

INITIATION AND PROLIFERATION OF PAPAYA (*Carica papaya* L.) SOMATIC EMBRYOS FROM ZYGOTIC TISSUE

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ABSTRACT

The study on the initiation and proliferation of somatic embryos from papaya (*C. papaya* L.) zygotic embryo explants was undertaken. Best results regarding initiation of embryogenic callus formation was obtained with 90 days after pollination (DAP) zygotic embryos. The medium were liquid and solid MS (0.5) medium containing glutamine (2.7 mM), 2,4-D (9 μ M) and sucrose (170 mM) at 6 and 10 weeks after the start of the cultures. In this condition 90% explains produced embryogenic callus formation. The best medium for proliferation of somatic embryos was a liquid MS (0.5) medium containing glutamine (2.7 mM), sucrose (170 mM) in the presence of 2,4-D (9 μ M). Using this medium, somatic embryos proliferated three times higher within two weeks.

INTRODUCTION

Papaya (*Carica papaya* L.) is a popular breakfast fruit, as a component of fruit salads and desert fruit. The production of papaya in Indonesia has been decreasing recently. An alternatives method to produce papaya is somatic embryogenesis.

The formation of somatic embryos on papaya has been reported previously (Litz and Conovers, 1982 ; 1983; Fitch and Manshardt, 1990, Rojas and Kitto, 1991. Fitch, 1993) however the mass production of somatic embryos, to produce content and good quality papaya remain difficult (Drew, Griffith Univ., Brisbane, personal communication). This problem of plant production prevent this method from being used for micropropagation of elite papaya germplasm. It is unknown if these techniques will work on Australia - Indonesia grown papaya cultivars.

The present study was undertaken to develop a reliable protocol for initiation and proliferation of somatic embryos from papaya zygotic embryos explants. The specific aims were to investigate the effect of a) adenine sulphate and 2,4 - D (experiment 1 and 2), b) explant age

(experiment 2), c) sucrose concentration (experiment 3), d) medium constitution (Liquid as compared to solid, experiments 1 and 2) and e) plant growth regulator combinations and concentration (0 and 9 μ M 2,4-D; 0.01 μ M NAA; 0.05 μ M BAP) (experiment 3) on somatic embryogenesis.

MATERIALS AND METHOD

The experiments were carried out at the Laboratory of Plant Molecular Biology, Department of Agriculture, The University of Queensland and Research Centre of Queensland Department of Primary Industries, Redlands, Cleveland, Australia from July 1994 to May 1995

Experiment 1

The sexual embryos (5 per vessel) were placed onto one of two basic culture medium type consisted of 0.5 MS nutrients, glutamine (2.7 mM), sucrose (87 mM), 2,4 - D (0, 9 or 45 μ M) adenine sulphate (0 or 800 μ M), agar (0.8%, Difco Laboratories, MI, USA) and was contained 20 ml medium in sterile plastic petri dishes (9 cm diam x 12 cm tall). The second medium type consisted of the same additives, but without agar, and contained (20 ml medium in conical flasks (200 ml), that were

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shaken on an orbital shaker at 90 rpm. After 4 weeks of incubation on these media embryos were assessed as to whether they had produced callus. Incubation of both forms of culture was undertaken at $25^{\circ} \pm 2^{\circ}$ C using a 16 h photoperiod with a photosynthetic photon flux density (PPFD) of $500 \text{ mmol m}^{-2}\text{s}^{-2}$.

Experiment 2

Four weeks after the start of Experiment 1 the callus was transferred to a embryogenesis induction medium which was identical to the callus induction medium but modified by the addition of adenine sulphate (0 or $800 \mu\text{M}$) and/or 2,4 - D (0, 9 or $45 \mu\text{M}$). Somatic embryo proliferation was undertaken on a range of different media containing sucrose (87 mM or 170 mM), 2,4 - D ($9 \mu\text{M}$), NAA ($0.01 \mu\text{M}$) and BAP ($0.05 \mu\text{M}$), with the medium physical condition either solid (agar 8 g l^{-1}) or liquid. After a further 4 weeks of incubation on these media the percentage of explants producing somatic embryos and the number of somatic embryos produced per explant were determined.

