Kasetsart J. (Nat. Sci.) 35 : 368 - 377 (2001)

Effects of Culture Media and Growing Media on Kluai Bep

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ABSTRACT

In vitro, culture of Kluai Bep was conducted in MS media supplemented with BA at 4 levels of concentration of 1,3,5 and 7 ppm. Subcultures were prepared every month. At high concentration of BA, more proliferation and shoot were obtained. Average proliferation time of BA cultured, on media of 1,3,5 and 7 ppm, were found to be 45, 41, 36 and 35 days respectively. It was also noticed that the numbers of shoot, increasing at the seventh month in 7 and 5 ppm BA, were 5.71 and 5.57, while those of 3 and 1 ppm BA 4.29 and 3.86 shoots/plant respectively. Shoots of 5 and 7 ppm BA were not significantly different from those of 1 and 3 ppm BA. All the plantlets were transferred to $^1/_2$ MS without hormone for rooting. The survival rate of K. Bep after transplanting was 81.71 per cent. The horticultural characteristics such as height, size of plant, size of pseudostem and leaf were recorded after growing in 4 and 6 inches pots with different growing media. K. Bep growing in 4 inches pots with 2 parts soil mix : 2 parts of sand and 1 part of coir dust showed the best development on the ninth month according to the above character . Morphology of the 7th generation of mature K. Bep were solid mutant of K.Khai.

Key words: Kluai Bep, culture media, growing media

INTRODUCTION

In vitro propagation of banana is the most useful procedure in rapid multiplication. Success *in vitro* culture depends on the choice of nutrient medium including its chemical composition and physical form. MS medium (Murashige and Skoog, 1962) is the most widely used for *in vitro* culture of banana and plantain. However, most scientists use slightly modified MS medium supplemented with alteration to carbon or vitamin, amino acid and growth regulator supplement. Plant growth regulators are essential for growth and development of explants *in vitro*. The most commonly used auxin are IAA (indole-3-acetic acid), NAA (α-napthalene acetic acid) and IBA (indole-3 butylic acid). BA or BAP (6-benzylamino purine) is the cytokinin, inducing shoot and bud proliferation *in vitro*. 2 mg/l BA and 30 mg/l of adenine sulphate supplemented to MS medium could produce 2-3 shoots in 3-4 weeks of Basrai banana (Rao *et al*., 2000) and 0.17 μM-10μM BAP were found in good proliferation of various genome groups of Indian banana (Patil and Singh, 2000). BA at 5 ppm. was suitable for supplementing to MS medium for culturing of banana (Vuylsteke, 1989). Similarity works in Thailand; 5 ppm BA were found to be good in proliferation of Kluai Khai (Kriengyakul, 2001), Carvendish banana (Attachat, 1992), and Red banana (Tinsirisuk, 1994).

The plantlets have been grown in a high humidity and low light intensity. They may lose

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water rapidly upon transfer to natural conditions. Moreover, *in vitro* produced plants are believed to have a limited photoautotrophic capacity, so their energy demand must initially be met by reserves of starch accumulated during culture (Vuylsteke, 1989). Plantlets are transplanted into soil mix. Soil mix must be kept moist. Control of soil content is a critical factor in successful plantlets establishment. Maintenance of a high humidity is very important. Besides of this, soil mix must contain macro and micro nutrients with good aeration (Suriyapananon, 1990). The material that can be used as growing media are peat moss, saw dust, corn cob mill, filter cake, peanut hull (Suriyapananon, 1990; Nelson, 1991), coir dust, rice hull and black rice hull (Lhakchaiyakul, 1997).

Kluai Bep (K.Bep) is a mutant of Kluai Khai which has been registered at the Ministry of Agriculture since the year 2000 (Silayoi, 2000, Maka, 2001, Trakullertsathien, 2001). To prove that K.Bep is a solid mutant, tissue culture of K.Bep has been made and morphology of MV_7 was observed. Since K.Bep is a new cultivar, the culture media and growing media should be learned, thus this study.

