The Bioscan 13(2): 541-544, 2018
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

IN -VITRO REGENERATION OF LASORA (*Cordia myxa* Roxb.) USING NODAL SEGMENTS

MINAKSHI PADHI*, S. P. SINGH, PRIYANKA SINGH AND NIRAJ MISHRA
Department of Horticulture,
Institute of Agricultural Sciences,Banaras Hindu University, Varanasi – 221 005, U.P., INDIA
e-mail: minakshipadhi@gmail.com

KEYWORDS
In-vitro
Nodal segments
Explant
Shoot proliferation

Received on :
20.01.2018

Accepted on :
17.05.2018

***Corresponding author**

ABSTRACT
To develop a reliable protocol for *in-vitro* propagation of lasora, an experiment was conducted in Plant Tissue culture Laboratory, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University,varanasi during the year 2015-16. The explants were dipped in 50 mg/L citric acid + 100 mg/L ascorbic acid for 20 min to avoid explant browning. These Explants were cultured on medium consisting of different concentrations of benzyladenine (BAP) and *á*-naphthalene acetic acid (NAA) was used to culture the explants. The highest average number of shoots (5) and highest average length (3.75 cm) of shoot were shown on medium supplemented with 3.5 mg/L BAP + 0.07 mg/L NAA. There was no shoots proliferation in control (without growth regulators). IBA was used for rooting of proliferated shoots. The rooting medium fortified with IBA (2mg/L) was found beneficial for days to root initiation, number of roots and root length.

INTRODUCTION

Cordia myxa Roxb. commonly known as lasora is one of the important members of the family Boraginaceae. The plant is useful in many ways as its leaves are used for treatment of cough and urinary disorder. Kaushik *et al*. (2010) reported the antimicrobial activity of *Cordia myxa*. The fruit contains ab out 80% pulp~ pulp contain s per 100g dry weight: ash 6.7 g, crude protein 8.32g, lipid 2.2 g, crude fibre 25.7g and carbohydrates 57.08 g and 281.4 k calorie (Aberoum and, 2011).The unripe fruits are used for making of pickle, while ripe fruits with mucilaginous pulp are eaten as fresh fruits.

Lasora is quick growing drought tolerant fruit tree favoured for cultivation in marginal eco-system due to several xerophytic characteristics. There is no well-defined cultivar in lasora. However, great variation exists in the size of fruits and their pulp content. Large fruited cultivars are preferred to cultivate to have average fruit weight not less than 8g, and suitable for consumption whereas small fruited ones (Sharma *et al*., 1983). It is generally multiplied by seeds, which results in considerable variation in plant type and its growth, yield and fruit quality. Plant tissue culture being essential component of plant biotechnology which can be an alternative tool for rapid multiplication of elite genotypes with limited number of mother plants. This method can be a alternative way for vegetative propagation of horticultural crops (Alizadeh *et al*., 2010).

Clonal propagation through tissue culture (popularly known as micro-propagation) is relatively rapid and can be performed within a small space (Krishna *et al*., 2008; Eftekhari *et al*., 2012). The major benefit of clonal cultivars in commercial production is the uniformity of individual plants within a clone population (Krishna and Singh, 2013).

Keeping these in view, the present investigation was carried to produce elite planting material of lasora through micro-propagation.

MATERIALS AND METHODS

The present investigation was carried out during the year 2015-2016 in Tissue Culture Laboratory, Department of Horticulture, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. *In-vitro* propagation was carried out by taking the nodal segments of the plant which were selected from the mother plant with early maturing, free from insect pests and diseases and were vigorous. Spraying of SAAF (0.2%) was done 3-5 days before the collection of explants. The explants, *i.e*., nodal segments or intermodal segments in 1.5-2.0 cm (length) and 0.2 cm (width) with a healthy bud excised from the healthy and vigorous branches of field grown lasora plant. The explants were thoroughly washed under running tap water for 7-10 minutes, and then washed with Tween-twenty for 2-3 times and transferred to fungicide solution for different time durations. After washing, the explants were dipped in 50 mg/L citric acid + 100 mg/L ascorbic acid for 20 min to overcome the problem of explant browning. Generally, the time of sterilization depends on the type of tissue for instance; leaf tissue requires shorter sterilization time than the seeds with a tough seed coat (Ndakidemi *et al*., 2013). The explants were then subjected to surface sterilization with 0.1% $HgCl_2$ (Mercuric chloride) for different durations and washed with autoclaved distilled water. Maximum survival with minimum

