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In vitro Conservation Approach of Rare Medicinal Plant *Ceropegia juncea* Roxb. (Asclepiadaceae)

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***In vitro* conservation protocol for rare medicinal plant *Ceropegia juncea* was developed using nodal explants by culturing on Murashige and Skoog (MS) medium. The maximum number of greenish nodular callus was induced in MS + 6-benzylaminopurine (BAP) 1.5 mg/l + 2,4-dichlorophenoxy acetic acid (2,4-D) 1.5 mg/l and massive callus induction was in MS + Indole-3-Butyric Acid (IBA) 1.0 mg/l + BAP 1.0 mg/l + 2,4-D 0.5 mg/l. The greenish nodular calli were raised on the MS medium supplemented with BAP 3.0 mg/l + kinetine (Kin) 0.5 mg/l + Indole-3-Butyric Acid (IBA) 0.5 mg/l + Naphthalene Acetic Acid (NAA) 0.5 mg/l for shoot induction. Root induction was achieved in MS + Indole-3-Acetic Acid (IAA) 0.5 mg/l + NAA 0.5 mg/l + IBA 0.5 mg/l + BAP 1.0 mg/l. The plantlets were established, acclimatized and thrived in greenhouse and then in natural environmental condition. The *in vitro* conservation protocol developed in this study provides a basis for germplasm conservation of this medicinal plant.**

Key words: Callus, *Ceropegia juncea*, *in* vitro conservation, MS medium.

India has a rich and varied heritage of biodiversity, encompassing a wide spectrum of habitats from rasin forests to alpine vegetation and from temperate forests to costal wetlands. In India, about 1500 rare and threatened species of both flowering and non-flowering plant groups were identified during the last two decades. The threat to a majority of them could be attributed to anthropogenic factors like habitat destruction due to grazing, urbanization and other developmental activities and exploitation (Myers, 1988).

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Ceropegia L. is an old world tropical genus containing about 200 species of which 48 *Ceropegia* species found in India (Bruyns, 2003). Twenty eight species of *Ceropegia* are endemic to the Peninsular India (Ansari, 1984 Ahmedulla and Nayar, 1986). The existence of the *Ceropegia* species has become restricted to remote pockets in the Himalayas and the Western Ghats, the two biodiversity hot spots. *Ceropegia* genus has now been placed under the category of rare and endangered plants (Nayar and Sastry, 1987; Chaturvedi et al., 1996; Jagtap et al., 2004)

Ceropegia juncea Roxb. (Asclepiadaceae) is an important medicinal herb, which is used as a source of 'soma' a plant drug of the Ayurvedic medicine with a wide variety of uses. The fleshy stem and root tubers used as a raw material for traditional and folk medicines for the treatments of stomach and gastric disorders (Nikam and Savant, 2009). *C. bulbosa* and *C. tuberosa*

root tubers contain an alkaloid called Ceropegin (Nadkarni, 1976), consumed after cooking (Mabberley, 1997). The root tubers also contain starch, sugars, albuminoids, fats, gum, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against many diseases especially diarrhea and dysentery. The ceropegin is an analgesic drug, tranquilizer and acts against ulcers and inflammation (Adibatti et al., 1991). It is important to prevent the extinction of *C. juncea* for its taxonomic and ethnobotanical values. The present study was aimed at *in vitro* conservation approach of this rare medicinal plant.

MATERIAL AND METHODS

Plant material and explants preparation

The plants of *C. juncea* were collected from a naturalized population in Nartharmalai (Pudukottai District), Tamil Nadu, India (Figure 1, a,b,c). For periodic harvest of explants, stocks were maintained in the herbal garden at Department of Botany, National College, Tiruchirappalli, Tamil Nadu. Nodal portions of succulent stem were tested for culture initiation. The young succulent stems were excised from the plants and kept under tap water with Tween 20 (4-5 drops) for 20 min. Nodal segments (1-2 cm long) was cut from these stems and was treated with fungicide Bavistin (0.5-1.0% w/v) and antibiotic streptomycin (0.05-0.1% w/v) for 10 min each. Each time the nodal segments were rinsed thrice in distilled water and then they were surface sterilized with aqueous solution of $HgCl_2$ (0.1% w/v) for 2-3 min and washed thrice with sterilized distilled water. The explants were cut gently with a sterilized blade and explants were inoculated in culture tubes with modified MS media under aseptic conditions.

