Mysore J. Agric. Sci., 57 (3) : 248-254 (2023)

Response of Geographical Indication Tagged Jasmines of Karnataka to *In vitro* Propagation

G. J. Ranganath, K. S. Nirmala and P. Venkatesha Murthy
Department of Horticulture, College of Agriculture, UAS, GKVK, Bengaluru - 560065
e-Mail : drnirmalaks@uasbangalore.edu.in

Authors Contribution

G. J. Ranganath : Conceptualization, investigation and data analysis;
K. S. Nirmala : Framing research proposal, data curation and draft correction;
P. Venkatesha Murthy : Experiment and supervision

Corresponding Author :
G. J. Ranganath
Department of Horticulture, College of Agriculture, UAS, GKVK, Bengaluru

Received : July 2022
Accepted : June 2023

Abstract

The Geographical Indication registered jasmine cultivars of Karnataka - Udupi mallige, Hadagali mallige and Mysore mallige produce flowers with unique fragrance. A study on use of leaf sections for rapid multiplication of jasmines *viz.,* Mysore mallige (*Jasminum azoricum* L., syn. *Jasminum trifoliatum* Moenc.) Udupi mallige (*Jasminum sambac* L. Aiton) and Hadagali mallige (*Jasminum auriculatum* Vahl.) was conducted at Plant Tissue culture Laboratory, Department of Horticulture, UAS, GKVK, Bengaluru. All the three species of jasmines showed early callus initiation on MS medium consisting of 0.2 mg L-1 2,4-D with cent percent callusing on MS medium with growth regulators. Callus intensity was also very high on MS medium supplemented with 2, 4-D alone. Colour and texture of callus varied with the cultivar. Callus appeared non embryogenic in Udupi mallige, while it was embryogenic in Hadagali and Mysore mallige. Regeneration of shoots was not achieved in any of the combinations of auxins or cytokinins.

Keywords : Geographical indication, Jasmine, Karnataka, *in vitro* propagation

Jasmines (*Jasminum* spp.) are commercially important flower crop belonging to family Oleaceae and are cultivated in the Southern and Eastern parts of India. A native of tropical and subtropical regions, jasmine is esteemed for its attractive fragrant flowers and is highly valued for essential oil. Indo-Malayan region being the center of origin diversity noticed in *Jasminum* spp is enormous in India.

Jasminum species show enormous morphological variations in their vegetative and floral characters. Such morphological variations among 48 genotypes of Jasmines have been recorded by Nirmala *et al.* (2017). Some of the jasmine cultivars produce flowers with unique fragrance due to specific soil and climatic conditions prevailing in that region. The Department of Horticulture, Government of Karnataka, India has obtained the Geographical Indication registration to protect some unique cultivars *viz.,* Udupi mallige, Hadagali mallige and Mysore mallige. Udupi mallige is a cultivar of *Jasminum sambac* and Hadagali mallige is *J. auriculatum* and these species are commercially cultivated in Tamil Nadu and Karnataka states while Mysore mallige cultivar of *J. azoricum* is commonly grown in home gardens and area under cultivation of this species has reduced due to urbanization.

This paper investigated the use of leaf sections for rapid multiplication of *Jasminum* sps. *viz., J. sambac* (Udupi mallige), *J. auriculatum* (Hadagali mallige) and *J. azoricum* (Mysore mallige),

Material and Methods

The present study was carried out in the Plant Tissue culture Laboratory, Department of Horticulture, UAS, GKVK, Bengaluru. The mother plants of GI tagged *Jasminum* sps. *viz.,* Udupi mallige (*Jasminum sambac*

(L.) Aiton), Hadagali mallige (*Jasminum auriculatum* Vahl.) and Mysore mallige (*Jasminum azoricum* L. syn. *Jasminum trifoliatum* Moenc.) (Fig.1) were maintained in 'D' Block, ZARS, GKVK.

Explant Collection and Surface Sterilization

The immature light green leaves were collected from the healthy mother plants and washed in running tap water for 60 m followed by soaking in 3 per cent of Tween 20 for 15 m, then rinsing in distilled water, disinfected by treating with 0.2 per cent carbendazim for 3 minutes, washed thrice with sterile distilled water followed by treating with Mercuric chloride (0.05%) 4 m and again washing 3-4 times with sterile distilled water.

