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ARTICLE

Indirect organogenesis from various explants of *Hildegardia populifolia* (Roxb.) Schott & Endl. – A threatened tree species from Eastern Ghats of Tamil Nadu, India

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Micropropagation;
Tissue culture;
In vitro

Abstract *Hildegardia* species are an important resource for fiber industry. This investigation was conducted to develop a plant regeneration protocol for *Hildegardia populifolia* (Roxb.) Schott & Endl. via indirect organogenesis Callus was obtained from leaf, internode and petiole explants, among these explants internode explant gave best result on MS medium supplemented with different concentrations of 2,4-Dichlorophenoxy acetic acid (2,4-D). The highest percentage (100%) of regeneration was obtained with benzyladenine (BA) (2.0 mg/l) + indole-3-acetic acid (IAA) (0.1 mg/l) + glutamine (25 mg/l) + thidiazuron (TDZ) (0.5 mg/l) from internode explants. Shootlets were highly rooted on MS medium supplemented with 3.0 mg/l indole-3-butyric acid (IBA). *In vitro* rooted seedlings were successfully acclimatized. This *in vitro* regeneration system will facilitate further development of reliable procedures for this genus.

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1. Introduction

Hildegardia populifolia (Roxb.) Schott & Endl. is a medium sized deciduous tree of the family Sterculiaceae confined to tropical forests of Tamil Nadu and Andhra Pradesh in India. A narrow endemic [2], *H. populifolia* was known to be represented by a sole surviving population comprising about 20 trees in Kalrayan Hills of Tamil Nadu [1]. This species is under threat due to factors not apparent at present. But it is assumed that anthropogenic interference, habitat losses and other intrinsic and extrinsic factors might have been the reason for their poor regeneration ability and low seed viability [21]. According to IUCN [14] list of threatened species, *H. populifolia* is critically endangered. Sarcar and Sarcar [29] located 13

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mature trees and 11 young natural regenerations of this species in dry mixed deciduous forests of Parigam Reserved Forests in Kalrayan Hills of Kallakurichi Forest Division, Tamil Nadu and they assessed its status as threatened. This endemic species was evaluated in its entire range and placed under IUCN category, critically endangered [27]. This tree is easily recognizable by its pale green bark. The fiber extracted from the bark is used for domestic purposes and leaf extract is known to have healing properties [28].

In vitro regeneration is an efficient means of *ex situ* conservation of plant diversity [9,15,5] because with this technology many endangered species can be quickly propagated and preserved using minimal of plant material. Moreover this technique has the unique advantage of propagating the desired taxon, independent of season, reproducing barriers and germination hurdles. *H. populifolia* is ideal candidate for *in vitro* regeneration with its intrinsic poor seed viability and regeneration capabilities. Available literature [3,16,8] reveals that an *in vitro* protocol for this vulnerable tree has been carried out through distinct *in vitro* regeneration approaches i.e. indirect organogenesis. Protocols for organogenesis and somatic embryogenesis of this plant from seedling derived axillary meristem and mature tree derived nodal meristem are standardized [3]. The present study describes successful plant regeneration via adventitious indirect organogenesis from internode, petiole and leaf segments of *H. populifolia*.

2. Materials and methods

2.1. Seed germination and explant preparation

Seeds of *H. populifolia* were collected on November 2004 from Kalrayan Hills which 300–600 m, Eastern Ghats of Tamil Nadu. Chemical scarification was carried out by soaking the seeds in concentrated H_2SO_4 (98%) for 5, 15, 30, 45, 60 and 90 min and subsequently rinsing with tap water for 30 min. The scarified seeds were then disinfected with 70% ethyl alcohol for 5 min and followed by aqueous solution of 0.1% mercuric chloride for 10 min. Then the seeds were rinsed 4–5 times with sterile distilled water. Sterilized seeds were aseptically inoculated in MS medium supplemented with GA_3 (1.0 mg/l) and cotton soaked with sterile water. The inoculated seeds were incubated under optimal culture condition. The *in vitro* raised seedlings were transferred to earthen pots containing soil and sand (1:1) maintained in the garden. The explants were collected from 45 day old seedlings and cut into 10–15 mm fragment as explants.

2.2. Media and culture condition

The basic culture medium (BCM) consists of Murashige and Skoog [20]. MS medium was fortified with 30 g/l sucrose (Himedia, India) and gelled with 0.8% agar (Himedia, India), and the pH of the medium was adjusted to 5.7 ± 0.2 with 0.1 N NaOH or 0.1 N HCl after addition of the growth regulators. The medium was dispensed in culture tube and autoclaved at 121 °C, for 30 min. All the cultures were maintained in a sterilized culture room at 26 ± 2 °C, under 16 h photoperiods provided by cool white fluorescent light (60 $\mu mol^{-2} s^{-1}$) and with 55–60% relative humidity. The cultures were subcultured on the fresh medium after 15–20 days.

2.3. Callus initiation

Explants including internode, (1 cm in length), petiole (0.5 cm in length), and leaf (0.5 × 0.5 cm) were excised from 45 day old *in vitro* germinated seedlings and placed horizontally on MS medium. In this experiment, the effects of cytokinins and auxins, both separately and in combination were studied on callus initiation and its proliferation. Auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) (0.1, 0.5, 1.0, 2.0 and 3.0 mg/l), and cytokinins benzyladenine (BA) and (kinetin) Kin (0.5, 1.0, 2.0 and 3.0 mg/l) either alone or in combinations of IAA + BA, IAA + Kin, and Kin + BA. For further callus proliferation supplements such as adenine, adenine sulfate, glutamine and sodium citrate (10, 25 and 50 mg/l) were used. These culture conditions were used in all the experiments mentioned below unless stated. Data of frequency (%) of callus formation and its fresh weight were recorded after 45 days of culture.

2.4. Shoot organogenesis from callus

Well-established hard and compact callus (~0.5 g fresh weight) were grown on MS medium supplemented with BA (2.0 mg/l) + IAA (0.1 mg/l) + glutamine (25 mg/l) for shoot organogenesis. Calli were transferred to shoot organogenesis media, consisting of MS basal media supplemented with TDZ (0.1, 0.5, 1.0, 2.0 and 3.0 mg/l) and activated charcoal (5, 10 and 25 mg/l), in combinations of TDZ at 0.5 mg/l. Cultures were transferred on fresh media after 20th day of inoculation. The percent of shoot organogenesis from callus and average number of shoots per inoculum were recorded on the 35thday after transferring the callus on shoot organogenesis media.

2.5. Formation of adventitious root

Regenerated shoots (2–4 cm long) obtained from micropropagated plantlets were cultured on MS basal medium fortified with either of IBA, NAA, or IAA (0.1, 0.5, 1.0, 2.0, and 3.0 mg/l) for adventitious rooting. Data were recorded on percentage of rooting, number, and length of the roots after 4 weeks of transfer onto the rooting media.

2.6. Hardening of regenerated plants

Rooted plantlets were removed from the medium and washed in sterile distilled water to remove all the traces of agar and basal callus. The plantlets were then transferring to plastic pots (5–6 cm diameter) containing garden soil mixed of vermiculite and sand (2:1:1). The plastic pots were covered with polyethylene bags to maintain the relative humidity of about 70–80%. These pots were maintained at 25 ± 2 °C with a 16-h photoperiod and a light intensity of 25 $\mu mol\,m^{-2} s^{-1}$for 2 weeks in the culture room, and the pots were then transferred to a shade (60 $\mu mol\,m^{-2} s^{-1}$) in the third week. Plantlets were then transferred to glass house in the fourth week.

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