JARQ 38 (1), 55 – 59 (2004) http://www.jircas.affrc.go.jp

# Effects of Complex Organic Extracts on Plantlet Regeneration from PLBs and Plantlet Growth in the *Doritaenopsis* Orchid

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## Abstract

Potato extract (PE), corn extract (CE) and papaya extract (PAE) significantly enhanced plantlet regeneration from PLBs (protocorm-like bodies) when 50 mL $L^{-1}$ of an extract was supplemented to the New Phalaenopsis (NP) medium containing sucrose. Among the treatments, CE 50 mL $L^{-1}$ induced the highest rate (66.63%) of plantlet regeneration from PLBs. Organic extracts of potato, corn and papaya at 50–100 mL $L^{-1}$ in NP medium also enhanced the subsequent growth of mini-plantlets. However, a higher level (200 mL $L^{-1}$) of extract in the medium inhibited the growth of the plantlets. Among these extracts, PE at 100 mL $L^{-1}$ showed the optimum effect on the acceleration of shoot growth while CE at the same concentration was optimum for root growth.

**Discipline:** Biotechnology
**Additional key words:** potato extract, corn extract, papaya extract

## Introduction

In the preceding report[5], it was demonstrated that the addition of potato extract, corn extract and papaya extract at various concentrations to the New Phalaenopsis medium used as basal medium, enhanced the growth of the calli of the *Doritaenopsis* orchid, an intergeneric hybrid of *Phalaenopsis* (hybrid of *Phalaenopsis* and *Doritis*) and promoted the regeneration of protocorm-like bodies (PLBs) from the calli.

In the present report, experiments were carried out to determine whether the addition of extracts of the same crops as those used for the culture of calli to the New Phalaenopsis medium (hereafter referred to as BM), would be suitable for the plantlet regeneration from PLBs through embryogenesis and for the subsequent growth of the plantlets of *Doritaenopsis*, in view of the economic importance of this orchid worldwide and the need for developing simple culture media for reducing the cost of production.

Major references of the literature relating to the micropropagation *in vitro* of various genera of orchids were cited in the preceding report[5] and some were added in the present report.

## Materials and methods

### 1. Preparation of organic extracts

Potato (cv. Lal pakhri) and papaya (cv. Shahi) produced locally and collected from a local market in Mymensingh, Bangladesh were peeled and cut into about 1 $cm^3$ pieces in size. These 500 g pieces were boiled for 20 min with 500 mL of distilled water and the hot supernatant was filtered through a kitchen steel mesh (1.0 × 1.0 mm ) and adjusted up to 500 mL by addition of distilled water. Corn (cv. Barnaly) grains (also a local product) were ground with a grinder and 500 g ground corn was boiled for 30 min with 500 mL of distilled water. Then 500 mL extract was collected in the same way as that for the potato extract. These extracts were stored at –4ºC and added to the medium as required after thawing.

### 2. Media and culture conditions

NP medium[3,4] was used as basal medium and supplemented with the extracts. Sucrose (20 g $L^{-1}$) and gel-

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Received 4 June 2003; accepted 11 October 2003.

rite (3 g $L^{-1}$) were added and the pH was adjusted to 5.6 to 5.7. After dissolution of the solidifier, 20 mL of hot medium was dispensed into 100 mL conical flasks. The conical flasks containing the medium were autoclaved at a pressure of 1.16 kg $cm^{-2}$ at 121ºC for 21 min. After autoclaving, the flasks containing the medium were allowed to cool and PLBs were cultured. Cultures were maintained in a growth room and allowed to grow at 25 ± 1ºC under a 16 h photoperiod and a photosynthetic photon flux of 50 mol $m^{-2}$ $s^{-1}$ provided by fluorescent tubes.

### 3. Plant materials and treatments

Callus-like masses consisting of small granular tissues which developed occasionally at the cut surface of the PLB segments during PLB subcultures were selected and subcultured[1]. Calli established have long been maintained by subculturing monthly on BM supplemented with 20 g $L^{-1}$ of sucrose. These calli were the source of the plant materials. Yellow, translucent and friable calli (0.5 g) were cultured on 20 mL of BM in 100 mL flasks supplemented with 20 g $L^{-1}$ of maltose. After 8 weeks of culture, friable calli turned green and a small mass of PLBs was produced. After 8 weeks of culture by subculturing of 1.0 g of the mass of PLBs, many larger PLBs were produced. Of these, PLBs larger than 2 mm in diameter were used as plant materials. Effects of potato, corn and papaya extracts on the growth of PLBs and PLB-derived plantlets were investigated. BM was supplemented with 0, 25, 50, 100 or 200 $mL^{-1}$ of the extracts to investigate their effect on the growth of PLBs as well as plantlets. In each flask, 8 PLBs of the same size were cultured on 20 mL BM and the number of PLB-derived plantlets was counted after 2 months of culture. Again to estimate the growth of the plantlets *in vitro*, all the miniplantlets derived from PLBs were cultured on the same fresh medium with the same treatments as those mentioned above for 12 weeks, and several growth-contributing characters of the plantlets were recorded.

