In the present study, efforts were made to develop micropropagation protocols for efficient and rapid multiplication of *Tinospora cordifolia* and *Buddleja madagascariensis*.

### Tinospora Cordifolia

In order to select the most suitable medium for shoot formation, cultures were raised on MS and WP basal Medium as well as with different concentrations of growth regulators.

#### 4.1. MS basal medium:

In *T. cordifolia*, none of the tried explants (shoot tip, internode, node, leaf and petiole) showed any response on MS basal medium.

#### 4.2. WP basal medium:

In contrast to MS basal medium, single shoot appeared in nodal as well as shoot tip explants on WP basal medium. However, internode, leaf and petiolar explants did not show any response. In case of nodal explants, buds were initiated in 23.3 % cultures after 21 days of inoculation (Table 5). In shoot tip explants response was 20 %, buds were initiated after 25 days of inoculation (Table 8). In nodal as well as shoot tip explants, single shoot appeared on each explant with average shoot length of 1.2 ± 0.05 and 0.5 ± 0.03 cm, respectively.

To study the effect of various growth regulators on shoot induction, callus formation and plant regeneration, the explants were inoculated on media containing various growth regulators individually and in different combinations. Effects of these treatments are being described in the following account:

#### 4.3. Cytokinins:

Effects of BAP and Kinetin were studied on various explants.
4.3.1. 6-Benzylaminopurine (BAP):

To study the effect of different concentrations of BAP on regeneration potential of different explants viz, shoot tips, internodes, nodes, leaf and petioles; these were inoculated on MS as well as WP medium supplemented with 0.5, 1.0, 2.0 and 4.0 mg/l BAP.

4.3.1.1. MS Medium supplemented with BAP:

In nodal explants, bud initiated after 19 days of inoculation at 0.5 mg/l BAP with 50 % response which resulted in the formation of 2.5 ± 0.13 shoots with shoot length of 1.3 ± 0.05 cm (Table 4, Plate 3.A). As the concentration of BAP increased, the percent response also increased. The explants raised on 1 and 2 mg/l BAP produced 3.2 ± 0.16 and 3.8 ± 0.16 shoots on nodal region after 18 days of inoculation with 56.7 and 60 % response, respectively. The best results in terms of time taken for bud initiation, percent response, number of shoots and shoot length were observed at 2 mg/l BAP (Plate 3.C, D). But, at 4 mg/l BAP the response decreased and 2.4 ± 0.12 buds appeared after 20 days. At this level shoot length also decreased (Table 4).

In case of shoot tip explants, at 0.5 mg/l BAP percent response was 43.3 % having 2.3 ± 0.13 shoots initiated after 22 days. But bud initiation increased (50 and 53.3 %) and buds appeared after 21 and 20 days of inoculation on 1 and 2 mg/l BAP, respectively (Table 7). Shoot number also increased (3.0 ± 0.15 and 3.5 ± 0.15) at these concentrations (Plate 8.A, B). However, response decreased at 4 mg/l BAP (40 %). At this level average shoot number was 1.8 ± 0.10 and buds appeared after 22 days. Best response in terms of time taken for bud initiation, percent response, number of shoots and shoot length was observed on 2 mg/l BAP (Plate 8.B).

Internode, leaf and petiolar explants failed to produce bud or callus at all the concentrations of BAP.

4.3.1.2. WP Medium supplemented with BAP:

In nodal explants, at 0.5 mg/l BAP percent response was 56.7 and 4.5 ± 0.23 shoots appeared from each explant after 16 days (Table 5, Plate 5.A). However, shoot number was maximum at 2 mg/l BAP (5.6 ± 0.23) (Plate 5.C, D). At this concentration,
bud was initiated after 15 days of inoculation with 70 % response. At higher level (i.e. 4 mg/l BAP) bud initiation was delayed and bud appeared after 18 days. Further, the percent response (53.3 %), shoot number (3.6 ± 0.15) and shoot length (2.1 ± 0.05 cm) decreased at this level.

Shoot tip explants at 0.5 mg/l BAP produced 3.6 ± 0.16 shoots with 46.7 % response after 20 days of inoculation and the response increased with increasing concentration of BAP (Table 8). The best results were observed at 2 mg/l BAP (Plate 9.B). At this concentration, shoot tips showed 60 % response within 19 days of inoculation with maximum average number of shoots (4.8 ± 0.23) and shoot length (3.1 ± 0.15 cm) per explant (Table 8).

Internode, leaf and petiolar explants failed to produce any bud or callus at any of the concentrations of BAP tried.

4.3.2. 6-Furfurylaminopurine (KIN):

To study the effect of KIN on shoot induction, different explants viz, shoot tips, internodes, nodes, leaf and petioles were inoculated on KIN enriched MS and WP medium. Four different concentrations of KIN i.e. 0.5, 1.0, 2.0 and 4.0 mg/l were tried. Cultures raised on basal medium without any growth regulators served as control.

4.3.2.1. MS Medium supplemented with KIN:

Nodal explants on MS medium showed the formation of 1.2 ± 0.07 shoots (having shoot length 2.4 ± 0.12 cm) at 0.5 mg/l KIN after 20 days of inoculation with 43.3 % response (Table 4, Plate 4.A). Response increased with increase in concentration and the best results were obtained at 2 mg/l KIN, where nodal explants gave 53.3 % response producing 2.1 ± 0.10 shoots (shoot length 3.0 ± 0.12 cm) after 19 days of inoculation (Plate 4.C, D & E). Response decreased at 4 mg/l KIN (40 %) and only single shoot appeared at this concentration.

Of the four concentrations of KIN tried on shoot tip explants on MS medium, 2 mg/l KIN proved most effective (Table 7, Plate 8.D). It induced 1.7 ± 0.10 shoots after 20 days of inoculation with 50 % response. At this level average shoot length was 2.4 ± 0.12 cm. Single shoot with shoot length 1.7 ± 0.07 cm appeared at 0.5 mg/l KIN after 23
days of inoculation with 40 % response. The response increased at 1 mg/l KIN to 46.7 % with 1.2 ± 0.05 shoots with average shoot length 2.0 ± 0.10 cm (Plate 8.C). The bud response, shoot number and shoot length decreased at 4 mg/l KIN and single shoot appeared at this level.

Internode, leaf and petiolar explants failed to produce any bud or callus at any of the concentrations of KIN.