Experiment 3

At the somatic embryo proliferation stage, one somatic embryogenic cell line (line E) was selected as it was vigorous in its growth. Embryos of this line were inoculated into a basal medium containing sucrose (87 mM or 170 mM) with 2,4 - D ($9 \mu\text{M}$), NAA ($0.01 \mu\text{M}$) and BAP ($0.05 \mu\text{M}$), with or without agar (8 g l^{-1}). After 2, 4, 6, 8 and 10 weeks of incubation on these media types the number of globular, heart, torpedo and cotyledonary somatic embryos were counted.

Experimental design and statistical analysis.

In experiments one and two (initiation stage) there were 25 replications per treatment for the initiation of somatic embryogenesis (five Petri dishes, each contained five zygotic embryos) and in experi-

ment three (proliferation stage), there were six replication per treatment (two plates, contained three clumps). The experimental design was Completely Random Design. A Wilcoxon test was used to determine the significance somatic embryos formation per zygotic embryo explant.

RESULTS

Experiment 1

The effect of adenine sulphate, 2,4 -D physical medium ion on initiation of somatic embryogenesis.

Adenine sulphates did not significantly increase the initiation of somatic embryos (Fig. 1) but it did promote the greening of the embryos at a later date (data not shown). In contrast, 2,4 - D significantly promoted the rate of somatic embryogenesis with 9 and $45 \mu\text{M}$ being the best concentrations tried. A liquid medium was better for somatic embryo production when compared to a solid medium.

Experiment 2

The effect of explant age, 2,4 - D and medium physical composition on initiation of somatic embryogenesis.

Somatic embryogenesis occurred on callus that had formed on zygotic embryos. Most somatic embryos formed on callus that had been generated from the embryos shoot (90% of them forming embryos) and roots (30% of them forming embryos). Some callus formed on cotyledons (10%) but no somatic embryos were ever produced on this type of callus. The development of somatic embryos on the callus from the shoot tip was first observed between 4 and 10 weeks after inoculation, and at this time somatic embryogenesis was very prolific.

The rate of somatic embryogenesis produced on zygotic embryos explants placed onto the solid medium was significantly affected by their age. Ninety-day-old zygotic embryos were the only age to pro-

