BCSIR

Available online at www.banglajol.info

Bangladesh J. Sci. Ind. Res. **53(4)**, 277-282, 2018

BANGLADESH JOURNAL OF SCIENTIFIC AND INDUSTRIAL RESEARCH

E-mail: bjsir07@gmail.com

In vitro micropropagation of *Jasminum grandiflorum* L.

Md. S. Rahman[1]*, N. J. Mouri[1], N. C. Nandi[1], S. Akter[2] and Md. S. Khan[2]

[1]*BCSIR Laboratories Chittagong, Chittagong-4220, Bangladesh*

[2]*BCSIR Laboratories Dhaka, Dhaka-1205, Bangladesh*

Abstract

An efficient protocol was developed for *in vitro* plant regeneration of *Jasminum grandiflorum* L. The highest elongated shoots (60%) were achieved from axillary meristems using MS (Murashige and Skoog) basal medium supplemented with 1 mg/l 6-benzyladenine (BAP) and 60 mg/l coconut water. After adding 1.0 mg/l BAP and 45 mg/l coconut water in the culture medium, the highest rate of shoot proliferation was exhibited after 4 weeks of culture. Rooting was found within 14-23 days after the cut end of shoots was soaked in 1.5 mg/l IBA solution for 3-7 minutes. The regenerated healthy rooted plantlets were transferred to small plastic pot containing garden soil and compost in a ratio of 2:1. Maximum (75%) *in vitro* rooted plants were survived in the shade house and finally survived naturally in soil condition. The successful protocol for *in vitro* regeneration was developed which will facilitate the conservation and propagation of the important medicinal plant.

Received: 12 June 2017
Revised: 15 February 2018
Accepted: 23 May 2018

DOI: http://dx.doi.org/10.3329/bjsir.v53i4.39191

Keywords: *in vitro* regeneration; mass propagation; *Jasminum grandiflorum* and explant

Introduction

J. grandiflorum L. (Family: Oleaceae; local names: Jasmine or Jatiful) is a medicinal plant of Bangladesh that has certain therapeutic properties against various psychiatric disorders, skin diseases such as conjunctivitis and dermatitis and different types of cancer. Previously, iridoid-type compounds secoiridoidglucosides, triterpenes, flavonoids, lignans, etc., were isolated from this herb (Ahmed *et al.*, 2010). The plant is glabrous twining shrub widely grown in gardens throughout India (Nayak and Mohan, 2007). It is useful in treating various diseases (Nayak and Mohan, 2007). Its flowers and leaves are widely used in folk medicine to prevent breast cancer and stop uterine bleeding (Sharma *et al.,* 2012). The callus of the plants contains alkaloids, glycoside, flavanoid, terpines, tannin, resin, and salicylic acid that showed *in-vitro* antimicrobial activity against selected disease (Joy and Raja, 2008). Biochemical and histological findings suggested *J. grandiflorum* flower extract have wound healing properties (Vidyalakshmi and Selvi, 2013). The ethanolic extract of leaves of these plants showed antisecretory and antiulcer activity (Mahajan *et al*., 2009). Without significant side effects, the plants can be used for its antifungal properties (Alka and Jain, 2010). Ethanol extract of *J. grandiflorum* flowers have chemo preventive as well as anti-lipid peroxidative potentiality (Kolanjiappan and Manoharan, 2005). The methanol, chloroform and aqueous extracts of leaves of the plant showed better anthelmintic activity compared with the standard drug albendazole and showed *in vitro* antibacterial activity (Sandeep *et al*., 2009). Hydro alcoholic extract of leaves showed analgesic and anticonvulsant activities (Gupta and Reddy, 2013). In spite of the availability of this plant in Bangladesh, pharmaceutical companies largely depend upon material collected from foreign country as their need. This is because, commercial cultivation of this plant is rare in this country.

*Corresponding author e-mail: k2salim@yahoo.com

In addition, foreign sources are also depending on the natural resources and the natural stock of this species has been markedly decreased. Because of poor germination under natural conditions as well as death of many young seedlings, propagation from seed is unreliable (Gupta and Reddy, 2013). Propagation in large scale as well as conservation of this species, *in vitro* culture is essential as an alternative method. That is why the present study was to investigate and to develop a suitable protocol for micropropagation of *J. grandiflorum*. This finding may be helpful for the establishment of micro-propagation techniques to produce rapid and clean clones of *J. grandiflorum*. It may be of great value for the future research studies.