MATERIALS AND METHODS

Removed leaf sheath of K.Bep , sterilized 2 times in 10% chlorox solution and then rinsed in sterile distilled water for 3 times in bio-clean cabinet. Then, longitudinally by cut the explants into 4 pieces, every piece with apical meristem.

Experiment 1. Culture Media

The explants were cultured in media of Murashige and Skooge (1962) with 30 gm. agar, pH5.8, and 1,3,5 and 7 ppm BA. CRD was designed with 4 treatments and 10 replications. Subcultures were made every month until MV_7. All cultures were maintained at 25-28°C, 80-85% RH and 1500-2000 Lux of light.

Proliferation and shoot multiplication were observed.

Experiment 2. Growing Media

Plantlets were transferred to 1/2MS without hormone for rooting before transferred to soil. They were grown in planting material, which consisted of mixture of mixed soil and composted manure (1:1) for one month. Survival data were recorded. Then they were transferred into 4 inches pots with 5 different growing media which were, mixed soil[2/] : sand: composed manure (1:1:1) or T_1, mixed soil: sand, coir dust (2:2:1) or T_2, peat moss: sand (1:1) or T_3, mixed soil or T_4, peat moss or T_5. CRD was designed with 5 different growing media, 8 replications (5 pots per one replication). Four grams of 15-15-15 fertilizer per pot were to be supplied before growing and 5 grams of 15-15-15 fertilizer per litre of water were applied every 2 weeks. Growth such as height, number of leaf and size of psuedostem were recorded for 9 months.

Experiment 3. Morphology

Morphology of mature plants were studied.

RESULTS AND DISCUSSIONS

Experiment 1. Culture Media

Proliferation: The culture media of MS with 7 ppm BA was found to have the shortest time in proliferation of 35 days while culture of 5,3 and 1 ppm BA had 36, 41 and 45 days, respectively (Table 1). After cultured in various media for 6 weeks, it was revealed that the MS with 7 ppm produced the largest amount of shoot. This was not different to 0.45 shoot on MS with 5 ppm. Shoot production in MS with 3 and 1 ppm were 0.15 and 0, respectively (Table 2). The earlier proliferation produced less shoots the same as Kluai Hom Thong reported by Atthachat (1992). After cultured over 4

2/ mixed soil = 1 burn soil: 2 composed manure: $^1/_4$ sand

weeks, the explants produced phenolic compound which blocked the media. This was similar to the work of Ratanopas (1986) on culturing of Kluai Khai Pra Ta Bong.

After subculturing the explants every month for 7 months (MV_7). BA at 7 ppm was noticed to give more shoot multiplication in every advanced month. The result was different from those at 3 and 1 ppm, but not at 5 ppm and 7 ppm on the 5-7thmonths. The 3 ppm BA was different from 1 ppm on the 3rd and 4th months (Table 3). That implied the high concentration of BA to give more shoots in regeneration, since cell division depended on higher doses of BA, which caused higher development. However, the development of the plant was not so high as cell division. Therefore the shoots produced from 7 ppm BA medium were not so healthy as those at the lower doses as 5 ppm BA. This was similar to K.Khai Pra Ta Bong, K. Hom Thong, and K. Tani reported by Ratanopas (1986), Atthachat (1992) and Tinsirisuk (1994) respectively. Culture media without BA would not produce shoot but the root. Therefore it could be concluded that the proper culture media for Bep was found in MS with 5 ppm BA, because number of shoot regenerated was not significantly different to MS with 7 ppm BA.

Table 1 Proliferation of K. Bep in various modified MS media.

Treatment	Proliferation time (days)
MS + 1 ppm BA	45
MS + 3 ppm BA	41
MS + 5 ppm BA	36
MS + 7 ppm BA	35

Table 2 Numbers of shoot of K. Bep from various modified MS media after 6 weeks of culturing.

