

Rapid multiplication of Geographical Indication (GI) tagged Mysore Mallige (*Jasminum azoricum*L.) using single node through *in vitro* culture

ABSTRACT

Aims: The present study was undertaken to rapid multiplication of (GI) tagged Mysore Mallige (*Jasminum azoricum*L.) using single node through *in vitro* culture

Study design: The present study follow Completely Randomized Design (CRD)

Place and Duration of Study: The study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru, from 2020 to 21.

Methodology: Single node cutting from the young shoots of field growing plants were used asexplants to conduct the experiment. The explants were sterilized and placed on MS mediums supplemented with different concentrations and combinations of growth regulators, namely BAP and Kinetin

Results: Among the different combinations of growth regulators, the combination of BAP 1.5 mg L⁻¹ and Kin 1.5 mg L⁻¹ produced maximum number of shoots from single node. Very low intensity of callus formation was observed from the cut ends of single node explants that were cultured on half strength MS medium consisting of 1.5 mg L⁻¹ BAP + 0.0 mg L⁻¹ Kin and 1.5 mg L⁻¹ BAP + 1.0 mg L⁻¹ Kin.

Conclusion: The use of growth regulators such as BAP and KIN is reliable for shoot regeneration even when the field explants are used.

Keywords: BAP, KIN, single node cuttings, *Jasminum azoricum*

1. INTRODUCTION

“Jasmine (*Jasminum* spp.) is a climbing, trailing and erect flowering shrub. A native of tropical and subtropical region and Indo-Malayan region being its center of origin, the diversity existing in jasmine is enormous in India. The distribution of *Jasminum* genus is pan-tropical but a large number of species are centered around India, China and Malaya. Belonging to family Oleaceae, genus *Jasminum* comprises of more than 200 species of which many are synonyms and 90 are true in existence” [1-3].

Jasminum species show enormous morphological variations in their vegetative and floral characters. Such morphological variations among 48 genotypes of Jasmines have been recorded by [6]. 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, Hadagalimallige and Mysore mallige. Udupi mallige is a cultivar of *Jasminum sambac* and Hadagalimallige 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.

“The traders in the Mysuru market mislead the buyers by addressing the Dundu Mallige from Tamil Nadu area as Mysore Mallige. This species was listed under endangered species, and there is no information available on area and production of Mysore Mallige. The crop is vegetatively propagated hindering the largescale multiplication. Hence, there is an urgent need for the developing rapid multiplication technique to meet the demand as well as to maintain them for further crop improvement programme”. [3] The present study aimed at developing a propagation technique for rapid multiplication of Mysoremallige.

2. MATERIAL AND METHODS

The study was conducted at the Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru, from 2020 to 21. The media used in the in vitro study were full-strength solid MS (Murashige and Skoog, 1962) media for both shoot and root initiation studies.

The field explants (nodal segments) were subjected to running tap water for 20 minutes and further sterilization was carried out under aseptic conditions. The explants were treated with 0.5 per cent bavistin followed by three sterile water washes (5 minutes each). The explants were treated with 0.3 per cent of mercuric chloride (HgCl_2) for 4 minutes, washed with sterile distilled water three times (5 minutes each), and transferred to 70 per cent alcohol for 1 minute. The explants were then cut to a length of 1 cm, comprising one node each, and transferred to a solution containing 0.1 per cent streptomycin for half an hour. Then the explants were immediately placed on the medium. The explants were placed on medium consisting of MS medium [5] salts supplemented with 3 per cent (w/v) sucrose, and the media were solidified by 0.6 per cent agar with different concentrations of growth regulators, viz., cytokinin with concentration of 0.5, 1.0 and 1.5 mgL^{-1} BAP (6 Benzyl amino purine), Kinetin (KIN) with concentration of 0.5, 1.0 and 1.5 concentration for shoot proliferation.

The culture bottles were maintained in a growth room at a temperature of 24 ± 2 °C. A light intensity of 2000 lx was provided using white fluorescence tubes for eight hours of light and 16 h of dark chamber was fumigated with potassium dichromate and formaldehyde weekly.

STATISTICAL ANALYSIS

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

3. RESULTS AND DISCUSSION

Establishment of cultures from single node cuttings

The single node cuttings measuring 1-2 cm with a pair of leaves having buds in their axils were cultured as explants on half strength MS medium in the presence of different concentrations of auxins and cytokinin.

Days taken for shoot initiation

With respect to days taken for shoot initiation, significant differences were noticed among the different culture medium used for shoot initiation.

