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^{-1}) 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^{-1} 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

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

The experiment was conductedinPlantTissueCultureLaboratory, Department of Horticulture, University of Agricultural Sciences, Bangalore.

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 of mercuric chloride ($HgCl_2$) 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. Excessmoistureadheringtotheexplantsweredrainedwiththehelpofsterilefilterpaperinsidethelaminar airflowchamber.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 agarhaving different concentrations of growth regulators 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.

STATISTICAL ANALYSIS

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

RESULTS AND DISCUSSION

Establishmentofculturesfromsinglenodecuttings

Thesinglenodecuttingsmeasuring1-2cmwithapairofleaveshavingbudsintheiraxilwereculturedasexplantsonhalfstrengthMSmediuminthepresenceofdifferentconcentrationsofauxinsandcytokinin.

Daystakenforshootinitiation

Withrespecttodaystakenforshootinitiation,significantdifferencewerenoticedamongthedifferentculturemediumusedforshootinitiation.

Thedaystakenforshootinitiationwereobservedearlyincombinationof BAPandKinetinat1.5mgL^{-}

^{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

Theeffectofdifferentconcentrationsandcombinationof BAPandKinonnumberofshootsperexplantarepresentedinthe(Table1).Therewasasignificantdifferencenoticedbetweenthecombinationof BAPandKinonnumberofshootsperexplant.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}Kinwhichsuggestthatthisconcentrationisoptimumforproductionofmaximumnumberofmultiplesho ots.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-1(4.24),BAPaloneat0.5mgL^{-1}(3.84)andKinetin0.5mgL-1(3.16).ThedeviationinresponsenoticedwithvariationingrowthregulatorconcentrationapartfromthetreatmentwithequalratioofBAandKinthatproducedmoreshootlengthmaybeduetotheendogenoushormones/duetopresenceofalreadydevelopedaxillarybuds.Salim.(2016)studiedeffectofdifferentconcentrationofBAonnodalcuttingof*J.azoricum*.Maximumshootnumberof2.5recordedat2.0mgL^{-1}BAP.However,hehasfurtherobservedthatBAhadnoinfluenceonshootlengthandnumberofnodesasthebasalmediumrecordedbestresults.However,incontrasttothistheseObservationareinlinewiththeresultsofthestudyconductedby[9],whoreportedmaximumshootlengthinBA1.0mgL^{-1}whichgave1.82cmcomparedtoothertreatments.

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

ItwasobservedthatfromthetherewasverylowintensityofcallusformationfromthecutendsofsinglenodeexplantsthatwereculturedonhalfstrengthMSMediumconsistingof1.5mgL^{-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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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
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%.

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

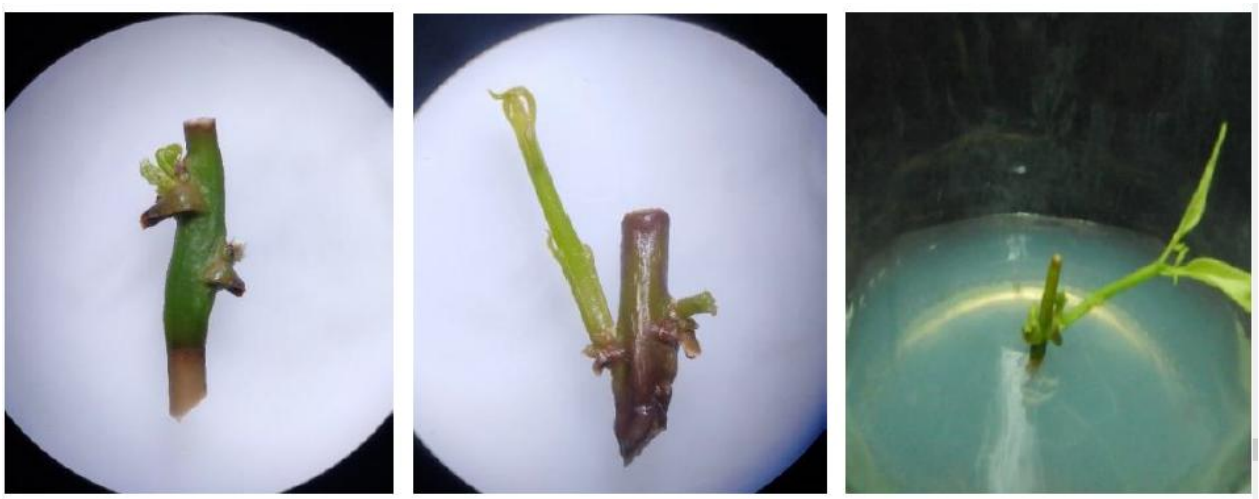

Plate 2: Sprouting of axillary bud, development of shoot and formation of leaves under *in vitro* condition
(a) Bulging of axillary bud (b) Initiation of shoot from sprouted axillary bud (c) Development of leaves

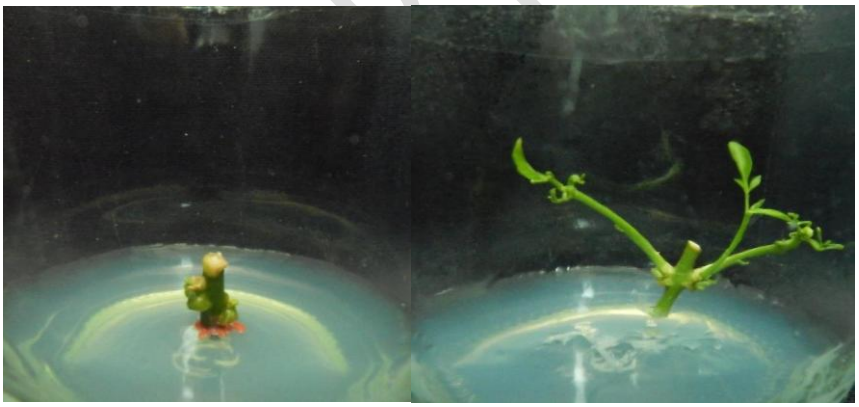

Plate 3 :Sprouting of axillary buds from single node cuttings and formation of multiple shoots in *J. azoricum* L under *in vitro*condition