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Full Length Research Paper

Enhancement of organic supplements and local fertilisers in culture medium on growth and development of *Phalaenopsis* 'Silky Moon' protocorm

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One-leaf shoots from protocorms of *Phalaenopsis* 'Silky Moon' were used as explants for protocorm-like bodies (PLBs) and plantlets induction. Culture media contained Hyponex (6.5N-6P-19K) in the presence or absence of Saturn fert (20N-20P-20K) supplemented with 22.2 µM N^6-Benzyladenine (BA), 100 g l^{-1} potato juice, 2.0 g l^{-1} peptone, 30 g l^{-1} sucrose, 1.0 g l^{-1} activated charcoal and 2.0 g l^{-1} Phytagel. A local commercial fertiliser, Viking Ship (10N-20P-30K), with or without Saturn fert was used to compare the effects of organic supplements and fertilisers on growth and development of explants. All explants were cultured for 10 weeks. The medium containing 0.75 g l^{-1} Viking Ship with 1.0 g l^{-1} Saturn fert provided significant higher number of PLBs per explant (24.4) with new plantlets per explant (8.3) than values (17.0 PLBs and 2.4 new plantlets per explant) obtained from the medium containing 1.0 g l^{-1} Hyponex with 1.0 g l^{-1} Saturn fert. An organic supplement, either yeast extract or biotin or folic acid, in the Viking Ship medium [1 g l^{-1} (10N-20P-30K) Viking Ship without Saturn fert) and Hyponex medium [1 g l^{-1} (6.5N-6P-19K) Hyponex with (20N-20P-20K) Saturn fert] enhanced numbers of PLBs and/or numbers of plantlets. The most significant organic supplement was 0.05 mg l^{-1} biotin in the medium containing 2.75 g l^{-1} Viking Ship. Effects of organic supplements in appropriate culture medium components on PLBs and plantlets formation were discussed.

Key words: *Phalaenopsis*, protocorm, protocorm-like bodies, hyponex medium, viking ship medium, yeast extract, biotin, folic acid.

INTRODUCTION

Phalaenopsis (moth orchids), a member in the family *Orchidaceae*, is one of the world economic cut flowers and pot plants. *Phalaenopsis* 'Silky Moon', a hybrid of *Phalaenopsis* 'Musashino' *and Phalaenopsis* 'Paper Moon', is an attractive pot plant characterized by its large and almost round white-colored flowers with yellow lips.

A number of *in vitro* culture techniques for *Phalaenopsis* have been developed for potential mass propagation in order to produce plants on a large-scale to supply the world mass-market. Protocorm-like bodies (PLBs) and plantlets were induced from various types of explants. PLBs have been induced from shoot tips (Intuwong and Sagawa, 1974), young flower stalks (Ichihashi, 1992), nodal segments from inflorescences (Chen et al., 2003), floral stalk-derived leaves (Park et al., 2000, 2002a, b; Kuo et al., 2005) and protocorms (Chen and Chang, 2004). Various culture media, Vacin and Went (VW) medium (Vacin and Went, 1949), Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and Knudson C (KC) medium (Knudson, 1946), have been used for *Phlaenopsis* micropropagation (Tanaka and Sakanishi, 1977, 1980; Griesbach, 1983; Lin, 1986; Bhattarcharjee, 1999; Alam et al., 2002; Park et al.,

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2002a; Chen and Chang, 2004). Hyponex medium (Nishimura, 1982), originally containing a commercial Hyponex fertiliser supplemented with sucrose and agar in basis and/or added with some other organic additives such as peptone or boiled potato juice, is an effective culture medium for orchids, especially *Phalaenopsis* (Amaki and Higuchi, 1989; Park et al., 2000; 2002a,b; Alam et al., 2002). However, the simpler medium makes the micropropagation procedure easier and more economical.

This study reports effective organic supplements in an efficient easy culture medium using a local commercial fertiliser as the main component in comparison to Hyponex medium for PLBs and plantlets induction from shoots, seed-derived protocorms with 1 leaf, of *Phalaenopsis* 'Silky Moon'.

MATERIALS AND METHODS

Plant materials

A Three-month-old capsule of *Phalaenopsis* 'Silky Moon' (*P.* 'Musashino' × *P.* 'Papermoon') was surface-sterilised in 30% (v/v) Clorox (6% sodium hypochlorite; Mfd. for The Clorox Company, Oakland, CA 94612, USA) containing 0.02% (v/v) Tween-20 for 10 min, followed by three rinses in sterile distilled water. Then, a capsule was dipped in 95% ethyl alcohol and flamed. Seeds in the capsule were cultured for three months in modified liquid Hyponex medium containing 3.5 g l^{-1} Hyponex (6.5N-6P-19K; Hyponex Co., Marysville, Ohio, U.S.A.), 2 g l^{-1} peptone, 100 g l^{-1} potato juice (a supernatant of boiled potatoes) and 20 g l^{-1} sucrose. Prior to autoclaving, media were adjusted to pH 5.4. Protocorms, approx. one-leaf shoots with 0.03 g average fresh weight (Figure 1L), were chosen to use as explants for protocorm-like bodies (PLBs) and plantlets induction.

