RESULTS
Studies were carried out on micropropagation of three firewood species, viz. *Cleistanthus collinus*, *Lagerstroemia parviflora* and *Pongamia pinnata*. The results of these studies have been described in this chapter.

Shoot bud cultures were initiated from nodal explants taken from seedlings, adult trees and basal sprouts of *C. collinus*, *L. parviflora* and *P. pinnata*. In case of *P. pinnata*, explants obtained from rooted cuttings were also used to study micropropagation. Plantlet regeneration through axillary shoot proliferation has three developmental stages, namely explant establishment, shoot proliferation and rooting of microshoots. The results of each developmental stage of cultures derived from various types of explants of all the three firewood species have been described separately.
A: *Cleistanthus collinus*:

1. **Seedling Explants**:
   
a. **Explant Establishment**:

   The effects of basal medium, cytokinin(s) and season of explant collection on explant establishment were investigated.

i. **Effects of Basal Medium and BA**:

   The data on the effects of basal medium and BA have been given in Table 1.

   All the four types of basal media without PGR induced shoot elongation from axillary buds of juvenile nodal segments. WPM and MS medium, induced bud break on 100% explants. B5 and White's media induced bud break on 80 and 60 percent explants, respectively. On all the four media without growth regulator, only one shoot elongated from each nodal segment. The shoots elongated on different media, showed considerable variation in length. On B5, White, WPM and MS media, average shoot length (in cm) were 0.6 ± 0.1, 0.9 ± 0.1, 2.5 ± 0.4 and 2.2 ± 0.2, respectively. On B5, White, WPM and MS media, average number of nodes per explant were 1 ± 0, 1.6 ± 0.1, 4.1 ± 0.4 and 5 ± 0.4, respectively.

   Addition of BA in B5 and White's media increased 20% bud break response. White's medium supplemented with 2.2, 8.9 or 17.8 μM BA decreased shoot elongation and node number. B5 medium supplemented with lower concentration (2.2 μM) of BA increased shoot length and node number but with higher concentration (8.9 or 17.8 μM) of
BA, it reduced shoot length and node number considerably.

WPM supplemented with 2.2 μM BA increased the number of shoots and nodes. On this medium, average number of shoots and nodes per explant were 1.8 ± 0.2 and 7.0 ± 0.5, respectively. Higher concentrations of BA (8.9 or 17.8 μM) in WPM decreased shoot and node number.

Seedling explants placed on MS medium supplemented with 2.2 μM BA produced 1.8 shoots per explant, 3.8 cm ± 0.4 average shoot length and 10.1 ± 0.8 nodes per explant. Higher concentrations of BA (8.9 or 17.8 μM) in MS medium inhibited shoot elongation and node number.

ANOVA showed that the effects of medium, concentration of BA and interaction of medium X concentration of BA were significant.

On the basis of these results, it is concluded that the MS medium with 2.2 μM BA is the best medium for establishment of seedling explants of C. collinus (Plate 1, 2 & 3).

ii. Effects of Kinetin:

The data on the effects of Kinetin have been given in Table 2. At 9.3 μM concentration, kinetin enhanced shoot length and node number. However, at 2.3 and 18.5 μM concentrations, it inhibited shoot length and node number.
For explant establishment, Kinetin was found to be less effective than BA.

iii. Effects of Season of Explant Collection:

The data related to seasonal variation in bud break response and contamination have been given in Figure 1.

The time of the year that explants were collected from seedling stock plants had an influence on axillary shoot out growth and contamination. Maximum contamination (73.33%) occurred during July to September, then gradually decreased 20% and 6.6%, during October to December and January to March, respectively. During April to June, contamination did not occur. Minimum bud break (10%) was observed during July to September, which increased up to 40% during October to December. Highest bud break response (90%) was observed during January to June. During April to June, average shoot number was 1.4 ± 0.2, while in rest of the time of the year explant produced only single shoot; and the shoots were also longer (2.4 cm ± 0.3) than during other time of the year. Thus, on the basis of minimum contamination (P < 0.0001), highest bud break response (P < 0.0001), maximum shoot number (P = 0.0020) and production of longest shoot (P < 0.0001), it is concluded that the best period for initiating shoot bud culture from seedlings of *C. collinus* is April to June.

b. Propagule Proliferation:

The original explants were placed on MS medium supplemented with 2.2 µM BA. The micronodes of microshoots that had elongated
from original explants were used for further shoot multiplication. The data related to shoot multiplication have been given in Table 3.

The micronodes subcultured on MS medium supplemented with 2.2 μM BA produced 1.8 ± 0.4 shoots. Subculturing of their micronodes on MS medium with lower concentrations (1.1 or 0.56 μM) of BA increased shoot number but the differences were not significant. The micronodes subcultured on MS medium without plant growth regulators caused significant reduction in shoot number.

Maximum shoot length (4.1 cm ± 0.5) and number of nodes (11.2 ± 0.7) were observed in micronodes subcultured on MS medium supplemented with 1.1 μM BA. At 2.2 or 0.56 μM concentration of BA, shoot length and node number were significantly lower than those at 1.1 μM concentration of BA. The number of nodes and shoot length have direct relationship with propagule multiplication.

It is concluded that the MS medium supplemented with 1.1 μM BA is the best medium for shoot multiplication (Plate 4).

c. Rooting:

For rooting, 3 to 4 cm long microshoots were placed on the rooting medium. Effects of auxins combinations of auxins and IAA pulse treatment on rooting were studied. The data on rooting of microshoots have been given in Table 4.
i. Effects of Auxins:

Rooting did not occur in microshoots placed on half-MS medium without growth regulators.

On half-MS medium supplemented with 2.4, 4.9 or 19.6 μM IBA 20 to 30% shoots developed roots with intervening callus. Thus, IBA proved to be an unsuitable auxin because it did not induce roots directly from the microshoots.

Microshoots placed on half-MS medium with 2.6 and 5.4 μM NAA showed 20 & 40% rooting with intervening callus, respectively. On higher concentrations (21.5 μM) of NAA microshoots produced only callus. Thus, NAA also failed to induce direct rooting in microshoots.

Continuous culturing of microshoots half MS medium with 2.8 μM IAA, did not produce any rooting or callus. On half-MS medium with 5.7 and 22.8 μM IAA, 20% microshoots developed roots. These roots were without intervening callus. Higher concentration (22.8 μM) of IAA reduced root length (0.4 cm ± 0.1). On 5.7 μM IAA, root elongation was proper (1.5 cm ± 0.1). Thus, IAA was found to be the only auxin which induced direct roots though with poor rooting percentage.

ii. Effects of Combination of Auxins:

Combination of IAA, IBA and NAA (0.7 + 0.6 + 0.6 or 1.4 + 1.2 + 1.3 μM) produced 20% rooting with intervening callus. Combination of higher concentrations of these auxins in medium produced only callus.
iii. **Effects of IAA Pulse Treatment**:

Microshoots were exposed to IAA enriched medium for 7 days, and then transferred to PGR free basal medium. On 2.8 μM IAA pulse, microshoots did not produce roots or callus. Sixty percent of microshoots formed roots without intervening callus when they were exposed to 5.7 μM IAA pulse. Best rooting response was obtained when a pulse treatment of 22.8 μM IAA was given. These roots were without intervening callus. On this treatment, rooting was 80% with $2.1 \pm 0.2$ roots per shoot and $2.3 \text{ cm} \pm 0.2$ root length.

It is concluded that the pulse treatment with 22.8 μM IAA for 7 days, the first 72-h in darkness, was the best treatment for rooting of microshoots derived from seedling explants of *C. collinus* (Plate 5).

d. **Hardening:**

The rooted microshoots were transferred to pots containing soil:sand mixture and covered with polyethylene bags, where only 30% plants survived for 5 weeks.

2. **Tree Explants**:

Nodal segments of a 15-year-old tree were used to initiate shoot bud cultures.

a. **Explant Establishment**:

Responses of explants to BA, medium and seasonal variation were studied.
i. Prevention of Leaching:

Explants exuded excessive phenolics into the culture medium from their cut ends and eventually died. This problem was overcome by suspending explants in a sterile solution of PVP 40 (3.75 μM) and citric acid (520.5 μM) for 10 minutes before inoculation. These chemicals were also added in the explant establishment medium. Finally, 25 μM PVP 40 and 104.1 μM citric acid in the establishment medium controlled the leaching of phenolics from explants.

ii. Effects of Basal Medium and BA:

The data for the effects of medium and BA on explant establishment have been given in Table 5.

All the three types of basal media induced shoot elongation from axillary buds of mature explants. On WPM, 90% explants produced bud break response, while MS and B5 media induced bud break on 100% explants. On all the three types of basal media, explants produced single shoot, however, shoot length and nodes per explant showed considerable differences. On WPM, B5 and MS media, the mean shoot lengths were 1.03 cm ± 0.11, 1.94 cm ± 0.1 and 3.07 cm ± 0.29, respectively, and number of nodes per explant were 1.9 ± 0.2, 4.1 ± 0.3 and 4.1 ± 0.7, respectively.

Addition of low concentration (0.44 μM) of BA in all the three basal media increased shoot length and node number considerably. While the mean shoot length and number of nodes per explant decreased with increase in concentration of BA in all the three media.
There was no change in average number of shoots. Higher concentration of BA in B5 and WPM reduced bud break percentage from 100% to 90% and 80%, respectively.

In all the three media tested, 0.44 μM BA was the most suitable concentration for axillary shoot growth from explants. All the three media supplemented with 0.44 μM BA induced bud break in 100% explants. On WPM and B5 medium supplemented with 0.44 μM BA, explants produced 2.3 cm ± 0.16 and 2.78 cm ± 0.12 long shoot, respectively; average number of nodes per explant on both the media was 4.7. However, on B5 medium with 0.44 μM BA, explants produced 100% bud break, 4.47 cm ± 0.17 mean shoot length and 5.7 ± 0.3 nodes per explant. Thus, it is concluded that MS medium with 0.44 μM BA is the best combination for establishment of adult tree explants of *C. collinus* (Plate 6 & 7).

iii. Effects of Season of Explant Collection:

The data related to seasonal variation in bud break response and contamination have been given in the form of a graph in Figure 2.

Seasonal variation in bud break response was studied by inoculating explants on MS medium supplemented with 0.44 μM BA, throughout the year. Contamination was 80% in August and 100% during December to March. Contamination was completely absent during April to July. In the month of August and September, bud break response was 40% and 30%, respectively. During October to March, explants did not exhibit any bud break. Explants showed 100% bud
break response during April to July.

