

# Development of a Callogenic Protocol in Papaya (*Carica papaya* L.). 1. *In vivo* Grown Vegetative Explants

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## ABSTRACT

Different explants from *in vivo* grown seedlings of papaya (*Carica papaya* L.) were appraised for callogenesis on half strength organic and inorganic salts of Murashige and Skoog (MS) media. It was supplemented with 6% of sucrose and growth regulators like 2,4-D (2.5, 5.0 & 7.5 mg L<sup>-1</sup>) and kinetin (0.5, 1.0 & 1.5 mg L<sup>-1</sup>) for callogenesis, separately. Media for regeneration and proliferation consisted of MS media modified with NAA (1.0, 3.0 & 5.0 mg L<sup>-1</sup>). Earliest callus induction was observed in leaf blade explant at control on 15th day of culture. Callus initiation (%) revealed leaf blade with midrib as the best explant with 70% callus induction in all the treatments employed. Maximum callus growth was noticed in leaf blade with midrib explant (0.1817 g). Leaf blade with midrib and petiole explants showed earliest callus multiplication on NAA (3 & 1 mg L<sup>-1</sup>) on 25th day, respectively. Maximum callus multiplication (%) was yielded by petiole explant derived callus (20% cultures) on NAA (1 mg L<sup>-1</sup>). The protocol may help to propagate male and female plants swiftly by subsequent embryogenesis and organogenesis. It may further be contributive in developing synthetic seeds and transgenic plants of papaya.

**Key Words:** Callogenesis; Papaya; *In vitro*; 2,4-D; Kinetin; NAA

## INTRODUCTION

Papaya (*Carica papaya* L.), family Caricaceae, is a fruit of tropics. Pakistan is contributing only about 8,500 metric tonnes in the total papaya production of the world, (Anonymous, 2000). It is commercially produced in the districts of Karachi and Thatta on an area of about 550 hectares. Though papaya is easily propagated through seed but enormous variation exists due to sexual propagation in commercial plantings. Clonal propagation procedures like grafting and root cuttings do exist but are slow and impractical on large scale. *C. papaya* being dioecious in nature bears a dilemma of sexual characterisation at seedling stage which imbalances proportion of male and female plants drastically.

Propagation of papaya through tissue culture presents an alternative. Several scientists have worked callogenesis from tissues like anthers (Litz & Conover, 1979), cotyledon (Jordan, 1989), hypocotyl (Fitch, 1993), lamina and midrib explants (Prahardini & Sudaryono, 1992), ovules (Rojas & Kitto, 1991), shoot tips (Yang & Ye, 1992) and petiole (Mosella & Iligarray, 1985); root, stem and leaf segments from *in vitro* grown seedlings (Mondal *et al.*, 1994), buds (Jordan & Velozo, 1995) and suspension cultures (Ye *et al.*, 1993).

Callus has successfully been induced on different media formulations supplemented with several different growth regulators. Half strength MS media used for callus induction from various explants employed is verified in pawpaw (Litz *et al.*, 1983), and species like *C. papaya* and *C. cauliflora* (Moore & Litz, 1984); babaco, *C. pentagona* (Rojas & Kitto, 1991), Hawaiian papaya cvs. Kapoho, Sunset, Sunrise and Waimanalo (Fitch, 1993; Mondal *et al.*,

1994). Certain media employed other than MS involve Wood and Braun media (Medora *et al.* 1984), De Fossard medium (Drew, 1987) and NN media (Jordan & Velozo, 1995). Induction of callus in media containing 2, 4-D is supported in pawpaws (Medora *et al.*, 1979), *C. papaya*, *C. monoica*, and *C. candamarcensis* (Kumar *et al.*, 1992), in *C. papaya* (Yang & Ye, 1992) and Hawaiian papaya cvs. Kapoho, Sunset, Sunrise and Waimanalo (Fitch, 1993). Addition of kinetin to the media for callus induction is supported in sp. *C. candamarcensis* (Jordan *et al.*, 1983), *C. papaya* cv. Honey Dew (Mondal *et al.*, 1990) and *C. pubescens* Lenne et Koch 'highland papaya' (Jordan & Velozo, 1995). The contrary findings are with addition of NAA to MS media in *C. candamarcensis* (Jordan *et al.*, 1983), in pawpaw with ammonium nitrate (Medora *et al.*, 1979), BA (Yang & Ye, 1992) and NAA (Litz *et al.*, 1983; Jordan, 1989; Prahardini & Sudaryono, 1992), NAA and GA (Mosella & Iligaray, 1985), adenine sulphate and CH (Winnaar, 1987), GA (Mosella & Iligaray, 1985), and NAA in combination with zeatin and benzyl adenine (Mondal *et al.*, 1994).