Inoculation of Explants and Incubation

Leaf sections of 1 cm^2 were prepared by trimming the excess tissues. The explants were cultured on MS medium (Murashige and Skoog, 1962) containing 3 per cent sucrose solidified with 8 per cent Agar. pH of the medium was adjusted to 5.8 before autoclaving. The nutrient medium was incorporated with different concentrations of Auxins (0.2 and 0.4 mg L-12,4-D; 0.25 and 0.5mg L-1 NAA) and cytokinin (2.0, 4.0 and 6.0 mg L-1 BAP; 2.0, 4.0 and 6.0 mg L-1 Kinetin) either alone or in combination. All the treatments were replicated 10 times. The cultures were incubated in growth room at 24±2^{0}C under light intensity of 2000 lux using white fluorescence tubes for 8 hours of light and 16 hours dark period.

Callus Subculturing

The callus was subcultured on the MS medium containing BAP (2.0 mg L^{-1} to 6.0 mgL^{-1}), Kinetin (2.0 mg L^{-1} to 6.0 mg L^{-1}) and NAA (0.25 mg L^{-1} to 0.5 mg L^{-1}) to study the proliferation and regeneration.

Statistical Analysis

The data recorded was analyzed according to CRD (Completely Randomized Design) using OPSTAT software.

Udupi mallige (*Jasminum sambac* (L.) Aiton)

Hadagali mallige (*Jasminum auriculatum* Vahl)

Mysore mallige *Jasminum azoricum* L.

Fig.1 : GI Tagged *Jasminum* sps. of Karnataka

Results and Discussion

Establishment of Aseptic Cultures

Aseptic cultures were established by sterilizing the leaf sections with 0.05 per cent mercuric chloride for 4 minutes followed by for 3-4 times with sterile distilled water which resulted in 95 per cent success, beyond which with increase in treatment time resulted in browning of tissues. Sodium hypochlorite was not very effective in surface sterilization of the explants.

Table 1

Influence of auxins and cytokinins on time taken for callus initiation in GI tagged jasmines

Treatments	Number of days		
	Udupi mallige	Hadagali mallige	Mysore mallige
T_0: Basal MS media	0.00	0.00	0.00
T_1: 0.2 mg L^{-1} 2,4-D	10	11.6	9
T_2: 0.4 mg L^{-1} 2,4-D	10	11.6	9
T_3: 2.0 mg L^{-1} BA +0.25 mg L^{-1} NAA	12	13.7	11.4
T_4: 4.0 mg L^{-1} BA +0.5 mg L^{-1} NAA	13	13	11
T_5: 6.0 mg L^{-1} BA +0.25 mg L^{-1} NAA	13	13.9	11
T_6: 2.0 mg L^{-1} BA +0.5 mg L^{-1} NAA	13	13	12
T_7: 4.0 mg L^{-1} BA +0.25 mg L^{-1} NAA	13	15	11
T_8: 6.0 mg L^{-1} BA +0.5 mg L^{-1} NAA	13	14	12
T_9: 2.0 mg L^{-1} Kn +0.25 mg L^{-1} NAA	13	15	11
T_{10}: 4.0 mg L^{-1} Kn +0.5 mg L^{-1} NAA	13	14	11
T_{11}: 6.0 mg L^{-1} Kn +0.25 mg L^{-1} NAA	13	14	12
T_{12}: 2.0 mg L^{-1} Kn +0.5 mg L^{-1} NAA	12	14	12.9
T_{13}: 4.0 mg L^{-1} Kn +0.25 mg L^{-1} NAA	13	14	13
T_{14}: 6.0 mg L^{-1} Kn +0.5 mg L^{-1} NAA	13	14	13
T_{15}: 2.0 mg L^{-1} BA+0.2 mg L^{-1} 2,4-D	12	13.8	12
T_{16}: 4.0 mg L-1 BA+0.4 mg L^{-1} 2,4-D	14	12	11
T_{17}: 6.0 mg L^{-1} BA+0.2 mg L^{-1} 2,4-D	13.6	13	12
T_{18}: 2.0 mg L^{-1} BA+0.4 mg L^{-1} 2,4-D	11	12	11
T_{19}: 4.0 mg L^{-1} BA+0.2 mg L^{-1} 2,4-D	11	13.6	12
T_{20}: 6.0 mg L^{-1} BA+0.4 mg L^{-1} 2,4-D	11	14	13
T_{21}: 2.0 mg L^{-1} Kn+0.2 mg L^{-1} 2,4 D	12	13	12
T_{22}: 4.0 mg L^{-1} Kn+0.4 mg L^{-1} 2,4 D	12	13	13
T_{23}: 6.0 mg L^{-1} Kn+0.2 mg L^{-1} 2,4 D	13	14	12
T_{24}: 2.0 mg L^{-1} Kn+0.4 mg L^{-1} 2,4 D	13	14	12
T_{25}: 4.0 mg L^{-1} Kn+0.2 mg L^{-1} 2,4 D	13	13	13
T_{26}: 6.0 mg L^{-1} Kn+0.4 mg L^{-1} 2,4 D	13	13	13
p=0.05	*	*	*
SEm±	0.03	0.07	0.03
CD	0.08	0.19	0.10