Materials and methods

Plant material

Elongated healthy shoots (8-10 cm long) of *J. grandiflorum* were collected from Bangladesh Council of Scientific and Industrial Research (BCSIR) Laboratories, Chittagong campus and used as sources of explants for this experiment. Shoot tips and stem nodes with a single axillary bud were used. The explants were surface sterilized with soft detergent for three times followed by washing with a few drops of Tween 20 and thoroughly washed in running tap water for 20-25 minutes. To remove surface contamination the stem segments were put in a 0.1% (m/v) aqueous mercuric chloride solution for 12 min. Thereafter, stem segments were washed 4-5 times with autoclaved distilled water to remove all traces of $HgCl_2$ and finally cut into smaller segments (about 1.3 cm long) each with one node.

Shoot regeneration

The sterilized fresh nodal explants were transferred to MS medium (Murashige and Skoog, 1962) supplemented with various concentrations of cytokinins, i.e. 6-benzylaminopurine (BAP: 0.0, 0.5, 1.0 and 1.5 mg/l), kinetin (Kn: 0.0, 0.5, 1.0 and 1.5 mg/l) and coconut water (30, 45 and 60 ml/l for shoot regeneration. The pH of the medium was adjusted to 5.8 before autoclaving. One excised stem segment was cultured in each tube (25 × 150

Table I. Effects of different concentrations of cytokinins (BAP, Kn) and coconut water added to the MS medium on shoot proliferation from axillary meristems of *Jasminum grandiflorum* L.

BAP	Kn (mg/l)	Coconut water (mg/l)	No of explants inoculated	Response percentage (%)	Time of response (day)	No of double shooted planlet	No. of single shooted plantlet	Mean shoot length (cm)	Mean No. of leaves
(mg/l)	0.0	00	20	00	-	-	-	-	-
0.0	0.0	00	20	30	11-13	4	2	3.23±0.98	4.72±0.43
0.5	1.0	00	20	55	12-14	6	5	4.78±1.2	3.82±0.78
1.0	0.0	00	20	60	11-12	7	5	5.88±0.75	3.67±0.54
1.0	0.0	30	20	55	10-12	7	5	5.43±0.96	4.32±0.76
1.0	**0.0**	**45**	**20**	**75**	**8-10**	**12**	**3**	**6.05±0.80**	**5.12±0.87**
1.0	**0.0**	**60**	**20**	**60**	**10-11**	**7**	**5**	**5.65±1.30**	**3.43±0.54**
1.0	1.0	45	20	55	11-14	6	5	4.65±0.76	3.87±0.76
1.0	0.0	00	20	45	10-13	7	2	4.43±0.87	5.08±0.16
1.5	0.0	60	20	50	10-13	6	4	5.76±0.76	4.21±0.34
1.5	1.5	60	20	45	10-12	6	3	3.88±1.50	2.67±0.32
1.5	0.5	45	20	10	13-15	1	1	5.67±2.43	4.43±0.43
0.0	0.0	45	20	15	13-15	2	1	3.00±00	5.21±0.23
0.0	0.5	00	20	00	-	-	-	-	-
0.0	1.0	00	20	25	12-14	3	2	3.87±0.87	4.32±0.63
0.0	1.5	00	20	40	10-14	5	3	4.23±0.54	2.76±0.85
0.0	0.0	30	20	10	12-15	1	1	3.80±0.83	3.65±0.84
0.0	0.0	60	20	20	11-14	2	2	4.23±0.65	3.83±0.87

mm) with about 20 ml of the culture medium. The cultures were maintained at 16 hrs photoperiod at 25 ± 2°C temperature with white fluorescent lamps having a light intensity of 55 μE/m^2/s. For shoot regeneration 20 replicates per treatment were used in each experiment. The percentages of shoots formation from segments were examined periodically up to 4 weeks of culture and the work was repeated three times. Subculture was also carried out in a 2-week interval.

Root regeneration and acclimatization

After development, elongated shoots (5-6 cm long) were excised and separated and the cut ends of separated shoot were soaked into different concentration (5, 10 and 15 mg/l) of IBA solution for 3-7 minutes. With the help of sterilized blotting paper extra IBA solution attached onto each shoot was removed and finally the treated shoots were transferred into previously autoclaved plastic pot containing garden soil and compost in a ratio of 2:1 and moistened them adequately for proper hardening. At one week interval, 2-3 ml MS solution was supplied in each pot. After 4 weeks of culture, the number of roots per shoot and the percentage of shoots forming roots were examined. Finally the well rooted plantlets were transfered into the field.

