Tissue culture is one of the basic tools for multiplication of endangered medicinal plants. Conventional propagation poses problems like improper germination of seeds and loss of viability with passage of time. Plant tissue and cells in culture show variation and this appears to be an unexpectedly rich and noble source of genetic variability. Tissue culture may provide chromosomal variation allowing for the regeneration of plants with different phenotypes which can be used to increase genetic variability through the sexual crosses or to obtain cells or tissues with a high degree of variability. Application of plant tissue and organ culture has immense potential in the large-scale propagation and conservation of the unexplored biodiversity of plants all over the world and especially in huge country like India. This is especially true for plants with medicinal properties.

Efforts have been made to identify natural drugs of plants origin which are effective against various diseases and have no side effects. Most of these plants have poor regeneration in wild. Members of Asclepiadaceae occupied top place in their medicinal value. Destructive collection techniques and requirement of raw material in large quantities of these plant species have led to alarming depletion of resources and consequent extinction of species. In vitro propagation is the best viable option to conserve these potent medicinal and endemic plants. In the present study an attempt has been made to propagate two important species *Ceropegia juncea* Roxb. and *Ceropegia elegans* Wall.

**Seed Germination Studies of Ceropegia juncea**

- Among three different surface sterilants HgCl₂, NaOCl and H₂O₂ used for surface sterilization of seeds, 20% H₂O₂ and 6 minutes exposure was found to be effective.

- Seeds cultured on ½ MS medium fortified with 0.6% agar 1% sucrose were found to be best for germination among ½ B₅ and ½ WPM.
Among the various combinations of hormones used for seed germination 0.25 mg/l BAP+0.01 mg/l NAA was found to be effective.

**Establishment of Aseptic Cultures of Ceropegia elegans**

- Out of three sterilants HgCl₂, NaOCl and H₂O₂ used for raising aseptic cultures HgCl₂ was found to be effective.
- Explants treated with HgCl₂ 0.1% about 6 minutes duration was found to be effective to obtain healthy shoot proliferation without contamination.

**Shoot induction and multiplication**

- The nodal explants cultured on MS medium fortified with BAP 1mg/l was found to be effective for shoot sprouting, number and length without callus formation followed by B5 and WPM in both Ceropegia juncea and Ceropegia elegans.
- Nodal explants showed better organogenic response than cotyledonary node and shoot tip explants in Ceropegia juncea and nodal explants than shoot tip in Ceropegia elegans.
- Shoot regeneration efficiency of nodal explants of Ceropegia juncea was maximum (79%) with BAP 2 mg/l, whereas in Ceropegia elegans Kn 5 mg/l was better than 2-iP, TDZ and Zeatin when used cytokinin alone.
- The effect of various cytokinins from nodal explants in Ceropegia juncea is in the order of BAP > TDZ > 2-iP > Kn > Zeatin and in case of Ceropegia elegans from nodal explants the order is Kn > BAP > 2-iP > Zeatin > TDZ.
- Combination of BAP 2 mg/l + TDZ 1 mg/l in Ceropegia juncea produced maximum number of 20.65 ± 0.20 shoots per explant and shoot length of 3.56 ± 0.03 cm with 76% response.
- Combination of Kn 5 mg/l + IAA 1 mg/l in Ceropegia elegans produced maximum number of 7.11 ± 0.07 shoots per explant and shoot length of 5.16 ± 0.09 cm with 86% response.
- Zeatin was less effective than 2-iP in Ceropegia elegans as well as in Ceropegia juncea.
Among various concentrations of growth adjuvants and antioxidants used Coconut milk at 20%, Casein hydrolysate at 50 mg/l, Yeast extract at 20 mg/l, Activated charcoal at 50 mg/l, Citric acid at 50 mg/l and PVP at 100 mg/l were found to be effective in regeneration of shoots from nodal explants of *Ceropegia juncea*.

In *Ceropegia elegans* 10% Coconut milk, 50 mg/l Casein hydrolysate, 50 mg/l Yeast extract, 20 mg/l Activated charcoal, 50 mg/l Citric acid, 200 mg/l PVP were found to be better in regenerating shoots from nodal explants.

Among different carbon sources such as glucose, fructose, galactose, sucrose and maltose, 3% sucrose was found to be optimum for shoot proliferation in *Ceropegia elegans* and in *Ceropegia juncea*.

**Callus culture studies**

Internode, cotyledonary node and leaf of *Ceropegia juncea* and mature internode, petiole and leaf of *Ceropegia elegans* used for callus induction, better response was observed on MS medium than B5 and WPM. Internode gave better response than others in both species.

Among all the auxins tested 2,4-D 2 mg/l, 2,4,5-T 2 mg/l, 2,4,5-TP 2 mg/l, Picloram 3mg/l were found to be effective for callus induction in *Ceropegia juncea*. Whereas in *Ceropegia elegans* 2,4-D 3 mg/l, 2,4 5-T 2 mg/l, 2,4, 5-TP 2 mg/l and Picloram 2 mg/l was found to be effective for induction of callus.

In *Ceropegia juncea* embryogenic callus proliferated on 2,4-D 2 mg/l combination with cytokinin (BAP, Kn and TDZ).

Subculture of embryogenic calli in the medium containing BAP 2 mg/l + NAA 0.5 mg/l failed to produced shoots.

Subculture of embryogenic calli in the medium containing 2,4-D 2 mg/l + BAP 0.25 mg/l + NAA 0.25 mg/l failed to produce shoots in both species.

**In vitro Rooting and Acclimatization**

Among the various media tested (full strength and half strength MS, B5 and WPM) ½ MS medium was proved to be effective in both the species.
Half strength MS medium fortified with IBA 1 mg/l + 0.25 mg/l NAA was effective for better rooting in *Ceropegia juncea* and IBA 1 mg/l was effective for better rooting observed in *Ceropegia juncea*.

The rooted plants were successfully established in soil with 78% survival rate in *Ceropegia juncea*, and 72% survival rate in *Ceropegia elegans*.

**Antimicrobial studies**

The crude water extract was found effective against most of the tested pathogenic microorganisms. Among all microorganisms tested *Escherichia coli* and *Klebsiella pneumoniae* exhibited highest zone of inhibition against three solvents extracts of the tested four in *Ceropegia juncea* and *Ceropegia elegans* respectively. Whereas *Candida albicans* and *Aspergillus fumigatus* did not form any zone of inhibition against any microorganism tested.