Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(1): 1070-1075
Received: 28-11-2020
Accepted: 30-12-2020

Swati Rani
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Deepti
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Kumari Anjani
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Vinay Kumar Sharma
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Corresponding Author:
Swati Rani
Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India

Effect of explant and phytohormone on *in vitro* regeneration of *Solanum indicum*, an important medicinal weed

Swati Rani, Deepti, Kumari Anjani and Vinay Kumar Sharma

DOI: https://doi.org/10.22271/phyto.2021.v10.i1o.13478

Abstract
The aim of this study was to observe the effect of different explants *viz*. apical shoot, nodal stem and leaf of *Solanum indicum* and the concentration of phytohormones on tissue culture responses during the *in vitro* regeneration and development of micropropagation protocol. For primary establishment of explants, 0.1% $HgCl_2$ solution for 5 minutes was found to be most effective for surface sterilization and Murashige and Skoog (MS) medium having 3 mg/l kinetin and 3 mg/l 6-benzylaminopurine was found to be the best for all the explants. Among all three explants, leaf explants showed the best primary establishment. The explants showed callus formation after 10 to 12 days of culture. The frequency of callus formation was higher on MS medium supplemented with 3 mg/l 3-indoleacetic acid and 5 mg/l 6-benzylaminopurine by the leaf explant. The highest frequency of shooting was recorded on MS medium with 3 mg/l kinetin and 2mg/l 6-benzylaminopurine. Apical shoot showed higher potential of shooting than nodal stem and leaf explants. The *in vitro* regenerated elongated shoots were then excised from shoot clumps and transferred to rooting medium containing Indole butyric acid at 1.5 mg/l. The *in vitro* regenerated plantlets were shifted to the polycups having mixture of sterilized soil and farm yard manure in 1:1 ratio. Initially, these plants were kept under high humidity and progressively acclimatized to reduced humidity for their hardening and acclimatization. Plantlets, thus developed, were successfully established and finally transferred to a greenhouse.

Keywords: Micropropagation, tissue culture, *Solanum indicum*, phytohormones

Introduction
Solanum indicum, a medicinally important weed, largely used as traditional and folk medicine worldwide. It belongs to the genus Solanum of the family Solanaceae, which is one of the largest and complex genera with cosmopolitan distribution. *S. indicum* is commonly known as Poison Berry, Indian night shed, Vrihati, Badi Kantakari, Vanbhantaa etc. It is a bushy herb containing prickles and spines on the stem and grows throughout India and all over the tropical and subtropical regions of the world [1]. The fruits and roots of this plant are used for treatment of many diseases such as loss of appetite, blood disorders, rhinitis, cough, asthma, sore throat and hiccup, abdominal pain, worm infestation, sexual disorders, fever, inflammation, insomnia, urinary complications, cardiac weakness and anti-obesity [2-3]. Fruits and roots of this plant also contain wax, fatty acids and alkaloids solanine, solanidine, disogenin, lanosterol, β-sitosterol, solasornine, solmargine and solaidine etc. [2, 4]. In Arunachal Pradesh, fruits and leaves of *S. indicum* are used in treatment against round and tape worms, leaves are also used as common growth supplements for preparation of fermentation starter cultures containing brewer's yeast [5]. It is also used as a component of traditional medicines like Dashamoolarista recommended for post-natal care to avoid secondary complications [6] and Chyavanprasha [7].

It grows as wild plant in many parts of India and due to lack of knowledge, destruction of natural habitat and overexploitation of these medicinal plants in ayurvedic industry as it forms basis of many polyherbal preparation their reserves are diminishing and facing danger of extinction. Hence, conservation of genetic diversity of medicinal plants resources are necessary, which can be accomplished by adopting alternative methods for rapid *in-vitro* multiplication through tissue culture technique. Micropropagation of these plants is a good way to conserve and multiply these amazing plant species. Though an extensive work has been done to explore the biochemical constitution and medicinal importance of these plants [1, 2, 8], not much effort has been made for micropropagation of these plants. Thus, there is an immense need for development of micropropagation protocol.

Materials and Methods

The tissue culture studies included selection, collection and preparation of explants, selection as well as preparation of medium, inoculation of the explants, incubation and multiplication of propagule, rooting and regeneration of plantlets, acclimatization of regenerated plantlet and finally observations of all the responses and their statistical analysis.

