International Journal of Research in Agriculture and Forestry
Volume 2, Issue 2, February 2015, PP 46-49
ISSN 2394-5907 (Print) & ISSN 2394-5915 (Online)

Micro Propagation of Papaya Variety CO-5

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ABSTRACT

Papaya cultivation is encountered with the problem of its dioecious nature Micro propagation represents the only economic way of continuously producing uniform planting materials of known sex. The propagation studies were carried out by enhanced release of axillary buds in Papaya variety CO-5. Apical buds and lateral buds from seedlings and mature plants were used as explant for *in vitro* propagation. Explants from papaya varieties and hybrids were subjected to different treatments of plant growth substances for culture establishment and shoot proliferation. The present study revealed that full strength MS medium supplemented with sucrose 30.00 g l^{-1} and agar 6.50 g l^{-1} under light condition produced highest shoot number and longest shoot in papaya variety CO-5. Application of BA 0.50 mg l^{-1} along with NAA 0.10 mgl^{-1} was found to be better for initial culture establishment and proliferation of papaya variety CO-5. *In vitro* rooting was best in full strength MS medium supplemented with IBA 3.00 mg l^{-1}, sucrose 30.00 g/l and activated charcoal 0.05 per cent.

Keywords: *Papaya, Propagation, Explants, Proliferation*

INTRODUCTION

Papaya has emerged as a main commercial crop during the last few years, because of the high nutritive value. One of the main advantages of papaya is that the fruits are available throughout the year. Unlike the other perennial crops, papaya is seed propagated and it yields early. Since seed propagated, due to lack of controlled pollination a lot of variability is seen. Papaya cultivation is encountered with the problem of its dioecious nature. The majority of plantations are established from seeds using dioecious cultivars. Hence seed propagation results in seedlings which are either male or female. Due to this mode of reproduction, papaya plants are not true-to-type and show significant variation in yield, fruit quality and disease susceptibility within cultivated populations (Drew, 1988). Moreover, as sex cannot be determined until the mid development stage, three seedlings are established in each planting position, till flowering. Then they are thinned, retaining only the most vigorous female plant with one male to every 10 to 20 female plants. This results in wastage of inputs. With a requirement to renew plantations every three year to ensure quality fruit production propagation by seed represents a significant cost to the producer. The multiplication rates are low in the vegetative propagation methods like mound layering. This field level problem necessitates the substitution of seedling progeny with tissue culture propagules developed from female or bisexual plants. Micro propagation represents the only economic way of continuously producing uniform planting materials of known sex. Papaya clones developed by in vitro method are uniform and produce high quality fruits similar to those of their mother plants (Chan and Teo, 2002).

MATERIALS AND METHODS

The studies on “In vitro propagation of papaya (Carica papaya L.)” were carried out at the Department of Pomology and Floriculture and Department of Plant Biotechnology, College of Agriculture, Vellayani. The propagation studies were carried out by enhanced release of axillary buds in Papaya variety CO-5. Apical buds and lateral buds from seedlings and mature plants were used as explant for in vitro propagation. Explants from papaya were subjected to different treatments of plant growth substances for culture establishment. The culture establishment medium is useful for conditioning of the explant and for stimulating its initial growth. In order to standardise a suitable hormone combination for better culture establishment, studies were carried out using BA, Kinetin and NAA at various concentrations. The treatments involved are different levels of cytokinins, viz; BA (0.20 -5.00

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mg l-1) and Kinetin (0.50-5.00 mg l-1) alone or in combination with auxin, viz., NAA (0.01, 0.10 and 0.50 mg l-1). Six replications were kept for each treatment. The basal media used for the study were Ms (Murashige and Skoog, 1962) and half strength Ms. Observations were recovered on the number of surviving cultures (percentage), number of cultures showing initial growth, number of days for bud initiation and number of buds per culture, after four weeks of culturing in the establishment media. Plant growth substances like BA (0.20-5.00 mg l-1) and Kinetin (0.50-5.00 mg l-1) alone or in combination with NAA (0.01, 0.10 and 0.50 mg l-1) were tried for shoot proliferation. Six replications were also kept for each treatment. The number of cultures survived (percentage), number of shoots per culture and length of the longest shoot and abnormalities in shoot growth if any, were recorded after six weeks of culturing in the shoot proliferation media. The cultures were kept in light or in darkness in order to assess the effect of light on multiple shoot proliferation. Light (3000 lux, 16 hours photoperiod) was provided using cool white fluorescent tubes. Darkness was provided by keeping the cultures in a temperature controlled dark room (25 ± 2°C). Trials on the in vitro rooting were conducted in full Ms medium. Individual shoots measuring 2.50-3.50 cm length, excised from shoot proliferating cultures were subjected to different rooting treatments with varying levels of IBA (0.50 - 4.00 mg l-1), IAA (0.10- 2.00 mg l-1) and NAA (0.10 – 3.00 mg l-1). Each treatment was replicated six times. Observations on the number of cultures initiating roots, number of days for root initiation, number of roots, root length and abnormality in root growth, if any, were recorded four weeks after culturing. Observations on number of days for planting out were also recorded.

