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Effect of Growth Regulators and Carbon Sources on Axillary Shoot Proliferation from Shoot-Tip Explant and Successful Transplantation of Papaya (*Carica papaya* L.)

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Abstract: The investigation was based on direct shoot formation from shoot tip explant, subsequent morphogenesis and rooting of the *in vitro* proliferated shoots and transplantation of regenerated plants under *ex vitro* condition. Through proper growth regulators, it was possible to differentiate multiple shoots from shoot tip of papaya (Shahi). In the present investigation, it was observed that shoot proliferation was best in MS medium containing BAP 1.0 mg L^{-1}+KIN 0.5 mg L^{-1} and BAP 1.0 mg L^{-1}+NAA 0.5 mg L^{-1}. Maximum number of shoot per culture (28.2) and length of the largest shoot (1.7 cm) was found on MS medium supplemented with BAP 1.0 mg L^{-1}+KIN 0.5 mg L^{-1}. Shoot proliferation efficiency was found the best in 11 weeks old explant in MS medium containing BAP 1.0 mg L^{-1}+KIN 0.5 mg L^{-1}. Sucrose in 30 g L^{-1} concentration as carbon source was proved to be best for shoot proliferation (82%). Maximum number of shoot per culture (41.2) and maximum length of shoot were obtained in the aforesaid concentration of carbon source. For adventitious rooting of regenerated shoot, MS medium containing IBA 1.0 mg L^{-1}+NAA 0.5 mg L^{-1} was found to be the best. In this growth regulator, treatment 92% of rooting was observed. Longest length of the root (7.9±0.121 cm) was found on MS medium supplemented with IBA 1.0 mg L^{-1}+IAA 0.5 mg L^{-1}. After transplanting the 20 days old rooted shoots into garden soil, compost and sand (2:1:1), 80% of survivability after 5 weeks was achieved.

Key words: Micropropagation, shoot-tip, morphogenesis, rooting, transplantation

INTRODUCTION

The papaya, *Carica papaya* L., is a member of the small family Caricaceae allied to the Passifloraceae. It is a native of tropical America and widely distributed in the tropical and subtropical regions of the world. In recent years papaya has assumed special significance in view of its high nutritive value and being more remunerative.

Papaya is commonly propagated from seeds. Papaya can also be propagated by cuttings or layers or grafting, but these methods are more expensive and laborious and not possible on a commercial scale due to the hollow and fragile nature of its stem. A method of vegetative multiplication of papaya *in vitro* by using different parts of the plant body and a reliable *in vitro* method for the propagation of papaya would have considerable benefits for the horticultural industry. This is the main objective of the present study. Moreover, selection and standardization of media composition, growth regulator requirement and culture environment for consistently high production of plantlets through shoot tip was included in the in the objective in this study. Now a days, application of plant cell and tissue culture may offer a valuable alternate to overcome all the problems concerned with papaya improvement program. It has been experimentally found that inbreed hermaphroditic papaya cultivars are more susceptible to disease and environmental stress (Litz *et al*., 1983). Papaya plants whether derived from somatic organogenesis, from shoot tip culture or from somatic embryos, may possess sufficient useful variability to be utilized in papaya improvement programme (Litz *et al*., 1983). *In vitro* clonal propagation of plants using tissue culture technique is popularly known as micropropagation and it is now being increasingly used for rapid clonal multiplication of many temperate fruit, forest and ornamental trees as well as other crop species (Hussey, 1983; Conger, 1981; Bhojwani, 1990; Debergh and Zimmerman, 1990; Hammerschlag and Litz, 1992). Micropropagation is also the most advanced and applied area of plant tissue culture (Murashige, 1978; Vasil and Vasil, 1980; Zimmerman, 1985). Micropropagation of papaya were also achieved from different explant using *in vitro* technique (Chowdhury, 1991; Hossain *et al*., 1991; Islam *et al*., 1993; McCubbin and Van Staden, 2003). This study describes a simple method of micropropagation of papaya through

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shoot tip culture for obtaining large-scale disease free seedlings. This can ensure adequate supply of uniform and disease free propagules of papaya with a view to manipulate for different purposes.