Media and culture conditions

The media used for protocorm-like bodies (PLBs) and plantlets induction were Hyponex medium containing Hyponex (6.5N-6P-19K) fertiliser as the main component and Viking Ship medium containing Viking Ship (10N-20P-30K), a local commercial fertiliser [Evergreenland Ltd., Bangkok; Yara (Thailand) Ltd., Bangkok, Thailand], as the main component. These two media were supplemented with 22.2 µM N^6-Benzyl adenine (BA), 20 g l^{-1} sucrose, 1 g l^{-1} activated charcoal, and 2.0 g l^{-1} Phytagel in the presence or absence of 1 g l^{-1} Saturn fert (20N-20P-20K), local fertiliser (Pitiporn Agriculture Co. Ltd., Bangkok, Thailand). Other organic additives, 100 g l^{-1} potato juice, 2 g l^{-1} peptone, 2 g l^{-1} yeast extract, 0.05 or 0.5 mg l^{-1} biotin, 0.1 or 0.2 mg l^{-1} folic acid, were added in different combinations. The pH of all media was adjusted to 5.4 prior to adding Phytagel and autoclaving. Explants were cultured in 120-ml glass jars containing 20 ml of culture medium for 10 weeks with five-week interval of subculturing. All cultures were incubated under a 24 ± 1°C with a 16 h photoperiod at 35 - 40 µmol m^{-2} s^{-1} provided by cool-white fluorescent lights.

Statistical analysis

Whole fresh weight, fresh weight of new regenerants (PLBs and new plantlets), number of PLBs and number of new regenerated plantlets were recorded after 10 weeks. Data were analysed by Duncan's New Multiple Range Test at $P = 0.05$ (Duncan 1955).

RESULTS

Effect of organic additives in Hyponex medium on growth and development of protocorms

For media containing Hyponex as the main component, 3.5 g l^{-1} Hyponex or 1.0 g l^{-1} Hyponex with 1.0 g l^{-1} Saturn fert supplemented with potato juice, peptone and yeast extract or biotin or folic acid from the media H3-H13 provided 0.74-1.78 g whole fresh weight, 0.23-0.80 g new regenerants fresh weight and 3.80-18.30 PLBs (Table 1; Figure 1A, C, E, G and I). The obtained values were significantly more than those obtained from 3.5 g l^{-1} Hyponex alone or Hyponex with potato juice and peptone from the media H1-H2, providing 0.34-0.47 g whole fresh weight with no new regenerant (Table 1). There were not significantly different among number of PLBs (12.30-18.30) and whole fresh weight (1.14-1.78 g) obtained from media containing 1.0 g l^{-1} Hyponex, 1.0 g l^{-1} Saturn fert, potato juice and peptone incorporated with yeast extract or biotin or folic acid (H8-H13), but significantly higher than other media (H1-H7). However, the medium containing 1.0 g l^{-1} Hyponex, 1.0 g l^{-1} Saturn fert, potato juice and peptone incorporated with 0.2 g l^{-1} folic acid (H13) resulted in less number of new plantlets. The maximum number of new plantlets (8.30 and 10.50 plantlets) were obtained from the media containing 1.0 g l^{-1} Hyponex and 1.0 g l^{-1} Saturn fert incorporated with yeast extract or 0.5 mg l^{-1} biotin (H9 and H11, respectively) (Table 1). Therefore, the media H8-H12 were chosen as the effective media to compare to media containing the Viking Ship fertilizer as the main component.

Effect of organic additives in Viking ship medium on growth and development of protocorms

For media containing the local fertiliser (Viking Ship) as the main component, 2.75 g l^{-1} Viking Ship with potato juice and peptone incorporated with yeast extract or biotin or folic acid from the media LF3-LF7 (Table 2; Figure 1B, D and F) provided 1.12-1.96 g whole fresh weight, 0.53-1.03 g new regenerants fresh weight and 15.10-25.90 PLBs. The obtained values were significantly greater than the vales obtained from 2.75 g l^{-1} Viking Ship alone or Viking Ship potato juice and peptone LF2, providing 0.55 and 0.72 g whole fresh weight, respectively with no and few new regenerants (Table 2). Similarly, number of PLBs (24.40 PLBs), whole fresh weight (1.53 g) and new regenerants fresh weight (0.82 g) obtained from the medium containing 0.75 g l^{-1} Viking Ship, 1.0 g l^{-1} Saturn fert, potato juice and peptone (LF8) were also significantly higher than those obtained from the media LF1 and LF2.

