academicJournals

Vol. 7(42), pp. 3098-3104, 10 November, 2013
DOI: 10.5897/JMPR11.1652
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Influence of cytokinins, sucrose and pH on adventitious shoot regeneration of *Polyscias balfouriana* (Balfour aralia)

Saiqa Ilyas[1]*, Shagufta Naz[1], Sumeera Javad[2], Kiran Shehzadi[2], Amna Tariq[2], Neelma Munir[1] and Aamir Ali[3]

[1]Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan.
[2]Department of Botany, Lahore College for Women University, Lahore, Pakistan.
[3]Department of Biological Sciences, University of Sargodha, Pakistan.

Accepted 9 January, 2012

A highly reproducible and efficient *in vitro* shoot regeneration system was developed for *Polyscias balfouriana* using apical shoot tips as explants. Explants after surface sterilization were cultured on Murashige and Skoog (MS) (1962) medium supplemented with different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mg/l) of 6- Benzylamino purine (BAP), Thidiazuron (TDZ) and Kinetin (Kin), alone as well as in combination with indole-3-butyric acid (IBA) (0.1, 0.5, 1.0, 1.5 and 2.0 mg/l). The highest rate of shoot multiplication (15.20 ± 0.86 for the average shoot number and 6.30 ± 0.84 cm for shoot length) was achieved on MS medium supplemented with 1.2 mg/ml BAP and 0.5 mg/ml IBA. The effect of sucrose and pH of MS medium on shoot proliferation was also tested. An average of 19.2 ± 0.34 shoots per explants with 6.90 ± 0.15 cm shoot length could be obtained by optimizing these two parameters. Rooting was achieved on micro shoots using MS medium with 3.0 mg/ml IBA. The *in vitro* developed healthy plantlets were successfully shifted to greenhouse for acclimatization.

Key words: Cytokinins, sucrose, pH, adventitious shoot, regeneration, *Polyscias balfouriana.*

INTRODUCTION

Polyscias balfouriana is an ornamental foliage shrub. These plants are popularly known in trades and horticulture nurseries as “aralias” since they belong to the family Araliaceae (Nayanar, 1985). The plant is also known as Scutellarium or dinner plate Aralia or Balfour Polyscias. It is a native of New Caledonia. It is also available in New Zealand, tropical Asia and the Pacific Islands. It can grow up to 25 feet (about 7.5 meters) tall, but is usually shorter. The stem is greenish-gray mottled color with leaves composed of three leaflets. It grows in an upright direction and is usually no wider than its container. These plants grow in temperatures above 13ºC) and in any good soil (equal parts of loam, peat and leaf mold, with a bit of sand and charcoal). Plants coming under the family *Araliaceae* are mainly constituted by triterpenoid saponins. The triterpenoid saponin content in this family play an important role in the pharmacological activity like stimulation of central nervous system (CNS), anti fatigue and enhancement of non-specific resistance (Sandhya et al., 2010). Sucrose is the main source of carbon for *in vitro* cultures. Plant cells and tissues in a culture medium lack autotrophic ability and therefore need external carbon for energy (Razdan, 1993). The addition of an external carbon source to the medium

*Corresponding author. E-mail: aamirali73@hotmail.com.

enhances the proliferation of cells and regeneration of green shoots (Nowak et al., 2004; Gurel and Gulsen, 1998).

The optimal sucrose concentration in a medium should be sufficient to satisfy the basic energy requirements for cell division/differentiation and not impose any negative osmotic effects on shoot formation (Stavarek et al., 1980). This indicates that sucrose acts not only as a carbon energy source in a medium but also as an osmoticum (Nowak et al., 2004) and that different concentrations of sucrose are one of the factors controlling the induction and growth of shoots (Gibson, 2000; Gurel and Gulsen, 1998). Plant cells and tissues require an optimum pH for growth and development in cultures. The pH affects nutrient uptake as well as enzymatic and hormonal activities in plants (Bhatia and Ashwath, 2005). The optimal pH level regulates the cytoplasmic activity that affects cell division and the growth of shoots and it does not interrupt the function of cell membrane and the buffered pH of the cytoplasm (Brown et al., 1979). The pH also influences the status of the solidifying agent in a medium (Bhatia and Ashwath, 2005). The change of pH in cells or organs is due to the ions absorbed from the medium (Sakano, 1990). Therefore, it is necessary to optimize the sucrose concentration and pH level for maximum shoot regeneration because the sucrose concentration and pH level directly influence shoot regeneration. There is not a single report on the *in vitro* regeneration of *P. balfouriana*. Therefore, the objective of this study was to develop an efficient and improved method for rapid *in vitro* propagation of *P. balfouriana* using apical shoot tips via tissue culture techniques which provide viable alternative methods for the mass production of healthy plants with uniform characteristics.