The plant parts *viz*. shoot apices, nodes and leaves of *S. indicum* were collected from the branches brought from field to be used as source of explants. The explants were excised from branches of plants and were cut to a size of about 1-2 cm initially with the help of sharp blade. These explants were washed under running tap water for few minutes to reduce the dust and then shifted in another flask containing distilled water added with 5-6 drops of tween 20 and a few drops of savlon (chlorhexidinegluconate plus cetrimide). Surface disinfection was done inside the laminar air flow cabinet with 0.1% $HgCl_2$ solution by shaking gently for five minutes and rinsed 3 times with sterile distilled water. The surface sterilized explants were treated with 70% ethanol for 30 seconds. After that the explants were washed many times with double distilled water to remove traces of sterilant.

Murashige and Skoog (1962) medium (MS) were the most favourable as well as widely accepted medium for the plant tissue culture study [9], it was selected as the basal medium throughout the study. For further experiment MS basal medium were supplemented with different combinations and concentrations of phytohormones to form MS modified medium. Total thirty four media having different combinations and concentrations of auxins (Indole butyric acid – IBA, 3-indoleacetic acid – IAA, α-naphthalene acetic acid – NAA) and cytokinins (kinetin – KIN, 6-benzylaminopurine – BAP) were used for the present work. The explants were cultured on all the media and responses were observed.

Before aseptic inoculation the pre-treated and surface sterilized explants were further trimmed to a size of approx 0.5 cm by incinerated sterilized scalpel and forceps. The explants were than cautiously inoculated into the culture tubes containing medium over the flame of sprit lamp. The inoculated culture tubes were shifted to the tissue culture room having controlled environmental conditions such as temperature 25±2°C, relative humidity 50 to 80% and continuous fluorescent light of about 2 kilo lux was maintained.

The established cultures were maintained under suitable environmental conditions for induction of multiple shoots. The established shoots were regularly transferred every 5-6 weeks by splitting shoot clusters with a sterilized scalpel on the most responsive medium, for further increase of multiple shoots. The callus was also shifted to shoot differentiation medium for multiple shoot formation. For sub culturing, the outer dead tissues from the base of explant were removed. The differentiated multiple shoots were separated into groups of 2-4 shoots and inoculated into separate culture tubes. The differentiated shoots were then shifted to root induction medium for root formation. The cultures were retained for three to four weeks on these media to regenerate plantlets.

When regenerated plantlets with well-developed root system attained 5-8 cm height with few leaves were removed from the culture vessels. Roots were washed gently and thoroughly under running tap water to remove traces of media and were transferred into polycups having sterilized sand and farm yard manure (1:1 ratio). They were initially kept under high humid conditions and progressively acclimatized to reduced humidity for their hardening and acclimatization in acclimatization chamber. The acclimatized plantlets were transferred to the field.

The frequencies of establishment of aseptic cultures, callus formation, differentiation of shoots and roots were the main responses observed during the study. The experiments were set up in completely randomized block design (CRD) with 70 tubes per treatment. All the data were analysed by running two way analysis of variance (two way ANOVA) using OPSTAT and Microsoft Excel 2013, to test the significance of the observed results. The means were compared using Duncan's multiple range test (Duncan, 1955) to find the difference at 5% ($P<0.05$) level [10]. The results were expressed as a mean ± SE of three replications.

The experiments were carried out at the Central Tissue Culture Laboratory, College of Basic Sciences and Humanities, RPCAU, Pusa, Bihar.

Results and Discussion

All the three explants shoot apices, nodal stem and leaves of *S. indicum* were cultured on the thirty media. The results of the experiment were evaluated for various tissue culture responses at regular intervals. The main responses were observed with respect to the frequencies of establishment of aseptic cultures, callus formation, differentiation of shoots and roots. The results for these observations were significant and dependent on the type of explant and composition of the medium used for the culture, as revealed by two way ANOVA. The establishment of cultured explants was evaluated with respect to the effect of the medium and type of explants (Table 1). Out of the 30 media, M28 (MS + 3 mg/l KIN + 3 mg/l BAP) showed the best establishment followed by M30 (MS + 4 mg/l KIN + 3 mg/l BAP) and M19 (MS + 1 mg/l NAA + 4 mg/l BAP) and the least was observed on M1 (MS basal). Among explants, leaf showed the best establishment followed by nodal stem and shoot apices (Figure 1 a-c). The establishment of explants was an index to show the frequency of cultures remaining sterile and viable as well as it was the first requirement of any tissue culture experiment. It depended upon the pre-treatment and surface sterilization of explants.