So, on the basis of the absence of contamination ($P<0.0001$) and maximum (100%) bud break response ($P<0.0001$), it is concluded that the best time for initiating shoot bud culture from mature tree of *C. collinus* is April to July.

b. Propagule Proliferation:

The nodes of microshoots that had elongated from the mother explants on MS medium with 0.44 μM BA, were used for further shoot multiplication. Relevant data have been given in Table 6.

i. Effects of BA:

Phenolic leaching was absent at this stage, therefore, citric acid and PVP were not added in the medium. Single shoot elongated from each micronode placed on MS with 0, 0.44, 2.2 or 11 μM BA. But, there was significant difference in mean shoot length. Higher concentration of BA reduced shoot length. On 2.2 and 11 μM BA, shoot lengths were 2.6 cm ± 0.18 and 2.4 cm ± 0.16, respectively. These shoots were having minute, unopened and albinic leaves. Growth regulator free MS medium induced 3.08 cm ± 0.15 long healthy-green shoots containing 2.9 ± 0.1 nodes. However, micronodes placed on MS with 0.44 μM BA, produced 3.96 cm ± 0.11 long shoots with maximum number of nodes 3.9 ± 0.7. Therefore, on the basis of maximum (3.96 cm ± 0.11) shoot length ($P<0.0001$) and maximum (3.9 ± 0.7) node number ($P=0.0022$), it is concluded that MS medium with 0.44 μM BA was the best medium for shoot elongation from micronodes.
derived from nodal explants of mature tree of *C. collinus*.

ii. Effects of Successive Transfers:

In this experiment, microshoots (> 0.5 cm long) developed from micronodes on multiplication medium (MS + 0.44 µM BA) were harvested after 1 month; and remaining part of the culture was transferred to fresh multiplication medium. Only single shoot elongated from remaining part of the culture upto the 2nd transfer (Table 7). After the third transfer, the number of shoots per culture gradually increased upto 7th transfer (P< 0.0001). On the 3rd, 4th, 5th, 6th, & 7th successive transfers, average no. of shoots per culture were 1.7, 3.1, 3.4, 4.1 and 5.3, respectively. During successive transfers, significant increase in shoot length (P< 0.0001) was also observed. On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th transfers, mean shoot lengths were 3.9, 4.0, 6.9, 7.2, 7.3, 7.1 and 6.2 cm, respectively. Sharp increase in number of nodes per culture was also recorded (P< 0.0001). On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th transfers, average node number per culture were 3.9, 4.4, 7.5, 9.5, 10, 11.6 and 20.2, respectively. Increase in node number is very important because each node can be used for further shoot proliferation.

Thus, after seven transfers, a total of 220 shoots could be obtained from a single micronode derived from tree explant of *C. collinus*. It is concluded that the harvesting of leader shoot(s) before next transfer of culture can be a useful for enhancing shoot multiplication (Plate 8).
c. Rooting:

For rooting, 3 to 4 cm long microshoots were placed on the rooting medium. Effects of auxin on rooting of microshoots were studied.

i. Effects of auxins:

The data on the effects of auxins on rooting of microshoots have been given in Table 8. Rooting did not occur in microshoots placed on half-MS medium without growth regulators. Profuse callus formation occurred at the base of microshoots placed on half-MS medium supplemented with 2.4, 9.8 or 39.2 μM IBA. IBA also induced shoot tip necrosis (Plate 9), which increased with the increased concentration of IBA. On 2.4, 9.8 & 39.2 μM IBA, the percentage of shoot necrosis were 40, 80 & 90, respectively.

It is concluded that IBA is not a suitable auxin for inducing roots in microshoots derived from tree explants of C. collinus. It induced callus formation at the base of the microshoots and caused necrosis at the top of the microshoots.

All the concentrations of NAA induced callus formation at the base of the microshoots. Shoot tip necrosis did not occur at lower (2.6 μM) concentration of NAA. Higher (10.8 & 43.2 μM) concentrations of NAA caused necrosis in 20 to 30% microshoots.

Thus, NAA also failed to induce root formation in microshoots derived from tree explants of C. collinus. All the concentrations of
NAA induced callus formation at the base of the microshoots. Higher (10.8 & 43.2 μM) concentration caused necrosis of microshoots.

Lower (2.8 μM) concentration of IAA induced slight callus at the base of microshoots without any rooting and caused necrosis in 25% shoots. At 45.6 μM concentration, IAA induced profuse callus formation at the base of the microshoots and necrosis in 25% microshoots. However, on 11.4 μM concentration of IAA, 10% microshoots formed direct roots and 20% microshoots died due to necrosis.

ii. Effects of IAA Pulse Treatment:

The data on the effects of IAA pulse treatment on rooting of microshoots have been given in Table 9. In the first set of experiments, microshoots were placed on IAA containing medium for 7 days and then transferred to PGR free basal medium. Lower concentrations, 2.8 & 14.5 μM, of IAA did not induce rooting in microshoots. At 14.5 μM concentration of IAA, 30% microshoots died due to necrosis. At 285 μM concentration of IAA, no rooting was observed; 40% microshoots formed profuse callus at their cut ends; and 70% microshoots showed shoot tip necrosis. Microshoots treated with 57 μM IAA produced 10% rooting but 40% of rooted microshoots died due to necrosis.

In the second set of experiments, bases of microshoots were dipped in 50% ethanol solution of IAA for 2 minutes and then 0.22 μM BA-agar drop was placed on each decapitated shoot tip. By the use of BA-agar drop, shoot tip necrosis was completely checked.
Microshoots treated with 5.7 & 11.4 mM IAA produced 10% rooting without intervening callus. Root number and root length were increased with the concentration. Microshoots on 5.7 & 11.4 mM IAA, formed 1.0 root of 0.6 cm ± 0.02 length and 1.2 ± 0.4 roots of 1.31 cm ± 0.49 length, respectively. However, shoots treated with 28.5 mM IAA, formed 40% direct rooting with 2.3 ± 0.5 roots per shoot and 1.75 cm ± 0.22 length.

It is concluded that the microshoots derived from adult tree explants of *C. collinus* are difficult to root. About 40% rooting can be achieved by dipping the microshoot base in 50% alcoholic 28.5 mM IAA for 2 min and placing a drop of 0.22 μM BA-agar on the decapitated microshoot tip (Plate 10).

3. Basal-Sprout Explants:

Nodal segments were cut from basal-sprouts of a 15-year-old tree and used to initiate shoot bud cultures.

a. Explant Establishment:

Control of leaching and effects of medium and BA were studied at this stage.

i. Prevention of Leaching:

Excessive leaching of phenolic substances from explants into the medium started within few hours of inoculation and the explants eventually died. The exudation of phenolics was completely checked in those explants which were first suspended in a sterile solution of
3.75 μM PVP 40 and 520.5 μM citric acid and then inoculated on a medium containing 12.5 μM PVP 40 and 104.1 μM citric acid.

ii. Effects of Basal Medium and BA:

The data on the effects of basal medium and BA have been given in Table 10.

All the three basal media induced shoot out growth from axillary buds of basal-sprout explants. Both MS medium and WPM induced bud break in 100% explants, whereas, B5 medium induced bud break in 90% explants.

All the explants that responded to different media showed elongation of single shoot from axillary bud. However, the shoots elongated on different media showed considerable difference in shoot length and node number. On B5, WPM and MS media, the average shoot length and number of nodes per explant were 0.25 cm ± 0.02 & 1 ± 0, 2.03 cm ± 0.1 & 3.7 ± 0.3 and 5.18 cm ± 0.6 & 7 ± 0.2, respectively.

All the three media with 0.44 μM BA induced 100% bud break response in explants. On WPM and B5 media supplemented with 0.44 μM BA, the explants produced 2.89 cm ± 0.13 and 3.05 cm ± 0.41 long shoots, respectively, and the average number of nodes were 4.3 ± 0.35 and 3.1 ± 0.22, respectively. The explants placed on MS medium with 0.44 μM BA showed 100% bud break, 6.18 cm ± 0.16 mean shoot length and 8.1 ± 0.3 number of nodes per explant. There
was no change in average number of shoots per explants (1 ± 0). Higher (2.2 & 11 μM) concentrations of BA reduced shoot length and number of nodes.

It is concluded that MS medium with 0.44 μM BA was the most suitable combination for establishment of explants from basal sprouts of C. collinus (Plate 11 & 12).

b. Propagule Proliferation:

The nodes of microshoots that had elongated from the original explants were used for further shoot multiplication. Effects of BA and successive transfers on shoot multiplication were studied.

i. Effects of BA:

Relevant data have been given in Table 11. Phenolic exudation was absent at this stage. The micronodes produced only one shoot when they were placed on MS medium with 0, 0.44, 2.2 or 11 μM concentrations of BA. MS medium without growth regulator induced 2.84 cm ± 0.06 long shoots and 2.6 ± 0.1 nodes per culture. These shoots were normal, healthy and green. Micronodes on MS + 0.44 μM BA produced 4.61 cm ± 0.2 long shoots with 4.7 ± 0.3 nodes. MS medium supplemented with higher (2.2 μM or 11.0 μM) concentrations of BA reduced shoot length. The leaves of these shoots were minute, unopened and albinic.

On the basis of longest (4.61 cm ± 0.2) shoot length (P < 0.0001) and maximum (4.7 ± 0.3) number of nodes (P < 0.0001), it is
concluded that MS medium with 0.44 μM BA was the best medium for proliferation of the propagules of basal-sprouts of *C. collinus*.

**ii. Effects of Successive Transfers:**

Relevant data have been given in Table 12. In this experiment, shoots (> 0.5 cm long) produced by micronodes on multiplication medium (MS + 0.44 μM BA) were harvested after one month and remaining part of the culture was transferred to fresh proliferation medium. The shoot proliferation gradually increased with the successive transfers. On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th transfers, the number of microshoots produced were 1, 1.6, 1.8, 2.5, 2.9, 3.5 & 5, respectively. The shoot length also increased during the first five consecutive transfers (*P* < 0.0001). On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th successive transfers, the shoot lengths (in cm) were 4.6, 4.1, 7.2, 7.1, 7.7, 6.2, and 6, respectively. A sharp enhancement in the number of nodes per culture was also observed during successive transfers (*P* < 0.0001). On the 1st, 2nd, 3rd, 4th, 5th, 6th and 7th transfers, average number of nodes per culture were 4.7, 6.5, 8.3, 11.1, 10.1, 12.1 and 23.8, respectively. Each node can be used to proliferate shoots *in vitro*.

It is concluded that after seven transfers a total of 270 shoots can be obtained from a single micronode derived from basal sprout explants of *C. collinus* (Plate 13).

**c. Rooting:**

Effects of continuous treatment with auxins and pulse treatment
with IAA on rooting of microshoots were studied.

i. Effects of Auxins:

The relevant data have been given in Table 13. The microshoots placed on half MS medium without growth regulators did not show rooting. On half-MS medium supplemented with IBA, the microshoots produced profuse callus at their bases. Besides callus formation, IBA caused severe shoot necrosis (Plate 14). The microshoots placed on half-MS with 2.4 μM IBA showed 60% mortality and with 9.8 or 39.2 μM IBA exhibited 100% mortality.

