The past decades of plant cell biotechnology has evolved as a new era in the field of biotechnology, focusing on the production of a large number of secondary plant products. During the second half of the last century the development of genetic engineering and molecular biology techniques allowed the appearance of improved and new products which have occupied an increasing demand in the productive systems of several countries worldwide (Vasil, 1994; Christou et al., 2006; Navarro, 2007; James, 2008). Nevertheless, these would have been impossible without the development of tissue culture techniques, which provided the tools for the introduction of genetic information into plant cells (Pareek, 2005).

*In vitro* culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells (Haberlandt, 1902) and unequivocally demonstrated for the first time in plants by Steward et al. (1964). Beyond the discovery of Kinetin (Miller et al., 1955), the major work on *in vitro* regeneration has been centered around tobacco (*Nicotiana tabacum* L.) tissue culture, culminating in the first convincing demonstration of the control of differentiation of shoots or roots or both by the Kinetin-auxin ratio (Skoog and Miller, 1957) followed by carrot (*Daucus carota* L.) tissue culture and birth of the concept of totipotency of plant cell with the regeneration of complete flowering plants of carrot from its phloem cells (Steward et al., 1964).

Reports concerning the recovery of plants from haploid cells began to appear in the 1960's. The first successes were obtained with *Datura* (Guha and Maheshwari, 1964) and tobacco (Bourgin and Nitsh, 1967). Plant protoplasts are isolated and began to be cultured in the 1960's (Cocking, 1960). The isolated protoplasts, under culture conditions are known, to form cell colonies (Kao et al., 1971), to form cell walls (Prat, 1972) and to regenerate into plants (Nagata and Takebe, 1971; Schuma and Koblitz, 1983).
The history of plant tissue culture and its application have been reviewed and discussed from time to time (Gautheret, 1983, 1985; Krikorion, 1982, 1988; Thorpe, 1990, 2007; Gamborg, 2002; Vasil, 2008). The problems and potentials of using tissue culture in micropropagation and biotechnology related to forestry improvement have been critically and elegantly analyzed in several reviews and discussions (Thorpe and Biondi, 1984; Dunstaan and Thorpe, 1986; Bonga and Durzan, 1989 and Hammat, 1992).

It is well known that micropropagation allows both rapid and massive clonal multiplication of plants; however, it does not ensure that material will be free of systemic agents, such as viruses, which can be present in tissues without manifesting symptoms and spread during the in vitro multiplication. However, among the in vitro techniques, shoot tip or meristem culture has been used for many decades to eliminate viruses in many species from vegetatively propagated plants (Ashmore, 1997; Faccioli and Marani, 1998). This is based on the uneven distribution of viruses in the youngest tissues of the shoot apex, as their concentration tends to decrease progressively toward the apical meristem of the stem, where the cells are in constant and rapid division (Abdelnour et al., 2006; Wang and Valkonen, 2009). Since not all cells in a shoot apical meristem are infected with pathogens (e.g., virus, phytoplasmas and endophytic bacteria), it is possible to dissect out a non-infected region and manipulate this explant in vitro to produce virus-free plants (Grout, 1990; Kane, 2005).

2.1 TISSUE CULTURE OF IMPORTANT LEGUMINOUS TREES

Leguminous forest trees are of special interest because of their economic and ecological importance. Therefore, the application of tissue culture technique for their clonal propagation and improvement is urgently warranted (Tomar et al., 1998). Tree legumes, once established, grow faster than other species even under poorly managed plantation-forestry system as they fix atmospheric nitrogen for sustenance and growth. Leguminous plants are difficult to propagate. Propagation through seed is unreliable as mature seeds are often damaged by pod borers followed by bacteria and fungi which cause secondary infections; healthy seeds are
rarely available for mass propagation. Poor germination and death of young seedling are also the causes of worry. Mass cloning via somatic embryogenesis can be an alternative and reliable system for plant multiplication (Ammirato, 1983). Somatic embryogenesis was reported for a number of dicotyledonous and monocotyledonous angiosperm but fewer woody species.