4.3.2.2. WP Medium supplemented with KIN:

In nodal explants, 2 mg/l KIN was optimum which resulted in the formation of 3.1 ± 0.15 shoots (Plate 6.D, E & F). At this level average shoot length was 5.3 ± 0.21 cm (Table 5). However, on medium supplemented with lower concentrations (0.5 and 1 mg/l) of KIN, 2.2 ± 0.13 and 2.7 ± 0.13 shoots appeared per explant, respectively (Plate 6.A, B & C). At 2 mg/l KIN, bud initiated after 16 days of inoculation with 60 % response. While at 0.5 and 1 mg/l KIN, shoot-bud formed at each nodal explant after 17 days of inoculation with 50 and 56.7 % response, respectively. At 4 mg/l KIN, response decreased (50 %) and buds appeared after 18 days with 1.8 ± 0.09 shoots.

In case of shoot tip explants, buds were initiated in 43.3 and 50 % cultures raised on 0.5 and 1 mg/l KIN after 20 and 19 days of inoculation, respectively (Table 8, Plate 9.C). The best response (53.3 %) was observed at 2 mg/l KIN. At this concentration, 3.1 ± 0.15 shoots with average shoot length 4.2 ± 0.16 cm were initiated after 20 days of inoculation (Plate 9.D). Bud initiation was delayed at higher concentration of KIN (4 mg/l) as bud appeared after 21 days of inoculation. Further, percent response (40 %), average shoot number (1.5 ± 0.09) and shoot length (2.6 ± 0.12 cm) also decreased at this concentration.

Internode, leaf and petiolar explants failed to produce any bud or callus at any of the concentrations of KIN.

From above observations it was clear that explants cultured on MS medium took longer time for bud initiation as compared to WPM. Also, the percent response was less than that of WPM. WPM also proved better in terms of time of bud initiation, number
of shoots, and shoot length. The results suggested that WPM is better as a basal medium for micropropagation of *Tinospora cordifolia*.

Of the two cytokinins tried, BAP was more effective than KIN for percent response of explants, time taken for shoot-bud induction and number of shoots on both media. However, in case of length of shoots KIN was better than BAP.

Further, nodal explants were best for direct shoot regeneration in *T. cordifolia*. Of the two explants (i.e. shoot tips and nodes), nodal explants gave better response in terms of time taken for shoot-bud induction, mean number of shoots and shoot length.

4.3.3. WP Medium supplemented with combination of BAP and KIN:

The effect of combination of BAP and KIN was studied on nodal and shoot tip explants on WPM as it was better than MS medium (Table 6 & 9, Plate 7). As, BAP as well as KIN elicited maximum response at 2 mg/l, therefore, in one set BAP (2.0 mg/l) was combined with varying concentrations of KIN (0.5, 1.0, 2.0 and 4.0 mg/l). In another set KIN at 2.0 mg/l was combined with different concentrations of BAP (0.5, 1.0, 2.0 and 4.0 mg/l). In both the types of explants, the combination increased the percent response and reduced the time taken for bud initiation. Of all the combinations tried, the combination with 2 mg/l BAP + 1 mg/l KIN was optimum in both the explants. At this combination nodal and shoot tip explants gave 80 and 70 % response within 12 and 15 days of inoculation, respectively. In nodal explants maximum shoots number at this combination was 5.4 ± 0.28 (Plate 7.C), whereas in shoot tip explant cultures, maximum shoot number was 4.5 ± 0.22.

4.4. Effect of Activated Charcoal (AC):

In *T. cordifolia*, the leaching of phenolic compounds from explants in media was observed in all the cultures which resulted in browning of the media as well as explants, rendering it unsuitable for proliferation. The frequent subculturating of explants after every 20 days was effective in reducing the problem, but increased the chances of infection. Addition of AC in the media prevented browning of medium and explants and resulted in regeneration. In order to select the most suitable concentration of AC for shoot regeneration, different concentrations *viz.* 1, 2, 3, 4 and 5 g/l of AC were tried in
both MS and WPM (Plate 10-14). The percent response of explants increased as the concentrations of AC increased from 1 to 5 g/l (Fig. 1-4). Among all the concentrations tried, 5 g/l AC was optimum for percent response which resulted in 73.3 % (+ 2 mg/l BAP on MS), 80 % (+ 2 mg/l BAP on WPM), 66.7 % (+ 2 mg/l KIN on MS), 73.3 % (+ 2 mg/l KIN on WPM) and 96.7 % (+ 2 mg/l BAP + 1 mg/l KIN on WPM) response of nodal explants.

4.5. Auxins:

Effects of three auxins (IAA, NAA and 2,4-D) were studied on shoot tip, internode, node, leaf and petiolar explants.

4.5.1. Indole-3-acetic acid (IAA):

To study the effect of IAA on callus induction from different explants, these were inoculated on IAA enriched MS and WP medium. Four different concentrations of IAA i.e. 0.5, 1.0, 2.0 and 4.0 mg/l were tried. Cultures raised on basal medium (without any growth regulator) served as control.

4.5.1.1. MS Medium supplemented with IAA:

In control cultures, neither callus nor shoots were induced on any of the explants tried. All the five types of explants produced callus at all the four concentrations of IAA on MS medium. Callus formation started at 0.5 mg/l and increased with increase in concentration of IAA from 0.5 to 4 mg/l. The maximum callus production was observed at 4 mg/l concentration in all the five types of explants (Fig. 5, Table 10).

In shoot tip explants on IAA supplemented medium percent response increased with increase in concentration. At 4 mg/l IAA percent response was 33.3 and callus was initiated after 25 days of inoculation with the formation of brown and soft callus. At this concentration, the fresh and dry weights of callus were 0.42 ± 0.03 and 0.06 ± 0.008 g, respectively.

Internodal explants also callused and callusing was higher at higher levels (2 & 4 mg/l). At 2.0 mg/l IAA 30 % explants showed callusing after 27 days whereas at 4.0 mg/l IAA percent response was 40 and callusing occurred after 25 days. At 4 mg/l of
IAA callusing was maximum and callus was brown and compact. At this concentration, the fresh and dry weights of callus were 0.48 ± 0.04 and 0.10 ± 0.01 g, respectively (Fig. 5 & Table 10). In internodes callus was initiated from both the sides of cut portion.

In nodal segments, callus was produced at the cut end of axillary bud and then spread throughout the portion. At 0.5 mg/l IAA percent response was 10 and at 4 mg/l it was 40 % (Fig. 5A). At 4 mg/l IAA, explants produced brown and compact callus within 23 days of inoculation whereas at 0.5 mg/l callusing occurred after 29 days. The fresh and dry weights of callus at this concentration (4 mg/l IAA) were 0.50 ± 0.06 and 0.10 ± 0.01 g, respectively.