Treatment	Number of shoot
MS + 1 ppm BA	0.00^{b} 1/
MS + 3 ppm BA	0.15^{b}
MS + 5 ppm BA	0.45^{a}
MS + 7 ppm BA	0.50^{a}
F-test	**
CV.(%)	147.47

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 3 Numbers of shoot produced in MS media with various concentrations of BA monthly measured for 7 months.

Treatment	Month						
	1st	2nd	3rd	4th	5th	6th	7th
MS + 1 ppm BA	0.86^{b} 1/	1.43^{b}	2.14^{c}	2.57^{c}	3.14^{b}	3.71^{b}	3.86^{b}
MS + 3 ppm BA	1.00^{b}	1.86^{ab}	2.71^{b}	3.14^{b}	3.57^{b}	4.00^{b}	4.29^{b}
MS + 5 ppm BA	1.14^{b}	2.14^{a}	3.00^{ab}	3.85^{a}	4.71^{a}	5.43^{a}	5.57^{a}
MS + 7 ppm BA	1.57^{a}	2.29^{a}	3.14^{a}	4.00^{a}	4.86^{a}	5.57^{a}	5.71^{a}
F-test	**	**	**	**	**	**	**
C.V.(%)	33.07	23.33	13.16	11.14	11.05	9.62	9.79

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

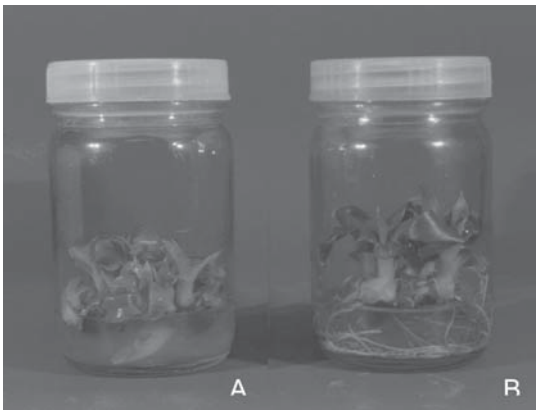

Figure 1 Kluai Bep in tissue culture
A. MS+5ppm BA medium
B. $^1/_2$ MS medium

Experiment 2. Growing Media

The plantlets were rooted on $^1/_2$ MS without hormone for one month, which were later grown on sand and composed manure (1:1). 81.71% survival plants were recorded after one month that was found to be lower than normal K.Khai reported by Saradhuldhat (1997), Suksom (2000) and Kriengyakul (2001). Leaf and petiole of K.Khai were thinner and longer than those of K. Bep. Leaf blades of K. Bep were quite big with short petiole and bending to ground. As the result, they became rotten after growing in nursery, making lower survival percentage.

The growth was recorded after growing in 4 inches pots in different growing media as shown in Table 4.

The height on the first month was found not to be different in every treatment. On the second month, seedling of K. Bep in peat moss media (T_5) was at the tallest of 1.44 cm.and different from the other treatments. Peat moss contained of 0.6-4% N, light medium, hygroscopic, good aeration and was very good in absorption of water and minor mineral nutrients according to Kasemsarp (1978), Suriyapananon (1979) and Sinsamut (1999). The mixed soil consisting of burnt soil, composted manure and sand (1:2:1/4) was not good because of less amount of sand and the tightly packed media making no room for air to go through causing poor drainage. These factors are important in relation to the absorption of water and mineral by the root, resulted in slow growth (Gusonsatit, 1999). On the 9th month, the treatment of mixed soil, sand and coir dust (2:2:1) (T_2) was revealed to give the highest plant that was different to peat moss and sand (3:1) (T_3) and peat moss only (T_5). The reason was that coir dust was organic matter that covered soil particle resulting in good absorbing, and good

Table 4 Height of Bep monthly measured in centimeter after transferred to 4 inches pots in the nursery.

