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EFFECTS OF VARIATIONS IN HORMONAL TREATMENTS UPON CALLUS INDUCTION POTENTIAL IN *Jasminum grandiflorum* L

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ABSTRACT

Plants are the real basis towards people's livelihood. A large number of medicinal plants is explored from the natural flora for Commercial drug production. Aromatic flower yielding plant has various contributions in performing various religious celebrations and has other multiple beneficiaries like medicine, human happiness as well as in treating deadly diseases. The growing demand for flower extracts in perfume trade can primarily be met by increasing flower production and multiplying planting material. A thrust upon developing advanced propagation technologies for *Jasminum grandiflorum* L., is needed in addition to conventional nature-depended agro-techniques as these floral crop is the major commercial aromatic flower yielding plant. In this study the effect of different (2,4-D, IAA, IBA, NAA) growth hormones and their combination on callus formation, callus texture and weight (fresh and dry) of *Jasminum grandiflorum* were investigated. In this investigation revealed that the best callus performance observed in individual MS medium containing 1.5 mg/L 2,4-D and combination 1.5 mg/L 2,4-D along with 1.5 mg/L IAA.

Keywords: *Jasminum grandiflorum;* callus; 2,4-D, IAA; IBA; NAA.

INTRODUCTION

Jasmines (family *Oleaceae*) are an important group of flowering plants and widely cultivated for their attractive fragrance. Moreover, different parts of the plant such as the leaf, stem, bark and roots are very useful in pharmaceutical industries. *Jasminum grandiflorum* has diuretic and emmenagogue properties. The fresh leave juice are applied to corns to prevent infections, and chewed in the treatment of mouth ulcer. The leaves contain resin, salicylic acid, and an alkaloid named jasmine [1]. The flowers are very fragrant used for production of perfumes, soap, and cosmetic industry. Pharmacology researches revealed anticancer activity – on jasmine extracts on human epidermoid carcinoma of nasopharynx, antimicrobial and anti-inflammatory effect against acute and chronic inflammation, while the oil is used externally to soothe dry or sensitive skin. Some of the phytoconstituents isolated from jasmines are iridoidal glycosides, oligomeric, sambacein I-III [2].

Jasmine occupies the first place among perfumery flowers. The odour of jasmine flower cannot be imitated by any known synthetic aromatic chemical or natural isolates, thus, giving it a unique status in the perfume world. Jasmine plants have medicinal value, because they are important sources of several compounds that are used in newer herbal drugs [3]. Therefore, it has attracted

the attention of researchers around the globe. Several studies have been done on aroma and oil of jasmines, but reports about the callus induction are few. So, the development of *Jasminum grandiflorum* tissue culture techniques permits to produce identical clones of parent plants, germplasm conservation and for production of secondary metabolites to meet the increasing demand of therapeutic and other industries.

MATERIALS AND METHODS

Plant Collection and Identification of Plants

The plants used in this study *Jasminum grandiflorum* L. (Fig. 1) – No.BSU/SRC/5/23/ 2011-12/Tech-132 (Oleaceae), was collected from Namakkal District, Tamil Nadu and it was authenticated at Botanical Survey of India, South Circle, Coimbatore, Tamil Nadu. The leaves from these plants were used as explants for the study.

Fig. 1. *Jasminum grandiflorum* L.

Preparation of the Explant

Plant tissues known to be free of the pathogen (viral, bacterial, or fungal) are physically selected as the explant for tissue culture. The garden or potted plant carries germs on its surface, this contaminated removed and selected explant carefully without any damage. The youngest and oldest leaves were avoided. About 1 cm sides of leaf square were selected as explants leaving short length of petiole attached from healthy plant.

