

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

### ABSTRACT

Mysore Mallige (*Jasminum azoricum* L. syn. *J. trifoliatum* Moench), a popular jasmine of Mysuru district of Karnataka State, India with Geographical Indication registration (GI) possess unique fragrance. Conservation of this unique cultivar needs immediate attention as it is endangered and area under this crop is decreasing. The present study aimed at rapid multiplication of this threatened species under *in vitro* culture. Single node cuttings were cultured on half MS medium supplemented with BAP and KIN (0.0 -1.5 mg L<sup>-1</sup>) for multiple shoot induction. Early shoot initiation, higher multiple shoot formation and maximum shoot length was observed in half MS medium supplemented with BAP and KIN at 1.5 mg L<sup>-1</sup> each.

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

### INTRODUCTION

Jasmine (*Jasminum* spp.) is a climbing, trailing and erect flowering shrub. It is widely grown in warm parts of Southern Asia, Europe, Africa and the Pacific regions. 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 [1] Belonging to family Oleaceae, genus *Jasminum* comprises of more than 200 species [2] of which many are synonyms and 90 are true in existence [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, 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.

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

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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. The present study aimed at developing a propagation technique for rapid multiplication of Mysore mallige.

## MATERIAL AND METHODS

### Study Location

The experiment was conducted in Plant Tissue Culture Laboratory, Department of Horticulture, University of Agricultural Sciences, Bangalore.

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### Explant Collection and Surface Sterilization

The immature light green leaves were collected from the healthy mother plants and washed in running tap water for 20 minutes followed by soaking in two per cent of tween 20 soap solution for 15 minutes and rinsed in distilled water to reduce contamination. The explants were treated with 0.5 per cent Bavistin followed by 3 sterile water washes (5 minutes each). This was followed by treating the explants with 0.3 per cent of mercuric chloride ( $\text{HgCl}_2$ ) for 4 minutes and washed with sterile distilled water for 3 times (5 minutes each), the explants were transferred to 70 per cent alcohol for a period of 1 minute. The explants were then cut to length of 1cm comprising of one node each, which were transferred to solution containing 0.1 per cent Streptomycin for half an hour. Excess moisture adhering to the explants were drained with the help of sterile filter paper inside the laminar airflow chamber. Then the explants were immediately placed on the medium.

The explants were cultured on MS medium [5] containing 3 per cent sucrose solidified with 8 per cent agar having different concentrations of growth regulators viz., cytokinin with concentration of 0.5, 1.0 and  $1.5\text{mgL}^{-1}$  BAP (6 Benzyl amino purine), KIN (Kinetin) with concentration of 0.5, 1.0 and 1.5 concentration for shoot proliferation.

### STATISTICAL ANALYSIS

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

## RESULTS AND DISCUSSION

### Establishment of cultures from single node cuttings

The single node cuttings measuring 1-2cm 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.

### Day taken for shoot initiation

With respect to day taken for shoot initiation, significant difference 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  $1.5 \text{ mg L}^{-1}$  ( $1.5 \text{ mg L}^{-1}$ ) among different concentrations of growth hormone used (Table 1). The early shoot initiation was followed by  $1 \text{ mg L}^{-1}$  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  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  Kin.

### Number of Multiple shoots proliferated

The effect of different concentrations and combination of BAP and Kin on number of shoots per explant are represented in the (Table 1). There was a significant difference noticed between the combination of BAP and Kin on number of shoots per explant. The results showed that, out of 16 different combinations of  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  Kin showed its superiority over other combination by producing maximum number of shoots per explant (2.60). Except control and  $1.0 \text{ mg L}^{-1}$  BAP +  $1.0 \text{ mg L}^{-1}$  Kin,  $1.0 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  Kin,  $1.5 \text{ mg L}^{-1}$  BAP +  $0.0 \text{ mg L}^{-1}$  Kin,  $1.5 \text{ mg L}^{-1}$  BAP +  $1.0 \text{ mg L}^{-1}$  Kin all the other treatments showed on par results. Best result was noticed in the treatment consisting of  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  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  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  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  $\text{mg L}^{-1}$  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  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  Kin of 5.06 cm on par results recorded in Kinetin alone treated cutting at  $1 \text{ mg L}^{-1}$  (4.24), BAP alone at  $0.5 \text{ mg L}^{-1}$  (3.84) and Kinetin  $0.5 \text{ mg L}^{-1}$  (3.16). The deviation in response noticed with variation in growth regulator concentration apart from the treatment with 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  $2.0 \text{ mg L}^{-1}$  BAP. However, he has further observed that BA had no influence on shoot length and number of nodes as 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  $1.0 \text{ mg L}^{-1}$  which gave 1.82 cm compared to other treatments.

## 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 from 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  $1.5 \text{ mg L}^{-1}$  BAP +  $0.0 \text{ mg L}^{-1}$  Kin and  $1.5 \text{ mg L}^{-1}$  BAP +  $1.0 \text{ mg L}^{-1}$  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  $0.4 \text{ mg L}^{-1}$  and 2,4-D [7].