Table 1: Establishment percentage of cultured explants of *S. indicum* with respect to the effect of different media and type of explants

Media	Composition	Shoot apex	Nodal stem	Leaf	Mean	SE
M1	MS Basal	90±2.89	91.67±1.67	93.34±1.93	91.67	0.97
M2	MS+0.1mg/l IAA	91.67±1.67	93.34±1.67	92.23±2.94	92.41	0.49
M3	MS+0.2mg/l IAA	90±2.89	95±2.89	91.12±2.94	92.04	1.52
M4	MS+0.1mg/l KIN	96.67±1.67	95±2.89	94.45±2.94	95.37	0.67
M5	MS+0.2mg/l KIN	95±2.89	91.67±3.34	95.56±1.12	94.07	1.22
M6	MS+1mg/l KIN	93.34±3.34	95±2.89	95.56±2.94	94.63	0.67
M7	MS+2mg/l KIN	95±2.89	91.67±1.67	96.67±1.93	94.44	1.47
M8	MS+3mg/l KIN	90±2.89	93.34±1.67	97.78±1.12	93.70	2.26
M9	MS+4mg/l KIN	93.34±3.34	91.67±1.67	95.56±2.23	93.52	1.13

M10	MS+5mg/l KIN	95±2.89	96.67±1.67	94.45±2.94	95.37	0.67
M11	MS+0.1mg/l BAP	91.67±4.41	96.67±3.34	93.34±3.34	93.89	1.47
M12	MS+0.2mg/l BAP	93.34±1.67	95±2.89	90±1.93	92.78	1.47
M13	MS+0.5mg/l BAP	91.67±3.34	96.67±1.67	93.34±1.93	93.89	1.47
M14	MS+1mg/l BAP	95±2.89	95±2.89	96.67±1.93	95.56	0.56
M15	MS+2mg/l BAP	93.34±3.34	93.34±1.67	95.56±2.94	94.07	0.75
M16	MS+5mg/l BAP	93.34±3.34	91.67±1.67	94.45±2.94	93.15	0.81
M17	MS+0.5mg/l IAA+5mg/l BAP	90±2.89	93.34±3.34	94.45±2.23	92.59	1.34
M18	MS+3mg/l IAA+5mg/l BAP	93.34±1.67	90±2.89	92.23±1.12	91.85	0.98
M19	MS+1mg/l NAA+4mg/l BAP	96.67±1.67	95±2.89	95.56±2.23	95.74	0.5
M20	MS+1mg/l NAA+5mg/l BAP	95±2.89	96.67±1.67	96.67±1.93	96.11	0.56
M21	MS+1mg/l IAA+5mg/l KIN	90±2.89	93.34±1.67	95.56±2.94	92.96	1.62
M22	MS+0.5mg/l NAA+2mg/l KIN	91.67±1.67	93.34±1.67	97.78±1.12	94.26	1.83
M23	MS+1mg/l NAA+3mg/l KIN	93.34±1.67	96.67±1.67	95.56±2.23	95.18	0.98
M24	MS+1.5mg/l KIN+2mg/l BAP	90±2.89	93.34±3.34	92.23±1.12	91.85	0.98
M25	MS+2mg/l KIN+1mg/l BAP	96.67±1.67	95±2.89	94.45±1.12	95.37	0.67
M26	MS+2.5mg/l KIN+2.5mg/l BAP	93.34±3.34	91.67±1.67	95.56±1.12	93.52	1.13
M27	MS+3mg/l KIN+2mg/l BAP	95±2.89	93.34±3.34	95.56±2.23	94.63	0.67
M28	MS+3mg/l KIN+3mg/l BAP	98.34±1.67	96.67±1.67	96.67±1.93	97.22	0.56
M29	MS+4mg/l KIN+2mg/l BAP	95±2.89	93.34±3.34	96.67±1.93	95.00	0.97
M30	MS+4mg/l KIN+3mg/l BAP	96.67±1.67	95±2.89	97.78±1.12	96.48	0.81
Mean		93.444	94	94.889		
SE		0.437	0.345	0.360		
Factors		C.D.	SE(d)	SE(m)		
Factor(A)		N/A	2.019	1.427		
Factor(B)		N/A	0.638	0.451		
Factor (AxB)		N/A	3.496	2.472		

