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Effect of plant growth regulators and silver nitrate on female papaya using shoot tip as explant

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ABSTRACT

The present study was carried out on Selection 1 cultivar of mature female papaya (*Carica papaya* L.) by using shoot tip as an explant and media supplementation with the main aim to assess the effect of growth regulators (auxins, cytokinins and silver nitrate) on *in vitro* regeneration of female papaya. 26 media were used for shoot regeneration while for root regeneration four media were tested supplemented with different concentration of IBA. Based on the results of this study, for shoot proliferation, MS basal medium supplemented with BAP (2.0 mg l^{-1}) + NAA (0.1 mgl^{-1}) was the best followed by MS medium fortified with BAP (2.0 mgl-1) + NAA (0.5 mgl^{-1}) while MS medium supplemented with IBA (2.0 mgl^{-1}) gave best rooting response. Besides, auxins and cytokinins, effect of silver nitrate ($AgNO_3$) on plant regeneration from shoot tip taken from mature female papaya plant was also carried out.

Key words: Auxins, cytokinins, proliferation, shoot tip, silver nitrate ($AgNO_3$).

INTRODUCTION

The papaya (*Carica papaya* L.) is a native of tropical America and belongs to family *Caricacea*. The plant starts fruiting in just one year and gives economically high yield per acre next to banana. But the tremendous yielding potential of this crop is left economically unexploited due to several problems associated with its cultivation. One of the major limiting factors is dioecious nature of the plant, varying degrees of sexes resulting into a large number of unproductive male plants in the progeny, which leads to continuous slackening of the improvement programme. It is well known that conventional asexual propagation techniques, such as grafting and rooting of cutting (Allan, 1; Soomark and Tai, 16) have not resulted in efficient method for large scale production of papaya plants. Thus, the only alternative left to overcome the above limitations is vegetative propagation of papaya through tissue culture. The plant tissue culture technique has been successfully employed in the regeneration of various fruit crops like almonds, strawberry, citrus, etc. (Bajaj, 2). A clonal propagation technique using mature female plants is therefore highly desirable for commercial practice, especially in sub-tropics where some dioecious lines perform better than hermaphroditic ones. Therefore, the present study was undertaken with a view to see the effect of different growth regulators on the *in vitro* regeneration of female papaya using shoot tip as explant.

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MATERIALS AND METHODS

In the present study, a shoot tip (3-4mm) was used as explant. They were collected from the fruit bearing plants about 6-8 mm in length. Healthy twigs were cut, leaves removed and they were surface sterilized with soap water and then treated with 0.5 percent Bavistin (fungicide) for 45 minutes. This was followed by 4-5 times rinsing with distilled water. Afterwards explants were given 70 percent alcohol treatment for 30 seconds followed by 50 percent sodium hypochlorite for 3 minutes. After this the explants were rinsed 4-5 times with sterilized double distilled water. Finally the explants were given 0.1% $HgCl_2$ treatment for 4 minutes and then the explants were again rinsed 5-6 times with autoclaved double distilled water. All the disinfections operations were carried out in the horizontal laminar flow cabinet. Different growth regulators namely indole-3-acetic acid (IAA), indolebutyric acid (IBA), naphthalene acetic acid (NAA), 2,4-dicholophenoxyacetic acid (2,4-D), kinetin, 6-benzylaminopurine (BAP), zeatin, silver nitrate ($AgNO_3$) were tested with Murashige and Skoog (MS) basic medium (Murashige and Skoog, 11) (Fig. 1a). Effectiveness of MS basal medium was also reported earlier in other crops (Banerjee 3). Standard procedure was followed for the preparation of media (Gamborg and Wetter, 6). The pH of the media was adjusted to 5.7 prior to sterilization done at 15 lbs/in^2 for 15 min. All media were solidified with 8g/l agar. Cultures were maintained at 26°C with 16 h light (light intensity 50$\mu^2 gms^-$

^{1})/8 h darkness. The data of all the experiments conducted under present investigations were presented as mean of the three repeats. Data were analyzed statistically using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

