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# *In vitro* mutagenesis in *Carica papaya* L. using oryzalin as a mutagen

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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. Highest multiple shoots has been obtained from 3.0 mg/l BAP, 0.50 mg/l IAA, 1.0 mg/l NAA. Shoot tips treated with oryzalin showed reduced survival percentage with increase in concentration and duration of oryzalin treatment. It was least at 50 µM oryzalin (17.6%) and the highest (70.0%) with control. $LD_{50}$ for oryzalin treatment of shoot tip was estimated to be 22.7 µM. Treated shoot tips on multiplication medium (MS + 3.0 mg/l BAP,0.50 mg/l IAA and 1.0 mg/l NAA) stimulated shoot initiation from the cut end of the shoot apices at lower doses which decreased with increase in oryzalin concentration.

**Keywords:** Mutagenesis in *Carica papaya* L., oryzalin as a mutagen

## 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, Oryzalin) mutagens were used for improvement programmes. Chromosome doubling is achieved by using antimitotic agents like oryzalin to obtain fertile plants (Lim and arle, 2008, 2009; Yetisirand Sari, 2003) [5, 6]. Various methods can be used to apply oryzalin *in vitro* and in vivo growth conditions like adding oryzalin 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) [14]. Papaya (*Carica papaya*) is one of the important fruit crops with nutritional and medicinal value. The use of direct shoot tip in mutation studies is a valuable method for reducing chimerism. A mutated cell in a multiple shoot 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 multiple shoot. (Broertjes and VanHarten, 1978) [1]. In the present study an attempt was made to find out the optimum dose required for inducing mutations in shoot tips derived cv. 'CO 8'.

Tissue culture work in papaya was started by De Bruigne et al. (1974) [2] 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) [2, 15]; 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) [15]. 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 B5 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) [3] cultured 10 days old hypocotyls sections on half strength MS medium to produce callus in 10-14 weeks.

## Materials and Methods

### Initiation of shoot tip culture

Shoot tip of *Carica papaya* L. cv. CO 8 were collected from the aspectic seedling grown in department of Agricultural Biotechnology, College of Agriculture, Orissa University of Agriculture and Technology. They were washed thoroughly with 2% (v/v) 'Teepol' (Qualigen, Bumbai, India) for about 15 min with constant shaking, and were then rinsed with water from a running tap. Further, its surface was sterilized with 0.1% (w/v) mercuric chloride solution for 30 min with constant shaking followed by rinsing with sterile distilled water thrice. The shoot tip explants were cultured on a semi-solid MS basal medium supplemented with 3.0 mg/l BAP, 0.50 mg/l IAA, 1.0 mg/l NAA Agar-agar (7 g/l) was added to the medium as a gelling agent after adjusting the pH to 5.7 to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving. Routinely, 20 ml of the molten medium was dispersed into the culture tube (25 x 15 mm) plugged with non-absorbent cotton wrapped in one layer of cheesecloth and autoclaved at 121 °C or 1.06 kg/cm2 for 15 min. Consequently, the explants were inoculated aseptically. The cultures were maintained at 25 ± 2 °C under 16 h photoperiod in cool white fluorescent lamps (Philips, Mumbai, India) with 3.0 Klux, and also in a fresh medium (MS + 3.0 mg/l BAP, 0.50 mg/l IAA, 1.0 mg/l NAA + 3% sucrose) at 4 weeks interval.

### Induction of mutation

After a successful development of the multiple shoots, the apical and axilary meristems were isolated aseptically and treated with different concentration (0, 10, 15, 20, 25, 30, 35, 40, 45, 50 µm) of oryzalin. After pretreatment, the survived axilary meristems were removed and shocked in the blotting paper aseptically. Subsequently, the meristems were transferred to the glass petriplate containing the basal MS medium (Murashige and Skoog, 1662) supplemented with 3.0 mg/l BAP, 0.50 mg/l IAA, 1.0 mg/l NAA and oryzalin of the same concentration in which the explants were pretreated and incubated under 16 h. As such, the photoperiod was provided by cool and fluorescent light (3000 lux). After two weeks, the treated explants were transferred to the test tube containing MS basal medium supplemented with 3.0 mg/l BAP, 0.50 mg/l IAA, 1.0 mg/l NAA without oryzalin. After another 4 weeks, the explants were transferred to the multiplication medium. After a successful multiplication, shoots from the shoot clumps were separated and inoculated into a semi-solid halfstrength MS medium supple-mented with 2% (w/v) sucrose and with rooting medium. Thus, one excised shoot was placed in each tube (25 x 150 mm) having 15 ml of the culture media. All the cultures were incubated at 25 ± 2 °C under 16 h photoperiod with 3.0 Klux intensity. Rooted micro propagules were thoroughly washed to remove the adhering gel and were planted in an earthen pot containing 100% sand for 2 weeks in the green house. After developing the good root systems, it was subsequently transferred to 6" earthen pots containing sterile mixture of sand, soil and cowdung manure in the ratio of 1: 1: 1 (v/v). The pots were kept in the green house for acclimatization and the experiment was repeated at least three times. The data pertaining to Number of dead shoot, number of micro shoot survived, survival percentage, corrected mortality and $LD_{50}$ were statistically analyzed by the Post-Hoc Multiple Comparison test (Marascuilo and McSweeney, 1977). Between the treatments, the average figures followed by the same letters were not significantly different at $P < 0.05$ levels.