Treatment	Month								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
T_1	0.97	1.04^{b} 1/	1.82^{a}	1.96^{a}	2.44^{a}	2.66^{a}	2.88^{a}	3.35^{a}	3.39^{b}
T_2	0.97	1.02^{b}	1.41^{bc}	1.59^{a}	2.36^{ab}	2.62^{ab}	3.14^{a}	3.63^{a}	3.87^{a}
T_3	0.98	1.11^{b}	1.43^{bc}	1.64^{a}	1.94^{cd}	2.06^{cd}	2.14^{c}	2.75^{b}	2.81^{c}
T_4	0.97	0.99^{b}	1.15^{c}	1.17^{b}	1.72^{d}	1.80^{d}	2.20^{c}	3.26^{a}	3.35^{b}
T_5	0.98	1.44^{a}	1.69^{ab}	1.96^{a}	2.09^{bc}	2.29^{bc}	2.47^{bc}	2.57^{b}	2.60^{c}
F-test	ns	**	**	**	**	**	**	**	**
C.V.(%)	20.14	23.47	29.34	32.77	22.51	22.30	23.31	20.25	15.65

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

aeration for growing media. Unlike peat moss, it could also hold water, make flexible soil and was slow in disintegrating.

On the 9th month, the peat moss was found to be disintegrated becoming compacted thus decreased aeration. However, the treatment of mixed soil: sand: coir dust (T_2) components should be good in forming structure. Peat moss appeared to be good for the first 4 months, after that it would disintegrate. It was also noticed not to last long as coir dust.

The circumference of pseudostem as height of K. Bep the first 4 months on peat moss (T5)was found to be better than the other treatments, which were 0.59, 0.98, 0.98, 1.01 and 1.08 cm. On the 7th, 8th, and 9th months, the circumferences of K. Bep were1.26, 1.29 and 1.36 cm. respectively on the treatment of mixed soil: sand: coir dust (T2), which were different from peat moss and sand growing medium (T3) (Table 5).

It was found that leaf width of K. Bep on peat moss growing media (T_5) were the largest on the first 5 months, which were 1.60, 2.14, 2.65, 2.71 and 2.82 cm. But on the 6th, 7th, 8th and 9th months the biggest leaf was found on the mixture of mixed soil, sand, and coir dust (T_2), the same as height and circumference. Leaf length was also similar to leaf width (Table 6 and 7)

Leaf thickness was measured at the middle of the leaf on the 9th month. It was found to be 0.028 cm in the mixture of mixed soil, sand and coir dust (T_2) and 0.027 cm in mixed soil: sand: composed manure (T_1) growing medium, but was not significantly different from each other while they were highly significant to peat moss and sand (T_3), peat moss (T_5), and mixes soil (T_4) growing media (Table 8).

In peat moss growing medium (T5), the numbers of leaf were 10.92, 15.39, 20.65 on the 2nd, 3rd, and 4th month, respectively which were different from those of the other treatments. On the 8thand 9th months, the numbers of leaf were 36.92 and 42.46 in mixed soil, sand and coir dust medium (T2) which were highly significantly different from those in the mixed soil medium (Table 9).

In the mixed soil medium (T4), leaf production were 14.57, 8.61, 7.08, 13.04 days on the 1st, 2nd, 3rd, and4thmonths respectively, which took longer period than the other treatments. On the 7th, 8th and 9th months, they were 5.63, 7.62, and 6.00 days in mixed soil, sand and coir dust (T2) (Table 10).

Table 5 Circumferences of Bep monthly measured in centimeter after planted in 4 inches pots in the nursery.

Treatment	Month								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
T_1	0.59	0.77^{c} 1/	0.91^{ab}	0.91^{ab}	1.07^{a}	1.17^{a}	1.17^{ab}	1.19^{ab}	1.21^{abc}
T_2	0.56	0.73^{c}	0.88^{ab}	0.87^{b}	1.06^{a}	1.16^{a}	1.26^{a}	1.29^{a}	1.36^{a}
T_3	0.56	0.86^{b}	0.89^{ab}	0.89^{ab}	0.99^{a}	0.99^{bc}	0.99^{b}	1.00^{b}	1.07^{c}
T_4	0.59	0.74^{c}	0.79^{b}	0.81^{b}	0.87^{b}	0.97^{c}	1.12^{ab}	1.18^{ab}	1.24^{ab}
T_5	0.59	0.98^{a}	0.98^{a}	1.01^{a}	1.08^{a}	1.13^{ab}	1.15^{ab}	1.15^{ab}	1.16^{bc}
F-test	ns	**	**	**	**	**	**	**	**
C.V.(%)	16.35	17.58	24.09	21.45	19.69	20.67	20.14	20.69	14.95

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 6 Leaf width of K. Bep monthly measured in centimeter after planted in 4 inches pots in the nursery.

