ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 143-147: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

CALLUS CULTURE OF A MEDICINAL PLANT VASAKA (*ADHATODA VASICA* NEES) FROM LEAF BASE EXPLANTS

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KEYWORDS

Adhatoda vasica Nees
Vasaka
MS media
Callus culture
Leaf explants

Proceedings of National Conference on
Harmony with Nature in Context of
Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

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ABSTRACT

The callus culture of a medicinal plant vasaka (*Adhatoda vasica* Nees) were obtained from leaf base explants. The present study was conducted with the objectives to find out the best media for callusing and shoot and root regeneration capacities of callus in *Adhatoda vasica*. Seven media's were tried under the present study. Out of them, five were of Murashige and Skoog's (MS) media and the other two media's were Gamborg B5 and White's media. Different combinations and concentrations of 2,4- Di chloro phenoxy acetic acid (2,4- D), Kinetin (Kn), benzyl amino purine (BAP) and Naphthalene acetic acid (NAA) were used. Among the seven media's tried M2 media contained MS + 1mgL^{-1} 2,4-D + 0.5mgL^{-1} Kinetin + 1mgL^{-1} IBA. The M3 media was Murashige and Skoog's (MS) media which contained 2mgL^{-1} BAP + 2mgL^{-1} Kinetin + 1mgL^{-1} IAA + 0.5mgL^{-1} NAA and 150mlL^{-1} Coconut water. The M4 media contained MS salts + 2mgL^{-1} 2,4-D + 1mgL^{-1} Kinetin + 1mgL^{-1} BAP. The best media found for callusing and shoot regeneration was M3 media followed by M4 and M2 media's respectively. The best media found for root regeneration was M2 media followed by M3 media. Doubling the amount of sucrose were found to be the best for root regeneration.

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INTRODUCTION

Adhatoda vasica Nees (Vasaka) of the family Acanthaceae is an important medicinal plant of India. It is native of India. It is distributed all over the plains of India and in lower Himalayan ranges. It is a small evergreen, sub-herbaceous bush which grows universally in open plains, especially in the lower Himalayas up to 1300 meters above sea level. Spread from the Punjab in the North, and Bengal and Assam in the South-East to the Ceylon, Malaysia and Singapore in the South. It is well known for its effectiveness in treating respiratory conditions. It is an antispasmodic and expectorant, used for centuries with much success to treat asthama, chronic bronchitis, and other respiratory conditions (Gangwar and Ghosh, 2014). Adhatoda vasica is an effective drug having bronchodilator, antitubercular, antiseptic, parasiticidal, anti-inflammatory, antiphlegmatic, antibilious, uterine stimulant, digestive, appetizer, emmenagoue, antihelmintic, diuretic and antihypertensive activities (Zainab *et al*., 2010). The main constituents of *Adhatoda vasica* Nees are pyroquinazoline alkaloids viz. Vasicine and Vasicinone. All parts of the plant are used in herbal medicine and particularly the leaves are credited with insecticidal and parasiticidal properties (Abhyankar *et al*., 2007). The root is useful in strangury, leucorrhoea, bronchitis, asthma, bilious vomiting, sore eyes, fever and gonorrhoea (Azad *et al*., 2003). This plant shows low germination and conventional propagation through cutting. Thus *in-vitro* propagation can be used as an effective alternative for conservation and multiplication of this plant. Thus to determine the possibility and potentiality of the tissue culture techniques in mass production of this crop species the present investigation has been undertaken with an objective, to determine the best suitable media for callusing and shoot and root regeneration in callus using leaf base explant.(Chomchalo and Sahavacharin, 1981) first attempted regeneration of Adhatoda vasica through tissue culture. (Khalekuzzaman *et al*., 2008) established an efficient protocol for *in-vitro* propagation of *Adhatoda vasica* Nees using shoot tip and nodal explants. (Shalaka and Parmeshwaran, 2009) developed callus cultures of *Adhatoda vasica* from leaf, petiole and nodes by using different PGR combinations. An efficient tissue culture system for regeneration of plants from cultured cells and tissues is the key in success of plant genetic engineering (Pua *et al*., 1996; Purnhauser *et al*., 1987; Bharose *et al*., 2014), enhancement in the regeneration frequency would be an added advantage in improving the genetic transformation protocols. Thus a mass multiplication protocol is to be developed for its better future supply.