MATERIALS AND METHODS

Establishment of aseptic cultures

Apical shoot tips (1 to 2 cm long) of *P. balfouriana* were used as the source of explants for establishing the *in vitro* cultures. These apical shoots were washed thoroughly with running tap water. Again washed with detergent and then rinsed five times with autoclaved distilled water. For surface disinfestation, the explants were immersed in 20% sodium hypochlorite (bleach) for 20 min and rinsed five times with autoclaved distilled water and inoculated in shoot proliferation media.

Culture media and culture condition

MS basal medium fortified with various concentrations of plant growth hormones was used in all the experiments. Different concentrations of sucrose (3.0, 6.0, 9.0, 12.0 and 15.0%, w/v) were used to optimize most appropriate one. The medium was adjusted to different pH levels viz 5.0, 5.4, 5.8, 6.2 and 6.6 using 1.0N NaOH or HCl and sterilized by autoclaving at 121°C and 15 lb/inch^2 pressure for 20 min. Cultures were incubated at 20±2°C under 16/8 h (light/dark) photoperiod provided by cool white fluorescent tubes (Phillips) with a photon flux density of 50 µmol.

Adventitious shoot formation and multiplication

Explants were cultured onto MS medium augmented with various concentrations of BAP, TDZ and Kn, (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 and 1.6 mg/ml) singly as well as in combination with different concentrations of IBA (0.1, 0.5, 1.0, 1.5 and 2.0 mg/ml) for induction and multiplication of shoots. Sub culturing was required periodically on the same fresh media after (21 days) week to avoid basal callusing. Data on the frequency of shoots formation, shoot number and shoot length (cm) were recorded.

In vitro rooting of shoots

Shoots with fully expanded leaves were transferred to MS Medium supplemented with either IBA or α-naphthyl acetic acid (NAA) at various concentrations including 0.5, 1.0, 2.0 3.0, 4.0 and 5.0 mg/l. Observations on percent of root formation, number of roots per shoot and root length were recorded.

Transfer of plant to soil

The rooted plants were transferred to small pots containing autoclaved sand for one month under greenhouse conditions. They were nourished with Hoagland solution daily. Then these plants were shifted to soil containing 50% of compost for one to two months. The hardened plants were transplanted for field trials.

Statistical analysis

A completely randomized design with 3 replicates was used for the experiment. The data for each parameter were subjected to analysis of variance (ANOVA) using the COSTAT V.63: statistical software (Cohort software, Berkely, California). The mean values were compared with the least significant difference test following Dancun's new multiple range test at 5% level.

RESULTS AND DISCUSSION

Effect of plant growth regulators on shoot formation and multiplication

The potential of various plant growth regulators on *in vitro* shoot formation from apical shoot tip was explored and is summarized in Tables 1 and 2. Data presented in Table 1 depicts that MS medium containing 1.2 mg/ml BAP showed higher rate of shoot formation (75.0%) as compared to other cytokinins used in this study (Table 1). In addition, significantly greater number of shoot buds was observed on this medium (Figure 1 a to f). At the same concentration, TDZ and Kin showed 60.0% and 57.0% response having an average of 7.20 ± 0.06 and 6.70 ± 0.21 shoot buds per culture vial. MS medium supplemented with BAP gave a better response than TDZ and Kin. Ovecka et al. (2000) reported that cell competence in the course of shoot bud regeneration is controlled by various internal factors such as genotype, endogenous level of auxin and cytokinin, basal medium,

Table 1. Effect of various cytokinins (BAP, TDZ and KIN) on shoot induction of *P. balfouriana* after four weeks of culture.

