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A N I N T E R N A T I O N A L J O U R N A L

The Effects of Different Media, Sucrose Concentrations and Natural Additives on Plantlet Growth of *Phalaenopsis* Hybrid 'Pink'

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ABSTRACT

The effects of various sucrose concentrations as carbon source and natural additives in different media on plantlet growth of Phalaenopsis hybrid 'Pink' were studied. Plantlets were cultured on two media (Murashige and Skoog [MS] and Vacin and Went [VW]) supplemented with 0, 10, 20, 30 and 40 g L^{-1} sucrose either with 0, 10 and 20% (v/v) coconut water (CW) or carrot juice (CJ) as natural additives. After four months of culture, the combination of sucrose and CW supplemented with both media affected plantlet growth where most of the plantlets showed slow growth and survival frequency (0–80%) with increasing concentrations of CW in all sucrose concentrations. However, plantlet growth on both media containing only 20 g L^{-1} sucrose without CW was optimal in terms of root number, root length, leaf number, leaf length, leaf width, fresh weight, dry weight and plant height. The combination of sucrose and CJ supplemented with MS medium resulted in overall good plantlet growth with 100% survival frequency. The combination of sucrose (20 g L^{-1}) and CJ (10%) supplemented with MS medium increased root length, leaf length, leaf width and plant height. Plantlet growth was also optimal in the combination of 20 g L^{-1} sucrose and 10% CJ supplemented with VW medium. The results of this study indicate that Phalaenopsis hybrid 'Pink' cultured on the combination of sucrose (20 g L^{-1}) and CJ (10%) supplemented with either MS or VW media can be used for plantlet growth of this species.

Key words: carrot juice; coconut water; natural additives; *Phalaenopsis*

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INTRODUCTION

Orchids are popular around the globe for their beautiful long lasting flowers and variety, and they are among the most diverse family of flowering plants in the world. Orchids are an important group of ornamental plants comprising several thousand species and hybrids, and are attractive to almost every individual including professional breeders, amateurs and normal collectors due to their natural beauty and uniquely shaped flower with a wide range of vibrant colour [1]. *Phalaenopsis* (Orchidaceae) is commonly known as moth orchid and is one of the widely acclaimed orchids due to its beautiful flowers [2]. *Phalaenopsis* is distributed throughout Southeast Asia, Taiwan, Australia and the Pacific, and has a great demand in flower markets in the world [3-4].

Phalaenopsis orchids are recognized as the most popular orchid genus in the horticultural industry due to their large, colourful, and durable flowers as well as their wider adaptability to room conditions. This orchid has a high economic value both as cut flowers and as potted plants in international flower markets [2, 5]. Although genotype and breeding technology largely impact the duration and intensity of flowering in *Phalaenopsis*, breeding technology is one of the most important factors influencing the vitality of the plants [6]. The characteristics of seedlings propagated by vegetative means are not uniform; therefore, propagation through tissue culture is desirable. Various tissue culture techniques have already been developed for *Phalaenopsis* orchids, where many types of explants such as flower stalks with auxiliary buds, meristems, internodal segments of flower stalks [6, 7-10], leaf segments [11-13] and root tips [14-15] were utilized.

The genus of *Phalaenopsis* is a monopodial orchid; therefore, mass vegetative propagation is limited for this orchid [6, 16]. Tissue culture techniques have been widely employed for rapid and large-scale propagation of this species [8, 17-18]. However, the effectiveness of micropropagation varies among plant species, types of explants and culture media [18]. The success of plant tissue culture in maximizing orchid's vigour is largely influenced by the presence of growth regulator and the nutritional components supplied in medium [19-20]. The medium used for orchid tissue culture is generally high in mineral salts, vitamins, growth regulators and water [20]. In addition, the carbon source in the form of carbohydrate (sugar) is another essential component in the culture medium, which can also influence the growth of the plants [21]. This is because carbohydrate supplies energy to the plants, especially when they are not ready to photosynthesize their own food during initial stage of tissue culture [22]. Carbon source can be in the form of simple or complex sugars [23]; however, sucrose is the most commonly used carbohydrate and carbon source in plant tissue culture medium [24-25]. Sucrose is considered as an important carbon and energy source in plant tissue culture as sucrose is the most common carbohydrate found in phloem sap and involved in controlling various development processes [26]. An appropriate type and concentration of sugar is needed in the medium to promote *in vitro* orchid seed germination and plant regeneration [25]. In vitro growth and regeneration can be improved by adding a small amount of organic additive to the orchid culture medium [16, 27-28]. A large number of complex additives such as coconut water (CW), banana extract, peptone, tomato juice, beef extract, potato extract are commonly added to orchid medium [20, 23, 29]. Organic additives help in germination, micropropagation and growth of many orchids [16, 23, 30-32]. The combination of sucrose with CW or CJ supplemented with either half-strength Murashige and Skoog (MS) or Vacin and Went (VW) medium on plantlet growth of *Phalaenopsis* hybrid 'Pink' has not been investigated. Therefore, the present study was carried out to evaluate the effects of culture media, sucrose and natural additives on plantlet growth of *Phalaenopsis* hybrid 'Pink'.

