In vitro Establishment of a Threatened Plant Species Adina Cordifolia

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A B S T R A C T
Adina cordifolia (Haldu) is tree species belonging to the family Rubiaceae. The paste of the stem bark or leaves heal deep wounds and jaundice, stomach ache, malarial fever, swelling in stomach and root is useful for dysentery and the direct seeding of Adina cordifolia failed due to low viability. Due to destructive mode of harvesting, Adina cordifolia is included in threatened species. Thus there is need to save this plant from being extinct. Adina cordifolia, apical buds were used as an explant for in vitro establishment. The explants collected in the month of March to June were surface sterilized by 5% (v/v) Tween-20 for 5 minute, 0.1% Bavistin for 5 minute, 0.1% (w/v) HgCl₂ for 1 minute and 70% ethanol for 1 minute and placed on the surface of Murashige and Skoog (MS) medium containing different combination of hormone BAP and NAA. The maximum survival rate of explants were observed 66.67% at 1 mg/L BAP. The shoot length was highest in MS medium supplemented 2mg/L BAP alone and 0.5mg/L NAA alone.

INTRODUCTION
Haldu (Adina cordifolia) is deciduous tree over 20m high belonging to the subfamily Cinchonoideae, family Rubiaceae. It is native to Southern Asia, from India and Sri Lanka east to southern China and Vietnam. It is found scattered in deciduous forests throughout the greater part of India, ascending to an altitude of 900 m in sub-Himalayan tract. It is also common in forests of South India (Iqbal et al., 2009). It have oppositely arranged leaves which are broadly oval in shape with heart shaped base and pointed tip. Flowering in A. cordifolia occurs from June to August, with yellow flowers shade of pink and bloomed together in balls with a circumference of 2 to 3cm. A. cordifolia is included in threatened species (www.fes.org.in). The plant A. cordifolia has been used in oriental medicine since ancient times as an essential component of various antiseptic and febrifuge prescriptions (Chopra et al., 1956). The bark is acrid, bitter pungent, tonic, vulnerary and aphrodisiac and is used in biliousness. The roots are used as an astringent in dysentery (Chadha, 1985). The A. cordifolia has been evaluated for its anti-ulcer potential active constituent showed interesting H⁺/K⁺ ATPase inhibitory activity (Kasinadhuni et al., 1999). The root
bark of *A. cordifolia* which is traditionally used in folklore medicine for the treatment of dysentery in different parts of India, especially in Pauri Garhwal region of Uttarakhand (Gaur, 1999). Antifertility properties of the leaf extract of *A. cordifolia* have been also examined (Sabir *et al.*, 1970). The seed of *Adina cordifolia* are not viable (about 11 million seeds/kg. *In vitro* plant regeneration from apical buds of *Adina cordifolia* has reported (Dubey *et al.*, 2004).

Vegetative propagation is an indispensable component of tree improvement programme, ensuring quick genetic gains by mass multiplication of selected genotype. The vegetative propagation of *Adina cordifolia* is difficult hence at present plants of *Adina cordifolia* become threatened species. Thus there is need to save this plant from being extinct. Hence vegetative propagation is an alternate for multiplication. *In vitro* propagation through apical buds is the best possible means of virus elimination and produces a large numbers of plants in a short span of time. It is a powerful tool for large-scale propagation. Most information on propagation and planting of *A. cordifolia* originates from India and Burma (Myanmar). Hence in vitro establishment of apical buds of *Adina cordifolia* was studied in this paper.

**MATERIALS AND METHODS**

**Plant material**

The explants apical buds of *Adina cordifolia* were collected freshly in the month of March, from Agroforestry Research Centre (AFRC), G.B. Pant University of Agriculture and Technology, Pantnagar. Murashige and Skoog (MS) medium (1962) supplemented with various concentration of plant growth regulators BAP and NAA was used for establishment of plant (Table 1).

**Establishment of explant and subculturing**

Apical buds were washed thoroughly with 5% (w/v) Tween-20 for 5 minute followed by rinsing with distilled water. Buds were then treated with 0.1% (w/v) Bavistin fungicide for 5 minute and washed thoroughly with autoclaved distilled water for 3 times. Apical buds were also surface sterilized by treating 0.1% (w/v) HgCl₂ for approximately 1 minute and again the buds were thoroughly washed with autoclaved distilled water followed by 70% ethanol for 1 minute and rinsed 3 times with autoclaved distilled water. Three buds per bottle were inoculated on surface of MS growth medium under aseptic conditions in the laminar flow and then kept in the tissue culture incubation chamber under 40W fluorescent tubes having a photon flux density of approximately 21,500 lux at a temperature of 25±1 °C and a relative humidity of ~ 70% with 16 h light 8 h dark photoperiods. The concentration of hormone (BAP and NAA), at which growth was maximum was selected.

