5. Discussion

5.1 *Cyathea gigantea* Wall ex. Hook

In this study spores were used as explant for *in vitro* culture. According to Linsay (1994) fern propagation by using spore is a reliable method and considered more advantageous than the vegetative propagation. To sterilize the spores 30% sodium hypochlorite for 7 minutes was used. Less concentration or more exposure to surface sterilants directly affect the germination rate of the spore. Fern propagation by using spore depends on many factors such as viability and storage of spores, media, sterilization of spores, temperature, pH range, gametophyte and sporophyte interaction (Kaur, 1991). Surface sterilize spores started to germinate within 25 – 27 days in MS medium (Full strength) and only 12 – 14 days required for germination in Fern Propagation medium (Full strength).Full strength MS medium was used during this study where half strength MS medium is preferred in previous experiment (Fernandez *et al*., 1997a, Kyte and Kleyn, 1996).Spore germination period vary from few days to a year (Doughlas and Sheffield, 1992). Smith and Yee (1975) observe *Nephrolepis* spores germinate in 3 – 4 days in culture while in ophioglossaceae spore germinate after 6 month of inoculation (Whittier,1981). In present study spores of *Cyathea gigantea* germinated after 25-27 days on full strength MS medium and germination percentage was 72% *Helminthostachys* species are known to take 8 month to germinate (Hedge and Dsouza, 2000). According to Page (1979) in the majority of Cyatheaceae species the spore germination abilities decrease after a few weeks of storage. In *Cyathea delgadii* spores lost their viability for germination during 2 months at 12°c in presence of low humidity (Randi and Felippe,1988).

Spores of *C. gigantea* was germinate in full strength MS medium without sugar and 72% germination was observed in MS medium while in Fern propagation medium 58% germination was observed. Growth and proliferation needs sugar supplementation but germination of spore in culture medium is inhibited by the sugar supplemention (Renner and Randi 2004) and

the result of the present work was similar with the earlier reported work. Low concentrations of micro and macro salts was suitable for spore germination and gametophyte development as reported earlier in *Cyathea australis(*Goller and Rybczynski,1995),*Dicksonia solviania*(Khoo and Thomas,1980).MS medium was taken for further culture of gametophyte and sporohyte because germination percentage was maximum in it. Knop and MS medium is best for growth and differentiation of fern as reported by Cheema (2005).

Media containing IAA at concentration 0.8mg/L and kinetin at 5 mg/L produced sporophyte with 20.29 cm (table 4.4) length. At higher concentration explants started to browning which confers detrimental effect of the dose (Higuchi and Amaki, 1989).For multiplication presence of auxin and kinetin in medium seems to be very important and the ratio between auxin and kinetin also very valuable for proper multiplication of plant (Bertrand *et al*.,1999, Fernandez *et al* ., 1999). Many reports are there which reveal that application of growth regulators enhance and suppress the growth of plant (Fernandez and Revilla, 2003). Media without auxin (NAA) produce much shoot and NAA is not essential for shoot production, only kinetin is necessary for shoot production of fishtail fern (Beck and Caponetti, 1983). Result of the present study was totally different from previously cited work where in present work IAA and kinetin was essential for shoot induction.

Survey of literature highlighted the point that *in vitro* propagation of *Cyathea gigantea* was not tried by any other worker till now so here I compare my work with other species of *Cyathea.*

Shukla and Khare (2012b) propagated threatened tree fern *Cyathea spinulosa* using Parker and Thompson medium. Highest number (12.5±0.45) of shoots were developed from callus on P&T media with 8.87µM BAP and 2.21µM 2,4-D and highest shoot elongation was observed on P&T media with 4.5µM BAP and 5.36µM NAA. Result of the experiment was completely different from present work. In present investigation MS media was used for *in vitro* propagation. MS media supplemented with 0.8mg/L IAA and 4mg/L Kinetin was highly efficient for shoot elongation. Maximum sporophyte length (20.29±0.23) was observed on this combination of growth regulators.

A micropropagation protocol of *Cyathea gigantea* is successfully established in this work in which spores are used as explants. It is the fastest and most economic method for *in vitro* propagation and using this propagation technique tree fern can be regenerated in laboratory conditions which are vanishing from their natural habitat.

5.2. *Dioscorea alata* L.