Values are expressed as mean (n=3)±SE

The initiation of callogenesis took place after 10 to 12 days of culture. Callus generally developed from the cut end of the explants that was in contact with the medium and gradually extended over entire surface of the explants as a result of cell division in epidermal and sub epidermal regions. The callus formation was influenced by the composition of the medium and explant type. Only 15 media showed callus formation out of the 30 (Table 2). The frequency of callus formation was significantly higher on M18 (MS + 3 mg/l IAA + 5 mg/l BAP) followed by M19 (MS + 1mg/l NAA + 4mg/l BAP) and M23 (MS + 1 mg/l NAA + 3 mg/l KIN), whereas the least on M13 (MS + 0.5 mg/l BAP). Leaf explants showed high percentage of callus formation than nodal stem and shoot apex respectively (Figure 1 d-f). Similar successful induction of callus from *S. nigrum* L. on MS basal medium supplemented with IAA and BAP were also observed [11]. Ray *et al.* (2011) observed the highest callusing on medium MS + 2.0 mg/l BAP and 0.5 mg/l NAA during *in vitro* cultivation and regeneration of *S. melongena* [12]. Alim *et al*. (2014) used the MS medium supplemented with different concentrations of auxin (2,4-D) and cytokinin (BAP) to assess the callus inducing potentiality of leaf explants of Brinjal [13].

Table 2: Callogenesis percentage of cultured explants of *S. indicum* with respect to the effect of different media and type of explants

Media	composition	Shoot Apex	Nodal Stem	Leaf	Mean	SE
M1	MS Basal	16.71^{d}_{z}±0.54	18.13^{e}_{y}±1.47	29.75^{e}_{x}±0.72	21.526	4.13
M2	MS+0.1mg/l IAA	21.84^{c}_{z}±0.39	24.96^{d}_{y}±1.37	34.94^{d}_{x}±0.4	27.24	3.96
M3	MS+0.2mg/l IAA	22.27^{c}_{z}±0.72	29.79^{c}_{y}±1.11	37.08^{c}_{x}±0.8	29.708	4.28
M4	MS+0.1mg/l KIN	12.02^{fg}_{y}±1.5	12.22^{g}_{y}±1.41	23.24^{g}_{x}±0.56	15.821	3.71
M5	MS+0.2mg/l KIN	10.55^{g}_{y}±0.33	10.94^{gh}_{y}±0.42	22.09^{g}_{x}±1.05	14.524	3.79
M11	MS+0.1mg/l BAP	14.48^{e}_{z}±1.44	15.56^{f}_{y}±0.56	25.07^{f}_{x}±0.93	18.367	3.37
M12	MS+0.2mg/l BAP	12.48^{f}_{z}±1.67	13.97^{f}_{y}±1.45	24.67^{fg}_{x}±0.84	17.038	3.85
M13	MS+0.5mg/l BAP	8.98^{g}_{z}±1.55	10.36^{h}_{y}±0.18	22.6^{g}_{x}±0.81	13.977	4.33
M17	MS+0.5mg/l IAA+5mg/l BAP	24.03^{b}_{z}±1.21	33.89^{a}_{y}±0.56	38.79^{b}_{x}±1.33	32.231	4.35
M18	MS+3mg/l IAA+5mg/l BAP	26.81^{a}_{z}±0.49	35.16^{a}_{y} ±1.02	45.77^{a}_{x}±0.67	35.909	5.49
M19	MS+1mg/l NAA+4mg/l BAP	25.88^{ab}_{z}±0.44	35.06^{a}_{y} ±1.02	39.53^{b}_{x}±0.24	33.488	4.02
M20	MS+1mg/l NAA+5mg/l BAP	24.52^{b}_{z}±1.21	34.48^{a}_{y} ±1.55	39.08^{b}_{x}±0.61	32.687	4.3
M21	MS+1mg/l IAA+5mg/l KIN	24.03^{b}_{z}±1.21	33.92^{a}_{y} ±1.55	38.45^{bc}_{x}±1.21	32.129	4.26
M22	MS+0.5mg/l NAA+2mg/l KIN	23.59^{bc}_{z}±1.37	32.17^{b}_{y}±0.59	38.63^{b}_{x}±0.69	31.457	4.36
M23	MS+1mg/l NAA+3mg/l KIN	24.96^{b}_{z}±1.37	34.48^{a}_{y} ±1.55	39.53^{b}_{x}±0.24	32.984	4.28
Mean		19.539	25.002	33.276		
SE		1.62	2.63	2.03		
Factors		C.D.	SE(d)	SE(m)		
Factor(A)		1.682	0.845	0.598		
Factor(B)		0.752	0.378	0.267		
Factor (A X B)		2.913	1.464	1.035		

Values are expressed as mean±SE. Mean value (n=3) in columns and rows bearing same letter (subscript and superscript respectively) are not significantly different at 5% level