### Conclusion

The present study reveals that the different growth regulators with combination BAP and Kin at higher concentration of  $1.5 \text{ mg L}^{-1}$  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  $1.5 \text{ mg L}^{-1}$  BAP +  $0.0 \text{ mg L}^{-1}$  Kin and  $1.5 \text{ mg L}^{-1}$  BAP +  $1.0 \text{ mg L}^{-1}$  Kin. The cytokinin in combination (BAP and Kin) at  $1.5 \text{ mg L}^{-1}$  in half MS medium produced more number of shoots and gave best result.

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**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 <sub>1</sub> : Basal medium (Control)	23.60	0.80	0.36
T <sub>2</sub> : 0.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> Kin	20.60	2.00	3.16
T <sub>3</sub> : 0.0 mg L <sup>-1</sup> BAP + 1.0 mg L <sup>-1</sup> Kin	21.00	2.20	4.24
T <sub>4</sub> : 0.0 mg L <sup>-1</sup> BAP + 1.5 mg L <sup>-1</sup> Kin	20.60	2.20	2.50
T <sub>5</sub> : 0.5 mg L <sup>-1</sup> BAP + 0.0 mg L <sup>-1</sup> Kin	20.40	2.20	3.84
T <sub>6</sub> : 0.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> Kin	20.60	1.80	1.16
T <sub>7</sub> : 0.5 mg L <sup>-1</sup> BAP + 1.0 mg L <sup>-1</sup> Kin	22.80	2.00	1.44
T <sub>8</sub> : 0.5 mg L <sup>-1</sup> BAP + 1.5 mg L <sup>-1</sup> Kin	21.00	1.80	1.16
T <sub>9</sub> : 1.0 mg L <sup>-1</sup> BAP + 0.0 mg L <sup>-1</sup> Kin	20.80	2.20	1.98
T <sub>10</sub> : 1.0 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> Kin	20.40	1.60	1.42
T <sub>11</sub> : 1.0 mg L <sup>-1</sup> BAP + 1.0 mg L <sup>-1</sup> Kin	20.20	1.20	0.84
T <sub>12</sub> : 1.0 mg L <sup>-1</sup> BAP + 1.5 mg L <sup>-1</sup> Kin	20.80	1.20	1.38
T <sub>13</sub> : 1.5 mg L <sup>-1</sup> BAP + 0.0 mg L <sup>-1</sup> Kin	20.40	1.20	1.52
T <sub>14</sub> : 1.5 mg L <sup>-1</sup> BAP + 0.5 mg L <sup>-1</sup> Kin	21.00	2.20	2.20

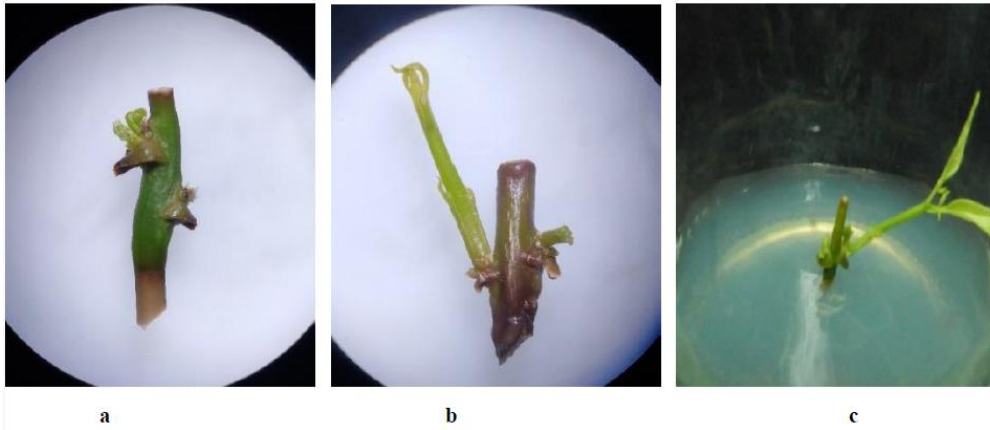
T <sub>15</sub> : 1.5 mg L <sup>-1</sup> BAP+ 1.0 mg L <sup>-1</sup> Kin	20.40	1.20	1.14
T <sub>16</sub> : 1.5 mg L <sup>-1</sup> BAP+1.5 mg L <sup>-1</sup> Kin	18.00	2.60	5.06
<b>P=0.01</b>	*	*	*
<b>SE.m±</b>	0.40	0.29	0.59
<b>CD</b>	1.50	1.07	2.22

Note : BA-6-Benzylaminopurine,Kin-Kinetin

SE.m±-Standarderrormean,CD-Criticaldifference,\*Significantat1%.



Plate 1 : General view of Mysore Mallige (*J. azoricum* L)



**Plate 2: 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



**Plate 3: Sprouting of axillary buds from single node cuttings and formation of multiple shoots in *J. azoricum* L under *in vitro* condition**