Micropropagatoion of various plant species including many medicinal plants has been described by many authors during last two decades (Skirvin *et al*., 1990). In the present investigation *in vitro* culture of *Dioscorea alata* was done and for those nodal segments were used as explants.MS media supplemented with IAA and kinetin was used for root and shoot growth. In this work we cultured *Dioscorea alata* in solid MS medium similarly *in vitro* propagation of other yam species is tried by Martine and Cappadocia (1992) using solid medium.

Nodal segments of *Dioscorea alata* was cultured on MS media supplemented with auxin (IAA), within 7-9 days .axillary bud proliferate in culture media supplemented with IAA (2mg/L) and results of many literature indicates that addition of either IAA or NAA in culture medium improved shoot growth in a number of species and it completely support the effect of IAA on bud breaking in present investigation.MS media was selected as culture media. Chen *et al.*,(1995) when work with *Eucommia colomoides* report that MS medium is effective than WPM (Woody Plant Medium) and their report support the findings of present work where also MS medium was used as culture medium and satisfactory growth was observed using nodal segments as explants. For axillary bud proliferation nodal segments culture is recommended (Narula *et al*.,2007) for rapid clonal propagation when work with *Dioscorea bulbifera.*

In the present work two growth regulators was added with full strength MS medium and results indicated that MS media supplemented with Kinetin (1.5mg/L) induce maximum number of shoot per explant (mean no. 7.7) with highest shoot length 9.90 cm. Effects of Kinetin and indole acetic acid concentration was showed in the table 4.6 and result of this experiment was opposed by the report which indicates for shoot proliferation cytokinin require

optimal quantity in many genotypes but addition of low concentration of auxin with cytokinin trigger shoot proliferation (Sengupta *et al* .,1984).In this study low concentration (1.5mg/L) Kinetin with high concentration (2.0 mg/L) IAA induce shoot proliferation, further increase of kinetin concentration after optimal concentration (1.5mg/L) decrease shoot length and result was supported by the work which shows that suppression of kinetin increase shoot length, node number and root length (Jha and Jha, 1998). In *Dioscorea composita* only the auxin NAA, IAA and IBA at 1.25mg/L and 2.5mg/L account for promotive effects on *in vitro* shoot growth (Ondo Ovono *et al*., 2007). Our experimental result was completely different from that the cited work. The effects of auxins and cytokinins on shoot multiplication of various medicinal plants are reported by many workers (Alizadeh *et al* .,1998; Ahuja *et al*.,1982;). 2mg/L kinetin + 1.0mg/L BAP+ 5mg/L NAA + 100Mg/L ascorbic acid is supplemented with MS media for shoot proliferation of *Dioscorea oppositifolia* and results shows 90% explants proliferate with highest rate of shoot multiplication (10.5shoot/explant)(Behera *et al*., 2009). In our study only one cytokinin (kinetin) was added with auxin (IAA)for shoot multiplication and IAA (2.0 mg/L) + Kinetin (1.5mg/L) induced highest root length 9.90 cm with 7.7 (mean) number of shoot per explant. Combination and interaction of BA and NAA plays an important role for in vitro propagation of nodal explants for multiple shoot induction is reported (Shin *et al.*, 2004). MS medium with 1.0 mg/L NAA and 0.5- 1.0 mg/L BA is best concentration for induction of multiple shoot bud in *Dioscorea opposita*. Present study revealed combination and interaction of Kinetin(1.5mg/L) and IAA (2.0mg/L)induced multiple shoot bud in MS media.

Half strength MS media supplemented with 2.5mg/L IAA was most efficient for root initiation and it produce roots (average 6.67) with average root length 8.33cm. Addition of IAA, IBA or NAA to MS medium produce rooting is observed (Barna and Wakhlu, 1988), result of this work support the observation of present work. Shoots culture on full strength MS medium with auxin produce callus at the base of the shoots is reported (Patra *et al*., 1998). The microshoots of various medicinal plants is rooted on only MS medium without growth regulators by many workers (Thomas and Maseena, 2006;

Saxena *et al*.,1998). Observation of this work showed difference with the result of recent study because no rooting was observed in auxin free MS medium. Rooting occur in presence and absence of NAA, IBA or IAA is observed and also recorded at higher concentration of NAA, IBA or IAA (5.0 - 10µM) induce root sooner than the lower concentration (0.1- 1.0µM/L) of IAA, NAA or IBA (Mao *et al* ., 1995). Findings of this experiment showed similarity with the result of the present work. Nodal segments cultured on MS medium with IAA at concentration 0.5mg/L required 60-62 days to initiate rooting but IAA at concentration 2.5mg/L require only 22-24 days for root initiation. No rooting is observed in shoots planted on auxin free basal medium, at lower concentration of auxin (NAA 0.5mg/L) produce very few or no root (Behera *et al*., 2009) and result of this work show similarity with present investigation because at low concentration of IAA (0.5mg/L) produced minimum number of root (average 2.56) with root length 1.33cm.

