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: 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 mediumsupplemented 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
Conclusion: The use of growth regulators such as BAP and KIN is reliable for shoot regenerationeven 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[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 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.

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. Themedia 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 furthersterilization was carried out under aseptic conditions. The explants were treated with 0.5 per centbavistin followed by three sterile water washes (5 minutes each). The explants were treated with 0.3per of mercuric chloride ($HgCl_2$) for 4 minutes, washed with sterile distilled water three times (5minutes each), and transferred to 70 per cent alcohol for 1 minute. The explants were then cut to alength of 1 cm, comprising one node each, and transferred to a solution containing 0.1 per centstreptomycin 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 growthregulators, viz., cytokinin with concentration of 0.5, 1.0 and 1.5mgL^{-1}BAP (6 Benzyl amino purine), KIN (Kinetin) 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 0 C. A light intensityof 2000 lx was provided using white fluorescence tubes for eight hours of light and 16 h ofdarkchamber was fumigated with potassium dichromate and formaldehyde weekly.

STATISTICAL ANALYSIS

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

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3. RESULTS AND DISCUSSION

Establishmentofculturesfromsinglenodecuttings

Thesinglenodecuttingsmeasuring1-2cmwithapairofleaveshavingbudsintheiraxilwereculturedasexplantsonhalfstrengthMSmediuminthepresenceofdifferentconcentrationsofauxinsandcytokinin.

Daystakenforshootinitiation

Withrespecttodaystakenforshootinitiation,significantdifferencewerenoticedamongthedifferentculturemediumusedforshootinitiation. ThedaystakenforshootinitiationwereobservedearlyincombinationofBAPandKinetinat1.5mgL^{-1}(18days)amongdifferentconcentrationofgrowthhormoneused(Table 1).Theearlyshootinitiationwasfollowedby1mgL^{-1}ofBAPandKinetin(20.20days).These observations were also reported previously byBiswal*etal.,*(2016)inastudyreportedthatthekindandconcentrationofgrowthregulatorsonfullstrengthMSmediumhaveinfluencedthenumberofdaysrequiredforbudbreakinshootapices/stemexplantswhichvariedbetween25.47daysin*J.sambac*.However,inthepresentstudyon*J.azoricum*,whensinglenodecuttingswereusedasexplant,thebudbreakwasnoticedasearlyas18daysonhalfMSmediumcontaining1.5mgL^{-1}BAP+1.5mgL^{-1}Kin.

NumberofMultipleshootsproliferated

TheeffectofdifferentconcentrationsandcombinationofBAPandKinonnumberofshootsperexplantarepresentedinthe(Table1).TherewasasignificantdifferencenoticedbetweenthecombinationofBAPandKinonnumberofshootsperexplant.Theresultshowedthat,outof16differentcombinationsof1.5mgL^{-1}BAP+1.5mgL^{-1}Kinshoweditssuperiorityoverothercombinationbyproducingmaximum 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 +0.0mg 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 noticedinthetreatmentconsistingof1.5mgL^{-1}BAP+1.5mgL^{-1}Kinwherein2.60shootswererecordedpersinglenodecuttingunderinvitroconditions.Inpresentstudythemaximumshootmultiplicationrateof2.60isnoticedathighestconcentrationof1.5mg L^{-1}BAP+1.5mgL^{-1}Kinwhichsuggestthatthisconcentrationisoptimumforproductionofmaximumnumberofmultipleshoots.These observations were also reported by [2]Roy (2008) stated that explantsfindingeffectofBAPandKinandAdswithincreasingBAPconcentrationfrom2-3mgL^{-1}theyhaveobservedthatthereisdeclineinrateofshootmultiplicationin*J.Sambac*[10].Theyareinopinionthatsuchresponsemaybeduetoionicconcentrationandbalancebetweendifferentnutrientionsintheculturemediumwhichiscrucialforoptimizationofshootmultiplicationoftheplant[8].

Meanshootlengthat45days

ThepresentstudyindicatedthattheshootlengthwasmaximuminhalfstrengthMSmedium(Table 1) containing1.5mgL^{-1}BAP+1.5mgL^{-1}Kinof5.06cmonparresultsrecordedinKinetinalonetreatedcuttingat1mgL-

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1(4.24),BAPaloneat0.5mgL^{-1}(3.84)andKinetin0.5mgL-1(3.16).Thedeviationinresponsenoticedwithvariationingrowthregulatorconcentrationapartfromthe treatmentwithequalratioofBAandKinthatproducedmoreshootlengthmaybeduetotheendogenoushormones/duetopresenceofalreadydevelopedaxillarybuds.Salim.(2016)studiedeffectofdifferentconcentrationofBAonnodalcuttingof*J.azoricum*.Maximumshootnumberof2.5recordedat2.0mgL^{-1}BAP.However,hehasfurtherobservedthatBAhadnoinfluenceonshootlengthandnumberofnodesasthebasalmediumrecordedbestresults.However,incontrasttothistheseObservationareinlinewiththeresultsofthestudyconductedby[9],whoreportedmaximumshootlengthinBA1.0mgL^{-1}whichgave1.82cmcomparedtoothertreatments.