In leaf explants callus initiation was earlier in comparison to shoot tip, internodal and nodal segments. Also, amount of callus produced on leaf segments was more than produced on shoot tip, internodal and nodal segments. Degree of callusing increased and time taken for callus initiation decreased with increase in concentration from 0.5 mg/l to 4.0 mg/l IAA. Leaf explant exhibited up to 46.7 % callus induction with the formation of white-brown and compact callus on 4 mg/l of IAA within 23 days of inoculation (Fig. 5). The fresh and dry weights of callus at this concentration were 1.32 ± 0.07 and 0.26 ± 0.04 g, respectively (Table 10). In case of leaf, the callus was initiated near the cut ends.

Petiolar segments were less responsive for callus initiation as compared to internode, node and leaf segments. At 0.5 mg/l IAA 10 % explants showed callusing after 30 days and with increase in concentration percent response increased and time taken for callusing was reduced (Fig. 5). At 4 mg/l IAA, 33.3 % explants produced brown and compact callus after 25 days of inoculation. At this concentration, the fresh and dry weights of callus were 0.45 ± 0.05 and 0.08 ± 0.007 g, respectively (Table 10). The callus was initiated from both the sides of cut portion and then spread throughout the length.

**4.5.1.2. WP Medium supplemented with IAA:**

WPM without IAA failed to induce callus from all the five types of explants tried. All explants produced callus at all the four concentrations of IAA (0.5, 1.0, 2.0 and 4.0 mg/l) on WP medium. The results of callus production are given in Fig. 6 and
The percent response and amount of callus varied with the concentration of IAA. Callus formation increased with increase in the concentrations from 0.5 to 4 mg/l. The maximum callus was produced at 4 mg/l IAA in all the five types of explants. Therefore, 4 mg/l concentration of IAA was optimum for all the explants tried.

In shoot tip explants, maximum callus induced on 4 mg/l IAA where 43.3 % explants showed callus induction after 20 days of inoculation. At this concentration, the fresh and dry weights of callus were 0.62 ± 0.05 and 0.12 ± 0.01 g, respectively. The callus was light-brown and soft (Plate 15.A).

In internodal segment percent response was 50 % and callus was induced within 20 days of inoculation on 4 mg/l of IAA. At this concentration callus was green and compact (Plate 16.A) and the fresh and dry weights of callus were 0.67 ± 0.06 and 0.14 ± 0.01 g, respectively. In internodes callus was initiated from both the sides of cut portion.

In nodal explants callus initiation was earlier in comparison to shoot tips and internodal segments. At 4 mg/l IAA, 50 % explants produced green and compact callus within 18 days of inoculation (Fig. 6, Plate 17.A). The fresh and dry weights of callus at this concentration were 0.70 ± 0.06 and 0.15 ± 0.02 g, respectively (Table 11).

Leaf explants produced maximum amount of callus in comparison to shoot tip, internodal and nodal segments. Sixty percent leaf explant exhibited callus induction with the formation of light-green and compact callus on 4 mg/l of IAA within 18 days of inoculation (Plate 18.A). The fresh and dry weights of callus at this concentration were 1.70 ± 0.08 and 0.32 ± 0.03 g, respectively. In case of leaf, the callus was initiated near the cut ends.

In petiolar segments, 50 % explants produced white-brown and compact callus at 4 mg/l IAA after 20 days of inoculation (Fig. 6, Plate 22.A). At this concentration, the fresh and dry weights of callus were 0.65 ± 0.05 and 0.12 ± 0.01 g, respectively (Table 11). The callus was initiated from both the sides of cut portion and then spread throughout the length.
4.5.2. α-Naphthalene acetic acid (NAA):

Different explants (shoot tips, internode, node, leaf and petioles) were inoculated on the different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of NAA fortified MS and WPM to study their potential for callus formation. Cultures raised on MS and WP basal medium without NAA served as control.

4.5.2.1. MS Medium supplemented with NAA:

No callus was observed in any of the explants on MS basal medium without NAA. All explants produced callus at all the four concentrations of NAA on MS medium. Callus formation increased with increase in the concentrations from 0.5 to 4 mg/l. In all the explants, maximum callus was produced at 4 mg/l NAA.

Shoot tip explants were least responsive for callus induction where only 46.7% explants induced callus at 4 mg/l NAA after 22 days of inoculation (Fig. 7). At this concentration, the fresh and dry weights of callus were 1.14 ± 0.05 and 0.20 ± 0.02 g, respectively (Table 10). The callus was brown and soft.

Internodal segment showed 50% callus induction within 20 days of inoculation on 4 mg/l of NAA with the formation of white-brown and compact callus (Fig. 7). At this concentration, the fresh and dry weights of callus were 1.25 ± 0.08 and 0.22 ± 0.03 g, respectively (Table 10). In internodes callus was initiated from both the sides of cut portion i.e. in contact with the medium and then spread throughout the length.

In nodal explants, maximum callus was induced at 4 mg/l NAA where 60% explants produced brownish-green and compact callus within 20 days of inoculation (Fig. 7). The fresh and dry weights of callus at this concentration were 1.30 ± 0.08 and 0.22 ± 0.04 g, respectively (Table 10). Callus was initiated at the cut end of axillary bud and then spread throughout the portion.

Leaf explants produced maximum amount of callus at 4 mg/l NAA. At this concentration, explants exhibited up to 60% callus induction with the formation of whitish-brown and compact callus within 19 days of inoculation (Fig. 7). The fresh and dry weights of callus at this concentration were 2.01 ± 0.07 and 0.35 ± 0.04 g, respectively (Table 10). In case of leaf, the callus was initiated near the cut ends.
In petiolar segments, callus was induced at all the levels and degree of callusing increased with increase in concentration of NAA. Fifty percent explants produced white and compact callus at 4 mg/l NAA after 21 days of inoculation (Fig. 7). At this concentration, the fresh and dry weights of callus were 1.18 ± 0.08 and 0.20 ± 0.02 g, respectively (Table 10). The callus was initiated from both the sides of cut portion and then spread throughout the length.

4.5.2.2. WP Medium supplemented with NAA:

In control cultures, neither callus nor shoot regeneration was observed in any of the explant. All types of explants produced callus at all the four concentrations of NAA on WP medium. The results are summarised in Fig. 8 and Table 11. Callus formation started at 0.5 mg/l and increased with increase in the concentration of NAA up to 4 mg/l. The maximum callus was produced at 4 mg/l concentration in all the five types of explants.