In the cultures of both PLBs and PLB-derived plantlets, complete randomized design was adopted where each treatment was replicated 6 times. Experimental data were analyzed statistically by using Duncan's Multiple Range Test (DMRT).

## Results and discussion

### 1. Effects of complex organic extracts on growth of PLBs

The growth of the PLBs was stimulated on culture media supplemented with potato, corn and papaya extracts added to BM. Responses of PLBs in terms of growth and plantlet regeneration varied with the quantity of extract in BM.

Potato extract added to BM at 25, 50 and 100 mL $L^{-1}$ significantly enhanced plantlet regeneration from PLBs by 50, 62.5 and 54.1%, respectively compared to that in the control (45.7%). Among the different concentrations of PE, 50 mL $L^{-1}$ of PE added to BM was the optimum concentration (Fig. 1). The present results agreed with the report of Ichihashi and Islam[2] who stated that PE enhanced the embryogenic growth of the callus of *Phalaenopsis*. However, a high concentration of PE (200 mL $L^{-1}$) inhibited plantlet regeneration.

Corn extract (CE) supplemented into BM stimulated plantlet regeneration from PLBs. With 0, 25, 50, 100 and 200 mL $L^{-1}$ of CE, the regeneration rate of PLBs were 45.7, 54.13, 66.63, 58.25 and 50%, respectively (Fig. 1). The optimum level of CE was 50 mL $L^{-1}$, which signifi-

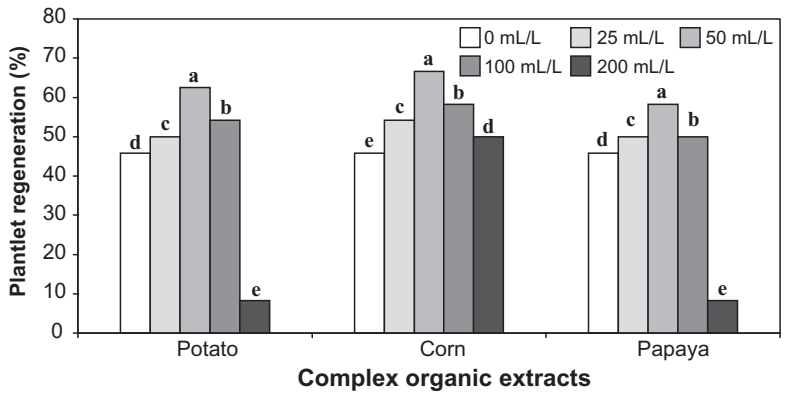


**Fig. 1. Effects of potato, corn and papaya extracts supplemented into NP medium on plantlet regeneration from PLB in *Doritaenopsis* after 2 months culture**

Different letters indicate significant difference by Duncan's multiple range test at 5% level.

cantly enhanced plantlet regeneration. However, higher or lower concentrations were less effective. A similar effect to that of PE was obtained with PAE where the highest percentage of plantlet initiation (58.25%) was observed at 50 mL $L^{-1}$ of PAE into BM and an inhibitory effect (8.25%) was observed at 200 mL $L^{-1}$ (Fig. 1). Among these three extracts, CE was the most suitable for plantlet regeneration from PLBs.

Complex organic extracts used in the present experiment contain carbohydrates, protein, fat, several vitamins, phenolic compounds, a lower level of some amino acids, and organic acids. Any of these or other yet unknown substances, alone or in combination might be a factor(s) enhancing plantlet regeneration compared to the control. Higher concentrations of organic additives also might reduce the balance of nutrients, their availability and water potential. Which factor(s) is particularly responsible for plantlet regeneration has not been determined yet and further studies should be carried out.