Results and discussion

The experiment was conducted to see the effect of cytokinin, BAP singly or in combination with auxin, IAA and coconut water on shoot induction and proliferation from meristematic shoot tip explants of *J. grandiflorum.* In the absence of growth regulators, axillary meristematic shoot growth could not be exposed on the medium. In this experiment, compared to BAP individually or BAP and coconut water containing media, kinetin alone or in combination with coconut water exhibited a low rate of shoot growth. The medium containing BAP in combination with coconut water enhanced the rate of shoot growth within short period of time in culture. The medium, having BAP and coconut water introduced a significant number of shoots growth as compared to the combination of BAP, Kn and coconut water. Individually, 1.0 mg/l BAP and 1.5 mg/l Kn were responsible to introduce 60% and 40% of shoot induction in *J. grandiflorum,* respectively (Fig. 1a-b). Whereas, 75% shoot proliferation occurred using MS supplemented with 1.0 mg/l BAP and 45 mg/l coconut water (Fig. 1c, Table-1). The similar concentration of BAP (1.0 mg/l) and Kn (1.5 mg/l) induced 65.8% and 36.2 % shoot proliferation in *Nyctanthes arbortristi* (Rout *et al.,* 2007). In this experiment, it was observed that 20% of shoot

Table II. Effect of different concentrations of IBA on rooting of in vitro regenerated shoot of *J. grandiflorum*

Growth regulator (mg/l) IBA	Soaked time of End cut (minutes)	No of explants inoculated	Number of rooting plants	Percentages of culture showing root	Mean Number of roots/plants	Mean of root length/ shoot	Days after rooting
0.0	0	20	0	0	0	0	0
0.1	3	20	10	50	3.67±1.2	3.87±0.65	19-22
0.5	5	20	11	55	2.54±0.67	3.13±0.56	20-23
1.0	7	20	7	35	3.65±0.65	4.14±0.98	18-22
1.2	3	20	10	50	5.76±0.54	4.98±0.75	20-23
1.5	**5**	**20**	**12**	**60**	**5.54±0.34**	**5.34±0.68**	**17-20**
2.0	7	20	8	40	3.76±0.54	4.98±0.47	17-20
2.5	3	20	9	45	3.98±0.78	5.34±0.54	19-21
3.0	5	20	9	45	3.43±0.65	4.17±0.91	19-21
3.5	7	20	6	30	4.62±0.65	5.12±0.58	17-23

induction occurred using coconut water alone at 60 ml/l (Fig. 1d). About 60% of cultures showed elongated shoots in the medium having 1.0 mg/l BAP and 60 ml/l coconut water. This observation revealed that, coconut water enhanced shoot induction of *J. grandiflorum*. Coconut water contains different types of plant growth regulator and mineral (Yong *et al*., 2009) which may be responsible for shoot induction of the plant. Efficacy of coconut water for shoot proliferation and callus induction has also been reported (Michael, 2012).

Different concentration of IBA for root development of the plant showed that 1.5 mg/l IBA exposed the highest percentages (80% and 60%) of root development due to 5 minutes soaked cut end of regenerated shoot of the plant (Fig. 1e, Table II). It was observed that higher concentration of IBA (>1.0 mg/l) and an increased number of soaking time of cut end increased root induction of the plant. In addition, low concentration of IBA (<1.0 mg/l) and less time (< 5 minutes) soaked of cut end decreased the percentages and number of root induction. The effect of IBA solution to introduce root induction of different woody plants has been published elsewhere (Paul and Aditi, 2009; Cai *et al*. 2007; Akter *et al.*, 2013; Khan *et al.*, 2012; Islam *et al.*, 2010) and this investigation also supported their works. *In vitro* rooting was achieved in medium containing 1.0 mg/l IBA by Siham (2016).