BA: 6-Benzy laminopurine, NAA: Naphthalene acetic acid, 2,4-D: 2,4-*Dichlorophenoxyacetic acid*, Kn: Kinetin

Influence of Growth Regulators on Time taken for Callus Initiation

Callus initiation occurred from cut end of leaf sections in all the cultivars *viz.,* Mysore Mallige (*J. azoricum*), Udupi mallige (*J. sambac*) and Hadagali mallige (*J. auriculatum*) on MS medium supplemented with any auxin or cytokinin either singly or in combination. All the three species of jasmines showed early callus initiation on the medium supplemented with 2,4-D alone at lower concentrations (Table 1). However, time taken for callus initiation was more when medium was supplemented with BA/Kinetin + NAA and BA/ Kinetin + 2,4-D. MS medium supplemented with

TABLE 2

Influence of growth regulators on callus intensity of GI tagged jasmines at 45 days of culturing

Treatments	Udupi mallige	Hadagali mallige	Mysore mallige
T_0:Basal MS medium	0.00	0.00	0.00
T_1:0.2mgL^{-1}2,4-D	3.30	2.60	2.60
T_2:0.4mgL^{-1}2,4-D	4.00	3.00	3.00
T_3:2.0mgL^{-1}BA+0.25mgL^{-1}NAA	1.50	1.40	1.50
T_4:4.0mgL^{-1}BA+0.5mgL^{-1}NAA	2.00	2.00	2.00
T_5:6.0mgL^{-1}BA+0.25mgL^{-1}NAA	1.00	2.00	2.00
T_6:2.0mgL^{-1}BA+0.5mgL^{-1}NAA	2.00	1.40	1.40
T_7:4.0mgL^{-1}BA+0.25mgL^{-1}NAA	2.00	1.90	1.90
T_8:6.0mgL^{-1}BA+0.5mgL^{-1}NAA	1.70	1.60	1.60
T_9:2.0mgL^{-1}Kn+0.25mgL^{-1}NAA	1.60	1.00	1.40
T_{10}:4.0mgL^{-1}Kn+0.5mgL^{-1}NAA	1.50	1.00	2.00
T_{11}:6.0mgL^{-1}Kn+0.25mgL^{-1}NAA	2.00	1.00	1.30
T_{12}:2.0mgL^{-1}Kn+0.5mgL^{-1}NAA	2.00	1.20	1.40
T_{13}:4.0mgL^{-1}Kn+0.25mgL^{-1}NAA	1.00	1.40	1.50
T_{14}:6.0mgL^{-1}Kn+0.5mgL^{-1}NAA	1.90	1.00	1.00
T_{15}:2.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.40	2.00	2.00
T_{16}:4.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.30	2.00	2.20
T_{17}:6.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.00	2.00	2.30
T_{18}:2.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.60	2.20	2.60
T_{19}:4.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.80	2.00	2.00
T_{20}:6.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	2.70	2.20	2.40
T_{21}:2.0mgL^{-1}Kn+0.2mgL^{-1}2,4D	1.90	1.70	1.70
T_{22}:4.0mgL^{-1}Kn+0.4mgL^{-1}2,4D	1.90	1.60	1.60
T_{23}:6.0mgL^{-1}Kn+0.2mgL^{-1}2,4D	2.00	2.00	2.00
T_{24}:2.0mgL^{-1}Kn+0.4mgL^{-1}2,4D	1.20	1.40	1.50
T_{25}:4.0mgL^{-1}Kn+0.2mgL^{-1}2,4D	2.80	1.40	1.40
T_{26}:6.0mgL^{-1}Kn+0.4mgL^{-1}2,4D	2.50	1.00	1.30
p=0.05	*	*	*
SEm±	0.11	0.13	0.14
CD	0.31	0.36	0.40