**Root Induction**

Half strength of MS basal medium supplemented with 0.25 mg/L and 0.5 mg/L of indole butyric acid (IBA) was used for root induction in the shooted plants. All the culture condition was same.

**RESULTS AND DISCUSSION**

*In vitro* survival of explants

It was observed that apical buds of *Adina cordifolia* led to the browning of the medium and impairment of establishment which was subside by subculturing of explant regularly. Browning and contamination was recorded quite often as the explants were taken in the month of March. Exudation of phenolics is a natural mechanism in plants (Vengadesan *et al.*, 2002). In the present study it was observed that on increasing the time of sterilization greater than 1 minute with 0.1% (w/v) HgCl₂ death of explants was observed. Survival of the explants was observed after 7 days of interval up to 21 days. Survival rate in *Adina cordifolia* was higher in MS medium supplemented only BAP was 66.67% (1 ppm), 62.96% (2 ppm) and 62.96% (3ppm) with significantly higher over the other combinations (Graph. 1).

The maximum survival rate was observed with 1mg/l BAP. However when 0.5 mg/L NAA was added with different combinations of BAP in MS medium the explants survival rate was decreased compared to without NAA composition. It showed that *in vitro* establishment of *Adina cordifolia* required 1mg/L BAP in MS medium for better survival rate i.e. 66.67%. Growth of explants was observed in different combination of phytohormones with time intervals (Graph. 2). The shoot growth was higher in presence of MS medium supplemented with 2 mg/L BAP alone and MS medium supplemented with 0.5 mg/L NAA alone after 21 days of inoculation.

The month of April to June was found to be the best period for establishment of apical buds and elongation of shoots. The explants establishment rate was higher in the month of April to June because on start of autumn season. The new apical buds secreted some milky substances which causes low rate of survival of explants due to fungal and bacterial contamination. It was also observed when the explants (apical buds) collected from 3-5 years old tree, the milky substance secretion was very low than the 30-40 years old explants. Hence the chances of contamination were very low in 3-5 years old trees.
The similar results have also been observed by (Dubey et al., 2004).

**Subculturings of explants in MS medium**

After 21 days, the explants were subcultured in the same MS medium with the same hormone concentration. The highest elongation of 2.5 cm in explants was observed in 0.5 mg/L NAA and 2 mg/L BAP supplemented in MS medium (Fig.1 and Graph 3). Dubey et al., (2004) showed the best shoot elongation on MS medium supplemented with 0.5 mg/L IBA and 0.5 mg/L NAA.

**Root Induction**

Half-strength MS medium with 0.25 and 0.50 mg/L IBA did not induce any rooting even after 30 days of culture. Dubey et al., (2004) reported that the rooting in *Adina cordifolia* at 0.5 mg/L IBA and 0.5 mg/L NAA in MS medium. However, in this combination no root induction was observed.

**CONCLUSION**

*In vitro* propagation through apical buds is the best possible means of *in situ* conservation of *Adina cordifolia*, a threatened species, to produce a large number of plants in a short span of time. It is a powerful tool for large-scale propagation. Hence *Adina cordifolia* can be very well established in vitro conditions in presence of MS medium supplemented with 2mg/L BAP or 0.5mg/L NAA alone.

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**Table 1** Various concentrations of phytohormones (BAP and NAA) with different combinations for establishment of explants

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<tr>
<td>NAA mg/L</td>
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**Graph. 1** Survival rate of explant *Adina cordifolia* after twenty one days of inoculation. Data represented the mean value of three replicates ± SE at P ≤ 0.05. Growth of explants in different time intervals

**Graph. 2** Shoot length of explants of *Adina cordifolia* in MS medium after 7, 14, 21 days. Data represented the mean value of length in fresh medium in cm SEm ± at P ≤ 0.05.
**Fig. 3** Shoot length of explants in fresh subcultured medium after 7, 14, 21, days in which data represents the mean value of length in fresh medium in cm SEm ± at P ≤ 0.5.
Fig. 1 *In vitro* establishment of *Adina cordifolia* A, B, C and D represented the inoculated apical buds on MS medium for 7, 14, 21 and 28 days respectively. E, F represented the elongated shoot after 35 and 45 days of subculture on the same MS medium.

REFERENCES