The days taken for shoot initiation were observed early in combination of BAP and Kinetin at 1.5 mg L^{-1} (18 days) among different concentrations of growth hormone used (Table 1). The early shoot initiation was followed by 1 mg L^{-1} of BAP and Kinetin (20.20 days). These observations were also reported previously by Biswa *et al.*, (2016) in a study reported that the kind and concentration of growth regulator on full strength MS medium have influenced the number of days required for bud break in shoot apices/stem explants which varied between 25.47 days in *J. sambac*. However, in the present study on *J. azoricum*, when single node cuttings were used as explant, the bud break was noticed as early as 18 days on half MS medium containing 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin.

Number of Multiple shoots proliferated

The effect of different concentrations and combination of BAP and Kin on number of shoots per explant are presented in the (Table 1). There was a significant difference noticed between the combination of BAP and Kin on number of shoots per explant. The result showed that, out of 16 different combinations of 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin showed its superiority over other combination by producing maximum number of shoots per explant (2.60). Except control and 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} Kin, 1.0 mg L^{-1} BAP + 1.5 mg L^{-1} Kin, 1.5 mg L^{-1} BAP + 0.0 mg L^{-1} Kin, 1.5 mg L^{-1} BAP + 1.0 mg L^{-1} Kin all the other treatments showed on par results. Best result was noticed in the treatment consisting of 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin wherein 2.60 shoots were recorded per single node cutting under in vitro conditions. In present study the maximum shoot multiplication rate of 2.60 is noticed at highest concentration of 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin which suggests that this concentration is optimum for production of maximum number of multiple shoots. These observations were also reported by [2] Roy (2008) stated that explants finding effect of BAP and Kin and Ad with increasing BAP concentration from 2-3 mg L^{-1} they have observed that there is decline in rate of shoot multiplication in *J. Sambac* [10]. They are in opinion that such response may be due to ionic concentration and balance between different nutrients in the culture medium which is crucial for optimization of shoot multiplication of the plant [8].

Mean shoot length at 45 days

The present study indicated that the shoot length was maximum in half strength MS medium (Table 1) containing 1.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin of 5.06 cm on par results recorded in Kinetin alone treated cutting at 1 mg L^{-1}

1(4.24), BAP alone at 0.5 mg L^{-1} (3.84) and Kinetin 0.5 mg L^{-1} (3.16). The deviation in response noticed with variation in growth regulator concentration apart from the treatment with the equal ratio of BA and Kin that produced more shoot length may be due to the endogenous hormones/ due to presence of already developed axillary buds. Salim. (2016) studied effect of different concentration of BA on nodal cutting of *J. azoricum*. Maximum shoot number of 2.5 recorded at 2.0 mg L^{-1} BAP. However, he has further observed that BA had no influence on shoot length and number of nodes as the basal medium recorded best results. However, in contrast to this these observations are in line with the results of the study conducted by [9], who reported maximum shoot length in BA 1.0 mg L^{-1} which gave 1.82 cm compared to other treatments.

Table 1: Influence of BAP and Kinetin on number of days taken for shoot initiation, number of shoots proliferated and mean shoot length at 45 days from single node cuttings of Mysore mallige (*Jasminum azoricum* L.) under *in vitro* culture.

Treatments	Number of days taken for shoot initiation	Number of shoots Proliferated	Mean shoot length at 45 days
T ₁ : Basal medium (Control)	23.60	0.80	0.36
T ₂ : 0.0 mg L^{-1} BAP + 0.5 mg L^{-1} Kin	20.60	2.00	3.16
T ₃ : 0.0 mg L^{-1} BAP + 1.0 mg L^{-1} Kin	21.00	2.20	4.24
T ₄ : 0.0 mg L^{-1} BAP + 1.5 mg L^{-1} Kin	20.60	2.20	2.50
T ₅ : 0.5 mg L^{-1} BAP + 0.0 mg L^{-1} Kin	20.40	2.20	3.84
T ₆ : 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} Kin	20.60	1.80	1.16
T ₇ : 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} Kin	22.80	2.00	1.44
T ₈ : 0.5 mg L^{-1} BAP + 1.5 mg L^{-1} Kin	21.00	1.80	1.16
T ₉ : 1.0 mg L^{-1} BAP + 0.0 mg L^{-1} Kin	20.80	2.20	1.98
T ₁₀ : 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} Kin	20.40	1.60	1.42
T ₁₁ : 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} Kin	20.20	1.20	0.84
T ₁₂ : 1.0 mg L^{-1} BAP + 1.5 mg L^{-1} Kin	20.80	1.20	1.38
T ₁₃ : 1.5 mg L^{-1} BAP + 0.0 mg L^{-1} Kin	20.40	1.20	1.52
T ₁₄ : 1.5 mg L^{-1} BAP + 0.5 mg L^{-1} Kin	21.00	2.20	2.20
T ₁₅ : 1.5 mg L^{-1} BAP + 1.0 mg L^{-1} Kin	20.40	1.20	1.14