tissue injury was found when explants were treated with mercuric chloride for 4 minutes (Rattanpal *et al.*, 2011). All the process of sterilization and inoculation of explants were carried out inside Laminar Air Flow Chamber (LAFC) with proper sterilization techniques. Culture tubes/flasks after inoculation were incubated in the growth chamber at 25 ± 2ºC temperature with 16/18 hours day/night regime at 3000-3200 lux light intensity supplied through fluorescent tubes. Sterilization of growth media and instruments were done by autoclaving at 121ºC at 15 psi for 20 minutes. Nodal segments were inoculated on MS medium supplemented with different concentrations of BAP and NAA.

RESULTS AND DISCUSSION

After five to seven days of inoculation of explants, the buds started responding by showing bud break and sprouting. Cytokinins promoted shoot proliferation as cytokinins induce cell division and organ enlargement (Devlin, 2000). Cytokinins concentration (BAP) increased the number of shoot up to certainl level and then decrease with further increase in cytokinins concentration (Singh *et al.*, 2016). The stimulating effect of BAP alone on shoot proliferation and *in-vitro* multiple shoot formation have been reported in *Curculigo orchioides* (Thomas, 2007), *Phaseolus lunatus* (Kanchiswamy and Maffei, 2008) and *Juncus effusus* (Xu *et al.*, 2009). The synergistic effect of BAP and NAA has also been demonstrated in many medicinal plants, e.g., *Santolina canescens* (Casado *et al.*, 2002) and *Bupleurum fruticosum* (Fraternale *et al.*, 2002). Keeping in view the beneficial effect of BAP and NAA in promoting shoot proliferation its combination was taken as treatment while conducting the experiment. Nodal segments were found to be better explants for initiation of *in vitro* culture in lasora. Various treatments with association of growth regulators were applied to different explants. For multiple shoot induction, the type and the concentration of the growth regulator in the medium were observed to be important factor (Bhat *et al.*, 2013; Pawar *et al.*, 2013).

No shoot proliferation was observed under control condition (without growth regulators). Treatment consisting of BAP (3.5 mg/L) and IBA (0.07 mg/L) gave best response with maximum of 89 per cent nodal segment sprouting, maximum number of 5 shoots, maximum shoot length of 3.75 cm and highest number of 6 leaves per explant as compared to rest of the treatments (Table 1). Maximum numbers of roots per explant along with more elongated roots were observed under IBA alone. The effectiveness of IBA on rooting has also been reported on a number of medicinal plants like *Heliotropium indicum* (Kumar and Rao, 2007), *Hemidesmus indicus* (Sreekumar *et al.*, 2000), *Cunilagalioides*(Fracaro and Echeverrigary, 2001) and *Aloe polyphylla* (Abrie and van Staden, 2001).

IBA was used for rooting of proliferated shoots of lasora. The shoots obtained from culture were transferred onto root induction medium containing IBA alone. It took about one month for root induction. The best result was obtained by

Table 1: Effect of BAP and NAA on shoot proliferation.

Concentration of growth regulators (mg/L) (BAP + NAA)	Sprouted shoot (%)	Number of shoots	Shoot length(cm)	Number of leaves
T_1 (0.00 + 0.00)	0.00	0.00	0.00	0.00
T_2 (0.5 + 0.01)	47.25	0.75	1.00	1.25
T_3 (1.0 + 0.02)	52.25	1.50	1.50	1.50
T_4 (1.5 + 0.03)	61.50	2.25	1.60	2.25
T_5 (2.0 + 0.04)	65.25	2.75	1.75	3.00
T_6 (2.5 + 0.05)	75.50	3.25	1.85	3.75
T_7 (3.0 + 0.06)	82.25	4.00	2.05	4.75
T_8 (3.5 + 0.07)	89.00	5.00	3.75	6.00
T_9 (4.0 + 0.08)	88.00	3.50	2.75	4.50
T_{10} (4.5 + 0.09)	79.25	2.75	1.80	3.50
S.E	2.68	0.219	0.269	0.387
C.D at 5%	7.77	0.635	0.782	1.124

Table 2: Effect of IBA on root proliferation.