Intensity of callus: Nil -0; Poor-1; Moderate-2; Good-3; Verygood-4and 5-Excellent

2,4-D showed callus initiation after nine days in Mysore Mallige followed by Udupi mallige and Hadagali mallige. As per the findings of Hurakadle *et al.* (2011) in *Jasminum malabaricum* Wight, a delayed response was noticed when leaf sections showed callus initiation after 23 days of culturing on MS medium supplemented with 2,4- D (2.0 mg L-1), BAP (2.5 mg L^{-1}), NAA (0.01 mg L^{-1}) and TDZ (0.5 mg L^{-1}). Inclusion of 2, 4-D has helped callus induction in leaf explants of *Jasminum grandiflorum* on MS medium supplemented with 2, 4-D (1.25 ppm) (Gomathi *et al.,* 2007).

Influence of Growth Regulators on Per cent Response of Explants

Explants of all the three species showed cent per cent callusing on MS medium supplemented with different hormonal combinations, while the response was very poor on basal MS medium with only 10 per cent of leaf sections showing callus initiation. Biswal *et al.,*

TABLE 3

Influence of growth regulators on nature of callus in GI tagged jasmines after 45 days

Treatments	Udupi mallige		Hadagali mallige		Mysore mallige	
	Colour	Texture	Colour	Texture	Colour	Texture
T_0:Basal MS medium	No callus	No callus	No callus	No callus	No callus	No callus
T_1:0.2mgL^{-1}2,4-D T_2:0.4mgL^{-1}2,4-D	White	Friable	Light brown	Friable	Creamish green	Friable
T_3:2.0mgL^{-1}BA+0.25mgL^{-1}NAA T_4:4.0mgL^{-1}BA+0.5mgL^{-1}NAA T_5:6.0mgL^{-1}BA+0.25mgL^{-1}NAA T_6:2.0mgL^{-1}BA+0.5mgL^{-1}NAA T_7:4.0mgL^{-1}BA+0.25mgL^{-1}NAA T_8:6.0mgL^{-1}BA+0.5mgL^{-1}NAA T_9:2.0mgL^{-1}Kn+0.25mgL^{-1}NAA T_{10}:4.0mgL^{-1}Kn+0.5mgL^{-1}NAA T_{11}:6.0mgL^{-1}Kn+0.25mgL^{-1}NAA T_{12}:2.0mgL^{-1}Kn+0.5mgL^{-1}NAA T_{13}:4.0mgL^{-1}Kn+0.25mgL^{-1}NAA T_{14}:6.0mgL^{-1}Kn+0.5mgL^{-1}NAA	Light green	Friable	Creamish green to Light green	Friable	Light green	Friable
T_{15}:2.0mgL^{-1}BA+0.2mgL^{-1}2,4-D T_{16}:4.0mgL^{-1}BA+0.2mgL^{-1}2,4-D T_{17}:6.0mgL^{-1}BA+0.2mgL^{-1}2,4-D T_{18}:2.0mgL^{-1}BA+0.2mgL^{-1}2,4-D T_{19}:4.0mgL^{-1}BA+0.2mgL^{-1}2,4-D T_{20}:6.0mgL^{-1}BA+0.2mgL^{-1}2,4-D	White to Light green	Friable	Brownish green	Friable	Creamish green	Friable
T_{21}:2.0mgL^{-1}Kn+0.2mgL^{-1}2,4D	White		Creamish green			
T_{22}:4.0mgL^{-1}Kn+0.4mgL^{-1}2,4D T_{23}:6.0mgL^{-1}Kn+0.2mgL^{-1}2,4D			Brownish green			
T_{24}:2.0mgL^{-1}Kn+0.4mgL^{-1}2,4D T_{25}:4.0mgL^{-1}Kn+0.2mgL^{-1}2,4D			Light green			
T_{26}:6.0mgL^{-1}Kn+0.4mgL^{-1}2,4D			Creamish green			

