Experimental Findings

4.1 *Cyathea gigantea* Wall. ex. Hook.

In vitro multiplication was investigated during this study and for this purpose, a series of experiments were performed.

Effect of sterilants

For sterilization three different kinds (mercuric chloride and sodium hypochlorite) of surface sterilants were used for spore sterilization. 30% (w/v) sodium hypochlorite was found to be best for sterilization of spores as spores are very sensitive. Spores were treated for seven minutes with 30% sodium hypochlorite to sterilize. Below 30% the spores were not sterile and high mortality was observed when more than 30% concentration was used or treatment time was more.

Germination percentage

After 25-27 days the spores on MS media (full strength) became swollen and a small heart like structure appeared called prothalli. Spores cultured on MS media showed 72% germination (table 4.1). Spores cultured on Fern propagation media (FPM) germinated within 12-14 days but germination percentage was not satisfactory only 58%. Observing both the parameter germination percentage and germination period MS media was selected for further multiplication of prothallus.

Table 4.1. Average germination percentage of spores of *Cyathea gigantea* Wall. ex. Hook.

Media	Period of germination (Days)	Percentage of germination
MS	25-27	72
FPM	12-14	58
Half MS	18-20	51
Half FPM	28-30	11

Prothallus development

In this series of experiments 5 different media formulation was tried. Prothallus developed after the spore germination and it produce a heart shaped structure.Spores divided into two parts – the prothallial initial which produce prothallus and rhizoidal initial which produce rhizoids. The prothallus contains male sex organ (antheridia) and female sex organ (female sex organ). After spore germination prothallus was transferred to the media containing different concentration of IAA (table 4.2) for growth of gametophytes.

Effect of Growth regulators on the development of gametophytes

To investigate the effect of growth regulators on gametophyte development five concentrations of IAA viz. 0.5mg/L,0.6mg/L,0.7mg/L,0.8mg/L,0.9mg/L was added with MS media. MS media with various concentration of IAA and a control (MS media without growth regulators) were tried to culture the prothallus. The growth of gametophyte recorded in terms of fresh weight (mg) of prothallus after 4 month. MS media containing 0.8mg/L IAA was found to be effective in case of gametophyte development. Gametophyte developed on MS + 0.8mg/L IAA showed maximum growth with highest fresh weight (136 mg). By observing the growth of gametophyte, 0.8mg/L concentration of IAA was considered as the optimum concentration for the growth of gametophyte. For the development of sporophyte this optimum concentration (0.8mg/L) of IAA was combined with various concentration (1.0mg/L, 2.0mg/L, 3.0mg/L, 4.0mg/L, 5.0mg/L, 6.0mg/L) of kinetin to observe the combined effect of growth regulators on sporophyte development.

Effect of Growth regulators on Sporophyte development

Sporophytic body did not develop from all germinating spores. Sporophytes developed on 15^{th} day of inoculation (Behera *et al.,* 2011). Young sporophytic bodies developed from prothallus (bearing both antheridia and archegonia).Spermatozoids originate from antheridia and archegonium produces one egg. The spermatozoids fertilize the egg to produce sporophyte (diploid). Growth of the sporophyte in terms of fresh weight and dry weight after 6 month was recorded and it was observed that MS media containing

Kinetin (5.0mg/L) and 0.8mg/L IAA was effective in sporophyte development (table 4.3).

MS media supplemented with kinetin and IAA showed profound effect on sporophyte initiation. MS media containing kinetin and IAA at concentration 5mg/L and 0.8mg/L respectively showed significant increase in multiplication of plant and length of the sporophyte was 20.29 cm (table 4.4) where high concentration of kinetin and IAA showed detrimental effect of the dose and low concentration reduce the number of plantlets.

Development of root-like rhizoid

For root induction experiments were also done in order to optimize the rooting medium. The sporophytes developed in culture bottle develop root like rhizoid. The highest length of the sporophyte (20.29cm) was observed on the MS media with 0.8mg/L IAA and 5.0mg/L KN.

Acclimatization of the cultured plants

Cultured plants were transferred in the sterile vermiculite mixture for one month. After full growth of the cultured plants were transferred to potting mixtures containing brick bats + leaf mold + charcoal + dried moss (1: 1 : 1 : 1).Surviving rate 81% was observed after one month.

Table 4.2 Growth of gametophyte (prothallus) after 4 month in terms of fresh weight (mg) for 10 nos. of prothallus in *Cyathea gigantea.*

Conc. of IAA in MS medium	Fresh weight of prothallus (mg)
0.5mg/L	95
0.6mg/L	111
0.7mg/L	123
0.8mg/L	136
0.9mg/L	110

Table 4.3. Growth of sporophytes of *Cyathea gigantea* Wall ex. Hook. after 6 month in terms of fresh and dry weight(g).

	IAA(mg/L)	KN (mg/L)	Fresh Weight (g)	Dry weight (g)
T0	0.00	0.0	1.13±0.05	0.49±0.12
T1	0.8	1.0	1.80±0.19	0.64±0.13
T2	0.8	2.0	2.52±0.18	1.13±0.13
T3	0.8	3.0	2.71±0.16	1.23±0.17
T4	0.8	4.0	2.81±0.11	1.25±0.15
T5	**0.8**	**5.0**	**3.25±0.21**	**1.63±0.13***
T6	0.8	0.6	2.55±0.13	1.11±0.11

*Each concentration consisted of mean value of 3 replications and each replication of 10 culture bottles.

T_0 = only MS

T_1=MS+0.8mg/LIAA+1.0mg/L KN

T_2 = MS +0.8mg/L IAA +2.0mg/L KN

T_3 = MS +0.8mg/L IAA+ 3.0mg/L KN T_4 =MS+0.8mg/L IAA + 4.0mg/L KN

T_5 = MS +0.8mg/L IAA + 5.0mg/L KN

T_6 = MS +0.8mg/L IAA + 6.0mg/L KN

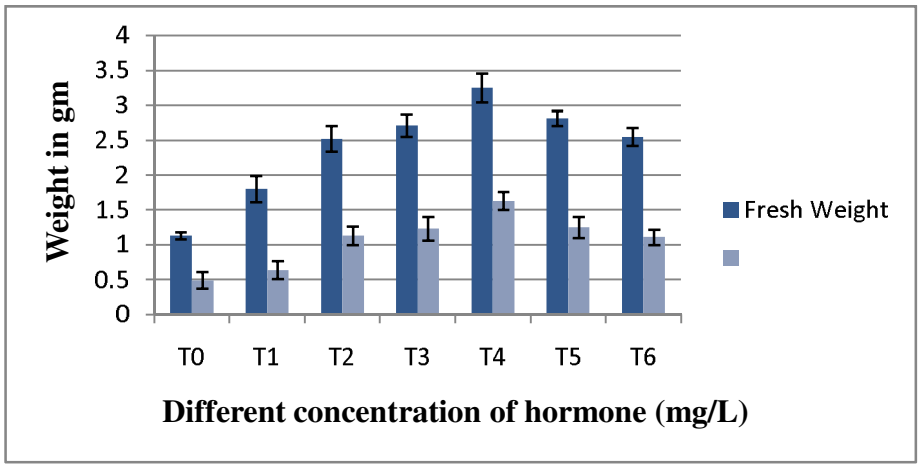


Fig.8. Growth of *Cyathea gigantea* Wall ex. Hook sporophytes in terms of fresh weight (mean) ± SE and dry weight (mean) ±SE after 6 month.

Table 4.4 Effect of growth regulators on sporophyte length (cm) of *Cyathea gigantea* after 10 month .

Treatments	IAA mg/L	KN mg/L	Mean Sporophyte Length (cm)	Sporophyte length ± SE
T0	0.00	0.00	1.89	1.89±0.13
T_1	0.8	1	9.74	9.74± 0.20
T_2	0.8	2	11.44	11.44 ± 0.25
T_3	0.8	3	13.78	13.78±0.29
T_4	0.8	4	16.88	16.88±0.38
T_5	0.8	5	20.29	20.29±0.23

*Each concentration consisted of mean value of 3 replications and each replication of 10 culture bottles.

T_0= MS

T_1= MS +0.8mg/L IAA +1.0mg/L KN

T_2 = MS +0.8mg/L IAA + 2.0mg/L KN

T_3 = MS +0.8mg/L IAA + 3.0mg/L KN

T_4 = MS +0.8mg/L IAA + 4.0mg/L KN

T_5 = MS +0.8mg/L IAA + 5.0mg/L KN

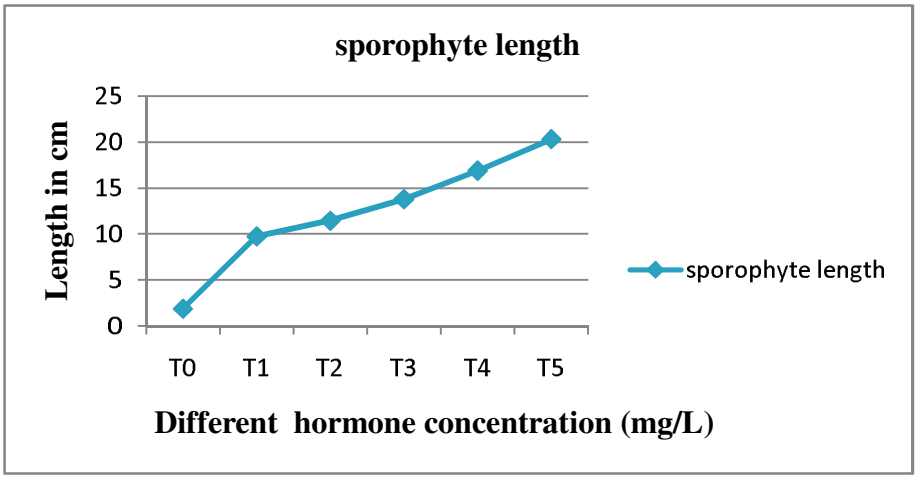


Figure 9. Effect of different concentration of hormones on sporophyte length(cm).