MATERIALS AND METHODS

Plant material and culture conditions

Laboratory experiments were carried out during June–November 2014 at the plant tissue culture laboratory, Rapee Sagarik Orchid Garden, Department of Horticulture, Faculty of Agriculture, Kasetsart University, Thailand. Three months *in vitro* young plantlets of *Phalaenopsis* hybrid 'Pink' (2 cm height) were used as donor plants and were purchased from the Salaya Orchid Company, Nonthaburi, Thailand. Flower stalk explants were used to produce donor plants that were cultured on VW medium supplemented with 10 g L^{-1} sucrose, 15 g L^{-1} banana extract and 15 g L^{-1} potato extract for regeneration and multiplication [16].

Plantlets were cultured on two media: (i) half-strength MS medium [33] and (ii) VW medium [34] on five sucrose concentrations (0, 10, 20, 30 and 40 g L^{-1}) supplemented with three concentrations (0, 10 and 20% [v/v]) of CW or CJ resulting in a total of 15 treatments. The pH of the medium was adjusted to 5.6 in half-strength MS medium and 5.2 in VW medium with 1 M KOH or 1 M HCl prior to autoclaving for 15 minutes at 121°C.

The media were filled in sterile vials (25 ml) prior to autoclaving. Subsequently, plantlets were placed on the surface of either half-strength MS or VW medium for four months and kept in the culture room at 25°C during the light and dark phase in a 15-h photoperiod under 40 µmol m^{-2} s^{-1} of light intensity provided by cool white florescent tubes.

Preparation of natural additives

Coconuts and carrots were purchased from local market. Coconut water was extracted directly from young coconut and filtered with Whatman filter paper No. 1 to remove unwanted debris [19]. Carrots were selected based on their smoothness and freshness, and were washed, peeled and cut into small sections to fit to the juice extractor. All natural additives were prepared fresh and immediately added to half-strength MS or VW medium as required [16].

Experimental design and data analysis

The experiments were laid out in a completely randomized design with 10 replications. Each experimental treatment combination consisted of two factors: sucrose concentration with CW or CJ concentration supplemented with either half-strength MS or VW medium. It was carried out with 10 glass vials each of which contained one *in vitro* young plantlet. Results were evaluated 120 days after cultures were initiated. The parameters recorded were root number, root length, leaf number, leaf length, leaf width, fresh weight, dry weight, plant height and survival frequency of explants. The data were subjected to analysis of variance (ANOVA) and significant differences among the treatments were tested using two-way ANOVA and means were separated by Duncan multiple range test (DMRT) at $P \leq 0.05$.

RESULTS

Effects of sucrose and coconut water supplemented with MS medium on plantlet growth

The combination of sucrose and CW supplemented with half-strength MS medium significantly ($P \leq 0.001$) affected plantlet growth (Table 1). After four months of culture, most of the plantlets exhibited slow growth and survival frequency (0–80%) with increasing concentrations of CW in all sucrose concentrations (0, 10, 20, 30 and

40 g L^{-1}). Half-strength MS medium containing 20 g L^{-1} sucrose without CW was suitable for plantlet growth in terms of increased root length (5.5 cm), leaf number (5), leaf length (3.16 cm), leaf width (1.83 cm), fresh weight (2.37 g), dry weight (0.12 g) and plant height (4.2 cm) with 100% survival frequency (Table 1). The combination of 10 g L^{-1} sucrose and 0% (v/v) CW showed good performance for leaf number (4.66), leaf length (3.33 cm), leaf width (1.5 cm), fresh weight (1.88 g), dry weight (0.08 g) and plant height (4.66 cm) with 100% survival frequency (Fig. 1A). The combination of 20 g L^{-1} sucrose and 10% (v/v) CW was shown to be optimal for leaf length (2.66 cm), leaf width (1.50 cm) and plant height (3.33 cm); however, the survival frequency was only 10%. No plantlet survived in the combination of 20 g L^{-1} sucrose and 20% (v/v) CW. The survival frequency was not satisfactory in the combination of 10, 30 and 40 g L^{-1} sucrose with either 10 or 20% (v/v) CW. Overall, plantlet growth of *Phalaenopsis* hybrid 'Pink' on half-strength MS medium containing 20 g L^{-1} sucrose without CW was the most optimal across the greatest number of recorded parameters.

