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Review Article

Ceropegia juncea–A Review on the Micropropagation Studies

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Abstract: *Ceropegia* belongs to family Apocynaceae, comprising one of the largest plant group. The plants in this genus have wide range of morphological characters, with leafless succulents or non-succulents mostly erect herbs, climbers or shrubs. *Ceropegia spp* are have high therapeutic value and are used for treating a number of diseases. Ceropegin is the bioactive compound present in this genus. *Ceropegia juncea* is having this compound and found to have analgesic, antiulcer, hepatoprotective and antioxidant activities. Increasing population and livestock are the main reasons for its rapid disappearance in the wild nature. So this endangered species plant need to be conserved using micropropagation methods. In the present review we have presented, a detailed study, of plant hormones used and their response in the shoot multiplication and rapid propagation. Protocols for the acclimatization used by different researchers were also discussed.
Keywords: *Ceropegia juncea*, micropropagation, shoot multiplication.

Introduction

Ceropegia genus belongs to family Apocynaceae, with more than 200 species. These are leafless succulent erect or climbing herbs or shrubs. They are distributed in Africa, Arabian peninsula, Madagascar and Indian subcontinent (Bruyns, 2003; Murthy *et al.*, 2012; Bruyns *et al.*, 2015). Among 57 species of *Ceropegia* in India we find 35 species which are endemic (Karthikeyan *et al.*, 2009). These species are over exploited due to pharmacological interest, and are under threat (Yadav and Kamble, 2006; Murthy *et al.*, 2012; Phulwaria *et al.*, 2013). *Ceropegia juncea* is having pharmacological significance in Ayurveda. It is used to treat Stomach disorders, fevers, ulcers, skin diseases, urinary tract disorders, and for kidney stones (Jain and Defillips 1991; Sukumar *et al.*, 1995; Rajan *et al.*, 2005; Reddy *et al.*, 2009; Sharma *et al.*, 2011). All these pharmacological applications are due to the presence of a number of phytochemicals like flavonoids, triterpenes, saponins, tannins, phenols etc., (Nadkarni, 1976; Adibatti *et al.*, 1991; Phulwaria *et al.*, 2013).

Micropropagation protocols

Nikam and Savant (2009) selected nodal and internodal explants for in vitro propagation. They have sterilized these explants by washing with sterile water followed by treatment with mercuric chloride ($HgCl_2$) (0.1% v/v) for 7 minutes (min) and rinsed with sterilized distilled water. For shoot induction they have used BA (6-benzyladenine), Kin (Kinetin), IAA (Indole-3-acetic acid), NAA (Naphthalene Acetic Acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) individually and in combinations. Best response for shoot induction was found to be MS (Murashige and Skoog's, 1962) medium supplemented with Kin (7.5 µM). They have reported 8.5±3 shoots per explants. For callus induction also they have used all 3 auxins and 2 cytokinins at different concentrations.

Better results were obtained with MS + 2,4-D (1µM) + BA (5µM). They have used this callus for cerpegin content estimation and found to contain (470 µg/g dry weight), when callus was developed on MS + IAA (10 µM) + BA (5µM). For rooting of the in vitro grown shoots (3cm), they have used half strength MS (1/2) containing IAA or NAA (0.5- 2.0 µM) and basal. Among the tested concentrations IAA (2.0 µM) was found to be best with 3.5±0.3 roots per shoot, 100% response and root length within 2-3 weeks, compared with NAA and IBA at the same concentrations. Rooted plants were washed and transferred to pots with garden soil in rainy and winter seasons. In summer, they placed these plantlets for one week in shade and reported 100% survival rates in field.

Krishnareddy *et al.*, (2011) sterilized the selected aseptic seedling nodal segments for the micropropagation of this plant. Seeds were collected from fruits after shade dry, washed with 2 drops of Tween 20 (1%) detergent for 20 min, then washed under tap water for half an hour. After transferring to the laminar chamber immersed in ethyl alcohol (70% v/v) for 30 seconds (s) again rinsed with 20% hydrogen peroxide (H_2O_2) for 6 min. Rinsed 5 times with sterilized water. These seeds were grown in basal ½ strength MS medium. Shoot tip, node and cotyledonary node were placed on MS medium containing various hormones for shoot induction. BAP (8.8µM) +TDZ (4.54 µM) (Thidiazuron), combination was found to be effective for shooting. This combination produced 20.65 ±0.20 shoots/explants with 76% response.