Concentration of growth regulators (mg/L)(IBA)	Days to root initiation (days)	Rooting (%)	Root length(cm)	Number of roots
T_1 (0.0)	0.00	0.00	0.00	0.00
T_2 (0.2)	29.25	26.25	1.01	1.25
T_3 (0.4)	25.75	31.25	1.30	2.25
T_4 (0.6)	21.75	42.50	1.74	2.50
T_5 (0.8)	18.75	50.00	2.28	2.75
T_6 (1.0)	15.75	72.75	2.82	3.00
T_7 (2.0)	10.15	86.50	3.16	3.75
T_8 (3.0)	13.75	71.50	2.13	2.00
T_9 (4.0)	17.25	51.25	1.60	1.50
T_{10} (5.0)	19.50	43.50	1.15	1.00
SE ±	1.616	2.531	0.275	0.303
C.D at 5%	4.689	7.347	0.799	0.879

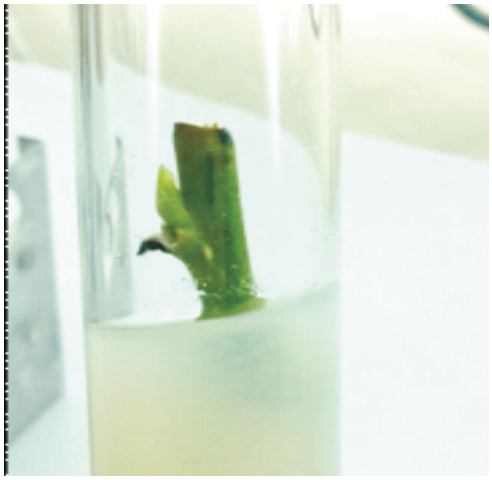

Figure 1: Initiation of shoot from a single nodal explants

Figure 2: Elongation of shoots in MS medium containing BAP (3.5 mg/L) and NAA (0.07 mg/L)

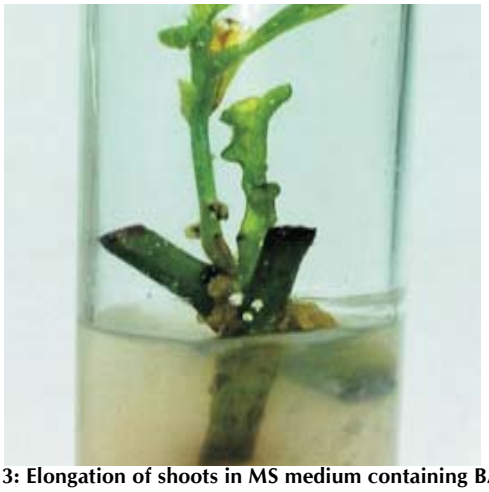

Figure 3: Elongation of shoots in MS medium containing BAP (3.5 mg/L) and NAA (0.07 mg/L)

applying IBA (2mg/L) which found maximum (86.50 per cent) rooting, minimum number of days for root initiation (10.15), produced maximum (3.75) number of roots and maximum (3.16 cm) root length (Table 2). The above results are in conformity with of Kumar and Rao (2007).

Figure 4: Proliferation of leaves in MS medium containing BAP (2.5 mg/L) and NAA (0.05 mg/L)

REFERENCES

Aberoumand, A . 2011. Determination and comparison of nutritive values and miner alelement of three important food edible plants of southern Iran. *Croatian J. Food Tech. Biotech. Nutr*. **6(3 4):** 148-151.

Abrie, A.L. and Van Staden, J. 2001. Micro-propagation of the endangered *Aloe polyphylla*. *Plant Growth Regulation*. **33:** 19–23.

Alizadeh, M., Singh, S.K. and Patel, V.B. 2010. Comparative performance of *in-vitro* multiplication in four grape (*Vitis* spp.) rootstock genotypes. *International J. Plant Production*. **4(1):** 41-50.

Bairu, M.W., Aremu, A.O. and Van Staden, J. 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulation*. **63(2):** 147-173.

Bhat, S.V., Jadhav, A.S., Pawar, B.D., Kale, A.A., Chimote, V.P. and Pawar, S.V. 2013. *In-vitro* shoot organogenesis and plantlet regeneration in brinjal (*Solanum melongena* L.). *The Bioscan*. **8(3):** 821-823.

