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Micropropogation of Papaya (*Carica Papaya* L.) By Tissue Culture Techniques

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SUMMARY

Papaya shoot growth & root induction process was studies by exposure of different plant growth hormone such as BAP, NAA in various concentrations. Bud were surface sterilized by the serial treatment of sterilization agents viz., mercuric chloride HgCl20.1% (3 min) mercuric chloride HgCl20.1% (6min) , mercuric chloride HgCl20.1% + 70% ethanol (4min.30 sec) , mercuric chloride HgCl20.1% + 70% ethanol (6min.30 sec) , sodium hypochlorite 0.5% (5min), sodium hypochlorite 0.5% (10min) , sodium hypochlorite 0.5% + mercuric chloride HgCl2 0.1% +70% ethanol (5min+30 sec+4min. Buds washed three times with sterile D/W after each treatment of sterilizing agent. Amongst, the various combinations of growth hormone tested, we observed the BAP (2mg/L) + NAA (2mg/L) combination has shown maximum shoot growth. Concentration NAA (2mg/L) + NAA (1mg/L) was found to be the best for root growth.

INTRODUCTION

Papaya (Carica papaya L.) belongs to the *Caricaceae* family. It is an important tree fruit crop (Evans & Ballen 2012). Papaya, native to Central America, has three sexual forms: male, female, and hermaphrodite (Storey 1938). Hermaphroditic plants are desired by the industry for their ability to self-pollinate and produce a marketable fruit in terms of size and quality. Papaya originated in Mesoamerica, likely in southern Mexico. The natural distribution of papaya, this has been suggested to range from the northern tropical limit of Mexico to Costa Rica in Central America (Aradhya et al., 1999; Carvalho and Renner, 2012). Total annual world production is estimated at 6 million tonnes of fruits. India leads the world in papaya production with an annual output of about 3 million tonnes. Other leading producers are Brazil, Mexico, Nigeria, Indonesia, China, Peru, Thailand and Philippines. In India, papaya is predominantly grown in Tamil Nadu, Karnataka, Andhra Pradesh, and Gujarat. In 2012-13 papaya production accounted for 1.9% of the total fruit cultivation area, and its production accounted for about 6.6% of India's total fruit crops. In Maharashtra: Sangli, Satara, Pune, Nasik, Sholapur, Nagpur, Amravati.

The plant grows well in I sandy loam soil having pH between 6.5 to 7. Papaya grows well in sun, warm & humid climate. Ideal temperature for papaya is between 25 to 30 degree centigrade. Papaya (Carica papaya L.) is an important fruit crop of Maharashtra and is one of the most popular versatile fruits, which is also used as vegetable. Papaya is a good source of pro-vitamin 'A' and ascorbic acid (Purnima and Sandhya 1988), important proteolytic enzyme such as papain and chymopapain with several commercial applications. Moreover, it also yields an alkaloid 'caprine', which is used as a heart depressant, amoebacide and diuretic (Litz 1984). Biologically papaya has three types such as male, female and bisexual but only female and bisexual types are productive. Conventional method of propagation like cutting or grafting has not been found successful in papaya. In this regard clonal propagation represents the economic way of continuously producing new uniform true-to-parental type planting materials of known superior lines. Cloning by in vitro technique has been proved as an excellent biotechnology of vegetative propagation especially for those, which are difficult to rooting (Conger 1981). In vitro micropropagation system of papaya was reported by many researchers (Hossain et al. 1993, Rahman et al. 1992, Winnaar 1988 and Islam et al. 1993). But their reports were not satisfactory for large scale propagation of plantlets as well as successful field transfer. Thus considering the above facts, the present study was undertaken to develop a suitable protocol for in vitro propagation of papaya.

Micropropagation can produce large numbers of elite homogeneous clones, allowing for the planting of a single hermaphrodite in each hole and eliminating the negative aspects Carica papaya L. (C. papaya) is considered one of the most important fruit trees, especially for tropical and subtropical regions. Ripe papaya fruits are commonly eaten fresh. Additionally, they can be made into candy, jelly or jam, and even be used as an element in fruit cocktails.

The unripe fruits, which contain latex, are the primary source of papain. Papain is used in different industries for instance; the pharmaceutical industry, in beer industry, in leather industry, in the cosmetics industry and industry of candy and chewing gum (Nakasone and Paull 1998). Papayas have earlier productions and higher yields compared to other perennial plant crops. They can produce flowers four months after being germinated and can produce fruits eight months after being planted. Every papaya tree can provide about 30-40 fruits annually.