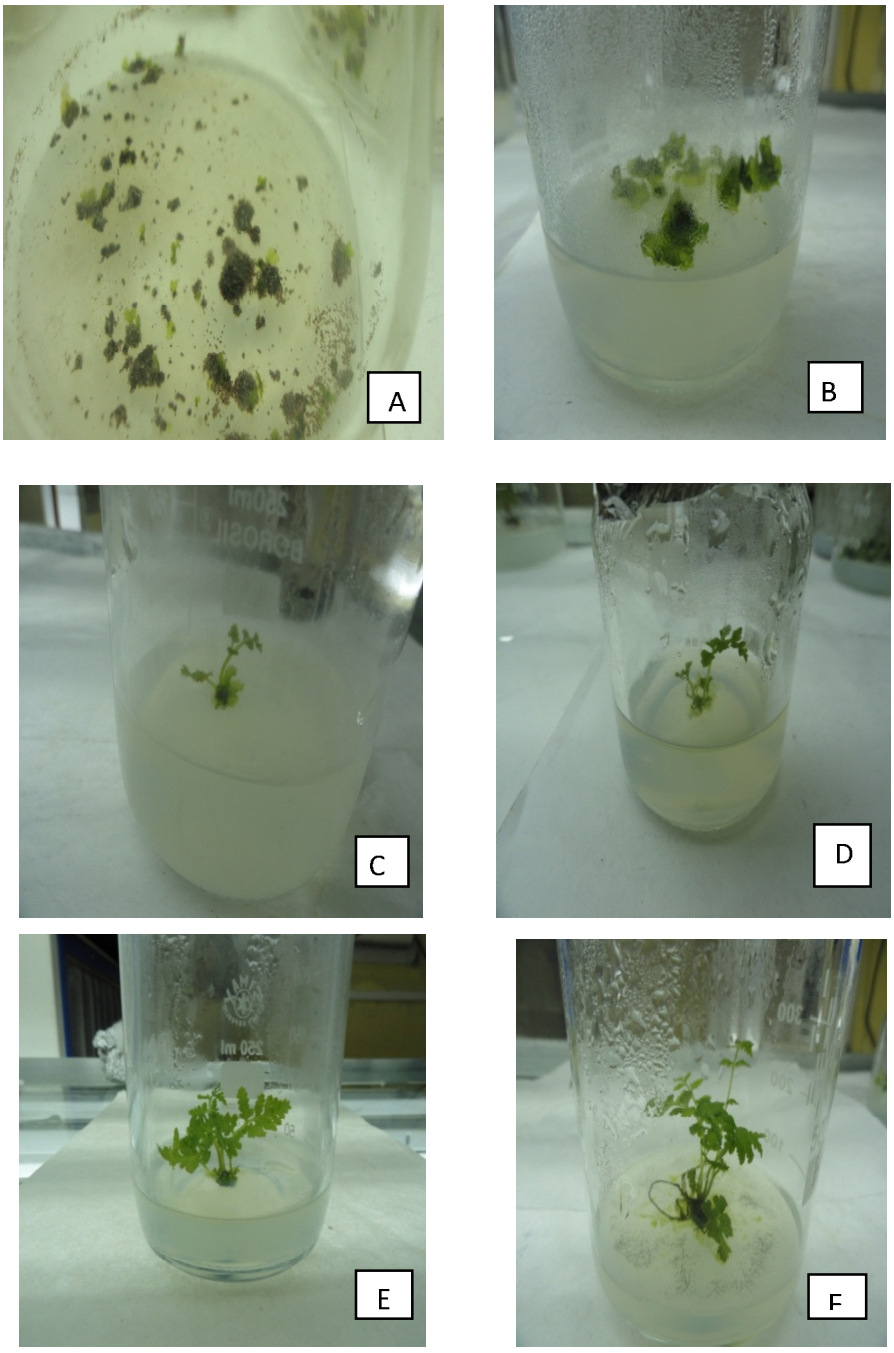


Plate 2. [A] Germinated spores of *Cyathea gigantea* Wall ex. Hook on MS basal medium[B] Gametophytes on same medium [c] sporophyte after 6 month [D] 7 month [E] 8 month [F] 10month.

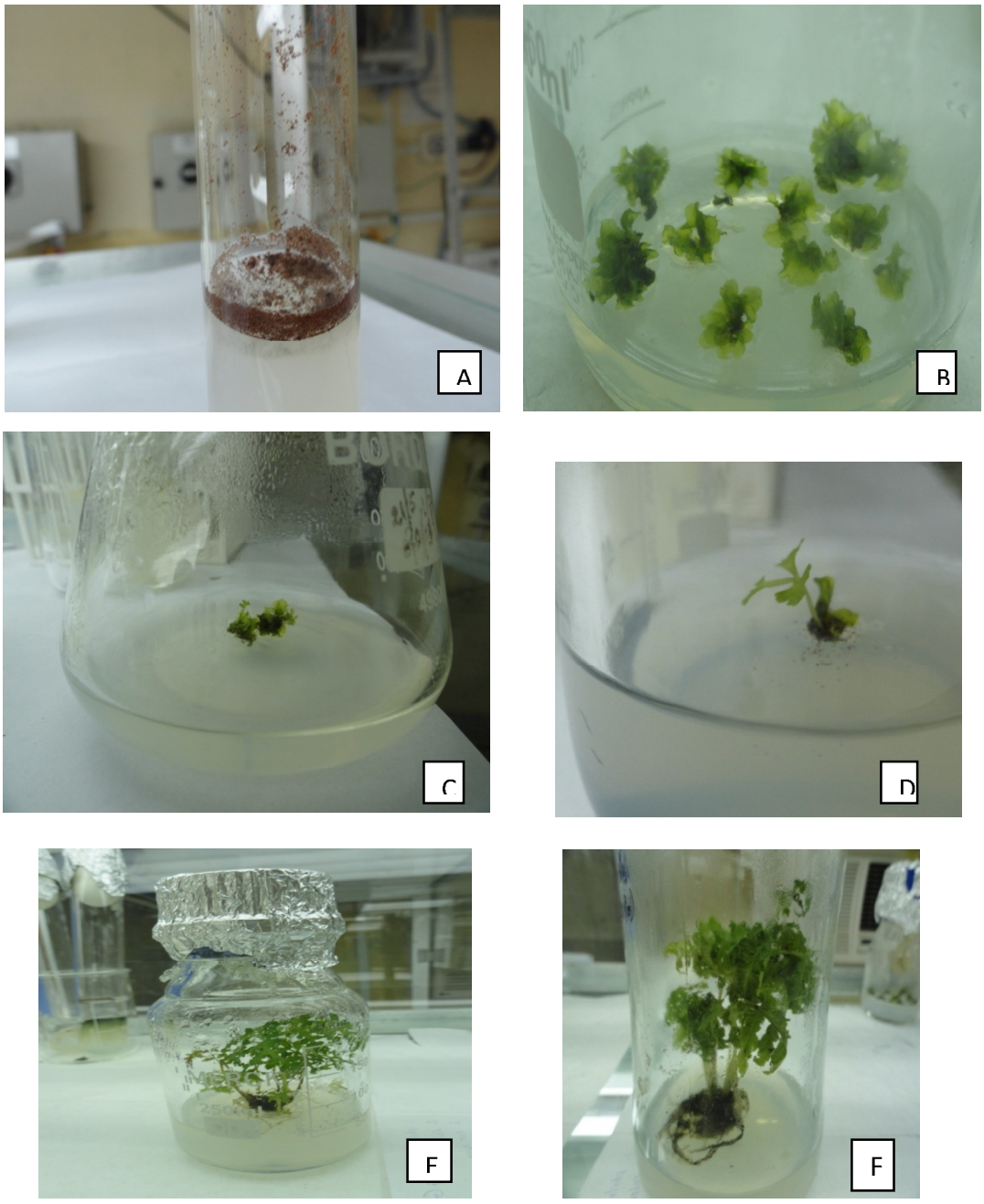
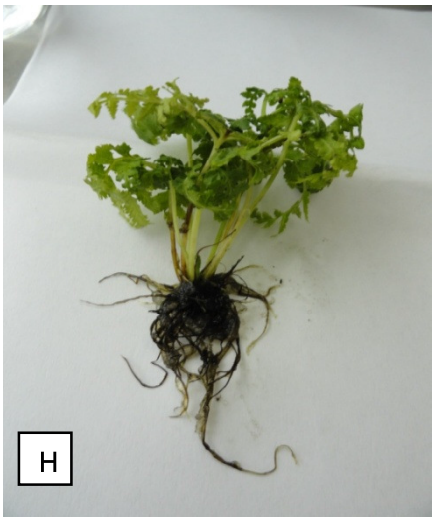


Plate 3:[A] Inoculated spores of *Cyathea gigantea* Wall ex.Hook [B]Gametophytes [C] Sporophyte developed from Gametophyte on T_5 treatment [D] Sporophyte after 5 month. [E] 8 month [F] 10 month.