This study was planned to evaluate *in vivo* and *in vitro* grown vegetative explants and growth regulators for callogenesis. This paper, however, reports the findings of study on *in vivo* grown vegetative explants. The results of similar research on *in vitro* vegetative grown explants have been reported by Usman *et al.* (2002). The modus operandi might be helpful in generating somatic embryogenesis and subsequent organogenesis from the explant derived callus. Keeping in view the dioecious nature of papaya, this protocol may be promising for commercial propagation of male and female plants. This system may also provide a tool to produce synthetic seeds and transgenic plants of papaya.

## MATERIALS AND METHODS

The basal medium contained half strength organic and inorganic salts of Murashige and Skoog (1962) supplemented with 6% of sucrose. The medium pH was adjusted to 5.7 and gelrite 1.6% was added in the media as solidifying agent. Media was autoclaved for 15 min at 121°C under 1.5 Kg/cm<sup>2</sup> pressure. For callogenesis, basal media was supplemented with 2, 4-D (2.5, 5.0 & 7.5 mg L<sup>-1</sup>) and kinetin (0.5, 1.0 & 1.5 mg L<sup>-1</sup>), separately. Regeneration and proliferation media contained MS modified with NAA (1.0, 3.0 & 5.0 mg L<sup>-1</sup>). Explant types employed for callogenesis were leaf blade (1 cm<sup>2</sup>), leaf blade with midrib (1 cm<sup>2</sup>), petiole (0.5 cm<sup>2</sup>) and leaf blade with midrib plus petiole (1 cm<sup>3</sup>). Explants were surface disinfested by submerging in 70% alcohol plus one to two drops of Tween 20 as surfactant for one minute and in 0.5% NaOCl for two to three minutes followed by 3-5 washings with autoclaved deionized water. Explants were excised and cultured in the callogenic media and were kept in growth room at temperature 25 ± 2°C and fluorescent light intensity of 2500 lux. Callus was weighed on fresh weight basis and subcultured on the regeneration and proliferation media.

Data were collected for callus induction (days), callus initiation (%), callus growth (g), callus multiplication (days) and callus multiplication (%). There were three levels used for each growth regulator with 10 replications in each treatment. The completely randomised design was applied in the experiment.

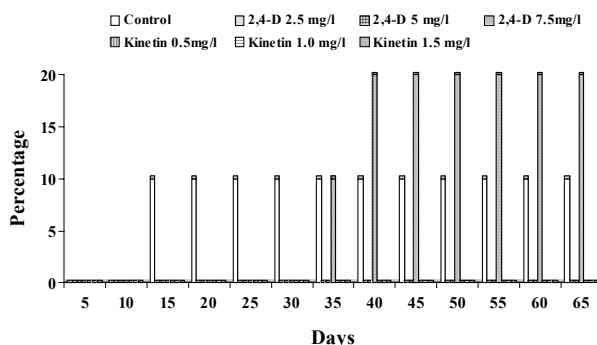
## RESULTS AND DISCUSSION

**Time span for callus induction (days).** MS media without growth hormones (control) took 15 days to induce callus from leaf blade explant ensued by 2, 4-D 7.5 mg L<sup>-1</sup> (30 days). Rest of the treatments did not produce any callus (Fig. 1). Leaf blade with midrib explant responded to media containing 2, 4-D (2.5 mg L<sup>-1</sup>) with earlier callus induction (10 days) whereas control and 2, 4-D (7.5 mg L<sup>-1</sup>) induced callus on 25th day (Fig. 2). Results obtained are contrary to the findings of Prahardini and Sudaryono (1992) who reported best callus induction in midrib explant in seven days on MS media supplemented with combinations of BA and NAA while our results depicted earliest callus induction on MS media with 2, 4-D (2.5 mg L<sup>-1</sup>) on 10th day. Though the basic salts employed are same, the growth regulators and their concentrations varied. Petiole induced callus on control on 20th day (Fig. 3). Leaf blade with midrib and petiole explant produced no callus on any treatment.

