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In Vitro Regeneration of *Jasminum Azoricum L* : an important Ornamental and Medicinal Plant

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Abstract

This study was conducted in order to investigate the ability of Jasminum azoricum L. to regenerate in vitro through two waiys; in the first way: nodal explants were cultured on MS medium containing different concentrations of BA (0.0 , 0.5 , 1.0 , 2.0 , 4.0 , or 6.0) mg/L for direct shoot proliferation. The results showed that BA in concentration 2.0 mg/L gave the best result in shoot number(2.5), while the control was the best in shoot length (2.51 cm) and number of nodes per shoot(4.5 node / shoot) than other treatments. In the second method: internodal explants were cultured on MS containing BA(0.0 , 2.0 , 4.0 , or 6.0) mg/L with 2,4-D (0.0, 0.01 , 0.05 or 0.1) mg/L for callus induction and indirect regeneration of shoots. The highest percent of callus induction (100%) was seen in MS supplemented with 4.0 mg/L BA + 0.1 mg/L 2,4-D and 6.0 mg/L BA + 0.1 mg/L 2,4-D. The proliferated callus was transferred into MS medium supplemented with BA (0.0 , 2.0 , 4.0 , or 6.0) mg/L with Kin (0.0 or 2.0) mg/L for adventitious buds regeneration. The highest number of buds (10.1) was seen in the combination 4.0 mg/L BA + 2.0 mg/L Kin. In vitro rooting was achieved in medium containing 1.0 mg/L IBA and the rooted plantlets were successfully acclimatized.

Key words: *Jasminum azoricum*, in vitro, regeneration, cytokinins, auxins.

Introduction

Plants of Jasminum genus are evergreen climbing or erect shrubs with thin woody stems of older growth belonging to the family Oleaceae, they are distributed in the warmer parts of Asia, Europe and Africa (Ramdas et al., 1993). The flowers are very fragrant used for production of perfumes, soap, and cosmetic industry (Alikhan et al., 1989). Pharmacology researches revealed anticancer activity of the extracts of jasmines on human epidermoid carcinoma of nasopharynx and anti-inflammatory effect against acute and chronic inflammation, while the oil is used externally to soothe dry or sensitive skin. The extracts of flowers and leaves of jasmine species showed antibacterial (Kumar et al., 2007; Shekhar and Prasad, 2015) and as herbicides (Poonpaiboonpipat et al., 2011).

Jasminum azoricum is an ornamental and medicinal plant which is a climbing shrub (2 to 3 m in height) , leaves are trifoliate, flowers are fragrant formed in groups of 1-5 at the end of branches, with a small green calyx and a white tubular corolla expanding into 6 petal lobes.

Jasmine plants have medicinal value, because they are important sources of several compounds that are used in newer herbal drugs. Some of the phytoconstituents isolated from jasmines are iridoidal glycosides, oligomeric, sambacein I-III (Somanadhan et al., 1998) ; jasminanhydride (Sadhu et al., 2007) ; oleanolic acid, β-rutinoside, daucosterol and hesperidin (Kunhachan et al., 2012).

Several studies have been done on aroma and oil of jasmines, but reports about the micro propagation are few.

So, the development of plant tissue culture techniques permits to regenerate of uniform plants, germplasm conservation and for production of secondary metabolites to meet the increasing demand of therapeutic and other industeries. Plantlets were regenerated from leaf callus of J. grandiflorum after sub cultured on MS provided with BAP (2 ppm) with sucrose at concentration 1.5% (Gomathi et al., 2007) . Multiple shoots of J. officinale were produced from axillary buds cultured on MS containing 45% sucrose + 4mg/L BA + 0.1mg/L NAA (Bhattacharya and Bhattacharya, 2010). The present study aimed to determine an efficient protocol for in vitroregeneration of J. azoricum .

Materials and Methods

Preparation of plant materials

Nodes and internodes were used as explants which collected from a garden grow adult shrub of J. azoricum . After removing off leaves; explants were washed with water and liquid soap to remove dirt, and then they were washed under running tap water for 1 hr. Surface sterilization was achieved in the laminar – air flow cabinet with 2% (v/v) Clorox (6% NaOCl) solution containing 2 drops of tween 20 for 20 min., then they were rinsed for two times with sterile distilled water (two min. in each). Nodal and intermodal explants (1.5 – 2.0 cm) were prepared for culture.

Culture initiation and shoot multiplication

The nodal explants were cultured on MS medium (Murashige and Skoog, 1962) containing 30% sucrose, 0.7% agar, and supplemented with various concentrations of benzyl adenine (BA) (0.0, 0.5, 1.0, 2.0, 4.0, or 6.0) mg/L for shoot initiation. The pH of medium was 5.7 ± 0.1 before autoclaving at 121ºC and 1.04 kg/cm² for 15 min. 10 replicates were used for each concentration. Cultures were incubated under 16 h photoperiod with light intensity of 1000 lux at 25 ± 2ºC. Results were taken after five weeks of culture. The shoots that were proliferated in vitro were cut into nodal explants and recultured on the same basal medium with the best results of BA concentration from the previous experiment above in order to produce multiple shoots.