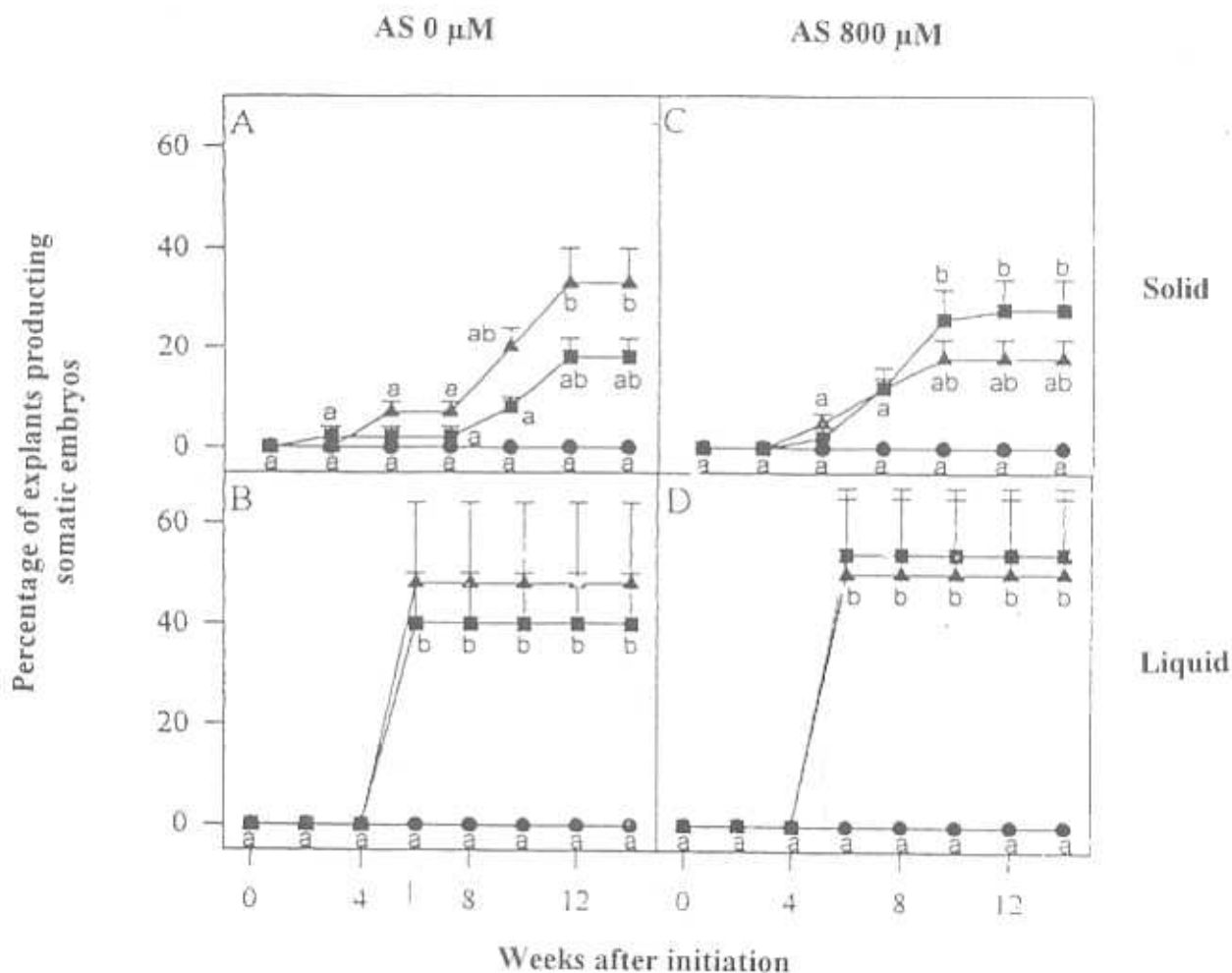


Figure 1. The effect of adenine sulphate (A and B 0 μM; C and D: 800 μM) and 2,4-D (1 : 0 μM; 5 : 9 μM; 1 : 45 μM) on initiation of somatic embryos. The explants were zygotic embryos taken from open pollinated fruit. The medium physical compositions were used. They both containing Murashige and Skoog nutrients but two were solidified with 0.8 % agar (Figs 1 A and 1 C) and two retained as liquid (Figs 1B and 1D). The results are for the mean of five plates containing five zygotic embryos per plate. Bars represent standard errors.

duce somatic embryos. It was found to be impossible to isolate younger zygotic, without severely damaging them. By contrast, the window of time for somatic embryogenesis on a liquid medium was much longer (90 – 120 days old) (Fig. 2). However, following a longer period of culture (10 weeks), 9 μM 2,4 – D in the solid culture system produced more somatic embryos than 45 μM (Fig. 3).

Experiment 3.

The effect of sucrose and plant growth regulator on the proliferation of somatic embryos

A high concentration of sucrose (170 mM) in the presence of 2,4 - D (9 μM) produced a higher number of globular embryos (Fig. 4) than was possible at other combinations of these substances. The maximum number of globular embryos was pro-

