

Prakash et al., (2007) stated that in conventional cultivation many plants do not germinate, flower and produce seed under certain climatic conditions or have long periods of growth and multiplication. Micropropagation insures a good regular supply of medicinal plants, using minimum space and time.

Sreelatha et al., (2007) used the different medium MS, B5 and MS macro salts + B5 micro salts with various hormones alone or in combinations. 0.1mg/l Kn + 0.1 mg/l TDZ + 2.0 mg/l 2-iP gave the best response on MS macro salts + B5 microsalts when nodal explants of *Cassia siamea* cultured.
Malik, (2007) stated that the micropropagation provides a fast and dependable method for production of a large number of uniform plantlets in a short time. Moreover, the plant multiplication can continue throughout the year irrespective of season and the stocks of germplasm and it can be maintained for many years.

Bin et al., (2007) reported a protocol for in vitro propagation of *Saussurea involucrata* Kar. Highest shoot regeneration frequency per leaf explant were achieved on MS media containing BAP and 2.5 µM NAA. The regenerated shoots were well rooted on a media containing 2.5 µM IAA. Regenerated plantlets survived and grew vigorously in greenhouse condition.

Nikam and Savant (2007) reported that the callus formed on the media augmented with IAA and NAA quite often showed fine hairy mass on the surface of calli, while the calli induced on the 2,4-D were soft, pale yellow and slight morphogenetic noticed in *Ceropegia sahyadrica*.

Faisal et al., (2007) described an efficient protocol for the rapid in vitro multiplication of an endangered medicinal plant, *Tylophora indica* (Burm. f.) Merrill, via enhanced axillary bud proliferation from nodal explants collected from young shoots of a two-year-old plant. The physiological effects of growth regulators (BAP, kinetin, thidiazuron, IAA, IBA or NAA, ascorbic acid) different strengths of Murashige and Skoog (MS) medium and various pH levels on in vitro morphogenesis were investigated.
Kottapalli and Prasad (2007) reported an efficient protocol for rapid in vitro multiplication of Drosera indica L. Maximum multiple shoots were developed on MS media supplemented with Zeatin (0.5 mg/l) and Kinetin (0.5 mg/l). Rooting was achieved on MS basal media.

Sivanesan and Byoung (2007) reported in vitro propagation of Sida cordifolia L. Multiple shoots were achieved on MS media supplemented with 2.0 mg/l BA, 0.5 mg/l NAA, 1.0 mg/l adenine sulfate, and 10% coconut milk. Regenerated shoots were successfully rooted on ½ strength MS media supplemented with 2.0 mg/l IAA and 3% sucrose. Rooted plantlets were established in the field.

Naika et al., (2008) reported an in vitro regeneration protocol through stem callus culture for the medicinal climber Clematis gouriana. The explant induced callus on MS-medium supplemented with BAP and NAA. The optimized callus induction occurred at the concentration of 1.0 mg/l BAP and 0.3 mg/l NAA. After initiation of callus, it was immediately transferred to MS medium containing FAP and indole-3-butyric acid (IBA). The microshoots rooted well on MS basal medium without growth regulators as well as on medium supplemented with 0.5 mg/l IBA.

Thomas (2008) reported that promotary effects of Activated Charcoal on morphogenesis may be mainly due to its irreversible adsorption of inhibitory compounds in the culture medium and substantially decreasing the toxic
metabolites, phenolic exudation and brown exudate accumulation. In addition to this activated charcoal is involved in a number of stimulatory and inhibitory activities including the release of substances naturally present in AC which promote growth, alteration and darkening of culture media, and adsorption of vitamins, metal ions and plant growth regulators, including abscisic acid and gaseous ethylene.

Verma (2008) achieved *in vitro* regeneration of *Trichodesma indicum* L. through embryo culture. The zygotic embryos placed on MS media with different hormones like Kinetin, BA and NAA for the induction of callus and adventitious shoots. About 60% of micro shoots developed roots on MS media supplemented with IBA. The regenerated plants were successfully acclimatized and transferred to the soil.