Multiple shoot formation was also affected by the medium and explant. It was observed after 8 weeks of culture and both callus mediated and direct differentiation of shoots were obtained. Medium and explant played an important role in multiple shoot formation (Table 3). Out of 30 media, only 22 showed multiple shoot formation. The significantly highest frequency of shooting was recorded on M27 (MS + 3 mg/l KIN + 2 mg/l BAP) followed by M26 (MS + 2.5 mg/l KIN + 2.5mg/l BAP) and M28 (MS + 3 mg/l KIN + 3 mg/l BAP) and the least on M16 (MS + 5mg/l BAP). Due to the best performance of shoot regeneration, M27 was used for the entire experiment and afterwards during subculture. Apical shoot showed high potential of shooting then nodal stem and leaf respectively (Figure 1 g-i) for *in vitro* organogenesis. Shivaraj and Rao (2011) obtained the highest number of shoots on MS medium containing 2.0 mg/l BAP + 0.5 mg/L KIN, during the development of high frequency and rapid regeneration protocol of eggplant [14]. The highest frequency of shoot induction was observed on MS medium supplemented with 10-15 µM KIN and BAP by Padmapria *et al.* (2011) during development of efficient protocol for *in vitro* propagation of *S. nigrum* [15]. Bardhan *et al* (2012), during the study, to develop an efficient protocol for establishment of *in vitro* plant regeneration of eggplant (*S. melongena* L.) reported the higher shoot regeneration (77.46%) on MS medium containing 2.5 mg/l KIN and 0.4 mg/l IAA [16]. The highest regeneration was observed on medium MS +2.0 mg/l BAP and 0.5 mg/l NAA from stem and leaf explant during *in vitro* cultivation and regeneration of *S. melongena* [12].

Table 3: Caulogenesis percentage of cultured explants of *S. indicum* with respect to the effect of different media and genotypes

