Pak J Agri. Sci. 1'01, 39(3), 2002

SOMATIC EMBRYOGENESIS IN PAPAYA

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Reproductive explant derived callus of papaya *(Carica papaya* L.) was subcultured on Murashige and Skoog (MS) media supplemented with different levels of NAA for embryogenesis and subsequent organogenesis, Embryo and ovary derived callus yielded maximum multiplication (80%) on NAA 5 and 3 mgL'1 in first week. Ovary (80%) derived callus multiplied at the highest rate while NAA 5 rngl." proved to be better treatment than others, Embryogenesis started on NAA 3 mgL" in first week of subculture and was highest (40%) on NAA 1 mgl." and control. Organogenesis initiated on both NAA I mg L" and control in fifth week and was better on control (30%), Only embryo derived callus developed somatic embryos and subsequent root and shoots, The protocol might be promising to propagate female plants swiftly. developing synthetic seeds and transgenic plants of papaya.

Key words: Callus, embryogenesis. papaya. in *vitro,* NAA, reproductive explants, embryo

INTRODUCTION

A detailed introduction on the subject has already been given (Usman *et al.*. 2002c), Several researchers have worked somatic embryogenesis from tissues of female plants which could avoid the problem of sexual identification at seedling stages, Successful somatic embryogenesis have been obtained from various reproductive explant derived callus on different media supplemented with activated charcoal (Litz and Conover, 1979), NAA and kinetin (Jordan *et al.. 1983;* Usman *et al.* 2002 a, b). BA (Jordan. 1989). half strength (Moore and Litz, 1984; Usman *et al.* 2002 a. b) and full strength MS media (Chen *et al.,* 1991). 2.4-D (Dhir and Yadava, 1995: Usrnan *et al.* 2002 a. b). GA). CH and IAA (Rojas and Kitto, 1991). NN medium (Jordan and Velozo, 1995), The study was initiated to induce somatic embryogenesis in callus derived from various reproductive explants on MS media (Murashige and Skoog, 1962) supplemented with various growth regulators. Author have already discussed results of callogenesis from reproductive explants (Usrnan *et al.,* 2002c), This paper. however. includes only development of somatic embryo of callus derived from various reproductive, explants of papaya *(Carica pa/JU.\'u* L.), The protocol may be promising for propagation of male and female plants. developing synthetic seeds and transgenic plants in papaya,

MATERIAL 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 to 5,7 and gelrite (1,6%) was used as solidifying agent in the media, Media was sterilized in autoclave for 15 minutes at 121°C under \.5 Kg/cm" pressure (Usman *et al.,* 2002 a. b), After nine weeks of culture. callus derived from various reproductive explants of papaya (Usman *et al.,* 2002c) was subcultured on MS media supplemented with NAA (\.0. 3,0 and 5.0 mgl,") for embryogenesis. regeneration or proliferation, Callus was weighed on fresh weight basis. divided and subcultured in the media. Cultures were kept in growth room at temperature 25 + 2 "C with fluorescent light intensity of 2500 lux, Data was collected as time taken to multiply callus and percentage. time taken to induce embryogenesis and percentage and time taken to induce organogenesis and percentage at weekly intervals, Experiment was laid out in completely randomized design (CRD), There were three replications with ten observations per treatment per replication, Data was analyzed by Duncan's Multiple Range test (DMR) at probability> 5% (Steel and Torrie, 1980),

RESULTS AND DISCUSSION

Time taken to multiply callus and percentage

Explant derived callus was subcultured on embryogenesis media as described earlier. Callus showed proliferation and multiplication instead of embryogenesis, In embryo. callus showed the highest proliferation (80%) on NAA (5 mgL'I) in first week of subculture while control gave minimum (10%) multiplication (Fig, I), Petal derived callus initiated multiplication (10%) on NAA 3 mgl." in third week (Fig, 2) while in stigma callus started proliferation (50%) on control in third week (Fig, 3). Stigma plus ovary derived callus multiplied (15%) at the earliest on NAA 3 mgl," and control in second week (Fig, 4) while callus from ovary proliferated (80%) on NAA 3 mgL'I(Fig. 5). Callus from floral bud explant showed the most delayed multiplication (25%) on NAA 1 rngl." (Fig, 6), The findings are in line with findings of Usman *et al,* (2002a. b) who reported callus multiplication from vegetative explants of papaya obtained from *in vivo* and *in vitro* sources on MS media supplemented with NAA. Mondal *et al.* (1990) also examined callus multiplication on media supplemented witf NAA and benzyl adenine.