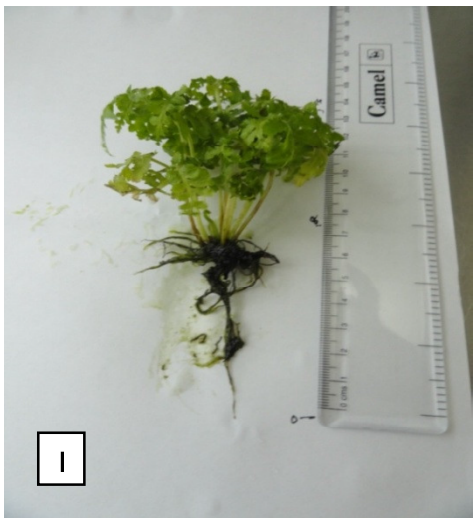


Plate 4 : [G] Sporophyte during transfer. [H] Showing length of Sporophyte. [I] complete sporophyte on T_5 treatment after 10 month.

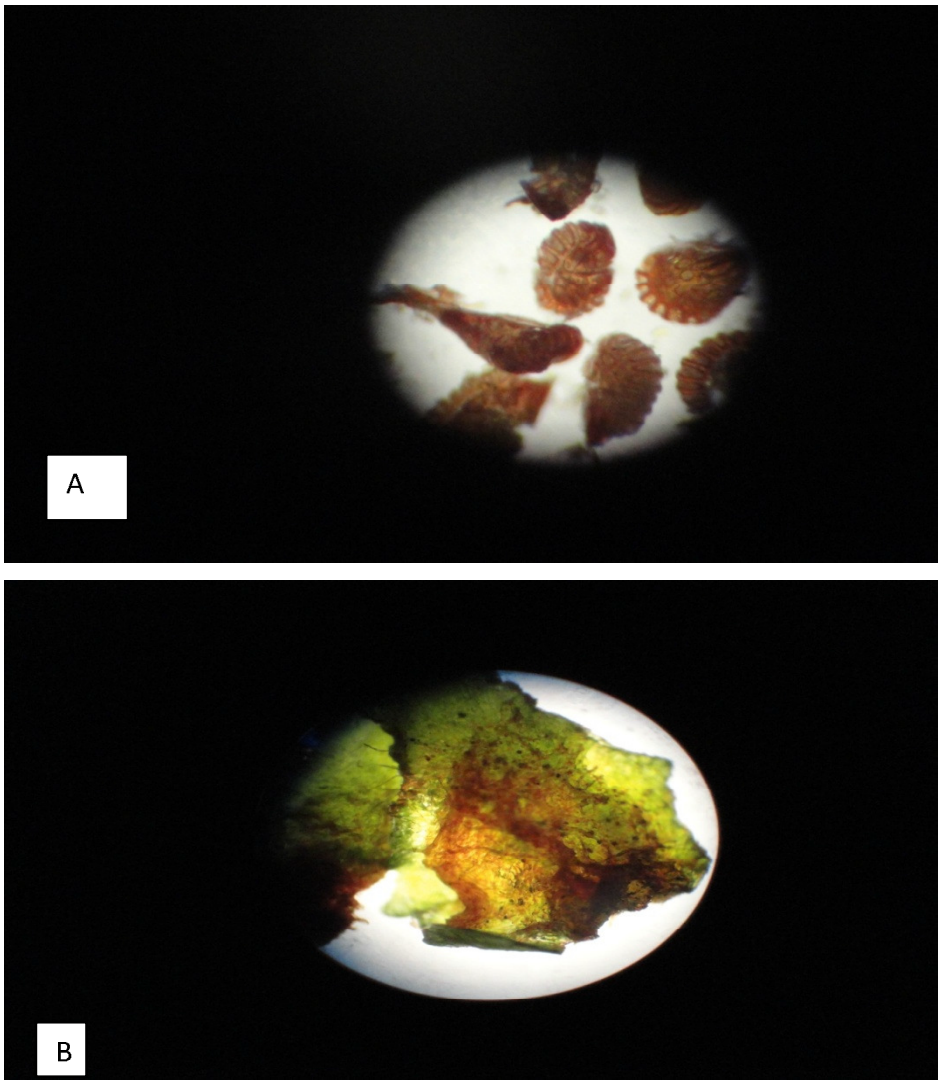


Plate 5: [A] Structure of Sporangium of *Cyathea gigantea* Wall ex. Hook. [B] Microphotograph of prothallus.

4.2 *Dioscorea alata* L.

The effect of various concentration of IAA (0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L) on axillary bud breaking of *Dioscorea alata* L. was listed in table 4.5. Explants cultured on MS medium without IAA showed proliferation of axillary bud but it required maximum time (35-37 days) for it. Addition of low concentration (0.5mg/L) of IAA was less effective in bud breaking and it took 28 – 30 days for proliferation, which one was second highest time period for bud proliferation. Explants cultured on media with IAA concentration (2.0mg/L) proliferate within 7-9 days and percentage of explants response (68) also very satisfactory. Nodal segments cultured on MS medium with 1.5mg/L IAA take second minimum time period (12 – 14) for bud proliferation and it showed impressive response (62%) of explants also.

Bud proliferation was enhanced by addition of IAA. After bud proliferation, for further growth, cultured plants were transferred to the media supplemented with kinetin and auxin (IAA). Response of different concentration of kinetin with 2.0mg/L concentration of IAA was recorded in terms of number of shoot and shoot length. 2.0mg/L IAA when supplemented with MS media it showed effective result in bud breaking for that this concentration of IAA was selected for further work and with this different concentration of kinetin was combined to study the effect of it on shoot proliferation in terms of shoot length, number of shoot per explants and data obtained from the study is tabulated in table 4.6

MS medium with growth regulators produced better result in terms of percentage of explats response, no. of shoots/explants, average shoot length. Of the combination tested MS+ kinetin (1.5 mg/L)+ IAA (2.0mg/L) elicited optimal response in which an average 7.7± 0.29 shoot lets with a mean shoot length of 9.90 ± 0.11cm per explant was recorded. Second highest shoot proliferation in terms of shoot number and shoot length was observed in the MS medium +Kinetin (1.0mg/L)+ IAA (2.0mg/L)in which 5.6±0.30 shootlets per explants with shoot length 7.09 ± 0.12 cm was recorded.

The well grown shoots were transferred to half strength MS medium containing IAA. The rooting responses of shoots on different concentration of IAA was measured in terms of days required for root initiation, mean no. of

roots / shoots and mean root length and data is represented in table 4.7. In different concentration of IAA (0.5mg/L, 1.0mg/L,1.5mg/L, 2.0mg/L, 2.5mg/L) the response of shoots was recorded.

IAA enhanced rooting and data was recorded. Shoots cultured on half strength MS media without any hormonal supplementation unable to produce root but in some shoots rooting was observed but in very negligible amount. Media supplemented with lower concentration (0.5mg/L) of IAA produce few (mean 2.6±0.16) number of root with minimum root length (mean1.39cm). Media treated with higher concentration of IAA (2.0mg/L, 2.5mg/L, 3mg/L) respond well .Half strength MS + 2.5mg/L showed more impressive result where about 62% explants responded with average root length 8.14 cm. In terms of response of explants two concentration 2.5mg/L, 3.0mg/L showed more or less similar result 62%, 59% respectively. Shoots treated with 2.0mg/L IAA showed second highest response with root number 5.7 and mean root length 7.7 cm.

Plants were found to be ready for transplanting in hardening medium after five month. Rooted plants were removed from culture tube and washed thoroughly to remove adhering gel then transplanted to sterile plastic cups containing vermiculite and kept inside the growth chamber. During this period the plants were sprayed with liquid MS medium without agar and sugar. The plants were dipped in 2mg/L Diethane for two minute as precaution to resist fungal infection. After 1 month they were transplanted to earthen pots containing mixture of Brick bats + soil + Charcoal +Dried moss + Leaf mold (1:1:1:1:1).