Chitra (2009) developed protocol for indirect organogenesis of *Phyllanthus amarus* using leaf explants and internodes. High frequency of callus proliferation was obtained when the callus was on MS medium supplemented with BAP (1.0 mg/l) and glycine (50.0 mg/l). Complete plantlets were obtained when the callus was sub-cultured on MS medium supplemented with BAP (2.0 mg/l) and GA3 (0.5 mg/l). Rooting (87.09%) of the shoots was best achieved on half strength MS medium supplemented with IBA (0.5 mg/l) and IAA (0.5 mg/l). Regenerated plants were successfully transferred to soil after acclimatizing them in the plant growth chamber.
Balaraju et al., (2009) reported the micropropagation of *Swertia chirata* Buch. Highest numbers of multiple shoots were induced from shoot tip explant on MS media with BAP at 1.0 mg/l and Kn 0.1 mg/l. Shoots were transferred to half-strength MS media with NAA 0.1 mg/l for rooting. Plants were hardened within the culture room.

De Pinto et al., (1999) studied the effect of Vitamin C (L-ascorbic acid) as a component of culture media will be used during explant isolation and to prevent blackening. Besides, its role as an antioxidant, ascorbic acid is involved in cell division and elongation, e.g., in tobacco cells.

Lim et al., (2009) reported callus induction from leaf explants of *Ocimum sanctum* on MS medium supplemented with 2,4-D, picloram, and IBA at 0, 1, 3, and 5 mg/l as well as the combination of 3 mg/l picloram with different concentrations (0, 0.5, 1.0, 1.5, and 2.0 mg/l) of BAP or kinetin. Results obtained from the studies revealed that all the leaf explants incubated on phytohormone supplemented medium formed callus.

Sood et al., (2009) established callus from different explants such as leaf, nodal and root segments of *Picrorhiza kurroa*. Callus induction was highest (70%) in root segments followed by leaf discs (56.3%) and nodal segments (38.3%) on MS medium supplemented with 2,4-D (2 mg/l) + IBA (0.5 mg/l) + sucrose 3% (w/v) + agar-agar 0.8% (w/v). The callus cultures derived from different
explants were differentiated into multiple shoots on MS medium containing different concentrations and combinations of BA, KN and IBA.

Mungole et al., (2009) obtained callus of the leaves, node and bud of *Ipomoea obscura* (L) was initiated on MS basal media supplemented with various combinations of auxins 2,4-D and NAA with cytokines kinetin and BAP. Callus initiation was observed in all media but with varied mass. Highest percentage of callus response was obtained in combination of 2,4-D (0.8 mg/l) with kinetin (0.8mg/l); NAA (0.2mg/l) with BAP(0.2 mg/l); NAA (0.8 mg/l) with kinetin (0.8 mg/l) for explants leaves, node and bud, respectively.

Kondamudi et al., (2010) evaluated the most suitable concentration of plant growth regulators and perfect explant (node, internode and thin cell layer explants-TCLs) for callus induction and subsequent organogenesis in an endangered medicinal *Ceropegia pusilla*. The best callus induction was found on the MS medium supplemented with BAP + 2,4-D from TCLs. After the initiation of the callus, it was immediately transferred to MS medium supplemented with BAP along with other auxins like 2,4-D, IAA, IBA, NAA. The regenerative calli were raised on the MS medium supplemented with 2,4-D.

Theriappan et al., (2010) made an attempt to develop a simple, reliable and reproducible protocol for micropropagation from different explants of *Aristolochia indica*. Shoot tip and nodal segments showed elongation without multiplication when either NAA or KN was used in MS medium. Shoot multiplication was
obtained when cytokinins like BAP was used. BAP alone also induced multiple shoots. The regenerated individual shoots were rooted on MS medium containing 1 mg/l IBA. Regenerated plants grew well when transferred to soil.

Sharma et al., (2011) tested powdered roots of *Heliotropium indicum* L. for preliminary phytochemical screening. The qualitative chemical examinations revealed the presence of various phytoconstituents like alkaloids, carbohydrates, phytosterols, tannins and saponin in the root extracts through the chromatographic separation technique i.e. TLC . These observations therefore support the use of *Heliotropium indicum* in herbal cure remedies.