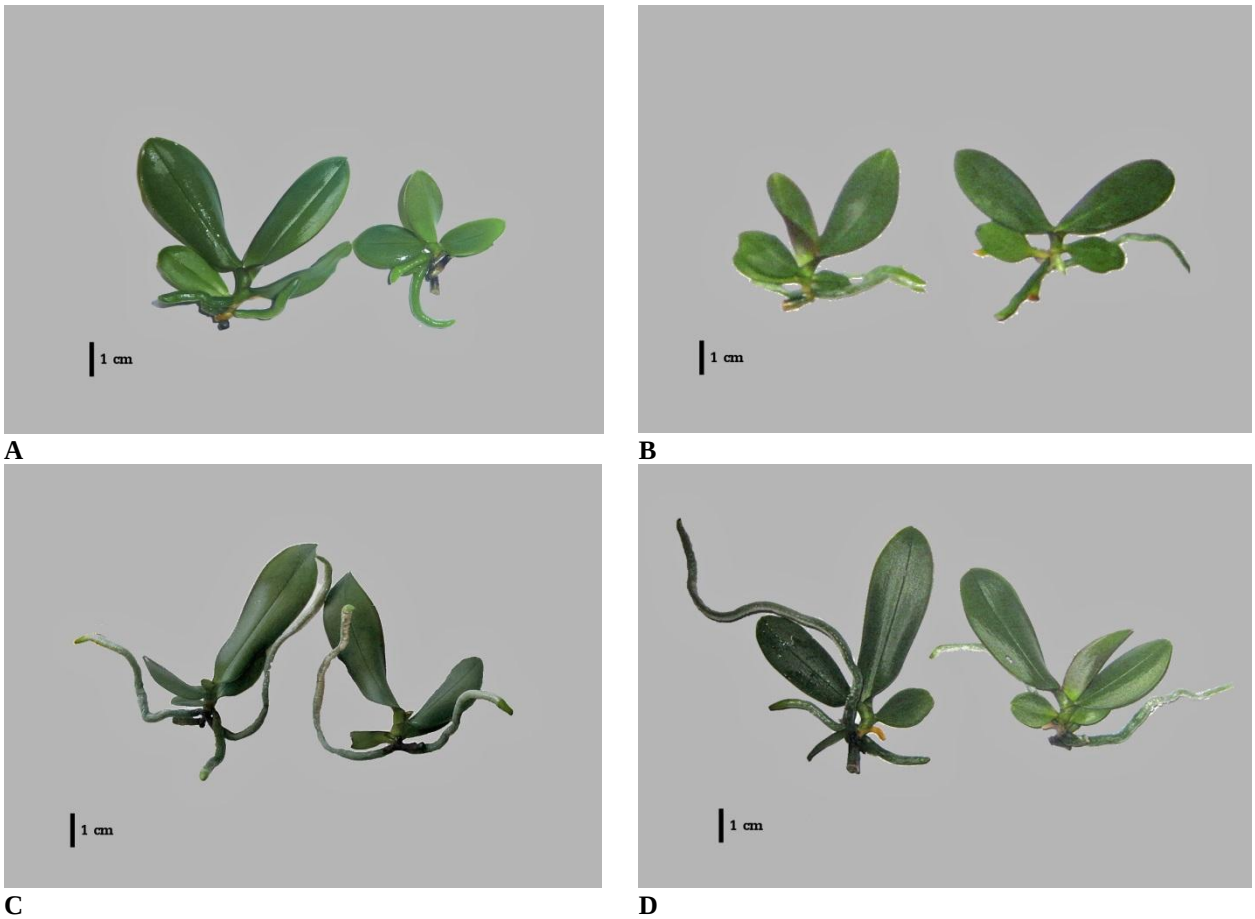

Figure 1- Effects of sucrose and coconut water (CW)/carrot juice (CJ) on plantlet growth of *Phalaenopsis* hybrid 'Pink' after four months of culture; (A) plantlets on 10 g L^{-1} sucrose + 0 % (v/v) CW supplemented with half-strength MS medium, (B) plantlets on 10 g L^{-1} sucrose + 0 % (v/v) CW supplemented with VW medium, (C) plantlets on 20 g L^{-1} sucrose + 10 % (v/v) CJ supplemented with half-strength MS medium, (D) plantlets on 10 g L^{-1} sucrose + 10 % (v/v) CJ supplemented with VW medium.

Table 1- Effects of sucrose and coconut water (CW) supplemented with half-strength MS medium on the production of rooting, leaf, fresh weight, dry weight, plant height and survival rate of *Phalaenopsis* hybrid 'Pink' shoots after four months of culture.