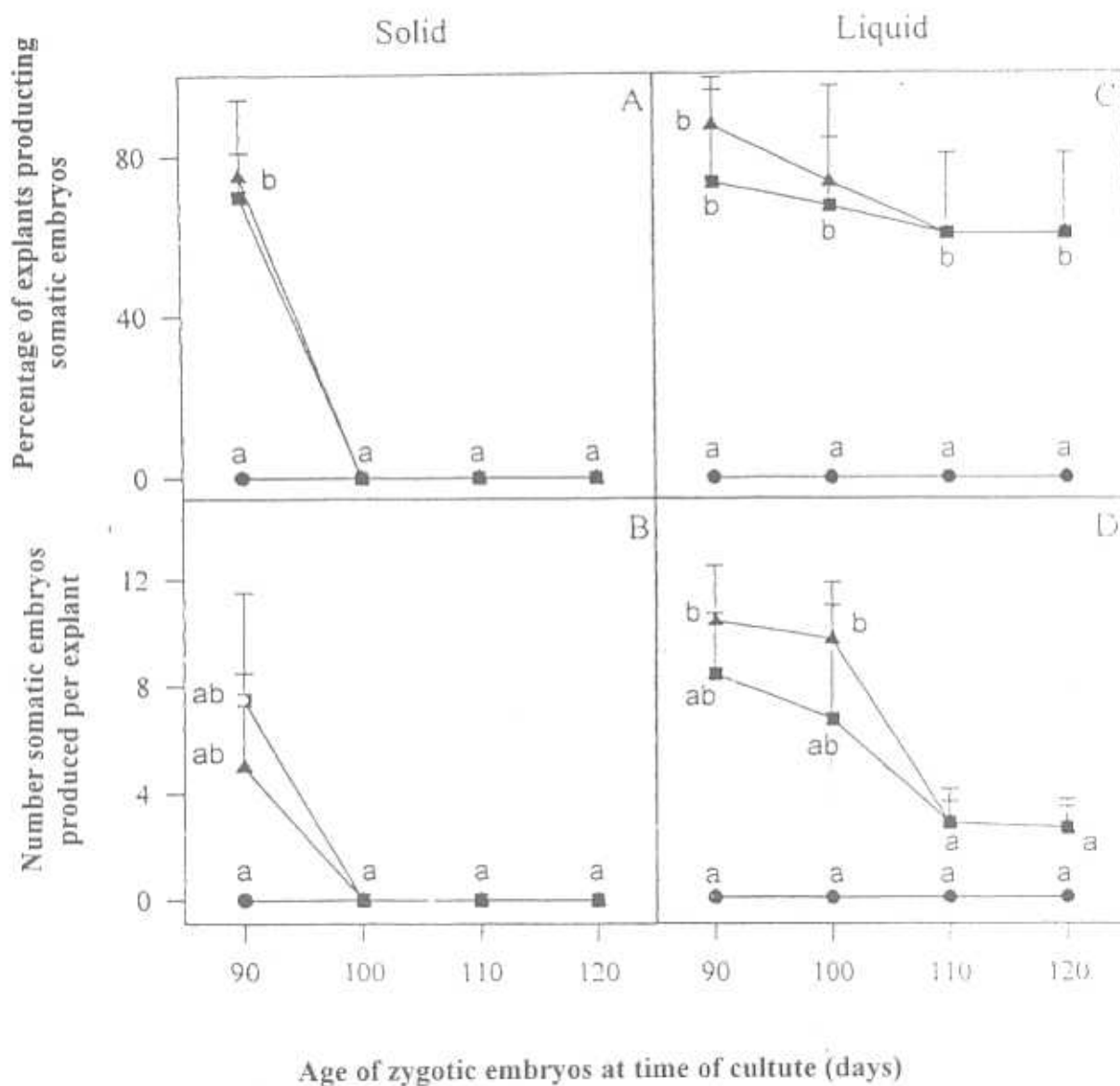


Figure 2 The effect of zygotic embryo age at the time of their culture and 2,4-D concentration on the percentage of explants producing somatic embryo and the number of somatic embryos produced per explant. The results are presented for six weeks after the start of culture. Two medium physical composition werw used both containing half strength MS nutrients, sucrose (170 mM), glutamine (2.7 mM) and 2,4-D (0(1); 9 μM (s) or 45 μM (n). Two mwdia were solidified with 0.8% agar (A and B) while the other two were liquid (C and D). The results are for the mean of five replicate plates each containing five xygotic emryos each. Bars represent standard errors.

duced 4 weeks after the start of culture, and this was true for both concentrations of sucrose tried. In the case of plant growth regulators, the response was slightly different to that of sucrose, however

there was no significant difference at 2, 4 and 6 weeks after initiation, for control and NAA/BAP treatments.

