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INVITRO MULTIPLICATION OF CRATEVA ADANSONI ON MURASHIGE AND SKOOG MEDIUM (MS)

Yogesh Ugale & Mukul Barwant

Research Scholar, Department Botany, Sanjivani Arts, Commerce and Science College Kopargoan Ahmedhnagar, Maharashtra, India

ABSTRACT

Invitro multiplication of Crateva adansonii is done with help of aseptic invitro methods. Tissue culture is in vitro aseptic culture of cells, tissue organ or whole plant is controlled under national condition to produce clone plants. These conditions are proper supplied with nutrients, ph, proper gasses and proper environment. This technique small pieces of tissues can be used to produced hundred plant produce single explant are multiplied into several thousand plants in short time period under controlled condition. A method of micropropagation through multiple shoot formation from axillary buds of mature tree and rootstock growths of Crataeva adansonii (DC) Prodr. (A multipurpose tree) has been developed. The successfully growth Crateva adansonii of done on artificial nutrient media (MS) and hormone and vitamin in aseptic condition it achieved .In this paper we are describing that the plant material sterilized the artificial media are prepared and on that media explant are inoculated then its feeding of different types of growth hormone such as auxin and cytokinin for the root and shoot formation.

***KEYWORDS**: Crateva Adansonii, Invitro Multiplication, MS Medium, BAA, IAA, NBA, Auxin, Gibbrelin*

Article History

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INTRODUCTION

Crateva adansonii is a deciduous tree developing a rounded crown as it grows older; it usually grows from 3 - 10 metres tall, but with occasional specimens to 16 metres. Tropical Africa - Mauritania to Ethiopia, south to Tanzania, Zimbabwe and Madagascar. The plant can tolerate occasional short-lived light frosts. The tree is harvested from the wild for local use as a food and medicine. A small, handsome tree, it is worthy of cultivation as an ornamental for its dense masses of white flowers borne at the ends of all the shoots. Tissue culture is in vitro aseptic culture of cells, tissue organ or whole plants are controlled under national condition to produce clone plants. These condition are proper supply of nutrients ,ph., proper gasses and proper environment .Plant tissue culture techniques is useful for the industrial importance ,plant propagation, disease elimination and production of secondary metabolite in this techniques small pieces of tissues can be used to produced hundred plant produce single explant are multiplied into several thousand plants in short time period under controlled condition .The some plants danger, threatened and rare a species have been successfully be grown by micro propagation. Commercial production of two recent advances in plants in vitro culture plants through micro propagation techniques has several advantages over the traditional methods of propagation through seed cutting, grafting, and air layering etc. Medicinal plants are important source of life saving drugs for majority of world population in vitro regeneration holds tremendous potential for the production of high quality plant base medicine. In vitro production of

secondary metabolites in plant cell suspension culture has been reported from various medicinal plants. Bioreactors are the key step towards commercial production of secondary metabolites by plant biotechnologies. This article discusses the application of biotechnology for regeneration and genetic transformation for enhancement for secondary metabolite production invitro from medicinal plants

A method of micro propagation through multiple shoot formation from axillary buds of mature tree and rootstock growths of Crataeva adansonii (DC) Prodr. (A multipurpose tree) has been developed. Factors affecting multiplication rate included season, age of explant source, explant type, type of bud, position of bud on the foliage twig, type of medium, various additives, and explant implantation on the medium. The maximum number of buds was produced from the sixth to 10th auxiliary buds taken from foliage twigs of 40–50-d-old rootstock growth in the months of October to December.

According to the International Union of Conservation of Nature (IUCN), the major threat of rapid loss and extinction of genetic diversity is due to habitat destruction, pollution, climate change, invasion of exotic species, human population pressure, ever increasing agricultural pressure and practices life style changes we well known. The industrial product of secondary metabolites using cell culture was initiated during the period 1955-1960 by the Pfizer Company with assistance of G.N.nickill, A distinguished expert on plant tissue culture. These early attempts were disappointing and little progress was made of this aspect on plant tissue Culture (Dodd's 1979). From 1975, the fundamental aspects of the subject were explored carefully and intensively. Rapid progress in these investigation attracted many species for engineering cell for secondary metabolite production (verpoorte et al. 1998). The technologies now available to the industry; the Commercial production of shionin, ginsehg saponins and berberidine has been particularly encouraging (Hosoki and Sagawa 1977).Plant are traditional source of many chemical used as pharmaceutical. Most valuable phytochemicals are product of plants secondary metabolism. The production of secondary metabolism in-vitro can be possible through plant cell culture. Successful establishment of cell lines capable of producing high yield of secondary compound in cell suspension culture has been reported by Argolo et al. (1995). The accumulation of secondary products in the plant cell culture depend on the composition of the plant cell culture depends on the composition of culture medium and on environmental condition. Strategies for improving secondary product in suspension culture, using different media for different species have been reported by Robins.