Fig. 2 : The colour and texture of callus produced from leaf after 45 days of culturing

a : White coloured friable callus from leaf explant on MS medium with 0.4 mg L^{-1} 2,4-D in Udupi mallige
b : Light brown coloured friable callus from leaf explant on MS medium with 0.4 mg L^{-1} 2,4-D in Hadagali mallige
c : Creamish green coloured friable callus from leaf explant on MS medium with 0.4 mg L^{-1} 2,4-D in Mysore mallige

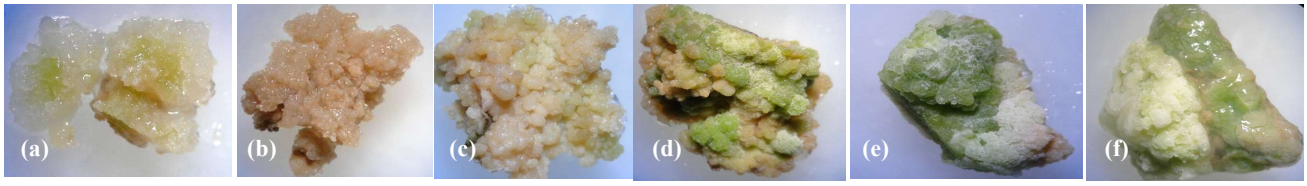

Fig. 3 : Callus produced from leaf sections after 60 days of culturing

3a : White friable translucent mucilaginous 'non-embryogenic' callus on MS medium with 2.0 mg L^{-1} BA in Udupi mallige
3b : Brownish friable 'non-embryogenic' callus on MS medium with 6.0 mg L-1 BA+0.5 mg L-1 NAA in Udupi mallige
3c : Brownish green nodular embryogenic friable callus on MS medium with 2.0mg L^{-1} Kn+0.25mg L^{-1} NAA in Hadagali mallige
3d : Light green nodular embryogenicfriable callus on MS medium with6.0 mgL^{-1} BA in Hadagali mallige
3e : Green nodular embryogenic callus onMS medium with 2.0 mg L^{-1} BA in Mysore mallige
3f : Whitish mass of callus on greenish nodular embryogenic callus on MS medium with 4.0 mg L^{-1} Kn+0.25 mg L^{-1} NAA in Mysore mallige

(2016), however, has reported that only 86.7 per cent leaf explants showed callus initiation on MS medium supplemented with BAP (3.0mg/l) and IAA (2.0mg/l) in *J. sambac* Aiton. Further, higher concentration of cytokinins or auxins did not promote callus initiation. Davallo *et al.* (2014) also reported only 68 per cent leaf section explants of *J. sambac* showing callus initiation when cultured on MS medium supplemented with 2,4-D at 0.3mg L^{-1}. In our study, callus initiation was achieved in the presence of 2,4-D alone (0.2 mg L^{-1} to 0.4 mg L^{-1}) in MS medium or in combination with BA (2.0 mg L^{-1} to 6.0 mg L^{-1}), kinetin (2.0 mg L^{-1} to 6.0 mg L^{-1}) and NAA (0.25 mg L^{-1} to 0.5 mg L^{-1}) in all the three jasmine species.

Influence of Growth Regulators on Callus Intensity

Very high intensity of callus was noticed in leaf sections after 45 days of culturing on MS medium supplemented with 2, 4-D alone while the intensity was significantly low in other combinations and concentrations of growth regulators (Table 2). Also, in the absence of any growth regulator, the basal MS medium showed no callusing. MS medium supplemented with 0.4 mg L^{-1} of 2,4-D resulted in maximum callus intensity in all the three species of Jasmine. The callus intensity was maximum in Udupi mallige (*J. sambac*) than in Hadagali mallige (*J. auriculatum*) or Mysore mallige (*J. azoricum*) (Table 2). Shubham *et. al.* (2019) also noticed that leaf explants of *J. sambac* produce callus when cultured on MS medium supplemented with a higher concentration of 2.0 mgL^{-1} each of 2,4-D and BAP.

