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RESPONSE OF REPRODUCTIVE ORGANS OF PAPAYA TO CALLOGENESIS

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Different explants excised from reproductive organs of papaya *(Carica papaya* L.) were appraised on half strength Murashige and Skoog (MS) medium. It was supplemented with 2,4-D (2.5. 5.0 & 7:5 rngl.") and kinetin (0.5, 1.0 & 1.5 mgl.") for callogenesis. separately. Highest initial callus induction (100% and 75%) was found in ovary explants on Kinetin (1.5 and 0.5 mg L") in first week of culture, respectively. Ovary and embryo proved to be the best explant for callus induction (68.57% and 67.1%) and callus growth (68.72 mg and 62.7 mg), respectively. Use of these two as explant source might be suggested as the best among various reproductive parts explored. Kinetin proved better growth hormone for both callus induction and callus growth than 2,4-D. This protocol can be helpful 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; reproductive explants; embryo; nucellus.

INTRODUCTION

Papaya *(Carica papaya* L.), family Caricaceae, is a fruit of tropics. Pakistan is contributing only about 8,500 metric tonnes in the total production of the world (FAO, 2000). It is commercially produced in Karachi and Thatta districts on an area of 550 hectares. Papaya being dioecious bears 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 methodology for plant propagation. Several researchers have worked on callogenesis from tissues as: anthers (Litz & Conover, 1979), cotyledon (Jordan, 1989), ovules (Rojas & Kitto, 1991), buds (Jordan & Velozo, 1995) and suspension cultures (Ye *et al.,* 1993). Successful callusing have been obtained on different media supplemented with activated charcoal (Litz & Conover, 1979), kinetin (Jordan *et al*., 1983; Usrnan *et al.* 2002a; b), BA (Jordan. 1989), half strength (Moore & Litz, 1984; Usman *et al.* 2002a; b) and full strength MS media (Chen *et al.*, 1991), 2,4-D (Dhir & Yadava, 1995; Usman *et al.* 2002a; b), GA_3, CH and IAA (Rojas & Kitto, 1991), NN medium (Jordan & Velozo, 1995). The study was initiated to evaluate various reproductive explants and efficacy of growth regulators for callogenesis on MS media (Murashige & Skoog, 1962) supplemented with certain growth regulators. We have already demonstrated the results on callogenesis from *in vivo* and *in vitro* grown vegetative explants of papaya (Usman *et al.* 2002 a,b). This paper, however, includes the development of protocol for callogenesis from field grown reproductive organs of papaya. The modus operandi might be promising for commercial propagation of male and female plants. The system explained here may also help to improve the understanding in developing synthetic seeds and transgenic plants in papaya.

MA TERIALS AND METHODS

The basal medium contained half strength organic and inorganic salts of Murashige and Skoog (1962) medium supplemented with 6% of sucrose. The medium pH was adjusted at 5.7 and gelrite (1.6%) was used as solidifying agent in the media. Media was autoclaved for 15 minutes at 121QC under 1.5 Kg/crn^2 pressure. For callogenesis basal media was supplemented with 2,4-D (2.5, 5.0 & 7.5 mg/") and kinetin (0.5, 1.0 & 1.5 rng/"), separately (Usman *et al.* 2002 a, b). Explant types employed were embryo. nucellus, petal (1 cm), sepal (I cm), stigma (0.4 cm^2), stigma plus ovary (1 ern"), ovary (0.5 cm^2) and floral bud (0.5 cm^2). Explants were surface sterilized by submerging in 70% ethanol plus one to two drops of Tween 20 (surfactant) for one minute and in 0.5% NaOCl for two to three minutes. Explants were then rinsed three to five times with double distilled water.. Explants were excised, cultured in the media and were kept in growth room at temperature 25 ± 2 °C with fluorescent light intensity of 2500 lux. Callus generated was weighed on fresh weight basis and data were collected as effect of growth hormones on callus induction, callus induction percentage and callus growth (mg). The experiment was laid out in completely randomized design (CRD). There were three replications with ten observations per treatment per replication. The data was analyzed by Duncari's Multiple Range test (DMR) at probability >5% (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Effect of growth hormones on callus induction