Sucrose (g L^{-1})	CW (%)	Root number	Root length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Survival frequency (%)
0	0	2.0 bc	3.66 bcd	5.00 a	2.33 ab	1.66 a	1.17 bcde	0.05 bcd	3.33 abc	100
	10	1.33 cd	3.00 cde	3.33 b	1.83 ab	1.33 b	0.93 cde	0.02 bcd	2.66 bc	80
	20	1.50 c	1.00 ef	2.66 b	1.66 ab	1.00 b	0.62 ef	0.06 bc	2.50 bc	20
10	0	2.66 bc	3.00 cde	4.66 a	3.33 a	1.50 a	1.88 ab	0.08 ab	4.66 a	100
	10	1.66 c	2.33 def	3.33 b	2.66 ab	1.33 b	1.57 bc	0.08 ab	3.66 abc	20
	20	1.33 cd	1.66 def	2.33 b	2.00 ab	1.00 b	1.16 bcde	0.01 cd	2.33 c	10
20	0	2.66 bc	5.50 abc	5.00 a	3.16 a	1.83 a	2.37 a	0.12 a	4.2a b	100
	10	2.00 bc	3.00 cde	2.33 b	2.66 ab	1.50 a	1.53 bc	0.05 bcd	3.33 abc	10
	20	0.00 d	0.00 f	0.00 c	0.00 c	0.00 c	0.00 f	0.00 d	0.00 d	0
30	0	3.20 ab	5.80 ab	5.60 a	2.50 ab	1.30 b	1.85 ab	0.12 a	3.70 abc	100
	10	2.00 bc	2.50 def	5.00 a	2.00 ab	1.66 a	1.44 bcd	0.05 bcd	3.00 abc	50
	20	1.50 c	2.50 def	5.00 a	2.00 ab	1.25 b	1.26 bcde	0.01 cd	2.50 bc	10
40	0	2.66 bc	6.33 a	5.25 a	3.12 a	1.62 a	1.68 abc	0.13 a	3.50 abc	90
	10	4.33 a	2.33 def	5.00 a	1.16 bc	1.00 b	0.72 def	0.05 bcd	2.16 b	50
	20	0.00 d	0.00 f	0.00 c	0.00 c	0.00 c	0.00 f	0.00 d	0.00 d	0
Sucrose effect		*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = *	*F* = NS	*F* = *	
CW effect		*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	
Sucrose × CW		*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	

Means followed by the same letter in each column do not differ by Duncan's multiple range test at $P < 0.05$.
NS: not significant. * and *** indicate significance at $P < 0.05$ and $P < 0.001$, respectively.

Effects of sucrose and coconut water supplemented with VW medium on plantlet growth

Plantlet growth in terms of rooting, leaf, fresh weight, dry weight, plant height and survival frequency was significantly ($P \leq 0.001$) affected by the combination of sucrose and CW supplemented with VW medium (Table 2). After four months of culture, most of plantlets had slow growth and survival frequency (0–70%) with increasing CW concentrations. The combination of 20 g L^{-1} sucrose and 0% (v/v) CW was suitable for plantlet growth in terms of increased root length (7.33 cm), leaf number (5.33), leaf length (4.0 cm), fresh weight (1.84 g), dry weight (0.29 g) and plant height (5.0 cm) with 100% survival frequency. The combination of 10 g L^{-1} sucrose and 0% (v/v) CW showed good performance for root number (3.20), root length (6.34 cm), leaf number (4.20 cm), leaf width (2.10 cm), fresh weight (1.89 g) and plant height (5.30 cm) with 100% survival frequency (Fig. 1B). The combination of 10 g L^{-1} sucrose and 10% (v/v) CW was shown to be optimal for root length (6.0 cm) and fresh weight (1.83 cm); however, the survival frequency was only 30% (Table 2). The survival frequency of plantlets was not satisfactory in the combination of 10, 20, 30 and 40 g L^{-1} sucrose with either 10 or 20% (v/v) CW. Overall, plantlet growth of *Phalaenopsis* hybrid 'Pink' on VW medium containing 20 g L^{-1} sucrose without the addition of CW showed satisfactory results across the greatest number of recorded parameters for plantlet growth.

Table 2- Effects of sucrose and coconut water (CW) supplemented with VW medium on the production of rooting, leaf, fresh weight, dry weight, plant height and survival rate of *Phalaenopsis* hybrid 'Pink' shoots after four months of culture.

