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Full Length Research Paper

Plant regeneration from nodal explants of *Adhatoda vasica* Nees.

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A simple micropropagation protocol for the important medicinal plant species *Adhatoda vasica* (Vasaka) from nodal explants was developed. The young juvenile nodal explants cultured on Murashige and Skoog's (MS) medium supplemented with CW (15% v/v) + BAP (5 mg/l) started proliferating multiple shoot buds (14±2) in 3 to 4 weeks and the shoots became 3 cm long in 6 to 8 weeks. The regenerated plants produced well developed roots on transfer to MS + IBA 1 mg/l in 10 to 12 days. The rooted plantlets were acclimatized before transfer to soil and 80% of the transferred plants survived.

Key words: *Adhatoda vasica*, Vasaka, nodal explants, micropropagation.

INTRODUCTION

The plants are important natural sources of medicines and pharmaceutical products (Kumar, 2004; Patwardhan et al., 2004). The ever increasing global interest in Ayurveda way of life has resulted not only in demand for a huge raw material of medicinal plants but also the right stage of the plant or plant part when the active principle(s) are available in optimum quantities for herbal preparations (Chaturvedi, 2001; Shinwari, 2010). The application of plant biotechnology for clonal propagation of medicinal plants, has the potential to meet the demand of raw materials, is well established fact (Pierik, 1987). *Adhatoda vasica* belonging to family Acanthaceae is an evergreen woody shrub distributed from the Punjab in the north and Bengal and Assam in the south–east to the Ceylon, Malaya and Singapore in the south. The plant is useful for the remedy of asthma, respiratory tract disorder and cough (Jaiswal et al., 1989; Azad et al., 2003; Nath and Buragohain, 2005; Abhyankar and Reddy, 2007; Khalekuzzaman et al., 2008). All parts of plant are used as an herbal medicine. The leaves contain vasicine and vasicinone which are well known for their bronchodilator activity (Kumar et al., 2007). The plant shows inhibitory action against *Mycobacterium tuberculosis* and its potential for controlling multidrug resistant tuberculosis (MDR-TB) and human immunodeficiency virus (HIV) infection (Grange and Snell, 1996), the species is very important to cure asthma and other throat infections (Shinwari et al., 2009). Generally, the plant is propagated by seed as well as stem cutting but very low seed germination and labour intensive vegetative propagation by stem cutting are constrains for large scale production of *A. vasica*. Attempts have been made in the past for regeneration of *A. vasica* through tissue culture (Jaiswal et al., 1989; Azad et al., 2003; Nath and Buragohain, 2005; Abhyankar and Reddy, 2007; Khalekuzzaman et al., 2008; Kumar et al., 2007). The alkaloid content of the plant has been reported to vary with the genotype and environment, so clonal propagation has been recommended (Duster, 1985). The present study was undertaken to establish a simple protocol for rapid plant regeneration from nodal explants in *A. vasica*.

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MATERIALS AND METHODS

The nodal, internodal and leaf explants were collected from mature and kinetin) either individually or in various combinations. The healthy plants growing in Sanskrit College, Muzaffarpur (Figures 1 and 2). The explants were cut into small pieces of 1.5 to 2 cm, thoroughly washed in running tap water followed by washing with liquid detergent. For surface sterilization the explants were treated with 0.2% $HgCl_2$ and washed with distilled water and finally washed with sterile distilled water for 4 to 5 times. The surface sterilized nodal explants were cultured on MS (Murashige and Skoog's, 1962) medium supplemented with coconut water (CW) as well as

Figure 1. A close photograph of plant of *Adhatoda vasica* growing in nature.

Figure 2. A close photograph of a flowering twig of *A. vasica.*

different concentrations of auxins (Naphthalene Acetic Acid, 2,4-dichlorophenoxy acetic acid and cytokinins (6-benzylaminopurine regenerated shoots were transferred to MS medium supplemented with IBA for root differentiation. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were maintained at 25±2°C under cool fluorescent light and 16 h photoperiod. All experiments were repeated 5 to 7 times. The regenerated plants were acclimatized under laboratory conditions and transferred to pots filled with sterilized soil: sand mixture (1:3).

