Sustainable Production of a Therapeutically Important Tree 
(*Holarrhena antidysenterica*) of India

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**Abstract**

*Holarrhena antidysenterica* (Linn.) Wall is an important medicinal plant of the Indian System of Medicine, the Ayurveda. The stem bark of the tree is used for antidiarrhoeal activity, owing to the presence of the bioactive compound conessine, a steroidal alkaloid. Due to the difference in environmental conditions or in genotype, the plant shows much variation in its therapeutic efficacy; hence there is a need to have more plants with an optimum and uniform therapeutic efficiency. In this study, we established a protocol for clonal multiplication of this important medicinal tree. The superior mother plant was selected on the basis of a higher concentration of the bioactive constituent, conessine, by phytochemical analysis. The maximum multiplication rate of axillary buds was observed in 8 μM 6-benzyladenine on Murashige and Skoog (MS) medium. Multiplied shoots of 7-9 cm were transferred to rooting medium (MS medium with 0.5 μM indole-3-acetic acid). The rooted plantlets were then transferred for primary and secondary hardening and finally transferred to the field. A 70-80% field survival was observed in regenerated plants. The established protocol offers a sustainable method for consistent production of this elite, medicinally-important tree.

**Keywords:** axillary bud multiplication, conessine, Kada Chhal, Kurchi, plantlets

**Abbreviations:** BA, 6-benzyladenine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; KIN, kinetin; MS, Murashige and Skoog medium; NAA, α-naphthaleneacetic acid; PGR, plant growth regulator

**INTRODUCTION**

*Holarrhena antidysenterica* (Family: Apocynaceae) is an important Indian medicinal tree locally known as Kada Chhal or Kurchi. The medicinal properties of the plants were known as early as 1000 B.C. Many traditional systems of medicine all over the world include the use of stem bark as a principal remedy of various types of dysentery (Gopal and Chauhan 1996). The bark is also reported to have astringent, anthelmintic, stomachic, febrifugal and tonic properties (Anonymous 2001). Water decoction of the bark showed significant antifulvic activity in albino rats at a dose of 400 mg/kg (De et al. 1997). Steroidal alkaloids (~4%) were reported as the major chemical constituents of the plant (Bhutani et al. 1984) and are responsible for the medicinal properties of the plant. A total of 48 alkaloids were reported to be present in the plant while conessine (0.4%) was reported to be one of the major bioactive compounds of the plant (Siddiqui 1936). Phytochemical studies on the plant reveal a high degree of variation in the chemical constituents, especially in the concentrations of the bioactive constituents (Srivastava et al. 2004) which in turn affects its medicinal properties. These variations could be genotypic or geographic. Hence, there is a need to have a superior quality, genetically uniform plant of this important tree of Ayurvedic Medicine. Conventional vegetative propagation methods, e.g. airlayering, grafting or cutting, are not sufficiently rapid and efficient, and thus *in vitro* techniques like micropropagation via multiple axillary branching offers a rapid means for multiplication of elite and rare germplasm and an alternative method for *ex situ* conservation (Anand et al. 1998; Boggetti et al. 1999).

The present study describes an efficient protocol for axillary bud multiplication and plantlet regeneration of *H. antidysenterica* from a superior mother plant selected on the basis of its phytochemical evaluation.

**MATERIALS AND METHODS**

**Selection of mother plants**

The mother plant was selected on the basis of total alkaloid as well as conessine content in the stem bark of the plant collected from various geographical locations of India.

**Culture media and incubation conditions**

Murashige and Skoog (1962), or MS medium with 20 g l⁻¹ sucrose and 8 g l⁻¹ agar (S.D.Fine-Chem Ltd., Mumbai, India) at pH 5.7 was used as the basal nutrient medium. Cultures were incubated at 24±2°C with 80-90% humidity under cool white fluorescent lights (Philips, India with total light intensity of 1300 lux) for a 16:8 h photoperiod. The cultures were transferred at four weeks interval onto fresh medium. Changes occurring in the explant during the incubation period were recorded on a daily and weekly basis. All experiments were repeated three times with 10 replicates in each case.