Culture medium and conditions

Basal Murashige and Skoog medium (Murashige and Skoog, 1962). (MS medium) was prepared using Hi media and Sigma chemicals. Sucrose (30% w/v) was added to medium and pH of medium was adjusted to 5.8 before adding agar-agar (8% w/v). Different growth regulators (BAP, 2, 4-D, IBA, Kin, NAA and IAA) at different concentrations and combinations were added to the medium. In the present study, the media were autoclaved at 121° C and 15 lbs pressure for 20 min. All the *in vitro* cultures were maintained at 24 ± 2° C and illuminated for 16 h with fluorescent light (18-24 µ mol/m/sec) followed by 8 h dark period and the relative humidity was about 60-80% within the 25×150 mm culture tubes covered with cotton plug.

Callus, shoot and root induction and acclimatization

The healthy and normal nodal segment explants were inoculated in MS medium with different combinations of growth regulators. When the growth regulators failed to induce a specific response in callus, direct organogenesis and multiple shoot production at the end of the first cycle, it was marked as inappropriate combination. Twenty cultures were raised for each treatment and all experiments were repeated thrice. BAP, 2, 4-D, IAA and IBA combinations were tested to estimate the callus production. BAP, Kin, IAA, IBA and NAA combinations were used for shoot induction. The regenerated shoots were inoculated for rooting medium supplemented with IAA, IBA, NAA and BAP combinations. The rooted shoots were washed with sterile distilled water to remove the traces of medium. The *in vitro* rooted plantlets were transferred to cups containing autoclaved sand. Those cups were covered initially with polythene bags to maintain humidity and placed in mist chamber. After every alternative day, quarter strength MS medium salt solution was supplied to the plantlets. After two weeks of growth, the complete plants were established, acclimatized and thrived in green house condition. The acclimatized plants after two weeks were then transferred to natural environment and established into healthy plants (Figure 1, l)

Statistical analysis

All statistical analyses were performed at 0.05% level, using the statistical software (SPSS inc. Chicago; USA).

RESULTS AND DISCUSSION

The combination of BAP, IBA and 2,4-D induced an excellent amount of massive callus from the nodal segments of *Ceropegia juncea* and the morphology of the callus was friable, yellowish green and greenish in colour and nodular in its nature (Table 1).

Callus initiation was observed in MS+ 2,4-

D 1.5 mg / l or MS + BAP 1.5 mg /l, produced in MS +2,4-D + BAP 1.5mg / l+ 1.5 mg/ l combination. (Figure 1, d,e). MS + IBA 1.0 mg/l + BAP 1.0 mg/l + 2,4-D 0.5 mg/l produced the maximum number of massive calli induction (Figure 1, f) followed by MS + IBA 0.5 mg/l + 2,4-D 0.5 mg/l., MS+ IAA 1.0 mg/l + BAP 1.0 mg/l., whereas MS + 2,4-D, MS + 2,4-D + IAA, MS + IAA or MS + IBA, failed to induce a specific response. The maximum greenish nodular calli was induced in MS + BAP 1.5 mg/l + 2, 4-D 1.5 mg/l followed by MS + BAP 2.0 mg/l + 2, 4-D 2.0 mg/l combinations (Fig. 1, g). Callus induction in *Ceropegia* spp. has been studied by many workers *viz. C. jainii* and *C. bulbosa* (Patil, 1998), C. *candelabrum* (Beena et al., 2003), *C. pusilla* (Kondamudi et al., 2010) and *C. hirsute* (Nikam et al., 2008). In the present study the callus induction in *C. juncea* showed variations with the findings of those workers.

MS medium supplemented with BAP, Kin, IBA, IAA and NAA responded variable effects on shoot induction in nodal explants (Figure 1, h and Table 2). The maximum number of shoots was recorded on MS + BAP 3.0 mg/l + Kin 0.5 mg/l +

Table 1. Callus induction from nodal explants of *C. juncea* cultured on MS medium supplemented with various concentrations of growth regulators

Concentrations of Plant Growth Regulators	No. of explants inoculated	No. of explants forming callus	Morphology of callus
MS + BAP 1.5 mg/l +2,4-D 1.5 mg/l	20	15±0.49	G, N, F
MS + BAP 2.0 mg/l +2,4-D 2.0 mg/l	20	10±0.99	G, N, F
MS + IAA 1.0 mg/l + BAP 1.0 mg/l	20	5±1.48	YG, M, F
MS + IBA 1.0 mg/l + BAP 1.0 mg/l + 2,4-D 0.5 mg/l 2017±0.49YC, M, F	20	17±0.49	YG, M, F
MS + IBA 0.5 mg/l + 2,4-D 0.5 mg/l	20	10± 1.48	YG, M, F