Figure 3. A photograph of nodal explants cultured on MS+CW (15%)+BAP (1 mgl^{-1}).

RESULTS AND DISCUSSION

The surface sterilized young juvenile nodal explants of *A. vasica* ranging in size from 1 to 1.5 cm long explants were cultured (Figure 3) on MS medium supplemented with coconut water and different concentration of auxins and cytokinins either alone or in various combinations. The nodal explants cultured on MS medium supplemented individually with CW, 2,4-D, NAA, IAA, BAP or KN did not show any response. The nodal explants cultured on MS+2,4-D (0.1 mgl^{-1})+NAA(0.1 mgl^{-1})+BAP(1 mgl^{-l}), MS+ NAA(0.1 to 1 mgl^{-1})+BAP(1 to 2.5 mgl^{-l}) did not show any morphogenetic response and turned dark in 12 to 15 days. Also, the increased concentration of BAP (5.0 mgl^{-l}) could not induce any effect on the cultured explants and turned black. The nodal explants cultured on MS+CW (15%)+BAP(1 mgl^{-l}) remained green and viable for 18 to 20 days and the axillary buds developed into green leafy structures (Figure 4). Significantly, the increase in concentration of BAP to 2.5 mgl^{-l} induced formation of shoot buds (3±1) from nodal regions in 82% of cultured explants (Figures 6).

However, the nodal explants culture on MS medium supplemented with CW (15% v/v)+BAP (5 mgl^{-l}) induced proliferation of 14±2 shoot buds (Figure 5) in 72% of the cultured explants in 20 to 25 days. It was interesting to observe the morphological differences in the nodal region during shoot bud proliferation from the nodal explants cultured on MS+CW (15%v/v)+BAP(2.5 mgl^{-l})+MS+CW(15%v/v)+BAP(5mgl^{-l}). In the presence of low

Figure 4. A photograph of green leafy structures developing from nodal region cultured on MS+ CW (15%)+BAP (1 mgl^{-1}).

Figure 5. A photograph showing induction of multiple shoot buds on MS++ CW (15%)+BAP (5 mgl^{-1}).

BAP (2.5 mgl^{-1}) the nodal region did not show any swelling, became dark colored and produced 2 to 4 shoot buds. On the other hand, the cultured nodal explants showed bulging of the nodal region and remained green while sprouting shoot buds. The number of explants cultured on MS+CW(15%)+BAP(5.0 mgl^{-1}) producing 14±2 shoot buds has been given in Table 1. The *in vitro* regenerated plants produced well developed roots (Figure 7) on MS medium supplemented with IBA (1 mgl^{-1}). *A. vasica* is very important traditional and pharmaceutically important plant. Several major alkaloids such as quinazoline alkaloid (vasicine) along with other minor alkaloids are present in the leaves. Even today the plant is propagated conventionally by seeds and stem cuttings. The attempts on tissue culture of nodal explants made earlier resulted in very limited number of plants (Jaiswal et al., 1989; Chomchalow and Sahavacharin, 1981). The presence of BAP (0.5 mgl^{-1})+NAA(0.1 mgl^{-1}) in the MS medium has been found to induce 7± 0.53 usable shoots in *A. vasica* although, 93% of cultured explants were responsive (Azad et al., 2003). In another study 13 shoots were reported to be formed in the presence of BAP (2 mgl^{-1})+NAA(0.2 mgl^{-1}) (Khalekuzzaman et al., 2008).