Casado, J.P., Navarro, M.C., Utrilla, M.P., Martinez, A. and Jimenez, J. 2002. Micro-propagation of *Santolina canescens* Lagasca and *in-vitro* volatiles production by shoot explants. *Plant cell,Tissue and Organ Culture*. **69(2):** 147-153.

Currais, L., Loureiro, J., Santos, C. and Canhoto, J.M. 2013. Ploidy stability in embryogenic cultures and regenerated plantlets of tamarillo. *Plant Cell, Tissue and Organ Culture*. **114(2):** 149-159.

Devlin, R.M. and Witham, R.H. 2000. Plant Physiology, CBS publishers and Distributors, New Delhi.

Eftekhari, M., Alizadeh, A., Mashayekhi, K. and Asghari, H.R. 2012.*In-vitro* propagation of four Iranian grape varieties: Influence of genotype and pretreatment with arbuscular mycorrhiza. *VITIS-J. Grapevine Research*. **51(4):** 175.

Fracaro, F. and Echeverrigaray, S. 2001. Micro-propagation of *Cunilagalioides*, a popular medicinal plant of south Brazil. *Plant Cell, Tissue and Organ Culture*. **64:**1-4.

Fraternale, D., Giamperi, L., Ricci, D. and Rocchi, M.B.L. 2002. Micro-propagation of *Bupleurumfruticosum*: The effect of Triacontanol. *Plant cell, Tissue and Organ Culture*. **69(2):** 135-140.

Kanchiswamy, C. N. and Maffei, M. 2008. Callus induction and shoot regeneration of *Phaseolus lunatus* L. cv. Wonder Bush and cv. Pole Sieva. *Plant Cell, Tissue and Organ Culture*. **92(2):** 239-242.

Kaushik P. and Kaushik D. 2010. Evaluation of antioxidant and antimicrobial activity of *Madhuca indic*. *Pharmacology Online*. **2:**1-8.

Krishna, H. and Singh, D. 2013. Micro-propagation of lasora (*Cordia myxa* Roxb.*). Indian J. Horticulture*. **70(3):** 323-327.

Krishna, H., Sairam, R K., Singh, S.K., Patel, V.B., Sharma, R.R., Grover, M. and Sachdev, A. 2008. Mango explant browning: effect of ontogenic age, mycorrhization and pre-treatments. *Scientia Horticulturae*. **118(2):** 132-138.

Kumer, M.S. and Rao, M.V. 2007. *In-vitro* micro-propagation of *Heliotropicum indicum* Linn- an ayurvedic herb. *Indian J. Biotechnology*. **6:** 245-249.

Ndakidemi, C.F., Mneney, E. and Ndakidemi, P.A. 2013. Development of sanitation protocol for leaf explants of *B. huillensis* for *in vitro* culture. *American J. Plant Sciences*. **4(12):** 2425.

Pawar, B.D., Jadhav, A.S., Kale, A.A., Chimote, V.P. and Pawar, S.V. 2013. Effect of explants, bacterial cell density and over growth control antibiotics on transformation efficiency in tomato (*Solanum lycopersicum* L.). *J. Appl. Hort*. **15(2):** 95-99.

Singh, P., Singh, S., Shalitra, R., Samantaray, R., Singh, S. and Tiwary, A. 2016. *In-vitro* regeneration of cape gooseberry (*Physalis Peruviana* L.) through nodal segment. *The Bioscan*. **11(1):** 41-44.

Sharma, H. C., Pundir, J. P. S. and Rawat, T. S. 1983. Top working, a success in Indian cherry. *Indian Horticulture*. **28:** 17-18.

Rattanpal, H. S., Kaur, G. and Gupta, M. 2011. *In-vitro* plant regeneration in rough lemon (*Citrus jambhiri* Lush) by direct organogenesis. *African J. Biotechnology*. **10(63):** 13724-13728.

Thomas, T.D. 2008. The role of activated charcoal in plant tissue culture. *Biotech.Adv*. **26:** 61 31.

Xu, L., Najeeb, U., Raziuddin, R., Shen, W. Q., Shou, J. Y., Tang, G. X. and Zhou, W. J. 2009. Development of an efficient tissue culture protocol for callus formation and plant regeneration of wetland species *Juncus effusus* L. *In-Vitro Cellular & Developmental Biology-Plant*. **45(5):** 610-618.