Plant tissue culture offer advantages over conventional methods for multiplication and large-scale production of woody plants (Thorpe et al., 1991). Micropropagation of *P. marsupium* (Das and Chatterjee, 1993; Kalimuthu and Lakhsmanan, 1994; Chand and Singh, 2004; Anis et al., 2005; Husain et al., 2007, 2008, 2010) has been reported. *In vitro* plant regeneration from seedlings are available on *P. marsupium*, but *in vitro* plant regeneration from nodal segment of mature tree of *P. marsupium* has not yet been reported. The present research work is an attempt to study the *in vitro* propagation of *P. marsupium* from nodal segment of mature plant.

### 2.2 MICROPROPAGATION THROUGH AXILLARY SHOOT PROLIFERATION

Micropropagation is the process of vegetative growth and multiplication from plants tissues. It is carried out in aseptic and favourable conditions on growth media, using various plant tissue culture techniques (Zhou and Wu, 2006). Tissue culture is based on concept of totipotency; the ability of plant cells and tissues to develop into whole new plant (Fowler et al., 1993). Gottlieb Haberlandt (1854-1945), a German botanist, considered as the father of plant tissue culture, was the first to separate and culture plant cells on Knop's salt solution in 1898.

Micropropagation work has been carried out on many leguminous plants and trees. Generally nodal explants, shoot tip, cotyledonary nodes, coppice shoots and auxillary meristem are used as explants for micropropagation of legumes. Besides these, hypocotyl part, epicotyl part, leaves and embryonic axis etc. can also be used. These explants are inoculated on culture medium supplemented with cytokinin/s or combination of auxins and cytokinins resulting into shoot bud morphogenesis. For elongation of shoot buds they are either sub cultured on the same medium or transferred to a medium with different hormonal combinations. Micropropagation of some important leguminous tree species has been discussed below:
Acacia species

**Acacia chundra**

Rout *et al.*, (2008) reported micropropagation of *Acacia chundra* from shoot tip and nodal explants derived from *in vitro* grown plants. MS medium supplemented with BA (1.0 mg/l) and 20 mg/l adenine sulfate (Ads) showed maximum bud break. Multiplication was obtained on MS medium supplemented with 1.5 mg/l BA, 0.01 to 0.05 mg/l IAA and 50 mg/l Ads. *In vitro* shoots were rooted on half-strength MS basal salts supplemented with 0.25 mg/l IBA or IAA and 20 g/l (w/v) sucrose after 10 to 12 days of culture.

**Acacia mangium**

Douglas and Mcnamara, (2000) observed that when wounded cotyledonary nodes obtained from seedlings of *Acacia mangium* were cultured on, DKW (Driver and Kuniyuki, 1984) medium with 2% sucrose and 10 µM BA, regeneration of adventitious shoots occurred in 96% of explants with an average of 2.03±0.27 shoots per explant cultured. Using 0.5 µM thidiazuron (TDZ) along with 0.5 µM N-(2-chloro-4-pyridyl)-N-phenylurea (CPPU) gave 96% shoot bud regeneration frequency with 1.8±0.21 shoots.

Nanda *et al.*, (2004) reported induction of bud sprout (74.6%) from mature nodal explants of 10-years-old tree of *Acacia mangium* on MS medium supplemented with 1.0 mg/l BAP, 1.0 mg/l GA₃, and 0.05 mg/l IAA after 7 days of culture. An average of 8 shoots per explant with average shoot length of 3.22 cm was obtained on MS shoots supplemented with 1.5 mg/l BAP, 0.05 mg/l IAA and 100 mg/l Ads after 4 weeks of culture.

**Acacia nilotica**

Abbas *et al.*, (2010) reported a micropropagation system for nodal segments obtained from *in vitro* raised seedlings of *Acacia nilotica*. MS medium proved more appropriate than B₅ medium and produced 4.23 average number of shoots with
43.2% shoot regeneration frequency in the presence of 2.0 mg/l BAP and 0.5 mg/l NAA.

Dhabhai et al., (2010) achieved the highest number of shoots (4.6 ± 0.7) on MS medium augmented with NAA (0.6 mg/l) from nodal segments from in vitro raised seedlings of Acacia nilotica (L.) with 90% shoot regeneration frequency. Within 4 weeks of inoculation, axillary shoots elongated up to 3.5 – 4.6 cm in height.

