Pak.J.Bot., 26(1): 191-195, 1994.

PLANT REGENERATION FROM *CARICA PAPAYA* CV. MALIR GROWN IN TISSUE CULTURE

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Abstract

Petiole segments and excised epidermal thin cell layers of *Carica papaya* cv. Malir were cultured on full strength MS medium containing a range of concentrations of nephthalene acetic acid (NAA) and isopentenyl adenine (2iP) used seperately and in combination. Petiole segments and epidermal thin cell layers formed maximum callus on medium containing 2.5 mg/l NAA plus 0.5 mg/l 2iP and 1 mg/l NAA plus 1 and 5 mg/l 2iP respectively. Enhanced callus growth and shoot formation occurred on half strength macro inorganic salts of MS medium containing 2.5 mg/l NAA plus 0.5/1 mg/l 2iP alongwith 15% coconut milk. Regenerated shoots produced roots on half strength macroinorganic salts containing MS medium supplemented with 5 g/l glucose. The method may be useful for the mass propagation of female papaya plants for large scale cultivation.

Introduction

The crop improvement programmes of papaya are faced with problems due to its heterozygosity, dioecious nature, solitary habit and a large number of viral diseases. Tissue culture techniques appear to be an alternative approach where mature tissues from field grown plants can be used for multiplication and large scale utilization. Tissue culture studies conducted on different cultivars of *Carica papaya* showed that callus growth and adventitious shoot development from seedling stem segments (Arora & Singh, 1978), from cotyledon lamina (Litz *et al.*, 1983), shoot growth and callus induction from the apical and lateral buds of 2-4 month old plants has been achieved. (Drew & Smith, 1986). Somatic embryogenesis has been reported from seedling petiole-derived callus (DeBruijne *et al.*, 1974), from seedling stem-derived callus (Yie & Liaw, 1977) and from ovular callus (Litz & Conover, 1981, 1982). In the present study petiole segments and epidermal thin cell layers excised from petiole segments of young leaves of field grown female plants were used for the regeneration of plants from *C. papaya* cv. Malir.

Materials and Methods

Leaf petioles and epidermal thin cell layers excised from young leaves of mature female field-grown plants of *Carica papaya* cv. Malir, raised from seeds were used. Young leaves, 8-12 cm long, growing around the shoot apex were removed, delaminated, washed free of dust under tap water and sterilized with 0.1% mercuric chloride containing 1-2 drops of Tween-20, for 15 min, with constant shaking. Sterilized petioles washed 3-4 times with sterile water were cut into 1 cm long segments under sterile water in a Petri dish. Longitudinal strips of epidermal thin cell layers (6-8 cells thick and 3-4 mm wide) were excised from the surface of these explants. Both excised petiole segments and epidermal thin cell layers, excised from similar explants, were placed on Murashige & Skoog (1962) basal medium containing growth hormones

Table 1. The effect of different concentrations of NAA and 2iP on callus induction and its growth from petiole segments of *Carica papaya* cv. Malir grown in culture for 4 weeks.

NAA (mg/l)	2iP (mg/l) 0.5	1.0	5.0
0	++	++	+++
2.5	+	++++	++++
6.25	+++	+	-
12.5	+	+	-
25.0	-	-	-

Degree of callusing: + = negligible; ++ = little callus; +++ = moderate; ++++ = good.

and other addenda (coconut milk and casein hydrolysate wherever used), adjusted at pH 5.5 and solidified with 1% agar. A range of NAA (1,2.5,5 and 10 mg/l) and 2iP (0.5, 1 and 5 mg/l) were added before autoclaving the medium. Wide mouthed jars of 75 ml capacity were used throughout the experiments. Cultures were maintained at 25°C with a light regime of 16 h light and 8 h dark period. Observations were recorded weekly under a stereomicroscope. Regenerated shoots were excised and transferred to the root induction medium. Regenerated plants were then transferred to pots.

Results

Petiole segments of *C. papaya* when cultured in the MS medium containing 0.5 and 1.0 mg/l 2iP (Table 1) produced small patches of brownish white spongy callus on the surface of the explants. More callus of the same texture developed with 5 mg/l 2iP and almost half of the surface of the explants was covered with 2-3 mm thick layer of callus. Maximum callus formation occurred when 2.5 mg/l NAA was used with 5 mg/l 2iP. It was brownish white and spongy. Higher concentrations of NAA produced some callus provided low concentrations (0.5 and 1.0 mg/l) of 2iP were present in the medium. Higher concentrations of 2iP inhibited callus growth.