MATERIALS AND METHODS

Plant material: Shahi, a local variety of *Caria papaya* L. (Papaya) was used as experimental material in this investigation. Shoot tips of field grown seedlings of *Carica papaya* L. (Shahi) were collected from Regional Horticultural Research Station, Rajshahi. Shoot tips collected from 10-12 weeks old field grown plants were disease free and healthy. This research was carried out in central science laboratory at the University of Rajshahi from the period of March, 2005 to July, 2006.

Surface sterilization: The shoot tips of papaya were washed thoroughly under running tap water and then treated with 1% Savlon and 4-5 drops of Tween-80 for about 15-20 min with constant shaking. Then explants were washed 3-4 times with distilled water to make the material free from Savlon. Subsequently the explants were transferred to laminar airflow cabinet and transferred to 250/500 mL sterilized conical flask. After rinsing in 70% ethanol for 1-3 min the explants were immersed in 0.1% $HgCl_2$. The sterling with explants was constantly shaked during sterilization according to necessity of time to increase the release of free Cl_2. To remove every trace of sterilent, the explant was then washed with sterile distilled water at least 4-5 changes.

Preparation of explants, inoculation and initiation of culture: The surface sterilized shoot tip materials were taken in sterilized petridish. Shoot tips were trimmed to (1.0-1.5 cm) terminal parts of lightly furled leaves and 1.2 node of shoot tip explants. The above explants were inoculated singly in culture tubes containing 15-20 mL of different growth regulators supplemented agar-gelled semi-solid media for induction of multiple shoots. The culture were transferred to fresh medium when necessity. The cultures were maintained at 25±2°C with light intensity varied from 2000-3000 lux. The photoperiod was maintained generally 14 h light 10 h dark.

Axillary shoot proliferation: These explants were cultured on MS nutrient medium supplemented with BAP, KIN, NAA alone or in combination at different concentration. Days required to shoot initiation, percentage of explants showing shoot proliferation, number of total shoots, per explant, length of the longest shoots were considered as parameters for evaluating this experiment. At this stage, the proliferating cultures were subcultured again in the same initial medium in order to increase budding frequency. After another 5 weeks of incubation the proliferating cultures were transferred to different media for shoot elongation.

The elongated shoots were excised (> 2.0 cm long) from the proliferated cultures and transferred individually to the rooting media. Some of the shoots, after removing leaves, were cut into pieces having axillary buds and again cultured to freshly prepared medium for multiplication of axillary shoot. These cultures again produced usable axillary shoots within 6 weeks of subculture. The process was repeated for several times in order to establish continuous production of shoots. In this experiment, shoot tip explants from 10, 11 and 12 weeks old field grown seedlings were also used. Explants were cultured on MS nutrition medium supplemented with 1.0 mg L^{-1} BAP and 0.5 mg L^{-1} KIN for proliferating shoots. Local sugar, sucrose and glucose with three different concentration viz., 20, 30 and 40 g L^{-1} for each were used for this experiment. The shoot tips were used as explants for this experiment and they were cultured on MS medium containing BAP 1.0 mg L^{-1}+KIN 0.5 mg L^{-1} with three different concentration of each carbon source separately.

Microcutting preparation for rooting: The shoots proliferated from shoot tip explants in parent culture or subsequent subculture was induced for adventitious root formation to get full-fledged plantlets *in vitro*. The usable shoots (with length> 2 cm) were collected aseptically from shoot culture for rooting and cuttings were cultured individually in 150×25 mm tubes with 20 mL of rooting medium having different strength and combination of auxin. The observations on development pattern of roots were made throughout the entire culture period.

Transplantation of plantlets under *ex vitro* condition: Rooted plants were taken out from the culture tubes and washed carefully under running tap water for complete removal of remains of the medium. Pots (9×6 cm) were kept ready filled with garden soil, compost and sand in the proportion of 2:1:1, respectively. All the pots were covered with wet polythene bag for providing high humidity to the plants. After 45 days, the plants were transferred to the soil.

RESULTS AND DISCUSSION

Multiplication of shoots, from shoot tip explants were found best on MS medium containing BAP 1.0+KIN 0.5 mg L^{-1} and BAP 1.0+NAA 0.5 mg L^{-1} (Table 1).