Fig. 1. Time Taken to Multiply Embryo Explant Derived caUus in Papaya

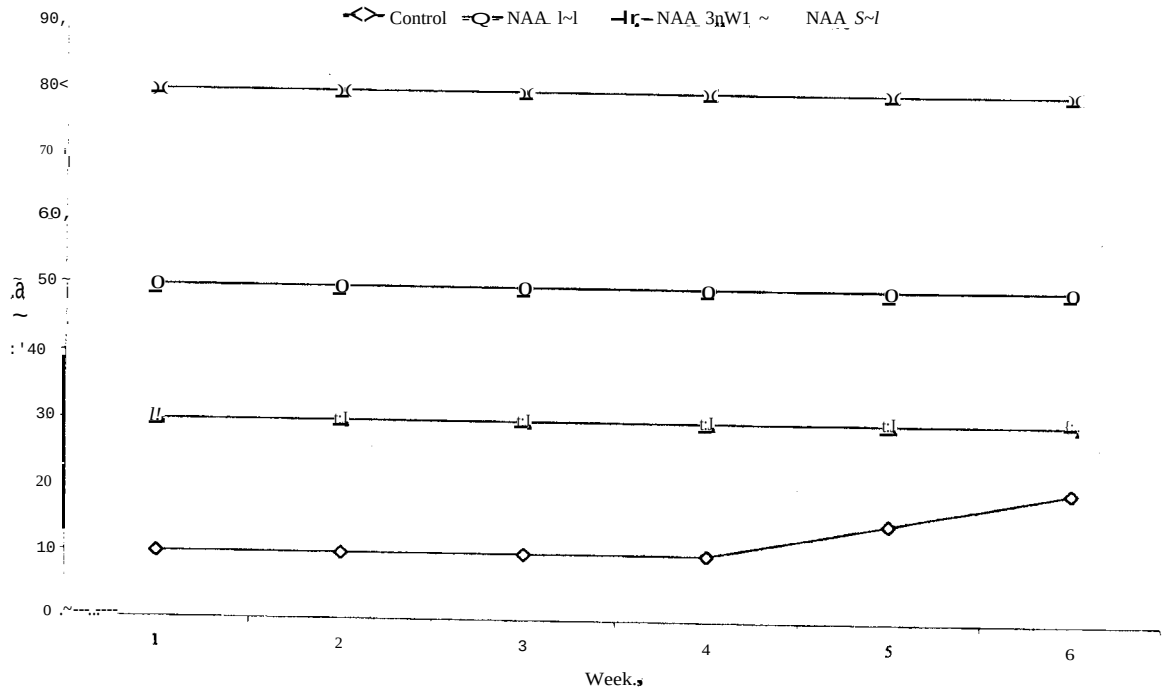


Fig. 2. Time Taken to Multiply Petal