Plant growth hormones mg/l			% Response	Mean no. of proliferated shoots
BAP	TDZ	KIN		
0.2			49.0	$3.20^{l} \pm 0.07$
0.4			55.6	$5.20^{hi} \pm 0.04$
0.6			59.3	$7.20^{c} \pm 0.04$
0.8			66.0	$7.40^{c} \pm 0.07$
1.0			70.2	$8.20^{b} \pm 0.06$
1.2			75.0	$10.0^{a} \pm 0.24$
1.4			72.1	$8.10^{b} \pm 0.03$
1.6			67.7	$7.20^{c} \pm 0.06$
	0.2		35.4	$3.40^{l} \pm 0.04$
	0.4		41.0	$3.90^{k} \pm 0.13$
	0.6		44.1	$4.80^{ij} \pm 0.11$
	0.8		51.2	$5.70^{fg} \pm 0.14$
	1.0		54.4	$6.60^{d} \pm 0.13$
	1.2		60.0	$7.20^{c} \pm 0.06$
	1.4		55.4	$6.40^{de} \pm 0.10$
	1.6		50.0	$6.00^{ef} \pm 0.17$
		0.2	33.7	$2.80^{m} \pm 0.11$
		0.4	36.4	$3.30^{l} \pm 0.08$
		0.6	40.0	$3.90^{k} \pm 0.17$
		0.8	47.1	$4.50^{j} \pm 0.14$
		1.0	55.2	$5.20^{hi} \pm 0.09$
		1.2	57.0	$6.70^{d} \pm 0.21$
		1.4	54.2	$6.10^{ef} \pm 0.13$
		1.6	50.0	$5.40^{gh} \pm 0.11$
LSD				0.38

pH, carbohydrate uptake, etc. By increasing the level of BAP from 1.2 to 1.6 mg/ml, the rate regeneration as well as number of shoots per culture vial was drastically reduced.

Similar observations are reported by Husain and Anis (2004) in *Melia azedrach*, where BAP at 10 µM showed a decrease in the rate of shoot multiplication. Such an inhibitory effect at higher concentration of BAP has also been reported by other workers irrespective of explants used (Chaudhuri et al., 2004; Ahmad et al., 2008). For further enhancement in shoot multiplication response, the optimal concentration of BAP, TDZ and Kin (1.2 mg/ml) in combination with IBA at different concentrations (0.1 to 2.0 mg/ml) was also analyzed (Table 2). The inclusion of IBA in the optimal medium enhanced the shoot multiplication response in *P. balfouriana*. Kim et al. (2001) suggested that the shoot forming ability of the explants is related to the *in vivo* level of endogenous auxin and cytokinin and that the differential response to different cytokinins may be because of the chemical and structural differences. Among various combinations of cytokinins- auxin combination used, the combined effect of BAP (1.2 mg/ml) and IBA (0.5 mg/ml) was best for shoot multiplication where the highest number of shoots (15.20 ± 0.86) and shoot length (6.30 ± 0.0.84 cm) were recorded.

Effect of different conc. of sucrose on shoot formation

Sucrose as a carbon source supports growth of plant cells in culture (Gamborg and Phillips, 1995). A sucrose concentration of 1 to 5% is generally used for *in vitro* culture, since it is also synthesized naturally by the tissue (Pierik, 1987). The optimum sucrose concentration as an efficient carbon source has been examined in tissue cultures of some plant species, such as *Paederia foetida* (Amin et al., 2003) and *Elaeocarpus robustus* (Rahman et al., 2004), in which 30 g/l sucrose enhanced shoot development. In our experiment, for shoot formation different concentrations of sucrose (3, 6, 9, 12 and 15 gm) were tested on MS medium containing 1.2 mg/l BAP and 0.5 mg/l IBA (Figure 2). Gradual increase in sucrose concentration resulted better increase in shoot initiation response. MS medium supplemented with 9 g/l sucrose promoted the best shoot development with highest number of shoots (17.4 ± 0.00) per culture vial and shoot