The number of shoots was further multiplied by using nodal segments of in vitro-regenerated shoots as micro cuttings and repeated sub culturing of stumps after excising the micro shoots. In vitro rooting on growth regulator-free MS medium was possible with 70% of micro shoots after 4 weeks. From one nodal segment, 150 plantlets were produced within 14 weeks.

Crateva adansonii the name Crateva is given in the honor of Crateva. A Greek botanist who was living in the time of Hippocrates and the name religious indicates its growth near the place of worship. Crateva religiosa is much branched deciduous tree belonging to the family capparidaceae, commonly called as Varuna. The trade name given for this tree is three leaved capper. The leaves are trifoliate glabrous, and ovate. Flowers are whitish to milky white in color in terminal corymbs. Fruit is berry, globose or sometimes oblong with woody rind, embedding seeds in the yellow pulp. The outer surface of bark is wrinkled and gray white in color, covered with large number of lenticels. Tree flowers and fruits in the month of Dec-May. Distribution Crateva relegiosa is globally distributed in India, Myanmar, Sri Lanka, Malaysia. Indonesia and China. In India, it is found in Peninsular India, Western India, Genetic Plains, and Eastern India, up to Tripura and Manipur. It is also found in Sikkim and Andman and Nicobar Island, It is found mostly along the bank of the river and streams and near to temple side.

Taxonomy of Crateva adansonii

Plantae: Kingdom

Division: Angiosperms

Class: Eudicots :

Sub-class: Resides

Order: Brassicales

Family: Capparidaceae

Genus Crateva

Species: C. Adansonii (Odora) (DC)

MATERIAL METHODS

The nutrient media contained inorganic and organic constituents according to Murashige and Skoog (12). Phytohormones were also added to the Murashige and Skoog medium in different concentrations and combinations. The pH of the medium was adjusted to 5.7 with either 1 N KOH or 1 N HCL. The culture media were autoclaved at 15 1b/in2 at 121oC for 15 minutes, then maintained at 27±2oC in the culture room. Actively growing shoot tips (5 to 10 mm in length) were cut from shoots. Excised shoot tips were used for isolation of meristem (under microscope). Shoot tips were washed with Tween 80 and then rinsed with distilled water 3-4 times. Meristems were isolated from these shoot tips and surface disinfested with a 0.15% solution of mercuric chloride for 1 min. Under a laminar flow hood, they were rinsed with sterile distilled water. All the tools required for dissection were previously sterilized. Disinfected meristems were placed separately in culture tubes (25 mm) containing 15 ml of MS medium with various growth regulators. The culture tubes containing the explants were maintained in the laboratory at 25±2oC. They were exposed to artificial illumination of 2000-3000 lux by placing them at 25-30cm below fluorescent light for sixteen hours every day.

The preparation of MS media is most commonly used for all experimental works such as formation of callus and shoots multiplication. The MS media was first discovered by the scientist Murashige and Skoog in 1962. The medium was supplemented with different concentration and combination of growth regulators like BAP, KIN, GA, ZIA, NAA, IBA and various concentrations. In experiment of Crateva there is modified MS medium used

Preparation of MS Media for Crateva Adansonii

- Washing of Glassware’s
- Preparation of Media
- Sterilization of Media
- Innnoculation of Explant

Washing of Glassware’s

In this step, there is the glassware which are used for the medium preparation are wash with the help of lanolin and running tap water. There is beaker, stirrer, cultural bottle or test tube. Petriplate, conical flask etc. Are washed and then it was rinse

with alcohol and then distilled water and stored in the clean and dry place for drying purpose.