**Axillary bud multiplication and plantlet formation**

Green, healthy axillary buds (2⁻⁰ to 6⁻⁰ from apex) from the selected superior mother plant were collected to initiate cultures. The buds were washed thoroughly and cleaned with water and polysorbate 80, thereafter they were surface sterilized in mercuric chloride (0.1% w/v). The sterilized explants were then cut into the desired size (internode tissue was cut 0.3 cm above and 1.0 cm below the node having opposite axillary buds) and inoculated onto MS medium supplemented with various concentrations of plant growth regulators (PGRs) like 6-benzyladenine (BA), kinetin (KIN) and α-naphthaleneacetic acid (NAA), individually or in combination. For histological analyses, a thin section of the ex-
plant was cut from the site of axillary bud multiplication and stained with safranin and methylene blue.

For elongation, microshoot clusters (5-6 shoots per cluster) 1-2 cm long were excised, and transferred to fresh medium optimized for shoot multiplication, as the same medium was also found suitable for shoot elongation. After elongation, the shoots were excised from the base and transferred individually to full (MS salts with original concentration) and half (MS salts with half concentration) strength liquid and solidified MS medium supplemented with and without auxins and various concentrations (1, 2, 3%) of sucrose for rooting. Various concentrations (0.2-2.0 μM) and combinations of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) were also tested for induction and elongation of roots. The obtained plantlets (elongated shoots with roots) were washed with sterile distilled water to remove adhering culture medium and transferred to pots containing a hardening mixture of sterile sand, soil, vermiculite, perlite and cocopeat in different combinations and ratios [cocopeat: soil (3:1), cocopeat: sand: soil (1:1:1)]. For secondary hardening, the primary hardened shoots were transferred to a combination of soil and sand (1:1). The shoots were kept in the greenhouse at 28±2°C and 60-70% relative humidity. The hardened shoots were then transferred to net house and subsequently to the field.

RESULTS AND DISCUSSION

Selection of mother plant

The phytochemical analysis of the stem bark samples collected from various states of the country showed that the maximum amount of total alkaloid was present in the bark sample collected from Gujarat State of India. The conessine content was also found to be highest in the bark sample collected from various states of the country showed that the maximum amount of total alkaloid was present in the bark sample collected from Gujarat amongst all the samples analyzed (Srivastava et al. 2004). Hence, the explant material for tissue culture studies was used from plants growing in Gujarat region.

Axillary bud multiplication and plantlet formation

The induction of multiple shoots through axillary branching is now recognized as a useful technique for propagation. To reduce the risk of somaclonal variability during multiplication, preformed apical and axillary meristems, as sources of new plants are usually preferred over indirect organogenesis (Boggetti et al. 1999). Various concentrations (2-12 μM) of BA and KIN were attempted individually for the multiplication of axillary buds (Table 1). Activation of quiescent meristems and sprouting of axillary buds was observed within one week at all concentrations of BA as well as of KIN but the extent of response varied with concentration (Fig. 1). Multiplication of axillary buds (1–2) was observed after two weeks of incubation at all concentrations of BA except for 2 μM. The highest multiplication rate (1–5) was observed in medium supplemented with 8 μM BA. Scale bar = 200 μm.

<table>
<thead>
<tr>
<th>Growth regulators</th>
<th>BA (μM)</th>
<th>KIN (μM)</th>
<th>NAA (μM)</th>
<th>Number of axillary buds sprouted</th>
<th>Number of axillary buds multiplied</th>
<th>Callus Phase</th>
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+: Extent of callus formation

Table 1 Effect of growth regulator on bud sprouting and multiplication (n=20).

Fig. 1 Axillary bud sprouting in different concentrations of BA or KIN. Values are mean of 10 replicates (each replicate with two axillary buds) in each treatment. Bars indicate SD.

Fig. 2 Axillary bud multiplication in different concentrations of BA and KIN. Values are mean of 10 replicates (each replicate with two axillary buds) in each treatment. Bars indicate SD.

Fig. 3 Histology of axillary bud multiplication in medium supplemented with 8 μM BA. Scale bar = 200 μm.
Micropropagation of Holarrhena antidysenterica. Srivastava et al.

The present work demonstrates a simple procedure for the rapid clonal multiplication of Holarrhena antidysenterica through axillary buds. This approach offers a means for producing identical propagules from phytochemically superior mature trees selected for desired therapeutic properties.

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**Fig. 7 Flowchart of timelines for each of the stages of micropropagation.**

**Axillary bud**
- 1-2 week

**Sprouting**
- 2-4 weeks

**Multiplication**
- 4 weeks

**Elongation**
- 3 weeks

**Rooting**
- 2-4 weeks

**Primary hardening**
- 4-6 weeks

**Secondary hardening**
- 4-5 weeks

**Net house**
- 5-6 weeks

**Field**
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