Explant Derived CaDus in Papaya

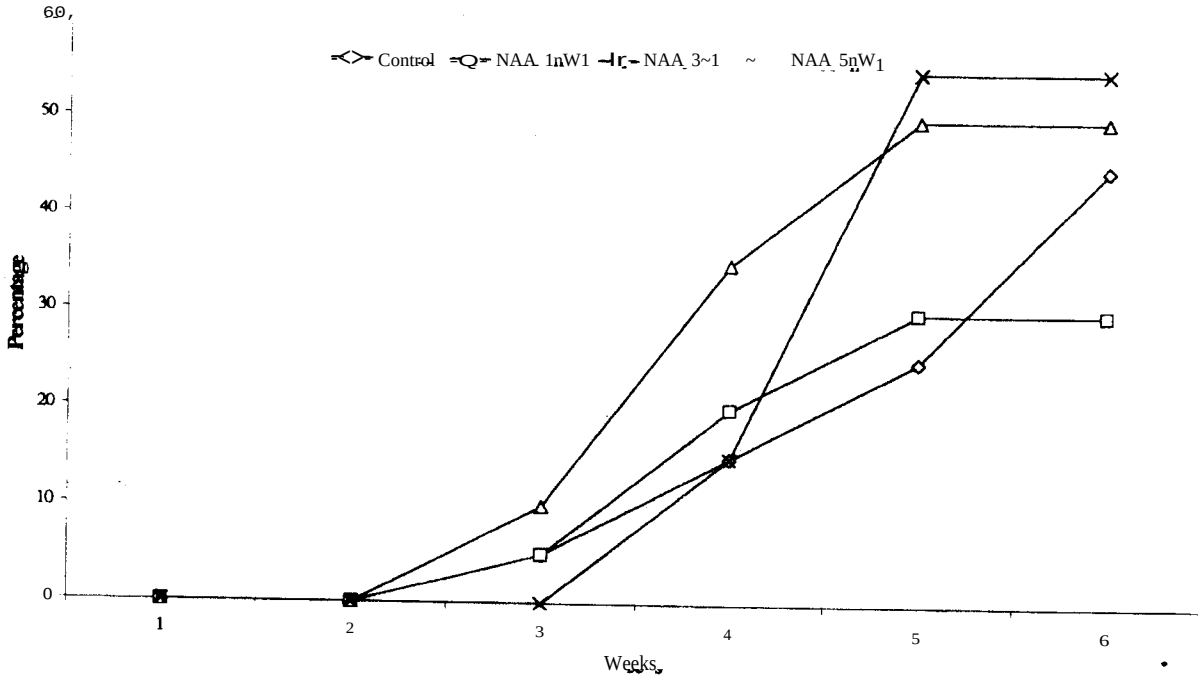


Fig. 3 Time Taken to Multiply Stigma Explant Derived Callus in Papaya

