Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2229

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **4 (1):** 291-295 (2016)

International Journal of Pure & Applied Bioscience

Research Article

OPEN ACCESS

Microprapogation of callus from *Syzygium cumini* explant

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Received: 18.02.2016 | Revised: 25.02.2016 | Accepted: 27.02.2016

ABSTRACT

Microprapogation of the syzygium cumini explant was tried by using the different kind of MS. medium, hormonal changes in the medium show variations in the result. At the appropriate quantity of the hormones the explant shows a significant amount of development; the basal medium does show the some kind of development into a bud & no effect on the leaf explant development. The auxin is generally root initiator and cytokinin are the shoot developers. Very low level of growth regulator concentration does not show the significant result. (0.1-mg/l -0.1 mg/l) Present work we got development of callus by using a MS medium of basal medium + 6 BAP with additional supplement of IBA and kinetin at concentration (0.60 mg/l – 0.60 mg/l) and at the (0.02-0.5 mg/l) of IBA & kinetin shoot development observed. IAA only shows some growth in explant but does not develop the tumor/ callus.

Key words: *Syzygium Cumini, Explant, Basal Medium, Callus, Growth Hormones*

INTRODUCTION

The importance of tissue culture approach propagation of woody plants, including conifers, for the tree improvement and reforestation has been discussed as burning issue by several workers. Significant progress has been achieving *in vitro* organogenesis in callus cultures of hardwood trees, only a few studies have led to complete plant regeneration[1,2,3]. *Syzygium cumini* (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc. trees of *Syzygium cumini* are found growing throughout the Asian subcontinent, Eastern Africa, South America, Madagascar and have also naturalized to Florida and Hawaii in the United States of America[4]. Different parts of the jambolan were also reported for its antioxidant, anti-inflammatory, neuropsycho-phar- macological, anti-microbial, anti-bacterial, anti-HIV, and antifugal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexi-genic, gastroprotective and anti-ulcerogenic and redio-protective activities[5]. Studies in the past one decade have also shown that Jamun possess antineoplastic[6] Radioprotective[7] and chemopreventive effects[8] all of which are useful in the prevention and treatment of cancer. The seeds have also shown anti-inflammatory effects in rats and antioxidant properties in diabetic rat[9]. *S.cumini* bark is used as astringent in dysentery, seeds are anti-diabetic, fruits are used to treat cough, diabetes, dysentery, inflammation[10].

Cite this article: Jejurker, S.H., Tanpure, U.R., Pawar, S.D., Joshi, R.R. and Wadikar, M.S., Micropropogation of callus from *Syzygium cumini* explant, *Int. J. Pure App. Biosci.* **4(1):** 291-295 (2016). doi: http://dx.doi.org/10.18782/2320-7051.2229

In vitro systems have been reported as an effective tool for obtaining genetically uniform plants, which can be the source for less variable pharmaceutical preparations[11] *Syzygium cumini.* (Black plum), a hard timber tree of tropics is also important for its edible fruit and medicinal value. Its fruit possess considerable nutritive value. It is a good source of iron apart from usual content e.g. Minerals, sugar, protein etc[12]. Efforts of plantlet regeneration from explants of mature trees have failed because of recalcitrant nature of this tree. However plantlets could be micro propagated from nodal and shoot tip explants of obtained from seedlings[13]. Depending on the auxin/cytokinin ratios of the induction medium, either shoots or roots can be regenerated[14]. Micropropagation is an advanced vegetative propagation techniques for producing a large number of genetically uniform and pathogen-free transplants in a limited time and space 2003[15]. Each plant species propagated *in vitro* has requirements different plant growth regulators and their concentrations. Also, optimization of culture conditions and media is significant for micropropagation studies. Transfer of useful traits such as resistance to diseases, insects and stresses between plant species has played an important role in crop improvement[16,17,18]. The crop improvement through genetic transformation requires an efficient *in vitro* regeneration system[19]. Studies on different crops have shown that growth of explants under laboratory condition is affected by many factors including genotype, media composition, growth regulators and growth conditions[20,21,22,23] .

MATERIAL AND METHODS

Plants Leaf of *Syzygium cumini* (Jamun) were collected from Botanical garden of Vinayakrao Patil Mahavidyalaya, Vaijapur, Dist Aurangabad and used as source of explant material. Explants were trimmed to around 2.5 cm. They were thoroughly washed in running tap water to remove dirt, followed by washing with detergent (1 drop of lyzol) for 5 minutes, again washed with double distilled water. Explants were washed with 0.5 % HgCl2 (*Syzygium cumini*) and again washed with distilled water. These were spread on sterilized petri dishes on sterile bloating paper inside laminar air flow hood. Explants were trimmed to about 1.5 cm[24].

CULTURE MEDIUM

Excised plant tissue and organ will only grow in vitro on a suitable culture medium. we have a use different kind of M.S. medium provided by HI media. The M.S. medium was prepared by autoclaving at 121^0C[15 lb. pressure] for 15 minute. And in without hormone medium the hormones added externally according to needed its concentration. (Hormones sterilized by the filter sterilization).The pH of the medium should have 5.6- 5.8 range it is adjusted by using 0.1 N NAOH, and 0.1 N HCL. The M.S. medium was prepared by autoclaving at 121^0C[15 lb. pressure] for 15 minute. And in without hormone medium the hormones added externally according to needed its concentration. (Hormones sterilized by the filter sterilization).The pH of the medium should have 5.6- 5.8 range it is adjusted by using 0.1 N NAOH, and 0.1 N HCL. The stock solution are not specific for M.S. medium; so stock solution of hormones are general and can be used in any medium at any combination(kalyan kumar de.)Auxin and cytokinin are not directly dissolved in distil water, so they are at first made soluble in water miscible solvent and then water is added to get final volume. Hormones stock solution prepared as per Table 02.Then explant smoothly transfers on M.S. medium with the help of fine forceps. Then kept it for 16-20 ^{0}c incubator for an about several days to weeks. In the present work the auxin source are used IAA, IBA and the cytokinin source was 6- BAP and kinetin.