In embryo cultures earliest callus induction was observed in the first week on MS media supplemented with kinetin 0.5 mgl." (20%) followed by 2, 4-D 5 mgl." (15%). While 2, 4-D (2.5 rngl.") and kinetin (I & 1.5 mgl.") depicted only 5%

callus induction in the first week. Control induced callus (5%) in second week while no callusing was found on 2, 4-0 (7.5 mgl.") till ninth week (Fig. I). Nucellus explant generated callus was observed in the first week in control (5%) whereas other treatments did not show any growth throughout the experiment (Fig. 2). Moore and Litz (1984) and Rojas and Kitto (1991) found similar results as callus was generated only from the reproductive tissues (ovule) on half strength MS media with 6 gl." sucrose and glutamine plus 20% v/v coconut water in one month.

In petal cultures first callus induction was observed on kinetin 0.5 mg 1:1 (15%) in the first week followed by control (10%: Fig. 3). While petal explant showed no response on 2, 4- 0 (5 & 7.5 mgl.") and kinetin (I & 1.5 rngl."). No treatment induced callus in sepal explant at any time interval. Earliest callus induction was observed in stigma explant on kinetin (1.5 & 0.5 mgl.") in first week (10% and 5%), respectively. Control and 2,4-0 (2.5 mgl.") induced callus in third week. In stigma plus ovarian explant faster callus induction was observed in kinetin 0.5 mgl," (10%) ensued by 5% callus induction in 2,4-0 (5 mgl.") and kinetin (I & 1.5 mgl."), Control showed delayed response for callus induction. Higher levels of 2,4-0 (7.5 mgl,") induced no callus in both types of explant (Fig. 4 & 5). Ovary derived explants depicted earliest callus induction (100%, 75% and 40%) on kinetin 1.5, 0.5 & I mgl.", respectively. All the treatments employed induced callus in the ovarian explants with maximum induction (100%) at kinetin 0.5 and 1.5 mg L-' (Fig. 6).

Floral bud exhibited earliest callus induction on kinetin 0.5 mgl." (15%) in the first week followed by kinetin 1.0 mgl." (5%). Maximum time for callus induction (5 weeks) was taken on kinetin 1.5 rngl." (Fig. 7). Use of 2, 4-0 @ 5 and 7.5 rngl." depicted no response for callus regeneration. Mondal *cl al.* (1990) reported similar observations and generated callus from established buds of C. *papaya* on MS media supplemented with I mgL-1 NAA and 3 rngl." kinetin. There was slight variation in the concentration of the growth regulators used. Our findings proved little better compared to Jordan and Velozo (1995) who obtained callus from axillary buds of C. *pubesence* (Highland papaya) on NN medium with NAA plus kinetin after 3 months. The divergent behaviour might be due to media, kind of growth regulators and particularly the cultivar used.

Callus induction percentage

Highest callus induction (100%) was found in embryo explant on MS media supplemented with 2, 4-0 (2.5 & 5 rngl,") and kinetin (0.5 & I rngl.") followed by ovary explant which showed maximum callus induction (100%) on kinetin (0.5 & 1.5 rngl.") and 2, 4-0 2.5 rngl," (90%). Overall significant (P>0.05) callus induction was found in ovary explant (68.57%) followed by embryo (67.1 %) in all the treatments employed and were statistically at par. Floral bud, stigma and stigma plus ovary obtained intermediary position for callus induction showing significantly less callus induction. Minimum callus induction (2.85%) was observed in nucellus explant while sepals remains unresponsive (Table, I). These results are in line with Mondal *el al.* (1990) and differ with Jordan and Velozo (1995) for their findings in floral bud explant. Results in nucellus explant for callus initiation percentage are similar to Rojas and Kitto (1991) and contrary to the findings of Moore and Litz (1984). Among treatments used for callus induction in all the explants, kinetin 0.5 mgl," (36.25%) came forth as the best treatment followed by 2, 4-0 2.5 mg L-1 (31.25%). The minimum response in callus induction was observed on 2,4-O 7.5 rngl." (3.75%). These observations are in line with Usman *et at.* (2002b) who reported kinetin (0.5 mg L-1) as better treatment for callus induction than 2,4-0. Our findings are contrary to Kumar *et al.* (1992) and Usman *cl al.*, (2002a) who reported 2, 4-0 better for callus induction and kinetin essential for induction of morphogenetically active callus in C. *papaya*, C. *monoica* and C. *candamarcensis*. The findings of Fitch (1993) are not in accordance who reported callus induction on half strength MS media plus 2, 4-0 in dark.

