Simultaneous Shoot Regeneration and Rhizogenesis of *Wedelia Chinensis* for *in Vitro* Clonal Propagation

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**ABSTRACT**

This abstract describes a method for *in vitro* clonal propagation of *Wedelia chinensis* (Family: Asteraceae), a medicinal plant used by traditional medicinal practitioners of Bangladesh. This plant is used in the traditional medicinal system of Bangladesh for hepatic disorders, coughs, cephalagia, skin diseases, dyeing and promoting hair growth, cancer, and also during uterine hemorrhages and menorrhagia. Explants (shoot tips and nodes) from field grown plants were collected and treated with 0.1% HgCl₂ for surface sterilization. Sterile explants were cultured onto Murashige and Skoog (MS) medium containing different concentration of auxins, cytokinins and gibberellic acid. Tips were sprouted and regenerated on MS medium fortified with BAP (1.0 mg/l) and had an average length of 1.19 cm. [BAP (2.0 mg/l) + IAA (1.0 mg/l)] when used with MS medium showed the best response (100%) on shoot elongation. Simultaneous shooting and rhizogenesis (2.68 roots/shoot) was obtained when explants were cultured in [MS + BAP (1.0 mg/l) + NAA (1.5 mg/l)]. Shoot proliferation was obtained with profused lateral budding in [MS + BAP (1.5 mg/l) + GA₃ (0.5 mg/l)]. Auto-rooting (16 roots/shoot) with vigorous shoot growth was observed in the same medium. The rooted plantlets were successfully established in earthen potted media after proper hardening and acclimatization. After eight weeks of transplantation more than 70% plants survived and no morphological variation was observed with the donor plants. This method of clonal propagation could reduce the cost and time of plantlet production of this species.

**Key words:** Bhringaraj, anti-hepatotoxicity, BAP + NAA, BAP + GA₃

**Introduction**

Medicinal plants have a great importance due to their potential uses to treat diseases in traditional medicine and pharmaceutical industries. *Wedelia chinensis* (Family: Asteraceae) is one of them which is used by the traditional healers of Bangladesh for a long time. The plant is commonly known as ‘bhringaraj’ in Bengali. It is a scabrous procumbent perennial soft herb with high camphor like odor and has a gorgeous growth (Martin *et al.*, 2003). The plant is reported to contain six new acylated eudesmanoids, germacrene, α-humulene, caryophyllene, squalene, phellandrene, p-cymene, sitosterol and wedelia-seco-kaurenolide (Rastogi and Mehrotra, 1993); leaves contain isoflavonoids and wedelolactone. This plant is considered to have different pharmacological properties such as antihepatotoxicity (Kirtikogi and Basu, 1975; Yang *et al.*, 1987; Lin, *et al.*, 1994); leaves are used in cough, cephalagia, alopecia and skin diseases (Chopra *et al.*, 1956); dyeing hair and...
for promoting hair growth; roots yield a black dye, ethanolic extract of the herb has been shown to inhibit the growth of Ehrlich ascites carcinoma (CSIR, 1992); decoction of the plant is used as deobstruent and given in uterine hemorrhage and menorrhagia (Kirtikar and Basu, 1975). The compounds of this herb have been reported to have synergistically suppression activity on androgen and growth in prostate cancer cells (Lin et al., 2007).

This herb can be propagated by seeds and vegetative stem cuttings. Due to cross-pollination the germinated seedlings are not true to type and propagation through this way is not reliable due to low span of viability. Habitat destruction and use in large quantities has endangered this species. Although, in vitro propagation of W. chinensis has been published previously (Martin et al., 2003; Bhuyan et al., 2000; Sultana and Handique, 2004), the present investigation describes the establishment of a rapid and cost reducing protocol for this plant.

**Materials and methods**

**Explant source and preparation:**

Nodal segments of donor W. chinensis plants (1 year old), grown in the Garden of Medicinal Plant at the Department of Biotechnology and Genetic Engineering, University of Development Alternative were used in the study. Explants were first washed for 30 minutes in running water and then with Tween-80. Finally they were rinsed three times with sterile distilled water following surface sterilization with 0.1% HgCl₂ for 5 minutes. All the explants were cultured on basal MS (Murashige and Skoog) (Murashige and Skoog, 1962) medium containing different concentrations of 6-benzylaminopurine (BAP) alone and in combination with indole-3-acetic acid (IAA), a-naphthalenacetic acid (NAA), gibberellic acid (GA₃), sucrose (3%) and agar (0.7%). The pH of the medium was adjusted to 5.8 before autoclaving and media was sterilized by autoclaving for 20 min at 121°C and 1.05 kg/cm².