When yeast extract or biotin or folic acid were added in the media containing 0.75 g l^{-1} Viking Ship, 1.0 g l^{-1}

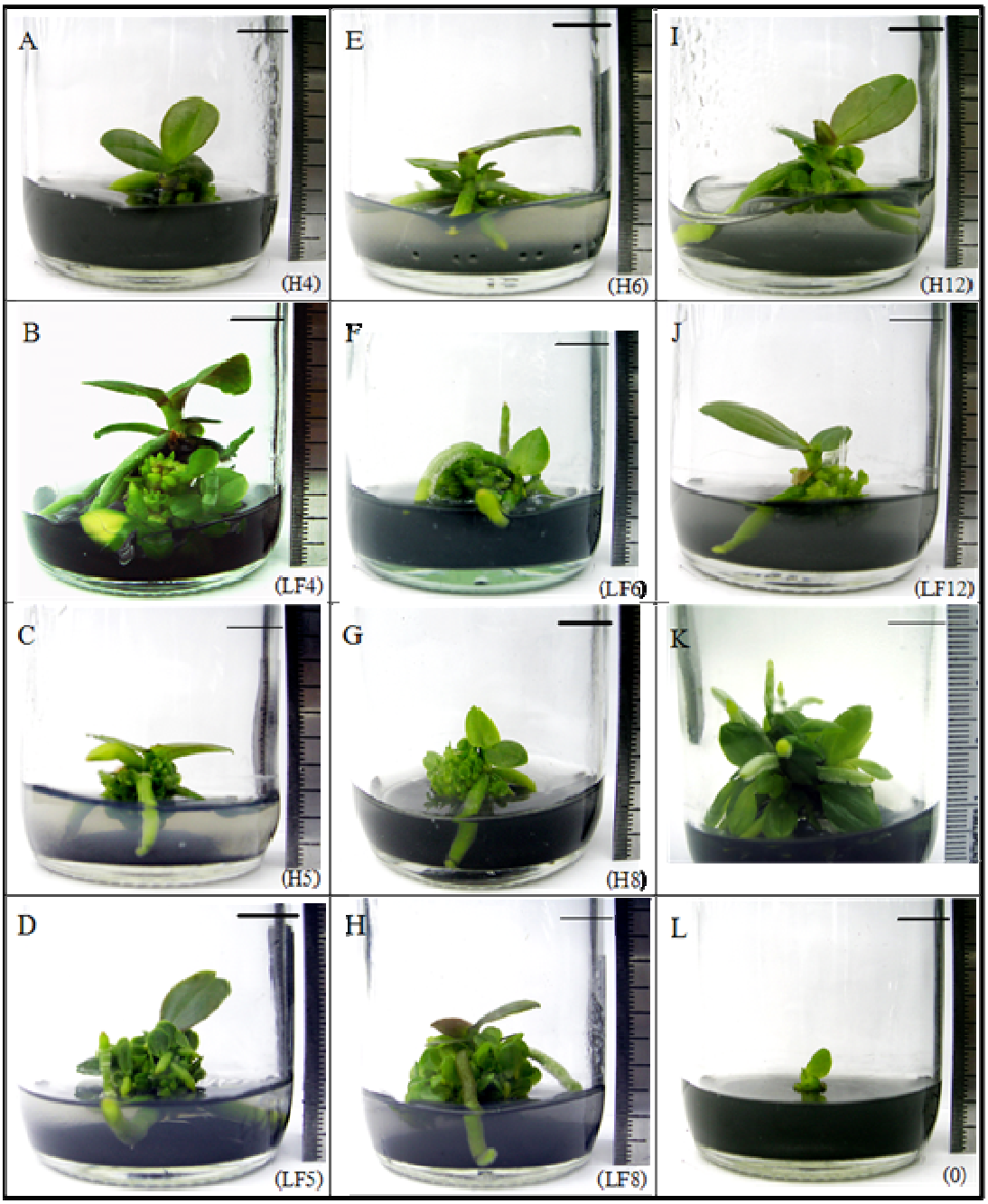

Figure 1. Growth of *Phalaenopsis* 'Silky Moon' protocorms, one-leaf shoots with approx 0.03 g, on media containing Hyponex or local commercial fertiliser (Viking Ship) in the presence or absence of local fertilizer Saturn fert supplemented with 22.2 µM BA, 20 g l^{-1} sucrose, 1 g l^{-1} activated charcoal, 2.0 g l^{-1} Phytagel and other organic additives, cultured for 10 weeks. A, C, E, G and I) medium containing Hyponex (Hy) as the main component supplemented with other organic additives. B, D, F, H and J) medium containing local commercial fertilizer (Viking Ship: LF) as the main component supplemented with other organic additives. A) H4 = Hy+Po+Pep+0.05Bi. B) LF4 = V+Po+Pep+ 0.05Bi. C) H5 = Hy+Po+Pep+0.5Bi. D) LF5 = V+Po+Pep+0.5Bi. E) H6 = Hy+Po+Pep+0.1FA. F) LF6 = V+Po+Pep+0.1FA. G) H8 = Hy*+S+Po+Pep. H) LF8 = V*+S+Po+Pep. I) H12 = Hy*+S+Po+Pep+0.1FA. J) LF12 = V*+S+Po+Pep+0.1FA. K) Conversion of PLBs into plantlets whereas PLBs were transferred to LF4 without BA and cultured for 5 weeks. L) An initial explant with 1 leaf and approx 0.03 g fresh weight. Hy = 3.5 g l^{-1} Hyponex (6.5N-6P-19K); V = 2.75 g l^{-1} Viking Ship (10N-20P-30K); Hy* = 1.0 g l^{-1} Hyponex (6.5N-6P-19K); V* = 0.75 g l^{-1} Viking Ship (10N-20P-30K); Po = 100 g l^{-1} potato juice; Pep = 2 g l^{-1} peptone; 0.05Bi = 0.05 mg l^{-1} biotin; 0.5Bi = 0.5 mg l^{-1} biotin; 0.1FA = 0.1 mg l^{-1} folic acid; S = 1.0 g l^{-1} Saturn fert (20N-20P-20K).