Sucrose (g L^{-1})	CW (%)	Root number	Root length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Survival frequency (%)
0	0	2.00 bc	3.30 d	5.60a	2.70 c	2.30 a	1.06 b	0.04 bc	4.00 b	100
	10	1.00 cd	2.00 d	3.33 bcd	1.66 e	1.00 c	0.92 b	0.06 bc	2.00 ef	70
	20	0.00 d	0.00 e	0.00 f	0.00 f	0.00 d	0.00 c	0.00 c	0.00 g	0
10	0	3.20 ab	6.34 ab	4.20 b	3.30 b	2.10 a	1.89 a	0.09 bc	5.30 a	100
	10	2.00 bc	6.00 ab	2.33 de	2.50 cd	1.33 bc	1.83 a	0.08 bc	3.00 cd	30
	20	0.00 d	0.00 e	0.00 f	0.00 f	0.00 d	0.00 c	0.00 c	0.00 g	10
20	0	2.00 bc	7.33 a	5.33 a	4.00 a	1.66 b	1.84 a	0.29 a	5.00 a	100
	10	2.00 bc	5.13 bc	3.66 bc	2.50 cd	1.00 c	1.13 b	0.08 bc	3.16 c	70
	20	1.00 cd	2.33 d	1.66 e	1.66 e	1.00 c	0.70 b	0.06 bc	1.66 de	30
30	0	2.33 bc	3.8 cd	4.00 b	2.00 de	1.00 c	0.75 b	0.03 bc	3.00 cd	60
	10	1.33 cd	3.00 d	2.66 cde	2.00 de	1.00 c	0.66 b	0.01 bc	2.00 ef	10
	20	0.00 d	0.00 e	0.00 f	0.00 f	0.00 d	0.00 c	0.00 c	0.00 g	10
40	0	3.25 ab	6.87 ab	4.25 b	1.50 e	1.25 c	1.79 a	0.14 b	2.50 de	70
	10	4.00 a	3.00 d	2.00 e	2.00 de	1.00 c	0.91 b	0.09 bc	2.50 de	30
	20	0.00 d	0.00 e	0.00 f	0.00 f	0.00 d	0.00 c	0.00 c	0.00 g	0
Sucrose effect		*F* = NS	*F* = NS	*F* = NS	*F* = **	*F* = NS	*F* = *	*F* = *	*F* = *	
CW effect		*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	
Sucrose × CW		*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	*F* = ***	

Means followed by the same letter in each column do not differ by Duncan's multiple range test at $P < 0.05$.
NS: not significant. *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Effects of sucrose and carrot juice supplemented with MS medium on plantlet growth

After four months of culture, all treatments with or without CJ showed 100% survival frequency (Table 3). The combination of sucrose and CJ supplemented with half-strength MS medium significantly ($P \leq 0.01$) affected root number, root length, leaf length, leaf width and plant height, but had no effect on leaf number, fresh weight and dry weight. Root number (7.75) was the highest in the combination of 40 g L^{-1} sucrose and 0% (v/v) CJ. The combination of 20 g L^{-1} sucrose and 10% (v/v) CJ was shown to be optimal for the longest root length (9.50 cm), leaf length (5.33 cm), leaf width (2.50 cm) and the highest plant height (6.33 cm) (Fig. 1C). Plantlets growth in the combination of 20 g L^{-1} sucrose and 20% (v/v) CJ was not optimal. In addition, the combination of 10, 30 and 40 g L^{-1} sucrose with either 10 or 20% (v/v) CJ also did not optimally enhance plantlets growth compared with plantlets growth in the combination of 20 g L^{-1} sucrose and 10% (v/v) CJ. Overall, the combination of 20 g L^{-1} sucrose and 10% (v/v) CJ showed good results across the greatest number of recorded parameters for plantlet growth.

Effects of sucrose and carrot juice supplemented with VW medium on the plantlet growth

In this experiment, the combination of sucrose and CJ supplemented with VW medium showed significant ($P \leq 0.05$) effect on plantlet growth except in terms of dry weight (Table 4). After four months of culture, all of the plantlets showed 100% survival frequency in all treatments. The combination of 20 g L^{-1} sucrose and 10% (v/v) CJ was shown to be optimal for most of the studied parameters such as root number (4.33), root length (6.83 cm), leaf number (6.0), leaf length (5.0 cm), leaf width (3.0 cm), fresh weight (3.60 g) and plant height (6.16 cm) with 100% survival frequency. The combination of 10 g L^{-1} sucrose and 10% (v/v) CJ was shown to be optimal for enhanced root length (7.0 cm), leaf number (5.60), leaf length (5.30 cm), leaf width (2.50 cm), fresh weight (3.13 g) and plant height (6.70 cm) with 100% survival frequency (Fig. 1D). The combination of 30 g L^{-1} sucrose and 10% (v/v) CJ was shown to be optimal for root length (8.66 cm) and plant height (6.40 cm) whereas the combination of 40 g L^{-1} sucrose and 10% (v/v) CJ was shown to be optimal for root number (4.0), root length (8.66 cm), leaf length (4.33 cm) and plant height (5.16 cm). Overall, the combination of 20 g L^{-1} sucrose and 10% (v/v) CJ showed good results across the greatest number of recorded parameters for plantlet growth.