Shoot tip explants exhibited 63.3 percent response after 19 days of inoculation on 4 mg/l NAA with the formation of light-brown and friable callus (Fig. 8, Plate 15.B). At this concentration, the fresh and dry weights of callus were 1.90 ± 0.07 and 0.36 ± 0.04 g, respectively (Table 11).

Seventy percent internodal segment showed callus induction within 15 days of inoculation on 4 mg/l of NAA with the formation of green and compact callus (Fig. 8, Plate 16.B). At this concentration, the fresh and dry weights of callus were 2.01 ± 0.07 and 0.40 ± 0.03 g, respectively (Table 11). In internodes callus was initiated from both the sides of cut portion and then spread throughout the length.

In nodal explants at 4 mg/l NAA, 70 % explants produced green and compact callus within 15 days of inoculation (Fig. 8, Plate 17.B). The fresh and dry weights of callus at this concentration were 2.10 ± 0.08 and 0.40 ± 0.03 g, respectively (Table 11).

In leaf explants callus initiation was earlier in comparison to shoot tip, internodal and nodal segments. Further, amount of callus produced on leaf segments was more than produced on shoot tip, internodal and nodal segments. In leaf explant percent response was 76.7 with the formation of greenish-brown and compact callus on
4 mg/l of NAA within 15 days of inoculation (Plate 19.B). The fresh and dry weights of callus at this concentration were 2.86 ± 0.08 and 0.50 ± 0.04 g, respectively. In case of leaf also, the callus was initiated near the cut ends.

At 4 mg/l NAA, 66.7 % petiolar explants produced whitish-green and compact callus after 18 days of inoculation (Fig. 8, Plate 22.B). At this concentration, the fresh and dry weights of callus were 1.95 ± 0.08 and 0.38 ± 0.03 g, respectively (Table 11). The callus was initiated from both the sides of cut portion and then spread throughout the length.

**4.5.3. 2,4-Dichlorophenoxyacetic acid (2,4-D):**

To study the effect of different concentrations of 2,4-D on callus induction in different explants viz. shoot tips, internode, node, leaf and petiole segments, these explants were inoculated on MS and WP medium supplemented with 0.5, 1.0, 2.0 and 4.0 mg/l 2,4-D. Cultures raised on MS and WP basal medium without any growth regulator served as control.

**4.5.3.1. MS Medium supplemented with 2,4-D:**

MS medium without 2,4-D failed to induced callus in any of the explants tried. Explants produced callus at all the four concentrations of 2,4-D. The results of callus production are given in Fig. 9 and Table 10. The percent response and amount of callus varied with the concentrations of 2,4-D. Callus formation increased with increasing the concentrations from 0.5 to 4 mg/l. The maximum callus was produced at 4 mg/l 2,4-D in all the five types of explants. Therefore, 4 mg/l concentration of 2,4-D was optimum for all the explants tried.

Shoot tip explants were least responsive for callus induction where only 56.7 % explants induced callus at 4 mg/l 2,4-D after 20 days of inoculation (Fig. 9). At this concentration, the fresh and dry weights of callus were 1.55 ± 0.07 and 0.25 ± 0.03 g, respectively (Table 10). The callus was white-brown and soft.

On medium with 4 mg/l of 2,4-D; 60 % internodal segment showed callus induction within 18 days of inoculation with the formation of brownish-green and compact callus (Fig. 9). At this concentration, the fresh and dry weights of callus were
1.67 ± 0.07 and 0.28 ± 0.04 g, respectively (Table 10). In internodes callus was initiated from both the sides of cut portion and then spread throughout the length.

In nodal explants, maximum callus was induced at 4 mg/l 2,4-D where 60 % explants produced brownish-green and compact callus within 16 days of inoculation (Fig. 9). The fresh and dry weights of callus at this concentration were 1.71 ± 0.07 and 0.30 ± 0.03 g, respectively (Table 10). In the nodal segments, callus was produced at the cut end of axillary bud and then spread throughout the portion.

Leaf explants produced maximum amount of callus in young leaf segments at 4 mg/l 2,4-D. At this concentration, 63.3 % explants exhibited callus induction with the formation of light-brown and compact callus within 15 days of inoculation (Fig. 9). The fresh and dry weights of callus at this concentration were 2.46 ± 0.08 and 0.43 ± 0.04 g, respectively (Table 10). The callus was initiated near the cut ends and then spread all over the explant.

In petiolar segments, 56.7 % explants produced white and compact callus at 4 mg/l 2,4-D after 18 days of inoculation (Fig. 9). At this concentration, the fresh and dry weights of callus were 1.60 ± 0.07 and 0.25 ± 0.02 g, respectively (Table 10). The callus was initiated from both the sides of cut portion and then spread throughout the length.

4.5.3.2. WP Medium supplemented with 2,4-D:

No callus was induced from any of the explants in control cultures. On 2,4-D supplemented WP medium explants produced callus at all the four concentrations (0.5, 1.0, 2.0 and 4.0 mg/l). The effects of 2,4-D on callus production are summarised in Fig. 10 and Table 11. Callus formation increased with increase in the concentration from 0.5 to 4 mg/l. The maximum callus was produced at 4 mg/l 2,4-D in all the five types of explants.

In shoot tip explants callus was induced in 66.7 % explants at 4 mg/l 2,4-D after 16 days of inoculation (Fig. 10). At this concentration, the fresh and dry weights of callus were 2.38 ± 0.08 and 0.45 ± 0.03 g, respectively (Table 11). The callus was greenish-brown and compact (Plate 15.C).
Internodal segments were more responsive in callus induction than shoot tips where 70% explants induced callus within 15 days of inoculation on 4 mg/l of 2,4-D with the formation of green and compact callus (Fig. 10, Plate 16.C). At this concentration, the fresh and dry weights of callus were 2.74 ± 0.08 and 0.50 ± 0.04 g, respectively (Table 11). In internodes callus was initiated from both the sides of cut portion and then spread throughout the length.

In nodal explants, maximum callus was induced at 4 mg/l 2,4-D where 80% explants produced green and compact callus within 12 days of inoculation (Fig. 10, Plate 17.C). The fresh and dry weights of callus at this concentration were 2.90 ± 0.08 and 0.56 ± 0.05 g, respectively (Table 11). In the nodal segments, callus was produced at the cut end and then spread throughout the portion.