Samake et al., (2011) obtained axillary bud initiation rate at 0.1 mg/l NAA with an average of 8 shoots within 2 weeks of culture. Epicotyl buds of Acacia nilotica (L.) Wild., multiplied on B5 medium supplemented with BA (0.5 mg/l), produced the average number of shoots (5.8) and shoot length (55.83 mm).

Rathore et al., (2014) developed micropropagation protocol for mature Acacia nilotica (L.) Del. ssp. Indica using liquid culture medium. Nodal segments obtained from 15 to 20 years old mature trees were used as explants and cultured on 0.8 % agar-gelled MS medium containing BAP for shoot bud induction. Once culture got established, explants were transferred to MS liquid medium containing BAP or Kn for shoot multiplication. MS liquid medium containing 4.4 μM BAP was found to be the best for shoot multiplication. The performance of liquid and agar-gelled medium for shoot multiplication was compared. About ten times increase in shoot number in liquid culture medium was achieved. Micropropagated shoots were rooted ex vitro. Shoots treated with 2.46 mM IBA solution for 1 h followed by 1.41 mM chlorogenic acid for 5 min exhibited highest percent of rooting in the greenhouse. This process of micropropagation of A. nilotica, can be utilized for plant production on a large-scale.

Acacia senegal

Khalafalla and Daffalla (2008) developed a reproducible method for in vitro multiplication of gum Arabic tree (Acacia senegal (L.) Wild). Multiple shoots were regenerated from cotyledonary node derived from 7-days old in vitro raised
seedlings and nodal segment derived from 12-months-old plant growing in a greenhouse. Explants were cultured on MS medium supplemented with 0.5–5.0 mg/l BA or Kn alone or in combination with 0.5 mg/l NAA. The maximum number of shoots per cotyledonary node (8.3 ±0.3) and nodal segment (5.3 ±0.7) explants were obtained on MS medium supplemented with 1.0 mg/l BA after 4 weeks of culture. Only 25% of the shoots formed roots after being transferred to MS medium containing 1.0 mg/l IBA after 28 days of culture under dark condition.

**Acacia sinuata**

Vengadesan et al., (2003) obtained maximum shoot proliferation (75.2%) with highest number (6.1) of shoots and average shoot length of 1.8 cm from nodal explants of a mature ‘elite’ tree of *Acacia sinuata* on MS medium supplemented with 8.9 μM BA, 2.5 μM thidiazuron (TDZ). After first transfer in the same medium a maximum of 10.4 shoots were obtained. Addition of 135.7 μM adenine sulfate in the above mentioned medium improved the multiplication rate with 12.6 shoots per explant, 72.4% shoot development and an average shoot length of 2.2 cm. GA3 at 1.8 μM promoted shoot elongation showing 65% response with shoots attaining a height of 6.7 cm.

**Dalbergia sissoo Roxb.**

Singh et al., (2002) observed 52% response for shoot regeneration and a mean number of 4.1 shoots per explant from proximal region of the semi-mature cotyledons of *Dalbergia sissoo* Roxb. within 15-20 days on MS medium supplemented with 4.44 μM BA and 0.26 μM NAA. Elongation was achieved in similar medium containing half-strength macro-nutrients. Mature cotyledons when pretreated with 8.88 μM BA for 48 hours exhibited 45% shoot bud differentiation on MS medium containing 22.20 μM BA without NAA within 20-22 days of culture. Each explant produced on an average 3.8 shoots and an average length of 4.2 cm in 35-40 days.

Joshi et al., (2003) collected young shoots from clonal seed orchard (New forest, Dehradun) of 60 year old (clone no. 36) superior tree of *D. sissoo*, during
March to September for initiation and establishment of cultures. Bud break was achieved within 6-8 days on MS and B₅ medium supplemented with 0.25 mg/l BA + 0.25 mg/l NAA. Establishment of cultures and shoot length was higher in B₅ medium as compared to MS medium. BA has given best response as compared to Kinetin. MS medium produced large amount of callus than those raised on B₅ medium. During shoot multiplication maximum 8 number of shoots were observed on 0.25 mg/l NAA + 1 mg/l BA supplemented medium. The regeneration capacity of the shoot has been decreased after 150 days of subculturing. Half strength MS medium supplemented with 1 mg/l IBA was found best for in vitro rooting (76.9 %), root length (25.1 mm) and root number (4.47).