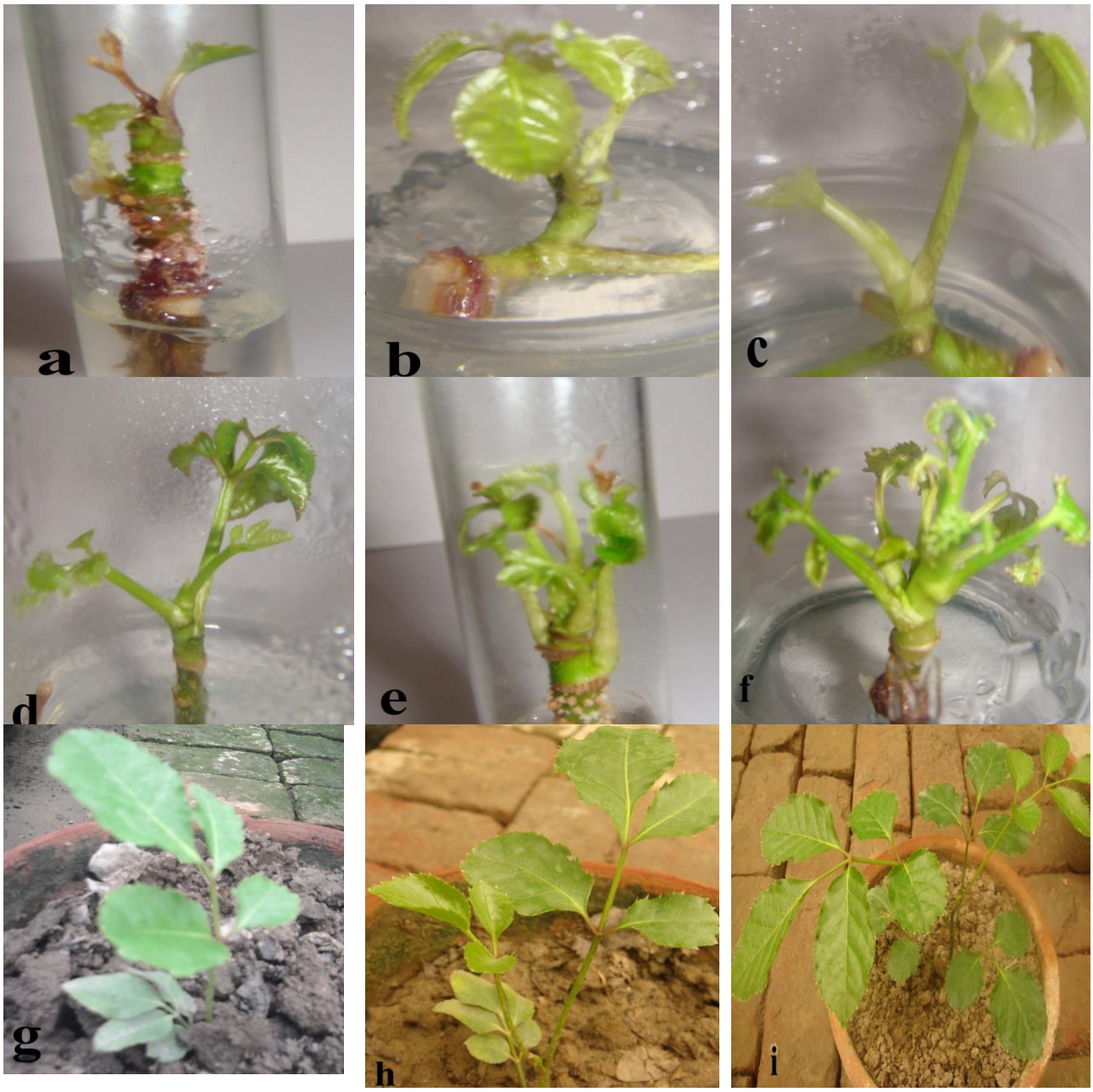

Figure 1. (a-i) Different stages of micro propagation**: a** Initiation from explants **b** Shoot proliferation **c** Multiple shoot induction. **d, e** and **f** Shoot multiplication on MS medium augmented with 1.2 mg/ml BAP and 0.5 mg/ml IBA. **g, h** and **i** Acclimatization of mericlones in green house.

length (6.50 ± 0.00 cm) but shoot development gradually decreased by increasing further sucrose concentrations in medium. High concentrations of sucrose seem to inhibit shoot growth (Nowak et al., 2004).

Effect of pH value on shoot regeneration

The pH of the culture medium is an important factor for promoting shoots *in vitro*. In the absence of pH regulation, the ionization of acidic and basic groups causes considerable changes in structure that affect their function at the cellular level (Sakano, 1990). The pH of tissue culture media decreases by uptake of NH_4^+ and increases by uptake of NO_3^- (Schmitz and Lorz, 1990). Any change in pH of medium may have various effects that may influence performance and development of explants (George et al., 2008). In this study, a better

Table 2. Effect of various concentrations of IBA with optimal concentration of BAP, TDZ and KIN on shoot multiplication and elongation of *P. balfouriana* after four weeks of culture.