Table 1. Effect of Hyponex medium supplemented with 22.2 µM BA, 20 g l^{-1} sucrose, 1 g l^{-1} activated charcoal, 2.0 g l^{-1} Phytagel and other organic additives in culture medium on growth and development of *Phalaenopsis* 'Silky Moon' shoots after culturing for 10 weeks.

Medium	Component [δ]	Average growth and development [#]			
		Whole FW (g)	New regenerants FW [Ψ] (g)	No. PLBs	No. new plantlets
H1	Hy	0.34 ± 0.03 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
H2	Hy+Po+Pep	0.47 ± 0.03 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
H3	Hy+Po+Pep+Ye	0.98 ± 0.18 c	0.23 ± 0.09 bc	11.80 ± 4.46 c	0.40 ± 0.27 a
H4	Hy+Po+Pep+0.05Bi	0.74 ± 0.07 bc	0.05 ± 0.03 ab	3.80 ± 1.97 b	0.00 ± 0.00 a
H5	Hy+Po+Pep+0.5Bi	1.16 ± 0.06 d	0.42 ± 0.07 de	16.80 ± 3.46 cd	1.80 ± 0.71 b
H6	Hy+Po+Pep+0.1FA	0.88 ± 0.08 bc	0.11 ± 0.04 bc	8.30 ± 3.80 bc	0.00 ± 0.00 a
H7	Hy+Po+Pep+0.2FA	1.27 ± 0.08 de	0.31 ± 0.07 bcd	12.30 ± 3.03 c	2.70 ± 0.86 b
H8	Hy*+S+Po+Pep	1.14 ± 0.12 d	0.35 ± 0.13 cd	17.00 ± 6.03 d	2.40 ± 0.68 b
H9	Hy*+S+Po+Pep+Ye	1.78 ± 0.15 f	0.80 ± 0.19 f	14.30 ± 4.19 c	8.30 ± 1.52 d
H10	Hy*+S+Po+Pep+0.05Bi	1.45 ± 0.15 e	0.39 ± 0.11 cd	15.40 ± 2.64 cd	4.60 ± 1.13 bc
H11	Hy*+S+Po+Pep+0.5Bi	1.45 ± 0.18 e	0.53 ± 0.15 e	12.30 ± 3.83 c	10.50 ± 2.61 d
H12	Hy*+S+Po+Pep+0.1FA	1.27 ± 0.07 de	0.33 ± 0.06 cd	15.50 ± 2.06 cd	2.70 ± 0.92 b
H13	Hy*+S+Po+Pep+0.2FA	1.20 ± 0.09 de	0.46 ± 0.06 de	18.30 ± 3.19 d	0.20 ± 0.13 a

[δ] Hy = 3.5 g l^{-1} Hyponex (6.5N-6P-19K); Po = 100 g l^{-1} potato juice; Pep = 2 g l^{-1} peptone; Ye = 2 g l^{-1} yeast extract; 0.05Bi = 0.05 mg l^{-1} biotin; 0.5Bi = 0.5 mg l^{-1} biotin; 0.1FA = 0.1 mg l^{-1} folic acid; 0.2FA = 0.2 mg l^{-1} folic acid; Hy* = 1.0 g l^{-1} Hyponex (6.5N-6P-19K); S = 1.0 g l^{-1} Saturn fert (20N-20P-20K).
[#] Values are mean ± SE (n = 20). Means followed by the same letters are not significantly different at $P = 0.05$ by Duncan's New multiple Range Test.
[Ψ] fresh weight of PLBs and new plantlets excluding the original explant.