Papaya fruits normally have short shelf lives, particularly in tropical countries where the temperature and humidity are always high. The quality of the fruits may be reduced through handling, storage, transportation, and sales, resulting in an unfortunate appearance, texture, flavour and overall acceptability. Apical buds and lateral buds from seedlings and mature plants were used as explant. Explants were subjected to different treatments of plant growth substances for culture establishment and shoot proliferation. The study revealed that full strength MS medium supplemented with sucrose 30.0 g/l and agar 6.5 g/l under light condition produced highest shoot number and longest shoot in papaya. Application of BA 0.50 mg/l along with NAA 0.1mg/l was found to be better for initial culture establishment and proliferation.. In vitro rooting was more in full strength MS medium supplemented with IBA 3.0 mg/l, sucrose 30.0 g/l and activated charcoal 0.05 per cent.

Varieties Cultivated:

Honeydew, sunrise, CO-1, CO-2, CO-3, Pusa Nanha, Pusa delicious.

Uses of Papaya: Delicious and Loaded with Nutrients. Papaya is the fruit of the Carica papaya plant. Has Powerful Antioxidant Effects. Has Anticancer Properties. May Improve Heart Health. May Fight Inflammation. May Improve Digestion. Protects Against Skin Damage. Delicious and Versatile.

The present study was done by using following objectives.

1) To Standardization of Surface Sterilization technique of Papaya (*Carica Papaya* L.)
2) To standardization of MS Media for protocol in vitro-rapid propagation of Papaya (*Carica Papaya* L.)
3) To study of effect of growth regulator (BAP and NAA) on propagation of Papaya (*Carica Papaya* L.)

Material and Method

The present work Micropropogation of Papaya (Cacira Papaya L.) was carried out Department of Plant Biotechnology, College of Agricultural Biotechnology, Saralgaon, Tal. Murbad, Dist. Thane. Maharashtra (India). The research work is undertaken with aim of To Standardization of Surface sterilization techniques for the micro propagation of Papaya, and to study of the effect of growth regulators (BAP and NAA) on propagation of Papaya. The material used and the methods adopted are on hand below.

Material: -

Plant material -

The explant collected from the Shree Datta nursery Shirol , Tal- Shirol, Dist- Kolhapur 416101. Potted and maintained in the green house. These plants were used as A source of bud segment throughout the investigation.

Chemicals – Salts of macro and micro elements of analytical grade, Vitamins and amino acids**,** Sucrose as a carbon source**,** Myo-inositol**,** Sodium hydroxide **,**Agar-agar as a gelling agent**,**1N HCI and 1N NaOH for pH adjustment**.**

Chemicals for surface sterilization: Carbendazim, Ethanol, Mercuric chloride (HgCl2), Sodium hypochloride**.**

Plant growth regulators: Auxins: Indole acetic acid (IAA), Naphthalene acetic acid (NAA), Indole 3-buturic acid (IBA), Cytokines: Benzyl amino purine (BAP), kinetin.

Equipment: All glassware used during the course of experiment, namely: Weighing balances, pH meter, Autoclave, Refrigerator, Incubator, Laminar air flow, Magnetic Stirrer .

Glass wares: Beaker**,** Conicalflask**,** Measuring cylinder**,** Pipettes**,** Culture bottles, 1.5 Media - MS media**,** Sucrose**,** Agar.

Method

Surface sterilization

1) The laminar air flow cabinet was surface sterilized by using alcohol and then all glassware required during inoculation was kept in the cabinet.
2) UV light was switch on after closing the cabinet for sterilizing the surface of laminar air flow cabinet. Kept it ON for 20 minutes.

3) switched OFF the UV light and white fluorescent light was kept ON while working.

Glassware's and Miscellaneous sterilization:

1) All the glassware's were wrapped in autoclave bag and autoclaved at 121ºC for 15 minutes.
2) The Presterilized forceps, blades, test tubes, medium containing conical flask, petri plates were kept in the laminar air flow cabinet.

Explant Selection:

Bud of papaya were taken as explants. The buds were peeled off to the size of 4 cm. The excised bud were washed thoroughly under running tap water after that ex plant sterilized with 70% ethanol for 1 minute, 2% sodium hypochlorite solution for ten minutes and washed 3 to 4 times with distilled water. After sterilization, the outer level was removed 3 cm long bud were excised and trimmed and transfers to the aseptic condition.

Preparation of Media:

Murashige and Skoog medium were used for this experiment. A beaker was filled with 800 ml distilled water and MS medium in powdered form was added slowly to it. 30 mg sucrose is added in to it. The pH was maintained at 5.8. Then 8g of agar was added. The media was transferred to a 1 litter of volumetric flask and make up by adding distilled water. Then medium was autoclaved at 15 psi and 1210 C for 20 minute and culture medium were allowed to cool at room temperature and stored in culture room.