F = Friable, G = Greenish, M = Massive, N = Nodular, YG = Yellowish Green
Value represents means ± SD from 20 replicates

Table 2. Shoot induction from nodal explants of *C. juncea* cultured on MS medium supplemented with various concentrations of growth regulators

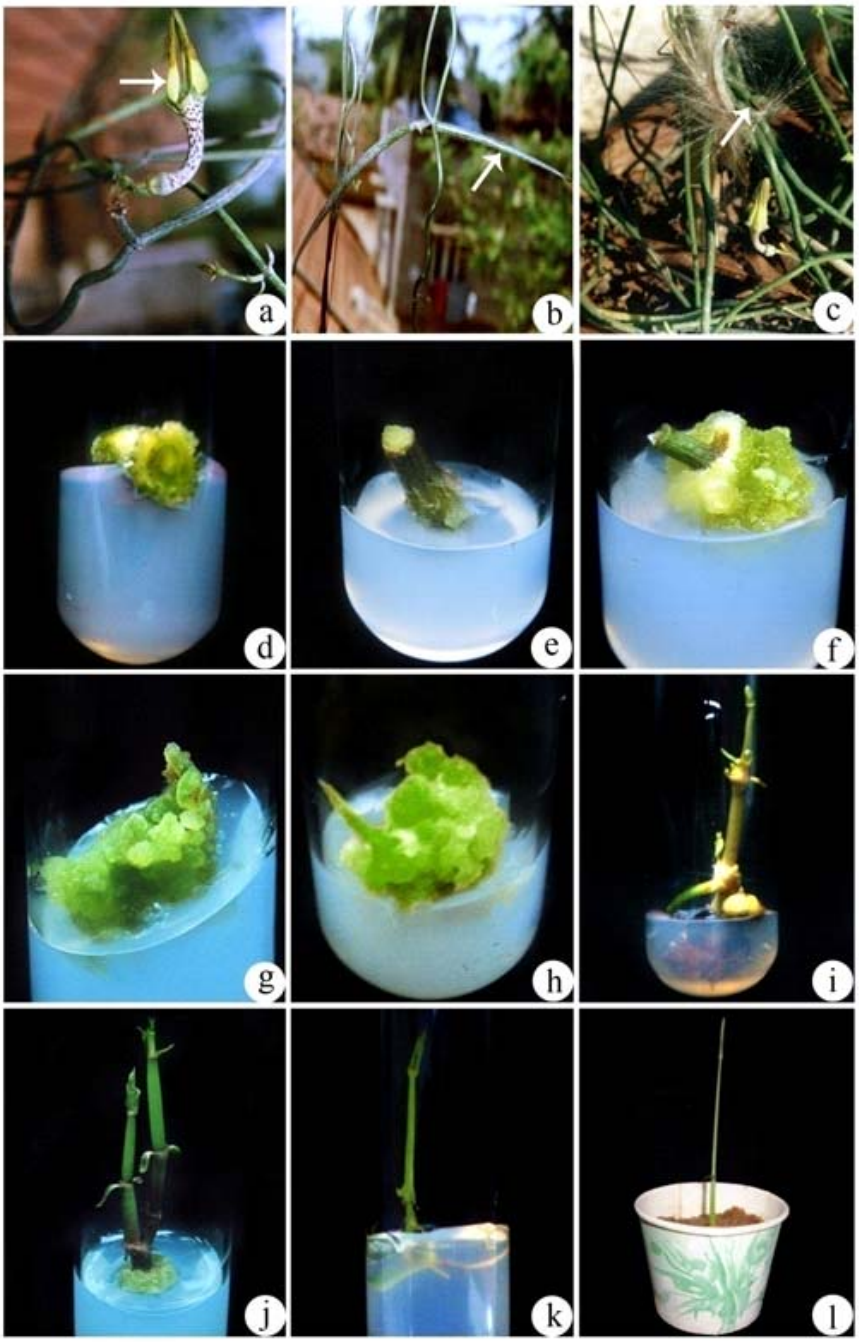
Concentrations of Plant Growth Regulators	No. of explants inoculated	No. of explants produced shoots	No. of shoots developed
MS + BAP 3.0 mg/l + Kin 0.5 mg/l + IAA 0.5 mg/l	20	4±0.49	1
MS + BAP 3.0 mg/l + Kin 0.5 mg/l + IBA 0.5 mg/l +NAA 0.5 mg/l	20	18±0.49	2
MS + BAP 3.0 mg/l + Kin 0.5 mg/l + IBA 0.5 mg/l	20	10± 1.48	2

