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Ex vitro rooting of micro-propagated intergeneric papaya through phloroglucinol

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ABSTRACT

Micropropagation techniques of papaya have been optimized worldwide. The standard rapid multiplication technique of papaya, which is economically viable still needs to be improved. Although establishing in vitro culture is easy, at the same time, in vitro rooting of papaya is still very difficult. However, in vitro rooting is costly, and the roots initiated in vitro do not have lateral branches and root hairs, making them difficult to acclimatize. Hence, the experiments on ex vitro rooting induction of micro-propagated plantlets and acclimatization were conducted. In vitro cultures were established using shoot tips from selected advanced intergeneric hybrid seedlings. They were inoculated onto Murashige and Skoog (MS) medium + 3% sucrose + 1 µM BAP + 1 µM GA3 + 0.1 µM IAA + 0.25% phytagel until plantlets were obtained. For ex-vitro rooting, plantlets were cultured with various concentrations of phloroglucinol (PG) to investigate the appropriate rooting condition. Direct rooting of papaya micro-cutting into potting medium (perlite + vermiculite + coco peat; in the ratio of 1:1:2) subsequent dipping into phloroglucinol was more effective than in vitro rooting and led to 82% survival of micro-cutting during acclimatization. PG at 1000 µM was more effective than IBA in encouraging root development. The Sachet method was found to be the most appropriate for ex vitro rooting and hardening of papaya with single-step acclimatization.

Key words: *Carica papaya* L., Acclimatization, Phloroglucinol, Micropropagation.

INTRODUCTION

Papaya (*Carica papaya* L.) is widely cultivated and one of the most important fruit crops of the tropical and subtropical regions of the World. The major producers include India, the Dominican Republic, Brazil and Mexico (FAO, 5). In India, the area under papaya cultivation is 0.149 mha with a production of 6.05 MT/ha and a productivity of 40.6 t/ ha (FAO, 5). Fruits are rich sources of vitamins A and C, relatively rich in minerals, and have high nutritive and medicinal values (Ming *et al.*, 11).

Commercial cultivation of papaya is mainly through seeds (Bhattacharya and Khuspe, 2), and vegetative propagation (layering, grafting and rooted cuttings) still needs to be a successful, profitable exercise in India. The tissue culture protocol of papaya has been demonstrated using explant from glasshouse-grown plants or the use of shoot-tip and axillary buds from the lateral shoots of field plants (Wu *et al.*, 21; Setargie *et al.*, 18). However, the most severe problem in papaya micropropagation is its vitro rooting and acclimatization, which results in poor rooting ability and root quality (McCubbin and Van Staden, 10; Sekeli *et al.*, 17). In addition, creation of more callus at the bottom of plantlet (Agnihotri *et al.*, 1; Sekeli *et al.*, 17) and abnormal roots on agar medium when IBA was used as a root promoting hormone (Suksa-Ard *et al.*, 19). Since in-vitro rooting through IBA is a challenging and tedious practice and unsuitable for hardening, ex-vitro rooting is a potential decision.

The addition of phloroglucinol (PG) increased the rooting percentage and eliminated callus formation during the propagation of apple rootstocks (Kim *et al.*, 9). PG (1, 3, 5-trihydroxybenzene), a phenolic compound which is a deprivation produce of phloridzin, is a growth regulator that acts as an auxin synergist and is a precursor in lignin biosynthesis (Perez *et al.*, 15; Kim *et al.*, 9). PG encourages root improvement, root length, and initiation of fresh roots in papaya (Perez *et al.*, 15), banana, apple (Modgil *et al.*, 12; Kim *et al.*, 9), wild cherry (Hammatt, 6), pear, plum, Rubus and Fragaria (James, 8), Cinchona, cocoa and Jatropha curcas L. (Daud *et al.*, 3). The establishment of micro-cuttings developed through direct rooting was meaningfully superior to micro-cuttings developed by in vitro rooting (Webster and Jones, 20). However, in vitro rooting also requires 2 to 3 steps (double inoculation and acclimatization) for proper rooting and establishment of plants (McCubbin and Van Staden, 10). Hence, ex-vitro rooting is an alternative to in vitro rooting using PG. This process also upgraded on a two-step technique where plants were cultured into an IBA encompassing medium and then moved to an IBA free medium. Therefore, an ex-vitro rooting experiment

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using PG was conducted to attempt an alternative to in-vitro rooting and improve ex-vitro rooting through IBA.

MATERIALS AND METHODS

The trials were laid out at the Indian Institute of Horticultural Research, Bengaluru, during the period 2017-2020 using the advanced generation intergeneric hybrid (IGH- *Carica papaya* cv. Arka Surya × *V. cauliflora* L.) of papaya developed at the Institute. Fruits were taken from the selected advanced intergeneric hybrids, which were self-pollinated, and seeds were removed from the ripe fruits and sown in the pro-trays holding coco peat after treatment with 100 ppm GA3.