**Culture of explants:**

Sterile explants were inoculated and cultured in the prepared medium (detailed in Result section). Thereafter they were maintained at 26 ± 2°C with a photoperiod of 16/8 h under an illumination of 40-50 μmol m⁻²s⁻¹ provided by cool white fluorescence lamps.

**Establishment into soil:**

The in vitro developed plantlets were acclimatized in polybags containing garden soil and cow dung at the ratio of 2:1 after proper hardening through gradual exposure to sunlight and relative humidity.

**Data assembly and scrutiny**

Weekly growth observations were made from 1st to 4th week of inoculation after setup of each treatment and experimental data were recorded. The parameters were:

a. Percentage response (%).
b. Average length of shoot in cm and number of leaves/shoot.
c. Average number of roots/shoot.

Recorded data were analyzed as mean ± SE.

**Results and discussion**

**Regeneration of shoots:**

Buds were sprouted in all the treatments of BAP supplemented in MS medium ranging from 0.5 to 2.0 mg/l (Table 1). A maximum of 90% explants responded when the medium contained BAP (1 mg/l) and the obtained average length of shoot was 1.19cm. Tallest shoot (1.64 cm) with massive lateral budding were produced in this phase from culture medium fortified with highest concentration of BAP (2.0 mg/l) within four weeks (Figure 1).

Nodal segments obtained from this experiment were used as a source of explants and cultured in the following sets of experiments.
Effect of BAP and IAA:

Different combinations of BAP and IAA were used to observe the synergistic effect of auxin and cytokinin. Shoot buds proliferated highly when they were cultured in MS medium containing BAP (2.00 mg/l) and IAA (1.00 mg/l). Good in-quality and elongated shoots with leaves were found in this composition (Figure 2). Relatively good responses (71.42 - 80.95%) were obtained also with other tested concentrations (Table 1).

Effect of BAP and NAA:

NAA added to the medium enriched with BAP also showed good performances. Shoots regenerated well (80%) and attained 1.43 cm length in MS medium with higher cytokinin (BAP at 1.0 mg/l) and lower auxin (NAA at 0.5 mg/l) (Table 1). This composition also resulted in a good percentage (75%) of simultaneous rooting of shootlets which became advantageous to produce in vitro plantlets (Figure 3).

| Table 1: Effect of plant growth regulators on explants of W. chinensis |
|------------------------|----------------------|------------------|-----------------|-----------------|----------------------|
| MS + Hormone(mg/l)     | Number of explants inoculated | Percentage of explants responded (%) | Average length of shoot (cm) | Average number of leaves | Percentage of root induction (%) | Average no of roots/shoot | Growth in vigor |
| BAP                   | 0.5                  | 20               | 75.00           | 1.42±0.20       | 3.20±0.40         | --                      | --          | +          |
|                       | 1.0                  | 20               | 90.00           | 1.19±0.17       | 4.10±0.43         | --                      | --          | ++         |
|                       | 1.5                  | 20               | 80.00           | 1.06±0.01       | 3.50±0.46         | --                      | --          | +          |
|                       | 2.0                  | 20               | 75.00           | 1.64±0.19       | 5.47±0.66         | --                      | --          | ++         |
| BAP+IAA               | 0.5+0.25             | 21               | 71.42           | 1.59±0.16       | 5.33±0.55         | --                      | --          | ++         |
|                       | 1.0+0.5              | 21               | 71.42           | 1.26±0.56       | 4.40±0.14         | --                      | --          | ++         |
|                       | 1.5+0.5              | 21               | 80.95           | 1.25±0.13       | 4.34±0.54         | --                      | --          | ++         |
|                       | 2.0+1.0              | 20               | 100.00          | 1.63±0.16       | 5.00±0.96         | --                      | --          | +++        |
| BAP+NAA               | 1.0+0.5              | 25               | 80.00           | 1.43±0.19       | 3.15±0.31         | 75.0                    | 3.55±0.83   | ++         |
|                       | 1.0+1.5              | 20               | 80.00           | 1.15±0.12       | 2.75±0.29         | 81.25                   | 2.68±0.45   | ++         |
| BAP+GA3               | 0.5+0.5              | 20               | 100.00          | 1.94±0.20       | 9.00±1.73         | --                      | --          | +          |
|                       | 1.0+0.5              | 20               | 90.00           | 1.89±0.23       | 68.00±4.04        | --                      | --          | +++        |
|                       | 1.0+1.0              | 20               | 90.00           | 2.53±0.32       | 62.00±11.16       | 80.0                    | 1.20±0.48   | +++        |
|                       | 1.5+0.5              | 20               | 100.00          | 2.38±0.63       | 18.54±4.04        | 100.0                   | 6.27±1.97   | ++         |
|                       | 1.5+1.0              | 20               | 90.00           | 2.65±0.54       | 22.37±3.16        | 90.0                    | 3.37±0.57   | ++         |