Pattar et al., (2011) reported *in vitro* vegetative propagation protocol has been developed for *Blepharis molluginnifolia*, Pers. (Acanthaceae) by using nodal explants. Direct shoot formation from nodal segments was obtained on MS medium with BAP (0.5 mg/l) within 4 weeks. *In vitro* shoots thus obtained were successfully rooted in MS supplemented with IAA (0.5mg/l) alone.

Sonappanavar et al., (2011) developed a protocol for rapid callogenesis in *Ionidium suffruiticosum*, Ging has been standardized using leaf explants. Among the explants, the rapid callogenesis and proliferation was obtained from leaf explants on Murashige and Skoog (MS) medium supplemented with 2,4-D (0.5 mg/l to 1.0 mg/l).

Sonappanavar et al., (2011) analyzed the effect of auxins and cytokinins to develop a standard protocol for in vitro propagation of *Ionidium suffruiticosum*
Ging. Hormones like 2,4-D, NAA, IBA, IAA, Kinetin, BAP and Zeatin were used in different combinations and concentrations for callogenesis and indirect plant regeneration.

Gajakosh et al., (2011) reported in vitro morphogenic response of leaf and shoot tip explants of Gardenia gummifera (L) f. Maximum number of advent has been obtained on MS medium augmented with 2.0 mg/l NAA and 2.0 mg/l IBA. From leaf explants maximum shoots from MS medium supplemented with 2.0 mg/l of KN and 5.0 mg/l BAP and 2.0 mg/l NAA.

Das et al., (2011) stated that to sustain the supply of quality propagules, develop a novel protocol for accelerated in vitro mass multiplication in Stevia (Stevia rebaudiana Bert.) through multiple shoot induction using shoot tips, nodal segments and axillary bud explants.

Bhagat (2011) In vitro Micropropagation of Acorus calamus Plant was achieved using Rhizome Explant. The Explant was inoculated in M.S Media supplemented with different concentration of phytohormones (0.5-1mg/l) IAA-BAP, (0.5-2mg/l) IAA-BAP or without any growth Hormones. The frequency of Shoot Organogenesis was highest at 73% response rhizome treated with (0.5-2mg/l) IAA-BAP and 30% response in treatment with (0.5-1mg/l) IAA-BAP, 18% response in any pytohormone. The micro shoots rooted well in M.S medium supplemented with 4.0mg/l (IBA) and hardened (60%) plants acclimatized in soil.
Corduk (2011) stated that inhibitory effects of different treatments such as Heller medium or adding activated charcoal, morpholine ethane sulfonic acid (MES) or ascorbic acid/citric acid combination to the medium, fast subculture passages or changing culture conditions to the dark were investigated against browning problem of *Sideritis trojana* Bornm. Adding a combination of 100 mg/l ascorbic acid and 50 mg/l citric acid to the Murashige and Skoog (MS) medium was found as the most effective treatment.

Loc *et al.*, (2011) developed tissue culture propagation system for *Solanum hainanense* Hance, a valuable medicinal plant, using shoot elongation culture and shoot regeneration cultures. MS medium containing 3% sucrose and 0.8%, supplemented with 0.7 mg/l BAP resulted in a good shoot elongation. Shoot regenerations were induced from leaf discs of plantlets on the MS medium containing 3% sucrose and 0.8% agar, supplemented with 0.1 mg/l IBA, 1.8 mg/l kinetin, and 3.8 mg/l BAP. Well-developed shoots (3-4 cm in length) were rooted on the MS medium with 3% sucrose, 0.8% agar and 0.5 mg/l IBA. Approximately 95% of rooted plants were established in soil after hardening.

Sathish Kumar *et al.*, (2011) studied micropropagation in *Solanum trilobatum*. MS medium supplemented with BAP 2.0 mg/l and Kinetin 2.0 mg/l was found to initiate the shoot from nodal explants within 10-15days. The root induction was obtained on MS media containing 2, 4-D (2.0 mg/l) and IBA (2.0 mg/l). For callus initiation node and leaf explants are used, NAA + Kinetin
(2.0+2.0 mg/l) was found good combination for induction of callus from node, NAA + Kinetin(2.0+2.0 mg/l) and NAA + BAP (2.0+2.0 mg/l) was found as good combination for induction of callus from leaf explants.

