

Effect on Shoot and Root Regeneration of Different Culture Medium through Different Explants in *Aristolochia indica*

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ABSTRACT

The present investigation was under taken to the study of Response of different culture medium on in vitro plant regeneration through different explants viz., Cotyledons, Stem segment and Leaf base in Ishwarmul (*Aristolochia indica* Linn). For In-Vitro regeneration of Ishwarmul, seven media were tested out of which five based on Murashige and Skoog's medium (MS), one on Gamborg's B₅ 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 Ishwarmul. 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 M₃ contained MS salt +5mg/l 2,4-D+1.6 BAP has shown superior performance of M₃ medium may be due to the presence of high concentration 2,4-D and BAP which are supported to be highly effective inducing multiple shoots from callus. 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%.

Key words: Ishwarmul, *Aristolochia indica*, Medium, Callus, Plant regeneration, Explants

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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, 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 rare endangered species. It is a twining herb, semi woody, 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 sub globose capsule. It is used in India to induce vomiting and to treat poisons, intestinal parasite, swelling, menstrual irregularities, dropsy, low appetite, ulcers and fever³. 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 ishwarmul done mainly through the seeds. Its seeds have poor germination and need specific care to grow, hence a effort has been made in this investigation to draw a protocol for In-Vitro plantlets of ishwarmul.

MATERIAL AND METHODS

The research experiment was carried out at Tissue Culture Laboratory, College of Agriculture, (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalyaya), Indore, (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 ishwarmul 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.

RESULT AND DISCUSSION

All the media under investigation responded for callusing percentage and fresh weight of callus irrespective of explants used, except B₅ 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 M₃ medium. On the basis of callusing efficiency M₃ media was the best medium among all the media tested in the present investigation. The M₃ 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 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.⁷ 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 Soniya⁶ and Wani et al.⁷ 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 M₃ 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 M₃ 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.*⁵ in their two different studies on ishwarmul 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.*¹ and Sahaya also suggested that combination of BAP and NAA in MS media enhance the shoot

multiplication and proliferation in calli derived from 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 ishwarmul. 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.*¹ and Chandraprabha *et al.*² reported in *Aristolochi atagala* 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.*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 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: ANOVA (mss) for cotyledons explant

Source	d.f.	Cotyledons	
		Callusing %	Fresh callus weight
Treatment	6	245.2548**	86094.59**
Error	28	2.78438	11.05714

** Significant at 5% level of significance

Table 2: ANOVA (mss) for stem segment explant

Source	d.f.	Stem Segment	
		Callusing%	Fresh callus weight
Treatment	6	364.0115**	58325.36**
Error	28	4.323817	5.014286

**Significant at 5% level of significance

Table 3: ANOVA (mss) for leaf base explant

Source	d.f.	Leaf Base	
		Callusing %	Fresh callus weight
Treatment	6	1279.215**	530207**
Error	28	8.881501	30.95714

** Significant at 5% level of significance

Table 4: ANOVA (mss) for explants on M₃ medium

Source	d.f.	M4 media	
		Callusing %	Fresh callus weight
Treatment	2	736.4096**	486498.6**
Error	12	1.731557	16.53333

**Significant at 5% level of significanc

Table 5: Composition of different media

MEDIA	COMBINATIONS AND CONCENTRATIONS OF HORMONES (mg/l)
M ₁	M S s a l t s + 5 m g / l 2 , 4 - D
M ₂	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 ₃	M S s a l t s + 5 m g / l 2 , 4 - D + 1 . 6 m g / l B A P
M ₄	M S s a l t s + 1 . 5 m g / l B A P + 1 m g / l I B A
M ₅	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 ₅	B ₅ S a l t s + 1 m g / l I B A 1 . 5 m g / l k i n e t i n
White's	White's s a l t s + 2 . 5 m g / l I B A + 1 . 5 m g / l B A P

Table 6: Callusing percentage and Fresh callus weight (mg) of different explants on different media

Media	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 (mg)	Callusing percentage*	Fresh callus weight (mg)	Callusing percentage*	Fresh callus weight (mg)
M ₁	11.80(20.03)	155.20	18.00(25.06)	117.00	21.80(27.74)	299.40
M ₂	15.00(22.76)	214.60	24.40(28.18)	169.80	47.40(43.49)	487.20
M ₃	31.60(34.19)	406.00	43.20(41.07)	340.60	71.60(57.79)	910.60
M ₄	25.40(30.22)	335.00	33.20(35.16)	274.60	66.40(54.58)	789.80
M ₅	17.80(24.91)	269.40	28.80(32.41)	226.60	55.40(48.09)	645.20
B ₅	6.40(14.52)	44.40	8.20(16.50)	47.60	09.00(17.24)	68.00
White'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 7: Shoot regeneration capacity of different explants on different media

Media	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 ₁	3	3	4			
M ₂	4	5	9			
M ₃	2	0	1	1	8	
M ₄	1	3	1	0	1	
M ₅	9	5	1	0		
B ₅	0	0	1			
White's	0	0	2			
S E m	0.231	0.478	0.589	0.589	0.9	
CD at (0.05)	4.324	3.244	3.651	4.542	2	

CONCLUSION

Ishwarmul is an important medicinal plant and it can be multiplied by *in-vitro* culture. Among Murashige and Skoog (MS), Gamborg's B₅ 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 (M₃) which supported with full strength of MS salts, 5mg/l 2, 4-D + 1.6mg/l BAP was 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 M₃ medium. The shoot regeneration capacity of MS media M₃ was proved to be the best irrespective of explants used but the highest number of shoots were induced through the leaf base. 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 Ishwarmul. On the basis of overall observation with regards to modification in concentration and combination of media and callusing efficiency of media it is concluded that: Small modification in concentration and combination of growth hormone may enhance the positive response of MS media. M₃ media containing MS + 5mg/l 2,4-D + 1.6 mg/l BAP was formed to be the best medium for callusing percentage, callus growth, and shoot regeneration *Aristolochia indica*. Leaf base was the best explants callusing in *Aristolochia indica*. The highest root regeneration was obtained M₃ media by reducing MS concentration by ½.

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