Plantlets were produced by culturing shoot tip explants on MS medium (Murashige and Skoog, 13) supplemented with 3% sucrose + 1 µM BAP + 1 µM GA3 + 0.1 µM IAA + 0.25% phytagel. The micro-cuttings were raised at 26 ± 2°C under a 16 h photoperiod (30–40 µE m–2 s –1) provided by white tubes. Shoot tips started sprouting within one week, and they became suitable for use as plant material for ex vitro root induction after three weeks. Subculturing was done after 20 to 25 days of sprouting (Fig. 1a).

The elongated plantlets (1-3 cm in length; leaves on top) were removed carefully from culture tubes with the help of sterile pincers. The agar adhering to the plantlets was completely removed by thoroughly washing in tap water. The callus was removed from the base of the plantlets by cutting the base part. The plantlets were dipped in various concentrations of phloroglucinol solution (0, 10, 50, 100,500 and 1000 µM) and planted in polybags (sachet) containing the potting medium (perlite + vermiculite + coco peat) in the ratio of 1:1:2 (Fig.1b, c). The sachet (polybags 200 gauge of 12×24 cm size filled to 1/3 height) method developed for grapes and watermelon, according to Ravindra and Thomas (16), was used for rooting and acclimatization. After planting, drenching was done with a fungicide solution containing bavistin + indofil (each at 2g/L). Polybags (sachet) were closed by stapler pin and misted. Polybags had holes at the bottom portion for drainage and aeration. For the first month, the polybags were kept under light (30–40 µE m–2 s –1) for 16 h under ambient situations (25-30°C) for rooting and thereafter, they were moved to the poly house for acclimatization. Polybags were retained inside plastic trays, and each tray contained 16 polybags. Watering was done at one-day intervals, and polybags were gradually opened after 20 days of planting. Rooting (%), number of roots, mean root length (cm) and number of secondary roots were recorded after one month and plant survival (%), days taken for hardening and height of plants (cm) after shifting in poly-house at monthly intervals were recorded.

All the trials were conducted in a completely randomized design (CRD) with five repetitions and five treatments. The collected data were analyzed using OPSTAT, an online agriculture data analysis tool, CCSHAU, Hissar.

RESULTS AND DISCUSSION

Based on their efficacy in encouraging adventitious roots, auxins containing IAA and IBA have been employed commercially to encourage rooting in numerous plants. High application of auxin at the initiation of the rhizogenesis stage is critical for adventitious root formation (El-Banna *et al.*, 4). Hammatt (6) also reported that phloroglucinol stimulates adventitious root establishment in tissue-cultured plantlets of wild cherry.

Current research outcomes are assisted by former findings, which state that PG significantly encourages the rooting of papaya plantlets (Perez *et al.*, 15). Plantlets are also rooted devoid of auxin, as described earlier with Rubus and Fragaria (James, 8). Hammatt (6) also reported that 1 mM PG was more operative than auxin for root development in cherry. A high percentage of rooting was recorded with less concentration of phloroglucinol, and it can be used as a substitute for IBA; hence, PG was taken as a rooting hormone in the present experiment.

The consequences achieved in the current research indicated that higher absorption of phloroglucinol had a significant optimistic effect on rooting (82%) with more numbers (7) of roots per shoot at 1000 µM PG (Table 1; Fig.1d). The results are in agreement with discoveries of Hammatt (6) and Webster and Jones (20) and Modgil *et al.* (12) who reported that higher concentration of phloroglucinol significantly increased rooting in apple. A probable foundation of endogenous

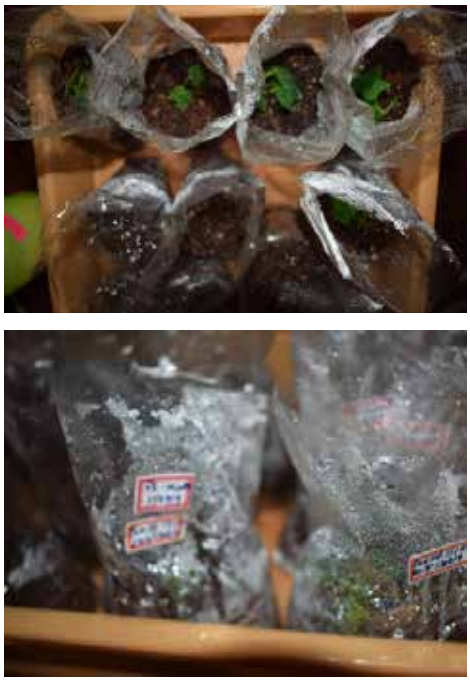
Table 1. Effect of Phloroglucinol on rooting parameters in papaya.

