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RESEARCH ARTICLE

MICROPROPAGATION OF TROPICAL MOKARA BY FLOWER-STALK CULTURE TECHNIQUE

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ABSTRACT

Mokara is atropical orchid species for flower cutting mainly in Ho Chi Minh City. Their flowers are beautiful and colorful diversity, harvesting 8-10 flower-stalks per plant per year, and it is not enough for demands in local and export. Their coefficient of propagation is low naturally after an economic cycle of 5-6 years. Each plant gives 1 main shoot and 1-3 lateral buds. Flower-stalks were favored as materials for *in vitro* cultivation. The second internodes (non-flower internode of stalk) cultivated on the medium MS + BA (1 mg/L) + coconut water (10%) + PVP (50 mg/L) gave 2.68 shoots/cluster. Single leaf was separated and cultivated on the medium MS + BA (1.5 mg/L) + coconut water (10%) reached 67.66 shoots/cluster. The multiple-shoot cluster was separated to small cluster with 2-3 shoots/cluster and cultivated on the multiple medium MS + BA (0.75 mg/L) + coconut water (10%) gave 103.33 shoots/cluster. Multiple-shoot clusters were separated to 2-3 shoots/cluster and cultivated on regenerated medium MS + coconut water (15%) formed 3 shoots/cluster and rooted on the medium MS + IAA (0.5 mg/L) + coconut water (10%) + activated charcoal (1 g/L) with 4.66 roots/shoot. The effect of genotypes on shoot regeneration is significant from internode and multiple-shoot propagation *in vitro*.

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INTRODUCTION

Mokaraorchid is a hybrid of three varieties including Ascocentrum, Arachnis and Vanda. Mokara is a single-stem orchid that is easy to grow and grows fast in tropical climates. Flowers are rich in shape and have many beautiful colors such as pink, red, lemon yellow, bronze, yellow, orange, violet, white ... (Xo, 2011). Mokara orchids are being planted in the area about 40-50% (200ha) of the total area of orchids in Ho Chi Minh City (2013) and the income of this orchid is up to 1 billion/ha. It has not yet met the demand of the domestic market and export. Mokara has low multiplication coefficients, almost no sprouted shoots on the stem nodes during the economic exploitation cycle (5-6 years). It was mainly propagated by asexual methods using apical shoots (50-80 cm) planting for the next crop. The base stem sprouts 1-3 young shoots reaching a height of 30-40 cm after 8 months used for growing on beds. 8 to 10 flower-stalksare harvested from a mother orchidper year (Xo, 2011). Therefore, the flower-stalk of mokarais a suitable material for *in vitro* propagation. Propagation of orchids by flower-stalk cultivation has been successful in many orchids (Phalaenopsis, Dendrobium, Cymbidium), (Arditti and Ernst, 2009), influenced by growth regulators (Wang and Lehmann, 2002), explants (Philips, 1988) and genotypes (Pierik, 1987; Sagawa, 1999b). In this paper, the propagation of mokara orchid through flower-stalk culture technique was studied.

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MATERIALS AND METHODS

Plant materials

The mokaravariety of Fullmoon (imported from Thailand), also known as lemon mokara, is used in this research. The mokara (and renanthera) breeding groups for business were used in research of gene source: Fullmoon, Newmoon, Banana, Chistie, Fuchs Delight, Charkuan Pink, Charkuan Orange, Chaopraya Sunset, Chaopraya Gold, Chaopraya Beauty, Ren Red, Kalsum x Ascocenda, Kalsum x Lena Rewol, Lena Rewol x Dr.Anek.

Culture medium

MS medium (Murashige and Skoog, 1962) was added plant growth regulators: BA (benzylaminopurine), kinetin (furfurylaminopurine), IAA (indole-3-acetic acid); Organic substances: sucrose, coconut water; and brown antioxidants: activated carbon and PVP (polyvynilpyrolydone).

Culture conditions

Light intensity of 22.2 µmol/m^2/s, humidity RH 70%, culture temperature 26 ± 2°C. Culture medium was sterilized at 121°C at 1 atmfor 45 minutes

Methods

Randomized block design (RBD) was repeated three times, each with 3 flasks (300 mL). Each sample has 2-3 shoots. Data were collected after 6 weeks of culture and processed using SPPSS v.16 software.