Ali et al., (2012) studied micropropagation of D. sissoo. Young, soft and mature and hard nodal explants of D. sissoo were taken as a source of explant and pre treatment with 1.5 % ascorbic acid + 1.5 % citric acid was used to avoid the exudation of phenolic compounds. MS medium supplemented with 1 mg/l BA + 0.25 mg/l NAA found best for bud induction. MS medium supplemented with 1.5 mg/l BA + 0.25 mg/l Kn was found to be best for shoot multiplication (4.0 shoot) with 1.8 cm average shoot length. Rooting was achieved on MS basal medium but with less number of roots (1.4) per plant. MS medium supplemented with 1 mg/l BA was found best for rooting with 3.4 roots per plant and average length of 2.8 cm.

Arya et al., (2013) studied in vitro responses of different clones of D. sissoo. For this study, three clones were investigated. In clone No. 9, maximum response of 92.35% was recorded, while in clone No. 59 and clone No. 79, it was 89.31 and 91.63% respectively. For in vitro shoot multiplication in clone No. 9 BAP at 2.5µM was found superior with multiplication rate of 2.41, while in clone No. 59 and clone No. 79 BAP at 5 µM was found superior with 2.35 and 2.37 multiplication rates respectively. In vitro rooting were induced on IBA supplemented medium. Effective IBA concentration was found to be 5 µM in clone No. 9 and clone No. 79, where 65.12% and 62.74% of root induction was obtained, while 7.5 µM IBA gave maximum of 60.50% of root induction in clone No. 59. Plants were hardened and acclimatized before field transfer in all the three clones.
**Pongamia pinnata**

Sugla et al., (2007) achieved multiple shoots induction (87%) from in vitro raised nodal segments of *Pongamia pinnata* on MS medium supplemented with 7.5 μM BAP which induced up to 6.8 shoots with an average shoot length of 0.67 cm. Incorporation of 2.5 μM GA₃ along with 7.5 μM BAP in the medium during the first subculture after establishment and initiation of shoot buds significantly improved the shoot elongation (43%) within 10 days with mean shoot number 6.85 per node. Thus, from a single cotyledonary node, about 16–18 shoots were obtained in 60 days.

Sujatha and Hazra, (2007) reported 64% bud break in shoots raised from mature-tree-derived axillary meristems of *Pongamia pinnata* on MS medium devoid of plant growth regulators (PGR). Bud break was completely suppressed in the presence of TDZ. At higher concentrations of TDZ (9.08 or 11.35 μM), a swelling developed at the axil. Primary explants after harvesting of shoots were identified as ‘stump’. Reculturing of stumps on 0.45 μM TDZ produced more shoots with 62.8% sprouting frequency, an average of 4.5 shoots per explant with mean length of 1.18 cm after 4 wk of culture.

Kesari et al., (2012) recorded that nodal segments of *Pongamia pinnata* when cultured on Woody Plant Medium supplemented with BA (5.0 mg/l) and Kn (0.5 mg/l) gave the bud break response of (99.78%) The multiplication rate of 11 shoots with an average shoot length of 3.0 cm was observed after 8 weeks of culture.

**Prosopis cineraria**

Shekhawat et al., (1993) reported microprogation of *Prosopis cineraria*. The maximum numbers of 10–12 shoots were induced from the nodal shoot segment from pruned thorny adult trees on MS medium containing 2.5 mg/l BAP and 0.1 mg/l IAA and additives. Differentiated shoots multiplied best on MS medium containing 1.0 mg/l BAP and 0.1 mg/l NAA + additives. Shoots were rooted by pulsing with 100 mg/l IBA for 4 h and then culturing on hormone-free half strength MS medium.
Kumar and Singh, (2009) observed shoot induction in mature nodal segment of *Prosopis cineraria* on MS medium supplemented with 5.0 mg/l BAP +1.0 mg/l IAA.