Callus induction and shoot regeneration

For callus induction , internodal explants were cultured on MS medium containing 30% sucrose, 0.7% agar, and supplemented with 2,4-Dichlorophenoxy acetic acid (2,4-D) at concentrations of (0.0, 0.01, 0.05, or 0.1) mg/L in combination with BA at concentrations of (0.0, 2.0, 4.0, or 6.0) mg/L . After four weeks, the induced embryogenic callus was transferred into MS free-hormone medium for two weeks, then cultured on medium containing BA at concentrations of (2.0, 4.0, or 6.0) mg/L with (0.0 or 2.0 mg/L) of kinetin . All cultures were incubated under the same conditions that mentioned previously. Results were evaluated after five weeks of culture.

Rooting of plantlets and acclimatization

The proliferated plantlets (1.5 – 5.0 cm height) were cultured in half strength MS medium supplemented with different concentrations of 1.0 mg/L IBA. The rooted plantlets were cultured in pots containing a mixture of river soil and peat moss (2 : 1) , then covered with beakers to prevent the loss of water in order to achieve acclimatization process.

Statistical analysis

Data were statistically analyzed in a Completely Randomized Design (CRD). Mean values were compared using Least Significant Difference (LSD) test at 0.05 (SAS, 2001).

Results and Discussion

Shoot proliferation from nodes

Results in table-1 show the effect of different concentration of BA on shoot proliferation from axillary buds. The majority of explants produced either short multiple shoots or only single long shoots. There was shoot formation (1.2) in basal MS medium without any addition of plant growth regulators (control). This average was appeared to be significant with the graduate increasing in cytokinin concentration until it was reached to the concentration 2.0 mg/L BA, which gave the highest shoot number gave (2.5) followed by the concentration 4.0 mg/L BA gave (2.3). However, there was a significant decreasing in shoot number with concentration of 6.0 mg/L BA gave (1.9), but it was remained significant as compared with control

Table 1: Effect of different concentration of BA in MS medium on shoot number, shoot length, and average number of nodes of Jasminum azoricum L.

BA(mg/L)	Shoot number	Shoot length(cm)	Number of nodes/shoot
0.0	1.2	2.51	4.50
0.5	1.9	1.78	3.19
1.0	2.0	1.82	3.10
2.0	2.5	1.62	2.60
4.0	2.3	1.55	2.20
6.0	1.9	1.32	2.20
L.S.D.(0.05)	0.41	0.13	0.10

Data also showed that there was a significant and best elongation of shoots of the control gave (2.51 cm) followed by the concentrations of 0.5 and 1.0 mg/L BA which gave (1.78 and 1.82 cm respectively) as compared with other treatments . The lowest shoot length was observed with 6.0 mg/L BA , which was 1.32 cm.

Significant difference was found in the average number of nodes per shoot (Table-1) in which the control gave the maximum number of nodes (4.50) than other treatments. The Figure-1 shows the shoot proliferation and multiplication in vitro . Cytokinins induced multiple shoot formation, but the proper type and concentration of these hormones are different for each plant species (Luo et al., 2009 ; Gantait et al., 2011 ; Wangren, 2011; Shen et al. , 2013).

(A): nodes cultured on free MS medium(right) and on MS+ 1.0mg/L BA (left)

(B): shoots multiplication on MS + 2.0 mg/L BA.

Figure(1): Shoots proliferation from nodes of Jasminum azoricum

Callus induction and buds regeneration

Results in table (2) revealed that there are significant differences in the effect of the different concentrations of BA and 2,4-D on the callus induction. The best concentrations of BA that gave the best percentage of callus induction were 4.0 and 6.0 mg/L, which gave 62.5 and 72.5 %, respectively, whereas 0.1 mg/L 2,4-D gave a significant difference (82.5 %) than other combinations.

The highest percent of callus induction (100 %) was seen in explants grown in MS medium containing 4.0 mg/L BA+ 0.1 mg/L 2,4-D and 6.0 mg/L BA+ 0.1 mg/L 2,4-D , whereas the control did not give any response for callus formation.

Table 2: Effect of different concentrations of BA and 2,4-D on the percentage of callus induction from internodes of Jasminum azoricum .