Plant growth hormones mg/l				%	Mean shoot length	Mean no. of shoots per explants
BAP	TDZ	KIN	IBA	Response	(cm)	
1.2			0.1	81.30	11.20^{b} ±0.76	5.50^{ab} ±0.44
1.2			0.5	85.00	15.20^{a} ±0.86	6.30^{a} ±0.84
1.2			1.0	78.8	10.10^{bc} ±1.02	4.70^{bc} ±0.43
1.2			1.5	76.0	9.40^{bc} ±0.92	4.10^{cde} ± 0.35
1.2			2.0	79.1	8.20^{cd} ±0.71	3.90^{cde} ±0.29
	1.2		0.1	66.1	8.00^{cd} ±0.89	3.70^{cdef} ± 0.38
	1.2		0.5	71.1	8.80^{c} ±0.65	4.40^{bcd} ±0.45
	1.2		1.0	56.3	8.30^{cd} ±0.48	3.10^{defgh} ±0.29
	1.2		1.5	51.4	7.80^{cde} ±0.59	2.90^{efgh} ±0.29
	1.2		2.0	47.5	6.10^{def} ±0.28	2.20^{ghi} ±0.33
		1.2	0.1	57.5	4.70^{fgh} ±0.59	2.50^{fghi} ±0.40
		1.2	0.5	64.3	5.60^{efg} ±0.15	3.40^{cdefg} ±0.26
		1.2	1.0	59.0	4.20^{fgh} ±0.52	2.10^{ghi} ±0.21
		1.2	1.5	43.0	3.40^{gh} ±0.82	1.90^{hi} ± 0.29
		1.2	2.0	31.3	2.80^{h} ±0.33	1.40^{i} ±0.21
LSD					2.14	1.21