Table No. 4.5 : **Effect of various concentration of IAA on axillary bud proliferation. (Data scored after 40 days, 10 replicates for each treatment, repeated thrice).**

Treatments	Hormonal supplementsIAA (mg/L)	% of explants response	Days to bud break
T_0	0	46	35 - 37
T_1	0.5	40	28 - 30
T_2	1.0	53	15 - 17
T_3	1.5	62	12 - 14
T_4	2.0	68	7 - 9
T_5	2.5	59	11- 13

Table No. 4.6: Shoot formation in nodal explants of *Dioscorea alata* L.cultured on MS medium supplemented with various concentrations of Kinetin and IAA. (10 replicates per treatment, data scored after three month, repeated thrice)

Treatments	Hormonal supplements (mg/L)		Mean no. of shoots/explants	Mean no. of shoots/explants ± SE	Mean shoot length cm	Mean shoot length ± SE
	IAA	KN				
T0	0	0	1.5	1.5±0.22	1.50	1.50±0.02
T1	2.0	0.5	1.9	1.9± 0.23	6.62	6.62±0.12
T2	2.0	1.0	5.6	5.6± 0.30	7.09	7.09±0.12
T3	2.0	1.5	7.7	7.7± 0.29	9.90	9.90±0.11
T4	2.0	2.0	3.8	3.8± 0.19	5.86	5.86±0.05
T5	2.0	2.5	3.1	3.1± 0.23	4.90	4.90±0.05

*Values represent mean± SE

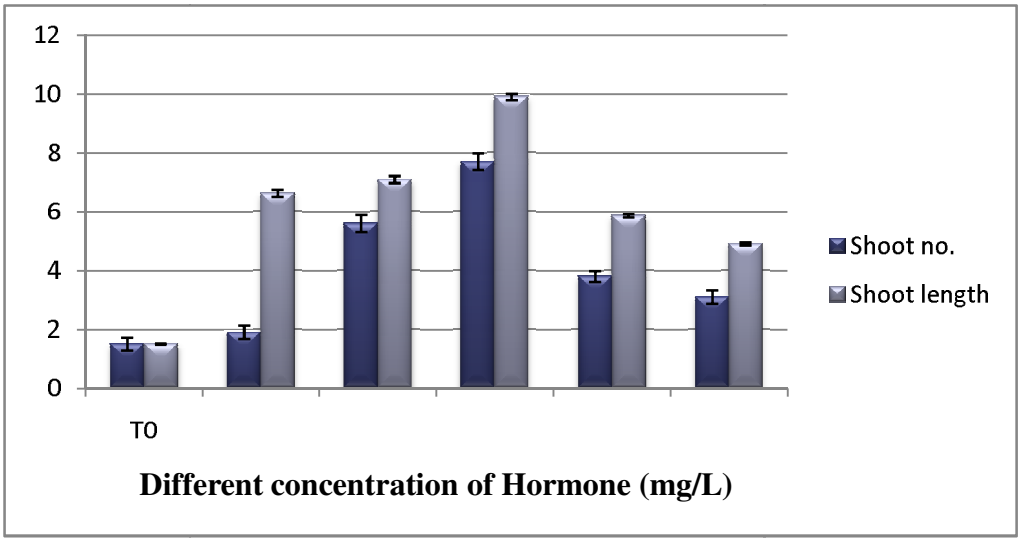


Figure 10. Effect of various concentration of growth regulator on shoot length and shoot number of *Dioscorea alata* L.

Table 4.7 Influence of different concentration of IAA on rooting of *in vitro* generated shootlets of *Dioscorea alata* L. (Data scored after 3 month of inoculation, 10 replicates per treatment, repeated thrice).

Treatments	Conc. of IAA (mg/L)	Days to root initiation	Percentage of explants response	Mean root no. ± SE	Mean root length ± SE
To	0	-	-	-	-
T1	0.5	60-62	46	2.6±0.16	1.39±0.02
T2	1.0	51-53	49	3.8±0.13	2.05±0.03
T3	1.5	43-45	58	4.9±0.17	3.73±0.07
T4	2.0	36-38	63	5.7±0.15	7.77±0.09
T5	2.5	22-24	62	6.6±0.16	8.14±0.06
T6	3.0	32-34	49	4.1±0.23	5.36±0.07

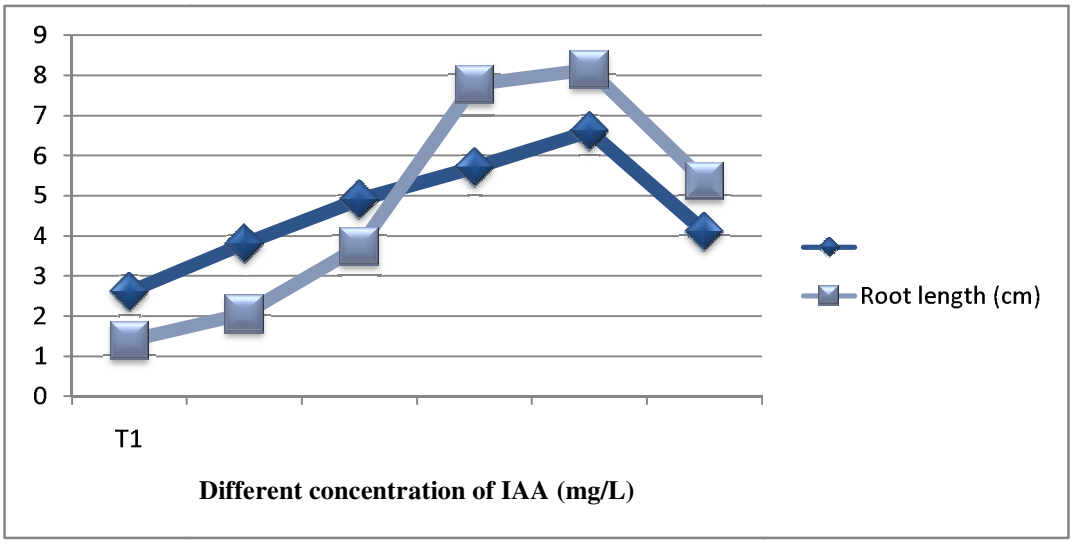


Figure 11: Effect of different concentration of IAA on root length and root number in *Dioscorea alata* L.

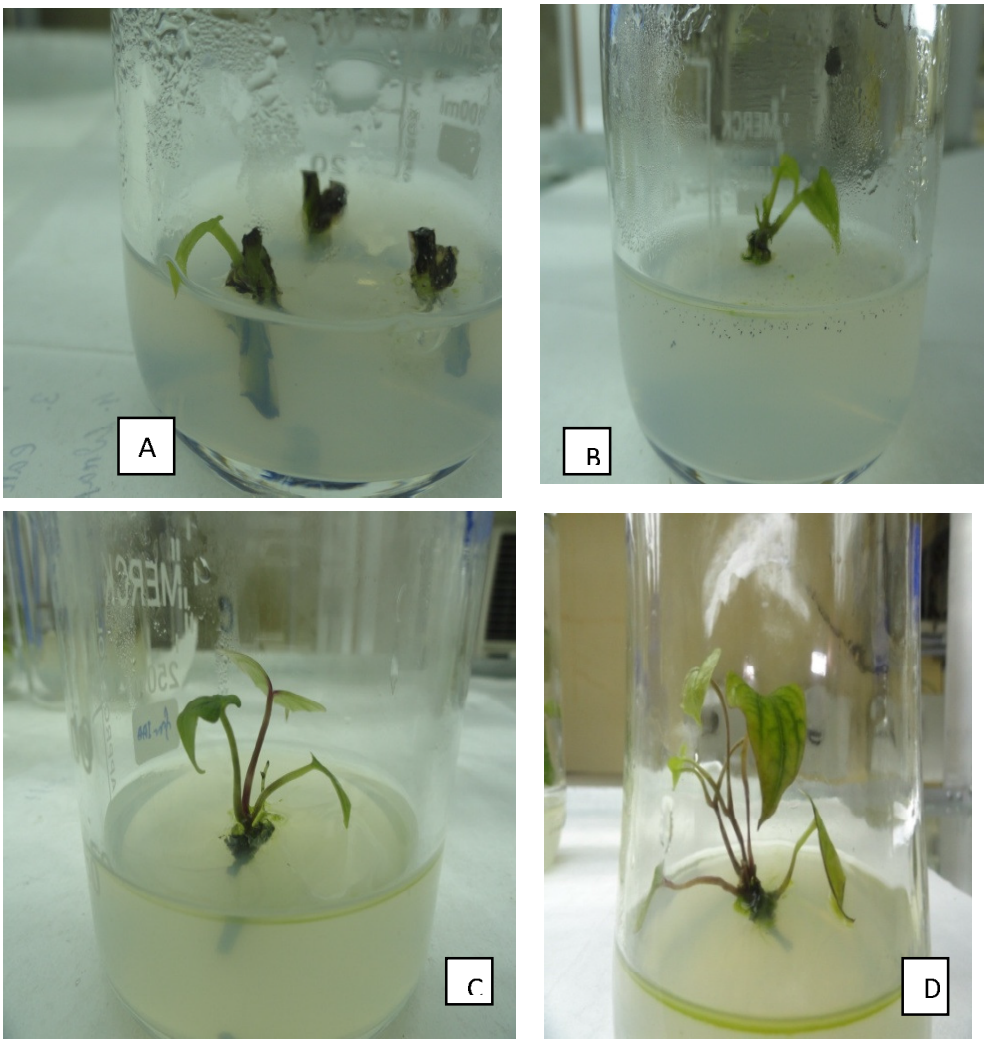


Plate 6: [A] Axillary bud proliferation of *Dioscorea alata* L. on basal MS media (control) after 35 days. [B] plant on same media after 2 month [c] after 3 month.[D] Plant on T2 treatment (2mg/L IAA+ 1.0mg/L KN) after 3 month.