In leaf explants callus initiation was earlier in comparison to shoot tip, internodal and nodal segments. Further, amount of callus produced on leaf segments was more than produced on shoot tip, internodal and nodal segments. Leaf explants exhibited up to 86.7% callus with the formation of whitish-brown and compact callus within 12 days of inoculation (Fig. 10, Plate 20.B). The fresh and dry weights of callus at this concentration were 3.75 ± 0.10 and 0.70 ± 0.05 g, respectively (Table 11). In case of leaf, the callus was initiated near the cut ends and then spread all over the explant.

In petiolar segments, 66.7% explants produced greenish-brown and compact callus at 4 mg/l 2,4-D after 16 days of inoculation (Fig. 10, Plate 22.C). At this concentration, the fresh and dry weights of callus were 2.44 ± 0.08 and 0.47 ± 0.04 g, respectively (Table 11). The callus was initiated from both the sides of cut portion and then extended throughout the length.

Above observations suggested that WPM was superior over MS medium for callus induction and proliferation. Further, all the five types of explants exhibited best response in terms of callus induction at 4 mg/l of IAA, NAA and 2,4-D. Moreover, among the auxins tried, 2,4-D was more effective than IAA and NAA for callus induction. Further, among all the five types of explants tried, leaf segments exhibited best response in terms of callus induction.
4.6. Callus growth:

The growth of callus was measured in terms of fresh and dry weights of callus formed by shoot tips, internode, node, leaf and petiolar explants on different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of IAA, NAA and 2,4-D are summarized in Table 10 and 11. Observations clearly show that the mean fresh and dry weight of callus increased as the concentration of IAA, NAA and 2,4-D increased from 0.5 to 4 mg/l. The maximum fresh and dry weights (3.75 ± 0.10 and 0.70 ± 0.05 g) were observed in callus induced by leaf explants cultured on WPM with 4 mg/l 2,4-D.

4.7. Shoot regeneration through callus:

The callus so obtained were divided into small pieces and cultured on WPM supplemented with different concentrations of BAP and KIN (0.5, 1.0, 2.0 and 4.0 mg/l) individually for shoot formation. The results are summarised in Table 12 and 13, it is clear that callus induced from nodal explants was most responsive for shoot formation as all the four concentrations of BAP as well as KIN induced shoots. The response increased with increase in the concentration from 0.5 to 2 mg/l BAP and KIN. The concentration of 2 mg/l BAP as well as KIN was optimum for callus organogenesis. BAP at this level resulted in 70 % response with 3.6 ± 0.16 shoots per culture (Plate 23) whereas KIN resulted in 60 % response with 3.0 ± 0.13 shoots per culture (Plate 24). Shoot regeneration delayed at higher concentration (4 mg/l) of both the growth regulator. Further of the two growth regulator tried, BAP was found to be more effective than KIN for shoot regeneration. But in contrast to BAP, the shoot length was better on KIN supplemented medium. There was no appreciable effect of addition of AC on shoot formation on calli was observed.

4.8. Rooting of in vitro developed shoots:

In vitro developed shoots on different media were excised when attained a height of 2-3 cm and inoculated on half strength MS and WPM without and with auxins (IAA IBA, NAA and 2,4-D) for rooting in different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l). The results are summarised in Tables 14 and 15. No rooting was observed on half-strength MS and WP basal medium without auxin. IAA induced rooting at 0.5-2.0 mg/l and 0.5 mg/l induced maximum roots. Higher concentration of NAA and 2,4-D
inhibited rooting and callusing was observed at the base of shoots. IBA was most effective in rooting as it induced healthy and elongated roots on MS and WPM. Among the four concentrations of IBA tried, 0.5 mg/l was optimum for rooting which resulted in to 70 % and 46.7 % response with 3.0 ± 0.13 and 2.1 ± 0.11 roots initiated in WPM (Plate 27.B) and MS medium (Plate 27.A), respectively. The response decreased as the concentrations of IBA increased from 0.5 to 4.0 mg/l on both the media. But on MS medium at 4.0 mg/l IBA callusing was observed at the base of shoot. Further, WPM found to be superior over MS medium for rooting also.

4.9. Effect of AC on rooting:

To study the effect of AC on rooting, the different concentrations (1, 2, 3, 4 and 5 g/l) of AC were tried in WPM. WPM supplemented with 0.5 mg/l IBA was served as control. The percent root induction and root length increased as the concentrations of AC increased from 1 to 5 g/l as compared to control culture (Table 16). Among all the concentrations tried, 5 g/l AC was optimum at which percent response was 80 with root length of 5.3 ± 0.16 cm (Plate 28).

4.10. Hardening and transfer of plantlets to the field:

Shoots with well-developed roots were gently pulled out of the medium and washed gently in running tap water to remove the medium sticking to the roots. These shoots were then transferred to plastic cups having sterile soil and sand mixture (1:1) and irrigated with ¼ strength WP salt solution (Plate 29.A-D). High humidity was maintained for initial 15 days with the help of polythene bags and thereafter, these pots were exposed to natural conditions for 3-4 hrs daily. After about a month the plants were shifted to pots in Polyhouse where they grew normally with 70 % survival rate (Plate 29.E). After one month these plants were transferred to the field from Polyhouse. The survived plants grew normally.
**Tinospora cordifolia**

Nodal explants

- **WPM + 5 g/l AC + 2 mg/l BAP**
  - Shoot bud initiation (15 days)
  - Sub-culturing
    - Shoot multiplication medium (after 25 days)
    - **WPM + 5 g/l AC + 2 mg/l BAP**
  - Multiple shoots (5.6 shoots)

- **WPM + 4 mg/l 2,4-D**
  - Callus initiation
    - (Green, compact callus) (12 days)
    - **WPM + 5 mg/l AC + 2 mg/l BAP**
  - Multiple shoot development from callus (3.6 shoots) (21 days)

- **WPM + 5 g/l AC + 0.5 mg/l IBA**
  - Rooting (21 days)
  - Plastic cups (Soil : Sand 1:1) 30 days
  - Pots in Polyhouse 30 days
  - Hardened plants transfer to field 30 days

**Flow Chart: 1. Protocol for micropropagation of Tinospora cordifolia.**
**BUDDLEGA MADAGASCARIENSIS**

To develop a protocol for rapid multiplication of *B. madagascariensis*; shoot tips, internodal, nodal, leaf segments and petiolar explants were cultured on MS medium supplemented with different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of growth regulators such as BAP, KIN, IAA, NAA and 2,4-D individually and in different combinations. In the present study shoot regeneration was achieved both directly from apical and axillary buds as well as indirectly through the callus induction and regeneration. The results are being summarized under the following headings:

4.11. Direct shoot regeneration
4.12. Indirect shoot regeneration
4.13. Rooting of *in vitro* regenerated shoots
4.14. Hardening and transfer of plantlets to field

**4.11. Direct shoot regeneration:**

**4.11.1. MS basal medium:**

Among the explants used, internode, leaf and petiolar explants did not show any response on MS basal medium. Nodal explant showed bud initiation after 19 days of inoculation and percent response was 41.7 %, while 33.3 % shoot tip explants showed response and buds were initiated after 20 days of inoculation. Single shoot appeared on both nodal and shoot tip explants with average shoot length of 1.5 ± 0.05 and 1.3 ± 0.06 cm, respectively (Table 17 & 19, Plate 30.A).