Influence of Growth Regulators on Nature of Callus

Nature of callus was influenced by the kind and concentration of growth regulators. The colour and texture of callus produced from leaf sections of Udupi

mallige was white or light green to green in colour (Fig. 2). Hadagali mallige leaf sections produced light green/creamish green to brownish green callus, while Mysore mallige leaf sections produced creamish green and light green callus. Texture of callus was friable in all the three cultivars until 45 days (Table 3). However, the colour and texture changed after 60 days. Callus of Udupi mallige cultures was friable, translucent, mucilaginous and appeared to be non-embryogenic. White/brownish callus was observed in the presence of BA kinetin, and NAA on MS medium (Fig. 3a and b). Nodular, embryogenic, friable, light green/ brownish callus formation was noticed from leaf sections of Hadagali mallige (Fig. 3c and d). However, the callus was compact in Mysore mallige leaf sections (Fig.3e and f). Reza *et al.* (2014) carried out double staining test to identify embryogenic cells in callus and embryogenic callus was detected in all callus formed in MS media supplemented with different concentrations of 2,4-D. But the entire callus did not show any development of organogenesis. The results of our study are similar to the report by Reza *et. al.* (2014). Regeneration studies carried by Dhanaraj *et al.* (1997) from the callus revealed that calli turned green on MS or Miller's medium with increased BA concentrations, but more than 10 mgL^{-1} resulted in browning of callus. Maximum percentage of cultures showing callus proliferation from leaf explants was observed by Palai, (2017) in the medium containing MS basal salts supplemented with 3.0 mg/l BAP and 2.0 mg/l IAA in leaf explants. White and friable callus was obtained in the medium containing MS basal salts supplemented with 2.0 mgL^{-1} l BAP, 2.0 mgL^{-1} Kn and 2.0 mgL^{-1} IAA from meristem explants. However, no regeneration was recorded.

Influence of Growth Regulators on Shoot and Root Regeneration

The callus was subcultured on MS medium supplemented with different concentrations and combinations of auxins and cytokinins. However, regeneration was not achieved in any of these combinations. Instead, production of friable callus continued in all the concentration of growth regulators tested even after 60 days. The growth regulators tested in this study, their concentration and combination do not support regeneration of multiple shoots in any of the species.

REFERENCES

BISWAL, M., PALAI, S. K., MISHRA, P., CHHURIA, S. AND SAHU, P., 2016, Standardization of protocol for shoot multiplication of jasmine (*Jasminum sambac* (L) Aiton). *Inter. J. Farm Sci.,* **6** (3) : 111 - 118.

DAVALLO, B. L., KAZEMITABAR, S. K., GHOLIPOUR, A. AND GHANBARI, S., 2014, Callus induction on *Jasminum sambac* L. by 2, 4- dichlorophenoxy acetic acid hormone. *Inter. J. Bio. Sci.,* **5** (2) : 114 - 118.

DHANARAJ, A. L., 1997, *In vitro* studies in Udupi mallige (*Jasminum sambac*). *M.Sc. Thesis* (Pub.), Univ. Agric. Sci., Bangalore.

GOMATHI, K. S., SAMBANDAMURTHY, S., SADASIVAM, S, RAMASAMY, N. M. AND RAJMOHAN, K., 2007, *In vitro* culture and biochemical analysis of *Jasminum* sp. ICAE National symposium on biotechnological interventions for improvement of horticultural crops : issues and strategies. *NIPA publication*, pp. : 149 - 153.

MURASHIGE, T. AND SKOOG, F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture, *Plant Physiol.,* **15** : 473 - 497.

NIRMALA, K. S., CHAMPA, B. V. AND MAHABALESHWAR, H., 2017, Morphological diversity of jasmine cultivars and wild species in Karnataka. *Mysore J. Agric. Sci.,* **51** (4) : 822 - 831.

PALAI, K. Y., MADHURI, G. AND BISWAL, M., 2017, *In vitro* propagation of jasmine. *Inter. J. Farm. Sci.,* **7** (1) : 185 - 189.

REZA, F., KAMALUDIN, R., ROSNA, M. T. AND KHAIRUL, A. I., 2014, Callogenesis and plant regeneration of *Jasminum sambac* L. (Jasmine). *Inter. Conference on Biol, Environ. Food Eng.,* (BEFE), August 4-5, Bali (Indonesia), pp. : 1 - 3.

SHUBHAM, D., JAGTAP, SHREEDHAR, S., OTARI, AINAPURE, M. D. R. AND NAGARAJA, T. G., 2019, *In vitro* study of effect of sodium azide on the callus of jasmine. *Inter. J. Innovative Sci. and Res. Tec.,* **4** (2) : 146 - 151.