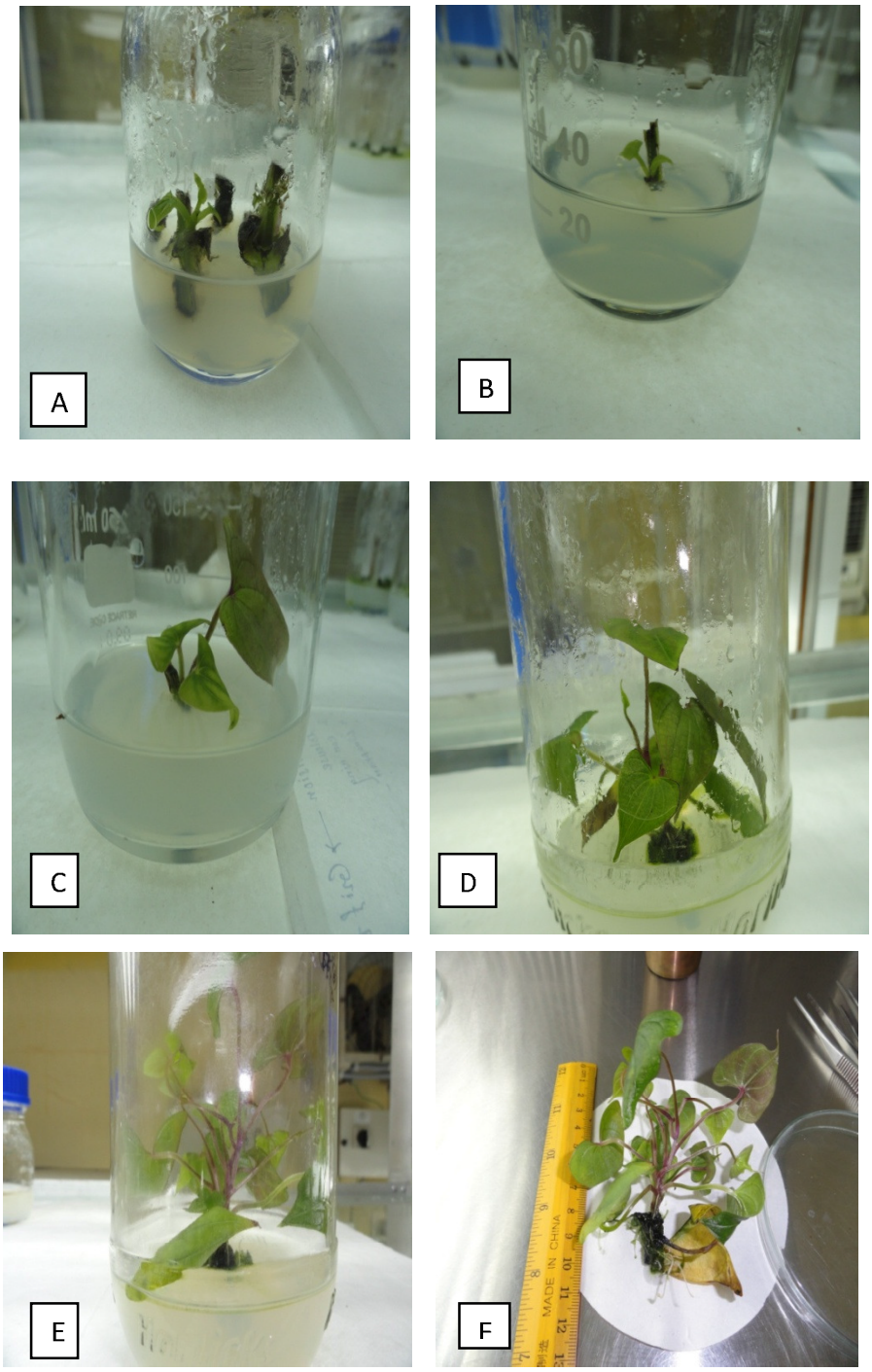


Plate 7 : [A]Axillary bud start proliferation of *Dioscorea alata* L. on MS + 1.5mg/L KN + 2.0 mg/L IAA [B] After 15 days [C] 1 month [D] 1month 15 days [E] 2 month 15 days[F] Showing length of shoot.

4.3 *Arundina graminifolia* (D. Don) Hochr.

In vitro multiplication of *Arundina graminifolia* was investigated during this study and for this purpose a series of experiment was performed.

Effect of Sterilants :

Mercuric chloride (different concentration) was used for explants sterilization. Nodal segments were first washed with tap water and then treated with 0.1% (w/v) for 5 minutes. Explants treated with less than 0.1% concentration not respond properly.

Axillary bud proliferation:

MS media supplemented with NAA (0.5mg/L, 1mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L) used for axillary bud proliferation and for this purpose sterilized nodal segments were inoculated in the medium.

Explants cultured on MS medium without NAA failed to proliferate axillary bud. Addition of low concentration of NAA (0.5mg/L) was effective in bud breaking and it took 38-40 days for axillary bud proliferation which one was the least time period required for bud proliferation but in case of explants response MS media containing 0.5mg/L NAA failed to show effective response. The explants cultured on MS+ 0.5mg/L NAA showed explants response 16% which one was not a satisfactory result. Increasing concentration of NAA increased the required time period for bud proliferation. Percentage of explants response was highest (50) in MS medium with 1.0mg/L NAA.

Media containing 1.0mg/L showed best result. Explants cultured on this medium proliferated within 45-47 days for bud proliferation.50% explants showed positive response cultured on this media.

Among the other treatments media containing 1.5mg/L NAA required 50-52 Days for bud proliferation and 43% explants showed positive response (table no. 4.8)

Multiple shoot proliferation:

Bud proliferation was enhanced by the addition of NAA. For the further growth, cultured plants were transferred to the media supplemented with NAA and kinetin. In this series of experiments different media formulation was tried for multiple shoot initiation. After bud proliferation explants were transferred to the media containing different concentration of NAA (0.5mg/L,1.0mg/L,1.5mg/L) and kinetin (1.0mg/L, 2.0mg/L, 2.5mg/L) and response of the cultured plants were recorded in terms of number of shoot per explants and shoot length. Data obtained from this experiment was tabulated in table no. 4.9.

Among the different combination, media supplemented with 1.0mg/L NAA and 2.5 mg/L KN elicited optimal response in which an average 5.33±0.26 number of shoot produced with shoot length 3.50 cm (mean). More concentration of NAA and kinetin reduce shoot length as well as shoot number.

Second highest shoot proliferation was observed in the MS media with 2.0mg/L kinetin and 0.5mg/L NAA in which 4.33 (mean) number of shoot was produced with shoot length 3.13 cm (mean).

***In vitro* Rooting:**

Experiments were also done in order to optimize the rooting medium. The number of root formed per shoot and time required for root initiation was significantly different among the different concentration of growth regulators.

To study the effect of auxins on root initiation two types of auxins (IAA,NAA) was added with half strength MS medium. IAA in different concentrations (2.0mg/L, 2.5mg/L, 3.0mg/L) combined with half strength MS medium. 3.0mg/L IAA when combined with half strength MS medium produced highest root length (4.7 cm) with root number 6 (table 4.10).

MS medium supplemented with NAA in different concentrations (0.5mg/L, 1.0mg/L,1.5mg/L, 2.0mg/L) and this medium was used for root induction. Media with 1.0 mg/L NAA produced root with root length (3.70 cm).

Acclimatization

Well developed plants were transferred in the sterile vermiculite mixture for one month. Cultured plants were transferred to the potting mixture containing Brick bats+ leaf molds +charcoal +Dried moss+ sand in 1:1:1:1:1ratio. After one month of field transfer 87% plants survived.

Table 4.8 Effect of various concentration of NAA on axillary bud proliferation of *Arundina graminifolia* (D.Don)Hochr. (Data collected after 70 days, 10 replicates for each treatments, repeated thrice).

Explant	Treatments	Days to bud break	Percentage of explants response
Nodal segments	MS+ o mg/L NAA	-	-
	MS+0.5mg/L NAA	38-40	16
	MS+ 1.0mg/L NAA	45-47	50
	MS+1.5mg/L NAA	50-52	43
	MS+2.0mg/L NAA	59-61	25
	MS+ 2.5mg/L NAA	68-70	18

Table 4.9 Effect of growth regulators on shoot formation in nodal explants of *Arundina graminifolia* (D. Don).Hochr.(Data collected after 6 month, 10 replicates for each treatment, repeated thrice).