T ₁₆ : 1.5 mg L ⁻¹ BAP+1.5 mg L ⁻¹ Kin	18.00	2.60	5.06
P=0.01	*	*	*
SE.m±	0.40	0.29	0.59
CD	1.50	1.07	2.22

Note :BA-6-Benzylaminopurine,Kin-Kinetin

SE.m±-Standard error mean,CD-Critical difference,*Significant at 1%.



Figure1 : General view of Mysore Mallige (*J. azoricum* L)

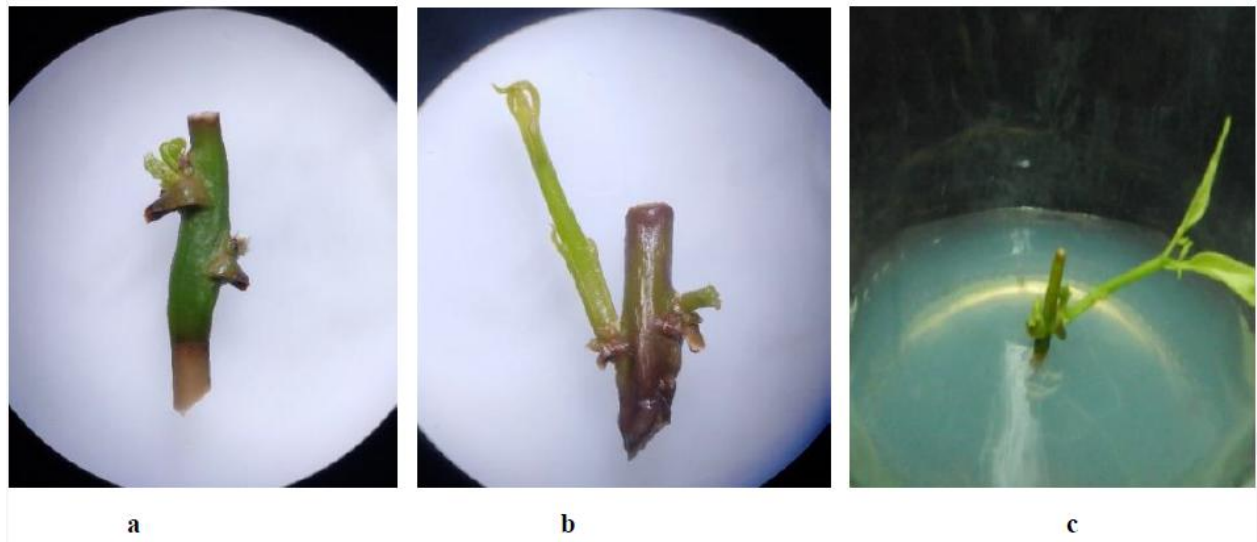


Figure2: Sprouting of axillary bud, development of shoot and formation of leaves under *in vitro* condition
 (a) Bulging of axillary bud (b) Initiation of shoot from sprouted axillary bud (c) Development of leaves



Figure3 :Sprouting of axillary buds from single node cuttings and formation of multiple shoots in *J. azoricum*L under *in vitro*condition

Influence of BAP and Kinetin on callus formation from single node cuttings of Mysore Mallige (*Jasminum azoricum* L.) under *in vitro* culture

It was observed that there was very low intensity of callus formation from the cut ends of single node explants that were cultured on half strength MS Medium consisting of 1.5mgL^{-1} BAP + 0.0mgL^{-1} Kin and 1.5mgL^{-1} BAP + 1.0mgL^{-1} Kin. Such formation of callus as very large clumps were noticed when leaf explants of *J. azoricum*, *J. sambac*, *J. auriculatum* were cultured on full strength MS medium containing 0.4mgL^{-1} and 2,4-D [7].

Conclusion

The present study reveals that the different growth regulators with combination BAP and Kin at higher concentration of 1.5mgL^{-1} showed best response for multiple shoot formation and shoot length.

Very low intensity of callus formation was observed from the cut ends of single node explants that were cultured on half strength MS medium consisting of 1.5mgL^{-1} BAP + 0.0mgL^{-1} Kin and 1.5mgL^{-1} BAP + 1.0mgL^{-1} Kin. The cytokinin in combination (BAP and Kin) at 1.5mgL^{-1} in half MS medium produced more number of shoots and gave best result.

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