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DETECTION OF BEST EXPLANT DURING CALLOGENESIS IN *ADHATODA VASICA* NEES

Sonal Tolwani *et al.,*

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SONAL TOLWANI[1]*, P. D. GAIKWAD[2] AND VIVEK KUMAR KURREY[3]
[*1]Department of Plant Breeding and Genetics,
College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya Jabalpur - 482 004 (M.P.) India
[2]Department of Plant Breeding and Genetics, College of Agriculture, Indore, RVSKVV, Gwalior - 452 001 (M.P.) India
[3]Department of Vegetable Science, Indira Gandhi Krishi Vishwavidyalaya Raipur - 492 012, Chhattisgarh, INDIA
e-mail: swty030490@gmail.com

ABSTRACT

Callus cultures were obtained from 3 explants of vasaka (*Adhatoda vasica* Nees) *i.e* Cotyledon, Stem segment and Leaf base. The main objective was to find out the best explant for callusing. The average callusing percentage of cotyledon explant is 11.2, 18.6, 31.2, 23.4, 14.8, 8.2 and 5.4 %. The average callusing percentage of stem segment explant is 18.6, 30.8, 48.2, 37.0, 24.4, 11.8 and 8.6 %. The average callusing percentage of leaf base explant is 27.2, 39.6, 67.4, 48.8, 32.2, 16.6, and 10.4%. The highest average callusing percentage is of leaf base (67.4 %) followed by stem segment (48.2 %) and cotyledon (31.2 %) respectively. The average fresh callus weight of cotyledon explant is 94.5, 134.3, 283.5, 174.2, 108.7, 61.8 and 42.6 mg. The average fresh callus weight of stem segment explant is 115.3, 232.2, 373.8, 284.6, 171.7, 74.2 and 52.1 mg. The average fresh callus weight of leaf base explant is 134.5, 343.6, 672.4, 486.3, 234.2, 92.7 and 63.2 mg. The highest average fresh callus weight is of leaf base (672.4 mg) followed by stem segment (373.8 mg) and cotyledon (283.5 mg) respectively. Among these 3 explants, leaf base gave best result.

***Corresponding author**

INTRODUCTION

Adhatoda vasica Nees (Vasaka) is an important medicinal plant of India, belonging to the family Acanthaceae. 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. *Adhatoda vasica* is an Indian herbal plant, which due to its quinazoline alkaloids, is traditionally used for treating respiratory diseases (Herrmann *et al*., 2006). *Adhatoda vasica* is a well-known herb in indigenous systems of medicine for its beneficial effects, particularly in bronchitis. Vasaka herb is used for treating cold, cough, chronic bronchitis and asthama (Kumar *et al*., 2010). 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). 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 flowers and fruits are bitter aromatic and antispasmodic. The flowers are used in opthalmia and jaundice (Kirtikar and Basu, 1994). 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 the present investigation has been undertaken with an objective, to determine the best explant for callusing, among the 3 different explants i.e cotyledon, stem segment and leaf base used. (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. (Maurya and Singh, 2010) established callus cultures of *Adhatoda vasica* from leaf explants. (Sil and Ghosh, 2010) regenerated *in-vitro* plant through organogenetic callus and embryogenic callus of *Adhatoda vasica* Nees using nodal segments, shoot apices, petioles and leaf discs. 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 cotyledon, stem segment and leaf base explants 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. The cotyledon is collected from the seeds. The stem segment and leaf base explants were collected from 2-3 years old plant of *Adhatoda vasica*. The explants were sterilized by thoroughly washing in running tap water for 20-25 minutes to remove any dirt or dead plant material. The explants were then cut into

pieces with sterilized razor and washed with liquid detergent (Teepol) by gently shaking for 10-15 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 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 $\pm$ 2°C 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°C and 16 hrs (light) and 8 hrs (dark) photoperiod day^{-1}. Average fresh callus weight and callusing % should be determined.

RESULTS AND DISCUSSION

The cotyledon, stem segment and leaf base were the explants inoculated in Murashige and skoog's (MS), Gamborg's B_5 and White's culture media with various concentration and combinations of growth hormones. The callus is initiated within four weeks of inoculation of explants in different culture media's. The callus induction was found in different quantity in different explants. The mean value of callusing percentage using cotyledon, stem segment and leaf base as explants averaged over five replications (Fig. 1). The data indicated that average callusing percentage for various media *viz;* M1, M2, M3, M4, M5, B_5 and White's media (Table1). The average callusing percentage using cotyledon as explant was found to be 11.2, 18.6, 31.2, 23.4, 14.8, 8.2 and 5.4 respectively. The average callusing percentage of stem segment was found to be 18.6, 30.8, 48.2, 37.0, 24.4, 11.8 and 8.6 respectively. The average callusing percentage of leaf base was found to be 27.2, 39.6, 67.4, 48.8, 32.2, 16.6 and 10.4 respectively. The highest average callusing percentage of cotyledon, stem segment and leaf base is 31.2, 48.2 and 67.4 % respectively (figure 3). The highest average callusing percentage is observed in leaf base explants followed by stem segment and cotyledons respectively. The bar diagram also depicts that the leaf base was superior to the explants followed by stem segment. The explants cotyledon was found as the most inferior one among the three explants taken under study.