Treatments	Conc. of Hormone		Percentage of explants response	Mean no. of shoot per explant	Mean Shoot length (cm)
	NAA	KN			
T_1	0.5	1.0	40	1.66±0.35	2.50±0.17
T_2	1.0	1.0	27	1.66±0.35	1.26±0.07
T_3	1.5	1.0	31	2.33±0.26	1.20±0.14
T_4	0.5	2.0	50	4.33±0.71	3.13±0.09
T_5	1.0	2.0	39	3.66±0.71	2.23±0.11
T_6	1.5	2.0	18	3.66±0.26	1.80±0.09
T_7	0.5	2.5	21	4.0±0.46	2.56±0.09
T_8	1.0	2.5	52	5.33±0.26	3.50±0.17
T_9	1.5	2.5	17	1.33±0.31	2.50±0.21

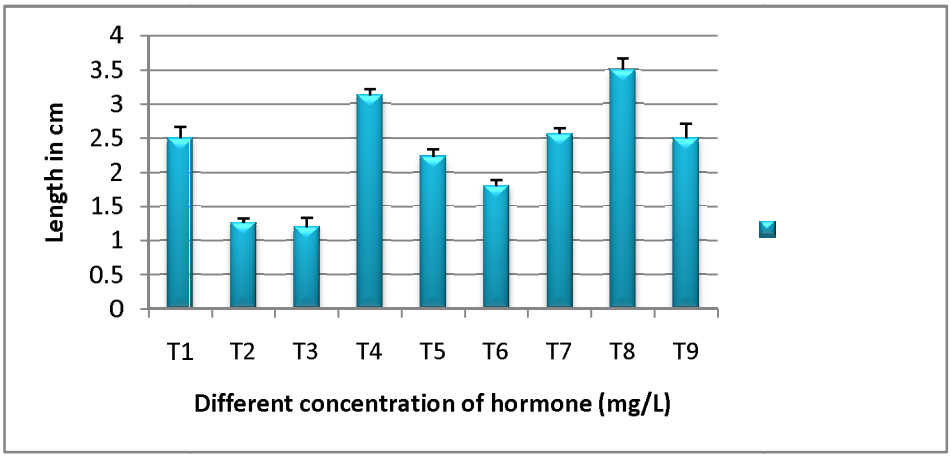


Fig. 12 : Effect of different concentration of hormone on shoot length of *Arundina graminifolia* (D.Don)Hochr.

Table 4.10 Influence of different concentration of IAA and NAA on rooting of *in vitro* generated shootlets of *Arundina graminifolia* (Data scored after 6 month of inoculation, 10 replicates per treatment, repeated thrice)

Treatments	Conc. of hormone mg/L	Days to root initiation	% of explants response	Mean root no.	Mean root length (cm)
T0	MS	-	-	-	-
T1	MS +2.0mg/L IAA	32-34	50	5.33±0.26	3.0±0.09
T2	MS+2.5mg/L IAA	30-32	52	4.33±0.26	3.53±0.07
T3	MS+3.0mg/L IAA	25-27	67	6.0±0.47	4.7±0.08
T4	MS+0.5mg/L NAA	31-33	43	3.66±0.26	2.53±0.11
T5	MS+1.0mg/L NAA	22-25	58	4.33±0.26	3.70±0.09
T6	MS+1.5mg/L NAA	38-40	62	4.0±0.46	3.2±0.21
T7	MS+ 2mg/L NAA	36-38	49	3.0±0.81	2.9±0.04

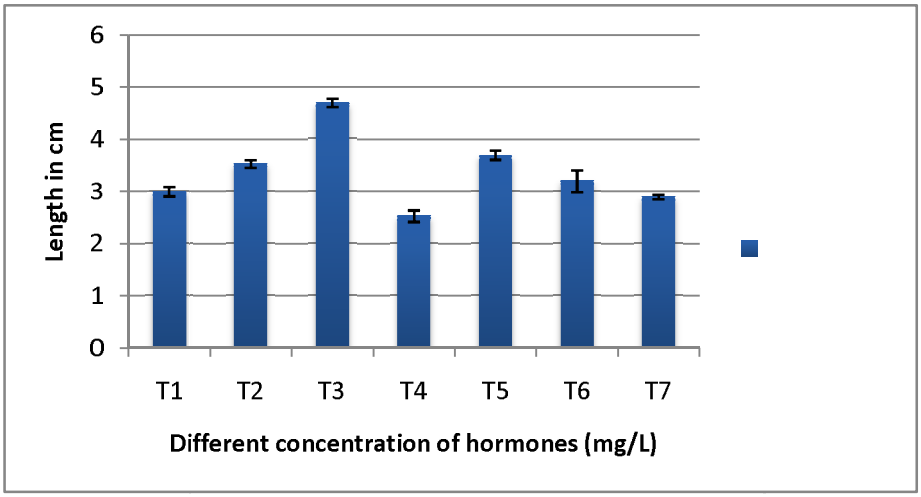


Figure13: Effect of different concentration of hormone on root length of *Arundina graminifolia* (D.Don)Hochr.

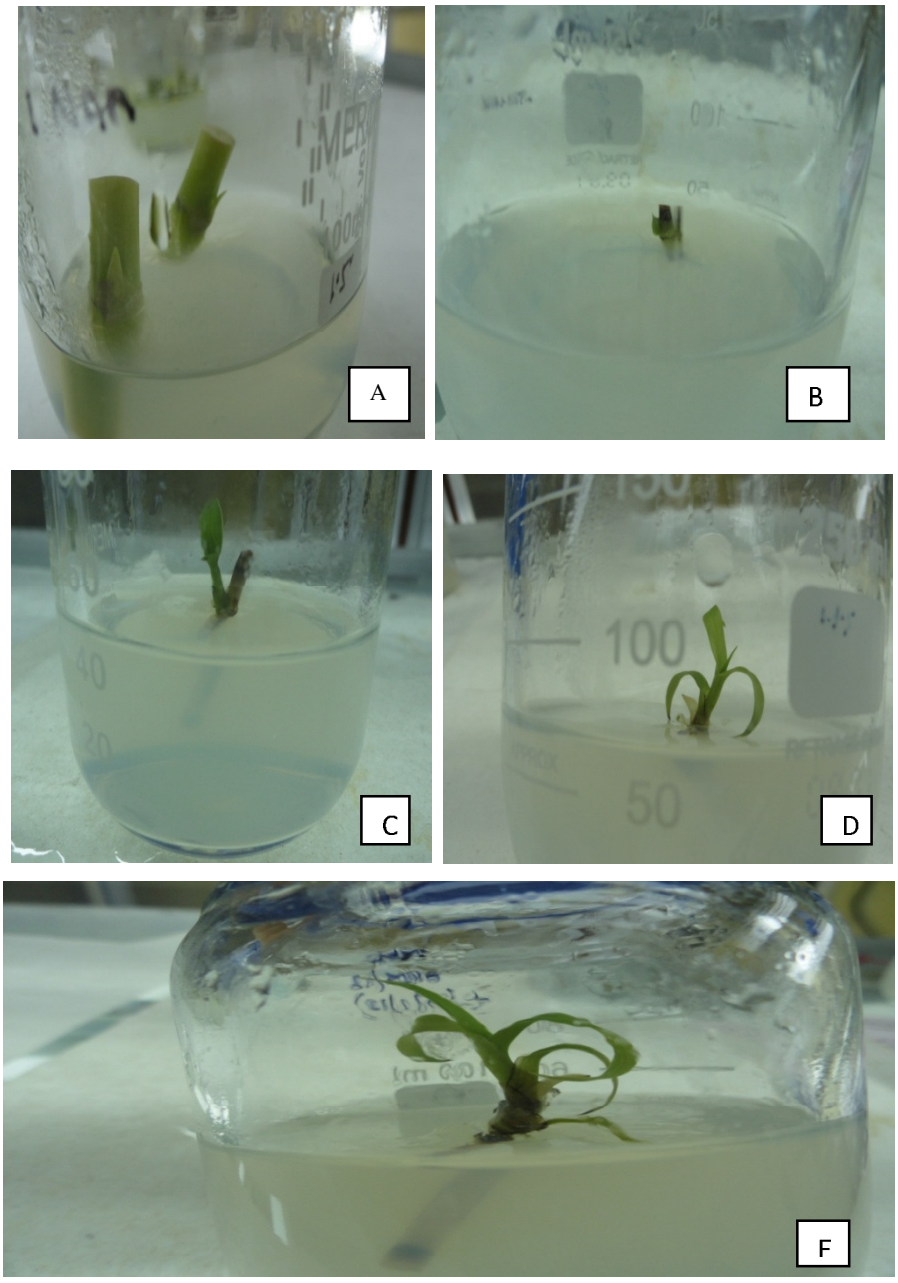


PLATE 8: [A] Inoculated nodal segment. of *Arundina graminifolia* (D. Don) Hochr. [B]Axillary bud start proliferation after 2 month on MS+ 2.0mg/L NAA. [C] Plant after 3 month on MS + 2.5 mg/L KN + 1.0 mg/LNAA [D] after 5 month on same media [E] after 6 month.