## Result and Discussion

A decrease in the survival of shoot tip culture was observed as the concentration of oryzalin in the medium increased. It was also observed that with the increasing pre-treatment time period, the percentage of survival decreases. At control there has been found to be highest percentage of survival (70.0%). Total 50 number of shoot tip has been inoculated in each treatment and replicated thrice. In control there has been 15 number of dead shoots and gradually number of dead shoots ioncresaes as concentration of oryzalin increases. At 10 µM it was found to be 21.3 and at 50 µM, the number of dead shoots were found to be 41.2 numbers out of 50 cultures. Likewise the survived microshoot were found to be highest in control that is 35 number out of 50 and least had been obtained from 50 µM (8.8 numbers). The survival percentage was highest at control (70%) and we got the fifty percent survivality had been obtained from at 22.7µM. After getting the 50% survivability, the treated shots were transferred to the different medium for multiplication and rooting, followed by acclimatization. However, it showed significant difference than that of the control for multiple shoot initiation and survival. As we have found some percentage of dead in control, so calculated corrected mortality and it was found to be highest at 50 µM. The mutagenic agent oryzalin has been found to be effective to induce mutation as different concentration of this chemical changes the survival rate as well as the growth of shoot tip.

Changes in DNA, caused by mutagens, result in a genetic variation (Teparkum and Veilleux, 1998; Lanteri, 2001) [13, 4]. However, it has to be noted that direct-acting alkylating agents, such as EMS, primarily cause point mutations which are the result of a single base pair deletion, addition or substitution (Schy, 1989) [12]. Changesin growth either cause change in sequence of the primer binding site, such as point mutations, or from changes altering the size or preventing successful amplification of a target DNA such as insertions, deletions and inversions (Rani, 1995) [11].

It is possible that these mutations have occurred in different loci, although it is not yet known whether these mutations have resulted in alterations of agronomical useful traits.

**Table 1:** Effect of Oryzalin on survival percentage of *in vitro raised Carica papaya* L. micro shoots

| Treatment | Concentration (µM) | Dead microshoots | Microshoots survived | Survival (%) | Corrected mortality | LD 50 |
|---|---|---|---|---|---|---|
| $T_1$ | Control | 15.0 | 35.0 | 70.0 | 0.0 | |
| $T_2$ | 10 | 21.3* | 28.7* | 57.4* | 7.5 | |
| $T_3$ | 15 | 22.5* | 27.5* | 55.0* | 8.8 | |
| $T_4$ | 20 | 23.5* | 26.5* | 53.0* | 9.9 | |
| $T_5$ | 25 | 28.8* | 21.2* | 42.4* | 16.2 | |
| $T_6$ | 30 | 30.0* | 20.0* | 40.0* | 17.7 | 22.7 µM |
| $T_7$ | 35 | 33.3* | 16.7* | 33.4* | 21.5 | |
| $T_8$ | 40 | 38.8* | 11.2* | 22.4* | 27.9 | |

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|---|---|---|---|---|---|---|
| $T_9$ | 45 | 40.0* | 10.0* | 20.0* | 29.4 | |
| $T_{10}$ | 50 | 41.2* | 8.8* | 17.6* | 30.8 | |
| SE (m) ± | | 1.46 | 1.82 | 2.80 | 1.95 | |
| CD (p=0.05) | | 4.4 | 5.4 | 8.3 | 5.8 | |
| CV (%) | | 9.1 | 8.1 | 7.4 | 10.1 | |

## Conclusion

From the above study it has been concluded that oryzalin though not commercially used in agricultural industry as a mutagenic but can used successfully as a chemical mutagen to create genetic variability in different fruit crops where conventional breeding takes longer time and non predicatable due to heterozygosity and cross pollinated behavior.

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