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## Organogenesis and *In vitro* plantlets production of ishwarmul (*Aristolochia indica* Linn)

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### Abstract

The *Aristolochia indica* is a rare and endangered medicinal plant and also known as Snakeroot or Ishwarmul in Aurveda. It is famous in Indian System of Medicine as an antidote in bites of poisonous insects particularly the snake bite. It is also used in treatment of intestinal parasite, swelling, menstrual irregularities, white leprosy, dropsy, low appetite, ulcers and fever. For *In Vitro* regeneration of *Aristolochia indica*, seven media were tested out of which five based on Murashige and Skoog's medium (MS), one on Gamborg's B<sub>5</sub> and one on White's media for their response on induction of callus from Stem segment, Leaf base and Cotyledons. Media based on MS responded well irrespective of explants used. Leaf base proved to be the best for callusing percent and fresh weight of callus in MS based media. The highest callusing efficiency was observed in MS medium with full strength of MS salts, 5mg/l 2, 4-D + 1.6mg/l BAP. Observation on shoot regeneration capacity suggested that MS medium contained MS salt +5mg/l 2,4-D+1.6 BAP has shown superior performance and was highly effective in inducing multiple shoots from callus. The MS medium with half strength of basic MS salts in combination with 1.2mg/l BAP/ 0.6 mg/l IBA was also found to be the best for root regeneration. The Darkness supports the fast root regeneration in *Aristolochia indica*. The rooted plantlets were successfully transplanted in pots in poly house, after 25 days these plants with pots were transferred in to green house for further acclimatization under natural environments. The survival rate was 63%.

**Keywords:** callusing induction, explants, ishwarmul, *Aristolochia indica*

### 1. Introduction

*Aristolochia indica* Linn. a divine herb which can fight against any poisonous bite. Indian Birthwort, Snakeroot, Ishwarmul, Iswarballi are the common names of *Aristolochia Indica*. It is a native of India and belong to family Aristolochiaceae. *Aristolochia indica* is distributed throughout the tropical, subtropical and Mediterranean countries. In India it is found in all most all types of forest throughout the country particularly in low hills and moist plains but due to indiscriminate harvesting from forest now it became arare endangered species. It is a twining herb, semiwoody, leaves are cordate or ovate, exstipulate; flowers are irregular, often offensively smelling, perianth is globose with a purple dilated and trumpet-shaped mouth with a strap-shaped brown purple appendage or lip behind; fruit is a subglobose capsule. It is used in India to induce vomiting and to treat poisons, intestinal parasite, swelling, menstrual irregularities, dropsy, low appetite, ulcers and fever (Dey (2011))<sup>[3]</sup>. The roots of plant are used as antidote in scorpion sting, bites of poisonous insects and snake bite. This plant is used both internally and externally. For white leprosy, the roots are rubbed with honey. The plant possesses emmenagogue, abortifacient, anti-spermatogenic, anti-fertility, anti-arthritic, anti-inflammatory, antiperiodic, diuretic and anti-bilious properties. The leaves of plant are applied externally in skin diseases. Though the plant is helpful in many ways, it has to be remembered that it is nephrotoxic and carcinogenic. Over dosage of the plant components may be lead to serious complications. The propagation of *Aristolochia indica* is done mainly through the seeds. Its seeds have poor germination and need specific care to grow, hence an effort has been made in this investigation to draw a protocol for In-Vitro plantlets of *Aristolochia indica*.

### 2. Material and Methods

The research experiment was carried out at Tissue Culture Laboratory, College of Agriculture, Indore, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior (M.P.) during the year 2015-2016. The experiment was conducted in Completely Randomized Design (CRD) with five replications for each explant under each medium.

Explants *viz.* Stem segment, Leaf base and Cotyledons were collected from pretreated mother plants of *Aristolochia indica* available in Medicinal plant nursery of College of Agriculture Indore. Seven combination and concentration of hormones with full strength basic salts and vitamins of MS, B5 and White's media were used to test the response of different explants for organogenesis, shoot regeneration and root regeneration capacity. Aseptic environment were maintained during the investigation period. Observations were recorded on various explants *viz.*, Cotyledons, Stem segment and leaf base for induction of callus, regeneration on different culture media, callusing percentage, weight of callus and regeneration capacity of callus into shoots and roots.