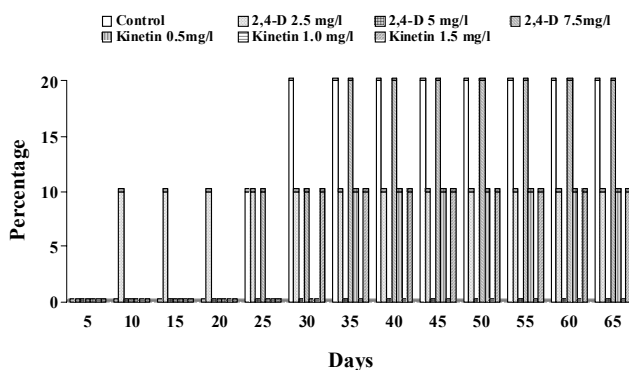
**Callus initiation (%).** Leaf blade with midrib explant proved to be the best on the basis of total callus initiation percentage in all the treatments employed (70%) followed by leaf blade (30%) and petiole (10%) (Fig. 4). The results displayed by leaf blade with midrib explants are in accordance with Prahardini and Sudaryono (1992) for callus induction. Further experimentation is needed for

rationalisation of the explants employed. Control and 2, 4-D (7.5 mg L<sup>-1</sup>) induced maximum callus (40%). 2, 4-D (2.5 mg L<sup>-1</sup>) and kinetin (0.5 & 1.5 mg L<sup>-1</sup>) produced 10% callus each. Kinetin at moderate concentration (1.0 mg L<sup>-1</sup>) could not induce any callus. The best results were obtained for employing callus induction with 2, 4-D (50%) growth regulator followed by control (40%) and kinetin (20%).

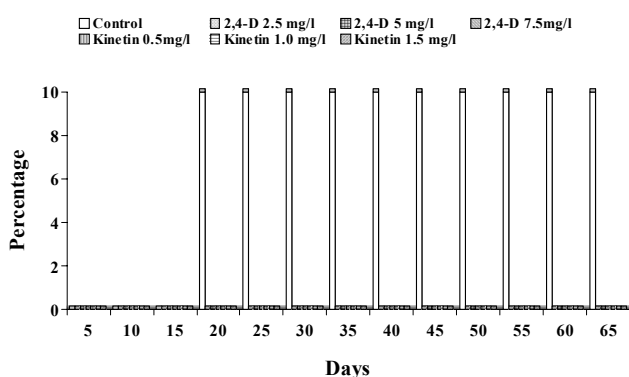
**Fig. 1. Time span for callus induction in leaf blade explant of papaya**



**Fig. 2. Time span for callus induction in leaf blade with midrib explant of papaya**

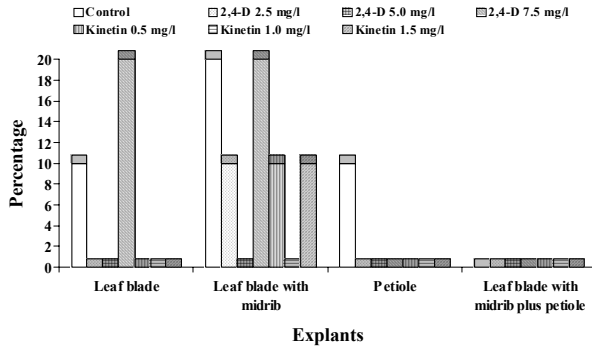


**Fig. 3. Time span for callus induction in petiole explant of papaya**

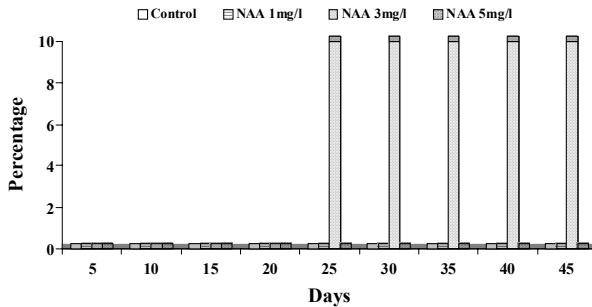


These results are compatible with the findings of Kumar *et al.* (1992). They found 2, 4-D beneficial for callus induction in *C. papaya*, *C. monoica* and *C. candamarcensis* while kinetin at low concentration was essential for growth of morphogenetically active callus. The findings of Fitch (1993) are also in accordance with the current results.

