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Research Article

IN VITRO MUTATION STUDIES IN PAPAYA (*CARICA PAPAYA* L.)

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ABSTRACT
Induction of variability using mutagens have been used for long by breeders A mutated cell in a mass of callus have a larger chance of surviving competition with other cells. The majority of the mutants produced will be solid if shoots are regenerated from a repeated subculture of mutated callus. Hence, mutation studies in papaya were conducted with callus and soot tips. Friable callus was induced from seedling derived shoot tips on MS medium supplemented with 6.0 mg/l of BAP and 2.0 mg/l of IAA. Survival percentage of callus decreased and growth was retarded with increase in gamma ray dosage. It was 81.88 per cent with control while 4 kR gamma irradiation recorded the least survival percentage (21.25%). LD_{50} was found to be 2.75 kR. Callus treated with colchicine showed reduced survival percentage with increase in concentration and duration of colchicine treatment. It was least at 0.1 per cent colchicine (37.50 %) and the highest (78.75 %) with control. Colchicine treatment for 40 m with 0.1 per cent concentration recorded least (33.75) survival percentage. LD_{50} for colchicine treatment of callus was estimated to be 0.05 per cent for 20 minute treatment and 0.027 per cent for 40 minute treatment. Irradiation of shoot tips on multiplication medium (MS + 0.1 mg/l NAA + 0.6 mg/l BAM) stimulated call us initiation from the cut end of the shoot apices at lower doses (0.5, 1 and 3 kR) which decreased with increase in gamma rays dosage, but in control and 4 kR treatment it was nil. LD_{50} was found to be 3 kR. Irradiation of shoot tips also reduced the survival percentage of shoot tips which decreased with increase in gamma ray dosage. In the control 90% of the shoot tips survived while in 4 kR treatment it was 20%. Significant difference was noticed in multiple shoot production with the lower dose (0.5 kR) which stimulated the production of more number of multiple shoot (6.25) compared to control (3.38) and 4 kR (2.25).

Key Words: *Mutation, Irradiation, Colchicine, Callus, Shoot Tips*

INTRODUCTION
Induced mutagenesis work was conducted from 1971 to July 2007, using both physical and chemical mutagens for improvement of a wide range of crops. Both physical (X-rays and Gamma-rays) and chemical (EMS, MMS, Colchicine) mutagens were used for improvement programmes. Chromosome doubling is achieved by using colchicine or other antimitotic agents like oryzalin to obtain fertile plants (Lim and arle, 2008, 2009; Yetisirand Sari, 2003). Various methods can be used to apply colchicine in *vitro* and in *vivo* growth conditions like adding colchicine to the growth media in *vitro* culture immersing roots, plants and single node cuttings into colchicine solution, application of colchicine to lateral buds by medicine dropper and immersing shoot tips of *in vivo* grown plants (Yetisir and Sari, 2003).
Papaya (*Carica papaya)* is one of the important fruit crops with nutritional and medicinal value. The use of callus in mutation studies is a valuable method for reducing chimerism. A mutated cell in a mass of callus seems to have a larger chance of surviving competition with other cells. The majority of the mutants produced by this method will be solid especially if shoots are regenerated from a repeated subculture of mutated callus (Broertjes and VanHarten, 1978). In the present study an attempt was made to find out the optimum dose required for inducing mutations in callus derived cv. 'Coorg Honey Dew'.

Tissue culture work in papaya was started by De Bruigne *et al.* (1974) with the induction of callus using seedling petioles as explants. Later, several workers developed callus from various explants types such as stem segments (De Bruigne *et al.*, 1974; Yie and Law, 1977); cotyledons, midribs and lamina (Litz *et al.*, 1983b and Rajeevan and Pandey, 1983); ovules (Litz and Conover, 1982). Papaya plants were regenerated from callus arising from seedling stem segments on a medium containing 1 mg/l NAA and 0.1 mg/l Kinetin (Yie and Law, 1977).

Callus was induced from lamina and midrib of cotyledons and maintained on modified MS with half strength major salts and chelated iron, 30g/l sucrose. The optimum concentration of growth regulators was found to be 0.3-2.0 mg BAP + 0.5 – 3.0 mg NAA per litre for midribs, 0.1 – 3.0 mg BAP + 1.2 – 5.0 mg NAA per liter for lamina (Litz and Conover, 1982).