Profuse callus formation occurred at the base of microshoots placed on half MS medium supplemented with different levels of NAA. The microshoots grown on lower (2.6 μM) concentration of NAA remained healthy, whereas, those placed on 10.8 or 43.2 μM NAA died due to necrosis.

The microshoots placed on half MS with 2.8 μM IAA showed 20% rooting without intervening callus and shoot tip necrosis. However, at 11.4 and 45.6 μM concentrations of IAA, necrosis was observed in 40% and 80% microshoots, respectively. Higher concentrations of IAA also induced slight callus at the base of microshoots. It is concluded that the most promising auxin for rooting of microshoots is IAA.

ii. Effects of IAA Pulse Treatment:

The data related to the effects of IAA pulse treatment on rooting have been given in Table 14.
First Set of Experiments:

7-day-pulse treatment with IAA: On 14.2 and 57 μM IAA, the microshoots showed 10% and 20% rooting, respectively. However, the microshoots rooted on 14.2 and 57 μM IAA exhibited 40% and 50% necrosis, respectively.

Second Set of Experiments:

Microshoots were dipped in 50% ethanol solution of IAA for 2 min, decapitated and applied 0.22 μM BA-agar drop and then placed on the basal medium. On this regime, the microshoots dipped in 5.7 and 11.4 mM IAA showed 5% and 50% rooting respectively. Higher (28.5 mM) concentration of IAA induced profuse callus and caused shoot necrosis. At 11.4 mM IAA, the mean root number and length were 1.6 ± 0.33 and 2.37 cm ± 0.21, respectively.

It is concluded that the treatment with 11.4 mM IAA for 2 min and placement of 0.22 μM BA-agar drop on decapitated microshoot is the best strategy for inducing roots in microshoots derived from basal-sprouts of C. collinus (Plate 15).

B. Lagerstroemia parviflora:

The seedlings, adult tree and basal-sprout explants were used to initiate shoot bud cultures of this species.

1. Seedling Explants:

Nodal segments of 1-year-old seedlings were employed to initiate shoot bud culture. There are three developmental stages of
plantlet regeneration through axillary shoot proliferation. The results of explant establishment, shoot multiplication and rooting stages have been given below:

a. Explant Establishment:

Responses of explants to basal media, BA and season of collection were studied during this stage.

i. Control on Phenolic Exudation:

Within few hours of inoculation, the nutrient medium turned brown due to excessive leaching of phenolic substances from cut ends of the explants; consequently, all the explants died within two days. This could be effectively checked by dipping the explants into a sterile solution of PVP 40 (25 µM) and citric acid (522.5 µM) for 15 minutes before inoculation and by adding these chemicals PVP 40 (100 µM) and citric acid (522.5 µM) in nutrient medium.

ii. Effects of Basal Medium and BA:

The data on the effects of basal medium and BA on bud break percentage, number of shoots and shoot length have been given in Table 15.

On all the three basal media, the explants showed 100% bud break; and only one shoot elongated from each explant. However, the shoot lengths slightly differed on different media. On B5, MS and WPM, the mean shoot lengths were 0.98 cm ± 0.11, 0.95 cm ± 0.12 and 1.11 cm ± 0.11, respectively.
Addition of BA in MS and WPM media did not affect bud break percentage. There was no change in average number of microshoots per explant.

Addition of 0.44 μM BA in all the three basal media significantly increased the shoot length. On WPM, B5 and MS media supplemented with 0.44 μM BA, the mean shoot lengths were 1.35 cm ± 0.14, 1.37 cm ± 0.11 and 1.45 cm ± 0.13, respectively. However, all the media supplemented with 2.22 μM or 11.1 μM concentrations of BA showed significant reduction in shoot length.

It is concluded that all the three basal media, viz. B5, MS and WPM supplemented with 0.44 μM BA were suitable for the establishment of explants from 1-year-old seedlings of L. parviflora (Plate 16, 17 & 18).

iii. Effects of Seasonal Variation:

The effects of seasonal variation on bud break response and contamination have been shown in Figure 3.

Effects of seasonal variation on bud break response were studied by inoculating explants on MS medium with 0.44 μM BA throughout the year.

Contamination occurred in 100% cultures raised during August to February; it was completely absent during March to June; and only 20% in the month of July.
Bud break response was absent during August to February. It was 100% during March to May, 80% in June and only 60% in the month of July.

On the basis of the percentage of contamination ($P < 0.0001$) and maximum bud break response ($P < 0.0001$), it is concluded that the best time for initiating shoot bud culture from 1-year-old seedlings of *L. parviflora* is March to May.

b. Propagule Proliferation:

The microshoots that had elongated on the original explants placed on MS medium with 0.44 μM BA were used for further shoot proliferation.

i. Prevention of Exudation of Phenolic Substances:

The micronodes started exuding phenolic substances into multiplication media within few hours of inoculation and eventually died. Addition of 100 μM PVP 40 and 522.5 μM citric acid in multiplication medium was found to be effective for controlling the exudation of phenolic substances.

ii. Effects of BA on Shoot Multiplication:

Relevant data have been given in Table 16. The micronodes showed significant increase in average shoot number with the increase in concentration of BA in MS medium. On 0, 0.44, 0.88 and 1.76 μM BA, the number of shoots per micronode were $1.5 \pm 0.1$, $2.4 \pm 0.2$, $2.0 \pm 0.2$ and $4.5 \pm 0.2$, respectively.
The number of shoots per micronode increased with successive transfer. However, the shoot length decreased up to 3rd transfer and then increased during the subsequent transfers (P < 0.0001). On 1st, 2nd, 3rd, 4th, 5th and 6th transfers, the mean shoot number and mean shoot lengths were 2.4 ± 0.2 and 2.1 cm ± 0.18, 2.4 ± 0.4 and 1.7 cm ± 0.15, 3.6 ± 0.5 and 0.7 cm ± 0.06, 3.5 ± 0.5 and 0.9 cm ± 0.09, 3.4 ± 0.6 and 1 cm ± 0.08 and 3.5 ± 0.4 and 1.1 cm ± 0.12, respectively.

After 1st transfer some green shoot buds also appeared. After 1st, 2nd, 3rd, 4th, 5th and 6th transfer, the number of shoot buds per micronode were 0, 2.3 ± 0.3, 2.6 ± 0.5, 2.4 ± 0.4, 2.4 ± 0.6 and 2.2 ± 0.3, respectively.

Thus, after six transfers approx. a total of 150 shoots can be obtained from a single micronode derived from seedling explants of L. parviflora. It is concluded that successive transfers increased the number of leader shoots which can be used for further multiplication of shoots (Plate 19 a & b).

c. Rooting:

For rooting, 1 to 1.5 cm long microshoots were placed vertically on rooting medium. At this stage also microshoots exuded phenolic substances. Therefore, 522.5 μM citric acid and 100 μM PVP 40 were added in the rooting medium. Relevant data have been given in Table 18.

Microshoots placed on half MS medium without growth regulators and half-MS medium supplemented with IAA (2.8, 5.7 or 11.4 μM) or NAA (2.6, 5.4 or 10.8 μM) did not produce rooting and died within 3
weeks of inoculation.

On 4.9 μM IBA, microshoots produce 10% rooting with 1.67 cm ± 0.1 long 2.5 ± 0.2 roots. Lower (2.4 μM) and higher (9.8 μM) concentrations of IBA failed to induce rooting in microshoots.

It is concluded that IBA is the most promising auxin for inducing roots in microshoots derived from 1-year-old seedlings of L. parviflora.

2. Tree Explants:
Nodal segments of a 50-year-old tree were used for initiating shoot bud cultures.

a. Explant Establishment:
Control of phenolic exudation and the effects of medium, BA and season of explant collection were studied at this stage.

i. Control of Phenolic Exudation:
Explants exuded excessive phenolic substances into the culture medium from their cut ends and died within two days of inoculation. Leaching was checked by suspending explants in a sterile solution of PVP 40 (25 μM) and citric acid (522.5 μM) for 15 minutes before inoculation and by adding PVP 40 (100 μM) and citric acid (522.5 μM) in the explant establishment medium.

ii. Effects of Basal Medium and BA:
Relevant data have been given in Table 19.
All the three types of basal media induced bud break in 100% explants. On B5, MS and WPM, average number of shoots per explant were $1 \pm 0$, $2.0 \pm 0.2$ and $2.2 \pm 0.2$, respectively.

Both the basal media, MS and WPM with 0.44 μM BA induced bud break in 100% explants, while B5 with 0.44 μM BA induced bud break in 90% explants. In general higher (2.22 and 11.1 μM) levels of BA in all the three basal media reduced bud break percentage. On MS, WPM and B5 media supplemented with 2.2 μM BA, bud break responses were 100%, 90% and 70%, respectively and with 11.1 μM BA, bud break responses were 90%, 90% and 30%, respectively.

On MS medium with 0.44, 2.22 and 11.1 μM BA, the number of shoots per explants were $2.5 \pm 0.2$, $1.2 \pm 0.1$ and $1.9 \pm 0.1$, respectively. On B5 medium supplemented with 0.44, 2.22 and 11.1 μM BA the number of shoots per explant were $1.1 \pm 0.1$, $1.6 \pm 0.2$ and $1.6 \pm 0.2$ respectively. WPM supplemented with different concentrations of BA did not show any significant change in shoot number as compared to control.

On all the three types of basal media, with or without BA, the explants produced cluster of shoots without normal leaves. These shoots remained stunted in growth. Increase in BA concentration in the medium caused further decrease in shoot elongation. On MS medium with 0.44, 2.22 and 11.1 μM BA, mean shoot lengths (in cm) were $0.65 \pm 0.05$, $0.61 \pm 0.04$ and $0.45 \pm 0.03$, respectively. On 0.44, 2.22 and 11.1 μM BA in WPM, mean shoot lengths (in cm) were $0.75$.
± 0.05, 0.65 ± 0.03 and 0.51 ± 0.03, respectively. On B5 medium with 0.44, 2.22 and 11.1 µM BA, mean shoot length (in cm) were 0.63 ± 0.06, 0.65 ± 0.03 and 0.47 ± 0.04, respectively.

It is concluded that WPM supplemented with 0.44 µM BA and MS medium are most suitable media for establishment of explants from 50 year old tree of *L. parviflora* (Plate 20 & 21).

**iii Effects of Seasonal Variation:**

The effects of seasonal variation on explant establishment were studied by inoculating explants on MS medium with 0.44 µM BA, throughout the year. The effects of seasonal variation and contamination have been shown in Figure 4.

Contamination occurred in 100% cultures raised during August to April. Whereas those initiated during May to July showed 10% contamination.