The maximum number of torpedo – shaped

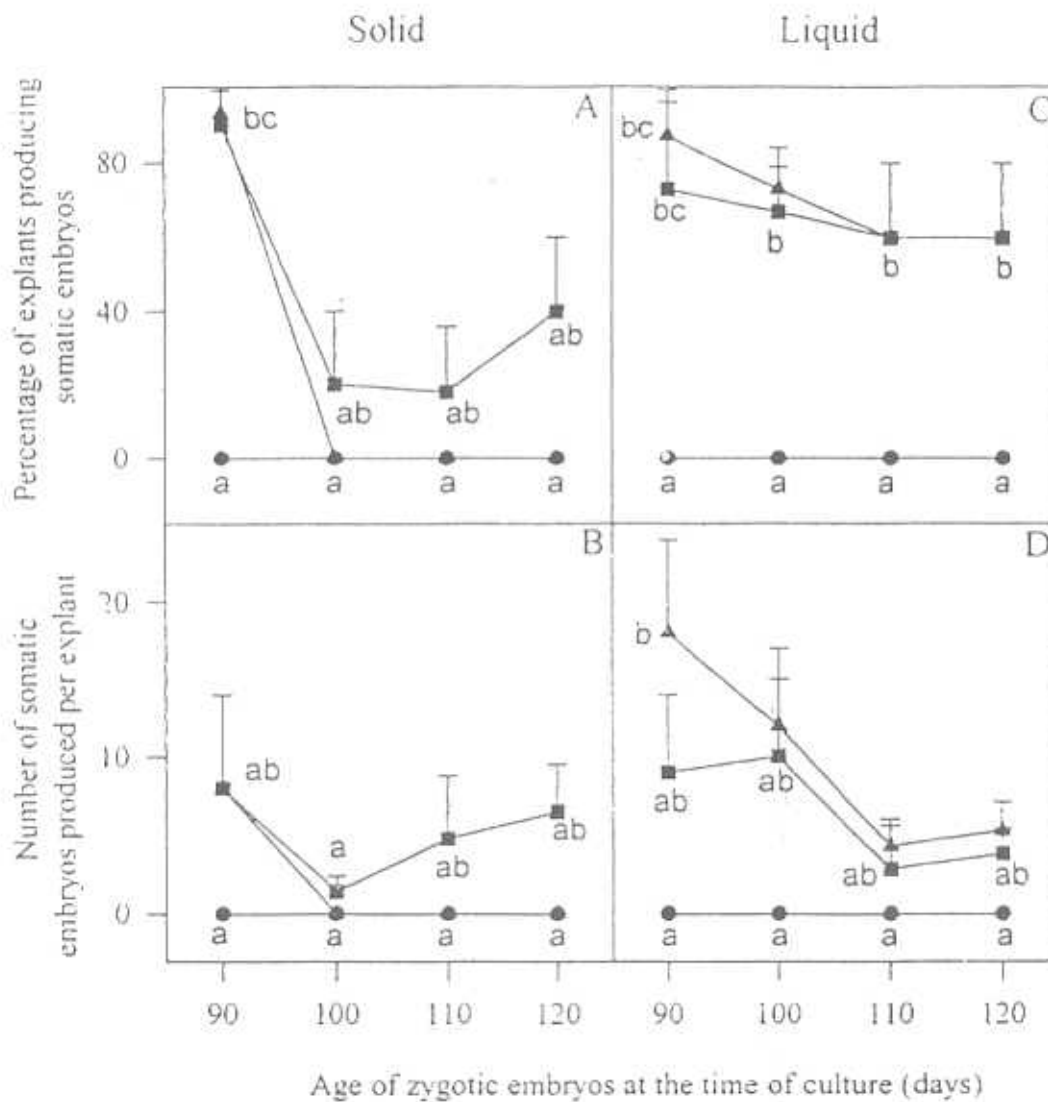


Figure 3 The effect of zygotic explant age at the time of their culture and 2,4-D concentration on the percentage of explants producing somatic embryos and the number of somatic embryos produced per explant. The results are presented for ten weeks after the start of culture. Two media systems were used both containing half strength MS nutrients, sucrose (0.17 mM), glutamine (2.7 mM) and 2,4-D at either 0(1); 9 μM (s) or 45 μM (n), however one medium was solidified with 0.8 % agar (3A and B) while the other was liquid (3C and D). The results are the mean of five plate which contain five zygotic embryos per plate.

embryos, in all of the treatments was observed 8 weeks from the start of culture. This was the case for all treatments except the low 2,4-D concentration. In the presence of the high sucrose concentration, which reached its maximum 2 weeks earlier. The highest number of torpedo-shaped embryos

was produced in the presence of the low 2,4-D (9 μM) concentration, the low sucrose (87 mM) concentration, and without other plant growth regulators (Fig. 4). There were no significant differences between low and high sucrose concentration in their ability to produce mature cotyledon embryos.