The present findings indicated that 50 mL $L^{-1}$ of organic extract of PE, CE or PAE was suitable for rapid, mass initiation of plantlets from PLBs. At this concentration, the level of organic acids, vitamins or other unknown substance(s) which may be responsible for enhancing the growth of PLBs may be optimum. High concentrations might be supra-optimum as a supplement.

**2. Effects of different organic extracts on growth of plantlets**

The addition of 100 mL $L^{-1}$ potato extract to BM significantly enhanced the number of leaves/plantlet, as well as length and width of leaves compared to other treatments (Fig. 2a). Higher concentration of PE in BM inhibited leaf growth. Leaf and shoot length as well as fresh and dry shoot weight were significantly enhanced with 100 mL $L^{-1}$ of PE compared to the other treatments


**Fig. 2. Growth of *Doritaenopsis* plantlets *in vitro* on NP medium**
Supplemented with (a) potato extract, (b) corn extract and (c) papaya extract after 12 weeks culture. Scale = 1.0 cm

**Table 1. Effects of potato extract on the subsequent growth and development of initiated *Doritaenopsis* plantlets after 12 weeks culture**

| *Organic extract supplements (mL $L^{-1}$) | No. of leaves/ plantlet | Length of leaf (cm) | Width of leaf (cm) | Length of shoot (cm) | Fresh wt. of shoot (g) | Dry wt. of shoot (g) | No. of roots per plantlet | Length of root (cm) | Diameter of root (mm) | Fresh wt. of root (g) | Dry wt. of root (g) | Root/shoot ratio (fresh wt.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PE 0 | 5.0a** | 1.25d | 0.50e | 1.55d | 0.092e | 0.004c | 3.0b | 1.35d | 1.90d | 0.047d | 0.001d | 0.514d |
| PE 25 | 4.0c | 1.55c | 0.65c | 1.65c | 0.101d | 0.004c | 2.5c | 2.35b | 2.25b | 0.094b | 0.005b | 0.931b |
| PE 50 | 4.5b | 1.65b | 0.75b | 1.95b | 0.114b | 0.006b | 2.5c | 1.55c | 1.85e | 0.066c | 0.004c | 0.579c |
| PE 100 | 5.0a | 1.95a | 0.85a | 2.65a | 0.385a | 0.020a | 3.5a | 2.70a | 3.50a | 0.380a | 0.021a | 0.987a |
| PE 200 | 4.5b | 1.15e | 0.55d | 1.65c | 0.103c | 0.004c | 1.5d | 0.85e | 2.00c | 0.016e | 0.001d | 0.155e |

* Each treatment had 6 replications. PE = Potato extract.
** Different letters within columns indicate significant difference by Duncan's multiple range test at 5% level.

**Table 2. Effects of corn extract on the subsequent growth and development of initiated *Doritaenopsis* plantlets after 12 weeks culture**

| *Organic extract supplements (mL $L^{-1}$) | No. of leaves/ plantlet | Length of leaf (cm) | Width of leaf (cm) | Length of shoot (cm) | Fresh wt. of shoot (g) | Dry wt. of shoot (g) | No. of roots per plantlet | Length of root (cm) | Diameter of root (mm) | Fresh wt. of root (g) | Dry wt. of root (g) | Root/shoot ratio (fresh wt.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CE 0 | 5.0b** | 1.25e | 0.50d | 1.55e | 0.092d | 0.004d | 3.0a | 1.35e | 1.90e | 0.047e | 0.001e | 0.514e |
| CE 25 | 5.0b | 1.40c | 0.50d | 2.05c | 0.111c | 0.007b | 2.5b | 2.15d | 2.80d | 0.117d | 0.006d | 1.054d |
| CE 50 | 6.5a | 1.70a | 0.68b | 2.55a | 0.204a | 0.013a | 3.0a | 3.30a | 3.00b | 0.383b | 0.021b | 1.877c |
| CE 100 | 4.0c | 1.50b | 0.73a | 2.35b | 0.163b | 0.006c | 3.0a | 3.12b | 3.30a | 0.392a | 0.022a | 2.404a |
| CE 200 | 4.0c | 1.30d | 0.58c | 1.65d | 0.072e | 0.004d | 2.5b | 2.27c | 2.90c | 0.136c | 0.008c | 1.888b |

* Each treatment had 6 replications. CE = Corn extract.
** Different letters within columns indicate significant difference by Duncan's multiple range test at 5% level.