**4.11.2. 6-Benzylaminopurine (BAP):**

To study the effect of BAP on shoot induction from different explants *viz.*, shoot tips, internodes, nodes, leaf and petioles, these were inoculated on BAP enriched MS medium. Four different concentrations 0.5, 1.0, 2.0 and 4.0 mg/l of BAP were tried. Cultures raised on basal medium without BAP served as control.

In case of nodal explants, at 0.5 mg/l BAP response was 83.3 % having 7.7 ± 0.43 shoots initiated after 10 days (Plate 31.A). Percent response increased further at 1 mg/l BAP and buds were initiated after 8 days of inoculation (Table 17). Shoot number also increased (9.3 ± 0.41) at this concentration (Plate 31.B & C). However, further
increase in BAP concentration (2 and 4 mg/l) percent response as well as shoot number and shoot length decreased (Plate 31.D).

In shoot tip explants, bud initiated after 13 days of inoculation at 0.5 mg/l BAP with 72.2 % response which resulted in the formation of 5.1 ± 0.27 shoots with shoot length of 3.1 ± 0.09 cm (Table 19, Plate 34.A). The percent response increased up to 77.8 and number of shoots to 6.2 ± 0.33 per culture on 1 mg/l BAP (Plate 34.B). Shoots were initiated after 12 days of inoculation. The best results in terms of time taken for bud initiation, percent response, number of shoots and shoot length were observed at this concentration. But, the percent responses, number of shoots and shoot length decreased at higher levels (2 and 4 mg/l BAP).

Internode, leaf and petiolar explants showed slight increase in size but failed to produce any bud or callus at all the concentrations of BAP.

4.11.3. 6-Furfurylaminopurine (KIN):

To study the effect of different concentrations of KIN on regeneration potential of different explants viz, shoot tips, internodes, nodes, leaf and petioles; these were inoculated on MS medium supplemented with 0.5, 1.0, 2.0 and 4.0 mg/l KIN.

Of the four concentrations of KIN tried on nodal explants on MS medium, 1 mg/l KIN proved most effective. It induced 4.0 ± 0.23 shoots with average shoot length 5.5 ± 0.27 cm (Plate 32.B). The percent response was 83.3 and shoots were initiated after 9 days of inoculation (Table 17). The percent response, shoot number and shoot length decreased at higher levels. The average shoot number was 3.1 ± 0.21 and 2.6 ± 0.10 at 2 and 4 mg/l KIN, respectively. The percent response was 72.2 and 63.9 at 2 and 4 mg/l KIN, respectively.

In shoot tip explants, 1 mg/l KIN was optimum which resulted in the formation of 3.3 ± 0.21 shoots with average shoot length 4.7 ± 0.21 cm (Plate 35.B). These were initiated after 13 days of inoculation and percent response was 72.2 (Table 19). However, on medium supplemented with 0.5 mg/l KIN 2.5 ± 0.12 shoots appeared per explant after 14 days of inoculation and percent response was 69.4 (Plate 35.A). Bud induction was delayed further on 2 and 4 mg/l KIN with decreased percent response, number of shoots and shoot length.
From above observations it was concluded that of the two cytokinins tried in *B. madagascariensis*, BAP was more effective than KIN for percent response of explants, time taken for shoot-bud induction and number of shoots. However, KIN was more effective than BAP in case of length of shoots which was more in medium containing KIN.

4.11.4. BAP and KIN combination:

The effect of different combinations of BAP and KIN were also studied on nodal and shoot tip explants (Table 18 & 20). In one set 1 mg/l BAP was combined with 0.5, 1.0, 2.0 and 4.0 mg/l KIN, whereas in second set 1 mg/l KIN was combined with 0.5, 1.0, 2.0 and 4.0 mg/l BAP. In both the cases, the combination increased the percent response of explants and also the combination was effective in reducing time taken for bud initiation. In all the combinations tried, the concentrations of 1 mg/l BAP + 1 mg/l KIN was optimum in both the explants. At this combination in nodal explants percent response was 94.4 and buds were initiated within 7 days of inoculation (Plate 33.B), whereas in shoot tip explants percent response was 86.1 and buds appeared after 10 days (Plate 36.B).

Also among the explants cultured, nodal and shoot tip explant were suitable for direct shoot regeneration in *B. madagascariensis* and between the above two explants cultured, nodal explants were better in terms of percent response, time taken for shoot-bud induction, mean number of shoots and shoot length.

4.12. Indirect shoot regeneration:

4.12.1. Callus induction:

Effects of three auxins (IAA, NAA and 2,4-D) were studied on shoot tip, internode, node, leaf and petiolar explants for callus induction and proliferation.

4.12.1.1. Indole-3-acetic acid (IAA):

To study the effect of IAA on callus induction from different explants, these were inoculated on IAA enriched MS medium. Four different concentrations of IAA (0.5, 1.0, 2.0 and 4.0 mg/l) were tried. Cultures raised on basal medium without any growth regulators served as control.
In control cultures, no callus was observed in any of the explant. All the five types of explants produced callus on MS medium at all the four concentrations of IAA tried. The results are summarised in Fig. 11 and Table 21. Callus formation started at 0.5 mg/l and increased with increase in the concentration of IAA from 0.5 to 2 mg/l. Callus formation decreased at 4 mg/l IAA. The maximum callus was produced at 2 mg/l IAA in all the five types of explants.

Shoot tip explants showed 66.7 percent response after 21 days of inoculation on 2 mg/l IAA (Fig. 11). The callus was white and compact (Plate 37.A). At this concentration, the fresh and dry weights of callus were 3.00 ± 0.10 and 0.29 ± 0.03 g, respectively (Table 21).

