



Research Article

ORGANOGENESIS AND *IN-VITRO* PLANTLETS PRODUCTION OF *Aristolochia indica* (Ishwarmul)

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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 a 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 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 1.2 mg/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.

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Introduction

Seasonal changes in day length or photoperiod act as an external temporal clue to start a series of physiological processes. As a result, certain events like growth and spawning are restricted to specific times of the year. These photoperiodically controlled reactions suggests a capacity of the organisms to distinguish between short and long days and therefore to measure physical processes and phenomena. This measurement seems to be based, at least in some species, on originating rhythms [1]. The induction of ovarian maturation and spawning of female penaeid shrimps are mainly carried out by using the unilateral eyestalk ablation technique [2]. This technique is used worldwide in hatcheries, many difficulties, such as deteriorations in spawn, larval quality and quantity over time have been joined with it [3]. Other techniques to the eyestalk ablation method, such as temperature and/or photoperiod manipulations, hormone injections have been examined in different shrimp species. [4] with *Penaeus duorarum*, and [5], with *Penaeus semisulcatus* studied the effects of temperature changes on induced maturation and spawning with a high degree of success. In general, long photoperiods and high temperatures were reported to be required for reproduction in *Penaeus duorarum* [4]. Low temperatures less than 25°C are known to discourage mating, gonad development and spawning in *Penaeus stylirostris* [6] and *Penaeus semisulcatus* [7]. Cycling temperature fluctuations between 20 and 28°C induce maturation and spawning in *Penaeus duorarum* [4] and *Penaeus semisulcatus* [5]. The cycling temperature fluctuation has been suggested to be an effective technique in obtaining off-season reproduction in the green tiger shrimp *P. semisulcatus* [5].

A few thermal manipulating experiments have also been conducted to induce spawning in *P. trituberculatus* at 21°C [8] and *Menippe mercenaria* at 25°C [9]. However, several experiments combining altered temperature and photoperiod

conditions have been performed *Penaeus merguensis* 22°C and 27°C, 10L:14D and 14L:10D [10,11] *Penaeus semisulcatus* 20-28°C, 10L:14D and 14L:10D [5] *Penaeus esculentus* 26°C, 14L:10D [12] *Jasus edwardsii* natural vs. compressed 9 month treatment [13] *Homarus americanus* 9.8-15°C, 8L:16D and 16L:8D [14] and 13-14°C, 8L:16D [15] *Panulirus japonicus* 13°C, 19°C, and 25°C, 10L:14D and 14L:10D [16]. All cited manipulated environmental conditions resulted in some degree of successful gonadal maturation of the respected species.

The principal aim of this research was to elucidate the effects of photoperiodism and temperature mechanism that regulate the key physiological processes of maturation of gonads in *Macrobrachium dayanum* with respect to understanding reproductive biology and growth. Moreover, such knowledge is necessary reliably to egg production for aquaculture of the crustaceans. To clarify the factors affecting initiation of gonadal development, further studies on the developmental processes of gonads, particularly connected to the function of reproductive hormones, were needed because the development was primarily inhibited by the endocrine system. *Aristolochia indica* Linn., a divine herb which can fight against any poisonous bite. Indian Birthwort, Snakeroot, Ishwarmul, Iswar balli are the common names of *Aristolochia indica*. It is a native of India and belongs 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 a rare endangered species. It is a twining herb, semi woody, leaves are cordate or ovate, estipulate; 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 fever [1]. 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. Its roots are rubbed with honey for leprosy treatment. The plant possesses emmenagogue, abortifacient, anti-spermatogenic, anti-fertility, anti-arthritis, anti-inflammatory, antiperiodic, diuretic and anti-bilious properties. The crushed 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 a effort has been made in this investigation to draw a protocol for In-Vitro plantlets of *Aristolochia indica*.

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.

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 observed that the highest percentage of callus induction (82.3 ± 0.57) from inter-nodal segment on Murashige and Skoog's medium (MS) supplemented with 1.5mg/l of 2,4-D in *Aristolochia bracteata*. Wani [2] 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. Earlier work [2-3] 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.

Table-1 Composition of different media

Media	Combinations and concentrations of hormones (mg/l)
M ₁	MS salts +5mg/l 2,4-D
M ₂	MS salts +5mg/l 2,4-D + 0.5 mg/l Kinetin
M ₃	MS salts + 5mg/l 2,4-D + 1.6mg/l BAP
M ₄	MS salts + 1.5mg/l BAP+1mg/l IBA
M ₅	MS salts + 1.5mg/l kinetin+1mg/l NAA
B ₅	B ₅ Salts +1mg/l IBA 1.5mg/l kinetin
White's	White's salts + 2.5mg/l IBA + 1.5mg/l BAP

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.

Table-2 Callusing percentage and fresh callus weight (mg) of different explants on different media

Media	Cotyledon		Stem Segment		Leaf Base	
	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
SEm	0.746	1.487	0.930	1.001	1.333	2.488
CD 5%	2.161	4.307	2.693	2.900	3.860	7.207

*The figures in parentheses are angular transformed values.

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. Two different studies[4] 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. It was suggested [5] that combination of BAP and NAA in MS media enhance the shoot multiplication and proliferation in calli derived from nodal are inter-nodal segment.

Table-3 Shoot regeneration capacity of different explants on different media

Media	Number of shoots		
	Cotyledon	Stem Segment	Leaf Base
M ₁	3	3	4
M ₂	4	5	9
M ₃	20	11	18
M ₄	13	10	14
M ₅	9	5	10
B ₅	0	0	1
White's	0	0	2
SEm	0.231	0.478	0.589
CD 5%	4.324	3.651	4.542

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. 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%. Earlier worker [6] 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 [7] 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.

Conclusion

Aristolochia indica 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 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 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.

Abbreviations: None declared

Conflict of Interest: None declared

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