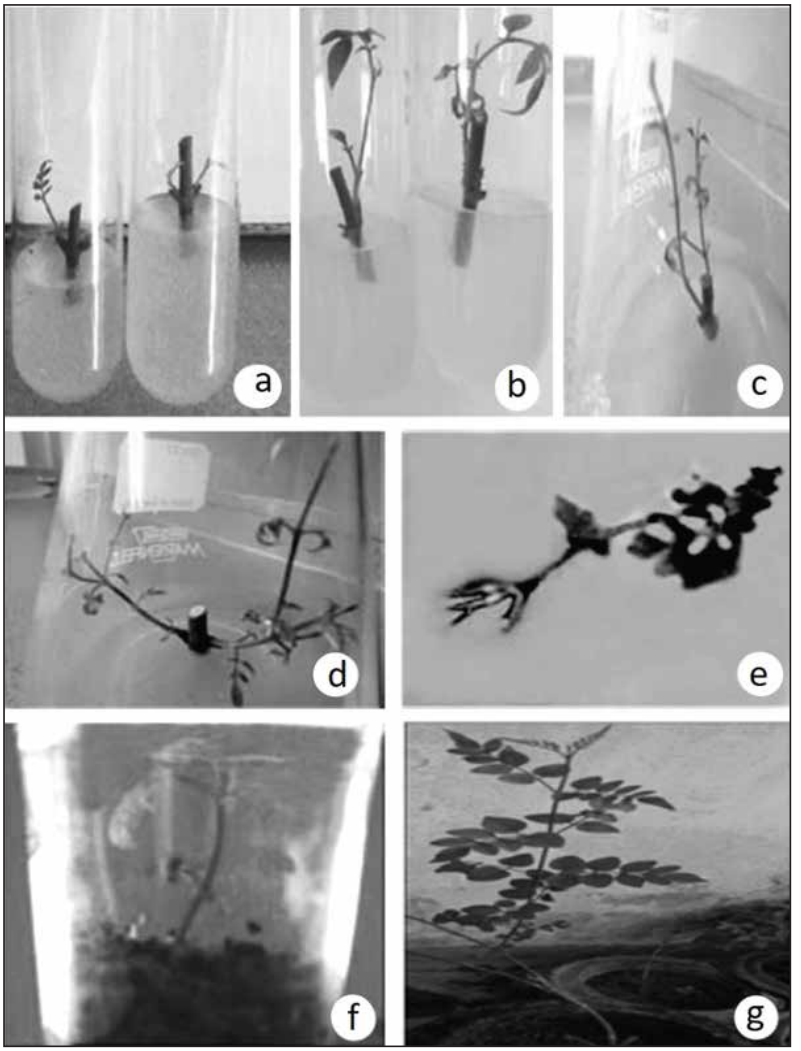

Fig. 1. Different stages of *in vitro* regeneration of *Jasminum grandiflorum* L., (a) Shoot formation after 2 weeks, (b) 3 weeks of culture containing MS medium + 1 mg/l BAP + 45 ml/l coconut water, (c-d) Shoot formation after 4 weeks of culture containing MS medium + 1 mg/l BAP + 45 ml/l coconut water, (e) Rooting and (f-g) Acclimatization into soil

It was observed that, a combination of soil and decomposed cow dungs at the ratio of 2:1 (m/v) introduced more percentages, earlier root induction and more root number than the ratio of 1:1:1 (m/v) of soil, decomposed cow dungs and sand (Fig. 1f-g). Similar effects were also observed in different other plants (Akter *et. al.*, 2013; Islam *et al.*, 2010; Jaheduzzaman *et al.*, 2012; Khan *et al.,* 2016).The increased amount of decomposed cow dung enriched Nitrogen (N) phosphorus (P), Potasium (K) in soil (Ewulo *et al.,* 2007) which are responsible for root induction of *Chrysanthemum* (Budiarto and Sulyo, 2013).

In conclusion, it can be said that, successfully production of shoots and *in vitro* root induction of *J. grandiflorum* were dependent on growth regulators and the culture conditions. This study might open a new concept for mass propagation and germplasm conservation of *J. grandiflorum*.

Acknowledgement

The authors are grateful to Industrial Botany Research Division, BCSIR Laboratories, Chittagong, Bangladesh for taxonomic confirmation of the plant. Due to the overall financial supports of the work the authors also thanks to the Director of the institute

References

Ahmed F, Sadhu SK and Ishibashi M (2010), Search for bioactive natural products from medicinal plants of Bangladesh, *J. Natl. Medi.* **64**(4): 393-401.

Akter S, Banu TA, Habib MA, Afrin S, Khatun A, Khan S and Islam S (2013), *In vitro* clonal multiplication of *Aeglemarmelos* (L.) Corr. through cotyledonary node culture, *Banladesh J. Sci. Ind. Res.* **48**(1): 13-18. DOI: http://dx.doi.org/ 10.3329/ bjsir.v48i1.15408

Alka M and Jain S (2010), Screening of Some Plant Extracts against *Alternariasp.* Isolated from Foot Infections in Cancer Patients, *Int. J. Pharm Tech Res.* **2**(2): 1165-1170.

Budiarto K, Sulyo Y and Maaswinkel R (2013), Effects of types of media and NPK fertilizer on the rooting capacity of chrysanthemum cuttings, *Indonesian J. Agri Sci.* **7**(2): 67-70.