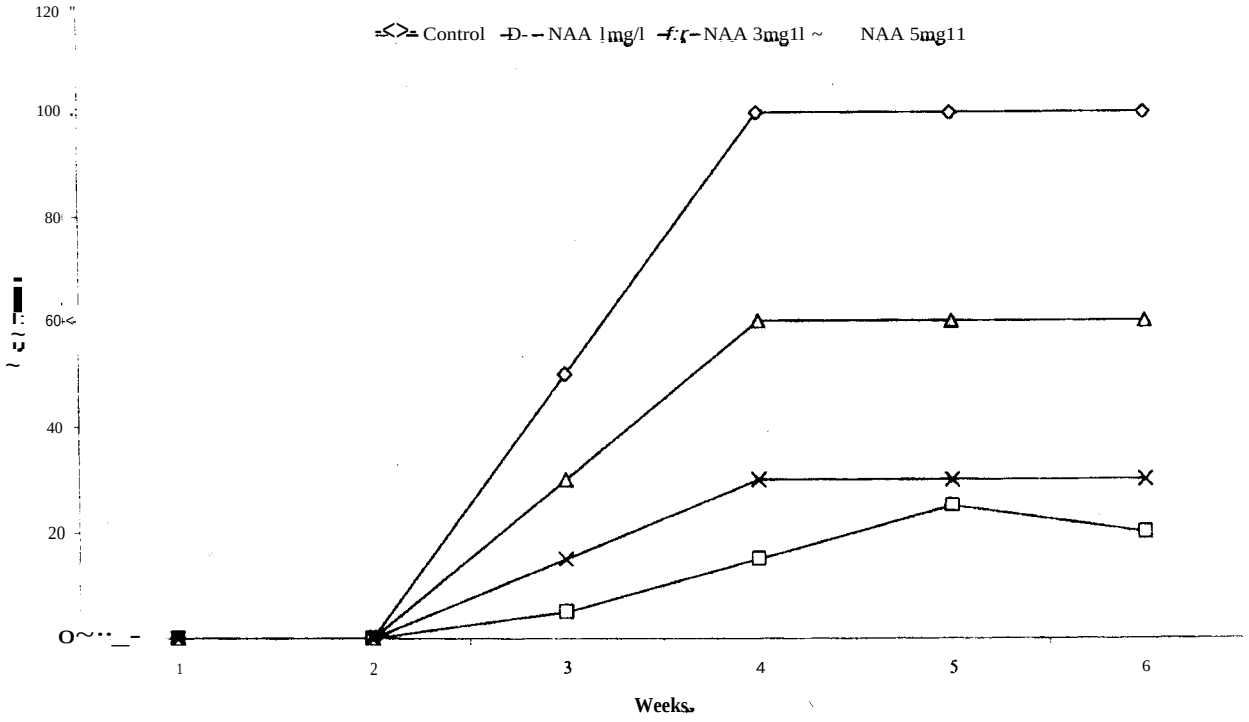


Fig. 4 Time Taken to Multiply Stigma plus Ovary Explant Derived Callus in Papaya

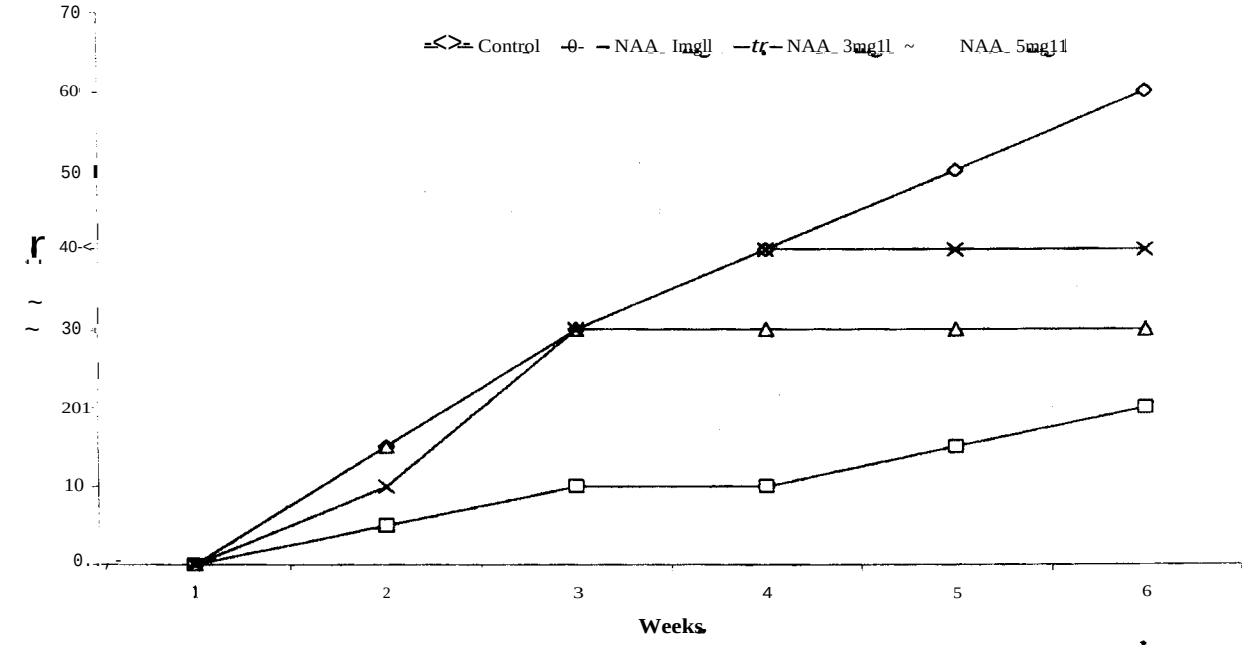