Days had taken 8-10 for both the medium BAP 1.0+KIN 0.5 and BAP 1.0+NAA 0.5 mg L^{-1} to initiate initial

shoots. But number of shoots per culture and the length of the longest shoots (cm) were showed some verification.

These variations were also found by Islam (2001), Hossain *et al.* (1991) and Islam *et al.* (1993). MS medium with BAP 1.0 mg L^{-1}, KIN 0.5 mg L^{-1}, was proved to be less effective for shoot multiplication in papaya (Shahi) comparatively. As a result, the combined concentration BAP 1.0+KIN 0.5 mg L^{-1} was the best hormonal treatment. Islam *et al.* (1993) also observed that BA at 0.5 mg L^{-1} and NAA at 0.1 mg L^{-1} gave best result in shoot proliferation in papaya. In papaya, Islam (2001), Islam *et al.* (1993) and Chowdhury (1991) observed that KIN and BA at various concentrations were inducing differentiations. However, Islam (2001) observed that BAP with IAA in MS medium produced highest number and length of shoots and also days to shoot initiation occurred within 7-9 days. Lai *et al.* (1999) found that exogenous ethylene enhanced axillary shoot proliferation in Papaya. Ethylene with 0.34, 0.20 and 0.15 ppm concentration for 1-3 weeks, respectively were proved to be best for shoot proliferation. Results of the current investigation are correlated with the previous findings.

In this experiment, shoot tips of 11 weeks old explants were found to be most suitable. Highest 96% of shoot proliferation was observed in 11 weeks old shoot tips. Lowest 56% of shoot proliferation was observed in 12 week old shoot tips. Among the three different concentration of local sugar, 30 g L^{-1} was found to be optimum for shoot proliferation (60%), number of shoots per culture (18.8) and length of shoot (1.2 cm). Highest 82% of shoot proliferation was recorded in 30 g L^{-1} sucrose concentration and lowest 48% in 20 g L^{-1} concentration (Table 2). Sucrose was proved to be the best among four carbon sources studied. Similar result was also found by Islam (2001).

Proliferating shoots obtained from shoot tip explants of papaya plants (Shahi) took maximum 9-12 weeks from the time of establishment to attain the size suitable for rooting (> 2 cm). Percentage of root formation ranged from 32-92%. The highest (92%) of shoots induced roots was observed in medium supplemented with IBA 1.0 mg L^{-1}+NAA 0.5 mg L^{-1}, followed by 76% in medium with IBA 1.0 mg L^{-1}+IAA 0.5 mg L^{-1} (Table 3). Similar result was also found by Islam (2001). Lowest 32% of root formation was recorded in NAA 1.0 mg L^{-1}+IBA 0.1 mg L^{-1}. The average number of roots per shoot ranged from 2.4+0.01±0.01-12.6±0.09. Highest (12.6±0.09) number of roots per shoot was recorded in IBA 1.0+IAA 0.5 mg L^{-1}, followed by (10.2±0.10) in

Table 1: Comparative effect of different hormonal treatments on highest % of shoot proliferation of papaya (Shahi)

Hormonal treatment (mg L^{-1})	Days to shoot initiation	% of explant showing proliferation	No. of shoot per culture	Length of the longest shoot (cm)
BAP 1.0	13-15	52.0	3.50	1.10
KIN 0.5	12-15	49.0	1.90	0.80
BAP 1.0+KIN 0.5	8-10	89.0	28.20	1.70
BAP 1.0+NAA 0.5	8-10	89.0	27.20	1.60
KIN 1.0+NAA 0.5	10-12	73.0	6.80	0.90
Mean		70.4	13.52	1.22
LSD at 5% level		7.54	2.13	0.09

Table 2: Effect of carbon source on *in vitro* shoot proliferation from shoot tips of papaya (Shahi) in MS medium supplemented with BAP 1.0 mg L^{-1} and KIN 0.5 mg L^{-1}. Each treatment consists of 8-10 explants. Data were recorded after 6 weeks of culture