MATERIALS AND METHODS

The leaf and leaf bases of Adhatoda vasica were collected from the nursery of Indore campus and present experiments were carried out at Tissue Culture Laboratory, College of Agriculture, Indore a constituent campus of Rajmata

Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) during year 2014-15.These explants were collected from 2-3 years old plant of *Adhatoda vasica*. To sterilize the explants, they were thoroughly washed in running tap water for 30 minutes to remove any remaining dirt or dead plant material. The explants were then cut with sterilized razor into pieces and washed with liquid detergent (Teepol) by gently shaking for 10 minutes. Then they were thoroughly washed with distilled water and transferred to 0.1% $HgCl_2$ solution for 5 minutes for surface sterilization. Finally, these were rinsed with sterile distilled water for three to four times before inoculation. Sterilized explants were inoculated onto three culture media viz. Murashige and skoog's (MS) 1962, Gamborg's B5 1968, and White's 1939, culture medium with various combinations and concentrations of 2,4- Dichloro phenoxy acetic acid (2,4- D), Kinetin (Kn), benzylaminopurine (BAP), Naphthalene acetic acid (NAA). Different phytohormones are added in different media's and pH is adjusted to 5.8. The inoculations were done under laminar air flow hood. While working in laminar air flow, hands should be washed with spirit. The forceps and scalpels should be sterilised with spirit and burned with spirit lamp. The inoculation should be done in front of spirit lamp in the laminar flow. After inoculating the explants in the test tubes, the tubes were tightly plugged with cotton plugs. All cultures were incubated at 25 ± 2^0C for a photoperiod of 16 hours day^{-1} under fluorescent light (about 1200 Lux). These inoculated explants will result into callus development after 4 weeks of culturing. The callus obtained from the explants were aseptically removed from the culture tubes and then divided into 5mm x 5mm x 5mm size pieces with the help of sterilized razor. Crumbs were then picked up by sterilized forceps and inoculated into the media. The culture tubes were then incubated at 25 $\pm$ 2^0C and 16 hrs (light) and 8 hrs (dark) photoperiod day^{-1}. Average fresh callus weight and callusing % should be determined. The shoot regeneration capacity of each medium was observed after 25 days. When shoots attained a height of 40-50 mm, they were carefully removed from the shoot regeneration medium and placed on sterilized petridishes. Dry leaves and adhering calli were removed with the help of forceps and scalpel. Shoots were then placed onto different rooting media at the rate of one shoot per tube. The rooting media contains the half strength of salts but double quantity of sucrose. The basal ends of shoots were slightly dipped into media and the cultures were incubated at 25ºC under a 16hrs photo period.