Fig. 5 Time Taken to Multiply Ovary Explant Derived Callus in Papaya

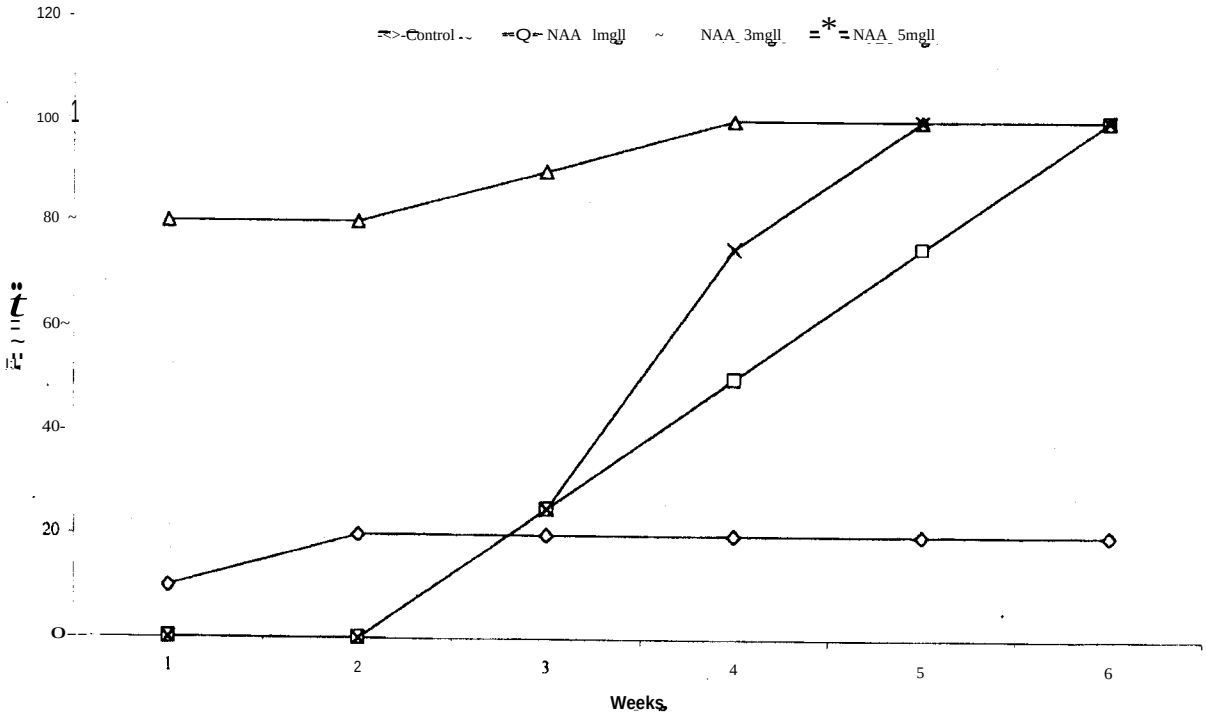
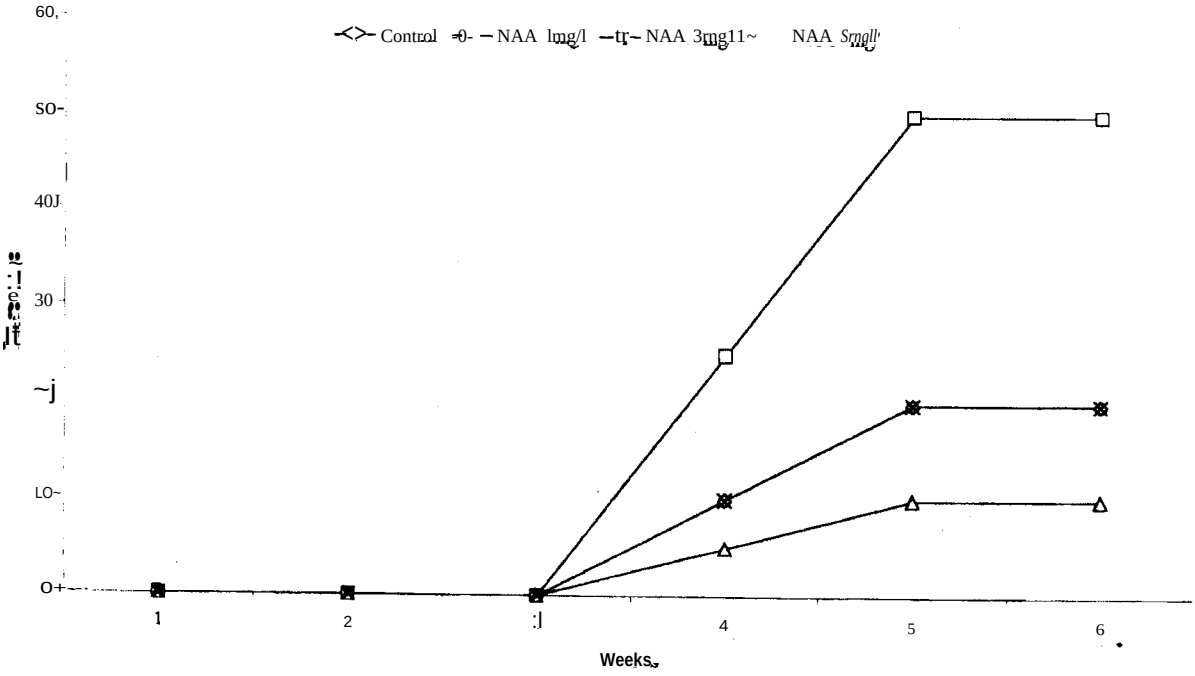


