6. Conclusion

A. *Cyathea gigantea* Wall ex. Hook

The total work of the present investigation systematically summarized and concluded as follows:

1. In case of *Cyathea gigantea* Wall Ex. Hook spores was selected as explants and spores were sterilized with 30% (w/v) sodium hypochlorite. Spores were treated with sodium hypochlorite for seven minutes.

2. After 25-27 Days of inoculation spores were start to germinate and produce heart- shaped prothallus on full strength MS medium and 72% spores germinate on MS medium.

3. MS medium was supplemented with different concentration of IAA (0.5mg/L,0.6mg/L,0.7mg/L,0.8mg/L, 0.9mg/L) for further growth of the gametophyte. Prothallus cultured on MS medium with 0.8mg/L IAA was best for gametophyte growth as highest fresh weight (136mg) after four month was observed on this media supplementation. The optimum dose of IAA concentration was determined by recording the growth of prothallus in terms of fresh weight (mg) for ten numbers of prothallus after four month. After that the optimum dose of IAA (0.8mg/L) was combined with various concentration of Kinetin (1.0mg/L, 2.0mg/L, 3.0mg/L, 4.0mg/L,5.0mg/L) with MS medium to observe effect of growth regulators on the growth of sporophyte.

4. MS medium supplemented with 0.8mg/L IAA and 5mg/L kinetin produced sporophyte with highest sporophyte length (20.29±0.23 cm) after 10 month.

5. MS media supplemented with different growth regulators like Indole-3 acetic acid (IAA) and kinetin was highly suitable for the propagation of *Cyathea gigantea* . This standardized protocol help to conserve this threatened tree fern from expected extinction.

B. ***Dioscorea alata*** **L.**

1. For *in vitro* propagation of *Dioscorea alata* nodal segments were used as explants. 0.1% mercuric chloride ($HgCl_2$) was used to sterilize the nodal segments for 7-8 minutes. MS medium was used for culture the explants.

2. The effect of various concentration of IAA (0.5mg/L, 1.0mg/L, 1.5mg/L,2.0mg/L,2.5mg/L) was recorded. Addition of low concentration (0.5mg/L) of IAA was less effective for bud breaking and it took 28-30 days for proliferation. Nodal segments cultured on MS medium supplemented with IAA (2.0mg/L) proliferate within 7-9 days and percentage of explant response (68) also very satisfactory.

3. After bud proliferation, for further growth, cultured plants were transferred to the media supplemented with kinetin and auxin. Response of different concentration of kinetin (0.5mg/L, 1.0mg/L, 1.5mg/L,2.0mg/L,2.5mg/L) with 2.0mg/L concentration of IAA was recorded in terms of number of shoot and shoot length. MS media supplemented with 1.5 mg/L kinetin and 2.0mg/L IAA elicited optimal response in which an average 7.7±0.29 shootlets with a mean shoot length of 9.90±0.11cm per explants was recorded.

4. The well grown shoots were transferred to half strength MS medium containing IAA. The rooting response of shoots in different concentration of IAA (0.5 mg/L, 1.0 mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L) was measured in terms of days required for root initiation, mean number of root per shoot, and mean root length. Half strength MS media supplemented with 2.5 mg/L IAA was effective for root initiation and it produce an average 6.6±0.16 number of shoot per explants with highest root length.

5. This work proposes an economic technique for propagation of *Dioscorea alata* which help to conserve the plant for expected extinction.

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

1. Nodal segments about 0.5-1cm length containing single node of *Arundina graminifolia* was used as explant for *in vitro* propagation.0.1% mercuric chloride was used for sterilization of explants and explants were treated for 5 minutes.

2. MS media supplemented with different concentration of NAA (0.5 mg/L, 1.0mg/L, 1.5mg/L,2.0mg/L, 2.5mg/L) to enhance the axillary bud proliferation. Nodal segment cultured on MS media with 0.5 mg/L NAA required minimum time (38-40 days) for axillary bud proliferation but highest percentage of explants response (50) was recorded in MS media supplemented with 1.0mg/L NAA.

3. After axillary bud proliferation cultured plants 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) for shoot induction and shoot growth. Highest number of shoot (5.33±0.26) with highest shoot length (3.50±0.17) was observed on MS media containing 1.0mg/L NAA and 2.5mg/L kinetin.

4. Two types of auxins (NAA, IAA) were used to observe the effect of both on root initiation. When MS media supplemented with 3.0mg/L IAA produced root with root length 4.7±0.02 cm.NAA at concentration 1.0mg/L was effective for root initiation and produced root with root length 3.70±0.09 cm.

D. *Kaempferia parviflora* Wall Ex. Baker

1. Rhizome bud was used as explants for *in vitro* propagation of *Kaempferia parviflora* and the explants was sterilize with 0.1% mercuric chloride (w/v). Explant was treated with mercuric chloride for 2 minutes to remove all types of microbial contamination.

2. For shoot induction two types of cytokinin (kinetin and BAP) combined with 1.0mg/L NAA and added with MS medium.MS medium supplemented with 1.0mg/L NAA and 3.0mg/L KN was best for shoot induction among the different treatment tried. Shoot with shoot length 7.50 cm was recorded on the medium containing 3.0mg/L KN+ 1.0mg/L NAA which produced 2.66 (mean) shoot and 8-10 Days required for shoot initiation.

3. Explant cultured on MS media supplemented with 2.5mg/L BAP+1.0mg/L NAA produced shoot with shoot length 11.20cm (mean) and it required only 6-7 Days for shoot initiation.

4. Two types of auxins (IAA, NAA) was tried to observe the effect of both 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.MS media with 2.0mg/L IAA induce root with root length 9.2cm.

5. Different concentration of NAA (0.5mg/L, 1.0mg/L,1.5mg/L,2.0mg/L)was added with MS medium to observe the effect of NAA on root growth.MS media with 1.5mg/L NAA was effective for root growth and shoots cultured on this media produced 12 (mean) number of root with root length 8.06cm(mean).