Sterilization and Inoculation of Explants

The explants were first thoroughly washed in running tap water for 30 min [4]. The explants were pretreated with Tween 20, an emulsifier and detergent (2-3 drops in 100 ml distilled water) for 20 min. Later the explants were sterilized with antimicrobial agents (Bactericide - streptomycin and Fungicide- bavistin) for 5 min [5]. Then, the explants were washed 2-3 times with distilled water and further sterilized in laminar airflow chamber. Further surface sterilized using 0.1% mercuric chloride solution for 1 min. After surface sterilization the explants were washed with sterile water 2 to 3 times, and inoculated. The leaves were trimmed into pieces of about 1 cm and inoculated on to the MS medium for callus induction studies. All these cultures were incubated at 25±2ºC [6]. Each experiment had 20 replicates with three explants in each bottle.

Preparation of Media for Callus Induction

The MS nutrient medium [7] was used for callus induction. MS basal medium supplemented with different hormonal concentrations like 2,4-D, (0.5, 1.0, 1.5, 2.0 mg/L), IAA (0.5, 1.0, 1.5, 2.0 mg/L), IBA (0.5, 1.0, 1.5, 2.0 mg/L), NAA (0.5, 1.0, 1.5, 2.0 mg/L) and combination of the hormonal concentrations were used. After addition of all the media constituents, the pH was adjusted to 5.8 using 0.1 N KOH or 0.1 N HCl. Gelling agent (agar-agar) at a concentration of 0.8% was added and the medium was steamed to melt the gelling agent. It was then dispensed into test tubes (10 ml per tube) or screw capped bottles (25 ml per bottle). The medium was autoclaved at 121°C at a pressure of 1.1 $kg.cm^{-2}$ for 20 min.

Culture Conditions

All the cultures were maintained in the culture room at 25±2°C at a relative humidity of 65-70%. The cultures were kept under white light at an intensity of 2000 Lux (at the level of culture tables) provided from white fluorescent lamps and a photo period of 16/8 h was maintained for all experiments. Each experiment was repeated three times and percentage of callus formation was observed. Simultaneously callus texture and weight (fresh and dry) were also calculated.

Effect of four different concentrations of 2,4-D, IAA, IBA and NAA (0.5, 1.0, 1.5, and 2.0 mg/L) on callus initiation (days) and percentage of callus formation were assessed. The percentage of callus formation was recorded after 15 days of inoculation. The increase in mass as gain in weight was recorded as fresh weight of callus after 4 weeks of inoculation. The dried fresh callus weight was observed as dry weight. A significant difference was observed between treatments.

Callus induction (%) = (No. of Plants produced/No. of Plants inoculated) X 100

Statistical Analysis

The data obtained from these experiments were subjected to statistical analysis by using the statistical software AGRES, in completely randomized design (CRD). Each experiment was repeated twice with a minimum of 3 replicates in each. The SEd (Standard Error Deviation), CD (Critical Difference) and CV % (Co-efficient of Variation) values were calculated. Significant differences (*p* value) were determined using Duncan's multiple range test (DMRT).

RESULT AND DISCUSSION

Effect of Growth Regulators on Callus Induction, Texture and Weight

Effect of four different concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) of 2,4-D, IAA, IBA and NAA, and their combination (1.5 mg/L 2,4-D + 0.5, 1.0, 1.5 and 2.0 mg/L) on callus initiation was assessed using the explants. It was observed that 2,4-D strongly induced callus formation and the results are shown in the Table 1.

The role of plant growth regulators or hormones in tissue culture is very important. In the present study the explants inoculated on plant growth regulator free medium did not show any morphological response up to three weeks. In contrast, explants cultured on medium containing 0.5, 1.0, 1.5 and 2.0 mg/L growth hormone exhibited callus formation in almost all cultures, however, some plant growth regulators promoted the callus induction formation better than the others.

Among the different auxins used in the experiment only 1.5 mg/L 2,4-D was able to produce high percentage of callus and weight. The inclusion of 2, 4-D in the culture medium has been reported to be necessary for inducing callus in *Swertia lawii* Burkill [8]. The other hormones like IAA, IBA and NAA produced callus but the frequency was inadequate. In callus induction medium, when single auxin was used, only restricted response was observed, but combination of 1.5 mg/L 2,4-D with IAA, IBA and NAA at concentrations of 0.5, 1.0, 1.5 and 2.0 mg/L enhanced callus induction frequency. Similar responses have been reported by Pola et al. [9] in sorghum.