Internodal segment showed 75 percent response. Callus was initiated within 17 days of inoculation on 2 mg/l IAA (Fig. 11) and the callus was white-brown and compact (Plate 37.B). At this concentration, the fresh and dry weights of callus were 3.37 ± 0.12 and 0.36 ± 0.04 g, respectively (Table 21). In internodes callus was initiated on lower side along the length and then spread throughout the portion.

In nodal explants at 2 mg/l IAA, 80.6 % explants produced white-brown and compact callus within 16 days of inoculation (Fig. 11, Plate 37.C). The fresh and dry weights of callus at this concentration were 3.51 ± 0.10 and 0.39 ± 0.04 g, respectively (Table 21). In the nodal segments, callus was initiated laterally and then spread throughout the portion.

In leaf explants, percent response was 69.4 and callus was whitish and soft at 2 mg/l of IAA (Plate 37.E). Callus was initiated within 20 days of inoculation. The fresh and dry weights of callus at this concentration were 3.12 ± 0.12 and 0.32 ± 0.03 g, respectively (Table 21). In case of leaf, the callus was initiated near the cut ends.

Petiolar segments were less responsive for callus initiation as compared to internode, node and leaf segments. At 2 mg/l IAA, 66.7 % explants produced brown and compact callus after 20 days of inoculation (Fig. 11, Plate 37.D). At this concentration, the fresh and dry weights of callus were 3.10 ± 0.10 and 0.30 ± 0.03 g, respectively (Table 21). Callus was initiated on lower side along the length and then spread throughout the portion.
4.12.1.2. \(\alpha\)-Naphthalene acetic acid (NAA):

Different explants (shoot tips, internode, node, leaf and petioles) were inoculated on the different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of NAA fortified MS medium to study their potential for callus formation. Cultures raised on MS basal medium without NAA served as control.

No callus was observed in any of the explants on MS basal without NAA. All types of explants produced callus on MS medium at all the four concentrations of NAA tried. The results of these treatments are summarised in Fig. 12 and Table 21. Callus formation started at 0.5 mg/l and increased with increase in the concentration of NAA from 0.5 to 2 mg/l. At higher level (4 mg/l NAA) callus formation decreased. The maximum callus was produced at 2 mg/l concentration in all the five types of explants.

Shoot tip explants showed 69.4 % response and callus was initiated after 19 days of inoculation on 2 mg/l NAA (Fig. 12) with the formation of white and soft callus (Plate 38.B). At this concentration, the fresh and dry weights of callus were 4.85 ± 0.15 and 0.54 ± 0.04 g, respectively (Table 21).

Internodal segment showed 77.7 % response and callus was initiated within 16 days of inoculation on 2 mg/l of NAA (Fig. 12). Callus was green and friable (Plate 39.B). At this concentration, the fresh and dry weights of callus were 5.10 ± 0.13 and 0.62 ± 0.05 g, respectively (Table 21).

In nodal explants at 2 mg/l NAA, 80.6 % explants produced green and friable callus within 16 days of inoculation (Fig. 12, Plate 40.A, B). The fresh and dry weights of callus at this concentration were 5.24 ± 0.15 and 0.67 ± 0.05 g, respectively (Table 21).

Leaf explant exhibited up to 75 % response with the formation of whitish and soft callus on 2 mg/l of NAA (Fig. 12 A, Plate 41.D). Callus was initiated within 18 days of inoculation (Fig. 12 B). The fresh and dry weights of callus at this concentration were 5.01 ± 0.15 and 0.60 ± 0.05 g, respectively (Table 21). In case of leaf, the callus was initiated near the cut ends and then spread all over the explant.
In petiolar explants, at 2 mg/l NAA, 72.2 % explants produced green and compact callus after 18 days of inoculation (Fig. 12, Plate 41.A). At this concentration, the fresh and dry weights of callus were 4.92 ± 0.13 and 0.58 ± 0.05 g, respectively (Table 21).

4.12.1.3. 2,4-Dichlorophenoxyacetic acid (2,4-D):

To study the effect of 2,4-D on callus induction from different explants, these were inoculated on 2,4-D enriched MS medium. Four different concentrations of 2,4-D (0.5, 1.0, 2.0 and 4.0 mg/l) were tried. Cultures raised on basal medium without any growth regulators served as control.

In control cultures, no callus was observed in any of the explant. All the five types of explants produced callus at all the four concentrations of 2,4-D on MS medium. The results are summarised in Fig. 13 and Table 21. Callus formation started at 0.5 mg/l and increased with increase in the concentration of 2,4-D from 0.5 to 2 mg/l. Callus formation decreased at 4 mg/l 2,4-D. The maximum callus was produced at 2 mg/l concentration in all the five types of explants.

Shoot tip explants showed 69.4 percent response after 20 days of inoculation on 2 mg/l 2,4-D with the formation of white and soft callus (Fig. 13, Plate 38.D). At this concentration, the fresh and dry weights of callus were 4.41 ± 0.12 and 0.46 ± 0.04 g, respectively (Table 21).

In internodal segment percent response was 77.8. Callus was initiated within 16 days of inoculation on 2 mg/l of 2,4-D and callus was white-green and compact (Fig. 13, Plate 39.D). At this concentration, the fresh and dry weights of callus were 4.72 ± 0.14 and 0.53 ± 0.04 g, respectively (Table 21).

In nodal explants at 2 mg/l 2,4-D, 77.8 % explants produced brownish-green and friable callus within 15 days of inoculation (Fig. 13, Plate 40.C, D). The fresh and dry weights of callus at this concentration were 4.86 ± 0.14 and 0.58 ± 0.04 g, respectively (Table 21).

Leaf explant exhibited 72.2 percent response with the formation of brownish-green, hairy and soft callus on 2 mg/l of 2,4-D (Fig. 13 A, Plate 42.C). Callus was
initiated within 20 days of inoculation (Fig. 13 B). The fresh and dry weights of callus at this concentration were $4.63 \pm 0.16$ and $0.51 \pm 0.03$ g, respectively (Table 21). In case of leaf, the callus was initiated near the cut ends and then spread all over the explant.

In petiolar segments at 2 mg/l 2,4-D, 72.2 % explants produced brownish-green and compact callus after 18 days of inoculation (Fig. 13, Plate 43.B). At this concentration, the fresh and dry weights of callus were $4.50 \pm 0.15$ and $0.48 \pm 0.03$ g, respectively (Table 21).

