Indian J. Applied & Pure Bio. Vol. 38(2), 747-751 (2023). **ISSN: 0970-2091**
A web of Science Journal

Callus induction in *Carica papaya* from hypocotyl and leaf explants

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Abstract

The papaya is fruit of the plant *Carica papaya,* the sole species in the genus *Carica*of the plant family Caricaceae. It is a source for proteolytic enzymes papain and chymopapain. The crop improvement programmes of papaya are faced with problems due to its heterozygosity, dioceous nature. Therefore, the present investigation focused on to standardize a protocol for rapid callus induction in *Carica papaya* species (Honey Dew var.). Juvenile hypocotyls, leaf and mature midrib were cultured on full strength MS medium containing a range of Kn, NAA, 2,4-D and BAP with different composition. Hypocotyl and leaf formed maximum callus on medium containing 1 mg/l Kn followed by 0.25 mg/l NAA and Kn 5 mg/l fortified with NAA 0.25 mg/l respectively. Hypocotyl and leaf explants proved to be the best explants for callus induction. Midrib unresponsive towards the callus induction. Kinetin proved better growth hormone than BAP. The method may be useful for the mass propagation of female papaya plants for large scale cultivation.

Key words : *Carica papaya*, BAP, MS, NAA.

The Papaya is fruit of the plant *Carica papaya*, it belongs to family Caricaceae. It is the native to the tropics of the Americas and was first cultivated in Mexico. Papaya is an important fruit crop, widely grown up in the tropical and the subtropical areas of the world for its fruit and as a source for proteolytic enzyme papain and chymopapain[9]. Papaya is a fast-growing herbaceous tree that continuously bears fruit starting at 8-10 month. After transplanted to the field it is also popularly grown in home gardens or scattered among other crops in small family farm plots. It is a good source of vitamins A and C and is especially importance for subsistence farmers with limited resources. The crop improvement programmes of papaya are faced with problems due to its heterozygosity,

diecious nature, solitary habitat and a large number of viral diseases. Tissue culture techniques appear to be an alternative approach where mature tissues from grown plants can be used for multiplication and large-scale production[2]. Papaya is a polygamous diploid (2n = 18) plant species with three sex type, *i.e.*, male, female and hermaphrodite. Male plants are useless for economic purpose. As a food, pyriform fruits from hermaphrodite trees are preferred on the market over spherical fruits from female plants. It is therefore desirable that only hermaphrodite individuals are cultivated in the field. However, as sex determination of papaya plants at the seedling stage was not possible. Conventional techniques of asexual propagation cutting and grafting were tried but resulted in very limited success[4]. Clonal methods for propagation can overcome some of these difficulties in papaya cultivation and improvement. During the last few years micropropagation technique has emerged as a promising technique for rapid and large-scale propagation of vascular plants.

Therefore, considering all the importance of this valuable plants our present investigation based upon standardization of a reproducible and feasible protocol for callus induction in *Carica papaya* from the stem, leaf and hypocotyl explants.

Plant material and source of explant :

The juvenile hypocotyls, leaf and mature midrib explants were obtained from Central University of Bihar campus for present study. Explants were excised and then surface sterilized with 0.1 % mercuric chloride ($HgCl_2$) for 6 min and rinsed five times in sterile distilled water.

Callus induction:

The *in vitro* juvenile hypocotyls, leaf and mature midrib explants were cultured on MS [8] medium supplemented with different concentration and combination of BAP, NAA, 2,4-D and kinetin to find an optimum concentration of plant growth regulators for callus induction. One explant was taken on each test tube in three replicates in vertical position. The percentage of callus induction of leaf and hypocotyls were recorded after 12 weeks of culture.

Culture conditions :

The importance of maintaining a sterile environment during the culture of plant tissue were necessary. Most tissue culture procedures were conducted in sterile operations, such as laminar flow cabinet. Uniform culture conditions were applied in all experiments. The pH of the medium was adjusted to 5.7 using 1 N KOH or HCL, prior to autoclaving at 1.05 kg/cm^2 pressure at 121°C for 20 min. The cultures were maintained at 25 ± 2 °C under dark period of 12 week.

