Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 1576-1578
Received: 16-05-2017
Accepted: 17-06-2017

Anju Nehra
Department of Plant Breeding and Genetics, College of Agriculture (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya), Indore (MP), India.

DK Shrivastava
Department of Plant Breeding and Genetics, College of Agriculture (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya), Indore (MP), India.

Rajesh C Jeeterwal
Division of plant Breeding and genetics, Rajasthan agricultural research institute (SKN Agiriculture University), Durgapura, Jaipur, India.

Correspondence
Anju Nehra
Department of Plant Breeding and Genetics, College of Agriculture (Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya), Indore (MP), India.

Response of different culture medium on *in vitro* plant regeneration through different explants in Ishwarmul *(Aristolochia indica* Linn)

Anju Nehra, DK Shrivastava and Rajesh C Jeeterwal

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 B_5 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. 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: Medium, plant regeneration, explants, Ishwarmul, *Aristolochia indica*

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 arare 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 fever Dey (2011) [3]. 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 Vidyalaya), 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 B_5 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_3 medium. On the basis of callusing efficiency M_3 media was the best medium among all the media tested in the present investigation. The M_3 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) [7] in his study on *Tridex 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) [6] and Wani *et al.* (2010) [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 M_3 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*. (2006) [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.* (2007) [1] suggested that combination of BAP and NAA in MS media enhance the shoot multiplication and proliferation in calli derived 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 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 *at al*. (2007) [1] and Chandra prabha *et al.* (2010) [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. Whle 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). 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 M_3 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 significance

Table 5: Composition of different media

MEDIA	COMBINATIONS AND CONCENTRATIONS OF HORMONES (mg/l)
M_1	MS salts + 5 mg/l 2, 4 - D
M_2	MS salts +5 mg/l 2, 4 - D + 0.5 mg/l Kinetin
M_3	MS salts + 5 mg/l 2, 4 - D + 1.6 mg/l BAP
M_4	MS salts + 1.5 mg/l BAP + 1 mg/l IBA
M_5	MS salts + 1.5 mg/l kinetin + 1 mg/l NAA
B_5	B_5 Salts + 1 mg/l IBA 1.5 mg/l kinetin
White's	White's salts + 2.5 mg/l IBA + 1.5 mg/l BAP

Table 6: 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 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	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 at (0.05)	4.324	3.651	4.542

Conclusion

Ishwarmul is an important medicinal plant and it can be multiplied by *in-vitro* culture. Among Murashige and Skoog (MS), Gamborg's B_5 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 M_3 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.

Reference

1. Biswas A, Bari MA, Mohashweta R, Bhadra SK. *In-vitro* Regenration of *Aristolochia tagala* Champ. A rare Medicinal Plant of Chittagong Hill Tracts. J Bio. Sci. Res. 2007; 15:63-67.
2. Chandra Prabha A, Rama Sabbu R. Micropropagation of *Aristolochi atagala* Champ. A Rareand Enademic Medicinal Plant from Western Ghats. J. Bio. Sci. Res. 2010; 1(2):70-73.
3. Dey Abhijit, De. Nath Jitendra. *Aristolochia indica* L.: A Review. Asian J Plant Sci. 2011; 10:108-116.
4. Remya M, Narmathabai V, Mutharaian VN. Changes in bioactive components of Aristolochia tagala. Cham, a rare species of medicinal importance during its *in vitro* development through direct regeneration. Department of Biotechnology, Vinayaka Missions University, Aarupadai Veedu Institute of Technology, Kancheepuram Tamil Nadu, India, 2016.
5. Siddique NA, Kabir MH, Bari MA. Comparative *in vitro* study of plant regeneration from nodal segments derived callus in *Aristolochia indica* Linn. and *Hemi desmusindicus* (L.) R. Br. Endangered medicinal plants in Bangladesh. J Plant Sci. 2006; (1):106-118.
6. Soniya KV, Sujitha M. An efficient *in vitro* propagation of *Aristolochia indica*. Biol. Plant. 2006; (50):272-274.
7. Wani Minal, Pande Snehal, More Nitin. Callus Induction Studies in *Tridaxprocumbens* (L).Int. J. of Biotech. Applications. 2010; 2(1):11-14.