In general, shoot tips as explants performed better by giving good regeneration percentage and better growth of propagules (Fig. 1b). Maximum (70.0%) response was observed on medium supplemented with BAP (2.0 mg l^{-1}) + NAA (0.1 mg l^{-1}) (Table 1) while comparatively poor response was observed on the media supplemented with kinetin (Table 1). No shoot regeneration was observed on media devoid of any growth regulators. Good response was observed on MS supplemented with BAP (2.0 mg l^{-1}) + NAA (0.5 mg l^{-1}), BAP (1.0 mg l^{-1}) + IAA (0.2 mg l^{-1}) and BAP (1.0 mg l^{-1}), (60.2, 54.2 and 46.4%, respectively), while rest of media gave poor response (Table 1). In case of kinetin maximum shoot regeneration (27.5%) response was observed on medium supplemented with Kinetin (2.5 mg l^{-1}) + NAA (0.2 mg l^{-1}) and an average response was observed on medium supplemented with Kinetin (2.5 mg l^{-1}) (24.3%). When $AgNO_3$ was added in the media (Table 2) supplemented with other growth hormones, the maximum regeneration response was observed (62.0%) on MS medium supplemented with BAP (1.0 mg l^{-1}) + $AgNO_3$ (2.0 mg l^{-1}) + Zeatin (3.0 mg l^{-1}). Moderate (57.3%) regeneration response was observed on MS + BAP (2.0 mg l^{-1}) + $AgNO_3$ (2.0 mg l^{-1}) + Zeatin (2.5 mg l^{-1}) medium, while MS medium supplemented with $AgNO_3$ (2.0 mg l^{-1}) and $AgNO_3$ (1.0 mg l^{-1}) gave comparatively poor response

Fig.1a. Shoot tip explants cultured on regeneration medium
b. Multiple shoot formation from shoot tip explants
c. Regenerated roots on rooting medium
d. Transfer of shoot tip regenerated plantlets into pot

Table 1. Effect of Kinetin or BAP with 2, 4-D, NAA, IAA on plant regeneration with shoot tip in female papaya (*Carica papaya* L.).

2,4- D (mg/l)	NAA (mg/l)	IAA (mg/l)	Kinetin (mg/l) Mean (%) response (Mean ±S.E.)*				BAP (mg/l) Mean (%) response (Mean ±S.E.)*			
			0.5	1.0	2.5	5.0	1.0	2.0	2.5	5.0
-	-	-		00.0 (04.05 ± 0.01)	24.3 (29.85 ± 0.73)	17.4 (25.01 ± 0.52)	46.4 (43.22 ± 0.28)		37.8 (38.24 ± 0.41)	31.4 (34.37 ± 0.44)
2.5	-	-	00.0 (04.05 ± 0.01)	22.2 (28.44 ± 0.39) 1	-	-				
-	0.1	-						70.0 (57.27 ± 1.06)		
	0.2	-		12.4 (21.05 ± 0.64)	27.5 (31.96 ± 0.37)	17.2 (24.86 ± 1.09)	22.6 (28.67 ± 0.99)		18.5 (27.75 ± 0.58)	15.8 (23.77 ± 0.36)
	0.5							60.2 (51.26 ± 0.67)		
	0.2			00.0 (04.05 ± 0.01)	17.6 (25.16 ± 0.66)	10.2 (19.04 ± 0.50)	54.2 (47.66 ± 0.29)	(34.27 ± 0.27)	(28.99 ± 0.83)	31.2 23.03

CD: 1.755; CV: 2.976; *Transformed value

(27.0 and 17.6%, respectively). No response was observed on MS medium without any growth regulators.

The regenerated plantlets from micropropagation experiments grown *in vitro* were later on transferred to different rooting media for carrying out their rooting *in vitro.* Roots formed were thick and strong (Fig.1c). No response was observed when medium was devoid of any auxins. Maximum rooting frequency (67.2%) was observed on MS medium supplemented with IBA (2.0 mg l^{-1} mg l^{-1}) medium; however, it was minimum (22.2% and 27.8%) on medium supplemented with IBA (0.1) and IBA (0.5 mg l^{-1}) respectively (Table 3). The regenerated plantlets were later on transferred to pots containing F: Y: M in 1:1:1 ration (Fig. 1d). About 70 % plantlets survive in the pot house condition.

As outlined by Skoog and Miller (14) that the root and shoot initiation was basically regulated by the interaction between the two hormonal substances i.e. auxins and cytokinins. Best medium irrespective of the explants used. A proper ratio of cytokinins and auxins helped in both the shoot and root formation (Murashige, 10).