Tejavathi et al., (2012) developed mass multiplication of *Majorana hortensis* through nodal cultures. Explants were inoculated on Murashige and skoog’s and Phillips and Collins media fortified with BAP and KIN. Direct and indirect organogeneses were obtained depending on the hormonal concentrations and combinations.

Mehta et al., (2012) reported micropropagation of *Stevia rebaudiana*. Shootlets were regenerated from nodal explants of stem through auxiliary shoot proliferation. The induction of multiple shoots from nodal segments was the highest in MS medium supplemented with 0.5 mg/l BAP+2.0 mg/l Kn. For rooting different concentration of IBA were used and highest rooting was recorded on MS medium with 1.0 mg/l IBA. The rooted Plantlets were hardened initially in culture room conditions and then transferred to mist house.

Kavitha et al., (2012) studied *in vitro* direct multiple shoot regeneration of *Solanum nigrum* L. Shoot tip and nodal explants were inoculated on MS medium containing BAP (1.0–5.0 mg/l) and KN (1.0–5.0 mg/l) individually. MS medium supplemented with BAP (1.0 mg/l) from shoot tip and MS medium supplemented with BAP (3.0 mg/l) from nodal explants produced good number of shoots. The *in*
vitro regenerated shoots were rooted on MS medium supplemented with NAA (1.0 mg/l) and the regenerated plantlets could be successfully established in soil.

Promod et al., (2012) developed a protocol for in vitro multiplication of *Sida cordifolia* L. Callus induced on MS medium supplemented with 0.5mg/l KN. Multiple shoots regenerated on MS medium supplemented with KN and NAA.

Thangapandian et al., (2012) reported micro propagation technology to conserve this valuable medicinal herb *Centella asiatica* within a month of period. The combinations of BAP (1.0 mg/l) with IAA (0.5 mg/l) showed good callus proliferation. The combination of cytokinin with auxin (3:1) in modified MS media showed higher response in shoot elongation. Among the combinations of BAP with IAA (4:1) showed good response in shoots elongation. BAP (1.5 mg/l) with IAA (0.5 mg/l) showed higher shoot multiplication within a month and the difference was significant among the trials.

Sakthi et al., (2012) reported large-scale micropropagation of *Scoparia dulcis* on Murashige and skoog (MS) medium supplemented with 2,4-D and BAP (1.5 mg/l each). The maximum numbers of shoots were produced in the combination of BAP and IAA (1.5 mg/l each). Transfer of shoots (4-5 cm) in to MS solid and liquid medium favoured rooting in 4 weeks and rooted plants (9 cm) were hardened and established (70-85%).

Verma et al., (2012) Twenty-three pharmaceutically important plants, namely, *Elaeocarpus sphericus, Rheum emodi, Indigofera tinctoria, Picrorrhiza*
kurroa, Bergenia ciliata, Lavandula officinalis, Valeriana wallichii, Coleus forskohlii, Gentiana kurroo, Saussurea lappa, Stevia rebaudiana, Acorus calamus, Pyrethrum cinerariaefolium, Aloe vera, Bacopa monnieri, Salvia sclarea, Glycyrrhiza glabra, Swertia cordata, Psoralea corylifolia, Jurinea mollis, Ocimum sanctum, Paris polyphylla, and Papaver somniferum, were successfully established in vitro.

Velayutham et al., (2012) established a successful in vitro mass micropropagation from the stem explants of Hybanthus enneaspermus. MS medium supplemented with different concentrations of IAA, IBA and NAA. The highest frequency of 98.8% callus culture was obtained on MS medium supplemented with 15 µM NAA in combination with 5 µM BAP. The highest frequency of 100% shoot regeneration was observed on MS medium supplemented with all the concentrations of BAP and KIN.