4.4 *Kaempferia parviflora* Wall ex. Baker

Rhizome buds of *Kaempferia parviflora* was used as explants for *in vitro* propagation. 0.1% (w/v) mercuric chloride ($HgCl_2$) was used for 9 minutes to remove all microbial contamination. Different plant growth regulators are used for shoot and root initiation.

For shoot induction two types of cytokinin (Kinetin, BAP) combined with NAA and added with MS medium. Different concentration of kinetin (1.0mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L, 3.5mg/L) added with 1.0mg/L NAA and observed the effect of growth regulators on shoot initiation. MS media supplemented with 2.5mg/L KN and 1.0mg/L NAA was best for shoot induction among the different treatment tried. Shoot with shoot length 7.50 cm was recorded on the medium containing 2.5mg/L KN + 1.0mg/L NAA which produced 2 shoot and 8-10 days required for shoot initiation where 85% cultured explants showed positive response.

Media supplemented with 3.0mg/L KN and 1.0mg/L NAA produced shoot with shoot length 7.50cm with 2 number of shoot (Table 4.11). 72% explants cultured on this medium showed positive response and it required 10-13 days for shoot initiation.

BAP when combine with NAA also enhanced shoot induction. Different concentration of BAP (1.0mg/L, 1.5mg/L, 2.0mg/L,2.5mg/L,3.0mg/L) was added with 1.0mg/L NAA. Explants cultured on MS media supplemented with 2.5mg/L BAP + 1.0mg/L NAA showed best result. 11.20 cm (Table 4.12) long shoots produced on culture medium with BAP (2.5mg/L)and (1.0mg/L) NAA with shoot number 2 and it required only 6-7 days for shoot initiation where 89% cultured explants showed positive response.

For *in vitro* root induction two types of auxins (IAA, NAA) was tried to observe the effect of both growth regulators on root initiation and for this purpose different concentration of IAA (0.5mg/L,1.0mg/L,1.5mg/L,2.0mg/L) was added with MS medium. Satisfactory results were observed on media containing 2.0mg/L IAA. Within 5-7 days root induction was observed on media with 2.0mg/L IAA and produced roots were 9.2 cm (Table 4.13) long.

Different concentration of NAA (0.5mg/L1.0mg/L, 1.5mg/L,2.0mg/L) was added with MS medium to observe the effect of NAA on root growth. Micro shoots cultured on MS medium with 1.5mg/L NAA produced roots with root length 8.06cm with 12 number of root (Table 4.13). Among the various tried concentration 1.5mg/L NAA influenced the root growth effectively.This experiment help to developed plants were transferred in the sterile vermiculite mixture for one month. Cultured plants were transferred to the potting mixture containing soil+ leaf molds in 1:1ratio. After one month of field transfer 97% plants survived.

Table.4.11. Effect of growth regulators (KN and NAA) on shoot formation of *Kaempferia parviflora* Wall Ex. Baker (Data collected after 4 month of inoculation , 10 replicates per treatment, repeated thice.)

Treatment	Hormone conc.(mg/L)	Days to bud break	Percentage of explants response	Mean Shoot no.	Shoot length (cm)
T_0	MS+0mg/L KN+0mg/L NAA	-	-	-	-
T_1	MS+1.0mg/L KN+1.0mg/L NAA	14-12	70	1.33±0.31	5.16±0.14
T_2	MS+1.5mg/L KN+1.0mg/L NAA	14-12	54	1.66±0.26	5.90±0.12
T_3	MS+2.0mg/L KN+1.0mg/L NAA	15-18	50	2.66±0.54	6.56±0.11
T_4	MS+2.5mg/L KN+1.0mg/L NAA	10-13	85	2.66±0.54	7.43±0.19
T_5	MS+3.0mg/L KN+1.0mg/L NAA	8-10	72	2.66±0.54	7.5±0.30
T_6	MS+3.5mg/L KN+1.0mg/L NAA	12-15	54	2.33±0.26	6.63±0.16

Table 4.12.Effect of growth regulators on shoot formation in *Kaemferia parviflora* Wall ex.Baker (data collected after 3 month of inoculation, 10 replicates per treatment, repeated thrice)

Treatment	Hormone conc.(mg/L)	Days to bud break	Percentage of explants response	Shoot no. ±SE	Shoot length (cm) ± SE
T_0	MS+0mg/L BAP+0mg/L NAA	-	-	-	-
T_1	MS+1.0mg/L BAP+1.0mg/L NAA	12-14	51	1.33±0.26	5.52±0.25
T_2	MS+1.5mg/L BAP+1.0mg/L NAA	11-13	53	1.66±0.35	7.20±0.03
T_3	MS+2.0mg/L BAP+1.0mg/L NAA	9-10	61	3.0±0.47	8.86±0.21
T_4	MS+2.5mg/L BAP+1.0mg/L NAA	6-7	89	2.66±0.26	11.20±0.12
T_5	MS+3.0mg/L BAP+1.0mg/L NAA	9-10	76	2.33±0.26	10.46±0.24
T_6	MS+3.5mg/L BAP+1.0mg/L NAA	14-15	64	1.66±0.26	8.13±0.06

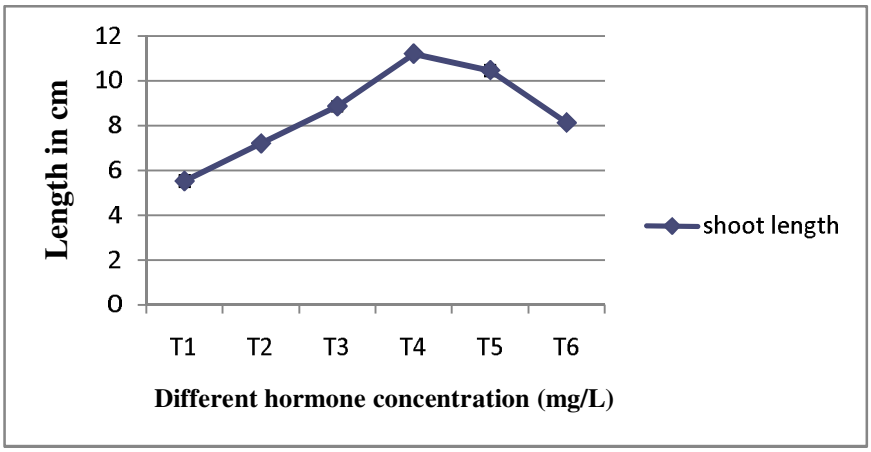


Fig. 14. Effect of different concentration of NAA and BAP on shoot length of *Kaemferia parviflora* Wall ex. Baker.

Table 4.13. Effect of growth regulators on root formation in *Kaemferia parviflora* Wall ex. Baker (Data collected after 3 month of inoculation,10 replicates per treatment, repeated thrice)

Treatment	Conc. of hormone	No. of root per explants ± SE	Root length ± SE (cm)	Days to root initiation
T_1	MS+0.5mg/L IAA	5.0±0.47	6.03±0.18	7-9
T_2	MS+1.0mg/L IAA	6.33±0.48	7.80±0.09	5-7
T_3	MS+1.5mg/L IAA	9.0±0.47	8.8±0.07	4-6
T_4	MS+2.0mg/L IAA	8.66±0.26	9.2±0.19	5-7
T_5	MS+0.5mg/LNAA	4.0±0.47	3.9±0.14	7-9
T_6	MS+1.0mg/LNAA	9.33±0.73	5.7±0.21	4-6
T_7	MS+1.5mg/LNAA	12.0±0.47	8.06±0.14	4-6
T_8	MS+2.0mg/LNAA	7.33±0.71	6.56±0.09	10-12

T_1 = MS + 0.5 mg/L IAA T_2 = MS + 1.0 mg/L IAA

T_3 = MS + 1.5 mg/L IAA T_4 = MS + 2.0 mg/L IAA

T_5 = MS + 0.5 mg/L NAA T_6 = MS + 2.0 mg/L NAA

T_7 = MS + 1.5 mg/L NAA T_8 = MS + 2.0 mg/L NAA

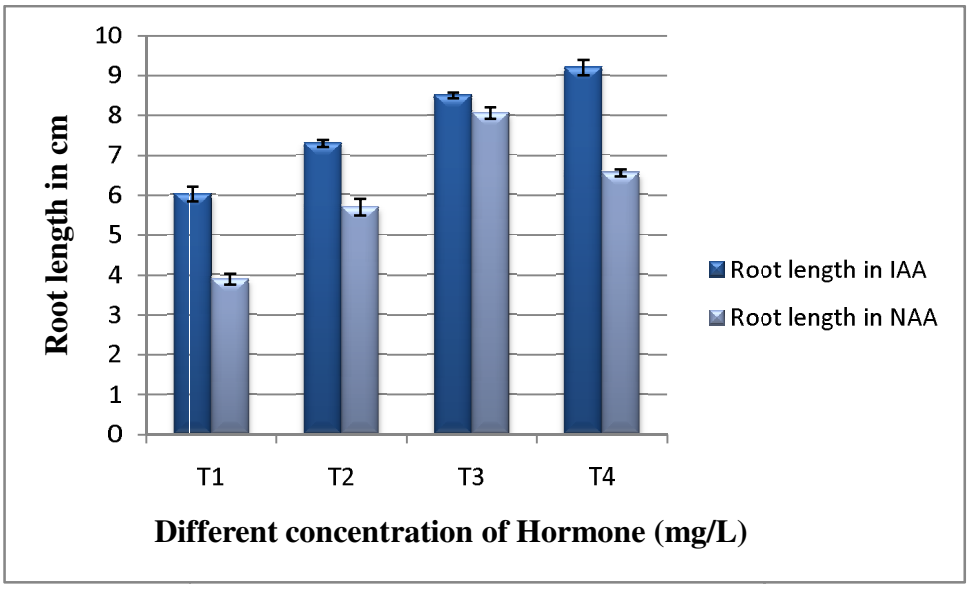


Figure. 15: Effect of different concentration of hormones in root length of *Kaemferia parviflora* Wall ex. Baker.