**Pterocarpus santalinus**

Prakash *et al.*, (2006) achieved clonal multiplication of *Pterocarpus santalinus* using mature nodal explants. 70% explants sprouted on MS liquid medium containing 4.4µM BA. The best rooting response was achieved on solidified half-strength MS medium supplemented with 4.9µM IBA. About 70% of the micropropagated plantlets were established in 20-cm pots containing a mixture of soil and farmyard manure (4:1 ratio) and formed new leaflets.

Rajeshwari *et al.*, (2008) developed a micropropagation protocol through axillary shoot proliferation from cotyledonary nodes of *Pterocarpus santalinus*. MS medium supplemented with 2.5µM BAP and 2µM 2-iP showed higher shoot multiplication rate.

Balaraju *et al.*, (2011) developed a protocol for *in vitro* propagation of *Pterocarpus santalinus* using shoot tip explants. The highest frequency of shoot regeneration (83.3%) with maximum number of shoot bud (11) per explants was obtained on MS medium supplemented with 1.0 mg/l of BAP along with 0.1 mg/l TDZ. Sixty percent of shoots produced roots when transferred to rooting medium containing MS salts and 0.1mg/l IBA.

Vipranarayana *et al.*, (2013) demonstrated the *in vitro* regeneration of *Pterocarpus santalinus* through shoot bud culture. Nodal segments from the seedlings were established on MS, B₅, and WPM medium supplemented with BAP, NAA, and TDZ, in which BA supplemented medium showed 85% of nodes. Then nodal segments from the developed shoots were cultured on MS medium with several BA concentrations. Best shoot multiplication was obtained with 1.0 mg BA+0.5 mg NAA and 0.8% Agar. Successful *in vitro* rooting was induced form cut end of the micro shoots when placed on half strength MS+IBA (1500ppm). The
regenerated shoots with well developed root system were successfully acclimatized and established in pots containing sterilized garden soil and garden manure (1:1:1) and grown under green house conditions 85.4% survival rates.

2.3 MICROPROPAGATION THROUGH CALLUS

Callus is referred to a mass of undifferentiated cells. Different explants such as apical buds, stem nodes, root segments, cotyledonary nodes, leaves, floral parts, epidermal tissue, seeds, cotyledons, immature embryo and zygotic embryos can be used for callus induction. Explants with mitotically active cells are generally good for callus induction. Immature tissues and organs are invariably more morphogenetically plastic in vitro than mature tissues and organs. Callus is produced on explants in vitro as a result of wounding and in response to hormones, either endogenous or exogenously supplied to the medium (Chawla, 2002).

Every explant has a certain endogenous level of auxin and cytokinin. This level depends upon species, physiological state of plant, explant type, season and on various ecological factors such as light, temperature etc. Manipulations of the endogenous level of these growth regulators can induce callusing. Exogenous supply of hormones results into changes in endogenous level of growth regulators, which subsequently into cell division, cell growth and differentiation. There are several reports of callus formation (dedifferentiation) followed by morphogenesis (redifferentiation) in case of leguminous species.

Vengadesan et al., (2000) observed that highest percentage calli (90%) were induced from hypocotyl explants excised from 7-day-old seedlings of Acacia sinuata on MS medium containing 3% sucrose, 0.8% agar, 6.78 µM 2,4-D and 2.22µM BA after 20 – 25 days of inoculation. Regeneration of adventitious buds (12-15 buds) from callus was achieved when they were cultured on MS medium supplemented with 10% coconut water, 13.2 µM BAP and 3.42 µM IAA after 25–30 days of culture. Addition of GA₃ (1.73 µM) favored shoot elongation (5–6 cm) within three weeks.
Pattnaik et al., (2000) observed that in *Dalbergia sissoo* percentage of callus induction from hypocotyl explant of *in vitro* raised seedlings was 88% within 8-10 days of inoculation when NAA at 2.0 mg/l (10.8 µM) was added in conjunction with 0.5 mg/l (2.2 µM) BA in MS medium. Friable hypocotyl-derived callus from the second subculture passage was used to initiate the cell-suspension culture on liquid MS medium with same hormonal composition as above (established in 2-3 months). Plating efficiency was highest (93%) with cells harvested at their exponential growth phase (day 4-10) and plated in 1.2 gm/l phytagel. The increase in cell volume was 12 fold with 1% inoculum cell density. 71% of cultures exhibited shoot-bud differentiation on a medium containing 3.0 mg/l BA and 0.5 mg/l NAA with 5.9 shoots per callus culture averaging 4.5 cm in length in 8 wk.