Rajeevan and Pandey (1983) used various explants *viz.*, stem segments, petiole, leaf segments and root from 45 days old plantlets for callus production and regeneration. Stem segments produced loose and friable callus pale green in colour on B_5 medium with NAA 10mg/l + K 5 mg/l while root segments produced loose and friable callus creamy white in colour on the same medium with NAA 10 mg/l + K 10 mg/l. Petiole and leaf segments produced slightly compact callus, creamy white in colour on MS medium with 0.5mg/l NAA + BAP 2.5 mg/l. Fitch (1993) cultured 10 days old hypocotyls sections on half strength MS medium to produce callus in 10-14 weeks.

MATERIALS AND METHODS

Various explants such as leaf sections, internodes, petiole segments, root segments, shoot tips were tried for their suitability to callus induction. These explants were collected from 30-45 days old seedlings grown in plastic trays containing vermiculite medium. Explants that showed good response in terms of callus growth were used for induction and maintenance. For irradiation, shoot tips were collected from 45 days old seedlings grown in vermiculite medium.

Leaf segments, internodes, petiole sections, root segments and shoot tips were disinfected with 0.1% mercuric chloride for 10 minutes. The explants were rinsed thrice with sterile triple distilled water. Leaves were cut into sections of 1 cm^2 after sterilization while internodes, petioles, roots and shoot tips were cut into 1 cm segments before inoculation. For shoot tip irradiation, 1 cm segments were disinfected with 0.1 per cent mercuric chloride for 10 minutes.

Callus Induction

Callus was induced from seedling shoot tips (showed good callus growth) of cv. Coorg Honey Dew on Murashige and Skoog medium containing 6 mg/l BAP and 2 mg/l IAA.

Colchicine Solution for Treatment of Callus

One per cent stock solution of colchicine was prepared by dissolving 200 mg colchicine in 20 ml triple distilled sterile water under aseptic condition. Then it was filter sterilized using a micro filter. To prepared colchicine solutions of desirable concentrations *viz.*, 0.025, 0.05 and 0.1 per cent, 2.5, 5.0 and 10 ml of the one per cent colchicine solution was transferred to previously sterilized bottles and the volume was made upto 100 ml. Callus was cut into small pieces and placed in these different concentrations of colchicine and agitated in a rotary shaker with 100 pm for 20 and 40 minutes. Later, the callus was rinsed in sterile distilled water and transferred to callus multiplication medium.

Establishment of Shoot Tips

Shoot tips established in the multiplication medium (MS + 0.1 mg NAA + 0.6 mg BAP) for one week, were irradiated with gamma rays at dose rates of 0.5, 1.0, 2.0 and 3.0 kR with a check to study their response to irradiation.

The cultures were incubated in an air conditioned room at a temperature of 25 ± 2^0 C with a relative humidity of 60 per cent under a photoperiodic regime of 16 h light and 8 h dark cycles. The illumination was provided by cool white fluorescent tubes at an intensity of 35-38 µ EM^{-2} S^{-1}.Starting from surface sterilization of various explants till inoculation on to the medium; all procedures were carried out under sterile laminar air flow cabinet.

Media Formulation

To elicit different responses such as establishment of explants, callus initiation and multiplication, establishment of ovules, initiation of growth, the media used with modifications are presented in the Table 1.

Table 1: Different modified media used in tissue culture study

Purpose	Explants	Media With Modification
Callus induction	Shoot tips	MS + 6 mg/l BAP + 2 mg/l IAA
Shoot tip irradiation	Shoot tips	MS + 0.6 mg/l BAP + 0.1 mg/l NAA (Rajeevan and Pandey, 1983)

Incubation

The cultures were incubated in an air conditioned room at a temperature of 25 ± 2^0 C with a relative humidity of 60 per cent under a photoperiodic regime of 16 h light and 8 h dark cycles. The illumination was provided by cool white fluorescent tubes at an intensity of 35-38 µ EM^{-2} S^{-1}.

RESULTS AND DISCUSSIONS

Callus Induction

Friable callus white or cream in colour was induced successfully within 30 days of culture from seedling derived shoot tips) on MS medium supplemented with 6.0 mg/l of BAP and 2.0 mg/l of IAA. Shoot tips were found to be the best explants for callus induction followed by hypocotyls segments which produced a little amount of callus Callus initiation in shoot tips occurred from the cut end in about one week and a good amount of callus was produced within 4 weeks of culture ((Figure 1). Per cent callusing with shoot tips was found to be 80 per cent and the colour of callus was white or cream or dull white (Table 2 and 3). The response to culture conditions is influenced by both the composition of the nutrient medium and physiological status of the explants tissue. These parameters would have been favourable in shoot tips which accounted for good amount of callus production and less favourable in the case of hypocotyls segments. Other explants such as root segments, leaf segments and petiole segments did not produce callus. This may be due to the limited morphogenetic potential of these explants which were collected from 30-45 days older seedlings and specific nutrient medium requirement of each explant.