Fig. 6. Time Taken to Multiply Floral Bud Explant Derived Callus in Papaya



Maximum multiplication in embryo derived callus was observed at NAA 5 and I rngl.$^{-1}$ (80% and 50% respectively). Nucellar and sepal derived callus exhibited no multiplication on any treatment. Maximum multiplication (70%) was observed on NAA 5 and 3 mgl.$^{-1}$ in petal derived callus. Stigma and stigma plus ovary explants generated best callus multiplication 100% and 70% respectively on control. Maximum multiplication (100%) from ovary explant was found on all levels of NAA succeeded by control while floral bud gave maximum multiplication (50%) on NAA 1 mgl.$^{-1}$ (Table I). Our findings are contrary to Mondal *et at* (1990) who reported callus multiplication on media with NAA and benzyl adenine differing in cv. Honey Dew and levels of growth regulators used. We examined callus multiplication on MS media supplemented with NAA.

Table I. Effect of growth regulators on callus multiplication percentage of papaya explants

Explants	Treatments				Means
	Control	NAA 1 mgl.$^{-1}$	NAA3 mgL^{-1}	NAA 5 mgL^{-1}	
Embryo	20 d	50 b	30 d	80 b	45 be
Nucellus	-	-	-	-	-
Sepal	-	-	-	-	-
Petal	50 c	30 c	50 be	70 b	50 b
Stigma	100 a	30 c	60 b	30 cd	55 b
Stigma with ovary	70 b	20 cd	30 d	40 c	40 c
Ovary	20 d	100 a	lOO a	100 a	80 a
Floral Bud	20 d	50 b	20 de	20 d	27.5 cd
Means	35 b	35 b	36.25 b	42.5 a	

Ovary yielded significantly better callus multiplication (80%) among explants employed on all treatments ensued by stigma, petal and embryo while minimum multiplication was yielded by floral bud (27.5%). NAA 5 rngl.$^{-1}$ (42.5%) emerged as the best level used while NAA 3 mgl.$^{-1}$, NAA I mgl.$^{-1}$ and control were statistically at par for callus multiplication percentage. Our findings are in line with Usman *et at* (2002a, b) who reported callus multiplication in papaya on MS media supplemented with NAA at various levels. Findings of Mondal *et at* (1990) and Kumar *et al.* (1992) are contrary who reported callus growth on NAA plus BA and kinetin, respectively.