Table 3- Effects of sucrose and carrot juice (CJ) supplemented with half-strength MS medium on the production of rooting, leaf, fresh weight, dry weight, plant height and survival rate of *Phalaenopsis* hybrid 'Pink' shoots after four months of culture.

Sucrose (g L^{-1})	CJ (%)	Root number	Root length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Survival frequency (%)
0	0	2.00 d	3.30 d	5.20	3.00 b	1.66 bc	1.15	0.04	4.00 d	100
	10	2.75 bcd	4.87 bcd	5.00	3.40 b	2.20 ab	1.73	0.07	5.10 abcd	100
	20	2.20 d	3.90 cd	4.20	4.10 ab	2.00 ab	1.67	0.08	5.20 abcd	100
10	0	2.40 cd	5.90 bc	4.20	4.50 ab	2.10 ab	1.89	0.23	6.10 ab	100
	10	2.80 bcd	6.50 b	4.40	4.98 a	2.20 ab	2.51	0.15	5.90 ab	100
	20	2.80 bcd	5.90 bc	4.20	4.26 ab	2.00 ab	1.98	0.13	5.20 abcd	100
20	0	3.80 bcd	5.60 bc	5.00	4.20 ab	2.10 ab	2.08	0.14	5.60 abc	100
	10	2.00 d	9.50 a	4.33	5.33 a	2.50 a	2.19	0.14	6.33 a	100
	20	2.80 bcd	5.90 bc	5.00	3.26 b	1.70 bc	1.90	0.13	4.20 cd	100
30	0	4.40 bcd	6.90 b	4.40	3.20 b	1.40 cd	1.71	0.13	4.20 cd	100
	10	3.20 bcd	5.40 bcd	4.20	4.00 ab	2.00 ab	1.68	0.14	5.30 abcd	100
	20	2.75 bcd	5.87 bc	4.25	4.00 ab	1.37 cd	1.59	0.13	5.25 bcd	100
40	0	7.75 a	3.25 d	4.75	1.50 c	1.00 d	1.77	0.15	2.50 e	100
	10	5.20 b	5.10 bcd	5.20	3.40 b	1.40 cd	1.94	0.16	4.60 bcd	100
	20	5.00 bc	5.75 bc	4.50	3.00 b	1.25 cd	2.08	0.22	4.00 d	100
Sucrose effect		*F* = ***	*F* = ***	*F* = NS	*F* = ***	*F* = ***	*F* = *	*F* = NS	*F* = **	
CJ effect		*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = NS	*F* = NS	
Sucrose × CJ		*F* = **	*F* = ***	*F* = NS	*F* = ***	*F* = ***	*F* = NS	*F* = NS	*F* = ***	

Means followed by the same letter in each column do not differ by Duncan's multiple range test at $P < 0.05$.
NS: not significant. *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Table 4- Effects of sucrose and carrot juice (CJ) supplemented with VW medium on the production of rooting, leaf, fresh weight, dry weight, plant height and survival rate of *Phalaenopsis* hybrid 'Pink' shoots after four months of culture.