Callus induction in hypocotyls :

This experiment was carried out to determine the suitable concentration of hormone (Table-1) to induce callus at different concentration and different composition of PGR. By referring to these previous researches, hormones treatment was designed. The concentration of Kn 1 mg/l and 5 mg/l were used followed by NAA 0.25 mg/l and 2,4-D 0.5 mg/l. Explant (hypocotyl) was taken from young plant and grown on fresh full strength MS media containing hormones. After the

explants were grown in different concentration of hormones for twelve weeks, the observation was done. The parameters used to determine the result were the number of calluses induced for different set of kinetin and BAP followed by NAA and 2,4- D, and type of explants were used for callus induction. Out of cytokinin (kinetin, BAP) tested for the purpose of establishing callus, kinetin in combination with NAA proved most effective. On MS + kinetin (1 mg/l) + NAA (0.5 mg/l), the hypocotyls explants swelled and 100 % them callused (Fig. 1A) along the entire surface. The callus obtained from hypocotyls (Fig. 1) was whitish brown, solid and slow growing. The response of hypocotyls segments to callusing with various growth regulators is summarized in (Table-1).

Callus induction in leaf :

This experiment was carried out to determine the suitable concentration hormone (Table-2) to induce callus. Different hormone with different composition and concentration were used. By referring to these previous researches, hormone treatment was designed. The concentration of Kn 1 mg/l and 5 mg/l were used followed by NAA 0.25 mg/l and 2,4-D 0.5 mg/l. The concentration of BAP 1 mg/l and 5 mg/l were used followed by NAA 0.1 mg/l, 0.25 mg/l and 0.5 mg/l. Explant (leaf) was taken from young plant and grown on fresh full strength MS media containing hormones. After the explants were grown in different concentration of hormones for twelve weeks, the observation was done. The number of callusesformed from explant was counted. Result was shown in (Table-2). The parameters used to determine the result were the number of calluses induced for different set of kinetin and BAP followed by NAA and 2,4-D. Out of cytokinin (kinetin, BAP) tested for the purpose of establishing callus, Kn in combination with NAA proved most effective. On MS+ Kn (5 mg/l) + NAA (0.25 mg/l), the leaf explant swelled (Fig. 2G). The callus obtained from leaf greenish white, solid and slow growing.

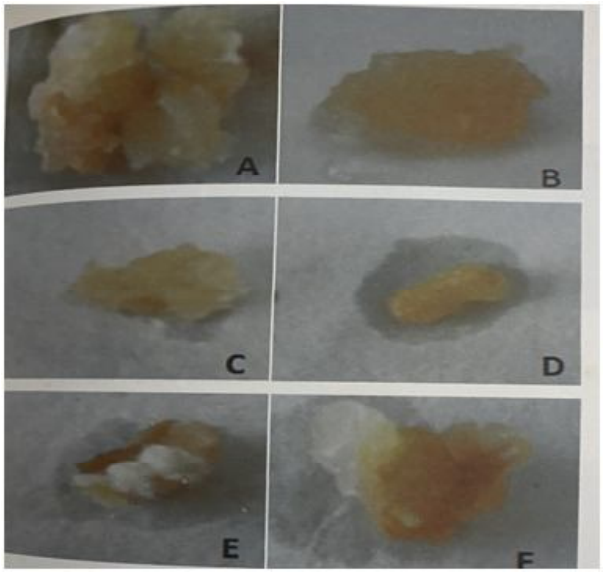

Fig: (A) MS + Kinetin (1 mg/l) + NAA (0.25 mg/l) (B) MS + Kinetin (5 mg/l) + NAA (0.25 mg/l) (C) MS + Kinetin (1 mg/l) + 2,4- D (0.5 mg/l) (D) MS + Kinetin (1 mg/l) + 2,4-D (0.5 mg/l) (E) MS + BAP (5 mg/l) + NAA (0.5 mg/l) (F) MS + BAP (5 mg/l) + NAA (0.1 mg/l)