Experimental design

Experiment 1: Flower-stalk culture: Flower-stalk after blossoming completely is used as a culture material. The nodes of flower-stalks at 1-2-3 positions were used for culture. Samples are sterile in two steps; the first step: explant is treated with hypochlorite Na (10%) for 20 minutes, and washed 3 times with sterile distilled water; the step two: it was disinfected with $HgCl_2$ (0.1%) for 10 minutes, and washed 3 times with sterile distilled water. MS medium was supplemented with BA (1 mg/L), 10% coconut water and PVP (50 mg/L).

Experiment 2: Induction of shoot clusters: shoots with 0.5-0.7 cm in high and 1-2 leaves were cultured on MS medium supplemented with growth regulators: BA (0.1-0.5-1-1.5-2 mg/L), Kinetin (0.1-0.5-1-1.5-2 mg/L) and coconut water (10%).

Experiment 3: Proliferation of shoot clusters on semi-solid media: the clusters with 2-3 shoots were cultured on semi-solid media. Clusters of shoots on MS medium supplemented with growth regulators: BA (0.1-0.5-1-1.5-2 mg/L) and Kinetin (0.1-0.5-1 -1.5-2 mg/L) and coconut water (10%).

Experiment 4: Regeneration of shoot clusters: the shoot clusters (2-3 shoots/cluster) were cultured on MS medium supplemented with coconut water (0-5-10-15-20-25%) and activated charcoal (1 g/L).

Experiment 5: Culture of root induction: shoots with 1.5 cm in height and 2-3 leaves were separated from the shoot clusters and cultured on root MS medium supplemented with growth regulators: IAA (0-0.25-0.5-0.75-1 mg/L), coconut water (10%) and activated charcoal (1 g/L).

Experiment 6: Effects of genotypes on *in vitro* multiplication: The Mokara (and Renantherahy brids) were grew for business in a large scale in Ho Chi Minh City as a source of gene for regeneration culture of the flower-stalk nodes and shoot multiplication on a semi-solid medium.

RESULTS AND DISCUSSION

Flower-stalk culture

Flower-stalk at full flowering stage was removed flowers and the internodes of flower-stalk (non-flowering) in the top-down at position of 1-2-3 was cultured on MS medium supplemented with BA (1 mg/L), coconut water (10%) and PVP (50 mg/L). The second node of flower-stalk reached survival rate was 78.45% and number of shoot was highest (2.68 shoots/internode) significantly different in comparison with the first internode culture (65.22% and 1.84 shoots/cluster) and internode 3 (28.14% and 1.15 shoots/cluster) (Table 1). The second one was used as the material for *in vitro* regeneration culture of Mokara cultivars, as compared to either top shoot or lateral shoots (when the shoots were damaged) (Figure 1).

Table 1. Cultivation of tropical Mokara flower-stalk

The internode position of flower-stalks	Survival rate (%)	Number of shoots Per internode
1	65.22 ^{b}	1.84 ^{a,b}
2	78.45 ^{a}	2.68 ^{a}
3	28.14 ^{c}	1.15 ^{b}
CV (%)	18.16	22.62

Figure 1. Shoot regeneration from (second internode) on medium MS + BA (1 mg/L) + coconut water (10 %) + PVP (50 mg/L)

Induction of shoot clusters

The culture medium supplied with BA and Kinetin at different concentrations had significantly different effects on shoot height, diameter of shoot cluster and shoot number/cluster compared to those of control with 28 shoots/clusters, bud diameters 0.91 cm, shoot height 0.86 cm (Table 2). The medium supplemented with BA (1.5 mg/L) stimulated the maximum number of shoots (67.66 shoots/cluster), as well as the shoot diameter (1.90 cm), and the height development of shoots decrease (1.04 cm). Similarly, Kinetin concentrations of 0.1 mg/L had a high shoot formation of 41.66 shoots/cluster with bud cluster diameter of 1.353 cm and a Kinetin concentration of 1.5 mg/L gave the highest shoot height (1.17 cm). In the treatments with higher Kinetin concentrations, the bud formation was inhibited. MS medium supplemented with BA (1.5 mg/L) stimulated rapid differentiation of bud formation and was more effective than that of Kinetin supplementation. Results of the study showed that BA and coconut water had an good effect on bud formation and medium suitable for cultivating Mokara shoots is MS + BA (1.5 mg/L) + coconut water (10%) (Figure 2).