2,4-D(mg/L) / BA(mg/L)	0.0	0.01	0.05	0.1	Mean of BA
0.0	0.0	10.0	30.0	60.0	25.0
2.0	10.0	20.0	60.0	70.0	40.0
4.0	30.0	40.0	80.0	100.0	62.5
6.0	30.0	70.0	90.0	100.0	72.5
Mean of 2,4-D	17.5	35.0	65.0	82.5	
L.S.D.(0.05)	BA=17.260		2,4-D=17.260		Interaction=24.720

Many researchers showed that cytokinins and auxins induced callus formation in many plants (Darion et al., 2010 ; Hesar et al., 2011). It was known that the cytokinins and auxins are used to promote the formation of callus in many excited and in vitro cultured explants or organs (Ibrahim et al., 2013) .

The induced callus was transferred into MS medium without any addition of plant growth regulators for two weeks , then it was transferred into fresh MS medium containing different concentrations of BA (0.0, 2.0, 4.0, or 6.0) mg/L in combination with Kin (0.0 or 2.0 mg/L) for adventitious buds regeneration. Data in Table (3) showed that there were significant differences of BA concentrations on the regenerated buds from callus (Figure- 2) . The highest number of buds was found in the concentration of 4.0 mg/L BA , which gave 8.45 buds, other concentrations of BA were comparatively better than the control. Results also showed that the effect of Kin in concentration 2.0 mg/L (7.05 buds) was significant than control (3.4 buds). The interaction between BA and Kin showed that the highest number of buds was (10.1) seen in the combination of 4.0 mg/L BA + 2.0 mg/L Kin

Table 3: Effect of different concentrations of BA and Kin on the buds regeneration from callus of Jasminum azoricum .

Kin(mg/L) / BA(mg/L)	0.0	2.0	Mean of BA
0.0	0.0	0.4	0.20
2.0	0.7	9.0	4.85
4.0	6.8	10.1	8.45
6.0	6.1	8.7	7.40
Mean of Kin	3.4	7.05	
L..D.(0.05)	BA=1.603	Kin=1.134	Interaction=2.268

Organogenesis in explants during micro propagation takes place either directly or after callus formation. Studies on many ornamental plants showed both kinds of organogenesis . Cytokinins are known to promote in vitro regeneration of organs or buds from callus tissues of many ornamental plants (Gomathi et al., 2007)

Figure (2)

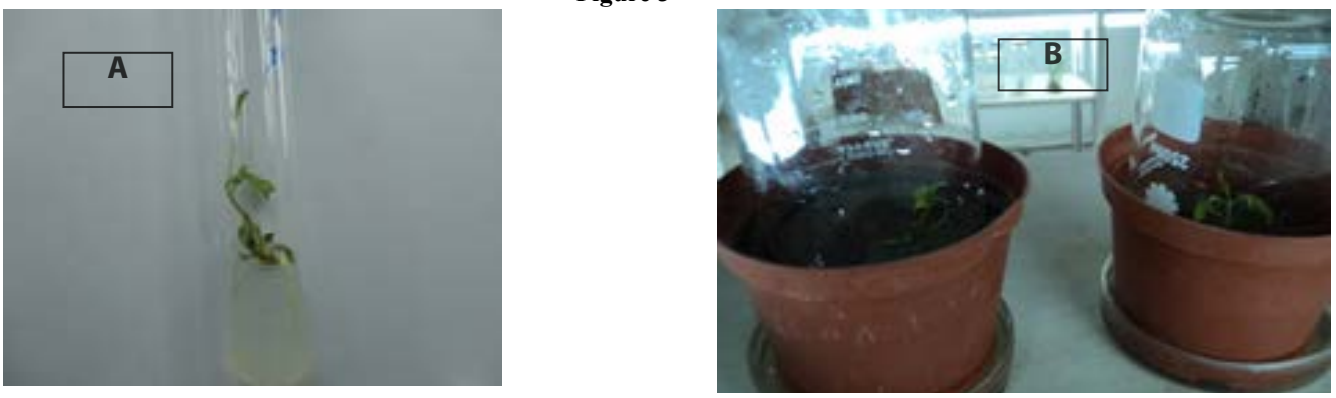
(A) Callus induction on internodes cultured on MS medium supplemented with BA and 2,4-D.

(B) Regeneration of buds from callus cultured on MS medium supplemented with BA and Kin.

Rooting and acclimatization

According to the conditions of the experiment, the plantlets rooted in vitro on medium containing 1.0 mg/L IBA (Figure- 3) and were successfully acclimatized. Most of recent studies focused on the rooting and hardening of in vitro micro propagated plantlets by treated them with different types and concentrations of auxins (Gantait et al., 2011).

Figure 3



(A) rooting of in vitro micro propagated plantlets. **(B) acclimatization of rooted plantlets.**

Conclusion

It can be concluded from this research that the method of micro propagation of ***Jasminum azoricum*** lead to efficient multiplication through large number of shoots and callus induction in large amounts in short period of time can help to promote the mass production of this valuable medicinal plant.

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