Table 1. Effect of growth hormones on callus induction percentage of papaya explants

Explants	Treatments							Means
	Control	2,4-0 2.5 mgL-1	2,4-0 5 mgL-1	2,4-0 7.5 mgL-1	Kinetin 0.5mgL,1	Kinetin 1.0mgL,I	Kinetin 1.5mgL"1	
Embryo	20 c	100 a	100 a	-	100 a	100 a	50 b	67.1 a
Nucellus	20 c	-	-	-	-	-	-	2.85 c
Sepal	-	-	-	-	-	-	-	-
Petal	10 d	20 c	-	-	20 c	-	-	7.14 c
Stigma	20 c	10 d	10 c	-	20 c	20 c	20 c	14.28 b
Stigma plus ovary	10 d	20 c	10 c	-	20 c	10 d	10 d	11.42 b
Ovary	50 a	90 b	70 b	30 a	100 a	40 b	JOO a	68.57 a
Floral Bud	30 b	10 d	-	-	30 b	20 c	10 d	14.28 b
Means	20 c	31.25 b	23.75 c	3.75 d	36.25 a	23.75 c	23.75 c	

Fig. 1. Effect of Growth Hormones on Callus Induction in Embryo Explant

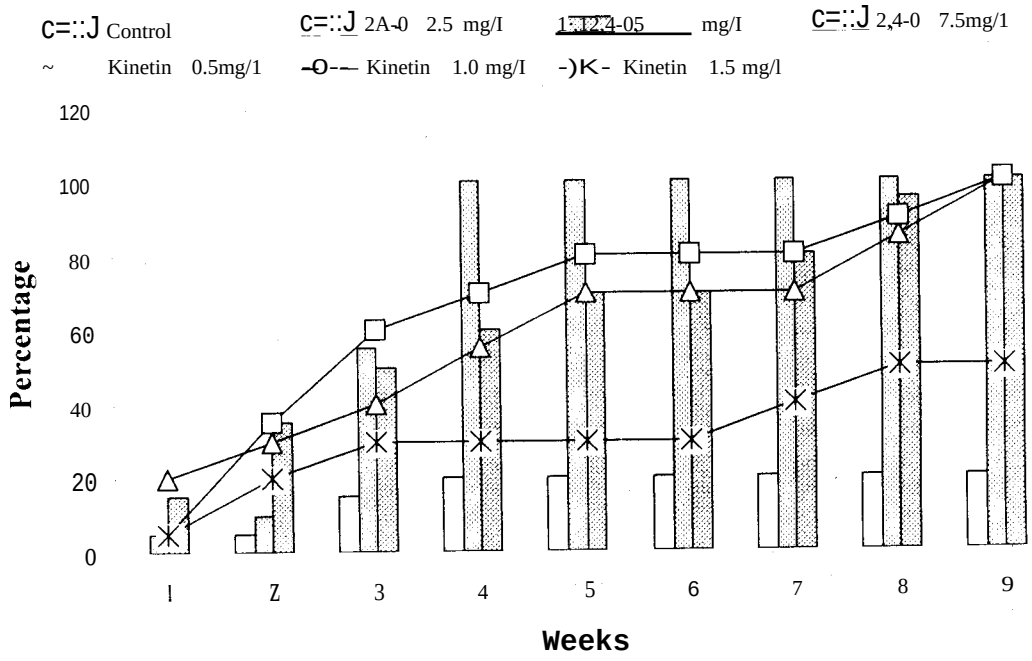


Fig. 2. Effect of Growth Hormones on Callus Induction in Nucellus Explant

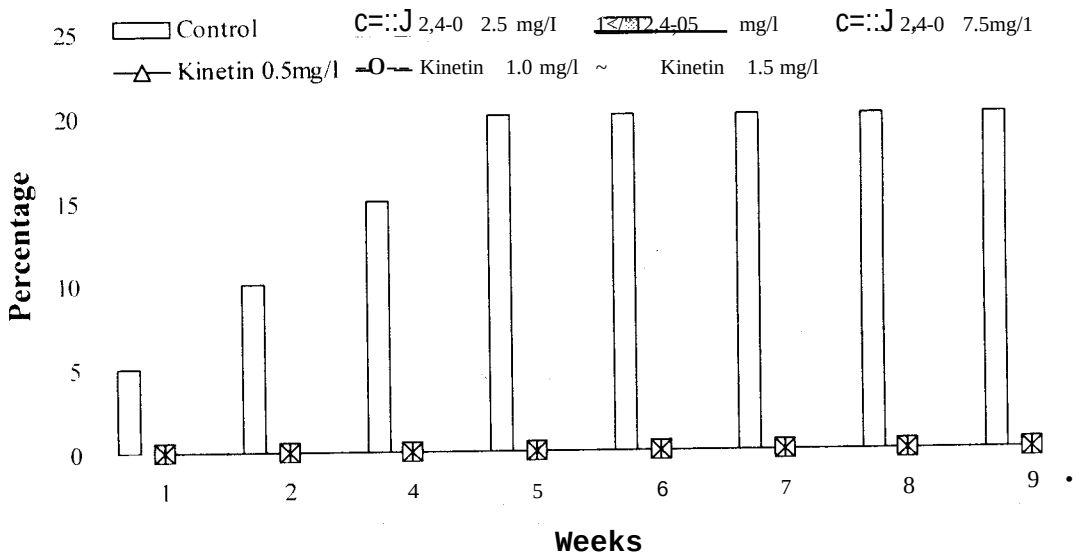


Fig. 3. Effect of Growth Hormones on Callus Induction in Petal Explant

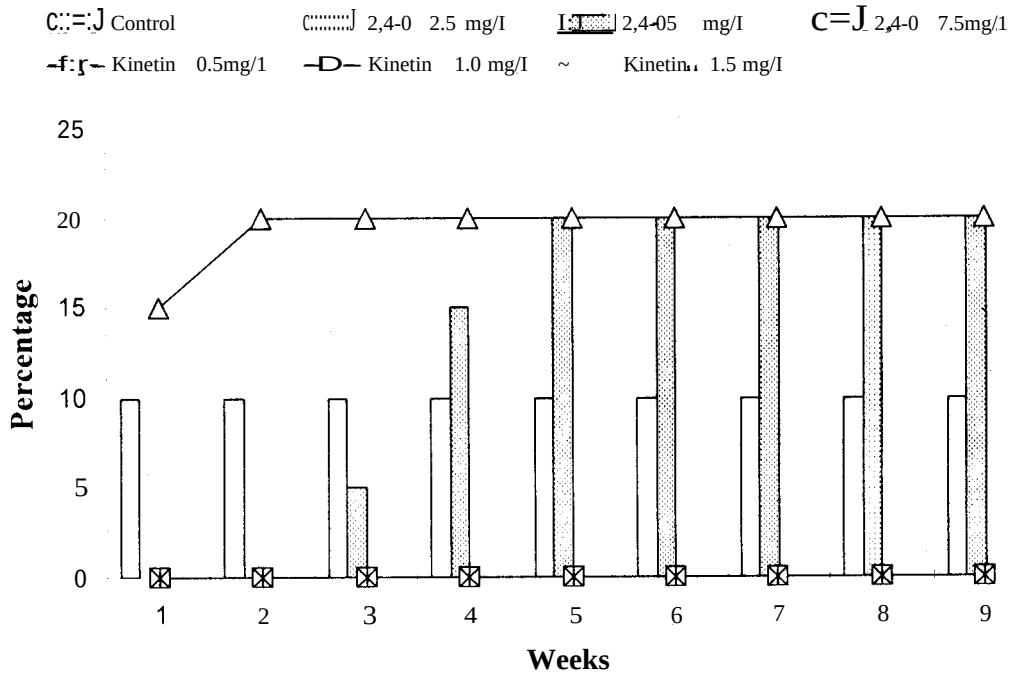


Fig. 4. Effect of Growth Hormones on Callus Induction in Stigma Explant

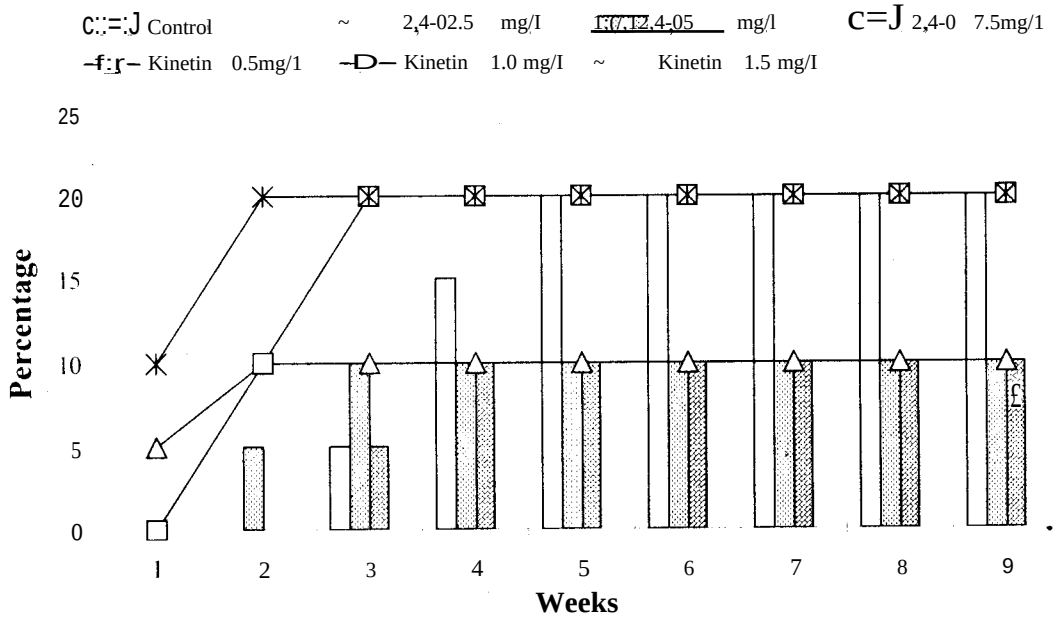


Fig. 5. Effect of Growth Hormones on Callus Induction in Stigma plus Ovary Explant