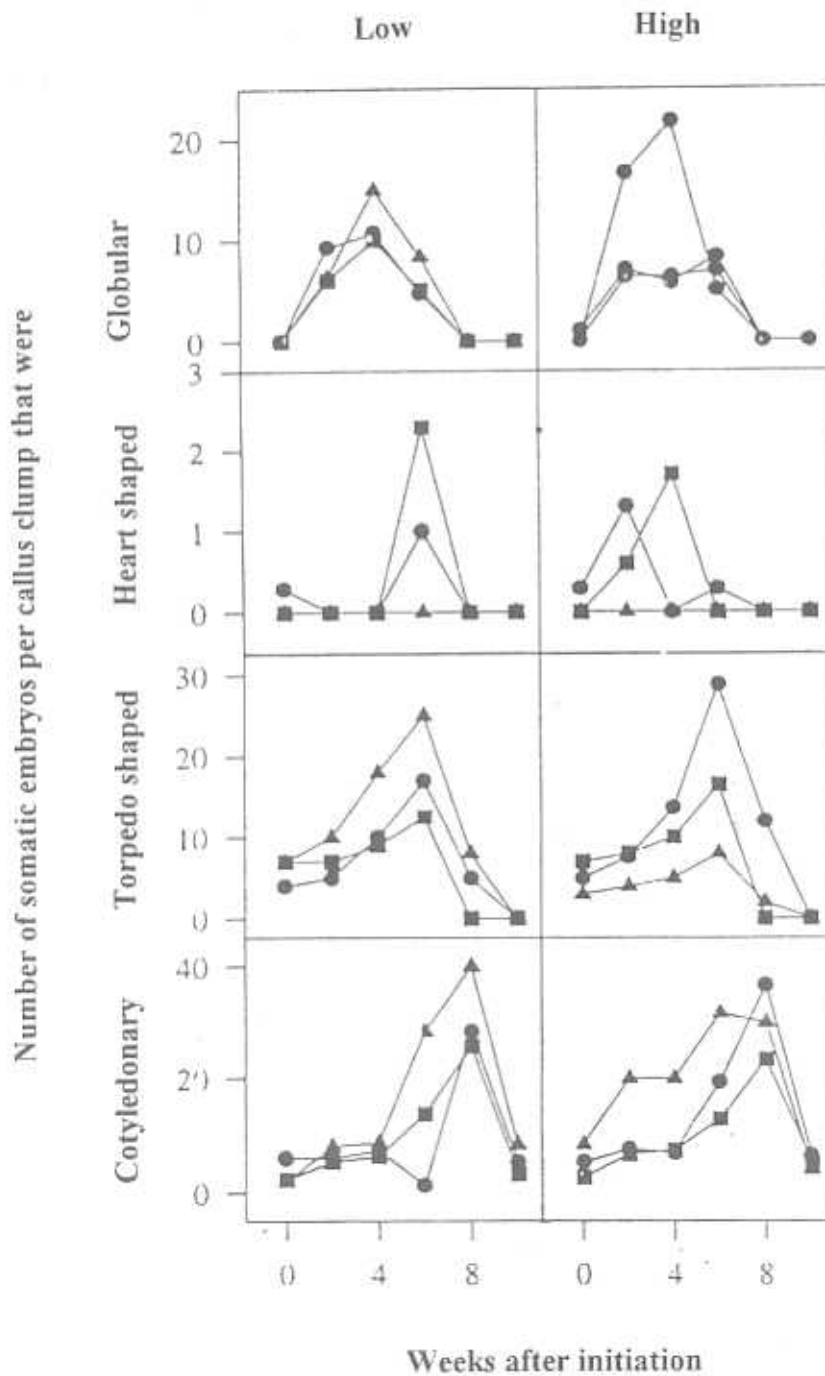


Figure 4 The effect of sucrose (low: 87 mM; high: 170 mM) on the proliferation of globular, heart, torpedo and cotyledon-shaped somatic embryos over time from somatic embryogenic Line E. The basic medium was half strength MS containing glutamine (2.7 mM) and agar (0.8%) with one of a number of plant growth regulator combinations including 9 μM 2,4-D (s), 0.01/0.05 μM NAA/BAP (n) and a control (1).

Table 1. Total number of cotyledon, torpedo, and heart-shaped somatic embryos produced in suspension culture 2 weeks after initiation. The medium contained either high or low concentrations and several plant growth regulators.

Treatments			Somatic embryos produced	
Sucrose (mM)	2,4-D (μ M)	NAA; BAP (μ M)	Start of culture	Two weeks in culture
87	9	0	10.0 \pm 1.0	25.0 \pm 3.0
	0	0.01 ; 0.05	10.0 \pm 1.0	14.6 \pm 2.0
170	9	0	10.0 \pm 1.0	29.6 \pm 2.0
	0	0.01 ; 0.05	10.0 \pm 1.0	20.0 \pm 1.0

The observed somatic embryos propagation rate produced by low 2,4-D (9 μ M) and high sucrose (170 mM) concentrations was the best for papaya and three times that seen in the control. The high sucrose and 0.01 μ M NAA/0.05 μ M BAP concentration combined induced two times the number of somatic embryos within 2 weeks than the control (Table 1).

DISCUSSION

Initiation of somatic embryos (Experiments 1 and 2)

The age of explants and the application of 2,4-D (9 – 45 μ M) were found to be the most important factors in initiating somatic embryogenesis in papaya. The youngest embryo that could be isolated (90 DAP) were found to be the best for initiating somatic embryos in both a solid or in a liquid medium (Fig. 2). The result to do with explant age is similar to that of Fitch and Manshardt (1990) who also found 90 to 114 days-old embryos to be best for somatic embryogenesis. Both concentrations of 2,4-D tried were equally good in inducing somatic embryogenesis (Fig. 2). This result is therefore similar to that of Fitch and Manshardt (1990)