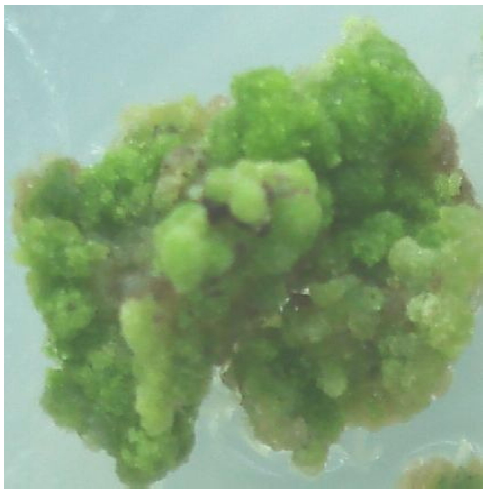

Fig. 2. *Jasminum grandiflorum* L. Callus
1.5mg/L 2,4-D + 1.5mg/L IAA

J. grandiflorum showed higher activity in callus formation, fresh weight and dry weight, in single hormone of MS media supplemented with 1.5 mg/L 2,4-D (1.5 mg/L 2,4-D). Khanam et al. [10] reported that callus induction of *Duboisia myoporoides* in medium supplemented with 2,4-D alone or various concentrations of cytokinins (Kin.) + 2, 4-D. Also, reported that callus induction took place after 7 to 10 days on 90.19% of the leaf explants. The same findings were reported by Khater et al. [11] which proved decided that 2, 4- D induced faster growing calluses in *Atropa belladonna* L.

Compared to all other hormones 2, 4-D produced increased callus formation with 1.5 mg/L 2,4-D. The same concentration was used for all further

Table 1. Effect of various concentrations of auxin on callus initiation, callus formation, fresh and dry weight of *Jasminum grandiflorum*

Treatment (mg/L)	Callus initiation (Days)	Texture of the callus	% of callus formation	Weight (mg)	
				Fresh	Dry
2,4-D					
0.5	11-14	C	37.67±1.53^{o}	361.24±1.12^{o}	199.19±1.88^{h}
1.0	11-14	C	45.33±3.51^{k}	451.51±4.66^{k}	251.37±4.26^{g}
1.5	**11-14**	C	**68.33±3.06^{c}**	**750.55±1.54^{c}**	**434.18±2.09^{b}**
2.0	11-14	C	52.67±3.06^{h}	542.68±1.27^{h}	294.88±3.54^{ef}
IAA					
0.5	8-12	F	24.67±4.04^{w}	245.36±1.80^{w}	136.53±2.16^{m}
1.0	8-12	F	27.33±3.51^{s}	288.72±4.73^{s}	163.29±3.67^{jk}
1.5	8-12	F	36.67±1.53^{p}	342.96±1.94^{p}	191.73±1.90^{hi}
2.0	8-12	F	29.33±4.51^{r}	314.24±4.05^{r}	174.70±3.39^{ij}
IBA					
0.5	12-15	C	21.67±2.52^{x}	210.99±2.03^{x}	118.04±2.37^{n}
1.0	12-15	C	26.33±3.06^{v}	251.25±4.65^{v}	140.99±1.19^{lm}
1.5	12-15	C	33.67±2.52^{q}	326.01±4.43^{q}	179.63±2.56^{ij}
2.0	12-15	C	28.33±3.06^{u}	256.53±2.31^{u}	143.81±3.93^{lm}
NAA					
0.5	10-14	C	22.33±3.51^{y}	201.79±2.29^{y}	112.45±1.91^{n}
1.0	10-14	C	26.33±3.06^{w}	245.07±3.72^{w}	136.73±4.01^{lm}
1.5	10-14	C	31.00±3.61^{t}	273.35±1.84^{t}	154.56±4.57^{kl}
2.0	10-14	C	24.67±4.16^{y}	201.75±1.64^{y}	115.36±4.38^{n}
2,4-D+ IAA					
1.5+0.5	9-11	F	46.00±3.00^{l}	446.98±1.47^{l}	247.45±3.40^{g}
1.5+1.0	9-11	F	58.67±2.52^{e}	608.32±2.94^{e}	336.87±3.74^{d}
1.5+1.5	**9-11**	F	**91.33±2.08^{a}**	**998.25±2.60^{a}**	**554.75±4.60^{a}**
1.5+2.0	9-11	F	74.67±1.53^{b}	788.95±1.43^{b}	438.06±3.89^{b}
2,4-D+ IBA					
1.5+0.5	10-13	C	44.67±4.04^{m}	433.42±3.11^{m}	244.76±4.08^{g}
1.5+1.0	10-13	C	55.33±3.06^{g}	550.20±1.02^{g}	304.75±2.77^{e}
1.5+1.5	10-13	C	77.33±1.53^{b}	791.35±1.00^{b}	445.80±3.20^{b}
1.5+2.0	10-13	C	64.00±3.00^{d}	625.41±2.57^{d}	351.85±3.85^{c}
2,4-D+ NAA					
1.5+0.5	10-12	C	41.33±2.52^{n}	420.35±3.67^{n}	234.74±3.57^{g}
1.5+1.0	10-12	C	48.33±2.08^{j}	495.43±2.86^{j}	278.19±4.25^{f}
1.5+1.5	10-12	C	57.33±3.06^{f}	595.64±2.56^{f}	343.08±2.70^{d}
1.5+2.0	10-12	C	52.67±3.51^{i}	516.75±2.90^{i}	292.42±2.73^{ef}
CD (0.05)			3.8729	3.8729	4.4621
SEd			1.9317	1.9317	2.2310
CV%			0.53	0.53	0.67