Cai H, Chen X-q, Xiong Z-m, Xie L-l and Zhao L-j (2007), Techniques of *in vitro* micro-propagation and sugar-free rooting of jasmine (*Jasminum sambac*), *Jiangsu J. Agri.Sci.* **23**(5): 464-468.

Ewulo BS, Hassan KO and Ojeniyi SO (2007), Coperative Effect of Cowdung Manure on Soil and Leaf Nutrient and Yield of Pepper, *Int. J. Agri Res.* **2**(13): 1043-1048.

Gupta RK and Reddy PS (2013), Antinociceptive and anticonvulsant activities of hydroalcoholic extract of *Jasminum grandiflorum* (jasmine) leaves in experimental animals, *Pharmacog Res.* **5**(4): 286-290. DOI: 10.4103/0974-8490.118813

Islam S, Zahan M, Akter S, Banu TA, Habib A, Khan S and Jahana MAA (2010), Mass propagation of *Feronialimonia* L. through tissue culture, *Bangladesh J. Sci. ind. Res.* **45**(1): 75-78.

Jaheduzzaman, Habib A, Banu TA, Rahman RB, Akter S, Banu TA, Khan S and Islam S (2012), *In-vitro* Regeneration of an Important Medicinal Plant *Centellaasiatica* (L.) Urban. *Banladesh J. Sci. Ind. Res.* **47**(3): 269-272.

Joy P and Raja DP (2008). Anti-Bacterial Activity Studies of *Jasminum grandiflorum* and *Jasminum sambac* Ethnobot Leaflets **12:** 481-483.

Khan S, Islam S, Akter S,Akhtar T and Habib A (2012), Admixture of Isubgol husk together with conventionally used agar as gelling agent for Potato and Stevia regeneration, *Bangladesh J. Sci. ind. Res.* **47**(2): 161-166.

Khan S, Akter S, Habib A , Ferdousi A and Islam S (2016), Establishment of *in vitro* regeneration protocol for *Adhatoda vasica* Nees, *Banladesh J. Sci. Ind. Res.* **51**(1): 75-80.

Kolanjiappan K, Manoharan S (2005), Chemopreventive efficacy and anti-lipid peroxidativepotentiala of *Jasminum grandiflorum* L. on 7, 12-dimethyben (a) anthacene-induced rat mammary carcinogenesis, Fundam. Clin. Pharmacol **19**(6): 687-693. DOI: org/ 10.1111/j.1472-8206.2005.00376.x

MahajanN, Sakarkar D, Anghai D (2009), Evaluation of anti-ulcer potential of leaves of *Jasminum grandiflorum* L. *Int J Pharm Sci.* **1**(2): 247-249.

Michael PS (2012), Effects of coconut water on callus initiation and plant regeneration potentials of sweetpotato, *J Proceed Royal Society New South Wales*. 144: 91-101.

Murashige T and Skoog, F (1962), A revised medium for rapid growth and bio assay with tobacco tissue cultures, *Physiol Plant* **15:** 473-497. DOI: org/10.1111/j.1399-3054.1962.tb08052.x

Nayak BS and Mohan K (2007), Influence of ethanolic extract of *Jasminum grandflorum* Linn. Flower on wound healing activity in rats, *Indian J Physiol Pharmacol.* **51**(2): 189-194.

Paul R and Aditi C (2009), IBA and NAA of 1000 ppm induce more improved rooting characters in air-layers of waterapple (*Syzygiumjavanica* L.) BulgJ Agric Sci. **15**(2): 123-128.

Rout GR, Mahato A and Senapati SK (2007), *In vitro* clonal propagation of *Nyctanthes arbortristis* L. – a medicinal tree, *Hort Sci.* **34**(2): 84–89.

Sandeep P, Paarakh M and Gavani U (2009), Antibacterial activity of *Jasminum grandiflorum* L. Leaves, *J Pharm Res.* **2**(7): 1206-1207.

Sharma P, Singh N and Verma OP (2012), First report of *Colletotrichum gloeosporioides* of *Jasminum grandiflorum* in India, *J Plant Protec Res.* **52**(1): 91-92.

Siham AAS (2016), *In Vitro* regeneration of *Jasminum azoricum* L: an important ornamental and medicinal plant, *Int J Pharm Sci&Scient Res.* **2**(3): 122-127.

Vidyalakshmi A and Selvi SE (2013), Protease Activity of Floral Extracts of *Jasminum grandiflorum* L., a Wound Healing Herb, *J. Medic. Plants.* **1**(4): 11-14.

Yong JWH, Ge L, Ng YF and Tan SN (2009), The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, *Molecules* **14**(12): 5144-5164. DOI: org/10.3390/molecules14125144