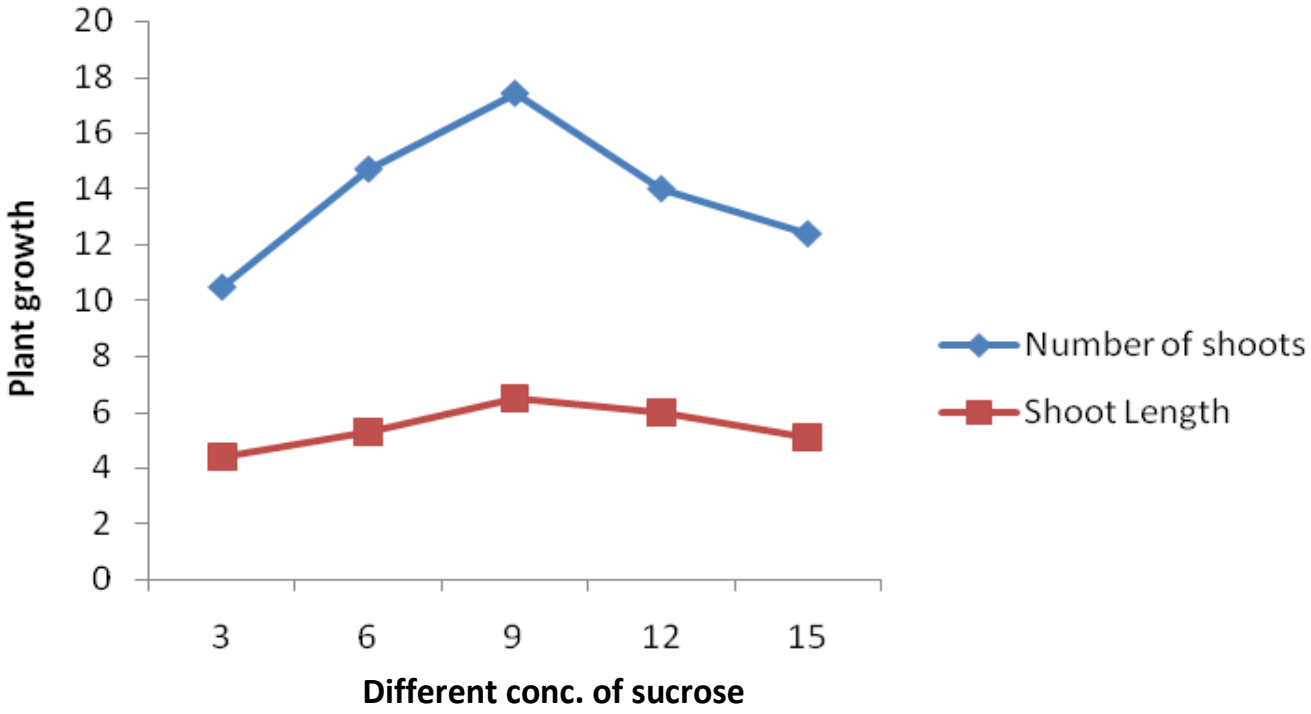

Figure 2. Effect of sucrose concentrations on shoot regeneration of *P. balfouriana* on MS medium fortified with BAP (1.2 mg/ml) + IBA (0.5 mg/ml).

performance in all parameters on shoot development was found at pH 5.8 among all pH levels tested (5, 5.4, 5.8, 6.2 and 6.6) on MS medium containing 1.2 mg/ml BAP and 0.5 mg/ml IBA (Figure 3).

Similar response has also been reported by Gautam et al. (1993) where the proliferation of shoots in *Azadirachta indica* was significantly increased when the pH of the medium was adjusted to 5.8. Karim et al. (2007) reported

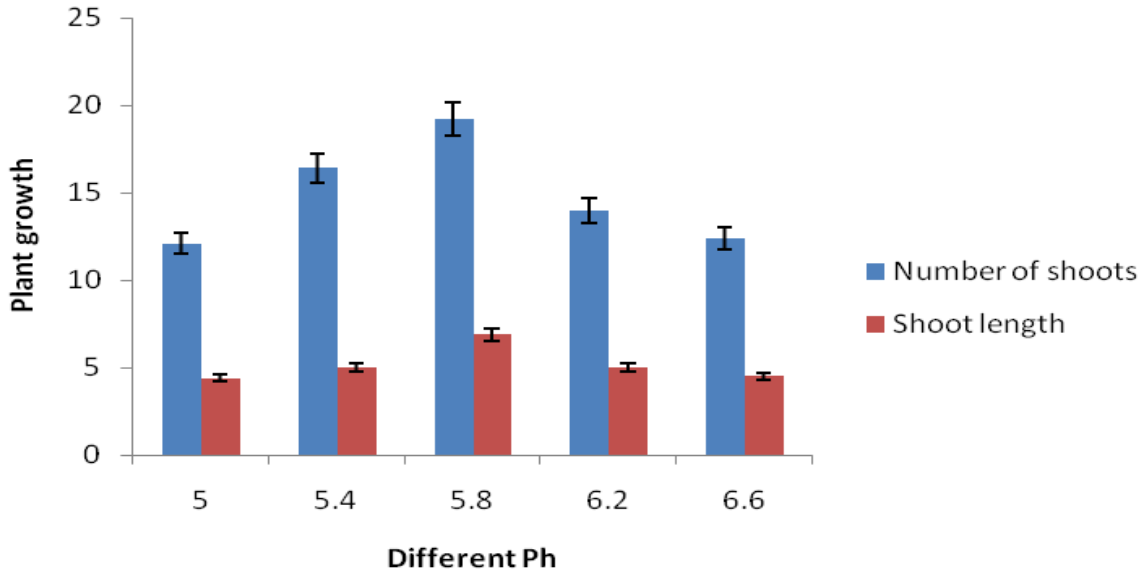

Figure 3. Effect of medium pH on shoot regeneration of *P. balfouriana* supplemented with BAP (1.2 mg/ml) + IBA (0.5 mg/ml).

Table 3. Effect of different auxins and its concentration on rooting of *in vitro* micro shoots of *P. balfouriana*.