Singh et al., (2002) reported micropropagation of *D. sissoo*. Mature and semi mature cotyledons were collected from 8-10 days old seedling. A set of mature cotyledons were given pre treatment for 24, 48 or 72 hours with 8.88 µM BA in liquid MS medium followed by treatment of 4.44, 8.88, 13.32 & 22.2 µM BA. Semi mature cotyledons induced 52 % of adventitious shoots within 15-20 days on ½ MS + 4.44 µM BA and 0.26 µM NAA with average of 4.1 shoot buds from each cotyledon. For shoot elongation the same medium with ½ strength macronutrients was used. For shoot bud regeneration from mature cotyledons incorporation of BA was found necessary. Pre treatment of mature cotyledons was found beneficial as it enhanced the adventitious shoot bud formation. 45% adventitious shoot regeneration was observed in pretreated cotyledons with average 3.8 numbers of shoots and average length of 4.2 cm in 35-40 days. 80 and 56 % rooting was observed in 1.23 µM IBA from semi mature and mature cotyledons respectively. 65% plant survival was recorded.

Vengadesan et al., (2003) reported callus induction (green, compact, nodular and organogenic) with 75% frequency in *Acacia sinuata* from cotyledon explants derived from 5-7 day old *in vitro* raised seedlings on MS medium containing 3% sucrose, 0.8% agar or 0.15% phytagel, 8.1µM NAA, and 2.2µM BA. High-frequency (75%) regeneration of adventitious buds from callus was achieved with maximum
number of 25.3 shoots per culture and highest shoot length of 2.1 cm when cultured on half-strength MS medium supplemented with 10% coconut water, 13.3 µM BA, and 2.5 µM zeatin. Among the various carbohydrates tested, sucrose at 87.6 µM was optimum for shoot-bud induction. Addition of 1.7 µM gibberellic acid along with 4.4 µM BA favored shoot elongation.

According to Cheepala et al., (2004) callus induced on cotyledonary explants of Sesbania drummondii when subcultured on 2.27 µM TDZ containing medium resulted in its mass proliferation having numerous embryoid-like structures. Hypocotyl and epicotyl explants from S. drummondii produced moderate to profuse callusing on the MS medium supplemented with 8.8 µM BA + 1.34 µM NAA (80% and 60% callus induction respectively.

According to Bari et al., (2008) the highest percentage of callus induction (100%) and highest fresh weight (5.4 g) of callus per culture was achieved from in vivo nodal segment of Dalbergia sissoo Roxb. on MS medium supplemented with 2.0 mg/1 BAP + 0.5 mg/1 NAA within 9-10 days. The highest number of shoots (2.4) per explant was recorded on MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/1 IAA for internode segment.

Kumar and Singh, (2009) reported 100% callus induction in Prosopis cineraria in MS medium fortified with 5 mg l⁻¹ BAP and 1 mg l⁻¹ NAA after 10 days of inoculation.

Rehman et al., (2012) developed a protocol for in vitro regeneration of D. sissoo. Callus formation and plantlet regeneration was achieved by culturing cotyledons, immature seeds, and mature embryos on a modified MS medium supplemented with plant growth regulators. Callus induction medium containing 2.71 µM 2,4-D and 0.93 µM. Kinetin produced better callus on all explants tested compared to other treatments. MS medium containing 1.4 NAA µM and 8.88 µM BA was better in general for regeneration regardless of callus induction medium and the
type of explant used. Rooting was best on half-strength MS medium with 7.35 μM IBA. Regenerated plantlets were acclimatized for plantation in the field.