Effect of Irradiation on Callus

Callus induced from seedling shoot tips of cv. Coorg Honey Dew, after first subculture, was irradiated with 0.5, 1.0, 2.0, 3.0 and 4.0 kR gamma rays with a check. Callus irradiated with gamma rays reduced the survival percentage of callus and also retarded callus growth. The same results were obtained when embryogenic calluses of orange were irradiated with gamma rays (Nito *et al.,* 1989).

Irradiation of callus with gamma rays reduced the survival percentage of callus after 30 days in culture. Survival percentage of callus decreased with the increase in gamma ray dose (Table 4). Highest survival (81.88%) was noticed with control callus while the lowest (21.25%) was noticed with 4 kR gamma rays. The LD_{50} dose for callus was found to be 2.75 kR. Callus growth was retarded by gamma rays at 3 and 4

kR while the growth was slow in 0.5, 1.0 and 2 kR treatments while the control callus continued its growth.

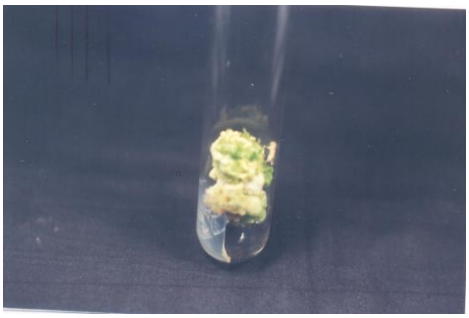

Figure 1: Callus induction from shoot tips

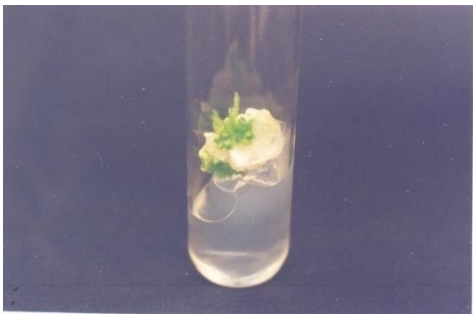

Figure 2: Differentiation of control callus into leafy shoots without subculture

Table 2: Callusing response of explants to MS+6 mg/l BAP + 2 mg/l IAA

Explants	Response
Root segments	-
Leaf segments	-
Shoot tip	+++
Hypocotyl segments	+
Petiole segments	-

+++ Good; + Fair; - Nil

Table 3: Callusing %, callusing frequency, callusing index from shoot tips cultured *in vitro* on MS + 6 mg/l BAP + 2 mg/l IAA

Shoot Tips	Response
Callusing %	80%
Callus appearance	Dirty white or Creamy white
Callusing frequency	30 days of culture

Table 4: Effect of gamma irradiation of callus on survival percentage and callus growth

Treatment	Survival %	Callus Growth
Control	81.88	+++
0.5 kR	74.63	+
1.0 kR	69.25	+
2.0 kR	59.50	+
3.0 kR	39.25	-
4.0 kR	21.25	-
SEM	1.17	
CD	4.59	

+++ Good; + Fair; - Nil

Regeneration of callus was not possible both in control and irradiated callus. But, differentiation of control callus into leafy shoots without subculture for ten weeks was achieved (Figure 2). This is because if sub-culturing is not done at a 4 week interval, it results in the development of roots or shoots (Aitchison *et al.*, 1978).

Effect of Irradiation on Shoot Tips

Shoot tips established in the multiplication medium (MS + 0.1 mg NAA + 0.6 mg BAP) for one week, were irradiated with gamma rays at dose rates of 0.5, 1.0, 2.0 and 3.0 kR with a check to study their response to irradiation. **S**hoot tips irradiated and maintained on multiplication medium induced callus from the cut end at lower doses (0.5, 1 and 3 kR) while control and 4 kR treatment did not produce any callus. . Similar results were reported by Shen *et al.*, (1990) when petiole explants of Chinese gooseberry were irradiated with gamma rays Callus induction was maximum at 0.5 kR which reduced with increase in gamma ray dose (Table 5, Figure 3).

Survival percentage of shot tips was reduced with increase in gamma ray dose with control plants recording the highest (90) while 4 kR treatment recorded the lowest (20) survival percentage. Mak *et al.*, (1995) also noticed a similar trend when banana shoot tips were irradiated. LD_{50} dose was estimated to be 3 kR.