Sucrose (g L^{-1})	CJ (%)	Root number	Root length (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Survival frequency (%)
0	0	1.80 d	3.30 d	4.20 b	2.70 efg	2.00 bc	1.19 e	0.37	3.90 def	100
	10	2.20 cd	5.46 bcd	5.20 ab	3.50 cde	2.20 b	1.67 de	0.39	5.00 abcde	100
	20	2.20 cd	4.10 cd	5.00 ab	3.80 bcde	2.10 bc	1.58 de	0.65	5.30 abcd	100
10	0	2.80 bcd	7.80 ab	4.60 bc	3.10 def	1.90 bc	1.86 cde	0.09	4.60 bcde	100
	10	3.00 bcd	7.00 abc	5.60 ab	5.30 a	2.50 ab	3.13 ab	0.17	6.70 a	100
	20	2.60 bcd	6.22 abc	5.60 ab	3.76 bcde	2.00 bc	1.85 cde	0.12	6.00 abc	100
20	0	2.75 bcd	7.50 ab	5.50 ab	1.75 g	1.50 cd	2.44 bcd	0.14	2.75 f	100
	10	4.33 a	6.83 abc	6.00 a	5.00 ab	3.00 a	3.60 a	0.70	6.16 abc	100
	20	3.25 bcd	5.75 abcd	4.25 b	4.10 abcd	2.00 bc	1.86 cde	0.12	5.25 abcd	100
30	0	3.00 bcd	7.12 ab	4.75 b	2.62 efg	1.50 cd	1.97 cde	0.13	4.25 cdef	100
	10	3.00 bcd	8.66 a	4.60 b	3.84 bcde	1.90 bc	2.69 bc	0.20	6.40 ab	100
	20	3.50 abc	6.62 abc	4.50 b	4.62 abc	2.00 bc	2.34 bcd	0.16	6.25 ab	100
40	0	4.00 ab	8.32 ab	4.20 b	2.00 fg	1.10 d	1.92 cde	0.17	3.40 ef	100
	10	4.00 ab	8.66 a	4.66 b	4.33 abcd	2.16 bc	2.73 bc	0.24	5.16 abcde	100
	20	2.66 bcd	7.33 ab	4.66 b	1.66 g	1.00 d	1.79 cde	0.18	2.50 f	100
Sucrose effect		*F* = **	*F* = ***	*F* = NS	*F* = *	*F* = **	*F* = **	*F* = NS	*F* = **	
CJ effect		*F* = NS	*F* = NS	*F* = NS	*F* = ***	*F* = **	*F* = **	*F* = NS	*F* = ***	
Sucrose × CJ		*F* = **	*F* = **	*F* = *	*F* = ***	*F* = ***	*F* = ***	*F* = NS	*F* = ***	

Means followed by the same letter in each column do not differ by Duncan's multiple range test at $P < 0.05$.
NS: not significant. *, ** and *** indicate significance at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

DISCUSSION

The inorganic salt formulation of MS medium represents one of the major achievements in the history of cell and tissue culture, facilitating widespread use in horticulture, agriculture and biology [33]. The modified MS basal medium is being used for overall plant tissue culture until now where the chemical compositions are formulated as needed for the basic nutrition with the addition of plant growth regulators or natural additives for growth [4, 6]. VW medium was especially formulated for orchid tissue culture by Vacin and Went [34] where all the chemical contents were calculated as needed for orchid growth. However, the efficacy of this medium could be enhanced with the addition of organic additives (e.g., CJ, tomato juice, banana pulp, CW) and probably would not be suitable for other plant tissue culture [29]. Another medium, known as New Phalaenopsis medium (NP medium), was developed by Ichihashi [35] for micropropagation of *Phalaenopsis* and tropical orchids. NP medium contains all chemical nutrients required by *Phalaenopsis* and this medium has been used in other orchids by some researchers [23, 36]. However, MS and VW medium as basal medium are still being widely used for orchid tissue culture including *Phalaenopsis*.

Sucrose has been widely used in plant tissue culture as the major carbohydrate source to supply the energy to cells because it can efficiently move across the cell membrane, plasmalemma [37]. The length of root was not influenced with the increase in sucrose concentration up to 20 g L^{-1} in *in vitro* culture medium of *Dendrobium nobile*; however, smaller plant height, shorter root length and lower rate of shoot multiplication were observed without sucrose [25]. Sucrose is considered as an important component in *in vitro* culture medium where it serves as a source of carbon and energy. It has been reported that the initial concentration of sucrose can affect growth and biomass accumulation [38]. In contrast, the development of cultured cells can be retarded by higher amount of sucrose by causing a termination of the cell cycle when nutrients are limited [39]. Sucrose concentrations of 20 and 30 g L^{-1} are the most commonly used carbohydrate source in orchid culture studies [25, 40-41]. Sugars enter into the metabolic pathways and breaking down of sugar produces the required energy for *in vitro* orchid seed germination, growth of cell, buds, shoots and even plantlets growth [42]. Other carbohydrates are also successfully used as a source of carbon; however, the effect of carbohydrates on micropropagation is species dependent [43].

Coconut water contains many nutritional and/or hormonal substances including diphenyl urea, which acts as cytokinin and this growth regulator induces the growth and cell division [19, 44]. Others also reported an effective growth and regeneration of plants in CW supplemented medium and this is due to the presence of natural contents of cytokinin and auxin [45]. In the present study, the concentrations of 10 and 20 g L^{-1} CW supplemented with either half-strength MS medium or VW medium reduced the production of rooting, leaf, fresh weight, dry weight, plant height and survival frequency of *Phalaenopsis* hybrid 'Pink' shoots after four months of culture in all sucrose concentrations. It was observed that the highest concentration of CW (20% [v/v]) in all sucrose concentrations showed the lowest survival frequency regardless of the medium. Our findings in this study are similar to Gnasekaran [19], Nambiar [1] and Sudipta [46], who also reported an inhibitory effect on the proliferation and regeneration of *Phalaenopsis* in half-strength MS medium and VW medium with higher concentration of CW (20–30% [v/v]). The inhibitory effect of higher concentration of CW on the plantlet growth could be due to the use of phytohormones above the optimum concentration.