C- Compact F-Friable, Data are expressed as mean ± SD of three replicates followed by a common superscript letter are significant at 5% level by using DMRT ($p<0.05$).

combination of IAA, IBA and NAA (0.5, 1.0, 1.5 and 2.0 mg/L). In combination MS media supplemented with 1.5 mg/L 2,4-D+1.5 mg/L IAA (Fig. 2) showed better activity in callus formation (91.33±2.08), similarly activity observed *Ocimum basilicum* and *Ocimum tenuiflorum* by Bhuvaneshwari et al. [12]. Correspondingly callus high fresh weight (998.25±2.60) and dry weight (554.75±4.60) were also observed in the same combination. However an efficient callus induction from leaf explants was observed on media supplemented with 2, 4-D. Very poor callus formations, fresh weight and dry weight was observed in NAA [13].

Among different combination of auxins tested, 1.5 mg/L 2,4-D+1.5 mg/L IAA proved to be better in terms of inducing high frequency of greenish

callus. Callus initiation was observed in single hormone IAA which showed callus formation between 8-12 days followed by NAA (10-14 days), 2,4-D (11-14 days) and IBA (12-15 days). A combination of IAA+2,4-D produced callus formation between 9-11 days followed by NAA+ 2,4-D (10-12 days), IBA+2,4-D (10-13 days). When texture of the callus in *J. grandiflorum* was observed it was found that IAA produced friable callus formation and where as other hormones (2,4-D, IBA and NAA) produced compact callus. In combination treatments IAA+2,4-D produced friable callus but all other hormonal combinations (IBA+ 2, 4-D and NAA+ 2, 4-D) showed compact callus formation. Similar result was observed in Zubeda et al. [14] in tomato callus formation.

CONCLUSION

It was concluded that use of leaf explants and MS medium supplemented with 2, 4-D and IAA is most suitable for callus formation in *Jasminum grandiflorum.* The manipulation of culture conditions using various combinations and concentrations of growth hormones can provide a reproducible protocol and reduce the high costs of plant production. It will meet the pharmaceutical requirements besides strengthening to the increasing concern to conserve biodiversity through preservation of germplasm of this medicinal shrub. Further studies should be established to determine the best concentrations and combinations of PGRs for *Jasminum grandiflorum* through a biotechnological approach. The results could be used for the large-scale commercial production of jasminoids and disease-free and healthy plants production.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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