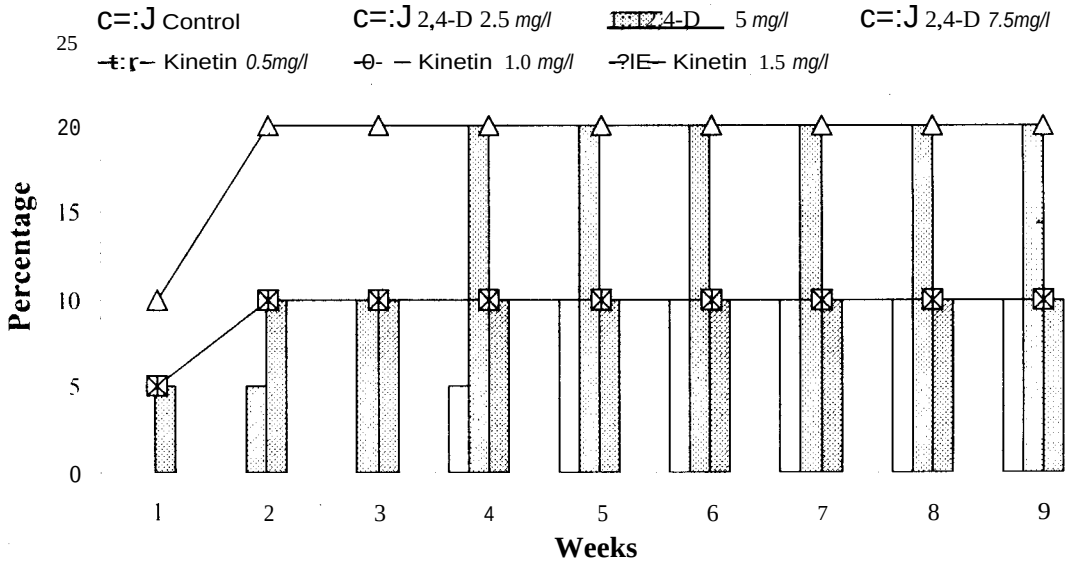


Fig. 6. Effect of Growth Hormones on Callus Induction in