Fig. 1: Regenerated shoot of W. chinensis in MS + BAP (2.0 mg/l). 2. Shoot elongation in MS + [BAP (2.0 mg/l) + IAA (1.0 mg/l)]. 3. Root induction of micro-shoot in MS + [BAP (1.0 mg/l) + NAA (0.5 mg/l)].

Fig. 4: Rhizogenesis was observed in MS + [BAP (1.5 mg/l) + GA3 (0.5 mg/l)]. 5. W. chinensis plant was established in to soil (after eight weeks of transplantation).
Effect of BAP and GA:

The inclusion of GA in MS medium containing BAP showed superior bud break and the length of shoots was longer than in all other trials. In all cases, bud response ranged from 90% to 100% and shoots were appreciably longer than all other combinations tested. That shoot elongation of *W. chinensis* depends upon the synergism of GA and BAP turned out to be clear from our data (Table 1) and increased concentration of GA showed increased length of shoots. Shoots attained an average length of 2.65 cm in [MS + BAP (1.5 mg/l) + GA (1.0 mg/l)]. Enough lateral buds flourished in each shootlet with strong morphological appearance. Shoot multiplication (2-3 shoots/explant) was also observed to be good in this experiment.

At the same time, root emerged at the tuft of shoots (16 roots/shoot) within 20 days when the composition of the medium was [MS + BAP (1.5 mg/l) + GA (0.5 mg/l)] in our present study (Table 1 and Figure 4). This result enabled us to produce *in vitro* plantlets within four weeks.

Establishment into soil:

*In vitro* raised plantlets obtained from the experiments of (BAP + NAA) and (BAP + GA) were transplanted into soil through successful acclimatization. More than 85% plantlets survived and resumed good growth without any morphological variation as compared with the mother plants after eight weeks (Figure 5).

Discussion:

Sultana *et al.* (2004) concluded that BAP as a plant growth regulator could not play an efficient role for *in vitro* regeneration of this medicinal herb but our finding was opposite to them and corroborated by Martin *et al.* (2003). BAP has efficient role in shoot regeneration for tree also (Azam *et al.*, 2009). That higher BAP to lower auxin ratio facilitates axillary shoot proliferation is a common event in auxin-cytokinin interaction (Staden *et al.*, 2007), which is also revealed by our results from the combination of BAP and IAA. Sudha and Seeni (1994) reported that BAP in combination with IAA was essential for the promotion of shoot regeneration, which is in agreement with the current findings. Shoot regeneration with concurrent rooting through the combination of BAP and NAA is the first reported data of this species. Combination of BAP and GA in MS for direct shoot regeneration and simultaneous rhizogenesis is the first such repot of *W. chinensis*. During *in vitro* culture of other plant species, Mulwa and Bhalla (Sudha and Seeni, 1994), Purohit and Singhvi (1998), Boggetti *et al.* (1999) and Dhaka and Kothari (2005) reported that addition of GA, with BAP-supplemented MS medium is beneficial and promoted shoot elongation of *Macadamia tetraphylla*, *Achras sapota*, *Anacardium occidentale*, *Eclipta alba*, respectively. Evidence on rooting inhibition was reported by Boggetti *et al.* when GA was added in the medium Boggetti *et al.* (1999). However, any such inhibition was not observed in the present study. This could be due to the fact that Boggetti *et al.* (1999) conducted their studies with *Anacardium occidentale*, while the present study was conducted with *Wedelia chinensis*.

Conclusion:

The developed method of *in vitro* clonal propagation could be a way of cost and time reducing protocol to produce plantlets of *W. chinensis*.

References


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