Time taken to induce embryogenesis and percentage

Embryogenesis was observed only in embryo derived callus. Embryogenesis (5%) initiated in first week on NAA 3 mgl.$^{-1}$ and second week on control while NAA I mgl.$^{-1}$ induced embryogenesis (10%) in second week. No embryogenesis was found on NAA (3 and 5 mgl.$^{-1}$) while it was one week earlier in NAA (I rngl.$^{-1}$) than control (Fig. 7). The results are better than Manshardt and WenslafT (1989a, 1989b) who reported somatic embryo initiation in I I weeks. Smaller but more number of embryos was found on control than NAA (I mg L^{-1}). Embryo derived callus might be more pre-embryogenic in nature leading to earlier and more response for embryo than other explantsemployed.

Findings of Fitch and Manshardt (1990) are in line for production of somatic embryos only from zygotic embryo derived callus on half strength MS media supplemented with 2, 4-D plus glutamine after 6 weeks and matured on MS media while kinetin (5 mgl.$^{-1}$) gave germination. Results differ for maturation and germination of embryos as MS media gave both while kinetin yielded callus induction only. Findings of Ch en *et at* (1991) and Dhir and Yadava (1995) are similar in occurrence of embryogenesis from zygotic embryo derived callus only. While findings of Dhir and Yadava (1995) are contrary who obtained somatic embryos from immature zygotic embryos on MS media with 2, 4-D and glutamine. Difference might be due to variation in strength of basal media and its modifications. Results are corroborative to Inglesis *et al.* (1997) regarding induction of somatic embryos and dissimilar to Moore and Litz (1984); Yang (1986); Litz (1986); Rojas and Kitto (1991) and Ye *et at* (1993) who obtained embryos from ovular callus in C *papaya* and C. *eau/iflora* on half strength MS media. Conclusions of Jordan and Velozo (1995) are incompatible who obtained somatic embryos from suspension cultures of bud derived callus on NN medium with NAA, IAA in combination with zeatin, benzyl adenine and kinetin.

Time taken to induce organogenesis and percentage

Earliest organogenesis (rooting) was observed from somatic embryos (5%) yielded by embryo derived callus on 5th week of subculture on NAA I mgl.$^{-1}$ and control (Fig. 8). Organogenesis percentage was significantly better on control (30%) than NAA I mgl.$^{-1}$ (10%). Results described are contrary to Inglesis *et at.* (1997) who obtained somatic embryos and germination on MS media with half strength salts, full strength vitamins, 6% sucrose and 2, 4-D. Contradictions might be due to difference in media formulation and growth regulators applied. No other explant derived callus generated organogenesis on any treatment and these findings are contrary to Rajeevan and Pandey (1983) who obtained rooting from established floral buds in papaya cv. Coorg Honey Dew on MS media with IAA. While our findings are supported by Burikam *et al.* (1988), Reuveni *et at.* (1990) and Mondal *et at.* (1990) who reported rooting of bud derived shoots on half strength MS media with IBA in cv. Khag Dum, Solo and Honey Dew while no organogenesis was detected on NAA. Major difference lies in media formulation making it most probably responsible for variation.

Fig. 7 Time Taken to Induee Embryogenesis from Embryo Explant Derived Callus in Papaya