The most striking influence on bud-break and shoot multiplication has been found with the use of auxins and cytokinins (Normanly, 12). The most commonly used cytokinins are BAP, kinetin, 2ip and zeatin, the latter two being natural cytokinins. Superiority of BAP over other cytokinins has been reported and discussed in relation to shoot proliferation in culture of trees (Bonga and Jvon Aderkas, 5). In the present study, lower level (1.0 mg l^{-1}) of BAP and of kinetin (2.5 mg l^{-1}) included highest frequency of shoot regeneration. Regeneration response of BAP was superior over kinetin in taking less time for induction of shoot regeneration. In the present study, highest frequency of shoot regeneration and axillary bud proliferation could be achieved on BAP (1.0 mg l^{-1}) without the need of subculture. In the present study, it was observed that an increase in the level of cytokinin from 1.0 to 5.0 mg l^{-1} produced a negative effect on all the parameters except shoot number (Tables 1).

In the present study, I found a promotive effect of cytokinin and auxin on shoot regeneration from shoot tip explant, however, an inhibitory effect was observed in the combination where levels of cytokinins were high (5.0 mg l^{-1}). Auxins (NAA, 2, 4-D and IAA) were also examined for their influence on multiple shoot production and growth of papaya shoot tip cultures. Auxins were found to affect only the growth of the cultures rather than proliferation rate. NAA (0.1 – 0.2 mg l^{-1}) resulted in better growth of the cultures and hence included in the medium. These results were similar when compared to the previous results in papaya (Renveni *et al*., 13). The promotive effect on shoot regeneration in combination of BAP/ kinetin with NAA, maximum shoot regeneration of frequency in shoot tip explant (70.0%) was observed on basal medium with BAP (2.0 mg l^{-1}) + NAA (0.1 mg l^{-1}) while kinetin found to have less promotive effect in combination with NAA. The complementary effect of cytokinin and auxin has been observed by Miller and Drew (9) during shoot proliferation in papaya. A combination of BAP (2.0 mg l^{-1}) and NAA (1.0 mg l^{-1}) was found to enhance shoot bud proliferation in cultured shoot tip in papaya (Litz and Conover, 8). Combination of cytokinin and auxin thus has been found to promote shoot bud proliferation.

Various auxins were tried with the objective of inducing roots in shoots. In this study, medium containing MS basal + IBA (2.0 mg l^{-1}) was found most appropriate for root induction. Plantlets produced with IBA were normal with regard to the shoot and root growth. IBA has been found to be effective for root induction in papaya-regenerated shoots by Bhattacharya *et al*. (4). Although IAA also induced root formation but the root development was poor as compared to IBA.

$AgNO_3$ is an ethylene inhibitor in plants which is

Table 2. Shoot induction response from axillary bud explants on media supplemented with $AgNO_3$ with Zeatin, BAP in papaya (*Carica papaya* L.).

Zeatin (mg/l)	BAP (mg/l)	$AgNO_3$ (mg/l) Mean (%) response (Mean ±S.E.)* 1.0	2.0
-	-	17.6 (25.19 ± 0.35)	27.0 (31.59 ± 0.56)
2.5	2.0	-	57.3 (49.47 ± 0.41)
3.0	1.0	-	62.0 (52.20 ± 0.39)

CD: 1.454; CV: 1.920; *Transformed value

Table 3. Effect of IBA on rooting of shoots in MS medium.

IBA (mg/l)	No. of shoots forming root	% of shoot forming root Mean (%) response (Mean ±S.E.)*
0.1	2	22.2 (25.04 ± 3.64)
0.5	2	27.8 (32.06 ± 1.74)
1.0	4	62.2 (52.37 ± 1.33)
2.0	7	67.2(55.46 ± 2.68)

CD: 1.431; CV: 1.436; *Transformed value

reported to help in somatic embryogenesis (Songstad *et al.* 15). $AgNO_3$ may also serve as stress agent inducing endogenous ABA accumulation, Ag+ being a metallic ion may also promote somatic embryo production *via* an increase in the endogenous ABA levels (Kong and Yeung 7). But in our experiments when $AgNO_3$ was added in the media (Table 3) supplemented with other growth hormones, the regeneration response was observed maximum (62.0%) on MS medium supplemented with BAP (1.0 mg l^{-1}) + $AgNO_3$ (2.0 mg l^{-1}) + Zeatin (3.0 mg l^{-1}). Thus, overall in conclusion media containing $AgNO_3$ has no promotive effect on shoot formation.

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