In the present experimental system, BAP + NAA combination neither proliferated neither callus nor shoot buds. BAP (2 mgl^{-1}) alone in the MS medium was reported to induce shoot formation (3±0.15), however 96.67% shoots developed callus at the cut basal end of explants and also turned brown and necrotic due to phenolic exudation (Nath and Buragohain, 2005). The callus formation at the cut end of the explants and browning of plantlets were overcome by culturing the explants on MS supplemented with Thiadiazuron (0.3 mgl^{-1}) and CW (15%v/v) (Nath and Buragohain, 2005). We obtained plantlets on MS+CW (15%v/v)+BAP (5 mgl^{-1}) but neither callus nor browning of plantlets were observed. In the split node culture technique, 7.75±0.392 shoots per nodal half on MS+BA(10mgl^{-1}) which, of course, on subculture produced 25-30 shoots in about 6 weeks (Abhyankar and Reddy,2007).The concentration of BAP above 5 mgl^{-1} was not found suitable in our experiment. Rooting in *A. vasica* has been reported to be 100% on ½ MS basal medium alone or ½MS+IBA (0.1 mgl^{-1}), ½MS+NAA (0.1 mgl^{-1}) and 60% only on ½ MS + IAA (0.1 mgl^{-1}). IBA (0.1 mgl^{-1}) and IBA (3 mgl^{-1}) supplemented MS medium have also been reported for rooting in *A. vasica by* different workers) (Nath and Buragohain, 2005; Abhyankar and Reddy, 2007). In the present study only MS medium containing IBA (1 mgl^{-1}) was found effective in inducing rooting as reported earlier (Khalekuzzaman et al., 2008). Although, ½ MS or MS basal medium have been found to induce 100% rooting , in our experiment MS basal medium was not favourable for root formation (Azad et al., 2003; Nath and Buragohain, 2005; Raageeva and Kaushal, 2008; Hussain et al., 2011). The roots produced in *A. vasica* on solidified medium were white in colour as also observed in a previous study (Abhyankar and Reddy, 2007). The regenerated plantlets were acclimatized under laboratory conditions before transferring to pots. The rate of

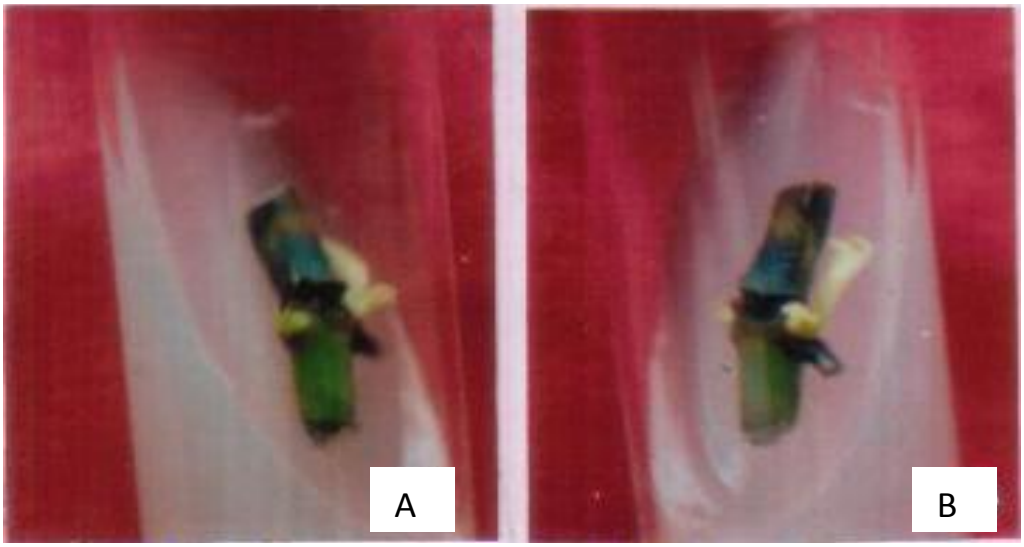

Figure 6. A and B. A shoot growing on MS + CW 15% + BAP 2.5 mg/l.

Table 1. Correlation between number of multiple shoot buds and the number of explants.

S/N	Number of shoot buds produced	Number of explants (out of 125 explants)	Percentage explants (%)
1.	16	25	20
2.	15	15	12
3.	14	55	44
4.	13	20	16
5.	12	10	8.0

Figure 7. Root formation in *in vitro* regenerated shoot of *A. vasica.*

survival was 80% (12 out of 15 plants).

The present study established the protocol for single step proliferation of 14±2 shoots in the presence of CW + BAP on MS nutrient medium which seems to be the highest number of shoots produced per node *in vitro* and has the potential for clonal propagation of *A. vasica*.

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