MODIFIED PROTOCOL FOR RAPID IN VITRO DEVELOPMENT CALLUS OF SYZYGIUM CUMINI (L.)

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Abstract
Earlier protocol for the callus development of syzygium cumini, comparatively longer periods. Various combination of tissue culture media were attempted in our laboratory. The combination a growth hormone IAA (0.5-3mg/ml) kinetin (0.5-1.5mg/ml) & 2-4 D(1-2mg/ml) 6-BAP (0.5-3mg/ml) resulted in development of green & brown calli from explants (stem &leaves) of syzygium cumini, a woody plant within 8-12 days. This may be prove useful for the callus development of woody plant, that can be used for micropropogation.
Keyword: IAA, BAP, Kinetin,2-4 D

Introduction:

The genus of syzygium cumini (L) forest tree is widely distributed in India & other tropical region of the world, commonly known as jamun is widely used in Ayurveda &other Indian folk medicines for the treatment of diabetes mellitus. A fast growing species, It can heights of up to 30 m & can live more than 100 years (1). Syzygium Cumini (L) is an evergreen tropical tree belonging to the Myrataceae family & native to india , Nepal Bangladesh(2) The leaves & extract of seed which is traditionally (2) used in diabetes has a hypoglycemic action antibacterial & antidiarrea effect.(1) Strengthen the teeth & gums to treat leucorrhea, stomachalgia, fever , gastropathy stangury, dermopathy (3,4,5,6,7,8),plant are the potential source of natural antioxidants, antibacterial or phytochemical antioxidant are the secondary metabolites of plants. Antioxidant & antibacterial agents like tannins,flavonoids, phenols, polyphenols & nitric acid, scavengers of free radicals such as peroxidase hydrogen or lipid peroxyl thus inhibits the oxidative mechanism that lead to degenerate disease.(Walton & Brown 1999). Production of calli formation from fragment stems, leaves, are mainly carried out to determine the culture condition in that growth hormone, micronutrients, macronutrients vitamins required by the explants to survive & grow, study cell development exploit products coming from primary & secondary metabolites (10). It fessibility the way for isolating/ separately economically valuable phytochemicals. It fessibility the way for isolating/ separately which can avoid collecting plant material from natural sources (12-13) In the area of plant Biotechnology callus /cell culture production may expedient to medicinal &bioactive compound in large-scale from medicinal plants. callus culture of syzygium cuminni prior reported by using same explants parts but there is no systematic study on the callus formation protocol by using same explants parts but different kind of media, with change/modification in growth regulator with medium contents, which give us bulk quantity of callus and in vitro plant regeneration. Therefore the present investigation is working on developing its in vitro regeneration protocol.

Material & Method: -

Collection of plant material: - The plants were collected from Dr. Babasaheb Ambedkar marathwada university sub campus area.

Surface Sterilization :- Both in vitro & in vivo explants were selected as culture explants, In vivo explants were washed with running tap water for 20 min to remove the traces of dust.etc followed by treatment with Bavistin at different concentration followed by treatment

10% teepol/tween 20 for 2 min. later on given treatment of 10 % teepol/tween 20 for 2min, then the explants were sterilized in 70% ethanol for 45 second / 1min. & finally with 0.1 %, 0.5%, 1%, Hgcl2 as well as 1.5% nacl2 for 2-3 min lastly washed 3-4 times with sterile double d/w water.

Culture medium: Later on the sterilization sterile glassware kept for 1-2 hrs. at Incubation period 80 c .after drying pour media in the bottle kept for overnight for checking contamination. Next day inoculate sterile young leaf inter -node segment (stem) small pieces (1 cm-1.5cm) were inoculated on MS (murashige skoog medium) M.S.G B5 medium without containing 3% Sucrose& gelled with 1% agar supplemented by Individualize concentration of auxins such as NAA,IAA,2-4-D & BAP, Cytokine, shoot & root initiation BAP, KIN, NAA, IBA & IAA were used. Adjust PH of the medium 5.8 to 6 before Addition of gelling agent agar & autoclave for 20 min. 121^{o} c. for 15 lbs pressure.

Culture Condition: The growth room condition maintained for in vitro culture were (25+- 2) & 60 % to 70 % relative humidity & the intensity is 3000 lux for calli formation , variation in the lux light depend on the experiment . Each experiment was carried at least three to four times with 10 replicate per treatment.

Table:Hormone concentration used in callus formation

SR. NO.	HORMONE	H.CONCENTRATION	MEDIA	DAYS	PLANT PARTS	CALLUS COLOR.W WITH WT.
1	IAA/Kin.	1.4µl,1.25 µl/ml	M.S.M with cacl2 w/o sucrose agar	18 days	leaf	Green .40g
3	IAA/Kin.	2µl,1.2µl /ml	M.S.M with cacl2 w/o sucrose agar	10-12 days	leaf	Green .80g
4	IBA/Kin.	2µl,2µl /ml	M.S.M with cacl2 w/o sucrose agar	8-10 days	leaf	Green 1 g
5	IAA/Kin.	1.6µl,1µl /ml	M.S.M with cacl2 w/o sucrose agar	20 days	leaf	Green 1g
6	2-4D /BAP	1.2µl,1.6µl /ml	M.S.M with cacl2 w/o sucrose agar	15 days	Leaf stem	Green 1g

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Photos:



Result:

Callus formation of selected sterilized plant parts (Inter-node leaf) not only particular period of cell development i.e. minimum 3-4 weeks but also cell development with proper growth between 8-12 days, by using shoot growth regulator for a callus formation. Also expedient when use equal concentration of shoot growth regulator i.e. IAA & kinetin callus formation within 8-12 days of brown color &callus growth regulator 2-4 D & BAP used for develop green callus in between 14 -15 days. Callus formation in short period by using shoot growth regulator than the callus growth regulator. Later on This callus helpful for isolating phytochemical compounds, which act as antimicrobial & antioxidant agent.

Conclusion:

Got prior reported result 3-4 weeks for callus development's by using callus growth regulator .but used shoot growth regulator as well as callus growth regulator callus formation with color variation in between two weeks. These callus used for further studies.

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