Value represents means ± SD from 20 replicates

Table 3. Root induction from nodal stem explants of *C. juncea* cultured on MS medium supplemented with various concentrations of growth regulators

Concentrations of Plant Growth Regulator	No. of shoot and clones inoculate	No. of clones produced roots	No. of roots Developed
MS + IAA 0.5 mg/l + NAA 0.5 mg/l + IBA 0.5 mg/l + BAP 1.0 mg/l	20	12 ± 0.99	2
MS + IBA 0.5 mg/l + NAA 0.5 mg/l + BAP 1.0 mg/l	20	6 ± 0.99	2

Value represents means ± SD from 20 replicates



a) Ceropegia juncea Roxb. Explant in flower (Natural habitat)
b) Ceropegia juncea Roxb. Fruit (Natural habitat)
c) Ceropegia juncea Roxb. Seed (Natural habitat)
d) Callus initiation (10 days)
e) Greenish callus (10 days)
f) Grenish hard callus(20 days)
g) Grenish nodular callsu (20 days)
h) Shoot induction (25 days)
i) Shoot induction at different nodoes (30 days)
j) Multiple shoot induction (30 days)
k) Root Induction (20 days)
l) Acclimatized young plant (40 days)

Fig. 1. In vitro conservation approach of rare medicinal plant Ceropegia juncea Roxb. (Asclepiadaceae)

IBA 0.5 mg/l + NAA 0.5 mg/l (Figure 1, i , j) followed by MS + BAP 3.0 mg/l + Kin 0.5 mg/l + IBA 0.5 mg/l and MS + BAP 3.0 mg/l + Kin 0.5 mg/l + IAA 0.5 mg/l. The other combinations MS+ IAA 3.0mg/l+ Kin 0.5 mg/l and MS+ IBA 3.0 mg/l+ Kin 0.5 mg/l+ NAA 0.5 mg/l are not significant for shoot regeneration.

Similar observations were noticed in *Ceropegia hirsute* (Nikam et al., 2008) in which BAP concentrations showed vital role in shoot induction. The present results are in agreement with previous reports on *C. bulbosa* and *C. jainii*(Nikam et al., 2008) and reveal that the BAP alone can induce axillary shoot multiplication from nodal segments(Patil, 1998). On the other hand, a synergistic effect of a range of growth regulators in combinations with BAP for shoot regeneration was well documented for members of Asclepiadaceae *viz., C.* candelabrum(Beena et al., 2003), *Holostemma ada-kodien* (Martin, 2008), *Hemidesmus indica* (Sreekumar et al., 2000) and *Leptadenia reticulate* (Arya et al., 2003).

The different concentration and combination of IAA, NAA and BAP induced rooting. One week old regenerated shoots were transferred to the different combinations of rooting medium MS + IAA 0.5 mg/l + NAA 0.5 mg/l + IBA 0.5 mg/l + BAP 1.0 mg/l was found to be best suited concentration and combination of growth regulators for root induction (Figure 1, k and Table 3) this is followed by MS + IBA 0.5 mg/l + NAA 0.5 mg/l + BAP 1.0 mg/l. The other combinations MS+ IAA 1.0mg/l., MS+ IBA 1.0mg/l and MS+ IBA1.0 mg/l+ NAA 1.0 mg/l did not respond for rooting.

The combination of BAP + IBA induced rooting in *C. pusilla* (Kondamudi *et al.,* 2010). Highest rooting percentage was reported with higher concentration of IAA 2.85-11.42 µm/l than NAA (2.69-10.74 µm/l) in *C. bulbosa* (Divya Goyal and Seema Bhadauria, 2003). However, the role of BAP + IBA in root induction is more significant from the present study as reported by earlier study (Kondamudi et al., 2010).

CONCLUSIONS

The present study reported successful callus , shoot and root induction protocol that can be employed in the *in vitro* propagation of rare medicinal plant *Ceropegia juncea* and helps in conservation and domestication, thereby minimizing the pressure on wild populations of the valuable medicinal plants of natural ecosystem of forest.

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