### 3. Result and discussion

All the media under investigation responded for callusing percentage and fresh weight of callus irrespective of explants used, except B<sub>5</sub> and White's media. All the three explants *viz.* Stem segment, Leaf base and Cotyledons showed their highest level of callusing percent and fresh weight of callus on M<sub>3</sub> medium. On the basis of callusing efficiency M<sub>3</sub> media was the best medium among all the media tested in the present investigation. The M<sub>3</sub> media was supported with full strength of MS salts, 5mg/l 2,4-D + 1.6mg/l BAP. Several scientist were also reported the presence of 2,4-D alone or with other hormones in MS basal media will enhance the callusing efficiency *viz.* Sahaya *et al.* (2011) observed that the highest percentage of callus induction (82.3 ± 0.57) from internodal segment on Murashige and Skoog's medium (MS) supplemented with 1.5mg/l of 2,4-D in *Aristolochia bracteata*. Wani *et al.* (2010) in his study on *Tridax procumbens* revealed that the Leaf and apical bud explants showed maximum callus induction by using MS media with the combination of 2, 4-D 0.5mg/lit and BAP 0.5mg/lit. The work of Soniya (2006) and Wani *et al.* (2010) is indirectly in support of the present study and suggested that the combination of 2, 4-D and BAP in different concentration may enhance the callusing efficiency of medium.

In present investigation three explants *viz.* Cotyledons, stem segment and leaf base were evaluated for their effectiveness for induction of callus on different media. The leaf base was

proved as the best explants on the basis of callusing percentage and averaged fresh weight of callus. Least effective explants was cotyledon.

The evaluation of media for shoot regeneration capacity suggested that medium M<sub>3</sub> contained MS salt +5mg/l 2,4-D+1.6 BAP has shown superior performance and was highly effective in inducing multiple shoots from callus. The efficiency of M<sub>3</sub> medium may be due to the presence of high concentration of 2, 4-D and BAP. The efficacy of 2,4-D and BAP in combination or alone in MS media for induction of shoot has already been reported by number of scientists. Siddiqui *et al.* (2006) in their two different studies on *Aristolochia indica* suggested that the highest percentage of shoot regeneration was obtained in MS medium fortified with 2.5mg/l Kn+ 1.0mg/l BAP and 1.0 mg L-1 BAP + 2.5 mg L-1 NAA respectively. Biswas *et al.* (2007) [1] and Sahaya *et al.* (2011) also suggested that combination of BAP and NAA in MS media enhance the shoot multiplication and proliferation in callus rived form nodal are inter-nodal segment. The MS medium with half strength of basic MS salts in combination with 1.2mg/l BAP/ 0.6 mg/l IBA was also found to be the best for root regeneration. The Darkness supports the fast root regeneration in *Aristolochia indica*. The rooted plantlets were successfully transplanted in pots in poly house, after 25 days these plants with pots were transferred in to green house for further acclimatization under natural environments. The survival rate was 63%. Biswas *et al.* (2007) [1] and Chandraprabha *et al.* (2010) reported in *Aristolochia tagala* Champ that excised shoot roots were cultured on half-strength MS medium containing 0.5 mg/l and 1.0 mg/l IBA respectively. While Remya *et al.* (2016) [4] in the same plant observed that well developed shoots were rooted on MS medium supplemented with indole acetic acid (1.5 µM), Kinetin (1.5 µM) and 6-benzylaminopurine (0.5 µM). Sahaya *et al.* (2011) reported in *Aristolochia bracteata* Retz that highest percentage, maximum number of rootlets/shoot let and mean length of rootlets were observed in ½ Murashige and Skoog's medium supplemented with 1.0 mg/L of IBA. Sixty eight percentages of plantlets were established in the earthen pots.