Plant growth hormones mg/l		% Response	Mean root length (cm)	Mean no. of roots
NAA	IBA			
0.5		35.3	1.50^{de} ±0.31	2.00^{cdef} ±0.40
1.0		47.0	2.60^{bcd} ±0.35	2.60^{bcd} ±0.26
2.0		58.1	3.00^{abc} ±0.40	3.10^{abc} ±0.35
3.0		62.0	4.20^{a} ±0.52	3.90^{a} ±0.29
4.0		54.0	3.30^{ab} ±0.38	3.50^{ab} ±0.31
5.0		51.0	2.80^{abcd}±0.52	2.40^{cde} ±0.38
	0.5	30.8	1.15^{e} ±0.21	1.20^{f} ±0.22
	1.0	37.4	2.00^{bcde}±0.48	1.40^{ef} ±0.26
	2.0	44.5	2.50^{bcde}±0.44	2.30^{cde} ±0.10
	3.0	51.0	3.10^{abc} ±0.57	2.50^{bcde} ±0.24
	4.0	47.0	2.80^{abcd}±0.22	2.20^{cdef} ±0.36
	5.0	39.0	1.70^{cde} ±0.18	1.50^{def} ±0.31
LSD			1.25	0.96

in-*Aralia elata* that pH levels lower and higher than 5.8 showed a low performance for the induction and elongation of shoots from callus of leaf. Nair and Seeni (2003) also found best shoot multiplication at pH 5.8 in *Calophyllum apetalum*. The main reason for such results seems to be the semisolid status of the medium at pH 5.8 as in semisolid media; the availability of many compounds is pH dependent. pH play important role in enhancing the activities of growth regulators and enzymes that affect the function of cells as well as whole plants (Scholten and Pierik, 1998; Van Winkle et al., 2003) and a lower pH resulted in unsatisfactory solidification of agar.

Rooting of micro shoots

The *in vitro* regenerated microshoots were transferred to MS basal medium supplemented with different concentrations of different auxins for example, NAA and IBA (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/ml) for rooting (Table 3). Root formation was noticed from the base of shoots after four weeks of culture. The best rooting was achieved in MS medium fortified with 3.0 mg/ml IBA where fairly good root number (4.20 ± 0.00) and root length per shoot (3.90 ± 0.00 cm) were obtained. IBA has been reported to have stimulatory effect on root induction in many tree species including *Morus indica* (Chand et

al., 1995), *Murraya koenigii* (Bhuyan et al., 1997), *Sterculia urens* (Hussain et al., 2008), *Balanites aegyptiaca* (Anis et al., 2010).

Acclimatization of plants

The rooted plantlets were transferred to green house under moderate temperatures and humid environment. After one month, surviving plants were transferred to pots containing normal garden soil and shifted to trial fields for further growth (Figure 1 g to I). They were indistinguishable morphologically from normal grown seedlings.

REFERENCES

Ahmad N, Wali SA, Anis M (2008). *In vitro* production of true-to-type plants of *Vitex negundo* L. from nodal explants. J. Hort. Sci. Biotechnol., 83(3): 313-317.

Anis M, Varshney A, Siddique I (2010). *In vitro* clonal propagation of *Balanites aegyptiaca* (L.) Del. Agroforestry Systems, 78: 151-158.

Amin MN, Rahman MM, Manik MS (2003). *In vitro* clonal propagation of *Paederia foetida* L. A medicinal plant of Bangladesh. Plant Tissue Cult., 13: 117-123.

Bhatia P, Ashwath N (2005). Effect of medium pH on shoot regeneration from the cotyledonary explants of Tomato. Biotechnol., 4: 7-10.

Bhuyan AK, Pattnaik S, Chand PK (1997). Micropropagation of Curry Leaf Tree [*Murraya koenigii* (L.) Spring.] by axillary proliferation using intact seedlings. Plant Cell Rep. 16: 779-782.

Brown DCW, Leung DWM, Thorpe TA (1979). Osmotic requirement for shoot formation in tobacco callus. Physiol. Planta, 46: 36-41.

Chand PK, Sahoo Y, Pattnaik SK, Pattnaik SN (1995). *In vitro* meristem culture – an efficient *ex situ* conservation strategy for elite mulberry germplasm. In: Mohanty RC (ed), *Environment: change and management*, Kamla Raj Enterprises, New Delhi, India, Pp. 127–133.

Chaudhuri KN, Ghosh S, Jha S (2004). The root: A potential source of competent cells for high frequency regeneration in *Tylophora indica*. Plant Cell Rep., 22: 731-740.

Gamborg OL, Phillips GC (1995). Media Preparation and Handling. In O.L. Gamborg and G.C. Phillips (Eds.). *Plant Cell, Tissue and Organ Culture*: Fundamental Methods, Springer, Germany, Pp. 21-34.