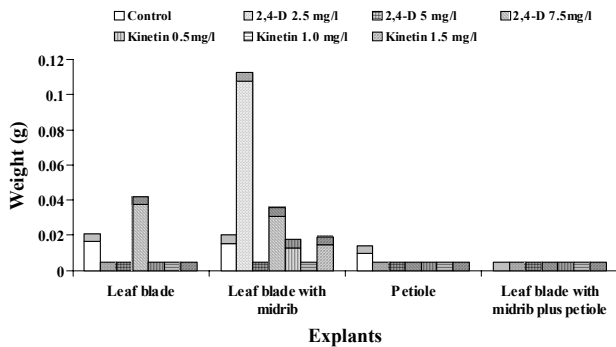
**Fig. 4. Effect of growth hormones on callus initiation (%) of papaya explants**



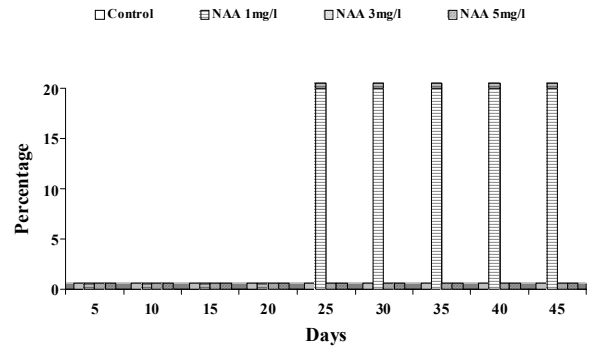
**Fig. 5. Effect of growth hormones on callus growth of papaya explants**



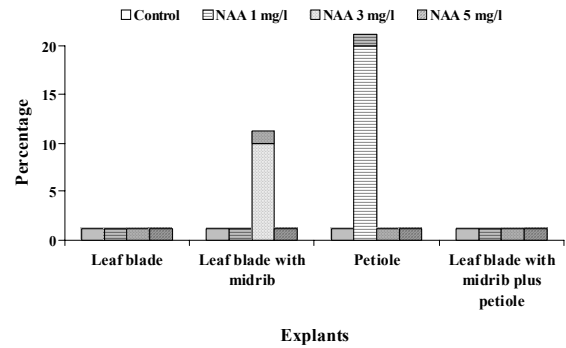
**Fig. 6. Time span to induce callus multiplication in leaf blade with midrib explant of papaya**



**Fig. 7. Time span to induce callus multiplication in petiole explant of papaya**



**Fig. 8. Effect of growth hormones on callus multiplication (%) of papaya explants**



**Callus growth (g).** Maximum callus growth was generated by leaf blade with midrib explant (0.1817 g) followed by leaf blade (0.0538 g) and petiole (0.0094 g) (Fig. 5). Little is known about the callus growth in terms of weight. Among treatments employed 2, 4-D (2.5 mg L<sup>-1</sup>) generated maximum callus weight (0.108 g) followed by 2, 4-D at 7.5 mg L<sup>-1</sup> (0.0685 g). As a whole 2, 4-D (0.1765 g) emerged better than kinetin (0.0273 g) for callus growth. The results of better performance of 2, 4-D are similar to Kumar *et al.* (1992) and Fitch (1993). Similar findings were obtained by Litz *et al.* (1983), Moore and Litz (1984), Rojas and Kitto (1991), and Mondal *et al.* (1994). The callus induction in media containing 2, 4-D is in accordance with various researchers (Medora *et al.*, 1979; Kumar *et al.*, 1992; Yang & Ye, 1992; Fitch, 1993). The addition of kinetin for callus induction is supported by Jordan *et al.* (1983), Mondal *et al.* (1990), and Jordan and Vellozo (1995).

**Time span to induce callus multiplication (days).** In leaf blade with midrib and petiole, callus multiplication was observed only on NAA (3 & 1 mg L<sup>-1</sup>) on 25th day, respectively (Fig. 6 & 7). Other explants displayed no callus multiplication.

**Callus multiplication (%).** Leaf blade alone and leaf blade with midrib plus petiole did not multiply callus on

any treatment. Leaf blade with midrib produced callus in 10% cultures on NAA (3 mg L<sup>-1</sup>). Petioles yielded callus in 20% cultures on NAA (1 mg L<sup>-1</sup>), (Fig. 8). There were better results for callus multiplication by NAA (1 mg L<sup>-1</sup>) than NAA (3 mg L<sup>-1</sup>). Control and NAA (5 mg L<sup>-1</sup>) did not respond as callus proliferation media. The results are contrary to Prahardini and Sudaryono (1992) who observed root formation from midrib explant derived callus of papaya cv. Dampit on MS media in combination with NAA and BA. The reason might be difference in concentration of the growth regulators used and their combination and the variety employed.

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