who found that a 2,4-D concentration range of 0.5 to 112.5 μ M was best for the initiation of somatic embryos, depending on the genotype.

Since other researchers have used solid media to initiate somatic embryos of papaya, there has been no past comparison of solid to liquid media. In the present study, initiation of somatic embryogenesis was significantly better on a liquid medium (Fig. 1 and 2) as compared to a solid medium. In experiment 1, the explants (zygotic embryos) used had come from open pollinated flowers growing under winter conditions, while in Experiment 2 the zygotic embryos used had been produced by flower involving controlled pollination and were grown during summer, consequently the formation of somatic embryos in Experiment 2 was faster taking between 4 and 6 weeks after initiation to form. This faster rate of formation is similar to that observed by Fitch and Manshardt (1990).

The effectiveness of the various plant growth regulators in proliferating the somatic embryos was difficult to determine but may have been higher in liquid culture than in solid medium culture. Litz and Conover (1983) were able to induce a high proliferation rate of somatic embryogenesis when a liquid culture medium containing 4.5 μ M to 9 μ M 2,4-D

was used for ovular callus culture. In the present study higher concentration of sucrose (170 mM) was better than the lower concentration (87 mM), probably because it increased the osmotic potential of the medium and this helped to induce secondary embryos to form.

CONCLUSION

For the initiation of papaya somatic embryogenesis a suitable explant is zygotic embryos taken at 90 DAP. These explants should be cultured in a liquid MS (0.5) medium containing glutamine (2.7 mM), sucrose (0.17 M) and 2,4-D (9 μ M). For their proliferation a suitable medium is a liquid 0.5 MS medium containing glutamine (2.7 mM), sucrose (85 or 170 mM) in the presence of 2,4 - D (9 μ M). Using such a system a very high production of somatic embryos is possible.

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