RESULTS AND DISCUSSION

The different combinations and concentrations of growth hormones were tried in Murashige and skoog's (MS), Gamborg's B_5 and White's culture media. Seven media's were tried under present study (Table 1.1). Of them, five were of Murashige and skoog's (MS) media with various concentration and combinations of growth hormones and other two media's were Gamborg's B_5 and White's culture media. The leaf base is used as explants. The callus is initiated within four weeks of inoculation of leaf base explants in different culture media's. The callus induction was found in different quantity in different combinations of growth hormones. Pawar *et al.* (2012) also observed that the type and varying phytohormones plays a major role in determining multiple shoot induction in case of tomato. The mean value of callusing percentage using leaf base as explants averaged over five replications (figure 2.1). The data indicated that average callusing percentage for various media *viz;* M1, M2, M3, M4, M5, B_5 and White's media (Table 1.2). The average callusing % of five replications was found to be 27.2, 39.6, 67.4, 48.8, 32.2, 16.6 and 10.4 respectively. The M2 media containing MS salts + $1mgL^{-1}$ 2,4-D + $0.5mgL^{-1}$ Kinetin + $1mgL^{-1}$ IBA. The M3 media containing Murashige and skoog's (MS) media + $2mgL^{-1}$ BAP + $2mgL^{-1}$ kinetin + $0.5mgL^{-1}$ NAA + $1mgL^{-1}$ IAA + 150 mlL^{-1} Coconut water. The M4 media containing MS salts + $2mgL^{-1}$ 2,4-D + $1mgL^{-1}$ Kinetin + $1mgL^{-1}$ BAP. The highest callusing percentage was observed in M3 media (67.4 %) followed by M4 media (48.8%) and M2 media (39.6%) respectively. This M3 media combination was found best for induction as well as for growth of callus. The least responding media was White's medium with 10.4% callusing.

The mean values of fresh weight of callus using leaf base as

Table 1.1: Different media used in experiment

Media	Combinations and concentrations of hormones($mg/l_)$
M_1	MS + 1mg/l 2,4-D
M_2	MS + 1mg/l 2,4-D + 0.5 mg/l Kinetin + 1mg/l IBA
M_3	MS + 2mg/l BAP + 2mg/l kinetin + 0.5mg/l NAA + 1mg/l IAA + 150ml/l Coconut Water
M_4	MS + 2mg/l 2,4-D + 1mg/l Kinetin + 1mg/l BAP
M_5	MS + 2mg/l NAA + 1mg/l BAP + 0.5 mg/l GA_3
B_5	B_5 Salts + 0.5mg/l NAA + 2 mg/l BAP
White's	White's salts + 2mg/l NAA + 2mg/l Kinetin + 1mg/l BAP

Table 1.2: Callusing percentage (%) obtained on different media-leaf base as explant

Media	Range of callusing	Average callusing percentage*
M_1	24-30	27.2 (31.44)
M_2	34-44	39.6 (39)
M_3	63-72	67.4 (55.18)
M_4	42-56	48.8 (44.31)
M_5	28-36	32.2 (34.57)
B_5	12-21	16.6 (24.04)
White's	7-14	10.4 (18.81)
SEm	=	1.06
CD at (0.05)	=	3.08

*The figures in percentage are angular transformed values

Table 1.3. Average fresh callus weight (mg) obtained on different media

Media	Range of fresh callus weight(mg)	Average fresh callus weight(mg)
M_1	128-139	134.5
M_2	336-352	343.6
M_3	663-680	672.4
M_4	482-494	486.3
M_5	228-240	234.2
B_5	87-98	92.7
White's	58-68	63.2
SEm		2.29
CD at (0.05)		6.65