RESULTS AND CONCLUSIONS

In the present study, in vitro propagation by enhanced release of axillary buds was attempted in papaya variety Co-5. Various workers reported micropropagation in papaya by enhanced release of axillary buds (Litz and Conover, 1978; Miller and Drew, 1990; Reuveni and Shlesinger, 1990; Lai et al., 1998; Chan and Teo, 2002). Explants used in the present study were apical buds and lateral buds of mature plants and one month old seedlings. Similar result was obtained by Rajeevan and Pandey (1983), who used shoot tips from seedlings and lateral buds from female plants for in vitro propagation of papaya variety Coorg Honeydew. Among the different plant growth substances used in the establishment medium, the earliest bud break was obtained with the application of BA 3.00 mg l-1 along with NAA 0.01 mg l-1 and highest bud initiation was recorded from BA 0.50 mg l-1 along with NAA 0.10 mg l-1. The result of the present study is in confirmity with the observations of reuveni et al. (1989) who observed that MS medium supplemented with BA 0.50 mg l-1 and NAA 0.10 mg l-1 gave the highest number of bud initiation of papaya in establishment medium. Full strength MS medium produced highest shoot proliferation rate, better survival of plantlets in papaya variety CO-5. Sucrose 30.00 g l-1 in shoot proliferation medium produced maximum number of shoots and also results in better survival. The study also revealed that agar 6.50 g l-1 produced highest shoot number and longest shoot in papaya .Application of BA 0.50 mg l-1 along with NAA 0.10 mgl1 was found to be better for shoot proliferation in papaya (Table 1). The results of the present experiment is in line with the findings of Islam et al. (1993) who reported that maximum number of shoots in papaya variety Rajshahi-red was produced from shoots cultured in MS medium supplemented with BA 0.50 mg l-1 along with NAA 0.10 mg l-1. Maximum shoot proliferation and better survival was noticed with the addition of amino acid glycine, 100.00 mg l-1. Addition of activated charcoal 0.05 per cent and Cobalt chloride 5.00 mg l-1 increased shoot proliferation rate and shoot length in papaya, while the highest survival percentage was obtained with the addition of Cobalt chloride 10.00 mg l-1. Cultures placed under light produced maximum number of shoots and increased shoot length in papaya. Better survival of papaya plants also was observed under light conditions. The positive effects of light was observed by Magdalitha et al., 1997; Chun et al., 1998; Rajeevan and Pandey, 1983; Suthamathi et al., 2002 also.

Among the various plant growth substances tried for in vitro rooting, MS medium supplemented with IBA 2.00 mg l-1 induced early rooting. Highest number of roots was obtained by the addition of IBA 3.00 mg l-1 in papaya variety CO-5 (Table 2). Bhattacharya et al. (1997) reported that in papaya variety Washington under in vitro conditions, application of IBA 3.00 mg l-1 promoted highest number of roots. The present experiment also showed similar result. Highest number of roots was noticed with the addition of sucrose 30.00 g l-1 to the rooting medium. Addition of activated charcoal 0.05 per cent to the rooting medium induced early rooting and highest number of root.