Media	composition	Shoot Apex	Nodal Stem	Leaf	Mean	SE
M6	MS+1mg/l KIN	$19.82^{n}{}_{x}\pm2.41$	$19.24^{m}{}_{xy}\pm1.33$	$18.63^{m}{}_{y}\pm1.17$	19.23	0.35
M7	MS+2mg/l KIN	$22.85^{m}{}_{x}\pm1.85$	$21.84^{l}{}_{y}\pm0.39$	$19.56^{m}{}_{z}\pm1.23$	21.42	0.98
M8	MS+3mg/l KIN	$27.84^{l}{}_{x}\pm0.9$	$24.96^{k}{}_{y}\pm1.37$	$23.87^{l}{}_{z}\pm0.27$	25.56	1.19
M9	MS+4mg/l KIN	$17.97^{n}{}_{x}\pm2.19$	$16.38^{n}{}_{y}\pm0.3$	$15.08^{n}{}_{z}\pm0.8$	16.48	0.84
M10	MS+5mg/l KIN	$13.97^{o}{}_{x}\pm1.45$	$12.02^{o}{}_{y}\pm1.5$	$11.72^{o}{}_{y}\pm0.82$	12.57	0.71
M14	MS+1mg/l BAP	$28.04^{l}{}_{x}\pm1.08$	$26.37^{k}{}_{y}\pm0.81$	$25.27^{l}{}_{z}\pm0.75$	26.56	0.81
M15	MS+2mg/l BAP	$32.23^{k}{}_{x}\pm1.12$	$32.17^{j}{}_{x}\pm0.59$	$30.31^{k}{}_{y}\pm1.67$	31.57	0.63
M16	MS+5mg/l BAP	$12.41^{o}{}_{x}\pm1.3$	$10.92^{o}{}_{y}\pm0.2$	$10.49^{o}{}_{y}\pm0.33$	11.27	0.59
M17	MS+0.5mg/l IAA+5mg/l BAP	$42.58^{j}{}_{x}\pm0.98$	$41.12^{i}{}_{y}\pm1.7$	$39.94^{j}{}_{z}\pm1.45$	41.21	0.77
M18	MS+3mg/l IAA+5mg/l BAP	$50.0^{i}{}_{x}\pm1.52$	$48.15^{h}{}_{y}\pm0.94$	$47.01^{i}{}_{z}\pm0.58$	48.39	0.88
M19	MS+1mg/l NAA+4mg/l BAP	$63.78^{f}{}_{x}\pm0.62$	$63.09^{e}{}_{xy}\pm1.13$	$62.78^{f}{}_{y}\pm1.07$	63.22	0.3
M20	MS+1mg/l NAA+5mg/l BAP	$56.16^{h}{}_{x}\pm0.89$	$55.18^{g}{}_{y}\pm1.53$	$54.03^{h}{}_{z}\pm0.58$	55.12	0.62
M21	MS+1mg/l IAA+5mg/l KIN	$59.28^{g}{}_{x}\pm0.96$	$58.97^{f}{}_{x}\pm1.08$	$56.95^{g}{}_{y}\pm0.9$	58.4	0.73
M22	MS+0.5mg/l NAA+2mg/l KIN	$63.65^{f}{}_{x}\pm1.63$	$62.48^{e}{}_{y}\pm0.69$	$61.38^{f}{}_{z}\pm0.69$	62.51	0.66
M23	MS+1mg/l NAA+3mg/l KIN	$67.84^{e}{}_{x}\pm0.59$	$67.29^{d}{}_{x}\pm1.15$	$66.27^{e}{}_{y}\pm1.05$	67.13	0.46
M24	MS+1.5mg/l KIN+2mg/l BAP	$81.55^{c}{}_{x}\pm1.33$	$78.52^{c}{}_{y}\pm0.75$	$78.31^{c}{}_{y}\pm0.27$	79.46	1.05
M25	MS+2mg/l KIN+1mg/l BAP	$82.81^{c}{}_{x}\pm1.41$	$82.52^{b}{}_{x}\pm1.29$	$81.2^{b}{}_{y}\pm0.95$	82.18	0.5
M26	MS+2.5mg/l KIN+2.5mg/l BAP	$85.75^{b}{}_{x}\pm1.65$	$85.48^{a}{}_{xy}\pm1.73$	$84.9^{a}{}_{y}\pm1.08$	85.37	0.26
M27	MS+3mg/l KIN+2mg/l BAP	$87.79^{a}{}_{x}\pm1.41$	$85.75^{a}{}_{y}\pm1.65$	$84.93^{a}{}_{z}\pm0.8$	86.15	0.86
M28	MS+3mg/l KIN+3mg/l BAP	$83.08^{c}{}_{x}\pm1.56$	$82.81^{b}{}_{x}\pm1.41$	$81.56^{b}{}_{y}\pm1.51$	82.48	0.47
M29	MS+4mg/l KIN+2mg/l BAP	$82.52^{c}{}_{x}\pm1.29$	$82.23^{b}{}_{x}\pm1.12$	$79.3^{c}{}_{y}\pm0.42$	81.35	1.03
M30	MS+4mg/l KIN+3mg/l BAP	$79.30^{d}{}_{x}\pm0.36$	$77.25^{c}{}_{y}\pm1.18$	$76.14^{d}{}_{z}\pm0.27$	77.56	0.93
Mean B		52.777	51.574	50.434		
SE		5.73	5.77	5.76		
Factors		C.D.	SE(d)	SE(m)		
Factor(A)		1.896	0.958	0.677		
Factor(B)		0.7	0.354	0.25		
Factor (A X B)		N/A	1.659	1.173		

Values are expressed as mean±SE. Mean value (n=3) in columns and rows bearing same letter (subscript and superscript respectively) are not significantly different at 5% level

After 20-25 days some plantlets of *S. indicum* were rooted (Figure 1k) and some others were needed to be subculture on fresh rooting medium for additional root development. Four medium containing IBA of different concentration (0.1, 0.5, 1.0 and 1.5mg/l) with MS basal were used for rooting. Out of these four, medium containing 1.5mg/l IBA showed the highest percentage of rooting than the other three and was used for rooting during the entire experiment. Similarly, Taghipour *et al*. (2015) observed in case of eggplant that after three weeks some differentiated shoots were rooted and some others were needed to be subcultured on fresh rooting medium for additional root development [17]. The best rooting was obtained on MS medium containing 0.5 mg/l IBA in *S. Nigrum* culture [11]. In *in vitro* study of *S. melongena*, MS medium with 3.0 mg/l IBA gave the best result for root induction for average number and mean length of roots [18].