Treatment	Month								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
T_1	1.58	2.16^{a}1/	2.16^{b}	2.48^{ab}	2.73^{a}	2.90^{a}	3.08^{a}	3.08^{ab}	3.30^{b}
T_2	1.57	2.00^{ab}	2.25^{b}	2.18^{bc}	2.66^{ab}	3.04^{a}	3.39^{a}	3.57^{a}	3.92^{a}
T_3	1.57	1.87^{b}	2.25^{b}	2.28^{bc}	2.28^{bc}	2.29^{b}	2.37^{b}	2.32^{c}	2.76^{c}
T_4	1.59	1.90^{ab}	1.92^{b}	1.95^{c}	1.96^{c}	2.02^{b}	2.53^{b}	2.95^{b}	3.43^{b}
T_5	1.60	2.14^{a}	2.65^{a}	2.71^{a}	2.82^{a}	2.94^{a}	3.00^{a}	3.03^{ab}	3.09^{bc}
F-test	ns	**	**	**	**	**	**	**	**
C.V.(%)	26.18	19.65	22.32	22.84	25.83	23.73	22.16	23.63	16.02

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 7 Leaf length of Bep monthly measured in centimeter after planted in 4 inches pots in the nursery.

Treatment	Month								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
T_1	2.95	3.85^{a}1/	3.88^{b}	4.28^{ab}	4.69^{a}	4.70^{a}	4.91^{a}	4.99^{ab}	5.41^{b}
T_2	2.93	3.53^{ab}	3.58^{b}	3.92^{bc}	4.54^{ab}	4.86^{a}	5.31^{a}	5.68^{a}	6.22^{a}
T_3	2.99	3.39^{b}	3.93^{b}	3.94^{bc}	3.99^{bc}	3.99^{b}	3.99^{b}	4.00^{c}	4.53^{c}
T_4	2.99	3.48^{ab}	3.53^{b}	3.58^{c}	3.76^{c}	3.76^{b}	4.23^{b}	4.76^{bc}	5.54^{b}
T_5	2.98	3.49^{ab}	4.55^{a}	4.56^{a}	4.79^{a}	4.99^{a}	5.08^{a}	5.09^{ab}	5.17^{b}
F-test	ns	**	**	**	**	**	**	**	**
C.V.(%)	21.87	17.42	21.39	19.86	21.52	19.67	19.36	21.31	14.01

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 8 Leaf thickness of Bep monthly measured after transferred to 4 inches pots in the nursery.

Treatment	Thickness (cm)
T_1	0.027^{a}1/
T_2	0.028^{a}
T_3	0.023^{b}
T_4	0.024^{b}
T_5	0.023^{b}
F-test	**
C.V.(%)	11.25

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 9 Numbers of leaf produced of K. Bep monthly measured after planted in 4 inches pots in the nursery.

Treatment	Month								
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
T_1	5.72		12.38^{bc}	17.77^{bc}	22.08^{b}	27.05^{a}	32.34^{a}	36.84^{a}	41.62^{ab}
T_2	5.62	7.95^{c}	12.00^{c}	17.24^{c}	21.51^{b}	27.05^{a}	32.57^{a}	36.92^{a}	42.46^{a}
T_3	5.72	9.55^{b}	13.27^{b}	18.78^{b}	22.86^{b}	27.82^{a}	32.52^{a}	36.50^{a}	41.19^{ab}
T_4	5.82	8.17^{c}	12.36^{bc}	16.50^{c}	19.12^{c}	23.78^{b}	29.00^{b}	33.50^{b}	37.95^{c}
T_5	5.77	10.92^{a}	15.39^{a}	20.65^{a}	24.51^{a}	28.83^{a}	32.85^{a}	35.79^{ab}	39.00^{bc}
F-test	ns	**	**	**	**	**	**	**	**
C.V.(%)	16.40	14.93	13.19	11.28	10.93	10.89	10.53	9.70	8.39

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

Table 10 The duration of leaf produced (days per leaf) of K. Bep monthly measured after planted in 4 inches pots in nursery.