The explants inoculated during August to April did not show any bud break. Those inoculated during May to June exhibited 90% bud break response. During July, only 80% explants exhibited bud break.

It is concluded that May to June is the best period for initiating shoot bud culture from adult tree explants of *L. parviflora*.

**b. Propagule Proliferation:**

The nodes of microshoots from original explants placed on MS
with 0.44 μM BA were used for shoot proliferation. Data concerning shoot proliferation have been given in Table 20.

i. Control of Phenolic Exudation:

PVP 40 (100 μM) and citric acid (522.5 μM) were incorporated in multiplication medium to control phenolic exudation from the cut ends of micrones.

ii. Effects of BA:

Micrones placed on MS medium with different concentrations of BA produced shoot buds (< 0.5 cm) and very rarely small shoots (> 0.5 cm) but never normal shoots with normal leaves.

On MS medium with 0, 0.44, 0.88 and 1.76 μM BA, the average number of shoot buds were 1 ± 0, 1.3 ± 0.6, 1.3 ± 0.3 and 1.1 ± 0.1, respectively. The shoot buds remained stunted on MS without BA and with 0.88 or 1.76 μM BA. Marginal elongation of shoots (0.75 cm ± 0.4) was observed on MS medium with 0.44 μM BA.

It is concluded that MS medium with 0.44 μM BA is comparatively better medium for shoot proliferation from nodes derived from 50-year-old tree of L. parviflora.

iii. Effect of Successive Transfers:

In this experiment, shoots (> 0.5 cm, if any) produced by micrones on multiplication medium (MS + 0.44 μM BA) were harvested after 4 weeks and basal parts with shoot buds were
transferred to fresh multiplication medium. Relevant data have been given in Table 21.

Shoot number did not increase with successive transfers. On 2nd transfer, mean shoot number per micronode and mean shoot length were 0.4 ± 0.02 and 0.62 cm ± 0.3, respectively. However, the number of shoot buds to increased up to 3rd transfer. On 1st, 2nd and 3rd transfer, average shoot buds per culture were 1.3 ± 0.6, 8.3 ± 0.8 and 10.0 ± 1.2, respectively.

After 3rd transfer, the cultures turned dark brown and the shoot buds died.

It is concluded that the shoot bud cultures derived from adult tree explants of *L. parviflora* are difficult to maintain after the 3rd transfer.

3. Basal-sprout Explant:

Nodal segments from basal-sprout of a 50-year-old tree were used to initiate shoot bud cultures.

a. Explant Establishment:

Responses of basal-sprout explants to medium and BA were studied during this stage.

i. Control of Exudation of Phenolic Substances:

Explants exuded phenolic substances into culture medium from their cut ends and died within few days of inoculation. This problem
was overcome by suspending explants in a sterile solution of PVP 40 (25 μM), and citric acid (522.5 μM) for 15 minutes before inoculation; and by adding 100 μM PVP 40 and 522.5 μM citric acid in nutrient medium.

ii. Effects of Basal media and BA:

Relevant data have been given in Table 22.

All the 3 types of basal media without growth regulators induced bud break from nodal explants of basal-sprouts. WPM induced bud break in 60% explants, whereas, B5 and MS media induced bud break in 90% explants. On all the three basal media, single shoot elongated from node of each explant. On B5, MS and WPM, mean shoot lengths were 0.69 cm ± 0.05, 0.93 cm ± 0.17 and 1.14 cm ± 0.01, respectively.

Lower concentrations of BA (0.44 and 2.22 μM) in B5 and MS media induced bud break in 100 percent explants; and these concentrations of BA in WPM induced budbreak in 90% explants. Higher concentration of BA (11.1 μM), slightly reduced the bud break percentage. At 11.1 μM concentration of BA in MS and WPM, 80% of explants showed bud break; and at 11.1 μM concentration of BA in B5, 90% explants exhibited bud break. All the concentration of BA in B5 medium induced development of single shoot from each explant. WPM with 0.44 or 2.22 μM BA induced elongation of single shoot per explant; and with 11.1 μM BA, it induced elongation of 1.3 ± 0.3 shoots per explants. On MS medium with 0.44, 2.22 and 11.1 μM BA, the
number of shoots per explant were 1.0 ± 0, 2.5 ± 0.9 and 2.3 ± 0.8, respectively.

Best shoot elongation (1.16 cm ± 0.22) occurred on explants placed on MS medium with 0.44 μM BA. On MS medium with at 2.22 μM and 11.1 μM BA, mean shoot lengths were 0.72 cm ± 0.04 and 0.39 cm ± 0.02, respectively.

It is concluded that MS medium with 0.44 μM BA is the most suitable medium for the establishment of nodal explants from basal‐sprouts of 50-year-old tree of L. parviflora (Plate 22 & 23).

b. Propagule Proliferation:

The microshoots that had elongated on the mother explants were harvested and their nodes were used for further shoot proliferation. Relevant data have been given in Table 23.

i. Prevention of Exudation of Phenolic Substances:

Leaching of phenolic substances from micronodes to the medium was observed in multiplication stage also. Therefore, 100 μM PVP 40 and 522.5 μM citric acid were incorporated in multiplication medium.

ii. Effects of BA:

Number of shoots increased with the increase in concentration of BA. On MS medium with 0, 0.44, 0.88 and 1.76 μM BA, average number of shoots were 1 ± 0, 1.3 ± 0.1, 1.5 ± 0.1 and 5 ± 0.3, respectively; and mean shoot lengths (in cm) were 1.2 ± 0.14, 1.5 ± 0.11, 1 ± 0.08
and 0.5 ± 0.03, respectively.

It is concluded that MS medium with 0.44 μM BA is the best medium for multiplication of shoots.

iii. Effects of Successive Transfers:

Microshoots (> 0.5 cm) produced by micronodes on multiplication medium (MS ± 0.44 μM BA) were harvested after 4 weeks; and basal part of the culture was transferred to fresh proliferation medium. During these transfers, formation of new shoot bud clusters (shoots < 0.5 cm) (Plate 24), development of leader shoots (shoots > 0.5 cm) (Plate 25) and death of shoot bud clusters were observed. Relevant data have been given in Table 24.

During successive transfers, average number of shoots continued to increase up to 3rd transfer and then remained constant up to the 6th transfer (P < 0.0001). On 1st, 2nd, 3rd, 4th, 5th and 6th transfers, the average number of shoots per culture were 1.3 ± 0.1, 1.4 ± 0.2, 6.2 ± 0.8, 6.0 ± 0.6, 5.8 ± 0.6 and 5.9 ± 0.7, respectively.

However, shoot length remained almost constant (1.2 to 1.5 cm) during 1st to 6th transfers, except on 3rd transfer, which showed significant decrease (P = 0.0043). Formation of shoot-bud clusters was observed on 1st transfer. The shoot bud cluster developed from the micronodes. On 1st, 2nd, 3rd, 4th, 5th and 6th transfers number of shoot buds, per culture were 0, 5.3 ± 0.7, 14.6 ± 1.0, 11.6 ± 1.1, 16.6 ± 1.5 and 12.5 ± 1.2, respectively.
Thus, after six transfers, a total of 170 shoots can be obtained from a single micronode derived from basal-sprouts of L. parviflora (Plate 25).

c. Rooting:

Relevant data have been given in Table 25.

Leaching of phenolic substances occurred at this stage also. Therefore, 100μM PVP 40 and 522.5μM citric acid were added in the rooting medium.

Half-MS medium supplemented with 4.9μM IBA induced rooting in 10% of microshoots. Average number of roots per microshoot and mean root length (in cm) were 2.1 ± 0.2 and 1.12 ± 0.2, respectively. Half-MS without growth regulators, half-MS with 2.4 or 9.8 μM IBA, and half-MS with NAA or IAA failed to induce rooting in microshoots.

It is concluded that half-MS medium with 4.9μM IBA is most promising medium for rooting of microshoots derived from basal-sprout explants of L. parviflora.

C. Pongamia pinnata:

The explants were obtained from an adult tree, basal-sprouts of an adult tree, rooted cuttings, field grown seedlings and aseptically grown seedlings of P. pinnata. The results of these studies have been described below.
1. Adult Tree Explant,

2. Basal-Sprout Explant,

3. Explants from Rooted Cuttings, and

4. Explants from Field Grown Seedlings.

These four types of explants of *P. pinnata* did not respond to any medium, cytokinin or season of collection. The various medium, cytokinin and season of collection attempted to initiate shoot bud culture have been given in materials and methods.

5. Explants from Aseptically Grown Seedlings:

Nodal segments, from aseptically grown seedlings were used to initiate shoot bud cultures. Two types of explants viz., cotyledonary node and the first node above the cotyledonary node (hereafter referred as upper node), from aseptically grown seedlings were used.

a. Explant Establishment:

i. Cotyledonary Nodes:

On MS medium, MS with 2.2, 8.9 or 35.6 µM BA, the bud break responses were 80, 100, 80 and 70 percent, respectively; and the mean shoot length (in cm) were 3.02 ± 0.2, 6.34 ± 1.0 (Plate 26), 4.41 ± 0.3 and 1.63 ± 0.1, respectively (Table 26). On MS medium without growth regulators, 50% of cotyledonary nodes showed rooting from their basal cut end, which was in direct contact with the medium.
ii. Upper Nodes:

On MS medium without growth regulators, 50% of upper nodes also showed rooting from their basal cut end (Plate 27). The upper nodes placed on MS medium with 2.2, 8.9 and 35.6 μM BA showed 70%, 90%, 90% and 80% bud break, respectively; and 0.95 cm ± 0.1, 0.98 cm ± 0.1, 1.34 cm ± 0.2 (Plate 28) and 0.25 cm ± 0.01 shoot lengths, respectively.

b. Propagule Proliferation:

i. Effects of BA:

Relevant data have been given in Table 27.

Microshoots that elongated from both explant types were excised and their micronodes were used for shoot production. Single shoot elongated from each micronode of both the types, placed on MS medium supplemented with 0, 2.2, 8.9 or 35.6 μM BA. But, higher concentration (35.6 μM) of BA, reduced shoot length (0.3 cm ± 0.01). On PGR free MS medium and MS medium with 2.2 μM BA, shoot lengths were 0.92 cm ± 0.1 and 0.95 cm ± 0.1, respectively. However, micronodes from both the sources, placed on MS medium with 8.9 μM BA, produced longest shoots (1.21 cm ± 0.2); therefore, it is concluded that MS medium with 8.9 μM BA was the best medium for shoot elongation from micronodes derived from microshoots of cotyledonary and upper nodes of *P. pinnata* seedlings.

ii. Effects of Subculturing:

Relevant data have been given in Figure 5.