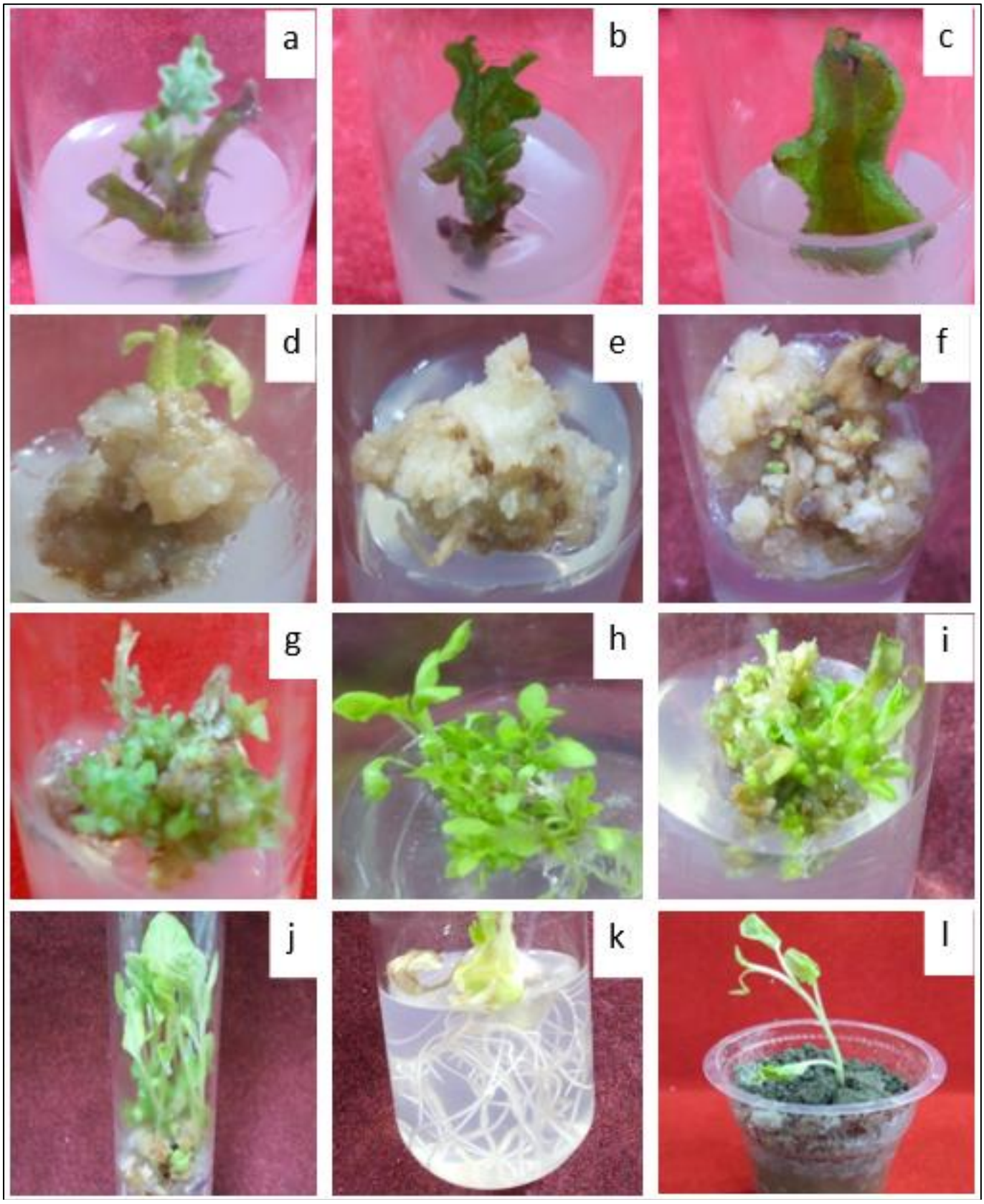

Fig 1: *In vitro* plantlet regeneration through apical shoot, nodal stem and leaf culture of *Solanum indicum*. (a. established nodal stem culture, b. established apical shoot culture, c. established leaf culture, d. callus formation in nodal stem culture, e. callus formation in apical shoot culture, f. callus formation in leaf culture, g. initiation of multiple shoots in nodal stem culture, h. development of multiple shoots through apical shoot culture, i. Initiation of multiple shoots in leaf culture, j. multiple shoots ready for sub culturing, k. development of roots, & l. hardening and acclimatization)

Rooted shoots were removed from the culture vessels. Roots were washed thoroughly but gently in running tap water and were transferred in polycups having sterilized soil and farm yard manure in 1:1 ratio (Figure 1l). These polycups were initially kept under high humid conditions and then plants were progressively acclimatized to reduced humidity for their hardening and acclimatization. Similarly, the regenerated plantlets with well-developed roots were transferred to plastic cups containing sand, soil and farmyard manure (1:1:1) and maintained in green house for acclimatization [19]. *In vitro*

rooted plantlets were planted in polar pots containing 1:2:1 ratio of sterile sand, soil and compost and acclimatized [18]. Shivaraj and Rao (2011) transferred the rooted plantlets to polycups containing vermiculate, soil and sand in 1:2:2 ratios [14].