Treatment	Month							
	1st	2nd	3rd	4th	5th	6th	7th	8th
T_1	13.07^{a}1/	7.60^{ab}	5.79^{b}	7.26^{b}	5.90^{b}	5.83^{b}	6.72^{b}	6.62^{bc}
T_2	13.53^{a}	8.05^{ab}	6.14^{b}	7.16^{b}	5.67^{b}	5.63^{b}	7.62^{ab}	6.00^{c}
T_3	7.92^{b}	7.64^{ab}	5.50^{b}	7.87^{b}	6.71^{b}	8.00^{ab}	8.47^{ab}	7.00^{bc}
T_4	14.57^{a}	8.61^{a}	7.08^{a}	13.04^{a}	7.73^{ab}	6.26^{b}	7.90^{ab}	7.37^{ab}
T_5	6.16^{b}	7.21^{b}	5.82^{b}	8.76^{b}	9.46^{a}	8.99^{a}	9.71^{a}	8.30^{a}
F-test	**	**	**	**	**	**	**	**
C.V.(%)	41.13	22.65	20.27	41.20	50.84	48.75	40.90	20.26

1/ = Means on the same row with the same letter showed no significant differences at 99% level by DMRT

It was also reported that on the 9th month, the K. Bep produced 37.95-42.46 leaves (Table 9). Normal K. Khai would flower after producing 39-41 leaves (Keawsompong, 1993). Kriengyakul (2001) found irradiated K.Khai flowered at 42.34 leaves. Therefore the K.Bep should have no flower at 9 months old as similar to Silayoi (2000) who reported no flower even at 4 years old. This character might result from the ploidy of plant.

At the end of the experiment or at 9 months old, the number of plants died in different media were collected as shown:

Peat moss (T_5)	30 plants
Mixed soil (T_4)	10 plants
Peat moss and sand (T_3)	21 plants
Mixed soil, sand and coir dust (T_2)	4 plants
Mixed soil, sand and composed manure (T_1)	2 plants