Table1. *Effect of plant growth substances on multiple shoot proliferation (shoots per culture, length of longest shoot) of papaya variety CO-5*

Treatments	Plant growth substances (mg l^{-1})	Number of shoots per culture	Length of longest shoot (cm)
MSP1	BA 0.00 + NAA 0.01	1.00	2.18
MSP2	BA 0.00 + NAA 0.1	1.17	1.28
MSP3	BA 0.00 + NAA 0.5	1.50	1.93
MSP4	BA 0.20	1.00	0.70
MSP5	BA 0.20 + NAA 0.01	1.00	2.15
MSP6	BA 0.20 + NAA 0.10	3.17	2.63
MSP7	BA 0.20 + NAA 0.50	1.67	1.68
MSP8	BA 0.50	2.17	2.90
MSP9	BA 0.50 + NAA 0.01	1.33	0.85
MSP10	BA 0.50 + NAA 0.10	6.50	5.78
MSP11	BA 0.50 + NAA 0.50	3.50	4.08
MSP12	BA 1.00	1.50	1.18
MSP13	BA 1.00 + NAA 0.01	1.17	2.15
MSP14	BA 1.00 + NAA 0.10	4.33	3.72
MSP15	BA 1.00 + NAA 0.50	1.00	2.05
MSP16	BA 3.00	1.50	1.53
MSP17	BA 3.00 + NAA 0.01	5.33	3.30
MSP18	BA 3.00 + NAA 0.10	1.00	0.87
MSP19	BA 3.00 + NAA 0.50	2.83	2.68
MSP20	BA 5.00	1.33	1.20
MSP21	BA 5.00 + NAA 0.01	2.67	2.05
MSP22	BA 5.00 + NAA 0.10	1.33	2.78
MSP23	BA 5.00 + NAA 0.50	1.00	1.37
MSP24	Kinetin 0.50	1.00	2.05
MSP25	Kinetin 0.50 + NAA 0.01	4.17	3.75
MSP26	Kinetin 0.50 + NAA 0.10	1.50	1.22
MSP27	Kinetin 0.50 + NAA 0.50	2.33	3.08
MSP28	Kinetin 1.00	2.17	2.22
MSP29	Kinetin 1.00 + NAA 0.01	1.00	1.05
MSP30	Kinetin 1.00 + NAA 0.10	1.00	1.40
MSP31	Kinetin 1.00 + NAA 0.50	5.33	2.07
MSP32	Kinetin 3.00	2.17	2.45
MSP33	Kinetin 3.00 + NAA 0.01	1.00	3.60
MSP34	Kinetin 3.00 + NAA 0.10	1.00	0.73
MSP35	Kinetin 3.00 + NAA 0.50	1.17	1.18
MSP36	Kinetin 5.00	1.00	1.28
MSP37	Kinetin 5.00 + NAA 0.01	1.00	2.62
MSP38	Kinetin 5.00 + NAA 0.10	1.67	1.18
MSP39	Kinetin 5.00 + NAA 0.50	1.00	3.10
Control		1.00	0.55
CD (0.05)		0.62	0.2

Table2. *Effect of plant growth substances on in vitro rooting (number of roots and length of longest root) of papaya variety CO-5*

Treatments	Plant growth substances (mg l^{-1})	Number of roots	Length of longest root(cm)
R1	IBA 0.50	-	-
R2	IBA 1.00	2.00	2.23
R3	IBA 2.00	1.17	2.70
R4	IBA 3.00	6.33	3.28
R5	IBA 4.00	2.50	1.13
R6	IAA 0.10	-	-
R7	IAA 0.50	1.00	0.88
R8	IAA 1.00	4.17	2.42
R9	IAA 2.00	2.33	1.60
R10	NAA 0.10	-	-
R11	NAA 0.50	3.17	1.37

R12	NAA 1.00	1.00	2.85
R13	NAA 2.00	1.33	2.08
R14	NAA 3.00	3.50	1.47
Control		-	-
CD (0.05)		0.47	0.13

It was concluded from the study that full strength MS medium supplemented with sucrose 30.00 g l^{-1} and agar 6.00 g l^{-1} under light condition produced highest shoot number and longest shoot in papaya. Application of BA 0.50 mg l^{-1} along with NAA 0.10 mgl^{-1} was found to be better for initial culture establishment and proliferation. Application of amino acid, arginine 50.00 mg l^{-1} resulted in highest shoot proliferation rate, while highest shoot length was obtained from arginine 100.00 mg l^{-1}. Addition of activated charcoal 0.05 per cent and Cobalt chloride 10.00 mg l^{-1} increased shoot proliferation rate and shoot length in papaya variety CO-5. *In vitro* rooting was best in full strength MS medium supplemented with IBA 3.00 mg l^{-1}, sucrose 30.00 g $l^{-1}$and activated charcoal 0.05 per cent.

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