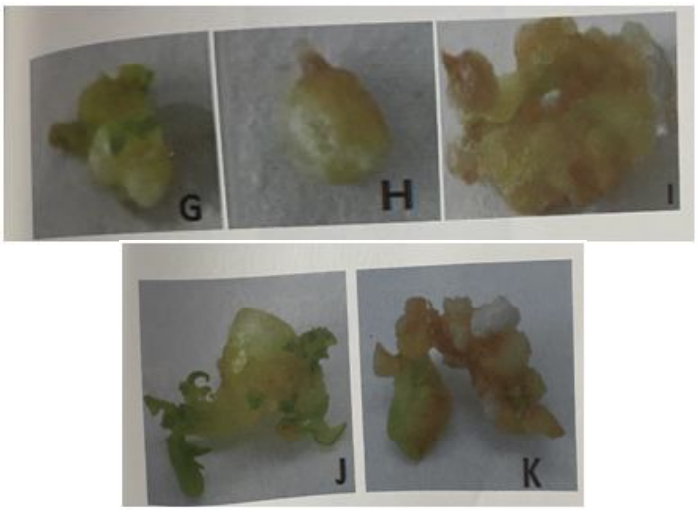

Fig. (G) MS + kinetin (5 mg/l) + NAA (0.25 mg/l) (H) MS + BAP (5 mg/l) + NAA (0.5 mg/l) (I) MS + BAP (1mg/l) + (I) MS + BAP (1 mg/l) + NAA (0.25 mg/l) (J) MS + kinetin (5 mg/l) + NAA (0. 25 mg/l)(K) MS + BAP (1 mg/l) + NAA (0.25 mg/l).

Table-1 Total percentage of calluses were generated from hypocotyls explants with various PGR

PGR				Response
BAP	KIN	NAA	2,4- D	
0	1	0.25	0	58.33
0	5	0.25	0	50
0	1	0	0.5	25
0	5	0	0.5	8.33
5	0	0.5	0	11
1	0	0.5	0	0
5	0	0.1	0	22
1	0	0.25	0	0

Table 1 Total percentage of calluses were generated from leaf explants with various

PGR				Response
BAP	KIN	NAA	2,4- D	
0	1	0.25	0	0
0	5	0.25	0	50
0	1	0	0.5	0
0	5	0	0.5	0
5	0	0.5	0	0
1	0	0.5	0	22
5	0	0.1	0	0
1	0	0.25	0	33

The present investigation was undertaken on *C. papaya* with a view to develop a reliable protocol for callus induction. In *C. papaya*, callus was induced from juvenile hypocotyls and leaf explants on full strength MS medium supplemented with different combination and composition of cytokinin and auxin. Several researchers reported callus induction in half strength[1,3,5,6,10-12]. However best result was obtained from kinetin with NAA over BAP with NAA [12] who reported kinetin (0.5 mg/l) as better treatment for callus induction than 2,4- D. It was also found that 2,4- D better for callus induction and kinetin

essential for for induction of morphogenetic ally[7,11]. In the present study callus induction occurred on both Kn (1 and 4 mg/l) with NAA (0.5 mg/l) and 2, 4- D (0.25 mg/l) but better response was obtained on Kn with NAA. Likewise Mondal *et al*.,[4] reported callus from established buds of C. papaya on MS media supplemented with 1mg/l NAA 3 mg/l kinetin. Callus were also obtained from BAP (1 mg/l and 5 mg/l) with NAA (0.1, 0.25, and 0.5 mg/l) but it was less significant than the Kn with NAA. Highest callus induction (58.33 %) was found in hypocotyls explant on MS media containing Kn (1 mg/l) followed by NAA (0.25 mg/l) among all treatment employed for callus induction. Leaf shows highest (50 %) callus induction on Kn (5 mg/l) followed by NAA (0.25 mg/l). Out of eight composition of different PGR hypocotyls was show callus induction on six compositions whereas leaf it was show only on three compositions of PGR. Overall juvenile hypocotyls and leaf explants emerged significantly better for callogenesis among the explants employed.

An efficient callus induction protocol was established for *C. papaya* using juvenile hypocotyls and leaf explants. The main observations made from this study are that juvenile hypocotyl is good explant over juvenile leaf for callus induction. Among the growth regulators employed kinetin proved significantly better than BAP. Kinetin 5 mg/l fortified with NAA 0.25 mg/l the best treatment among all the combination of PGR used.

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