T_1 = MS + 0.5 mg/L IAA/ NAA T_2= MS + 1.0 mg/L IAA/NAA

T_3 = MS + 1.5 mg/L IAA/ NAA T_4 = MS + 2.0 mg/L IAA / NAA

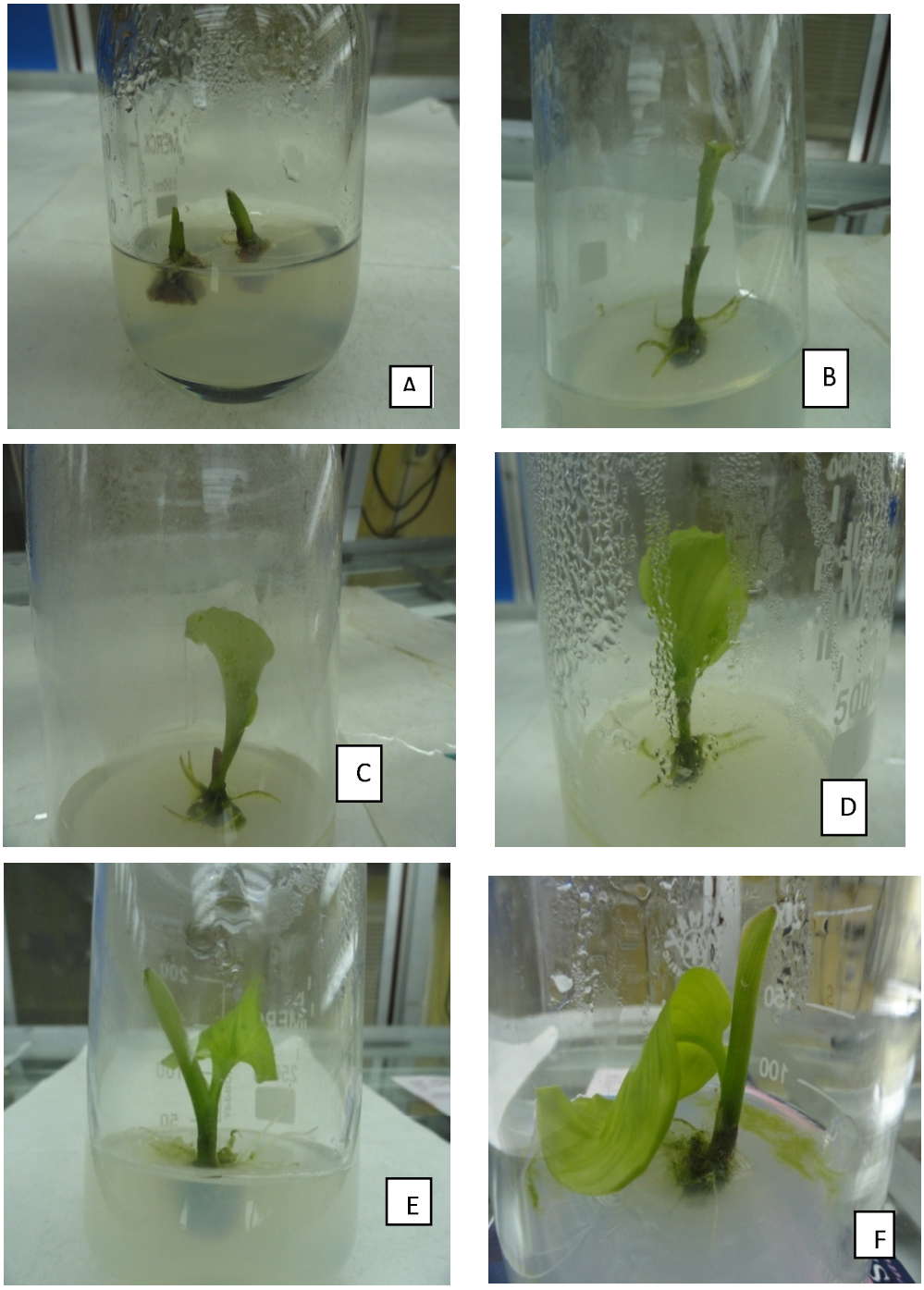


Plate 9 : [A]**Inoculated explants of *Kaemferia parviflora* Wall ex. Baker. on MS+ 2.5mg/L BAP+1.0mg/L NAA [B] plant after 15 Days on same media [C] After 30 Days of inoculation. [D] 45 Days [E] 60 Days [F] 90 Days.**

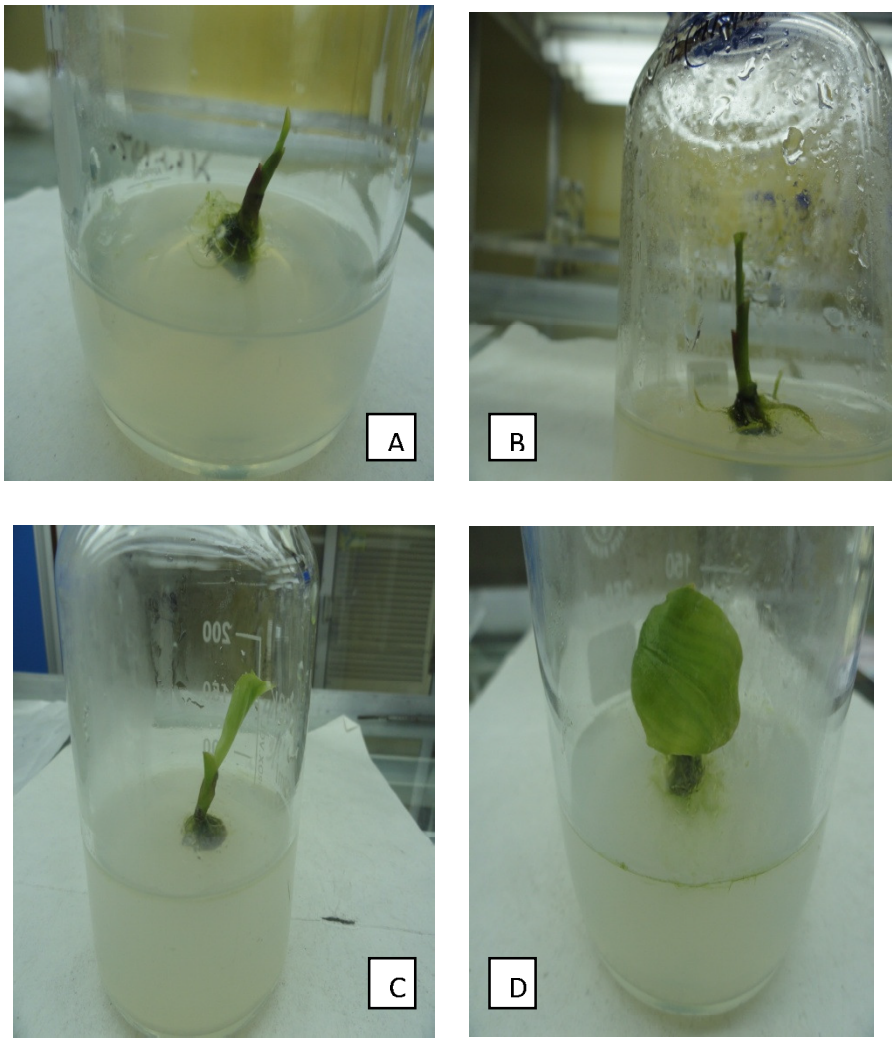


Plate 10: [A] Explant of *Kaemferia parviflora* Wall ex. Baker. after 1month on MS+2.5mg/L KN+ 1.0 mg/L NAA [B] plant after 1 month on same media [C] after 2 month [D] 3 month.

Plate 11. Plants After Field Transfer [A] *Cyathea gigantea* Wall. ex. Hook. (10 month) [B] *Dioscorea alata* L. (4 month) [C] *Arundina graminifolia* (D. Don) Hochr. (7 month) [D] *Kaempferia parviflora* Wall. Ex. Baker. (4 month)