Gautam VK, Nanda N, Gupta SC (1993). Development of shoots and roots in anther derived callus of *Azadirachta indica* A. Juss- A medicinal tree. Plant Cell Tissue Organ Cult., 34: 13-18.

George EF, Hall MA, De Klerk GJ (2008). Plant propagation by tissue culture. Vol 1: The background, 3rd edn. Springer, Dordrecht.

Gibson IS (2000). Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiol., 124: 1532-1539.

Gurel S, Gulsen Y (1998). The effects of different sucrose, agar and pH levels on *in vitro* shoot production of Almond (*Amygdalus communis* L.). Tr. J. Bot., 22: 363-373.

Husain MK, Anis M (2004). *In vitro* proliferation of Shoots of *Melia azedarach* L. from mature trees. In: D'Sauza L, Anutadha M, Nivas S, Hedge S, Ragendra K (eds) Biotechnology For A Better Future, SAC Publications, Manglore, India. Pp. 294-301.

Karim MZ, Yokota S, Rahman MM, Eizawa J, Saito Y, Azad M, Ishiguri F, Iizuka K, Yoshiza N (2007). Effect of the sucrose concentration and pH level on shoot regeneration from callus in *Araria elata* Seem. Asian J. Plant Sci., 6(4): 715-717.

Kim KH, Park HK, Park MS, Yeo UD (2001). Effects of auxin and cytokinin on organogenesis of soybean *Glycine max* L. J. Plant Biotech., 3: 95-100.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiologia Plantarum, 15: 473-497.

Nair LG, Seeni S (2003). *In vitro* multiplication of *Calophyllum apitalum* (Clusiaceae) an endemic medicinal tree of the Western Ghats. Plant Cell Tissue Organ Cult., 75: 169- 174.

Nayanar MP (1985). Meaning of Indian flowering plant names. p.280.

Nowak B, Milczzynski K, Hudy L (2004). Sugar uptake and utilization during adventitious bud differentiation on *in vitro* leaf explants of Wegierka Zwykla plum (*Prunus domestica*). Plant Cell, Tissue Organ Cult., 76: 255-260.

Ovecka M, Bobak M, Samaj J (2000). A comparative structure analysis of direct and indirect shoot regeneration of *Papaver somniferum* L. *in vitro*. J. Plant Physiol., 157: 281-289.

Pierik RLM (1987). *In vitro* Culture of Higher Plants. Martinus Nijhoff Publishers, Dordrecht, Netherlands.

Rahman MM, Amin MN, Ahmed R (2004). *In vitro* rapid regeneration from cotyledon explants of native olive (*Elaeocarpus robustus* Roxb.). Asian J. Plant Sci., 3: 31-35.

Razdan MK (1993). Introduction and techniques. In: An Introduction to plant tissue culture. Oxford and IBH publishing, New Delhi, Bambey, Calcutta, Pp.1-40.

Sakano K (1990). Proton/phosphate stoichiometry in uptake of inorganic phosphate by cultured cells of *Catharanthus roseus* (L.) G. Don. Plant Physiol., 93: 479-483.

Sandhya S, Vinod KR, Madhu DC, Nema RK (2010). Evaluation of immune-stimulant activity of the root and leaf of Polyscias Balfouriana Marginata. Int. J. Pharm. Clin. Res., 2(2): 61-62.

Schmitz U, Lorz H (1990). Nutrient uptake in suspension cultures of gramineae. 2. Suspension culture of rice (*Oryza sativa* L.). Plant Sci., 66: 95-111.

Scholten HJ, Pierik RLM (1998). Agar as a gelling agent: chemical and physical analysis. Plant Cell Rep., 17: 230-235.

Stavarek SJ, Croughan TP, Rains DW (1980). Regeneration of plants from long-term cultures of alfalfa cells. Plant Sci. Lett., 19: 253-261.

Hussain TM, Chandrasekhar T, Gopal GR (2008). Micropropagation of *Sterculia urens* Roxb, an endangered tree species from intact seedlings. Afr. J. Biotechnol., 7(2): 95-101.

Van WSC, Johnson S, Pullman GS (2003). The impact of gelrite and activated carbon on the elemental composition of two conifer embryogenic tissue initiation media. Plant Cell Rep., 21: 1175-1182.