**Table 1:** Composition of different media

M e d i a	C o m b i n a t i o n s a n d c o n c e n t r a t i o n s o f h o r m o n e s	
	( m g / l )	
M <sub>1</sub>	M S s a l t s + 5 m g / l 2 , 4 - D	
M <sub>2</sub>	M S s a l t s + 5 m g / l 2 , 4 - D + 0 . 5 m g / l K i n e t i n	
M <sub>3</sub>	M S s a l t s + 5 m g / l 2 , 4 - D + 1 . 6 m g / l B A P	
M <sub>4</sub>	M S s a l t s + 1 . 5 m g / l B A P + 1 m g / l I B A	
M <sub>5</sub>	M S s a l t s + 1 . 5 m g / l k i n e t i n + 1 m g / l N A A	
B <sub>5</sub>	B <sub>5</sub> S a l t s + 1 m g / l I B A + 1 . 5 m g / l k i n e t i n	
W h i t e ' s	W h i t e ' s s a l t s + 2 . 5 m g / l I B A + 1 . 5 m g / l B A P	

**Table 2:** Callusing percentage and Fresh callus weight (mg) of different explants on different media

M e d i a	C o t y l e d o n		S t e m S e g m e n t		L e a f B a s e	
	Callusing percentage*	Fresh callus weight ( m g )	Callusing percentage*	Fresh callus weight ( m g )	Callusing percentage*	Fresh callus weight ( m g )
M <sub>1</sub>	11.80 (20.03)	155.20	18.00 (25.06)	117.00	21.80 (27.74)	299.40
M <sub>2</sub>	15.00 (22.76)	214.60	24.40 (28.18)	169.80	47.40 (43.49)	487.20
M <sub>3</sub>	31.60 (34.19)	406.00	43.20 (41.07)	340.60	71.60 (57.79)	910.60
M <sub>4</sub>	25.40 (30.22)	335.00	33.20 (35.16)	274.60	66.40 (54.58)	789.80
M <sub>5</sub>	17.80 (24.91)	269.40	28.80 (32.41)	226.60	55.40 (48.09)	645.20
B <sub>5</sub>	6.40 (14.52)	44.40	8.20 (16.50)	47.60	09.00 (17.24)	68.00
W h i t e ' s	8.80 (17.20)	85.40	12.40 (20.54)	72.80	16.40 (23.62)	126.00
S E m	0.746	1.487	0.930	1.001	1.333	2.488
CD at (0.05)	2.161	4.307	2.693	2.900	3.860	7.207

\*The figures in parentheses are angular transformed values.

**Table 3:** Shoot regeneration capacity of different explants on different media

M e d i a	N u m b e r o f s h o o t s		
	C o t y l e d o n	S t e m S e g m e n t	L e a f B a s e
M <sub>1</sub>	3	3	4
M <sub>2</sub>	4	5	9
M <sub>3</sub>	2	0	1
M <sub>4</sub>	1	3	0
M <sub>5</sub>	9	5	1
B <sub>5</sub>	0	0	1
W h i t e ' s	0	0	2
S E m	0 . 2 3 1	0 . 4 7 8	0 . 5 8 9
C D a t ( 0 . 0 5 )	4 . 3 2 4	3 . 6 5 1	4 . 5 4 2

#### 4. Conclusion

*Aristolochia indica* is an important medicinal plant and it can be multiplied by *in Vitro* culture. Among Murashige and Skoog (MS), Gamborg's B<sub>5</sub> and White's media only MS media were responded for callusing percentage and fresh weight of callus. Among the MS media, the callusing efficiency of media(M<sub>3</sub>) which supported with full strength of MS salts, 5mg/l 2,4-D + 1.6mg/l BAP was the highest. The callusing percent and fresh weight of callus of all the three explants i.e. Stem segment, Leaf base and Cotyledons were high on M<sub>3</sub> medium. The shoot regeneration capacity of MS media M<sub>3</sub> was proved to be the best irrespective of explants used but the highest number of shoots were induced through the leaf base.

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