Conclusion

The present experiment led to standardization of a highly efficient protocol for the micropropagation of *S. indicum.* Among different hormonal treatments MS medium supplemented with 3 mg/l IAA and 5mg/l BAP was found to be the best for callus formation from leaf explant and for multiple shoot formation MS medium having 3 mg/l KIN and 2mg/l BAP was found to be the best. Explant shoot apex was found most suitable for multiple shooting. Indole butyric acid at 1.5 mg/l with MS medium was observed most effective for root formation. In addition, the type of method used for explant disinfections, was the right one, indicating a high survival rate. As well as the types of explant and nutrient medium used for the *in vitro* establishment and regeneration were giving satisfactory results.

Acknowledgements

The authors express their thanks to Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar for providing the study resources.

References

1. Sundari SG, Rekha S, Parvathi A. Phytochemical Evaluation of three species of *Solanum* L. Int. J Res Ayurveda Pharm. 2013;4(3):420-425.
2. Deb PK, Ghosh R, Chakraverty R, Debnath R, Das L, Bhakta T. Phytochemical and Pharmacological Evaluation of Fruits of *Solanum indicum* Linn. Int J Pharm Sci Rev Res 2014;25(2):28-32.
3. Epoh NJ, Dongmo OLM, Tchouanguep FM, Telefo PB. Ethnobotanical Study of Medicinal Plants used as Anti-Obesity Remedies in Foumban and Dschang Cities (West-Cameroon). European Journal of Medicinal Plants. 2020;31(9):54-70.
4. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi 1956, 256.
5. Namsa ND, Mandal M, Tangjang S, Mandal SC. Ethnobotany of the Monpa ethnic group at Arunachal Pradesh, India. J Ethnobio Ethnomed 2011;7(31):1-14.
6. Sharma V, Hem K, Seth A, Maurya SK. *Solanum indicum* Linn.: An ethnopharmacological, phytochemical and pharmacological review. Current Research Journal of Pharmaceutical and Allied Sciences 2017;1:1-9.
7. Bhutya RK. Ayurvedic Medicinal Plants of India. Scientific Publisher (India), Jodhpur. 2011;2:143-144.
8. Abdel-Aziz H, Fawzy N, Ismail AI, El-Askary H. Toxicological studies on a standardized extract of *Solanum indicum* ssp. distichum. Food Chem Toxicol 2011;49(4):903-909.
9. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 1962;15:473-497.
10. Duncan OD, Duncan BA. methodological analysis of segregation indexes. American sociological review 1955;20:210-217.
11. Kolar AB, Vivekanandan L, Basha MG. *In vitro* regeneration and flower induction on *Solanum nigrum L*. from Pachamalai hills of Eastern Ghats. Plant Tissue Cult. & Biotech 2008;18(1):43-48.
12. Ray BP, Hassan L, Sarker SK. In vitro cultivation and regeneration of *Solanum melongena* (L.) using stem, root and leaf explants. Nepal Journal of Biotechnology. 2011;1:49-54.
13. Alim MA, Biswas BK, Hasanuzzaman M, Bala P, Roy UK. Callus induction of brinjal by genotype and growth regulators. J. Expt. Biosci 2014;5:35-42.
14. Shivaraj G, Rao S. Rapid and efficient plant regeneration of eggplant (*Solanum melongena* L.) from cotyledonary leaf explants. Indian Journal of Biotechnology 2011;10:125-129.
15. Padmapriya H, Karthikeyan AVP, Jahir Hussain G, Karthi C, Velayutham P. An efficient protocol for *in vitro* propagation of *Solanum nigrum* L. from nodal explants. J Agric Technol 2011;7:1063-73.
16. Bardhan SK, Sharma C, Srivastava DK. *In vitro* plant regeneration studies in brinjal, Journal of Cell and Tissue Research 2012;12:3213-3218.
17. Taghipour F, Jamshidi S, Huyop F. High performant eggplant *in vitro* regeneration and organogenesis. Agroecology Journal 2015;11:24-29.
18. Rahman M, Asaduzzaman M, Nahar N, Bari MA. Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (*Solanum melongena* L.). Journal of Bio-Science 2006;14:31-38.
19. Muthusamy A, Vidya KS, Pratibha PK, Rao MR, Vidhu SB, Guruprasad KP. Establishment of an *in vitro* plantlet regeneration protocol for unique varieties of brinjal (*Solanum melongena* L.) var. Mattu Gulla and Perampalli Gulla. Indian Journal of Experimental Biology 2014;52:80-88.