Carrots are rich in carbohydrates and minerals such as Ca, P, Fe and Mg, which are very important for the plant energy [47]. The important chemical constituents of carrot have been reported by Gopalan [48] as moisture (86%), protein (0.9%), fat (0.2%), carbohydrate (10.6%), crude fibre (1.2%), total ash (1.1%), Ca (80 mg 100 g^{-1}), Fe (2.2 mg 100 g^{-1}) and P (53 mg 100 g^{-1}). Some other constituents of CJ include cytokinin, vitamins, amino acids and endogenous sterols [49].The presence of the auxin in CJ is reported to be responsible for promoting callus formation and the effectiveness is greater in medium supplemented with CJ than unsupplemented medium. Auxin is widely used in micropropagation and is incorporated into nutrient medium to promote the growth of callus, cell suspensions or organs such as meristems, shoot and root tips, especially in combination with cytokinin [49]. Higher auxin concentrations compared with cytokinin generally leads to root formation on the cutting edge, embryogenesis and adventitious root formation. Higher cytokinin concentration generally favours callus initiation, adventitious shoot formation and auxiliary shoot proliferation [50]. The use of CJ in half-strength MS medium also resulted in good results in micropropagation of *Leptadenia reticulate* [46]. The combination of 30 and 40 g L^{-1} sucrose and 10% CJ (v/v) supplemented with half-strength MS or VW medium, respectively, resulted in the highest plant height, leaf length and photosynthetic activity (CO_2 uptake, leaf greenness and total chlorophyll content) in *Phalaenopsis* hybrid 'Pink' [16]. The authors also observed that the uptake of CO_2 and chlorophyll content was slightly higher in VW medium with 40 g L^{-1} sucrose compared with half-strength MS medium with 30 g L^{-1} sucrose at the same concentration of CJ. The present study is the first protocol where the regeneration of *Phalaenopsis* hybrid 'Pink' was evaluated in the combination of sucrose and CJ in different media.

It has been reported that cytokinin content in some natural additives (e.g., CW) can induce the division of leaf cells [51]. According to Dixon and Gonzales [52], cytokinin without auxin was completely optimum for the division and growth of leaf cells. In addition, CW also contains some other elements such as Ca and vitamins, which help stimulate the production of new leaf [53]. However, cytokinin also appears in CJ. In the present study, sucrose and CJ in half-strength MS medium had no effect on the fresh weight of *Phalaenopsis* hybrid 'Pink'. However, the highest fresh weight (3.60 g) was obtained from the plantlets grown in combination of 20 g L^{-1} sucrose and (10% [v/v]) CJ supplemented with VW medium. It might be due to high concentration of sucrose in CJ (10.6% [v/v]) and supported by additional sucrose content (20 g L^{-1}), which increased the amount of carbohydrate in VW medium [36]. Baker [54] reported that *Phalaenopsis* developed better protocorms on VW medium. Goh and Wang [55] observed that protocorm-like bodies proliferation of *Aranda* orchid was better on liquid VW medium in combination with sucrose and CW.

CONCLUSION

Among all the treatments, most parameters recorded to evaluate plantlet growth of *Phalaenopsis* hybrid 'Pink' showed better performance in 20 g L^{-1} of sucrose in both media. Higher concentration of CW resulted in an inhibitory effect on plantlet growth. On the other hand, CJ showed better results compared with CW based on greater root number, root length, leaf number, leaf length, leaf width, fresh weight and plant height with 100% survival frequency in all treatments. An inclusion of CJ in the medium might have also caused an increase in the level of various substances such as vitamins, amino acid, purines, pyrimidines and carbohydrate providing a beneficial effect on plantlet growth in the presence of CJ [56]. Altogether this study showed that *Phalaenopsis* hybrid 'Pink' cultured on the combination of sucrose (20 g L^{-1}) and CJ (10% [v/v]) supplemented with either half-strength MS or VW media

can be used for plantlet growth of this species. The present study could be the first protocol of using CJ for plantlet growth of *Phalaenopsis* hybrid 'Pink'. Further research needs to be done to generate greater information regarding the effect of CJ in somatic embryogenesis of *Phalaenopsis* as CJ contains many important chemical substances which could help decrease the cost of micropropagation in the future.

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