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 +0.0mg 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

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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±-Standarderrormean,CD-Criticaldifference,*Significantat1%.

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

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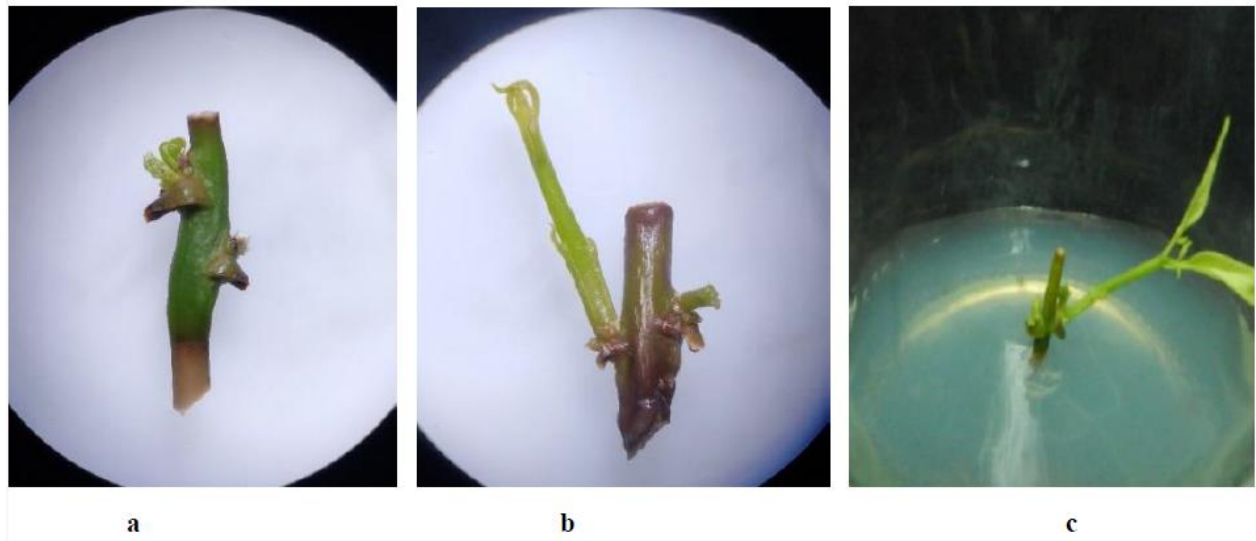

Figure2: Sprouting of axillary bud, development of shoot and formation of leaves under *in vitro* condition
(a) Bulging of axillary bud (b) Initiation of shoot from sprouted axillary bud (c) Development of leaves

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

InfluenceofBAPandKinetinoncallusformationfromsinglenodecuttingsofMysoreMallige(*Jasminumazoricum*L.)under*invitro*culture

ItwasobservedthattherewasverylowintensityofcallusformationfromthecutendsofsinglenodeexplantsthatwereculturedonhalfstrengthMSMediumconsistingof1.5mgL^{-1}BAP+0.0mgL^{-1}Kinand1.5mgL^{-1}BAP+1.0mgL^{-1}Kin.Suchformationofcallusasverylargeclumpswerenoticedwhenleafexplantsof*J.azoricum,J.sambac,J.auriculatum*wereculturedonfullstrengthMSmediumcontaining0.4mgL^{-1}and2,4-D[7].

Conclusion

The present study reveals thatthedifferentgrowthregulators with combinationBAPandKinathigherconcentrationof1.5mgL^{-1}showed bestresponseformultipleshootformationandshootlength. VerylowintensityofcallusformationwasobservedfromthecutendsofsinglenodeexplantsthatwereculturedonhalfstrengthMSmediumconsistingof1.5mgL^{-1}BAP+0.0mgL^{-1}Kinand1.5mgL^{-1}BAP+1.0mgL^{-1}Kin.Thecytokininincombination(BAPandKin)at 1.5mgL^{-1}inhalfMSmediumproducedmorenumberofshootsandgavebestresult.

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