Treatments	Rooting (%)	No. of roots	Mean root length (cm)	No. of secondary roots
T_1(Control)	0.00(0.00)^d	0.00^e	0.00^f	0.00^f
T_2(10 µM)	66.60(54.70)^c	3.00^d	2.80^e	8.00^e
T_3(50 µM)	70.00(56.70)^c	4.00^c	4.60^d	9.00^d
T_4(100 µM)	75.00(60.00)^b	4.00^c	4.70^c	11.00^c
T_5(500 µM)	80.00(63.40)^a	6.00^b	5.20^b	14.00^b
T_6(1000 µM)	82.00(64.90)^a	7.00^a	6.50^a	19.00^a
SEm±	1.97	0.02	0.02	0.06
CD at 5%	4.15	0.08	0.08	0.21

Note: Figures in parenthesis are angular transformed values and data followed by various letters are meaningfully diverse at a 5 % level of significance.

a. *In vitro* proliferating cultures of IGH papaya

b. Plantlet of IGH papaya dipped in PG solution and planted in polybags



c. Polybags kept under light for rooting

Rooted plantlets of IGH papaya with 50 µM PG

Rooted plantlets of IGH papaya with 100 µM PG

Rooted plantlets of IGH papaya with 1000 µM PG

d. rooted plantlets of IGH papaya above and acclimatized plants of IGH papaya

Figure 1: *Ex vitro* rooting and acclimatization of IGH papaya.

Table 2. Effect of Phloroglucinol on survival percentage of plantlets, plant height (cm) and days taken for hardening.

Treatments	Survival (%)	Days taken for hardening	Plant height (cm)			
			30 days	60 days	90 days	120 days
T_1(Control)	0.00(0.00)^f	0.00^e	0.00^d	0.00^e	0.00^d	0.00^e
T_2(10 µM)	66.60(54.60)^e	120.00^a	2.00^c	3.00^d	6.00^c	8.00^d
T_3(50 µM)	70.00(56.70)^d	120.00^a	3.00^a	5.00^a	8.00^a	10.00^b
T_4(100 µM)	72.00(58.00)^c	100.00^b	2.50^b	3.50^c	6.00^c	9.00^c
T_5(500 µM)	80.00(63.40)^b	95.00^c	2.50^b	3.50^c	7.60^b	11.60^a
T_6(1000 µM)	82.00(64.80)^a	90.00^d	3.00^a	4.00^b	6.00^c	10.00^b
SEm±	0.39	0.55	0.01	0.02	0.03	0.05
CD at 5%	1.22	1.73	0.04	0.06	0.11	0.16

Note: Figures in parenthesis are angular transformed values and data followed by various letters are meaningfully unlike at 5 % level of significance.

PG could be the breakdown of phloridzin (Hunter, 7), which also leads to the construction of phloretic acid [PA; 3-(4-hydroxyphenyl) propionic acid], and both PG and PA stimulate adventitious root formation.

In the current research, it was also perceived that considerable success was obtained in rooting (100 to 500 µM PG produced 75 to 80 per cent rooting and 4 to 6 roots per shoot) by the use of phloroglucinol of lower to medium concentration (Table 1). Related outcomes were described by Daud *et al.* (3), where they found that in the presence of 2.5 µM IBA and 238 µM PG, 83 per cent of the plantlets were rooted with an average of 3.1 roots per shoot in Jatropha curcas.

In the current study, PG also affected shoot growth, which caused 11.60 cm plant height within 95 days. Maximum (11.60 cm) plant height was recorded with 500 µM PG and other treatments also had plant height from 8.00 to 10.00 cm (Table 2). Daud *et al.* (3) reported that phloroglucinol promoted shoot growth in papaya. Pérez *et al.* (14) also documented new root development and elongation of shoots when papaya micro-cuttings were treated with phloroglucinol.

In the present study, a maximum (19.00) number of secondary roots with more root hairs were obtained with 1000 µM PG, and it was found that the establishment of plants was maximum with no microbial contamination owing to high aerated potting media, which gave positive results in other treatments too.

According to Perez *et al.* (15), phloroglucinol produces more root hairs without callus formation, increases photosynthesis and decreases total transpiration and stomatal conductance along with porous material allows the high suspension of the oxygen absorption and lowers the microbial contamination; a great number of root hairs helped to take up nutrients and water more.

The present investigation found that the concentration of PG had meaningfully influenced the survival percentage of micro-propagated intergeneric hybrid shoots. A strong correlation was observed between PG application and survival percentage during the range of applications confirmed. However, the survival percentage (82.00) and height of plants after 90 days (10 cm) were pointedly superior at 1000 µM PG than at further levels (Table 2). The results obtained in the study are in accordance with the findings of Pérez *et al*. (15) who reported optimistic effect of PG on rooting and adaptation of the papaya plantlets.

It was observed from the present research that the plantlets with well-established roots were hardened to 100 per cent using the sachet method of acclimatization (Fig.1d). Additionally, this method provides a single-step acclimatization process which is easy, cost-effective and gives better results as compared to protray or minipot method of hardening. Similar results have also been reported by Ravindra and Thomas (16) in grapes.

The results with Intergeneric hybrid papaya suggest that application of 1000 µM PG in the rooting media produced maximum rooting percentage (82.00) and number of roots (7.00). The results were closely followed by application of 500µM PG (80.00). Additionally, sachet method produced 100% survival rate with single-step acclimatization which was simple and reduced labour cost. The results clearly point out that papaya plantlets can be efficiently rooted *ex vitro* using PG with maximum rooting and survival percentage.

AUTHORS' CONTRIBUTIONS

Conduction research work and preparation of Manuscript (KR, VC), clarification of facts and rereading the manuscript (MRD), Revision of MS (PT)

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DECLARATION

The authors declare that they do not have any conflict of interest.

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