Ganthikumar (2013) Nodal segments of Coccinia indica Wight & Arn were cultured on MS supplemented with various concentration and combination of 6-BAP in combination with Kn stimulated shootlets formation with varied percentage. Highest percentage of rootlets formation was observed in half strength of IAA combined with 0.5 mg/l of Kn. 68% of plants were established in the field.

Pramod et al., (2012) developed a protocol for rapid callus induction and plant regeneration from young nodal explants of Sida cordifolia L. Maximum shoots were developed on MS medium supplemented with KN and NAA.
Pramod et al., (2012) developed a protocol for in vitro regeneration of plantlets from leaf and nodal explants of Aristolochia indica L. Leaf and nodal explants cultured on MS medium supplemented with BAP developed into mass of callus. Subcultures of calli on MS medium supplemented with BAP and NAA induces shoots.

Sahu et al., (2013) studied in vitro plantlet regeneration in Aerva lanata (L.) from nodal segments cultured on MS medium supplemented with BA, KN and NAA was best and produced the maximum multiple shoots via callus mediated organogenesis.

Kaul et al., in 2013 established a successful in vitro micropropagation protocol for Ajuga bracteosa. MS medium supplemented with IAA (2 mg/l) and BA (5 mg/l) induced 100 % shoot regeneration with an average of 41.4 shoots of 8.4 cm per culture. Of the three explants, leaf, petiole and root, leaf displayed quickest response followed by petiole while root was the slowest. Hardening of plantlets was achieved with 82 % survival.

Mandal (2013) established protocol for micropropagation of Hyptis suaveolens. A 100% shoot regeneration frequency and 16.9 shoots per node explant were achieved on Murashige and Skoog medium (MS) containing 1.0 mg/l BAP and 0.5 mg/l GA₃. Node explants of microshoots could be induced for proliferation of a large number of harvestable shoots on the same medium. The shoots developed in vitro inflorescences and 8.5 flowers per shoot on MS medium.
supplemented with 2.0 mg/l IBA. Rooting of microshoots was induced on MS medium containing 1.0 mg/l IAA. The plantlets were acclimatized, transplanted to natural soil, and evaluated.

Ceasar (2013) established an improved protocol was developed for the micropropagation of *Plumbago zeylanica* L. from nodal explants. The best response of shoot induction was observed on Murashige and Skoog (MS) basal medium supplemented with 1.0 mg/l thidiazuron (TDZ) and 1.0 mg/l KN. The regenerated shoots rooted best on half strength MS basal medium containing 1.0 mg/l IBA. *In vitro* propagated plants were transferred to soil with 90% survival rate.

Pramod et al., (2013) developed a protocol for the induction of somatic embryos from nodal callus of *Sida cordifolia* L. MS medium supplemented with KN individually and in combination s with NAA induces callus and radially develops somatic embryos.

Sebastian et al., (2013) established protocol micropropagaion of *Heliotropium keralense* from root explants. Murashige and Skoog's (MS) medium supplemented with 2,4-D (0.5 mg/l) and 2 i P (4.0 mg/l) was the most effective combination for the induction of callus from roots. MS medium supplemented with BA (3.0 mg/l) and IAA (0.5 mg/l) was the most suitable combination for callus regeneration. MS medium supplemented with 0.5 mg/l IBA was most
effective for rooting of shoots. Plantlets hardened and eventually established in the field.

Haque and Ghosh (2013) worked on *Bacopa chamaedryoides*. Shoot-tips and nodal segments explants were inoculated on M S basal medium containing BAP and IAA. Shoot-tips proved to be a better explant. *In vitro* rooting of multiplied individual shoots was achieved on half strength MS medium supplemented with 50% of ‘*Aloe vera* gel’, with a maximum of 18.3 ± 0.17 roots. Up to 66.7% of these multiplied shoots induced healthy flowers *in vitro* on MS medium containing low concentration of BAP (0.2 mg/l).

Sarma *et al.*, (2013) studied the effect of hormone on micropropagation and induction of callus in *Nyctanthes arbor- tristis Linn.* and *Tinospora cordifolia*. Murashige and Skoog’s (MS) media supplemented with 2.5 µM of BAP and 4.4 µM of BAP showed best response.