Table No 1: -Preparation of culture media

Sr. No.	Components	Concentration
1.	Macronutrients	25 ml
2.	Micronutrients	5 ml
3.	Iron stock	5 ml
4.	Vitamin stock	1 ml
5.	Amino acid	1 ml
6.	Myoinositol	100 mg
7.	Sucrose	30 g
8.	Growth regulators	As required
9.	Agar(forsolidmedium)	8 g

Media Autoclave:

The jars with media then autoclaved at 1.06kg/cm at 121℃ pressure for 25 mint after adjusting pH to 5.8.-6.2. and let it to set. The Explants then placed on autoclaved MS media under Laminar flow.

Bud inoculation:

The isolated and surface sterilized explants were collected carefully and inside the laminar air flow cabinet the explants cutting directly inoculated in taken test tube or jars with 20ml of MS medium supplemented with different concentrations of treatment to be given and cover it with Aluminium foil. The papaya bud are incubated at an optimum growth temperature of 28 ± 2° C for 16 hours .For the development of explants aseptic conditions are kept up inside the cultural room.

Preparation of Plant Growth Regulators:

The growth hormones were dissolved in few drops of the solvent and volume was made up to the required level with double distilled water, filter sterilized. The stock of growth hormones was prepared in different mg/ml for convenient use and stored at cold condition (4°C).

Table No 2.Growth regulators stock preparation

Growth regulators	Solution Preparation				
	Solvent	Diluents	Powder Storage	Liquid storage	Sterilization
BAP	1NNaOH	Water	RT	2-8°C	CA/F

NAA	1NNaOH	Water	RT	2-8°C	CA

RT-Room temperature, CA-Co-autoclavable, F-Filter sterilization

Result:

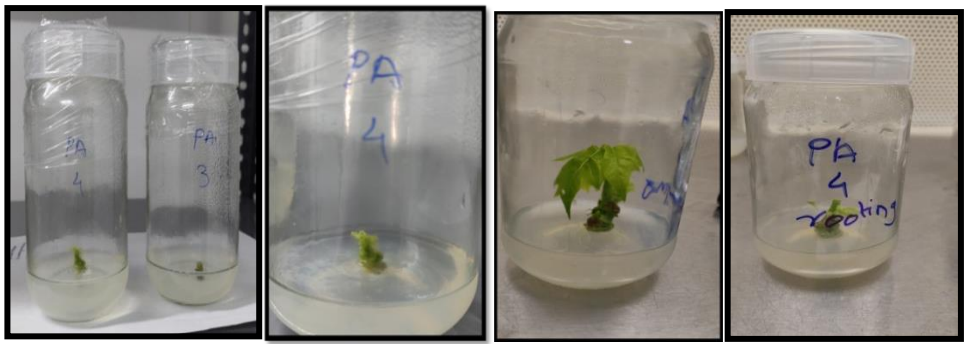
Results of the present Papaya Bud segment explants were cultured on MS basal media and MS medium supplemented with different concentration of growth hormones (BAP 0.5 mg/l, 1.0 mg/l, 1.5 mg/l, 2.0 mg/l) was successfully used. Data was obtained after 6 weeks of initiation of culture showed that Bud segment Papaya of could be established at all tested media including the control medium (free from growth regulators). The best results were obtained on MS medium supplemented with MS medium + BAP (6.00 mg/l) + NAA (2.00 mg/l).

Table 4. Effect of Cytokinins and Auxins on shoot proliferation of Papaya explants.

Treatment	PGRs (mg/l)		Growth response			
	BAP (mg/l)	NAA (mg/l)	No. of explants inoculated	Shoot emergence (%)	No. of shoot per explant	Shoot length (cm)
T0	0	0	10	0%	0	0
T1	2	0	10	0%	0	0
T2	4	0	10	20%	1	1cm
T3	6	0	10	0%	0	0
T4	2	2	10	0%	0	0
T5	4	2	10	50%	1	1.5cm
T6	6	2	10	80%	4	2 cm

Root Establishment of Papaya (C*arica Papaya* L.) *in-vitro:*

Root initiation and establishment from Papaya Bud segment explants were cultured on MS basal media and MS medium supplemented with different concentration of growth hormones (NAA 2.0 mg/l, 4.0 mg/l, 6.0 mg/l, +BAP 2.0 mg/l) was successfully used. Data was obtained after 6 weeks of initiation of culture showed that Bud segment Papaya of could be established at all tested media including the control medium (free from growth regulators). The best results were obtained on MS medium supplemented with **MS medium + NAA (4.00 mg/l) + BAP (2.00 mg/l).**



Shoot development , Root development

CONCLUSION

The surface sterilization treatment Sodium hypochlorite (5%) 5min + Ethanol (70%) 30 sec + Mercuric chloride (0.1%) 4 min.was found to be best combination to achieve highest percentage of contamination free healthy culture. MS medium + BAP (6.00 mg/l) + NAA (2.00 mg/l) treatment were found highest rate of shoot survival. MS medium + NAA (6.00 mg/l) + BAP (2.00 mg/l) treatment were found highest rate of root survival.

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