Saturn fert, potato juice and peptone (LF9-LF13) (Figure 1J), the obtained whole fresh weight (0.72-1.01 g) and new regenerants fresh weight (0.05-0.37 g) were not significantly different from the media LF1 or LF2 (Table 2). There were no significant differences in number of PLBs (15.10-25.90 PLBs) obtained from media containing 2.75 g l^{-1} Viking Ship, potato juice and peptone incorporated with yeast extract (LF3) or 0.05-0.5 mg l^{-1} biotin (LF4-LF5) or 0.1 mg l^{-1} folic acid (LF6) and the medium containing 0.75 g l^{-1} Viking Ship, 1.0 g l^{-1} Saturn fert, potato juice, peptone alone (LF8) or added with yeast extract (LF9) or 0.05 mg l^{-1} biotin (LF10) or 0.1 mg l^{-1} folic acid (LF12) (Figure 1B, D, F, H and J) . However, the highest whole fresh weight (1.96 and 1.53 g), new regenerants fresh weight (1.03 and 0.82 g), number of new plantlets (11.0 and 8.30 g) and number of PLBs (23.20 and 24.40 PLBs) were obtained from the media LF4 and LF8 (Table 2; Figure 1B and H). Moreover, the media LF3, LF5 and LF6 provided higher whole fresh weight, new regenerants fresh weight and number of new plantlets than the media LF9 and LF12 did (Table 2). Therefore, the media LF3 – LF6 and LF8 were chosen as the effective media to compare to the medium containing Hyponex as the main component.

Comparison of media containing hyponex and local fertiliser as the main components (Hyponex medium and Viking ship medium) on growth and development of protocorms

Comparing the medium containing 1 g l^{-1} Hyponex, 1.0 g l^{-1} Saturn fert, potato juice and peptone (H8) to the medium containing 0.75 g l^{-1} Viking Ship, 1.0 g l^{-1} Saturn fert, potato juice and peptone (LF8), it was shown that LF8 induced better growth and development of explants including fresh weight, number of PLBs and new plantlets (Figure 1G and H). LF8 provided 8.30 PLBs and 24.40 new plantlets while 2.40 PLBs and 17.00 new plantlets were obtained from H8 (Table 3). The significant higher whole fresh weight (1.78 and 1.96 g) was found from the media containing 1.0 g l^{-1} Hyponex, 1.0 g l^{-1} Saturn fert, potato juice, peptone, yeast extract (H9) and 2.75 g l^{-1} Viking Ship, potato juice, peptone, 0.05 mg l^{-1} biotin (LF4). There were no significant differences in whole fresh weight (1.12-1.53 g), new regenerants fresh weight (0.39-0.82 g) and number of plantlets (4.40-8.30 plantlets) obtained from the 5 media: H10 (1.0 g l^{-1} Hyponex, 1.0 g l^{-1} Saturn fert, potato juice, peptone, 0.05 mg l–1 biotin), LF3 (2.75 g l–1 Viking Ship, potato juice, peptone, yeast

Table 2. Effect of local fertilizer (Viking Ship medium) supplemented with 22.2 µM BA, 20 g l^{-1} sucrose, 1 g l^{-1} activated charcoal, 2.0 g l^{-1} Phytagel and other organic additives in culture medium on growth and development of *Phalaenopsis* 'Silky Moon' protocorms after culturing for 10 weeks.

Medium	Component [δ]	Average growth and development [#]			
		Whole FW (g)	New regenerants FW [Ψ] (g)	No. PLBs	No. new plantlets
LF1	V	0.55 ± 0.06 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
LF2	V+Po+Pep	0.72 ± 0.05 ab	0.18 ± 0.00 b	7.60 ± 2.22 b	1.00 ± 0.54 a
LF3	V+Po+Pep+Ye	1.12 ± 0.18 c	0.58 ± 0.19 de	16.40 ± 4.66 cd	5.10 ± 2.18 b
LF4	V+Po+Pep+0.05Bi	1.96 ± 0.09 e	1.03 ± 0.22 g	23.20 ± 4.19 de	11.00 ± 3.42 d
LF5	V+Po+Pep+0.5Bi	1.29 ± 0.14 c	0.53 ± 0.14 de	15.10 ± 3.44 cd	5.70 ± 1.61 b
LF6	V+Po+Pep+0.1FA	1.43 ± 0.20 cd	0.75 ± 0.23 def	25.90 ± 5.24 e	4.40 ± 1.15 b
LF7	V+Po+Pep+0.2FA	0.71 ± 0.08 ab	0.19 ± 0.09 b	10.20 ± 6.16 bc	1.00 ± 0.60 a
LF8	V*+S+Po+Pep	1.53 ± 0.10 de	0.82 ± 0.23 ef	24.40 ± 6.64 e	8.30 ± 3.61 c
LF9	V*+S+Po+Pep+Ye	1.01 ± 0.12 bc	0.34 ± 0.18 bcd	17.70 ± 7.79 cd	0.00 ± 0.00 a
LF10	V*+S+Po+Pep+0.05Bi	0.76 ± 0.12 ab	0.24 ± 0.09 bc	16.00 ± 6.16 cd	0.00 ± 0.00 a
LF11	V*+S+Po+Pep+0.5Bi	0.98 ± 0.17 bc	0.37 ± 0.15 bcd	10.50 ± 3.99 bc	3.10 ± 1.83 b
LF12	V*+S+Po+Pep+0.1FA	0.96 ± 0.10 bc	0.30 ± 0.08 bcd	18.90 ± 2.92 cd	0.00 ± 0.00 a
LF13	V*+S+Po+Pep+0.2FA	0.72 ± 0.09 ab	0.05 ± 0.03 ab	0.07 ± 0.47 a	0.07 ± 0.47 a