Above observations suggest that all the five types of explants exhibited best response in terms of callus induction at 2 mg/l of IAA, NAA and 2,4-D. Thus the concentration of 2 mg/l of these auxins was most favourable for callus induction. Moreover, of the auxins tried, NAA was more effective than IAA and 2,4-D for callus induction. Further, among the five types of explants tried, nodal segments gave best response in terms of callus induction.

4.12.2. Callus growth:

The growth of callus was measured in terms of fresh and dry weights of callus formed by shoot tips, internode, node, leaf and petiolar explants on different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of IAA, NAA and 2,4-D (Table 21). Results clearly show that the mean fresh and dry weight of callus increased as the concentration of auxin increased from 0.5 to 2 mg/l and decreased at 4 mg/l of these auxins. The maximum fresh and dry weights ($5.24 \pm 0.15$ and $0.67 \pm 0.05$ g) were observed in callus induced by nodal explants cultured on MS medium with 2 mg/l NAA.

4.12.3. Cytokinin and auxin combinations:

Different explants (shoot tips, internode, node, leaf and petioles) were inoculated on MS medium fortified with the combinations of cytokinin and auxin. In one set best concentration (2 mg/l) of auxin (IAA, NAA and 2,4-D) was combined with different concentrations (0.5, 1.0, 2.0 and 4.0 mg/l) of cytokinin (BAP and KIN) individually. In second set of experiment, best concentration (1 mg/l) of cytokinin was combined with different concentrations of auxin. Cultures raised on MS basal medium without any growth regulator served as control. No callus was observed in any of the explants on
MS basal without any growth regulators. Further, by addition of cytokinins, callus induction efficiency of auxins increased. The auxin and cytokinin combinations increased the percent response of explants and also reduced the time taken for callus induction (Fig. 14-19 and Plate 44 & 45). Further, the combination of 0.5 mg/l cytokinin (BAP/KIN) + 2 mg/l auxins was optimum in all the explants. Among the explants tried, nodal segments were most responsive for producing good amount of callus at a combination of 0.5 mg/l BAP + 2 mg/l NAA which produced green and friable callus in 97.2 % explants within 9 days of inoculation (Plate 44.B).

Fresh and dry weights of calli increased on the medium having combinations of cytokinin and auxin (Tables 22 & 23). Maximum fresh and dry weights (5.30 ± 0.15 and 0.70 ± 0.06 g) were observed in nodal explants at a combination of 0.5 mg/l BAP + 2 mg/l NAA. However, with increase in the concentrations of BAP and KIN in the combinations, fresh and dry weights decreased.

When leaf segments were inoculated on the combinations of 1 mg/l BAP or KIN with 0.5-4.0 mg/l IAA, NAA and 2,4-D individually on MS medium, small amount of callus was produced which further differentiated into shoots and roots (Tables 24). In the present investigation, 1 mg/l BAP/KIN + lower concentration of IAA, NAA and 2,4-D (0.5 and 1 mg/l) individually led to shoot formation in leaf segments, while 1 mg/l BAP/KIN + higher concentration of IAA, NAA and 2,4-D (2 and 4 mg/l) individually led to root formation on callus. Maximum shoots (2.8 ± 0.13) were produced at a combination of 1 mg/l BAP + 0.5 mg/l IAA in 62.5 % explants after 20 days of inoculation (Plate 46.A & B), while maximum roots (2.5 ± 0.13) were produced at a combination of 1 mg/l BAP + 2 mg/l IAA in 72.2 % explants after 16 days of inoculation (Table 24, Plate 46.C).

When nodal segments were inoculated on MS medium supplemented with the combinations of 1 mg/l BAP/KIN + 0.5 mg/l IAA, NAA and 2,4-D callus was formed which further differentiated in to shoots (Table 25, Plate 47). Maximum shoots (3.5 ± 0.16) were observed on a combination of 1 mg/l BAP + 0.5 mg/l IAA in 65.2 % explants after 15 days of inoculation.
4.12.4. Shoot regeneration from callus:

Effects of BAP and KIN were studied on callus produced by shoot tip, internode, node, leaf and petiolar explants for shoot regeneration.

The calli so obtained were divided into small pieces and cultured on MS medium supplemented with different concentrations of BAP and KIN (0.5, 1.0, 2.0 and 4.0 mg/l) for shoot formation. Callus induced from leaf explants was most responsive for shoot formation where maximum shoots (6.4 ± 0.32) were regenerated on medium supplemented with 1 mg/l BAP in 69.4 % cultures after 16 days (Table 26, Plate 51.A). Similarly, the concentration of 1 mg/l KIN was effective for callus regeneration resulted in to 61.1 % response with 4.6 ± 0.23 shoots per culture after 20 days were initiated (Table 27, Plate 51.B). Shoot regeneration delayed at higher concentration (2 and 4 mg/l) of both the cytokinins. Further, of the two growth regulator tried, BAP was more effective than KIN for shoot regeneration through callus, whereas KIN proved better for the shoot length.

4.13. Rooting of in vitro developed shoots:

In vitro developed shoots on medium were excised when attained a height of 2-3 cm and inoculated on half strength MS medium without and with auxins (IAA IBA, NAA and 2,4-D) in the concentration range 0.5, 1.0, 2.0 and 4.0 mg/l for rooting. The results are summarised in Table 28. No rooting was observed on half-strength MS medium without auxins. Higher concentration of NAA and 2,4-D inhibited rooting and callus was observed at the base of shoots. IBA proved best for rooting which induced healthy and elongated roots at all the concentrations (Plate 53.A). Among the four concentrations of IBA tried, 0.5 mg/l was optimum for rooting which resulted in to 94.4 % response. The average number of roots was 10.1 ± 0.53 and average root length was 5.2 ± 0.18 cm. Roots were initiated after 6 days of inoculation. The response decreased as the concentrations of IBA increased from 0.5 to 4.0 mg/l on the medium.

4.14. Hardening and transfer of plantlets to the field:

Shoots with well-developed roots were gently pulled out of the medium and washed gently in running tap water to remove the medium sticking to the roots. Then transferred them to plastic cups having sterile soil and sand mixture (1:1) and irrigated
with $\frac{1}{4}$ strength MS salt solution (Plate 54.A & B). High humidity was maintained for initial 15 days with the help of polythene bags and thereafter, these pots were exposed to natural conditions for 3-4 hours daily. After about a month the plants were shifted to pots in Polyhouse where they grew normally with 80 % survival rate (Plate 54.D). After one month these plants were transferred to the field from Polyhouse (Plate 54.E). The survived plants showed normal growth.