Table 2. Effects of BA and Kinetin on shoot cluster formation

BA (mg/L)	Kinetin (mg/L)	Survival rate (%)	Height of shoot (cm)	Diameter of bud cluster (cm)	Number of shoot/cluster
0.0	0.0	77.76 ^{a}	0.86 ^{b,c,d}	0.91 ^{c,d}	28.00 ^{d,e,f}
0.1		83.33 ^{a}	1.146 ^{b}	1.07 ^{c}	34.66 ^{c,d,e}
0.5		77.73 ^{a}	1.150 ^{b}	1.32 ^{b}	45.00 ^{b,c}
1.0		72.16 ^{a}	1.152 ^{a}	0.99 ^{c}	34.00 ^{c,d,e}
1.5		83.30 ^{a}	1.04 ^{b,c}	1.90 ^{a}	67.66 ^{a}
2.0		66.63 ^{a}	1.14 ^{b}	1.74 ^{a}	55.66 ^{a,b}
	0.1	83.33 ^{a}	0.99 ^{b,c,d}	1.35 ^{b}	41.66 ^{b,c,d}
	0.5	16.63 ^{b}	0.71 ^{c,d}	0.76 ^{d,e}	18.66 ^{f,g}
	1.0	49.96 ^{a,b}	1.04 ^{b,c,d}	1.06 ^{c}	33.66 ^{c,d,e}
	1.5	44.43 ^{a,b}	1.17 ^{a}	0.58 ^{e}	11.66 ^{g}
	2.0	55.53 ^{a,b}	0.69 ^{d}	0.88 ^{c,d}	24.00 ^{e,f,g}

There was no significant difference between the values with the same characters at $p < 0.05$

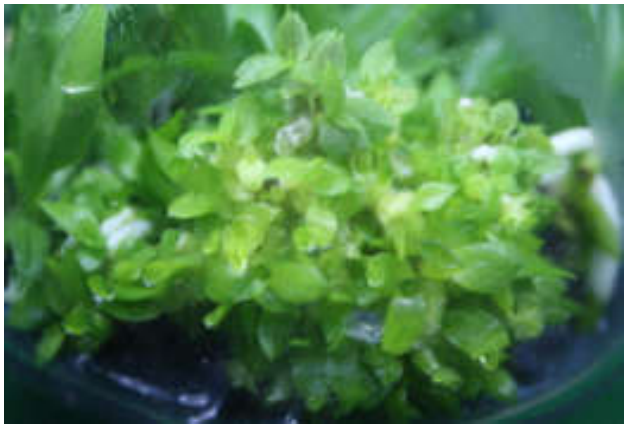

Figure 2. Bud formation on medium MS + BA (1.5 mg/L) + coconut water (10 %)

Multiplication of shoot clusters on semi-solid media

The multiplication of shoot cluster on growth medium supplemented with BA (0.75 mg/L) produced buds (103.33 shoots/cluster), bud diameter (2.78 cm) and shoot height (1.20 cm). Similarly, the culture medium supplemented with Kinetin (0.5 mg/L) produced 97.66 shoots/cluster, 2.64 cm bud diameter, and shoot height of 1.23 cm. The results showed that medium supplemented with BA (0.75 mg/L) or Kinetin (0.5 mg/L) was highly effective compared with control of 65.33 shoots/cluster, bud diameter 1.70 cm, height of shoots 1.23 cm (Table 3); BA has a positive effect on the formation and growth of shoots. The suitable medium for Mokara shoots multiplication in vitro was MS + BA (0.75 mg/L) + coconut water (10%) (Table 3) (Figure 3).