[δ] V = 2.75 g l^{-1} Viking Ship (10N-20P-30K); Po = 100 g l^{-1} potato juice; Pep = 2 g l^{-1} peptone; Ye = 2 g l^{-1} yeast extract; 0.05Bi = 0.05 mg l^{-1} biotin; 0.5Bi = 0.5 mg l^{-1} biotin; 0.1FA = 0.1 mg l^{-1} folic acid; 0.2FA = 0.2 mg l^{-1} folic acid; V* = 0.75 g l^{-1} Viking Ship (10N-20P-30K); S = 1.0 g l^{-1} Saturn fert (20N-20P-20K).
[#] Values are mean ± SE (n = 20). Means followed by the same letters are not significantly different at $P = 0.05$ by Duncan's New multiple Range Test.
[Ψ] fresh weight of PLBs and new plantlets excluding the original explant.

Table 3. Comparison of medium containing Hyponex and medium containing Local fertilizer (Viking Ship) supplemented with 22.2 µM BA, 20 g l^{-1} sucrose, 1 g l^{-1} activated charcoal, 2.0 g l^{-1} Phytagel and other organic additives in culture medium on growth and development of *Phalaenopsis* 'Silky Moon' protocorms after culturing for 10 weeks.

Medium	Component [δ]	Average growth and development [#]			
		Whole FW (g)	New regenerants FW [Ψ] (g)	No. PLBs	No. new plantlets
H8	Hy*+S+Po+Pep	1.14 ± 0.12 a	0.35 ± 0.13 a	17.00 ± 2.03 a	2.40 ± 1.28 a
H9	Hy*+S+Po+Pep+Ye	1.78 ± 0.08 c	0.80 ± 0.19 b	14.30 ± 3.09 a	8.30 ± 1.52 b
H10	Hy*+S+Po+Pep+0.05Bi	1.45 ± 0.15 ab	0.39 ± 0.11 a	15.40 ± 2.64 a	4.60 ± 1.13 ab
H11	Hy*+S+Po+Pep+0.5Bi	1.45 ± 0.10 ab	0.53 ± 0.15 ab	12.30 ± 3.83 a	10.50 ± 3.61 c
H12	Hy*+S+Po+Pep+0.1FA	1.27 ± 0.07 a	0.33 ± 0.06 a	15.50 ± 2.05 a	2.70 ± 1.12 a
LF3	V+Po+Pep+Ye	1.12 ± 0.18 a	0.58 ± 0.19 ab	16.40 ± 3.66 a	5.10 ± 2.18 ab
LF4	V+Po+Pep+0.05Bi	1.96 ± 0.09 d	1.03 ± 0.22 c	23.20 ± 3.19 b	11.00 ± 1.42 c
LF5	V+Po+Pep+0.5Bi	1.29 ± 0.12 a	0.53 ± 0.14 ab	15.10 ± 3.44 a	5.70 ± 2.61 ab
LF6	V+Po+Pep+0.1FA	1.43 ± 0.10 ab	0.75 ± 0.23 b	25.90 ± 4.24 b	4.40 ± 2.15 ab
LF8	V*+S+Po+Pep	1.53 ± 0.14 ab	0.82 ± 0.23 b	24.40 ± 2.64 b	8.30 ± 1.61 b

[δ] Hy* = 1.0 g l^{-1} Hyponex (6.5N-6P-19K); V = 2.75 g l^{-1} Viking Ship (10N-20P-30K); Po = 100 g l^{-1} potato juice; Pep = 2 g l^{-1} peptone; Ye = 2 g l^{-1} yeast extract; 0.05Bi = 0.05 mg l^{-1} biotin; 0.5Bi = 0.5 mg l^{-1} biotin; 0.1FA = 0.1 mg l^{-1} folic acid; V* = 0.75 g l^{-1} Viking Ship (10N-20P-30K); S = 1.0 g l^{-1} Saturn fert (20N-20P-20K).
[#] Values are mean ± SE (n = 20). Means followed by the same letters are not significantly different at $P = 0.05$ by Duncan's New multiple Range Test.
[Ψ] fresh weight of PLBs and new plantlets excluding the original explant.

extract), LF5 (2.75 g l–1 Viking Ship, potato juice, peptone, 0.5 mg l–1 biotin), LF6 (2.75 g l–1 Viking Ship, potato juice, peptone, 0.5 mg l–1 folic acid) and LF8 (0.75 g l–1 Viking Ship, 1.0 g l–1 Saturn fert, potato juice and peptone). Although significant higher number of PLBs occurred with LF4, LF6 and LF8 at 23.20, 25.90 and 24.40 PLBs, respectively (Table 3; Figure 1B, F and H), LF4 provided the maximum whole fresh weight, new regenerants fresh weight and number of PLBs.