Carbon source	Concentration	% of explant showing proliferation	No. of shoots per carbon	Length of the shoot (cm)
Local sugar	20	42.00	14.40	0.60
	30	60.00	18.80	1.20
	40	66.00	10.10	1.00
Mean		56.00	14.43	0.93
Sucrose	20	48.00	25.20	1.10
	30	82.00	41.20	1.90
	40	70.00	33.80	0.90
Mean		66.66	33.40	1.30
Glucose	20	44.00	11.80	0.70
	30	67.00	21.70	0.90
	40	72.00	11.90	0.80
Mean		61.00	15.13	0.80
Grand mean		61.22	20.98	1.01
Effect of	20g	44.66b	17.13b	0.8b
concentration	30g	69.66a	27.23a	1.33a
of sugar	40g	69.33a	18.6b	0.9b
Effect of carbon				
Source Local	56	11.43b	0.93b	0.93
Sugar sucrose	66.66	33.4a	1.3a	1.30a
Glucose	61	15.13a	0.8b	0.80b

The figure in a column with different letters are statistically different at 5% level

Table 3: Effect of different concentration and combination of auxin in MS medium on adventitious root formation from microcuttings in papaya Shahi. Data were recorded after 4 weeks of culture. Each treatment consisted of 10-12 microcuttings and the experiment was repeated three times

Growth regulators (mg L^{-1})	Days to root initiation	% of cuttings rooted	No. of roots/ cutting $\bar{X}$±SE	Length of the root (cm) $\bar{X}$±SE	Shoot length $\bar{X}$±SE increment (cm)	Morphology of root
OO	-	-	-	-	-	-
IBA 0.5	10.11	49	4.2±0.03	2.5±0.16	31±0.02	NL
IBA 1.0	10-11	56	0.5±0.03	2.9±0.28	2.5±0.19	NL
IBA 1.0+IAA 0.5	8-10	76	12.6±0.09	7.9±0.12	3.9±0.08	NL
IBA 1.0+NAA 0.5	8-10	92	10.3±0.10	7.2±0.08	4.1±0.02	NL
IAA 0.5	-	-	-	-	-	-
IAA 1.0	-	-	-	-	-	-
IAA 1.0+IBA 0.1	10-11	42	2.4±0.01	3.8±0.12	2.5±0.21	NL
IAA 1.0+IBA 0.5	98-11	91	7.1±0.09	5.8±0.12	3.5±0.01	NL
NAA 0.5	-	-	-	-	-	-
NAA 0.1	-	-	-	-	-	-
NAA 1.0+IBA 0.1	9-11	32	2.7±0.06	3.3±0.04	3.1±0.01	SR
NAA 1.0+IBA 0.5	9-11	54	5.0±0.03	5.4±0.14	3.2±0.22	TS

NL = Normal with lateral, SE = Spongy elongate, TS = Thick smooth, SR = Spongy radish shaped, - = No response

IBA 1.0+NAA 0.5 mg L^{-1}. Lowest number of roots (2.4±0.01) was observed in NAA 1.0 IBA 0.1 mg L^{-1}. Average length (cm) of root ranged from 2.5±0.16-7.9±0.12 cm. Highest (7.9±0.12 cm) length of root was recorded in IBA 1.0+0.5 IBA mg L^{-1}, followed by 7.2±0.08 cm in IBA 1.0+NAA 0.5 mg L^{-1}. Lowest (2.5±0.16) length of root was recorded in IBA 0.5 mg L^{-1}. Siriwan *et al.* (1988), Islam *et al.* (1993), Yang and Ye (1992) and Reuveni *et al.* (1990) reported that shoots were successfully rooted in MS medium with only IBA (1.5 mg L^{-1}).

Plantlets with actively growing roots were transferred to pots containing three different types of soil mixture. Survivability percentage of plantlets ranged from 38-80%. Highest 80% of survivability was recorded in garden soil: Compost: sand (2:1:1) where rapid shoot length was also observed. Of the three kind of ages 12 and 20 day-old rooted shoots performed better than 28 day-old rooted shoots although 28-day-old rooted shoots behaved in the same way as those of 12-day-old rooted shoots when transferred to garden soil conditions.

CONCLUSION

The present investigation was undertaken with a view to establish a standard micropropagation technique for *Carica papaya* (Shahi). The investigation was based on axillary shoot proliferation, multiple shoot induction, subsequent morphogenesis and rooting of *in vitro* proliferated shoots. Regeneration of complete and unique plantlets from *in vitro* culture and their successful transplantation and establishment under natural condition were achieved by this investigation.

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