The mean values of fresh weight of callus using cotyledon, stem segment and leaf base as explants have been presented in (Fig. 2). The data indicated the average fresh callus weight for seven media's *viz;* M1, M2, M3, M4, M5, B_5 and White's

Table 1: Callusing percentage (%) of different explants on different media

Media	Cotyledon	Stem segment	Leaf base
M_1	11.2	18.6	27.2
M_2	18.6	30.8	39.6
M_3	31.2	48.2	67.4
M_4	23.4	37.0	48.8
M_5	14.8	24.4	32.2
B_5	8.2	11.8	16.6
White's	5.4	8.6	10.4

Table 2: Average fresh callus weight (mg) of different explants on different media

Media	Cotyledon	Stem segment	Leaf base
M_1	94.5	115.3	134.5
M_2	134.3	232.2	343.6
M_3	283.5	373.8	672.4
M_4	174.2	284.6	486.3
M_5	108.7	171.7	234.2
B_5	61.8	74.2	92.7
White's	42.6	52.1	63.2

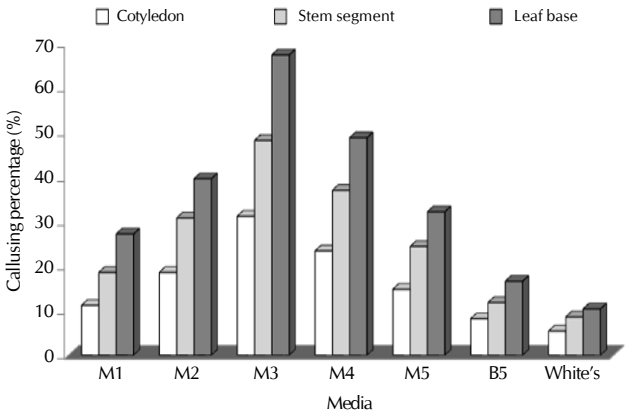

Figure 1: Callusing Percentage (%) of different explants on different media

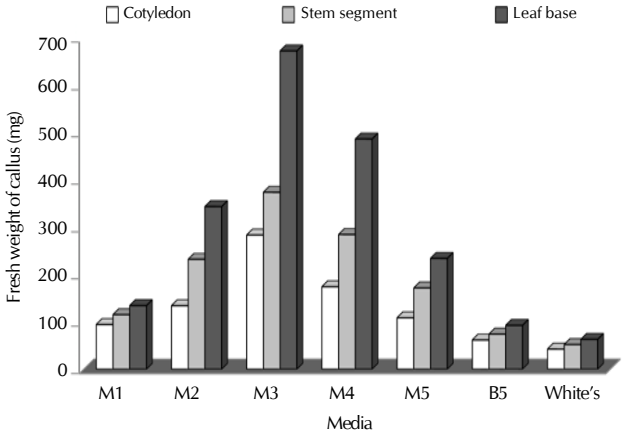

Figure 2: Average fresh callus weight (mg) of different explants on different media