Table 3. Effects of BA and Kin on shoot multiplication in Mokara orchids

BA (mg/L)	Kinetin (mg/L)	Survival rate (%)	Height of shoot (cm)	Diameter of bud cluster (cm)	Number of shoot/cluster
0.00	0.00	77.73 ^{a,b}	1.23 ^{b}	1.70 ^{d}	65.33 ^{d,e}
0.10		94.43^{a,b}	1.52 ^{a}	2.04 ^{c}	77.33^{b,c,d}
0.25		100.00 ^{a}	1.10 ^{b}	2.54 ^{b}	92.66^{a,b}
0.50		88.86^{a,b}	1.22 ^{b}	2.49 ^{b}	88.66^{a,b,c}
0.75		100.00 ^{a}	1.20 ^{b}	2.78 ^{a}	103.33 ^{a}
1.00		72.16 ^{b}	0.78 ^{d}	1.51 ^{d}	62.66^{d,e}
	0.10	72.16 ^{b}	1.07 ^{b,c}	2.02 ^{c}	77.66 ^{b,c,d}
	0.25	83.30 ^{a,b}	0.92 ^{c,d}	1.92 ^{c}	75.33 ^{c,d}
	0.50	77.73 ^{a,b}	1.23 ^{b}	2.64 ^{a,b}	97.66 ^{a}
	0.75	88.86 ^{a,b}	1.21 ^{b}	2.46 ^{b}	92.00^{a,b}
	1.00	72.16 ^{b}	1.57 ^{a}	1.53 ^{d}	58.66 ^{e}

Figure 3. Bud multiplication on medium MS + BA (0.75 mg/L) + coconut water (10 %)

Regeneration of shoot cluster

During bud propagation, the small bud size has not yet developed, due to the effect of growth regulators. Regeneration of the shoots aims to bring the shoots to maturity and grow in size before transferring to the nursery. Coconut water and activated charcoal are used in addition to the culture medium of shoot regeneration in orchids. The results showed that culture medium supplemented with coconut water (15%) reduced bud formation (3.00 shoots/cluster), bud diameter (1.07 cm) and shoot height (3.07 cm). Addition of activated charcoal (1 g/L) + coconut water (15%) to the culture medium found no significant difference. The high yielding shoot regeneration medium was MS + coconut water (15%) (Table 4) (Figure 4).

Table 4. Effects of CW and AC on shoot cluster regeneration of Mokara orchid

Coconut water (%)	Activated charcoal (g/L)	Survival rate (%)	Height of shoot (cm)	Diameter of bud (cm)	Number of shoot/shoot
0	0	100.00 ^{a}	1.76 ^{c}	1.64 ^{d}	24.66 ^{c}
5		100.00^{a}	1.52 ^{d}	2.07^{b,c}	27.00 ^{c}
10		100.00 ^{a}	2.04 ^{b}	2.26 ^{b}	37.33 ^{b}
15		100.00 ^{a}	3.07 ^{a}	1.07 ^{e}	3.00 ^{d}
20		94.43 ^{a}	1.59 ^{d}	2.64 ^{a}	45.33 ^{a}
25		100.00 ^{a}	2.02 ^{b}	2.55 ^{a}	41.33^{a,b}
	5	55.53 ^{b}	1.12 ^{e}	1.08 ^{e}	2.00^{d}
	10	38.86 ^{b}	1.17 ^{e}	1.09 ^{e}	3.00 ^{d}
	15	55.50 ^{b}	1.08 ^{e}	1.10 ^{e}	2.66 ^{d}
	20	94.43 ^{a}	3.05 ^{a}	2.04 ^{c}	6.66 ^{d}
	25	88.86 ^{a}	2.08 ^{b}	1.96 ^{c}	3.66 ^{d}

Figure 4. Shoot buds regeneration on medium MS + coconut water (15 %) + Activated charcoal (1 g/L)

Root formation

Auxin is commonly used in *in vitro* root formation studies, NAA is suitable for root induction and stimulates the formation of roots in nurseries, IBA is suitable for rooting on dendrobium orchids, IAA is used extensively in research on roots of Phalaenopsis, Rhynchostylis, Cymbidium, Vandal orchid. IAA is used as a supplement to the mokara root culture medium. MIAA concentrations (0.5 mg/L) were added to the MS medium for effective root formation (4.66 roots/shoot), leaf with (0.75 cm) and shoot height (3.57 cm). The results were significantly different from those of control of leaf growth, limiting the growth of leaf width (0.52 cm) and leaf length (3.04 cm) (Table 5) (Figure 5). The plantlets grows and develops normally in the nursery.