Nodal segments showed better response than shoot tip and cotyledonary node. BAP (8.87 µM) was found to be best with 6.37±0.18 shoots/explants and shoot length of 4.87±0.12cm. Order of response for selected cytokinin hormones was, BAP > TDZ > 2iP > Kin > Zeatin. For rooting, shoots (4-5cm) were place in different concentrations of NAA, IAA and IBA alone or in combinations on ½ MS medium. Finally for rooting ½ MS + IBA (4.90 µM) + NAA (1.27 µM) was found to be best. Plantlets with well-developed roots were washed to remove agar and placed in small (5cm) plastic cups with sterilized soil rite mix and covered with polythene bag and incubated for 15 days. In this period liquid quarter strength MS basal (1/4MS) was irrigated for the plants, until new leaves appeared. Later small holes are made polythene bag to reduce relative humidity inside polythene bag. After acclimatization 78% survival rate was recorded.

Abubacker and Dheepan, (2012) attempted for *in vitro* conservation using nodal explants. They have sterilized the explants under tap water using 4-5 drops of detergent Tween-20 for 20 min. Latter nodal segment (1-2) cm were treated with bavistin (0.5- 1.0% w/v) and streptomycin (0.05 – 0.1% w/v) for 10 min each, and rinsed with distilled water 3 times after each treatment. Later treated with $HgCl_2$ (0.1% w/v) for 2-3 min and rinsed thoroughly 3 times with distilled water. For callus induction MS+ BAP (1.5mg/l) + 2,4-D (1.5mg/l) was best with green nodular callus. Shoot induction was done by placing on MS medium containing different growth regulators (BAP, 2,4-D, IBA, Kin, NAA and IAA) at different concentrations and combinations. Maximum shoots were observed on MS+ BAP (3mg/l) + Kin (0.5mg/l) + IBA (0.5mg/l) + NAA (0.5 mg/l). Rooting was found to be best with MS + IAA (0.5mg/l) + NAA (0.5 mg/l + IBA (0.5mg/l) + BAP (1.0 mg/l). Rooted plantlets were transferred to cups containing sterilized sand. These cups were first placed in mist chamber and alternative day quarter strength MS salt solution was irrigated. Acclimatized plants after 2 weeks were placed in green house and transferred to soil after next 2 weeks.

Binish (2018) reported the *in vitro* propagation of *C. juncea* using nodal and shoot tips of mature plants. They cultured these explants on MS medium supplemented with different BAP

concentration and found BAP (1.5mg/l) as the best. Subculturing increased the shoot number in this concentration. After two weeks of culturing reported 8.28 ±1.11 shoots /explants with 86% of response and a length of 5.37±0.74 cm in third subculture on MS + BAP (1.5mg/l) + NAA (1.0 mg/l) reported 9.71±0.75 shoots and 76% of response. For callus induction nodes were placed on MS medium containing BAP (1.5mg/l) + NAA (2mg/l). On this medium they have reported yellowish green and friable callus. Root formation was observed; when shoots were placed on ½ MS + IBA (0.75mg/l) showed quick response with 6 roots formed within 5-14 days. Thus rooted plants were washed with tap water and 50% plants were transplanted in plastic cups containing Azolla, Soil and coir waste (1: 1: 1); while remaining 50% were placed in a potting mixture of cowdung : sterile river sand: garden soil (1: 1 :1). Plants were placed in high humid and low light intensity conditions. Gradually humidity was decreased; light intensities were increased over a period of 15-30 days. Pots irrigated with quarter strength MS solution and later changed to water alone. Cowdung: sand: garden soil mixture was found to be the best potting mixture; acclimatized plants were transferred to soil.

Conflicts of interest: There is no conflict of interest of any kind.

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