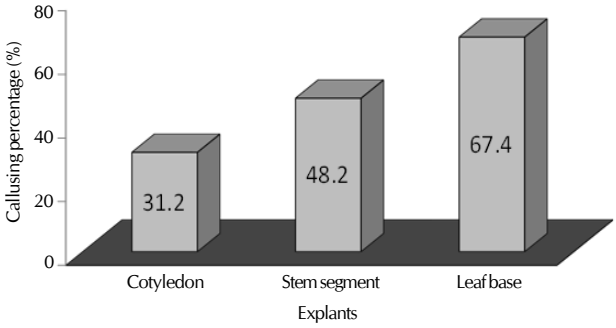

Figure 3: Highest average Callusing Percentage (%) of different explants

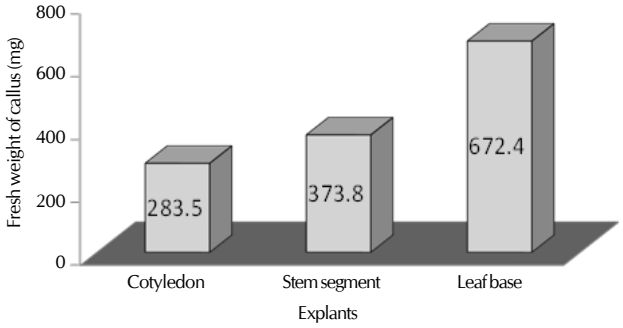

Figure 4: Highest average fresh callus weight (mg) of different explants

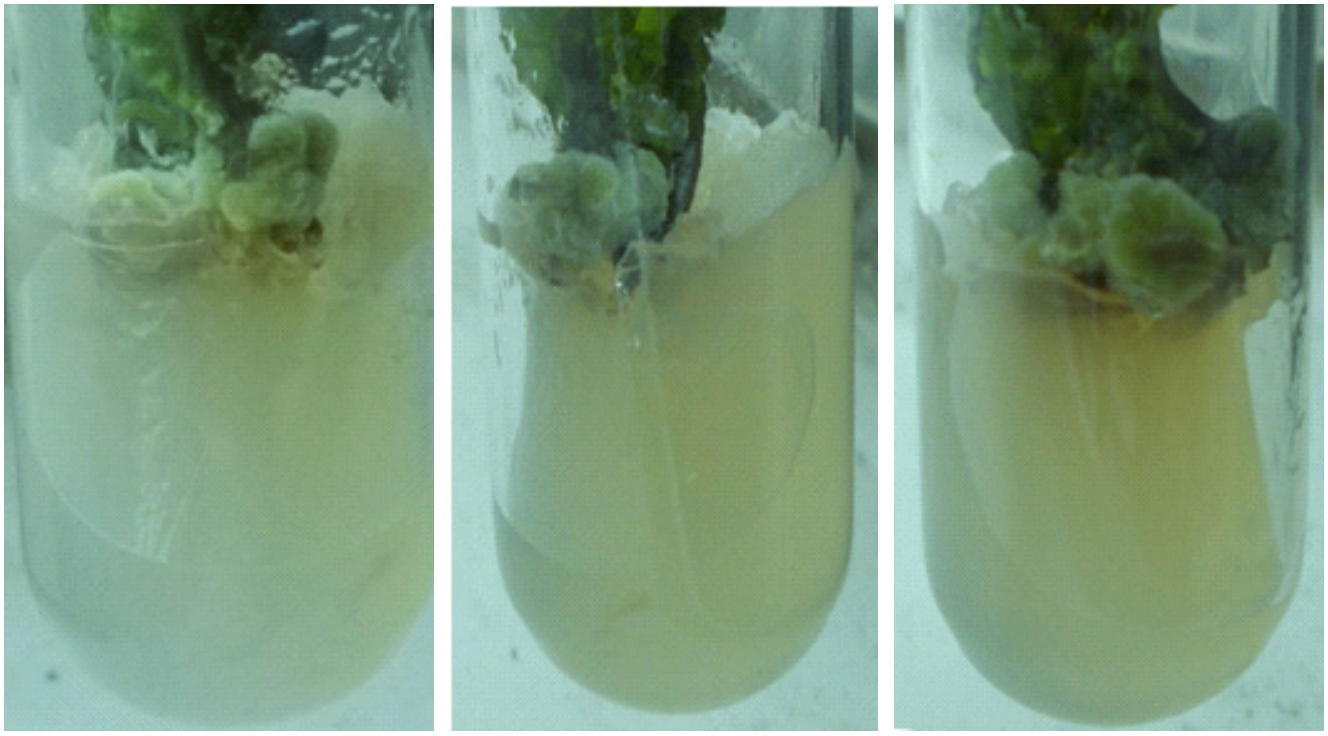

Figure 5: Callus formation from explants

media (Table 2). The average fresh callus weight of cotyledon was found to be 94.5, 134.3, 283.5, 174.2, 108.7, 61.8 and 42.6 mg respectively. The average fresh callus weight of stem segment explant was found to be 115.3, 232.2, 373.8, 284.6, 171.7, 74.2 and 52.1 mg respectively. The average fresh callus weight of leaf base explant was found to be 134.5, 343.6, 672.4, 486.3, 234.2, 92.7, and 63.2 mg respectively. The highest average fresh callus weight observed in cotyledon, stem segment and leaf base is 283.5, 373.8 and 672.4 mg respectively (figure 4). The data indicates that the maximum callus weight was noted on leaf base, followed by stem segment and cotyledon respectively.

The callogenesis was done in *Adhatoda vasica* Nees by the cotyledons, stem segment and leaf base explants are taken under present investigation (Fig. 5). It is supported by (Gaikwad and Prasad, 2003, Sariharan *et al.,* 2005, Li and Wang 2006). With regards to callusing percentage and callus growth, leaf base explants gave high callusing efficiency, as compared to other explants. This has also been reported best for callus induction and are supported by (Shalaka and Parmeshwaran 2009, Maurya and Singh 2010, Mungole *et al.,* 2011). These findings have also been supported by (Zachariah *et al.,* 2001, Mahato *et al.,* 2009, Sil and Ghosh 2010, Naz *et al.,* 2011). Thus, overall observations of the present investigation suggested that, among the explants tried, the maximum callusing efficiency was obtained on leaf base explant. The decreasing order of effectiveness of different explants tried was Leaf base > Stem segment > Cotyledon.

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