Conversion of PLBs into plantlets

After 10 weeks of culturing, obtained PLBs and small plantlets were transferred to the Viking Ship medium containing 2.75 g l^{-1} Viking Ship, 100 g l^{-1} potato juice, 2 g l^{-1} peptone and 0.05 mg l^{-1} biotin (LF4 medium without BA). All PLBs and small plantlets developed to healthy plants within 3 - 5 weeks (Figure 1K).

DISCUSSION

Generally, KC, VW and MS medium were used as main culture media for *Phalaenopsis* (Tanaka and Sakanishi, 1977, 1980; Griesbach, 1983; Lin, 1986; Bhattarcharjee, 1999; Park et al., 2002a, b; Chen and Chang 2004). Tanaka and Sakanishi (1977, 1980) used solid and liquid VW medium with coconut water for proliferation of PLBs. Griesbach (1983) used MS medium for production of plantlets from PLBs, whereas Lin (1986) used modified KC medium for conversion of PLBs into plantlets. Chen and Chang (2004) induced repetitive embryogenesis from seed-derived protocorms of *Phalaenopsis amabilis* var. 'Formosa Shimadzu' on a modified half-strength MS medium.

Hyponex is a commercial fertiliser used in culture medium for orchid-seed germination and plantlet regeneration from PLBs. Amaki and Higuchi (1989) obtained PLBs from leaf segments of *Doritaenopsis* cultured on Hyponex medium containing 3 g l^{-1} Hyponex (7N-6P-19K), 2 g l^{-1} Bacto-peptone, 15 g l^{-1} sucrose and 8 g l^{-1} Bacto-agar, at pH 5.5. Alam et al. (2002) reported that MS medium was the best for characters studied in *Dendrobium transparens* followed by Hyponex medium. To obtain plantlets, PLBs of *Phalaenopsis* were transferred to the modified Hyponex medium containing 3 g l^{-1} Hyponex, Nitsch microelements (Nitsch and Nitsch, 1967), 100 m g l^{-1} myo-inositol, 1 m g l^{-1} nicotinic acid, 2 g l^{-1} activated charcoal, 3% sucrose and 1% agar, and cultured for 5-7 months (Tanaka 1992). It was reported that the suitable medium for plantlet regeneration from PLBs of *Phalaenopsis* and *Doritaenopsis* hybrid was Hyponex medium containing 1 g l^{-1} of 6.5N-4.5P-19K, 1 g l^{-1} of 20N-20P-20K and 45 g l^{-1} sucrose at pH 5.7 (Park et al., 2000) or 1 g l^{-1} of 6.5N-4.5P-19K, 1 g l^{-1} of 20N-20P-20K, 2 g l^{-1} peptone, 30 g l^{-1} potato homogenate, 0.5 g l^{-1} activated charcoal and 30 g l^{-1} sucrose at pH 5.2 (Park et al., 2002a) or 1 g l^{-1} of 6.5N-4.5P-19K, 1 g l^{-1} of 20N-20P-20K, 2 g l^{-1} peptone, 30 g l^{-1} potato homogenate, 0.5 g l^{-1} activated charcoal and 20 g l^{-1} sucrose at pH 5.5) (Park et al., 2002b).

From the preliminary study, six culture media, MS, modified MS, VW, modified VW, Hyponex and modified Hyponex were compared for seed germination of *Phalaenopsis*. The best culture medium for developing seeds to plantlets was modified Hyponex medium. Tanaka and Sakanishi (1980) presented that addition of 22.2 µM BA or 20% coconut water to the medium for the culturing of *Phalaenopsis* flower-stalk cuttings promoted the subse-quent formation of PLBs. In this experiment, seed-derived explants of *Phalaenopsis* 'Silky Moon' from modified sta-tionary liquid Hyponex medium (containing 3.5 g l^{-1} Hyponex (6.5N-6P-19K), 2 g l^{-1} peptone, 100 g l^{-1} potato juice and 20 g l^{-1} sucrose) were used. Seeds developed to protocorms at the stage of one-leaf shoots within three months and further developed to small plantlets within five months (data not shown). Therefore, explants were cultured on solidified media containing Hyponex or Viking Ship with 22.2 µM BA supplemented with different organic additives to investigate the effects of media on PLBs and plantlets induction. Viking Ship (10N-20P-30K) used in this study is a local commercial fertiliser produced by Yara, a chemical company, which is the producer and marketer of mineral fertilisers that supplied to market around the world.

It was found that no new regenerants occurred in the H1 (Hyponex alone), H2 (Hyponex + potato juice + peptone) and LF1 (Viking Ship alone) medium. However, potato juice and peptone enhanced more fresh weight in H2 while promoted growth of new regenerants in LF2 (Viking Ship + potato juice + peptone) (Tables 1 and 2).