**Table 3. Effects of papaya extract on the subsequent growth and development of initiated *Doritaenopsis* plantlets after 12 weeks culture**

| *Organic extract supplements (mL $L^{-1}$) | No. of leaves/ plantlet | Length of leaf (cm) | Width of leaf (cm) | Length of shoot (cm) | Fresh wt. of shoot (g) | Dry wt. of shoot (g) | No. of roots per plantlet | Length of root (cm) | Diameter of root (mm) | Fresh wt. of root (g) | Dry wt. of root (g) | Root/shoot ratio (fresh wt.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PAE 0 | 5.0a** | 1.25d | 0.50e | 1.55c | 0.092b | 0.004b | 3.0c | 1.35c | 1.90e | 0.047d | 0.001d | 0.514c |
| PAE 25 | 4.5b | 1.45b | 0.58d | 2.15a | 0.083d | 0.004b | 2.5d | 1.30d | 2.25c | 0.050c | 0.003c | 0.614b |
| PAE 50 | 5.0a | 1.35c | 0.68b | 1.75b | 0.094b | 0.006a | 4.5a | 2.15b | 2.50b | 0.147b | 0.009b | 1.560a |
| PAE 100 | 5.0a | 1.85a | 0.75a | 2.15a | 0.105a | 0.006a | 4.0b | 2.45a | 2.75a | 0.162a | 0.010a | 1.540a |
| PAE 200 | 4.0c | 0.95e | 0.65c | 1.75b | 0.088c | 0.004b | 2.5d | 0.55e | 2.00d | 0.015e | 0.001e | 0.170d |

* Each treatment had 6 replications. PAE = Papaya extract.
** Different letters within columns indicate significant difference by Duncan's multiple range test at 5% level.

(Table 1). Root growth was significantly increased with PE supplement in the medium compared to the control and optimum growth was obtained with 100 mL $L^{-1}$ of PE. However, a higher concentration of PE inhibited root growth. Root/shoot ratio indicated that the potato extract enhanced shoot growth rather than root growth (Table 1).

Number of leaves/plantlet and length of leaves significantly increased at 50 mL $L^{-1}$ of CE compared to the other treatments (Fig. 2b). Similarly, the leaf width increased with CE. Shoot length as well as fresh and dry shoot weight were significantly enhanced with 25, 50 and 100 mL $L^{-1}$ of CE where the highest growth rate was obtained at 50 mL $L^{-1}$ of CE (Table 2). However, there was a pronounced inhibitory effect with 200 mL $L^{-1}$ CE compared to the control and other treatments. Corn extract did not increase the number of roots but the length, diameter and weight of roots were markedly increased by the supplementation of CE. Length and diameter of roots significantly increased at all the concentrations of CE compared to the control but the highest values for the length and diameter were recorded at 50 and 100 mL $L^{-1}$ of CE, respectively. The highest values for the fresh weight and dry weight of roots were recorded at 100 mL $L^{-1}$ of CE compared to the other treatments. Root/shoot ratio indicated that CE markedly increased root growth compared to shoot growth (Table 2).

Number of leaves/plantlet, leaf length, leaf width, as well as fresh and dry shoot weight significantly increased with 50–100 mL $L^{-1}$ of PAE compared to the control and the other treatments (Table 3). Similarly, root growth was also enhanced with 50–100 mL $L^{-1}$ of PAE (Fig. 2c). Root/shoot ratio was significantly higher at 25–100 mL $L^{-1}$ of PAE compared to the control and other treatments. The present results indicate that 50–100 mL $L^{-1}$ of PAE was the optimum concentration for the growth of plantlets.

Based on the above findings, it can be concluded that a concentration of 50 mL $L^{-1}$ of PE, CE or PAE was suitable for plantlet regeneration from PLBs. Among the extracts, corn extract (50 mL $L^{-1}$) was optimum for plantlet regeneration. Shoot growth of plantlets was remarkably enhanced at 50 mL $L^{-1}$ of CE or 100 mL $L^{-1}$ of PE or PAE. However, PE enhanced shoot growth, while CE supported root growth.

Supplementation of organic extracts to orchid culture medium is simple, practical, beneficial and a convenient method to improve culture media used for commercial production[2]. Local adjustment and optimization are important for extract supplementation. The present results indicate that there is a common factor(s) in all the extracts which might be responsible for supporting the growth of the culture. Promotive effects of the respective organic extracts varied with the growth stages of *Doritaenopsis* orchid, indicating that the requirements of organic compounds are not always constant. Which factor(s) is particularly responsible for better growth of PLB and plantlets remains to be determined.

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