Influence of Polyamine on Induction of Adventive Embryony in Papaya (*Carica papaya* L.)

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Abstract

Somatic embryogenesis has been reported in different papaya cultivars across the world. However, all report suggests indirect embryogenesis in papaya with minimum of 3-6 months required for embryo formation. We have investigated role of polyamines (spermidine, spermine and putriscine) on direct embryogenesis on immature zygotic embryos excised from papaya cultivar Pusa Delicious. A brief exposure (2-4 weeks) of putriscine on induction medium (100 µM) augmented direct embryogenesis on explant. However, prolonged exposure of putriscine was found detrimental.

INTRODUCTION

Papaya (*Carica papaya* L.) is one of the most widely growing fruit crop in the tropics and subtropics of the world. It is rich source of Vitamin A, C and papain. The genus *Carica* comprises 21 species (Purseglove, 1974) but only *Carica papaya* has economic importance. Papaya is agronomically, horticulturally and commercially important fruit crop. Somatic embryogenesis is an important pathway for in vitro plant regeneration of majority of fruit crops and a potential model system for studying regulatory events of plant morphogenesis in vitro and it allows for easy manipulation of tissues for genetic transformation. Polyamines such as spermidine, spermine and putriscine are small, aliphatic amines that are ubiquitous in all plant cells. Although the defined modes of action of polyamines are yet to be understood (Walden et al., 1997), studies support the role of polyamines in modulation of a variety of physiological processes like cell growth and differentiation to stress responses (Galston and Kaur-Sawhney, 1990; Bajaj and Rajam, 1996; Kumar et al., 1997; Rajam, 1997). They have also been considered as a new class of growth regulators (Bagni and Torrigiani, 1992) and being used to improve plant developmental processes, including somatic embryogenesis (Rajam, 1997). Frequency of embryogenesis in papaya is high however; it takes long incubation periods (3-6 months) for embryos to appear. Therefore, we have investigated role of polyamines on early induction of somatic embryoids in papaya.

MATERIAL AND METHODS

The study was conducted at Biotechnology Laboratory, Central Institute for Subtropical Horticulture, Lucknow. Ninety to 120 days old immature green fruit of Pusa Delicious cultivar of papaya maintained at germplasm block of Central Institute of Subtropical Horticulture, Lucknow was excised. The fruit was washed under running tap water and than soaked in 1.05% sodium hypochloride (NaOCl) solution containing 1 drop of Tween 20 for surface sterilization for one hour and than washed with autoclaved distilled water five times. The fruit was bisected under aseptic condition and than white, plump immature seeds were scooped out. The testa of seed was removed with the help of forceps and scalpel. Immature zygotic embryos were taken out by cutting one side of seed and pressing gently in the middle portion. Excised immature zygotic embryos were inoculated on petridishes containing induction media (½ strength MS medium containing 60 g/L sucrose, 400 mg/L L-glutamine, MS vitamins, 0.8% difco bacto-agar and 10 mg/L 2,4-D) supplemented with polyamines (spermidine, spermine and putricine) at different

concentration viz., (0, 25, 50, 75 and 100 µM) under dark for four to six weeks. All the embryos were later subcultured on MS medium devoid of 2,4-D and polyamine. All the polyamines were filter sterilized under aseptic condition and added in the medium. pH of the media was kept at 5.8. The cultures were incubated under dark at 25±2°C with 50% relative humidity. Ten Petri plates formed one replication and each treatment was replicated three times. Observations were recorded periodically.

RESULT AND DISCUSSION

Quick somatic embryogenesis was induced on immature zygotic embryos of papaya inoculated on ½ MS medium fortified with 10 mg/L 2,4-D, 400 mg/L glutamine, 60 gm/L sucrose and putriscine. It is clear from the data (Table 1) that three fold increase in embryogenesis was observed under the influence of 100 µM putriscine (70 embryos/explant) with significant increase in weight of embryonic clump (899 mg) followed by 75 µM putriscine (53.3 embryos/explant). All the three polyamines viz., spermine, spermidine and putriscine augmented production of embryos over control. Increasing concentration (50, 75, and 100 µM) of polyamines enhanced embryo production (Table 1) as well. However, it is interesting to note that callusing was reduced in explants treated with polyamine suggesting direct embryogenesis. Explants exposed to 100 µM putriscine were least (7.03%) callused whereas maximum callusing was observed on tissues which did not get polyamine during course of investigation. Polyamines are ubiquitos cellular compounds involved in the regulation of several developmental processes in plants such as cell growth stimulation, cellular multiplication, somatic embryogenesis, rooting, floral development and protection against stress (Evans and Malamberg, 1989). Polyamines play a positive role in tissue culture system such as morphogenesis in *Helianthus tuberosus* (Phillips et al., 1987), micropropagation of asparagus (Fiala et al., 1991) and somatic embryogenesis in *Hevea brassilensis* (El Hadrami et al., 1989). However, inhibitory effect of polyamine has been reported in coffee (Calheiros et al., 1994). Our results clearly indicate that polyamine had positive role in inducing embryogenesis in papaya and putriscine (100 µM) played significant role in inducing direct embryogenesis.

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Tables

Table 1. Mean influence of certain polyamines on induction of embryogenesis.

Polyamine	Conc (μM)	% Explant callused	No. of somatic embryos/explant	Weight of embryonic clump (mg)
Spermidine	0	72.66	18.60	328
	50	25.70	22.00	363
	75	19.20	25.00	380
	100	11.50	26.60	400
Spermine	0	72.66	18.60	328
	50	20.00	27.00	376
	75	14.30	35.30	425
	100	10.60	40.60	484
Putriscine	0	72.66	18.60	328
	50	16.00	45.30	463
	75	11.60	53.30	600
	100	7.03	70.60	899