Explants culture in 2.0mg/l Kn + 1.0 mg/l BAP + 0.5 mg/l NAA showed highest rate of shoot multiplication is reported when work with *Dioscorea alata* (Borges *et al*., 2009) and rooting is more profuse in half strength MS basal media with 2.0mg/l NAA but in present investigation, it was clearly observed that MS media supplemented with only auxin (IAA 2.0 mg/l) was effective in axillary bud proliferation and it initiated shoot formation within 7-9 days. Kinetin was used to increase the shoot length. Explants cultured on media (MS + 2.0mg/L IAA + 1.5mg/L Kn) showed highest shoot length within three month. Auxin (IAA) was very effective in case of bud breaking as well as shoots formation. Number of shoot induced in per explant by the effect of IAA (2.0mg/L) is also very satisfactory, Kinetin (1.5mg/L) with IAA (2.0 mg/L) enhanced shoot multiplication and it also increase number of node.

For hardening the well rooted plants were transferred to sterilized plastic cups containing vermiculite and kept under controlled condition. Production of plantlets with profuse rooting in *in vitro* is essential for successful establishment of regenerated plants in soil (Ohyma, 1970). Later Plants were transferred to earthen pots containing mixture of Brick bats + soil + Charcoal + Dried moss + leaf mold (1: 1: 1: 1: 1) and survival rate was 85% - 87% after one month of hardening.

Here an attempt was made to propagate *Dioscorea alata* using minimum number of growth regulators. In this investigation an efficient micropropagation technique (1.5 mg/L IAA + 2.0 mg/L KN for shoting and 2.5 mg /L IAA for rooting) was derived which may be useful for raising quality plants of *Dioscorea alata* for commercial purpose in lowest cost.This technique pave the way not only for *ex situ* conservation but also for the restoration of genetic stock of the species.

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

Micropropagation is currently used all over the world with different species from fern to orchid. Maximum *in vitro* propagation of orchid was done by using seed. Nodal segments of *Arundina graminifolia* was used as explants for *in vitro* propagation. MS media was selected as culture media and for axillary bud proliferation MS medium was supplemented with different concentration of NAA (0.5mg/L, 1.0mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L) and nodal segments were inoculated in it. 50% cultured explants showed positive response on MS medium containing 1.0mg/L NAA within 45-47 days. MS medium supplemented with 0.5mg/L NAA required minimum time(38-40 days) for axillary bud proliferation but percentage of explant response was also very minimum(16) (table no. 4.8).

MS medium supplemented with 2.5mg/L kinetin + 1.0mg/L NAA was recorded as best medium for shoot growth and average 3.50 cm long shoots produced in this media composition with average 5.33 number of shoots per explants. In micropropagation of many orchid species the ratio and type of auxin and cytokinin play an important role as reported by Arditti and Ernst (1993). George and Ravishankar (1997) cultured *Vanilla planifolia* on MS medium containing 2mg/L BA and 1mg/L NAA using axillary bud as explants. Second highest root length (3.13cm) with root number 4.33 obtained in media composition MS+ 2.0mg/L KN + 0.5mg/L NAA.

MS mediua (half strength) supplemented with different concentration of IAA(2.0mg/L , 2.5mg/L,3.0mg/L) and NAA (0.5mg/L, 1.0mg/L, 1.5mg/L,

2.0mg/L)was used for root initiation.Satisfactory result was obtained on MS medium with 3.0mg/L IAA supplementation. Average 4.70cm long roots with 6.0number of roots were observed in MS medium with 3.0mg/L IAA.MS medium containing 1.0mg/L NAA was effective for root growth and it induced root with root length 3.70cm with 4.33 number of root.

Yan *et al* ., (2006) cultured *Cypripedium flavum in vitro* using Havais media and reported that BAP was not beneficial for root induction of *C. flavum* In this experiment we used MS media for propagation of *Arundina graminifolia* and our result also support the view that cytokinin was not efficient for rooting because without cytokinin only auxin induce rooting in micropropagated plants.