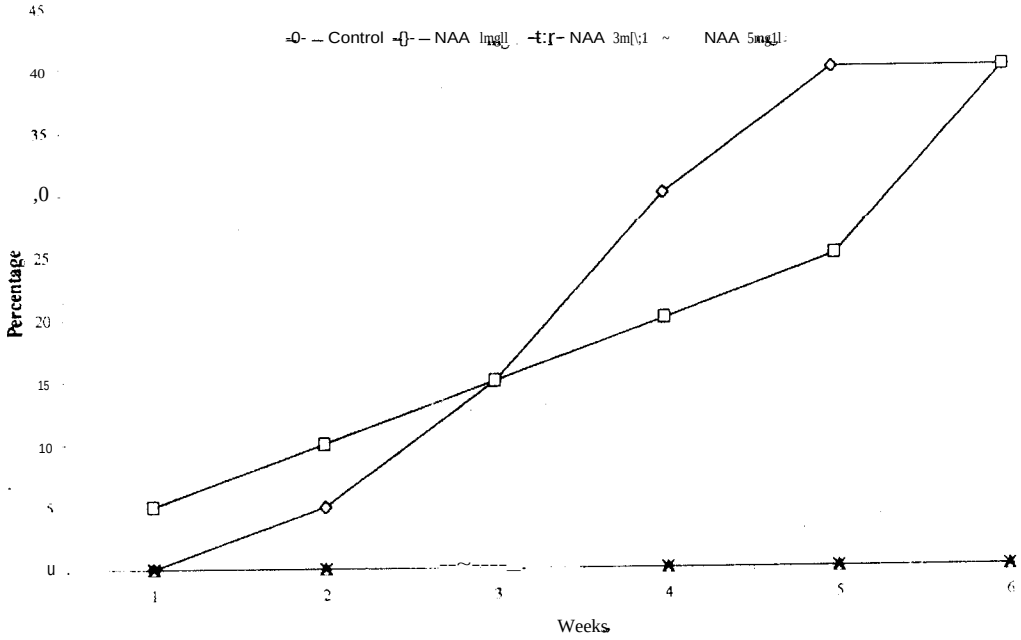
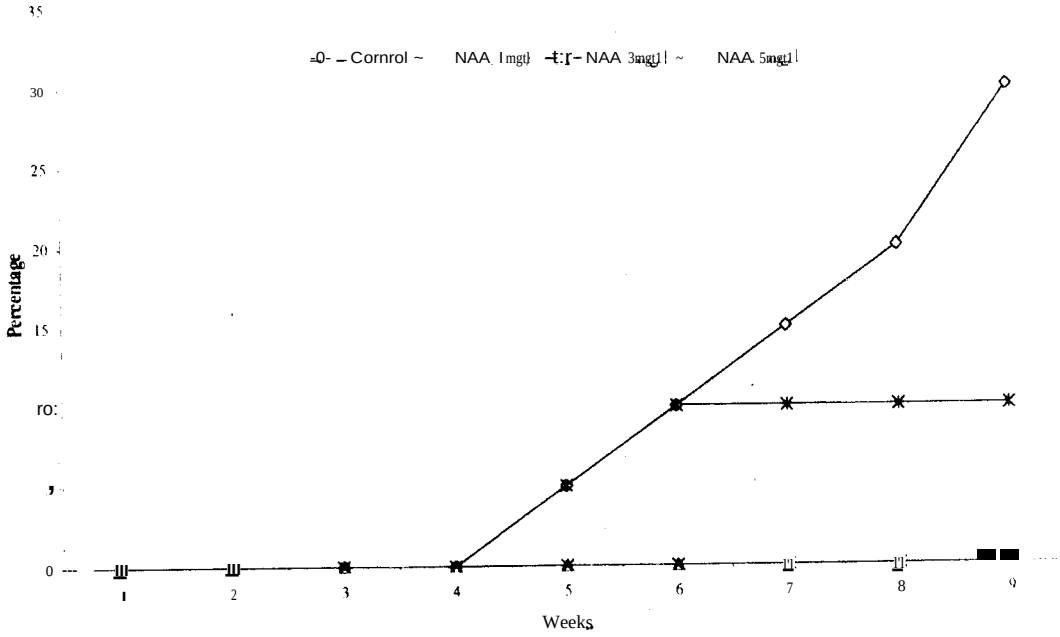


Fig. 8. Time Taken to Induce Organogenesis from Embryo Explant Derived Callus in Papaya



Overall, ovary and stigma derived callus have been better for multiplication while only embryo derived callus yielded the somatic embryos at control and low levels of NAA. Among the explants employed embryo might have the most proembryonic nature and callus would have more embryogenic potential than other explnts. It has given somatic embryos even on media without growth regulators and shows that callus was highly embryogenic. It might be suggested that only use of embryo/embryo derived callus has the potential to produce direct/indirect somatic embryos. The protocol might be promising to propagate male and female plants swiftly. developing synthetic seeds and transgenic plants of papaya.

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