Ovary Explant

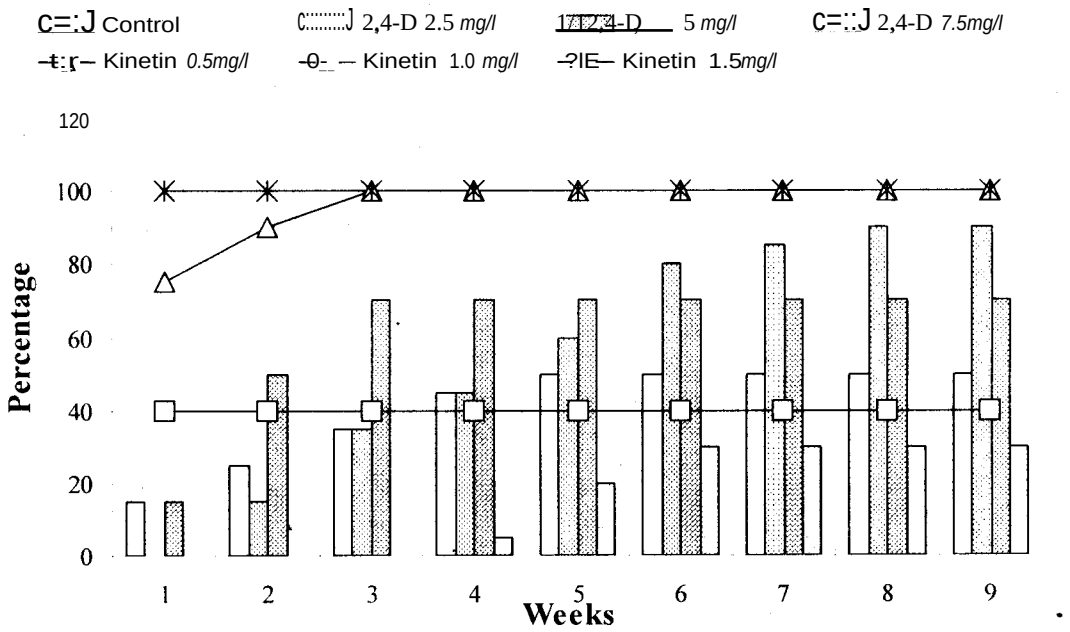
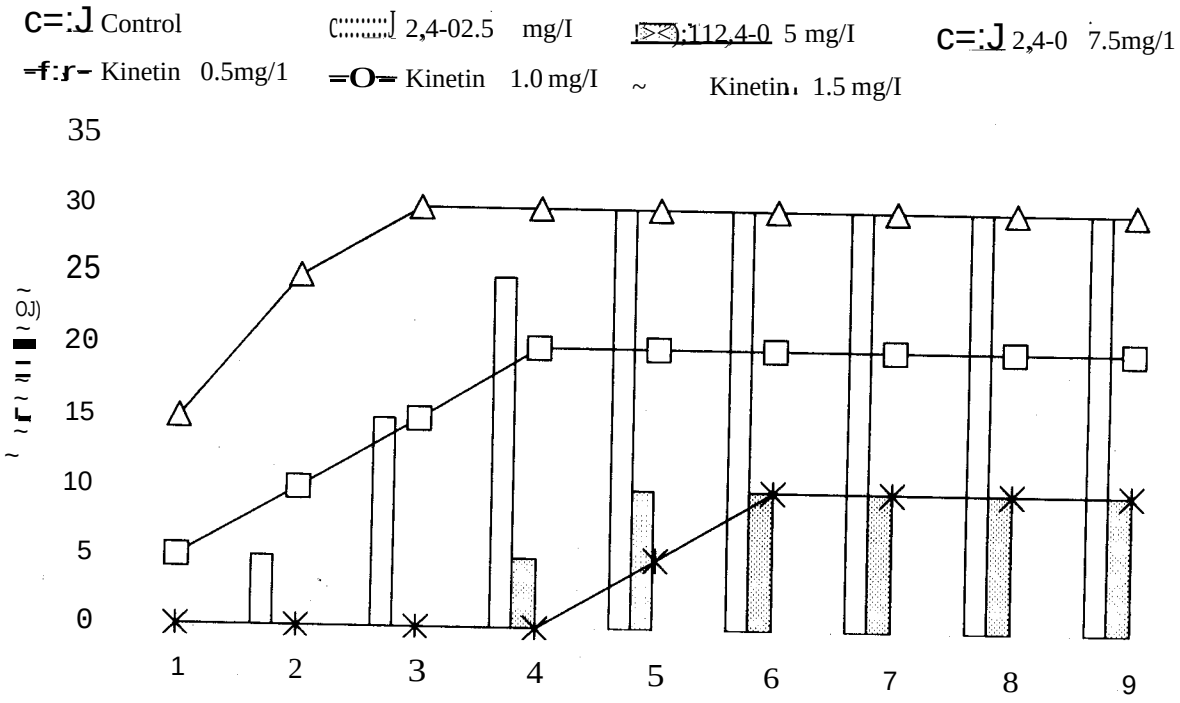