Chen *et al.,*(2002) used 3 mm long nodal segments of stem of *P. philippinese* for *in vitro* culture. According to the report half strength basal medium supplemented with 4.52µM 2,4-D induced axillary bud proliferation after 2 weekand same media was equally effective in shoot formation. After 22 week plants were ready for field transfer after 3 month of hormone free basal media. In the present work full strength MS medium was used for shoot proliferation and multiplication of shoot with different concentration of hormones. MS medum supplemented with different concentration of NAA was tried for axillary bud proliferation. MS media with 0.5mg/L NAA was highly effective for axillary ud proliferation in terms of time requirement. Axillary bud proliferate within 38-40 days. With the increasing concentration of NAA time required for bud breaking also increased. In terms of percentage of explant response 1.0mg/L NAA supplementation was more effective than 0.5mg/L NAA because 50% explant proliferated on 1.0mg/L NAA where only 16% explant showed positive response on 0.5mg/L supplementation (table no. 4.8). So the result of the earlier worker was completely different from the present findings. 4.52µM 2,4-D induced bud proliferation but result of the present work clearly indicated that MS media with NAA(0.5mg/L NAA, 1.0mg/L NAA) induced proliferation. Highest shoot length was observed on MS with 1.0mg/L NAA and 2.5mg/L Kinetin (table no. 4.9).

Kong *et al.,*(2007) worked with *Dendrobium strongylanthum* Rchb.f. and usedseed as explant. PLBs were formed on half MS medium with 0.2mg/L NAA. PLBs were cultured on half strength MS with 0.5 mg/L V-6 BA and shoot proliferation started within 30 days. Addition of 0.5mg/L NAA induced rooting according to the report. Findings of aforesaid worker support the result of present investigation. Here also NAA induced rooting and among the various concentration of NAA (0.5, 1.0, 1.5, 2.0mg/L) tried 1.0mg /L NAA was effective in terms of shoot length(3.70±0.09).

Kalimuthu *et al*., (2007) worked with *Oncidium* sp. using seed as explant on MS medium supplemented with BAP and NAA and reported that BAP and NAA enhanced shoot and root growth.

A procedure was formulated for *in vitro* propagation of *Dendrobium draconis* Rchb.f. using thin cross section of young stem and cultured on MS media with BA, NAA, Kinetin. Among two cytokinin was tested BA was found more effective than kinetin on PLBs formation and MS media with sucrose and coconut water was effective in shoot formation (Rangsayatron 2009). In this study shoot formation was achieved on MS media with NAA and Kn but without sucrose and coconut water.

Nayak *et al*., (1997) reported that TDZ (2.2-4.4µM) was more effective in shoot bud differentiation from shoot segment.MS media supplemented with 10.8µM IBA was reported as best in terms of rooting. In present work IAA and NAA was tried for root formation.

Sharma and Tandon(1992) worked with *D. wardianum* R. Warner and used MS+ 2.5mg/L BAP for PLBs production *in vitro.* Nagaraju and Parthasarathy (1995) propagated *Arundina bambusifolia* Lindl. and for shoot proliferation they used Raghavan and Torrey's (1964) medium without any growth regulators. The results of this experiment completely differ with the result of the present experiment. *Vanilla planifolia* Andr. was cultured by Kalimuthu *et al*.,(2006) using MS+ 1mg/L BAP + 150ml/L CW for shoot and root multiplication.

Mahendran and Narmathu (2009) propagated *Satyrium nepalense* . Don. and MS medium was highly effective in case of seed germination and PLBs formation was observed. Here for micropropagation of *A. graminifolia* MS medium was used.

Martin (2007) propagated *Arundina graminifolia* through protocorm-like bodies (PLBs) using node as explants. According to the report nodal explants were cultured on half strength MS medium + 6.97µM Kinetin effective for proliferation of the axillary bud or 13.3µM BA was also effective for axillary bud proliferation. PLBs (mean 5.4) formed when buds were cultured 44.4µM BA. Half-strength MS medium supplemented with 6.97µM kinetin enhanced shoot formation (89%) from PLBS. For rooting 1g/L activated charcoal was effective. In present experiment nodal segment was used as explant and for axillary bud proliferation NAA was used with MS medium. 1.0mg/L NAA was effective for axillary bud proliferation. 50% explant proliferate when nodal segments were cultured on MS medium supplemented with 1.0mg/L NAA within 45-47 days (table no.4.8).