Peptone is the product from digestion of protein by acid or enzyme such as meat casein and gelatin. Peptone was used as a source of organic nitrogen in VW medium (Arditti and Ernst, 1993). The addition of peptone or tryptone to the culture medium promoted growth of PLBs derived from seeds of *Phalaenopsis* 'Surfrider' × (*Phalaenopsis* 'Joseph Hampton' × *Doritaenopsis* 'Kaalan-Gleam'). The maximum stimulation was obtained from 2.0 g l^{-1} tryptone (Amaki and Higuchi, 1989). Potato juice, the supernatant of boiled potatoes, contains polyamine and biosynthetic enzyme that affects growth and development of plant cells especially to nucleic acid replication and cell division in mitosis. Moreover, potato consists of useful carbohydrate, sugar, protein and vitamin for plant growth. Addition of potato juice in orchid culture medium promoted seed germination and enhanced growth of seedling (Arditti and Ernst, 1993).

In general, the addition of yeast extract or biotin or folic acid, in either Hyponex medium or Viking ship medium, significantly promoted number of PLBs and new plantlets. Some vitamins, sources of B vitamin, such as biotin, folic

acid and yeast extract, play an important role in plant growth and development metabolism. Al-khayri (2001) reported that biotin and thiamine incorporated in culture medium enhanced date palm height. Yeast extract, a natural source of B complex vitamin, plays an important role as coenzyme in plant metabolisms, morphogenesis and development (Arditti and Ernst, 1993). While folic acid, found in leaves and other plant tissues, functions as a B vitamin and demonstrates coenzyme (an organic molecule associated with enzymes) activity in nucleic acid (RNA and DNA) synthesis (Arditti and Harrison, 1977; Kyte and Kleyn, 1996). Biotin, important in fat, protein and carbohydrate metabolism, promoted growth of leaves and stems in some *Cattleya*, *Odontoglossum* and *Paphiopedilum* while it enhanced root growth in *Cymbidium* (Arditti and Harrison, 1977; Kyte and Kleyn, 1996).

For Hyponex medium, the addition of 1.0 g l^{-1} Saturn fert (20N-20P-20K), to the media containing 1.0 g l^{-1} Hponex (6N-6.5P-19K) with potato juice, peptone, yeast extract and 0.05-0.5 m g l^{-1} or 0.1-0.2 mg l^{-1} folic acid (H8-H13), was found to produce more whole fresh weight, new growth fresh weight, number of PLBs and plantlets than those from the same component without Saturn Fert (H2-H7). Similar results were obtained from Park et al. (2000). It was shown that H8-H12 were not significantly different. However, H9 (1 g l^{-1} Hyponex + 1 g l^{-1} Saturn fert + potato juice + peptone + yeast extract) provided the highest whole fresh weight, new regenerants fresh weight.

On the other hand for Viking Ship medium, 2.75 g l^{-1} Viking Ship in the culture medium without Saturn fert presented more effective than 0.75 g l^{-1} Viking Ship with 1 g l^{-1} Saturn fert in the medium containing potato juice and yeast extract or 0.05-0.5 m g l^{-1} biotin or 0.1 g l^{-1} folic acid (LF3-LF6). However, LF8 (0.75 g l^{-1} Viking Ship + Saturn fert + potato juice + peptone) provided high growth and development. Although, there was no significant difference among LF3-LF6 and LF8, LF4 provided the highest whole fresh weight, new regenerants fresh weight and number of new plantlets.

In comparison, media H8-H12 from Hyponex medium and LF3-LF6, LF8 from Viking Ship medium, considered as the effective media, were chosen. The most efficient culture medium was LF4 (2.75 g l^{-1} Viking Ship + potato juice + peptone + 0.05 mg l^{-1} biotin) followed by LF8 (0.75 g l^{-1} Viking Ship + 1 g l^{-1} Saturn fert + potato juice + peptone) and H9 (1.0 g l^{-1} Hyponex + 1 g l^{-1} Saturn fert + potato juice + peptone), respectively.

From the study, it was demonstrated that local fertiliser can be used as the nutrients in *Phalaenopsis* 'Silky Moon' culture medium. Organic compounds, biotin, folic acid and yeast extract, provided the promising effect on PLBs and plantlets induction from seed-derived shoots. Moreover, the reported procedure can be applied to shoot-tip explants excised from flower stalk buds for micropropagation to reduce somaclonal variation. The advantages gained from this research will be in both short and long terms. For the short term benefits, the easy and simple biotechnology of *Phalaenopsis* micropropagation in order to respond the needs of growers at present will be obtained. For the long-term benefits, the development of techniques will be the efficient procedure for rapid propagation to minimize numbers of chemicals used, production cost and logistic impact. In addition the acquired knowledge will meet the government's policy following the philosophy on 'Sufficiency Economy', and elevate farmers' income leading to better economics and social development of the country.

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