Fig. 7. Effect of Growth Hormones on Callus Induction in Floral Bud Explant



Callus growth (mg)

Significantly high amount of callus growth was found in ovary explant on kinetin 1.5 mg L^{-1} (199.6 mg) followed by embryo explant on 2, 4-0 2.5 mg L'I and kinetin 0.5 mg L^{-1} (190.7 and 142.1 mg). Overall among explants employed ovary and embryo proved the best for callus growth on all the treatments employed (68.27 and 62.7 mg) and were statistically at par to each other. Minimum growth was observed in stigma explant (3.52 mg) with no callus growth in sepal explant. For treatments used 2,4-0 (2.5 mg L^{-1}) and control showed similar behaviour (43.25 and 42.23 mg) for callus growth while minimum growth was found on 2, 4-0 7.5 mg L^{-1} (1.37 mg). Among the growth regulators employed, kinetin (24.32 mg) proved significantly better than 2, 4-0 (19.10 mg) for mean callus growth at all the levels. The superiority of kinetin to 2, 4-0 is also reported by Jordan *et al.* (1983) and Mondal *et al*, (1990). Usman *et al.* (2002b) also reported kinetin as better growth hormone for callus growth than 2, 4-0. These findings are contrary to Kumar *et al.* (1992), Fitch (1993) and Usman *et al.* (2002a) who reported 2.4-0 as better growth hormone for callus induction in papaya. Use of half strength media for callus induction is in accordance with Litz *et al*, (1983), Moore and Litz (1984), Rojas and Kitto (1991), Fitch (1993), Mondal *et al.* (1994), Usman *et al.* (2002a, b). The callus induction in media containing 2, 4-0 is supported by Medora *et al.* (1979), Kumar *et al.* (1992), Yang and Ye (1992) and Fitch (1993). The addition of kinetin to the media for callus induction is supported by Jordan *et al*, (1983), Mondal *et al.* (1990) and Jordan and Velozo (1995).

Overall, ovary and embryo explants emerged significantly better for callogenesis among the reproductive explants employed. However, ovary initiated callus earlier than embryo. These reproductive parts are pro-embryogenic in nature and might have more amount of growth hormones there leading to an earlier and better response for callus induction. Therefore, use of ovarian tissues and embryo as explant source could be suggested as the best among various reproductive parts for callogenesis. The protocol might be helpful to propagate male and female plants swiftly by subsequent embryogenesis and organogenosis. It may further be contributive in developing synthetic seeds and transgenic plants of papaya.

Table. 2. Effect of growth hormones on callus growth of papaya explants

Explants	Treatments							Means
	Control	2,4-D 2.5 mg/l	2,4-D 5 mg/l	2,4-D 7.5 mg/l	Kinetin 0.5 mg/l	Kinetin 1.0 mg/l	Kinetin 1.5 mg/l	
Embryo	*69. I* c	*/90.7 a*	*13,2 b*	-	*/42./ a*	*20.9b*	*2.9c*	62.73
Nucellus	50.6 d	-	-	-	-	-	-	7.22 c
Sepal	-	-	-	-	-	-	-	-
Petal	23.7 e	14.5 c	-	-	20.5 b	-	-	8.38 c
Stigma	0.7 f	3.7 d	5.3 c	-	*5A* d	3.6 c	6c	3.52 d
Stigma plus ovary	115 a	IIA c	15.5 b	-	11,1 c	20.2 b	14.7 b	26.8 b
Ovary	24 e	119.8 b	67.5 a	Ila	6.9 d	52.3 a	199.6 a	68.72 a
Floral Bud	94.8 b	5.9 d	-	-	8.2 c	69.1 a	0.4 d	25,48 b
Means	42.23 a	43.25 a	12.68 c	1,375 d	24.27 b	20.76 be	27.95 b	

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