Table 5: Effect of irradiation of shoot tips on callus induction, survival and multiple shoots

Treatment	Callus Induction	Survival %	Multiple Shoots
Control	-	90	3.38
0.5 kR	+++	82	6.25
1.0 kR	++	78	5.13
3.0 kR	+	52	3.13
4.0 kR	-	20	2.25
SEM			0.170
CD			0.675

+++ *Good;* ++ *Fair;* + *Poor;* - *Nil*

Figure 3: Lower gamma rays dose (0.5 kR) showing the multiple shoots

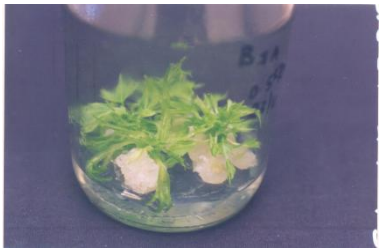

Figure 4: Irradiated shoot tip showing abnormal, narrow and mosaic leaves

Multiple shoots were significantly varied for irradiation of shoot tips. At lower gamma rays dose (0.5 kR) the multiple shoots produced were the highest (6.25) which decreased with increase in gamma rays and the lowest (2.25) was with 4 kR. Irradiated shoot tips also showed abnormal, narrow and mosaic leaves upon 4 weeks of culture (Figure 4).

Effect of Colchicine Treatment on Callus

Callus treated with colchicine showed reduced survival percentage with increase in concentration and duration of colchicine treatment (Table 6). It was least at 0.1 per cent colchicine (37.50) and the highest (78.75) with control. Colchicine treatment for 40 m with 0.1 per cent concentration recorded least (33.75) survival percentage. Callus growth was also retarded with increase in concentration of colchicine. LD_{50} dose for colchicine treatment of callus was estimated to be 0.05 per cent for 20 minute treatment and

0.027 per cent for 40 minute treatment (Figure 5 and 6).Reduced survival percentage of callus and retardation of callus growth upon colchicine treatment both at 20 and 40 minutes treatment is perhaps due to the direct action of colchicine on callus.

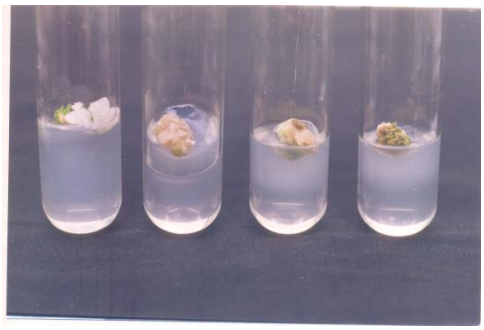

Figure 5: Colchicine treated callus for 20 minutes

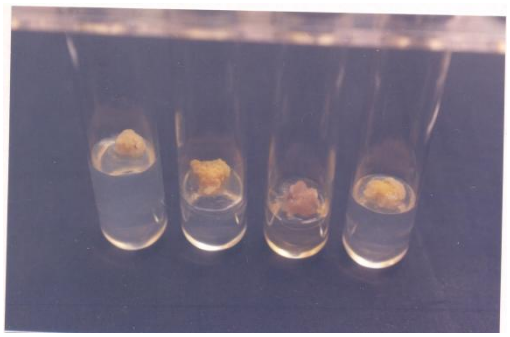

Figure 6: Colchicine treated callus for 40 minutes

Table 6: Effect of colchicine treatment of callus on survival % and growth of callus

Treatment (B)	Survival % Duration (A) 20'	40'	Mean	Callus Growth
Control	18.75	78.75	78.75	+++
0.025%	67.75	60.50	64.12	+
0.05%	48.25	43.25	45.75	+
0.1%	41.25	33.75	37.50	-
Mean	59.00	54.06		
	A	B	A x B	
SEM	0.708	1.001	1.415	
CD	1.961	2.773	3.922	

+++ *Good;* + *Fair;* - *Nil*

Treatment of callus with colchicine concentrations *viz.*, 0.025, 0.05 and 0.1 per cent for 20 and 40 minutes resulted in decreased survival percentage of the callus which decreased with increase in colchicine concentration and duration of treatment (Table 6). Survival percentage of callus was the lowest with 0.1 per cent colchicine for 20 minutes (41.25) and 40 minutes (33.75) treatment while it was highest (78.75) with control .LD_{50} value for survival percentage of callus was estimated to be 0.05 per cent at 20 minutes treatment and 0.027 per cent at 40 minutes treatment.

Callus growth was also retarded by colchicine treatment of callus. Control callus continued its growth while growth was completely retarded at 0.1 per cent colchicine and callus growth was slow at 0.025 per cent colchicine treatment.

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