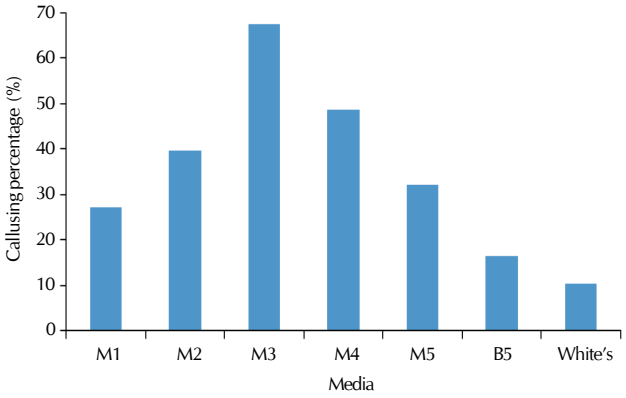

Figure 1: Callusing Percentage (%) obtained from leaf base as explant

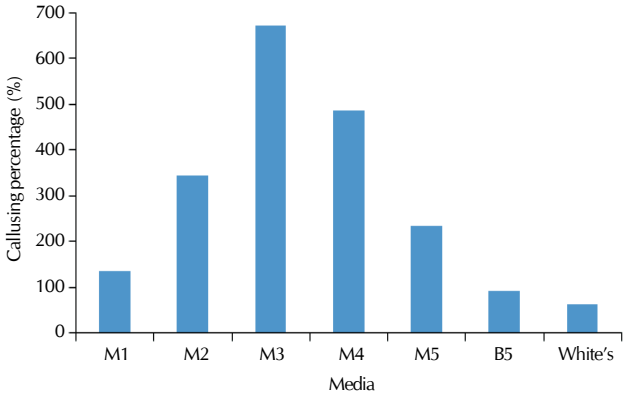

Figure 2: Average fresh callus weight (mg) obtained on different media - leaf base as explant

a. Inoculation of leaf base explant in culture media

b. Callus induction fom leaf base explants of *Adhatoda vasica*

c. Multiple shoots initiation from the callus

d. Rooting of *in vitro* shoots

e. Acclimatised plant after transferring to the garden

Figure 3: In vitro propagation of *Adhatoda vasica* Nees using leaf base explants

explants have been presented in (figure 2.2).This revealed that in M1, M2, M3, M4, M5, B_5 and White's media, the average fresh callus weight was found to be 134.5, 343.6, 672.4, 486.3, 234.2, 92.7, and 63.2 mg respectively (Table 1.3). The data in the bar diagram indicates that the maximum callus weight was noted on M3 media (672.4mg), followed by M4 (486.3mg) and M2 (343.6mg) media's respectively.

With regards to callusing percentage and callus growth, some modifications of MS medium gave high callusing efficiency, as compared to other MS combinations (Fig 2.3). Medium M3 was the best, which contained MS salts + 2mgL^{-1} BAP + 2mgL^{-1} Kinetin + 0.5 mgL^{-1} NAA + 1mgL^{-1} IAA and 150 mlL^{-1} CW. This medium has also been reported best for callus induction and shoot regeneration and are supported by (Naz *et al,* 2011, Sinha and Bandhopadhyay 2011).

The experimental performance of medium M3 may be attributed to the presences of IAA + Kinetin in the media. They are good growth hormones and stimulant for callus induction. M4 media contained MS + 2 mg/l 2,4-D + 1mg/l kinetin +1mg/l BAP. The media like M1, M5, B5 and White's although contained fairly good amount of auxins and cytokinins but not give favourable results comparatively. The present findings are in accordance with Mahato and Prasad 2009.

In the present investigation, so far as the shoot regeneration is concerned, medium M3 was found to be the best. The overall superior performance of M3 media can be attributed to the presence of BAP + Kinetin in the media which are supposed to be highly effective in inducing multiple shoots from callus. Several workers have reported the efficiency of BAP and Kinetin in inducing morphogenesis of callus. These findings have also been supported by (Madhumita and Raychaudhuri 2001, Zachariah *et al.,* 2001, Azad *et al*, 2003, Sarihan *et al.,* 2005, Soni *et al.*, 2009).

Thus, overall observations of the present investigation suggested that, among the media tried, the maximum callusing efficiency and shoot regeneration was obtained on Medium M3. The decreasing order of effectiveness of different media tried was M3 > M4 > M2 > M5 > M1 > B5 > White's.

In the present investigation rooting of individual regenerated shoots was best seen in M2 medium with 2.6 cm root length after 25 days of inoculation of shoot on rooting media. Out of these seven media M2 is the best medium for rooting which contained MS (1/2 strength salts) + double quantity of sucrose + 1mgL^{-1} 2,4-D + 0.5 mgL^{-1} kinetin + 1mgL^{-1} IBA. The outstanding performance of M2 media can be attributed due to the presence of IBA. Several workers have reported the efficiency of IBA in inducing morphogenesis of callus. Similar

results have been observed by (Gaikwad 2003) and were found to be favourable for root regeneration.

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