Table 5. Effect of IAA on rooting of Mokara orchid

IAA (mg/L)	Survival rate (%)	Leaf length (cm)	Leaf width (cm)	Number of roots/shoot
0.00	100.00	3.04 [b]	0.52 [b]	3.00[a,b]
0.10	100.00	2.99[b,c]	0.44[b,c]	3.00[a,b]
0.25	88.86	2.76 [c]	0.38 [c]	2.33 [b]
0.50	94.43	3.57 [a]	0.75 [a]	4.66 [a]
0.75	100.00	3.22 [b]	0.49[b,c]	2.66[a,b]
1.00	88.86	3.03 [b]	0.04[b,c]	1.66 [b]

Figure 5. Root creation on medium MS + IAA (0.5 mg/L) + coconut water (10 %) + activated charcoal (1 g/L)

Effect of genotype

The mokara orchids grown on this large scale now including Fullmoon, Newmoon, Banana, Chistie, Fuchs Delight, Charkuan Pink, Charkuan Orange, Chaopraya Sunset, Chaopraya Gold, Chaopraya Beauty, and Renanthera as Ren Red, Kalsum x Ascocenda, Kalsum x Lena Devol, Lena Devol x Dr.Anek.

Table 6. Effect of genotypes on flower-stalk culture and rapid multiplication in vitro

Cultivars	Number of Shoots per second internode	Number of Shoots per cluster
Mokara Fullmoon,	2.54	101.24
Mokara Newmoon	2.12	98.68
Mokara Banana	2.88	96.42
MokaraChistie	2.24	96.86
Mokara Fuchs Delight	2.02	88.16
Mokara Charkuan Pink	2.44	84.12
Mokara Charkuan Orange	2.62	82.66
Mokara Chaopraya Sunset	2.18	80.46
Mokara Chaopraya Gold	2.26	80.12
Chaopraya Beauty	2.82	76.44
Renanthera Red	2.16	42.64
Kalsum x Ascocenda	2.32	40.14
Kalsum x LenaRewol	2.24	38.42
Lena Rewol x Dr.Anek.	2,08	44,18
CV (%)	14,56	28,42

The mokara (and Renanthera) orchid are rapidly multiplied by the flower-stalk culture technique on MS + BA (1 mg/L) + coconut water (10%) + PVP (50 mg/L) and multiply on MS + BA (0.75 mg/L) + coconut water (10%) (Table 6) (Figure 6). The number of shoots regenerated through flower-stalk did not differ between genotypes. Mokara genotypes that produced bud growth were significantly higher than those of the Renanthera genotypes.

Figure 6. Two-month-old tissue culture tropical Mokara Fullmoon

Conclusion

The second internode of flower-stalk (non-flowering) is used as a suitable material for cultivating regeneration of shoots on MS + BA (1 mg/L) + coconut water (10%) + PVP (50 mg/L) generating 2.68 buds/cluster. Shoot regenerations were separated by individual leaves cultured on bud regeneration MS + BA (1.5 mg/L) + coconut water (10%) and 66.67 buds/cluster. Shoots were separated into 2-3 shoots/cluster, cultured on shoot multiplication MS + BA shoot (0.75 mg/L) + coconut water (10%) gave rise to shoots 103.33 shoots/bud. Shoots were regenerated on MS + coconut water (15%) to form 3 buds/cluster and rooted on MS + IAA (0.5 mg/L) + coconut water (10%) + charcoal active (1 g/L). Mokara genotypes that produced bud regeneration were significantly higher than those of the Renanthera genotypes.

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