In present study nodal segments were used for *in vitro* propagation through direct axillary bud proliferation and shoot multiplication, no PLBs formed during this propagation. MS media supplemented with 2.5mg/L KN and 1.0mg/L NAA was efficient for shoot induction (mean 3.5 cm) (table no.4.9).For root induction two types of auxin (IAA, NAA) with MS medium was used. 3.0mg/L IAA with MS medium induced root with highest root length 4.7 cm.MS medium with 1.0mg/L NAA induced highest root length with 3.2cm (table 4.10).

5.4 *Kaempferia parviflora* Wall Ex. Baker

Rhizome buds were selected as explant and 0.1% (w/v) mercuric chlorite was used to treat the explant for 9 minutes to sterilize the explant. Raju *et al*.,(2005) sterilized explant using 0.1% $HgCl_2$ for 15 minutes. To establish the asptic culture of turmeric 0.1% $HgCl_2$ was used by Rahman *et al.*,(2004) for 14 minutes. Explant sterilization by removing contaminants with chemicals

which are toxic to microorganism but non toxic to plants are the important factor for tissue culture (Hartmann *et al.*, 1997)

The rhizome buds were used as explants for *in vitro* culture of *Kaempferia parviflora* on MS medium supplemented with different concentration of (1.0mg/L, 1.5mg/L, 2.0mg/L, 2.5mg/L, 3.0mg/L, 3.5mg/L) BAP and Kinetin in combination with 1.0mg/L NAA for shoot multiplication. *In vitro* multiplication of Zingiberaceae already reported in *Alpinia galanga* (Inden and Asahir,1988)and *zingiber officinale* (Pillai and Kumar, 1982; Hosoki and Sagawa,1977; Bhagyalakshmi and Singh,1988).

Various concentrations of BAP and Kinetin were tried. MS media supplemented with 3.0mg/L KN +1.0mg/L NAA produced shoot with shoot length 7.5cm with mean number of shoot 2.66. 85% explant showed positive response within 8-10 days (table 4.11). Explants cultured on media containing 2.5mg/L BAP and 1.0mg/L NAA produced shoot with highest shoot length (11.20cm). The effect of BAP on shoot multiplication was reported in other Zingiberaceae species (Ikeda and Tambe,1989; Balchandran *et al*.,1990; Smith and Hamil,1996, Mohanty *et al*., (2011).Faridah *et al*.,(2011) observed highest shoot length on MS media containing 5.0mg/L BAP+2.0mg/L IAA and also 3.0mg/L BAP and 0.5mg/L IAA. In the present study we used NAA with kinetin and BAP in shoot multiplication media but Faridah *et al*., (2011) used IAA in media for shoot multiplication. MS media supplemented with 6mg/L BAP induced multiple shoot formation in *Z. zerumbet* (Stanly and Keng ,2007).

Plants cultured on MS media supplemented with 2.5mg/LBAP and 1.0 mg/L NAA produced highest shoot length (11.20 cm) (Table no.4.12) . BAP at 3mg/L was effective for shoot proliferation in turmeric (Balachandran *et al*., 1990). MS with 2mg/L BA produced maximum number of shoot in turmeric when shoot tips were used as explant.and 3mg/L BA was highly effective in shoot proliferation when rhizome buds were used as explant.(Dipti *et al*.,2005)

Dogra *et al.,*(1994) reported that large number of shoots were developed when rhizome buds was used as explant and cultured on 2.5mg/L BA and 0.5mg/L NAA.

Highest number of shoot was obtained on MS medium supplemented with 5-10 mg/L BAP from *Zingiber officinale* Roscoe (Noguchi and Yamakawa,1988).1.0mg/L BAP and 0.5mg/L IAA was found to be best for shoot and root multiplication in *Curcuma haritha* (Bejoy *et al*., 2006). MS medium with 0.50mg/L BAP and 3.0mg/L kinetin produced highest number of shoots per explant after 120 days of incubation in *Kaempferia galanga*(Vincent *et al*., 1992).

For root initiation two types of auxins NAA and IAA was added with MS medium. Various concentrations of NAA and IAA (0.5mg/L,1.0mg/L, 1.5mg/L, 2.0mg/L)was tried for root initiation. Media containing 2.0mg/L IAA produced 8.66 (mean) number of roots with highest root length 9.20cm. Raju *et al*., (2005) reported that MS media supplemented with 0.5 mg/L IAA induced maximum rooting in *Curcuma caesia* and *curcuma zedoaria.* Findings of the reported work and present work was completely different.