Epidermal thin cell layers did not respond to either NAA or 2iP applied individually over a range of concentrations (Table 2). Yellowish white spongy callus formed in the medium containing both NAA and 2iP at 1 mg/l concentration (increase in their concentration decreased growth) which formed rounded compact organized masses around the periphery of explants where the cut ends of the explants touched the medium. On extending the culture period for further two week these callus masses gradually turned brown and became necrotic. Subculturing on fresh medium also did not revive them. Epidermal explants cultured on half MS medium supplemented with 15% coconut milk (CM) also containing NAA (2.5 mg/l) plus 2iP (0.5 & 1.0 mg/l) exhibited profuse callus growth (Table 3). After 2 week compact rounded primordia developed which turned green in colour and produced shoots with characteristic pal-

Table 2. The effect of different concentrations of NAA and 2iP on callus induction and its growth from epidermal thin cell layers of *Carica papaya* cv. Malir grown in culture for 4 weeks.

NAA (mg/l)	2iP (mg/l) 0	0.5	1.0	5.0
0	-	-	-	-
1.0	-	-	++++	+++
2.5	-	++	++	++
5.0	+	+	++	++
10.0	-	+	+	-

Degree of callusing: + = negligible; ++ = little callus; +++ = moderate; ++++ = good.

mate leaves. Rooting of the excised shoots occurred on transfer to a medium containing 2.5 mg/l IBA. Rooted plants were successfully transferred to beakers containing sterile soil moistened with 1/2 strength MS inorganic salts plus 5 g/l glucose and then to the pots in a wet humid place.

Discussion

Petiole segments and epidermal thin cell layers showed varied response under similar culture conditions. Petiole segments produced callus even in the absence of NAA whereas for epidermal explants requirement of NAA for the callus induction was essential.

Optimum callus formation from both type of explants was obtained in a medium containing both NAA and 2iP. DeBruijne *et al.*, (1974) also obtained best callus yield in papaya in the presence of NAA and 2iP. Morphogenesis failed to occur on callus induction medium although other cultivars of papaya produced roots, shoots and embryos on callus induction medium (Litz *et al.*, 1983; Chen *et al.*, 1987).

Table 3. The effect of different concentrations of NAA and 2iP on callus induction, its growth and morphogenesis from epidermal thin cell layers of *Carica papaya* cv. Malir grown on half MS medium containing 15% coconut milk for 5 weeks.

NAA (mg/l)	2iP (mg/l) 0.5	1.0	5.0
0	+	+	+
2.5	+++++s	+++++s	+

Degree of callusing: + = negligible; ++ = little; +++ = moderate; ++++ = good; +++++ = extensive; s = shoot.

For shoot development actively growing calli were transferred to the MS medium in which major inorganic salts have been reduced to 1/2 strength suggests that a relatively low osmotic pressure may possibly be involved in the process. A beneficial effect of lowering the osmotic concentration of the medium, by varying the amount of different constituents of the medium, on somatic embryogenesis, has been reported for carrot (Wetherell, 1984), for *Psidium guava* (Ammirato & Steward, 1971) and for *C. papaya* suspension cultures subjected to osmotic stress. Coconut milk is a growth factor and contributes to stimulate rapid cell division.

Rooting of isolated regenerated shoots occurred in the medium supplemented with IBA 2.5 mg/l as has been reported for *C. papaya* (Litz & Conover, 1978; Rajevan & Pandey, 1986), however, other cultivars viz., Solo and Sunrise are shown to require NAA in the medium for root development (Chen *et al.*, 1987).

Acclimation of regenerated plants was performed following Singh & Pandey (1989) and Preece & Sutter (1991). A procedure for the *in vitro* propagation of female plants of *C. papaya*, through callus, is developed utilizing epidermal thin cell layers. The thin cell layers used in this study comprised of epidermal and subepidermal parenchyma (Fisher, 1980) and provided a relatively simple and refined system which retains morphogenetic potential and shows rapid reactions to external factors (Aghion-Prat, 1965; Tran Thanh Van & Drira, 1970). The system can serve as a very useful explant type in studies concerning papaya improvement through tissue culture methods. This technique can be employed for clonal propagation of mature papaya plants for commercial multiplication of desired genotype and sex.

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(Received for Publication 10 August 1993)