RESULT AND DISCUSSION

The Variations observed in different kind of MS medium;. basal M.S. medium (medium without growth regulators) does not show any significant growth after 4 weeks of incubation. The individual supply of cytokinin 0.1, 0.2,-1 mg/l concentration does not show the callusogenic or organogenic response; because of this reason the growth regulators are supply in the combination (auxin + cytokines) with a different concentration.

The Basal medium supplement IAA and kinetin (0.1-0.1 mg/l & 0.2 -0.2 mg/l respectively) which show a very little bet development in explant. The concentration of growth hormones added to the basal medium

about IAA & kinetin from [0.1-1 mg/l IAA] and checked the results and some concentration got a significant result as follows. After concentration of IAA &kinetin provided in 0.5-0.5 mg/l leaf explant show increase in size after a 6 week of incubation. In a next set the concentration of the IAA & kinetin (0.02 mg/l-0.5 mg/l) show the fast growth, but it does not developed in to callus or any organ. The IAA does not give an expected result, so by supplying the auxin in the form of IBA show some kind of them same results as that of IAA; but the IBA shows the rapid growth in bud within 4 week with a concentration (0.02-0.5 mg/l) of IBA & kinetin .Both the hormonal changes could not develop in to a callus, so by observing above result we was changing the medium basal medium with containing a 6-BAP are used; with an additional supplement of kinetin and IBA. In the next we use the IBA & kinetin (0.02-0.5 mg/l respectively) with a basal + 6- BAP medium which show a tremendous growth as compare to previous medium used. It will show the about 2-3 cm long bud development within the 4 week of incubation. And leaf explant also shows some yellowness an indication to callus development. Then after we trying to develop to callus by supplying only basal medium + 6 BAP which does not show growth, and then we will also give IBA & kinetin additional supplement from 0.2- 1.mg/l concentration the best result was found to 0.6 mg/l as follows. Basal medium+ 6 BAP is supplemented with the 0.6 mg/l - 0.6 mg/l IBA + KINETIN show a callus development.

In the present study the 6- BAP was found to be most suitable cytokinin source to develop a callus after 6BAP kinetin shows some kind of growth. IBA + kinetin to develop shoot buds mostly from the nodules[13]. IAA and kinetin also give a shoot development but required more time than that of IBA used. The greatest shoot development of explant obtained from medium containing IBA + kinetin ratio 0.5 mg/l- 0.025 mg/l. The basal medium+ 6-BAP with addition of growth regulators IBA & kinetin show fastest and higher growth as compare to other medium used. The higher concentration of auxin will inhibit the explant (bud /leaf /flower) growth; it was in shoot development required in low concentration for a significant callus or organ development.at the concentration of 0.02 mg/l of IBA shows a well development of shoot buds. The basal medium does not show the significant growth of explant, with a supplement of 0.1- 0.2 mg/l hormone does not create bigger change as required for callus development.

Table 1

S.No.	Medium type	Medium with	Medium without
1	M.S. medium type-1	Cacl2, vitamin, sucrose.	Agar
2	M.S.medium type-2	6-BAP,vitamins,sucrose,agar	Cacl2,IAA,cytokinin.
3	M.S.medium type-3	Vitamin, agar, sucrose.	Cacl2, IAA, kinetin..
4	M.S.medium type-4	Vitamins, sucrose, cacl2	Cacl2,IAA, agar.

Table 2

S.No.	Hormones	stock solution in ml	Solvent required to dissolved	Water to be added	Concentration mg/ml	Duration of storage [days]
1	Auxin					
a)	2-4 dichlorophenoxy acetic acid(2-4 D)	10	1 ml absolute alcohol	9 ml	0.5 mg/ ml	7
b)	Indole acetic acid (IAA)	10	1 ml absolute alcohol	9 ml	0.5 mg/ ml	7
c)	Alpha- naphthalene acetic acid (NAA)	10	1 ml absolute alcohol	9 ml	0.5 mg/ ml	7
d)	3- indole butyric acid (IBA	10	1 ml absolute alcohol	9 ml	0.5 mg/ ml	7
2	Cytokines					
a)	Kinetin	10	1ml of (1 N) HCL	9 ml	0.5 mg/ ml	7
b)	6-BAP (6-benzyl amino purine)	10	1ml of (1 N) HCL	9 ml	0.5 mg/ ml	7
c)	Zeatin	10	1ml of (1 N) HCL	9 ml	0.5 mg/ ml	7

CONCLUSION

Tissue culture technology offers an alternative method for the conservation of germplasm as well as micropropagation of medicinally important plant resources. From result and discussion it was concluded that the concentration of growth hormone shows the significant effect on growth of explant; also the woody plant are very difficult to microprapogate easily. The hormonal change will show the change in explant development i.e. when IAA used with kinetin dose show normal or some excised growth; but while the use of IBA show a significant highest shoot bud development with an short time of incubation period. Basal medium +6 BAP with additional supplement of IBA and kinetin show a significant development of callus within the four week of incubation

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