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After 4 weeks, micronodes from both the sources, were again excised from these elongated microshoots and placed on fresh MS medium with 8.9 μM BA; and repeated after every 4 weeks. Each micronode produced single shoot upto the 2nd subculture. On the 1st and 2nd subcultures, mean shoot lengths were 1.21 cm ± 0.2 and 1.1 cm ± 0.1, respectively. After the 2nd subculture, the micronodes from both the sources became dormant and did not elongate.

It is concluded that the shoot multiplication from micronodes derived from cotyledonary and upper nodes from *in vitro* grown seedlings of *P. pinnata*, can be achieved upto the 2nd subculture only.

c. Rooting:

For rooting, approximately 1 cm long microshoots derived from cotyledonary and upper nodes were placed on rooting medium. Relevant data have been given in Table 28.

Microshoots placed on half-MS medium without PGR or with 0.49 μM IBA showed 50% rooting. On half-MS medium with 2.46 μM IBA, 90 percent microshoots showed root initiation; the mean root number and mean root length were 2.0 ± 0.3 and 2.73 cm ± 0.3, respectively (Plate 29).

Half-MS medium with IAA failed to induce rooting. Half MS with NAA induced only callus at the base of shoots.

It is concluded that nodal explants from aseptically grown seedlings
of *P. pinnata* can be used for initiating shoot bud cultures. Best medium for establishment of cotyledonary nodes, shoot proliferation and rooting of microshoots are MS + 2.2 μM BA, MS + 8.9 μM BA and half-MS + 2.46 μM IBA, respectively.

d. Hardening:

Plantlets were removed from culture tubes, washed thoroughly with sterile water to remove agar-agar and then transplanted into pots containing sterile sand, soil and compost in equal volume. The plantlets were covered with polyethylene bags and the pots were placed in semicontrolled temperature (approximately 25-32°C), for 30 days; after removing polyethylene bags, the pots were placed in a shady place for another 30 days; and then these plantlets were transferred to field where they resumed normal growth within 15 days (Plate 30).
Table 1: Effects of different media and BA levels on axillary shoot growth from nodal segments of *C. collinus* seedlings, after 4 weeks.

<table>
<thead>
<tr>
<th>Medium</th>
<th>BA (µM)</th>
<th>Bud break (%)</th>
<th>Shoot number/ explant Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes/ explant Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS</td>
<td>0</td>
<td>80</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.3</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.4 ± 0.1</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>MS</td>
<td>0</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>2.2 ± 0.2</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>100</td>
<td>1.8 ± 0.2</td>
<td>3.8 ± 0.4</td>
<td>10.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>1.0 ± 0.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>White's</td>
<td>0</td>
<td>60</td>
<td>1.0 ± 0.0</td>
<td>0.9 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>80</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.04</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>80</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.04</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>80</td>
<td>1.0 ± 0.0</td>
<td>0.7 ± 0.04</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>WPM</td>
<td>0</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>2.5 ± 0.4</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>100</td>
<td>1.8 ± 0.2</td>
<td>2.5 ± 0.4</td>
<td>7.0 ± 0.5</td>
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<td></td>
<td>8.9</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.9 ± 0.04</td>
<td>1.4 ± 0.1</td>
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<tr>
<td></td>
<td>17.8</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.8 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
</tbody>
</table>

ANOVA: Shoot no: BA : df = 3; F = 72; P<0.0001  
Medium : df = 3; F = 24; P<0.0001  
BA X Medium : df = 9; F = 24; P<0.0001  
Shoot length: BA : df = 3; F = 379.85; P<0.0001  
Medium : df = 3; F = 456.90; P<0.0001  
BA X Medium : df = 9; F = 3.87; P<0.0001  
Nodes / explants: BA : df = 3; F = 146.4; P<0.0001  
Medium : df = 3; F = 122.0; P<0.0001  
BA X Medium : df = 9; F = 43.18; P<0.0001
Table 2: Effects of different levels of BA and Kinetin (in MS medium) on axillary shoot growth from nodal segments of C. colinus seedlings, after 4 weeks.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Cytokin</th>
<th>Bud break ( % )</th>
<th>Shoot no. per explant Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes/ explant Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.2 ± 0.2</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Kinetin</td>
<td>2.3</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.3 ± 0.2</td>
<td>4.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>9.3</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.0 ± 0.3</td>
<td>4.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>18.5</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>BA</td>
<td>2.2</td>
<td>100</td>
<td>1.8 ± 0.2</td>
<td>3.8 ± 0.4</td>
<td>10.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.0 ± 0.3</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>17.8</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

ANOVA:
- Shoot no: Concentration X Cytokinin: df = 2; F = 36; P<0.0001
- Shoot length: Concentration X Cytokinin: df = 1; F = 79.75; P<0.0001
- Shoot length: Cytokinin: df = 1; F = 7.89; P = 0.0064
- Nodes / explants: Concentration X Cytokinin: df = 2; F = 135.32; P<0.0001
- Concentration X Cytokinin: df = 2; F = 7.05; P = 0.0095
- Concentration: df = 1; F = 90.96; P<0.0001
- Cytokinin: df = 1; F = 88.55; P<0.0001
Table 3: Effects of different concentrations of BA on shoot proliferation from micronodes derived from seedling explants of C. collinus, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (μM)</th>
<th>Shoot no. per node Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes/micronode Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>1.0 ± 0.0 b</td>
<td>1.1 ± 0.3 d</td>
<td>2.6 ± 0.2 d</td>
</tr>
<tr>
<td>1.1</td>
<td>2.5 ± 0.7 a</td>
<td>4.1 ± 0.5 a</td>
<td>11.2 ± 0.7 a</td>
</tr>
<tr>
<td>0.56</td>
<td>2.3 ± 0.2 a</td>
<td>3.3 ± 0.4 b</td>
<td>8.3 ± 0.8 b</td>
</tr>
<tr>
<td>0.0</td>
<td>1.8 ± 0.4 a</td>
<td>2.7 ± 0.1 c</td>
<td>6.1 ± 0.4 c</td>
</tr>
</tbody>
</table>

Same letters followed by means in each column do not differ significantly, at 5% level (by DMR test)

LSD at 0.5 for Shoot number = 0.68
Shoot length = 0.23
Nodes/micronode = 0.84

ANOVA: BA: Shoot no: df = 3; F = 7.96; P = 0.0003
Shoot length: df = 3; F = 251.61; P<0.0001
Nodes/micronode: df = 3; F = 152.86; P<0.0001
Table 4: Rooting response of *C. collinus* seedling explant-derived microshoots to auxins (in half MS medium), after 4 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rooting (µM)</th>
<th>Rooting (%)</th>
<th>Root no. (per shoot) Mean ± SE</th>
<th>Root length (cm) Mean ± SE</th>
<th>Callusing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Effect of auxins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.7 IAA</td>
<td>20</td>
<td>1.0 ± 0</td>
<td>1.5 ± 0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22.8 IAA</td>
<td>20</td>
<td>1.0 ± 0</td>
<td>0.4 ± 0.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.4 IBA</td>
<td>30</td>
<td>9.3 ± 3.7</td>
<td>0.9 ± 0.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4.9 IBA</td>
<td>20</td>
<td>1.0 ± 0</td>
<td>0.6 ± 0.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>19.6 IBA</td>
<td>20</td>
<td>6.0 ± 1.0</td>
<td>0.4 ± 0.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2.8 NAA</td>
<td>20</td>
<td>2.0 ± 1.0</td>
<td>0.6 ± 0.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5.4 NAA</td>
<td>40</td>
<td>4.0 ± 1.0</td>
<td>0.7 ± 0.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>21.5 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.7 IAA</td>
<td>20</td>
<td>2.0 ± 1.0</td>
<td>0.5 ± 0.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+0.6 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+0.6 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.4 IAA</td>
<td>20</td>
<td>2.0 ± 1.0</td>
<td>0.5 ± 0.2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>+1.2 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+1.3 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>+2.4 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+2.6 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Effect of IAA (7 days) + darkness (first 72h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.7 IAA</td>
<td>60</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>22.8 IAA</td>
<td>80</td>
<td>2.1 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Callus +, present; -, absent.
Table 5: Effects of different media and BA concentrations on axillary shoot growth from nodal segments of *C. collinus* adult tree, after 4 weeks.

<table>
<thead>
<tr>
<th>Media</th>
<th>BA (µM)</th>
<th>Bud break (%)</th>
<th>Shoot number per explant</th>
<th>Shoot length (cm)</th>
<th>Nodes/explant</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS</td>
<td>0.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.94 ± 0.10</td>
<td>4.1 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.78 ± 0.12</td>
<td>4.7 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.16 ± 0.16</td>
<td>2.7 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>0.39 ± 0.01</td>
<td>1.0 ± 0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>0.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>3.07 ± 0.29</td>
<td>4.1 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>4.47 ± 0.17</td>
<td>5.7 ± 0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.66 ± 0.18</td>
<td>5.3 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>0.29 ± 0.02</td>
<td>1.0 ± 0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WPM</td>
<td>0.00</td>
<td>90</td>
<td>1.0 ± 0</td>
<td>1.03 ± 0.11</td>
<td>1.9 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.3 ± 0.16</td>
<td>4.7 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>90</td>
<td>1.0 ± 0</td>
<td>0.94 ± 0.14</td>
<td>1.8 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>80</td>
<td>1.0 ± 0</td>
<td>0.25 ± 0.02</td>
<td>1.0 ± 0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA: Shoot length: BA : df = 3; F = 674.71; P<0.0001
Medium : df = 2; F = 245.29; P<0.0001
BA X Medium : df = 6; F = 47.49; P<0.0001

Nodes / explants: BA : df = 3; F = 116.89; P<0.0001
Medium : df = 2; F = 38.74; P<0.0001
BA X Medium : df = 6; F = 11.24; P<0.0001
Table 6: Effects of different concentrations of BA on shoot proliferation from micronodes derived from adult tree explants of C. collinus, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (µM)</th>
<th>Shoot number per micronode Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes / micronode Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.0 ± 0</td>
<td>3.08 ± 0.15 b</td>
<td>2.9 ± 0.1 b</td>
</tr>
<tr>
<td>0.44</td>
<td>1.0 ± 0</td>
<td>3.96 ± 0.11 a</td>
<td>3.9 ± 0.7 a</td>
</tr>
<tr>
<td>2.20</td>
<td>1.0 ± 0</td>
<td>2.61 ± 0.18 c</td>
<td>2.5 ± 0.2 b</td>
</tr>
<tr>
<td>11.00</td>
<td>1.0 ± 0</td>
<td>2.44 ± 0.16 d</td>
<td>2.4 ± 0.2 b</td>
</tr>
</tbody>
</table>

Same letter followed by means in each column, do not differ significantly at 5% level (by DMR test).