2.4 Pterocarpus marsupium

Das and Chatterjee (1993) attempted micropropagation of Pterocarpus marsupium using seedlings and coppiced shoot explants without any response.

Kalimuthu and Lakshmanan (1995) studied the effect of different treatment on pod germination of Pterocarpus species. The highest germination percentage was showed by seed soaked in 40% HCl for 24 hrs.

Tiwari et al., (2004) reported in vitro propagation through nodal segment from seedlings of P. marsupium Roxb. on different media composition, viz. MS, B5 and White’s without growth hormones and medium supplemented with 3.0mg/ l BAP and 0.5 mg/ l NAA. MS medium was the best. Regenerated plants were acclimatized and successfully transferred under field conditions.

Chand and Singh (2004) developed a protocol for in vitro plant regeneration from cotyledonal nodes of Pterocarpus marsupium Roxb. Multiple shoots were induced from coteledonal nodes derived from 20-day-old seedlings grown on MS medium containing 2.22–13.32mM BA or 2.32–13.93mM Kn alone or in combination with 0.26mM NAA. The highest frequency of responding explants (85%) and maximum number of shoots per explant (9.5) were obtained on MS medium supplemented with 4.44 mM BA and 0.26mM NAA. Nearly 30% of the shoots formed roots after being transferred to half-strength MS medium containing 9.84 mM indole-3-butyric acid after 25 day of culture. About 52% plantlets rooted under ex vitro conditions were successfully acclimatized and established in pots.

Husain et al., (2007) developed a protocol of Pterocarpus marsupium for micropropagation from cotyledonal nodes derived from 18-d-old axenic seedling. MS medium supplemented with 0.4 μM TDZ showed highest shoot regeneration frequency (90%) and maximum number (15.2±0.20) of shoots. About 65% of in vitro
regenerated shoots produced a maximum number (4.4±0.2) of roots per shoot by a two-step culture procedure employing pulse treatment 200 μM IBA and subsequent transfer of treated shoots to a low concentration of 0.2 μM IBA along with phloroglucinol (3.96 μM). The in vitro raised plantlets were successfully acclimatized first under culture room conditions, then to greenhouse with 70% survival rate.

Husain et al., (2008) reported in vitro propagation of *Pterocarpus marsupium* Roxb. from nodal explants obtained by in vitro raised 18-day-old axenic seedlings. Multiple shoots were obtained from nodal explants on MS medium amended with 4.0 μM BA, 0.5μM IAA and 20μM Ads. Rooting was best induced in microshoots excised from proliferated shoot cultures on semisolid hormone-free half-strength MS medium, after a pulse treatment for 7 days in half-strength MS liquid medium containing 100 μM IBA and 15.84 μM phloroglucinol. The in vitro-raised plantlets were potted and acclimatized under culture room conditions for 4 weeks before their transfer to a greenhouse, where the established plants showed 75% survival.

Husain et al., (2010) achieved somatic embryo from hypocotyls-derived callus culture in *Pterocarpus marsupium* Roxb. Ninety percent of hypocotyl explants (excised from 12-day-old in vitro germinated axenic seedlings) produced callus on MS medium supplemented with 5 μM 2,4-D and 1 μM BA.

Tippani et al., (2013) developed a protocol for in vitro plantlet regeneration and *Agrobacterium tumefaciens*-mediated genetic transformation using immature cotyledon explants of Indian Kino tree (*Pterocarpus marsupium* Roxb.). Immature cotyledon explants excised from 9-day old axenic seedlings produced optimal callus on MS medium supplemented with 1.07 μM NAA, after 2 weeks of culture. When the above said callus was incubated on MS medium supplemented with 8.90 μM BAP, 1.07 μM NAA, a regeneration frequency of 60.41% with average shoot number 12.2 ± 0.85 and shoot length 1.4 ± 0.13cm was observed. For further shoot multiplication and elongation, these cultures were transferred onto MS medium with 4.40 μM BAP. Elongated shoots dipped in 19.60 μM for 24 h and then cultured on MS medium
supplemented with 2.85µM IBA, 75% shoots developed roots and 95% of plantlets survived in field condition.