1.5mg/L NAA supplemented with MS medium also very effective for root formation. 8.06 cm long roots produced with mean root number 12 (mean) on MS medium with 1.5mg/L NAA supplementation (table 4.13).Highest rooting was observed in turmeric on MS media with 0.5mg/L NAA Dipti *et al.,* 2005). Present study revealed media containing 1.5mg/L NAA enhanced root proliferation. Report of earlier work was not similar with present study.

MS medium containing 5mg/L BAP and 2.0mg/L IAA produced highest mean number (17.0) of roots. 1.0mg/L BAP produced longest root (Faridah *et al*., 2011).0.50mg/L of IAA in combination with BAP found to be optimum for rooting in *B. pulcherrima* (Anish *et al*., 2008). IAA and IBA were effective for rooting in herbaceous plants and NAA was more effective for rooting in case of woody plants (Sharma, 2006).

Yusuf *et al*.,(2011) obtained maximum number of shoots (5) in MS medium supplemented with 2.0mg/L and 0.5mg/L NAA and also reported that MS medium with 5mg/L BAP + 2mg/L NAA showed low frequency of micropropagation in *Boesenbergia rotunda* (L.). In the present study we obtained highest shoot length (11.20cm) in MS + 2.5mg/L BAP + 1mg/L NAA.

MS medium supplemented with 2.5mg/L kinetin and 1mg/L NAA produced shoot with shoot length 7.50 cm and when 3.0mg/L kinetin + 1.0mg/L NAA was added with MS medium it produced shoot with shoot length 7.43 cm. The findings of the present investigation was totally supported by the report of the Balachandran *et al*.,(1990) where they observed that in case of *Zingiber officinale* higher concentration of kinetin was not suitable for growth. Loe *et al*., (2005) observed that on MS+ 20%coconut water + 3mg/L BA + 0.5mg/L IBA induce shoot formation in *Curcuma zedoaria* with an average of 5.6 number of shoot per explants. MS medium supplemented with 4mg/L BAP and 1.5mg/L NAA was reported as best medium for shoot multiplication of *C. caesia* (average 3.5 shoot per explants) and MS+ 1.0mg/L BAP + 0.5mg/L NAA was best for *C. zedoaria* (Bharalee *et al*., 2005).

MS medium containing 3mg/L BAP produced average 3.4,2.8 and 2.7 number of shoot per explants in *C. domestica, C.caesia and C. aeruginosa* respectively. MS medium containing 5mg/L BAP was highly effective for shoot multiplication of *C. aromatic.* Palai *et al.*,(1997) observed that shoot multiplication rate increased in the combination of two cytokinin along with auxin within 4 weeks of culture.

Mongkolchaipak *et al*.,(2006) cultured *kaempferia parviflora* and observed that optimal proliferation was on MS media supplemented with 7mg/L BAP and average 4.2 and 4.5 shoots produced after 1 and 2 month respectively.Maximum rooting was observed on solid MS with 1mg/L NAA. Survival percentage was recorded 98% and 96.5% after 1 and 2 month respectively.

Here for shoot regeneration kinetin BAP and NAA was used to understand the combined effect of both of these growth regulators on shoot proliferation. Maximum shoot length was observed on MS+3.0 mg/L KN+1.0mg/L NAA where percentage of explant esponse was 85% (table 4.11). 2.5mg/L BAP+1.0mg/L NAA induced highest shoot length 11.20 cm(mean) (table 4.12). Result of present investigation was completely different from earlier worker because earlier worker tried to propagate the plant with BAP only. Here my study revealed the combined effect of auxin and cytokinin. They

reported maximum rooting was observed on MS with 1.0 mg/L NAA but in present study highest rooting was observed on 1.5mg/L NAA supplementation with 8.06 cm root length(table 4.13).

IAA also tried to know the effect on rooting and it was observed that highest root length (9.2 cm) induced on media supplemented with 2.0mg/L IAA. Among both the auxin IAA (concentration 2.0mg/L) was better in case of root length but maximum number of root was developed on 1.5mg/L NAA. So in case of highest number of root regeneration NAA was more effective than IAA for *Kaempferia parviflora* species.

The findings of the present experiment suggested that by manipulating plant growth regulators micropropagation of *Kaempferia parviflora* could be done by using rhizome buds as explants. This established protocol help to conserve this medicinally important plant species by mass propagation.