LSD at 0.05 for shoot length = 0.19
LSD at 0.05 for nodes = 0.81

ANOVA: Shoot length : df = 3 ; F = 107.46 ; P<0.0001
ANOVA: Node number : df = 3 ; F = 5.88 ; P = 0.0022
Table 7: Effects of successive transfers on shoot multiplication and shoot elongation from adult tree explant derived micronode cultures on MS medium supplemented with 0.44 μM BA. Observations were taken after every 4 weeks.

<table>
<thead>
<tr>
<th>No. of Transfer</th>
<th>Shoot / culture Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes/culture Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1.0 ± 0.0 d</td>
<td>3.9 ± 0.1 d</td>
<td>3.9 ± 0.7 e</td>
</tr>
<tr>
<td>2nd</td>
<td>1.0 ± 0.0 d</td>
<td>4.0 ± 0.3 d</td>
<td>4.4 ± 0.2 e</td>
</tr>
<tr>
<td>3rd</td>
<td>1.7 ± 0.3 d</td>
<td>6.9 ± 0.6 b</td>
<td>7.5 ± 0.6 d</td>
</tr>
<tr>
<td>4th</td>
<td>3.1 ± 0.3 c</td>
<td>7.2 ± 0.4 a</td>
<td>9.5 ± 0.5 c</td>
</tr>
<tr>
<td>5th</td>
<td>3.4 ± 0.3 bc</td>
<td>7.3 ± 0.5 a</td>
<td>10.0 ± 0.4 c</td>
</tr>
<tr>
<td>6th</td>
<td>4.1 ± 0.3 b</td>
<td>7.1 ± 0.5 ab</td>
<td>11.6 ± 0.7 b</td>
</tr>
<tr>
<td>7th</td>
<td>5.3 ± 0.3 a</td>
<td>6.2 ± 0.6 c</td>
<td>20.2 ± 0.8 a</td>
</tr>
</tbody>
</table>

Same letters followed by means in each column; do not differ significantly, at 5% level (by DMR test).

LSD at 0.05 for Shoot / Culture = 0.71
Shoot length = 0.20
Nodes / Culture = 1.21

ANOVA: Transfer :Shoot/ Culture : df = 6; F = 41.79; P<0.0001
Shoot length : df = 6; F = 433.12; P<0.0001
Nodes / explants : df = 6; F =162.93; P<0.0001
Table 8: Rooting response of *C. colinus* adult tree explant-derived microshoots to auxins (in half-MS medium), after 4 weeks.

<table>
<thead>
<tr>
<th>Auxin (μM)</th>
<th>Rooting (%)</th>
<th>Root No. per shoot Mean ± SE</th>
<th>Root Length (cm) Mean ± SE</th>
<th>Callusing</th>
<th>Shoot Tip Necrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.28 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C</td>
<td>25</td>
</tr>
<tr>
<td>1.14 IAA</td>
<td>10</td>
<td>1.0 ± 0</td>
<td>3.0 ± 0.8</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>4.56 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CCC</td>
<td>25</td>
</tr>
<tr>
<td>0.24 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>40</td>
</tr>
<tr>
<td>0.98 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>80</td>
</tr>
<tr>
<td>3.92 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>80</td>
</tr>
<tr>
<td>0.26 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>-</td>
</tr>
<tr>
<td>1.08 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>20</td>
</tr>
<tr>
<td>4.32 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>30</td>
</tr>
</tbody>
</table>

C, Slight Callus; CC, Moderate Callus; CCC, Profuse Callus
Table 9: Effects of IAA pulse and 72 h dark treatment on rooting of microshoots derived from adult tree explants of C. collinus. Half-MS medium. Observations recorded after 4 weeks.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rooting</th>
<th>Root no.</th>
<th>Root length (cm)</th>
<th>STN*</th>
<th>STN* in shoots</th>
<th>Callusing per shoot</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>( %)</th>
<th>( %)</th>
<th>( %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>Effect of IAA (µM) (7 days):</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>2.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>14.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>57.0</td>
<td>10</td>
<td>1.3 ± 0.3</td>
<td>1.8 ± 1.0</td>
<td>30</td>
<td>40</td>
<td>20</td>
<td>70</td>
<td>40</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>285.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Effect of 0.22 µM BA-agar cube at decapitated shoot tip + basal dip in 50% alcoholic IAA (mM) for 2 min:

<p>| | | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>10</td>
<td>1.0 ± 0</td>
<td>0.60 ± 0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.4</td>
<td>10</td>
<td>1.2 ± 0.4</td>
<td>1.31 ± 0.49</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28.5</td>
<td>40</td>
<td>2.3 ± 0.5</td>
<td>1.75 ± 0.22</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*STN*: Shoot tip necrosis
Table 10: Effects of different media and BA concentrations on axillary shoot growth from nodal segments of *C. collinus* basal-sprouts, after 4 weeks.

<table>
<thead>
<tr>
<th>Media</th>
<th>BA (μM)</th>
<th>Bud break (%)</th>
<th>Shoot number per explants</th>
<th>Shoot length (cm)</th>
<th>Nodes/explant</th>
</tr>
</thead>
<tbody>
<tr>
<td>B5</td>
<td>0.00</td>
<td>90</td>
<td>1.0 ± 0</td>
<td>0.25 ± 0.02</td>
<td>1.0 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>3.05 ± 0.41</td>
<td>3.1 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>80</td>
<td>1.0 ± 0</td>
<td>1.52 ± 0.20</td>
<td>2.2 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>70</td>
<td>1.0 ± 0</td>
<td>0.20 ± 0.01</td>
<td>1.0 ± 0.00</td>
</tr>
<tr>
<td>MS</td>
<td>0.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>5.18 ± 0.6</td>
<td>7.0 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>6.18 ± 0.18</td>
<td>8.1 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.87 ± 0.39</td>
<td>4.4 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>0.35 ± 0.3</td>
<td>1.0 ± 0.00</td>
</tr>
<tr>
<td>WPM</td>
<td>0.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.03 ± 0.1</td>
<td>3.7 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>2.89 ± 0.13</td>
<td>4.3 ± 0.35</td>
</tr>
<tr>
<td></td>
<td>2.20</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>1.80 ± 0.11</td>
<td>3.5 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>11.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>0.33 ± 0.01</td>
<td>1.0 ± 0.00</td>
</tr>
</tbody>
</table>

ANOVA: Shoot length: BA: df = 3; F = 511.77; P<0.0001
Medium: df = 2; F = 583.67; P<0.0001
BA X Medium: df = 6; F = 110.64; P<0.0001

Nodes/explants: BA: df = 3; F = 78.69; P<0.0001
Medium: df = 2; F = 92.90; P<0.0001
BA X Medium: df = 6; F = 16.80; P<0.0001
Table 11: Effects of different concentrations of BA on shoot proliferation from micronodes derived from basal-sprout explants of C. collinus, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (μM)</th>
<th>Shoot number per explant Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes/explant Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.0 ± 0</td>
<td>2.84 ± 0.06 b</td>
<td>2.6 ± 0.1 b</td>
</tr>
<tr>
<td>0.44</td>
<td>1.0 ± 0</td>
<td>4.61 ± 0.20 a</td>
<td>4.7 ± 0.3 a</td>
</tr>
<tr>
<td>2.20</td>
<td>1.0 ± 0</td>
<td>3.47 ± 0.26 b</td>
<td>3.5 ± 0.2 b</td>
</tr>
<tr>
<td>11.00</td>
<td>1.0 ± 0</td>
<td>3.09 ± 0.29 b</td>
<td>3.0 ± 0.2 b</td>
</tr>
</tbody>
</table>

Same letter followed by mean in each column, do not differ significantly, at 5% level (By DMR test, LSD at .05 = 0.5 and 0.94 for shoot length and node number, respectively).

ANOVA: Shoot length: BA: df = 3; F = 16.4; P < 0.0001
Node number: BA: df = 3; F = 7.2; P < 0.0001
Table 12: Effects of successive transfers on shoot multiplication and shoot elongation from basal-sprout explant-derived micronode cultures on MS medium supplemented with 0.44 μM BA, observation were taken after every 4 weeks.

<table>
<thead>
<tr>
<th>No. of Transfer</th>
<th>Shoot / culture Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes / culture Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1.0 ± 0.0 e</td>
<td>4.6 ± 0.20 d</td>
<td>4.7 ± 0.3 f</td>
</tr>
<tr>
<td>2nd</td>
<td>1.6 ± 0.1 d</td>
<td>4.1 ± 0.30 e</td>
<td>6.5 ± 0.3 e</td>
</tr>
<tr>
<td>3rd</td>
<td>1.8 ± 0.1 d</td>
<td>7.2 ± 0.50 b</td>
<td>8.3 ± 0.7 d</td>
</tr>
<tr>
<td>4th</td>
<td>2.5 ± 0.2 c</td>
<td>7.1 ± 0.50 b</td>
<td>11.1 ± 0.9 bc</td>
</tr>
<tr>
<td>5th</td>
<td>2.9 ± 0.2 c</td>
<td>7.7 ± 0.50 a</td>
<td>10.1 ± 0.7 c</td>
</tr>
<tr>
<td>6th</td>
<td>3.5 ± 0.2 b</td>
<td>6.2 ± 0.30 c</td>
<td>12.1 ± 0.6 b</td>
</tr>
<tr>
<td>7th</td>
<td>5.0 ± 0.3 a</td>
<td>6.0 ± 0.30 c</td>
<td>23.8 ± 0.9 a</td>
</tr>
</tbody>
</table>

Same letters followed by means in each column, do not differ significantly, at 5% level (by DMR test).

LSD at : 0.05 for Shoot / culture = 0.55  
Shoot length = 0.30  
Nodes / culture = 1.63

ANOVA : transfer : Shoot / culture : df = 6; F = 47.75; P<0.0001  
Shoot length : df = 6; F = 165.14; P<0.0001  
Nodes / culture : df = 6; F =115.98; P<0.0001
Table 13: Rooting response of *C. collinus* basal-sprout explant-derived microshoots to auxins (in half-MS medium), after 4 weeks.