Preparation of Media

For the 1000 ml of medium, there is 30gm sucrose was dissolved in 200-300 ml of distilled water. The stock solution is added into that solution in the following manner:

Stock solution A- 50 ml

Stock solution B- 50 ml

Stock solution C- 50 ml

Stock solution D- 50 ml

After adding the stock solution and growth hormone and make final volume 100 ml the ph. adjusted on 5.8 and kept beaker on hot plate for boiling medium, then solidifying agent was added into solution then it transferred to the cultural bottle and bottle are air tightly closed and transfer to bottle into the autoclave .

Table 1: Preparation of Stock Solution of MS Media

Sr. No.	Contents of MS Medium	mg	Dissolve in Water	1ltrs MS Media
	Macronutrients			
1	NH_4NO_3	33000		
2	KNO_3	38000		
3	$CaCl_{22}H_2O$	8800	1000 ml (A)	50 ml
4	$MgSo_{47}H_2O$	7400		
5	KH_2PO_4	3400		
	Micronutrients			
1	KI	166		
2	H_3BO_3	1240		
3	$MnSo_{44}H_2O$	4460		
4	$ZnSO_{47}H_2O$	1720	1000 ml (B)	5 ml
5	$Na_2MoO_{42}H_2O$	50		
6	$CuSO_{45}H_2O$	5		
7	$CoCl_{26}H_2O$	5		
	Fe- Versanate			
	EDTA	5560	1000 ml (C)	5 ml
	NAEDTA	7460		
	Vitamin and Hormone			
	Inositol	2000		
	Nicotinic acid	100		
	Pyridoxine -HCL	100	1000 ml (D)	5ml
	Thymine –HCL	20		
	Glycine	400		

Sterilization of Media

Media was sterilized in autoclave at 1210C at 15 lb. pressure, at the same time distilled water and petriplates and also sterilized. After the sterilization of media it was transferred into 70% alcohol clean laminar air flow

Preparation of Explants

The auxiliary bud, node and meristematic tissue of Crateva adansonii were collected from botanical garden, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad. All these explants were washed carefully by running tap water for 10 minutes. Explant was transferred in laminar air flow. In laminar airflow explants was sterilized by distilled Water

for 5 minutes. For surface sterilization, chemical HgCl2 (0.2 %) was used. Explant were surface sterilized for 5 min by 0.2 % mercuric chloride followed by 3 subsequent rinses with Sterilized double distilled water. All these explants were dissected into small pieces and treated so that maximum part can be exposed to media. These small pieces of explants were included on MS Media. After complete inoculation, all bottles were transferred into culture room.

Culture Room

The culture room should have following facilities:

- Controlled temperature usually at 25°c = 2 with the help of air condition and room heaters.
- Culture rakes fitted with light, generally 1000 lux or lower light generally by fluorescent tubes, automatic timers, can be used to regulate photoperiod.

RESULTS AND DISCUSSION

- When the Auxillary bud, nodal portion, young leaf was inoculated on MS medium for the in-vitro multiplication of plants Crateva adansonii.
- We generally took nodal portion of the Crateva adansonii
- The Micropropogation is done by sterilization by chemical, then surface sterilization that material used as explant
- The prepare MS medium prepare by using some organic and inorganic chemical constituent use as media
- With Nutrient media we has used Some Growth regulator like Auxin and Gibbrelin
- After inoculation of explant on growth media within first eight days shows callus formation
- After callus formation it shows seedling formation will take place within next 20 days
- The invitro multiplication of plants Crateva adansonii was shown growth in 2-3 week by formation of shoot.
- It was found that nodal segment of stem most suitable for invitro propagation.
- When the nodal segment of stem inoculated on MS medium supplemented with 3 mg/L BA and 0.05-0.1 mg/L NAA is most effective in direct inoculation of axillary buds from nodal explants.
- Table 1 shows the different concentration of growth regulator and their effect on the plant growth maximum effect shows 0.5 mg (auxin)+ 0.5 mg (gibbrelin) they shows effect early in callus formation as well as root shoot formation

Table 2: Growth Regulators and Ex. Plant Formation Information

Sr No.	Growth Regulators		Explant Formation		
	Auxin	Cytokinnin	Callus	Shoot	Root
1	0.5 mg	2.5 mg	-	✓	-
2	0.5 mg	0.5 mg	✓	✓	-
3	500 ml	1 ml	-	✓	✓
4	500 ml	1.5 ml	-	✓	-
5	0.2 mg	0.2 ml	✓	-	-

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