<table>
<thead>
<tr>
<th>Auxin (µM)</th>
<th>Rooting ( % )</th>
<th>Root no. per shoot</th>
<th>Root length (cm)</th>
<th>Callusing</th>
<th>Shoot tip necrosis ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>20</td>
<td>2.0 ± 0.18</td>
<td>1.8 ± 0.1</td>
<td>C</td>
<td>40</td>
</tr>
<tr>
<td>11.4 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C</td>
<td>80</td>
</tr>
<tr>
<td>45.6 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C</td>
<td>80</td>
</tr>
<tr>
<td>2.4 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CCC</td>
<td>60</td>
</tr>
<tr>
<td>9.8 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>100</td>
</tr>
<tr>
<td>39.2 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>100</td>
</tr>
<tr>
<td>2.6 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>-</td>
</tr>
<tr>
<td>10.8 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>100</td>
</tr>
<tr>
<td>43.2 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CC</td>
<td>100</td>
</tr>
</tbody>
</table>

C, slight callus; CC, moderate callus; CCC, profuse callus.
Table 14: Effects of IAA pulse and 72 h dark treatment on rooting of microshoots derived from basal-sprouts of *C. collinus* (Half-MS medium, observation recorded after 4 weeks).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rooting</th>
<th>Root no. (per shoot)</th>
<th>Root length (cm)</th>
<th>STN*</th>
<th>STN* in Callus (all rooted shoots)</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of IAA (µM) (7 days):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>30</td>
<td>2.0 ± 0.7</td>
<td>3.75 ± 0.25</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>14.2</td>
<td>10</td>
<td>2.0 ± 0.7</td>
<td>3.75 ± 0.25</td>
<td>40</td>
<td>1.5 ± 0.5</td>
<td>2.03 ± 1.23</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>57.0</td>
<td>20</td>
<td>1.5 ± 0.5</td>
<td>2.03 ± 1.23</td>
<td>50</td>
<td>-</td>
<td>1.6 ± 0.33</td>
<td>2.37 ± 0.21</td>
<td>-</td>
</tr>
<tr>
<td>285.0</td>
<td>-</td>
<td>-</td>
<td>90</td>
<td>-</td>
<td>2.1 ± 0.5</td>
<td>0.82 ± 0.07</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Effect of 0.22 µM BA-agar cube at decapitated shoot tip + basal dip in 50% alcoholic IAA (mM) for 2 min:

<table>
<thead>
<tr>
<th>Effect of 0.22 µM BA-agar cube at decapitated shoot tip + basal dip</th>
<th>Rooting</th>
<th>Root no.</th>
<th>Root length</th>
<th>STN*</th>
<th>STN* in Callus</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.7</td>
<td>5</td>
<td>2.1 ± 0.5</td>
<td>0.82 ± 0.07</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.4</td>
<td>50</td>
<td>1.6 ± 0.33</td>
<td>2.37 ± 0.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>28.5</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*STN*: Shoot tip necrosis
Table 15: Effects of different media and BA concentrations on axillary shoot growth from nodal segments of *L. parviflora* seedlings, after 4 weeks.

<table>
<thead>
<tr>
<th>Medium</th>
<th>BA (µM)</th>
<th>Bud break (%)</th>
<th>Shoot number per explant</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>0.00</td>
<td>100</td>
<td>1±0</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1±0</td>
<td>1.45±0.13</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>1±0</td>
<td>0.51±0.03</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>100</td>
<td>1±0</td>
<td>0.42±0.02</td>
</tr>
<tr>
<td>WPM</td>
<td>0.00</td>
<td>100</td>
<td>1±0</td>
<td>1.11±0.11</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1±0</td>
<td>1.35±0.14</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>1±0</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>100</td>
<td>1±0</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td>B5</td>
<td>0.00</td>
<td>100</td>
<td>1±0</td>
<td>0.98±0.11</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1±0</td>
<td>1.37±0.11</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>1±0</td>
<td>0.61±0.03</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>100</td>
<td>1±0</td>
<td>0.45±0.01</td>
</tr>
</tbody>
</table>

**ANOVA**: Shoot length: BA: df = 3; F = 143.48; P<0.0001
Medium: df = 2; F = 0.91; P = 0.4063 ns
BA X Medium: df = 6; F = 1.26; P = 0.2822 ns
Table 16: Effects of different concentrations of BA on shoot proliferation from micronodes derived from seedlings explants of *L. parviflora*, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (µM)</th>
<th>Shoot no. per micronode Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.5 ± 0.1 c</td>
<td>1.7 ± 0.11 a</td>
</tr>
<tr>
<td>0.44</td>
<td>2.4 ± 0.2 b</td>
<td>2.1 ± 0.18 a</td>
</tr>
<tr>
<td>0.88</td>
<td>2.0 ± 0.2 bc</td>
<td>0.8 ± 0.04 b</td>
</tr>
<tr>
<td>1.76</td>
<td>4.5 ± 0.2 a</td>
<td>0.5 ± 0.02 c</td>
</tr>
</tbody>
</table>

Same letters followed by means in each column do not differ significantly, at 5% level (by DMR test)

LSD at 0.05 for Shoot number = 0.84

LSD at 0.05 for Shoot length = 0.33

ANOVA: Shoot no: df = 3; F = 20.9; P<0.0001

ANOVA: Shoot length: df = 3; F = 36.6; P<0.0001
Table 17: Effects of successive transfers on shoot multiplication and shoot elongation from seedling explant-derived micronodes, on MS medium supplemented with 0.44 μM BA, observations were taken after every 4 weeks.

<table>
<thead>
<tr>
<th>No. of Transfer</th>
<th>Shoot number per culture Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Shoot buds per culture Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>2.4 ± 0.2 b</td>
<td>2.1 ± 0.18 a</td>
<td>-</td>
</tr>
<tr>
<td>2nd</td>
<td>2.4 ± 0.4 b</td>
<td>1.7 ± 0.15 a</td>
<td>2.3 ± 0.3 b</td>
</tr>
<tr>
<td>3rd</td>
<td>3.6 ± 0.5 a</td>
<td>0.7 ± 0.06 c</td>
<td>2.6 ± 0.5 a</td>
</tr>
<tr>
<td>4th</td>
<td>3.5 ± 0.5 a</td>
<td>0.9 ± 0.09 bc</td>
<td>2.4 ± 0.4 a</td>
</tr>
<tr>
<td>5th</td>
<td>3.4 ± 0.6 a</td>
<td>1.0 ± 0.08 bc</td>
<td>2.4 ± 0.6 a</td>
</tr>
<tr>
<td>6th</td>
<td>3.5 ± 0.4 a</td>
<td>1.1 ± 0.12 b</td>
<td>2.2 ± 0.3 a</td>
</tr>
</tbody>
</table>

Shoots > 0.5 cm

LSD at : 0.05 for Shoot number = 0.46
Shoot length = 0.40
Shoot buds = 0.40

ANOVA : transfer : Shoot number : df = 5; F = 12.08; P<0.0001
Shoot length : df = 5; F = 16.2; P<0.0001
Shoot buds : df = 5; F = 47.6; P<0.0001
Table 18: Rooting response of *L. parviflora* seedling explant-derived microshoots to auxins (in half-MS medium), after 4 weeks.

<table>
<thead>
<tr>
<th>Auxin (µM)</th>
<th>Rooting (%)</th>
<th>Root no. per shoot</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.7 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.4 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.4 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.9 IBA</td>
<td>10</td>
<td>2.5 ± 0.2</td>
<td>1.67 ± 0.1</td>
</tr>
<tr>
<td>9.8 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.6 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.4 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.8 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 19: Effects of different media and BA concentrations on axillary shoot growth from nodal segments of *L. parviflora* adult tree, after 4 weeks.

<table>
<thead>
<tr>
<th>Media</th>
<th>BA (µM)</th>
<th>Bud break (%)</th>
<th>Shoot number per explant</th>
<th>Shoot length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>0.00</td>
<td>100</td>
<td>2.0 ± 0.1</td>
<td>0.80 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>2.5 ± 0.2</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>1.2 ± 0.1</td>
<td>0.61 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>90</td>
<td>1.9 ± 0.2</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>WPM</td>
<td>0.00</td>
<td>100</td>
<td>2.2 ± 0.2</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>2.2 ± 0.3</td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>90</td>
<td>2.3 ± 0.2</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>90</td>
<td>2.2 ± 0.2</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>B5</td>
<td>0.00</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>0.50 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>90</td>
<td>1.1 ± 0.1</td>
<td>0.63 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>70</td>
<td>1.6 ± 0.2</td>
<td>0.65 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>30</td>
<td>1.6 ± 0.2</td>
<td>0.47 ± 0.04</td>
</tr>
</tbody>
</table>

ANOVA: Shoot no: BA: df = 3; F = 3.39; P = 0.0206
Medium: df = 2; F = 17.6; P < 0.0001
BA X Medium: df = 6; F = 4.01; P = 0.0011

Shoot length: BA: df = 3; F = 24.94; P < 0.0001
Medium: df = 2; F = 10.65; P = 0.0001
BA X Medium: df = 6; F = 5.38; P = 0.0001
Table 20: Effects of different concentrations of BA on shoot proliferation from micronodes derived from adult tree explants of *L. parviflora*, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (µM)</th>
<th>Shoot no. per micronode Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Shoot buds per micronode Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>-</td>
<td>-</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>0.44</td>
<td>0.4 ± 0.03</td>
<td>0.75 ± 0.4</td>
<td>1.3 ± 0.6</td>
</tr>
<tr>
<td>0.88</td>
<td>-</td>
<td>-</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>1.76</td>
<td>-</td>
<td>-</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Shoot > 0.5 cm
Shoot buds < 0.5 cm
Table 21: Effects of successive transfers on shoot multiplication and shoot elongation from adult tree explant derived micrornodes, on MS medium supplemented with 0.44 μM BA, observations were taken after every 4 weeks.

<table>
<thead>
<tr>
<th>No. of Transfer</th>
<th>Shoot number per culture Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Shoot buds per culture Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>0.4 ± 0.03 a</td>
<td>0.75 ± 0.4 a</td>
<td>1.3 ± 0.6 c</td>
</tr>
<tr>
<td>2nd</td>
<td>0.4 ± 0.02 a</td>
<td>0.62 ± 0.3 b</td>
<td>8.3 ± 0.8 b</td>
</tr>
<tr>
<td>3rd</td>
<td>-</td>
<td>-</td>
<td>10.0 ± 1.2 a</td>
</tr>
</tbody>
</table>

Shoots > 0.5 cm
Shoot buds < 0.5 cm

Same letters in each column followed by means do not differ significantly, at 5% level (by DMR test)

LSD at 0.05 for Shoot number = 0.38
Shoot length = 0.09
Shoot buds = 0.73

ANOVA: transfer: Shoot number: df = 2; F = 3; P = 0.0666
Shoot length: df = 2; F = 156.7; P<0.0001
Shoot buds: df = 2; F = 341; P<0.0001
Table 22: Effects of different media and BA concentrations on axillary shoot growth from nodal segments of *L. parviflora* basal-sprouts, after 4 weeks.

<table>
<thead>
<tr>
<th>Medium</th>
<th>BA (μM)</th>
<th>Bud break per explant (%)</th>
<th>Shoot number per explant Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>0.00</td>
<td>90</td>
<td>1.0 ± 0.0</td>
<td>0.93 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>1.16 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>2.5 ± 1.9</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>80</td>
<td>2.3 ± 0.8</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>WPM</td>
<td>0.00</td>
<td>80</td>
<td>1.0 ± 0.0</td>
<td>1.14 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>90</td>
<td>1.0 ± 0.0</td>
<td>1.94 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>90</td>
<td>1.0 ± 0.0</td>
<td>0.63 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>80</td>
<td>1.3 ± 0.3</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>BS</td>
<td>0.00</td>
<td>90</td>
<td>1.0 ± 0.0</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>0.44</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2.22</td>
<td>100</td>
<td>1.0 ± 0.0</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>11.10</td>
<td>90</td>
<td>1.0 ± 0.0</td>
<td>0.33 ± 0.01</td>
</tr>
</tbody>
</table>

ANOVA: Shoot length: BA : df = 3; F = 22.75; P < 0.0001
Medium: df = 2; F = 50.24; P < 0.0001
BA X Medium: df = 6; F = 17.64; P < 0.0001

Shoot length: BA : df = 3; F = 67.67; P < 0.0001
Medium: df = 2; F = 13.04; P < 0.0001
BA X Medium: df = 2; F = 5.57; P < 0.0001
Table 23: Effects of different concentrations of BA on shoot proliferation from micronodes derived from basal-sprout explants of *L. parviflora*, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (µM)</th>
<th>Shoot no. per micronode Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>1.0 ± 0.0 b</td>
<td>1.2 ± 0.14 a</td>
</tr>
<tr>
<td>0.44</td>
<td>1.3 ± 0.1 b</td>
<td>1.5 ± 0.11 a</td>
</tr>
<tr>
<td>0.88</td>
<td>1.5 ± 0.1 b</td>
<td>1.0 ± 0.08 b</td>
</tr>
<tr>
<td>1.76</td>
<td>5.0 ± 0.3 a</td>
<td>0.5 ± 0.03 c</td>
</tr>
</tbody>
</table>

Same letters followed by means in each column do not differ significantly, at 5% level (by DMR test)

LSD at 0.05 for Shoot number = 0.6554
Shoot length = 0.2963

ANOVA: Shoot no : BA : df = 3; $F = 65.5; P < 0.0001$
Shoot length : BA : df = 3; $F = 20.4; P < 0.0001$
Table 24: Effects of successive transfers of shoot multiplication and shoot elongation from basal-sprout explant derived micronodes of L. parviflora, on MS medium supplemented with 0.44 μM BA, observations were taken after every 4 weeks.

<table>
<thead>
<tr>
<th>No. of Transfer</th>
<th>Shoot number per culture Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Shoot buds per culture Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>1.3 ± 0.1 b</td>
<td>1.5 ± 0.11 a</td>
<td>0.0 f</td>
</tr>
<tr>
<td>2nd</td>
<td>1.4 ± 0.2 b</td>
<td>1.4 ± 0.20 a</td>
<td>5.3 ± 0.7 e</td>
</tr>
<tr>
<td>3rd</td>
<td>6.2 ± 0.8 a</td>
<td>0.8 ± 0.08 b</td>
<td>14.6 ± 1.4 b</td>
</tr>
<tr>
<td>4th</td>
<td>6.0 ± 0.8 a</td>
<td>1.2 ± 0.22 a</td>
<td>11.6 ± 1.1 d</td>
</tr>
<tr>
<td>5th</td>
<td>5.8 ± 0.8 a</td>
<td>1.3 ± 0.23 a</td>
<td>16.6 ± 1.5 a</td>
</tr>
<tr>
<td>6th</td>
<td>5.9 ± 0.7 a</td>
<td>1.3 ± 0.15 a</td>
<td>12.5 ± 1.2 c</td>
</tr>
</tbody>
</table>

Shoots > 0.5 cm
Shoot buds < 0.5 cm

Some letters followed by means in each column do not differ significantly, at 5% level (by DMR test)

LSD at 0.05 for Shoot number = 0.44
Shoot length = 0.40
Shoot buds = 0.74

ANOVA: Transfer: Shoot number
: df = 5; F = 234.4; P<0.0001
Shoot length
: df = 5; F = 3.9; P = 0.0043
Shoot buds
: df = 5; F = 549.5; P<0.0001
Table 25: Rooting response of *L. parviflora* basal-sprout explant derived microshoots to auxins (in half-MS medium), after 4 weeks

<table>
<thead>
<tr>
<th>Auxin (μM)</th>
<th>Rooting (%)</th>
<th>Root no. Mean ± SE</th>
<th>Root length (cm) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.8 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.7 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11.4 IAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.4 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.9 IBA</td>
<td>10</td>
<td>2.1 ± 0.2</td>
<td>1.12 ± 0.2</td>
</tr>
<tr>
<td>9.8 IBA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.6 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.4 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.8 NAA</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 26: Effects of different concentrations of BA (in MS medium) on axillary shoot growth from different nodal segments of *P. pinnata* seedlings, after 4 weeks.

<table>
<thead>
<tr>
<th>BA (μM)</th>
<th>Bud break (%)</th>
<th>Shoot number per explant</th>
<th>Shoot length (cm)</th>
<th>Rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledonary Node:</td>
<td>0.0</td>
<td>80</td>
<td>1.0 ± 0</td>
<td>3.02 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>100</td>
<td>1.0 ± 0</td>
<td>6.34 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>80</td>
<td>1.0 ± 0</td>
<td>4.41 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>35.6</td>
<td>70</td>
<td>1.0 ± 0</td>
<td>1.63 ± 0.1</td>
</tr>
<tr>
<td>Upper Node:</td>
<td>0.0</td>
<td>70</td>
<td>1.0 ± 0</td>
<td>0.95 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>90</td>
<td>1.0 ± 0</td>
<td>0.98 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>8.9</td>
<td>90</td>
<td>1.0 ± 0</td>
<td>1.34 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>35.6</td>
<td>80</td>
<td>1.0 ± 0</td>
<td>0.25 ± 0.01</td>
</tr>
</tbody>
</table>

ANOVA: Shoot length : BA: df = 3; F = 352.63; P < 0.0001
Explant: df = 1; F = 2335.05; P < 0.0001
BA X Explant : df = 3; F = 199.36; P < 0.0001

Bud break % : BA: df = 3; F = 6.28; P = 0.0051
Explant: df = 1; F = 0; P = 1
BA X Explant : df = 3; F = 2.85; P = 0.1178

Rooting % : BA: df = 3; F = 150; P < 0.0001
Medium: df = 1; F = 0; P = 1
BA X Explant : df = 3; F = 0; P = 1
Table 27: Effects of different concentration of BA on shoot elongation from micronode derived from seedling explants of *P. pinnata*, observation recorded after 4 weeks.

<table>
<thead>
<tr>
<th>BA (μM)</th>
<th>Shoot number per micronode Mean ± SE</th>
<th>Shoot length (cm) Mean ± SE</th>
<th>Nodes per micronode Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1.0 ± 0</td>
<td>0.92 b</td>
<td>1.0 b</td>
</tr>
<tr>
<td>2.2</td>
<td>1.0 ± 0</td>
<td>0.95 b</td>
<td>1.0 b</td>
</tr>
<tr>
<td>8.9</td>
<td>1.0 ± 0</td>
<td>1.21 a</td>
<td>1.8 a</td>
</tr>
<tr>
<td>35.6</td>
<td>1.0 ± 0</td>
<td>0.30 c</td>
<td>1.0 b</td>
</tr>
</tbody>
</table>

Each treatment consisted of 10 replicates and each experiment was repeated three times.

Same letters in each column followed by means do not differ significantly, at 5% level (by DMR test)

LSD at 0.05 for shoot length = 0.20
Nodes per micronode = 0.22
Table 28: Rooting response of *P. pinnata* seedling explant-derived microshoots to different concentrations of auxins in half-MS medium, after 4 weeks.

<table>
<thead>
<tr>
<th>Auxin (µM)</th>
<th>Rooting (%)</th>
<th>Root no. per shoot Mean ± SE</th>
<th>Root length (cm) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>50 b</td>
<td>1.2 c</td>
<td>2.03 c</td>
</tr>
<tr>
<td>IAA 0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.85</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.27</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>IBA 0.49</td>
<td>50 b</td>
<td>1.6 b</td>
<td>2.41 b</td>
</tr>
<tr>
<td>2.46</td>
<td>90 a</td>
<td>2.0 a</td>
<td>2.73 a</td>
</tr>
<tr>
<td>12.26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NAA 0.54</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.70</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Each treatment consisted of 10 replicates and each experiment was repeated three times.

Same letters in each column followed by means do not differ significantly, at 5% level (by DMR test)

- LSD at 0.05 for rooting % = 19.98
- Root number = 0.28
- Root length = 0.12

ANOVA: Rooting %: IBA : df = 2; F = 16; P = 0.0039
- Root no: IBA : df = 2; F = 14.57; P < 0.0001
- Root leng: IBA : df = 2; F = 77.92; P < 0.0001
Figure 1. Effect of seasonal variation on axillary shoot outgrowth from nodal segments of C. collinus seedlings, after 4 weeks.

Same letters followed by means do not differ significantly, at 5% level (by DMR test).

LSD at .05 for contamination % = 12.15; Bud break % = 16.30; Shoot no. = 0.23; Shoot length = 0.26

ANOVA: Season:
- Contamination % : df = 3; f = 79.73; p < 0.0001
- Bud break % : df = 3; f = 63.33; p < 0.0001
- Shoot no. : df = 3; f = 6; p = 0.0020
- Shoot length : df = 3; f = 61.41; p < 0.0001
Figure 2. Effect of seasonal variation on axillary shoot outgrowth from nodal segments of *C. collinus* adult tree, after 4 weeks.

Same letters followed by means do not differ significantly, at 5% level (by DMR test).

LSD at .05 for contamination % = 8.4;
Bud break % = 6.9

ANOVA: Season:
Contamination % : df = 11; f = 246.5; p < 0.0001
Bud break % : df = 11; f = 394.2; p < 0.0001
Figure 3. Effect of seasonal variation on axillary shoot outgrowth from nodal segments of *L. parviflora* seedlings, after 4 weeks.

Same letters followed by means do not differ significantly, at 5% level (by DMR test).
LSD at .05 for contamination % = 4.86;
Bud break % = 6.88

ANOVA: Season:
Contamination % : df = 11; f = 890.2; p < 0.0001
Bud break % : df = 11; f = 390.5; p < 0.0001
Figure 4. Effect of seasonal variation on axillary shoot outgrowth from nodal segments of *L. parviflora* adult tree, after 4 weeks.

Same letters followed by means do not differ significantly, at 5% level (by DMR test).

LSD at .05 for contamination % = 6.88;
Bud break % = 6.88

ANOVA: Season:
Contamination % : df = 11; f = 321.8; p < 0.0001
Bud break % : df = 11; f = 277.6; p < 0.0001
Figure 5: Effects of subcultures on shoot elongation from micronode derived from P. pinnata seedlings, on MS + 8.9 μM BA, observations were taken after every 4 weeks.

Same letters followed by means do not differ significantly, at 5% level (by DMR test).

LSD at .05 for shoot length = 0.1
Nodes per culture = 0.3