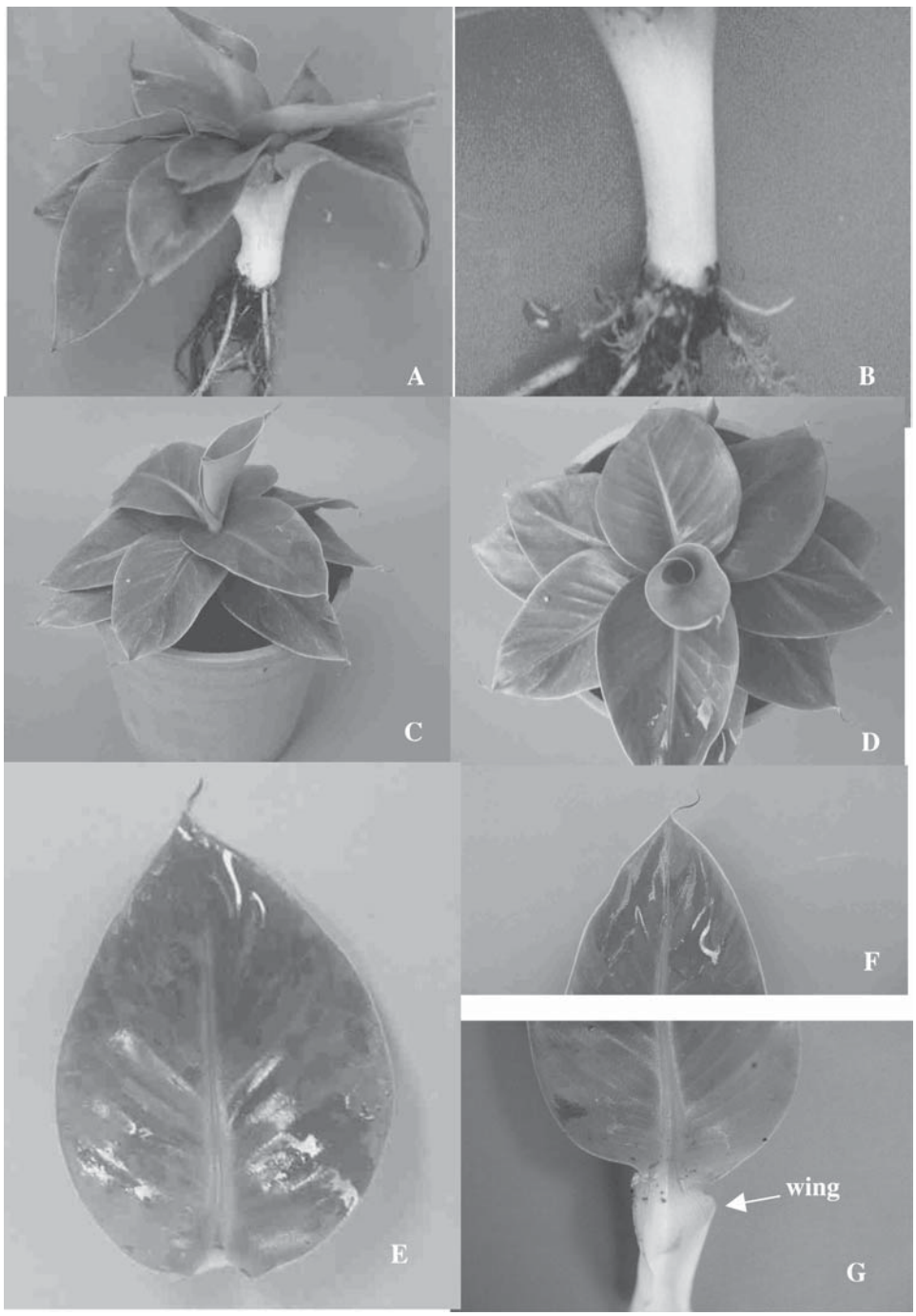

Figure 2 Morphology of Kluai Bep
A. psuedostem and roots B. Colour of mature psuedostem C. K. Bep in pot
D. rolled immature leaf E. Leaf blade (ovate) F. Leaf Apex (acuminate)
G. Leaf base (obtuse) with wing

For the first three months, peat moss (T_5) was found to be the best growing medium since during such time the temperature was at the optimum of 33-35°C, 73-85% RH. At high temperature, the rate of water absorption is also high. The efficient intake of water depends on good aeration. Peat moss is a light medium that can absorb and hold water, as well as composed minor element and 0.6-1.4%N. The plants can grow well in these kinds of medium (Sinsamut, 1999; Kasemsarp, 1978; Suriyapananon, 1979). For mixed soil, the construction of soil was compact, poor aeration, which is not good for growing plants. On the 4th month, peat moss disintegrated, growing media compact with no air going through. The growth of K. Bep appeared to slow down after 3 months in peat moss and 30 plants found dead in the last month because of poor aeration, and no nutrient in growing media. This was different from the mixture of mixed soil, sand and coir dust medium (T_2) where the nutrient could be released to plant. Coir dust and sand in this growing medium were noticed to make good structure and ventilation. Burnt soil, as the component of this growing media, was a small particle that could absorb and hold water and mineral (Khamlert, 1986; Wanaputi, 1994). This growing media was the best in the last three months. The height, circumference, width, length, thickness of leaf were better than those of the other treatments. It could be that coir dust did not disintegrated and could hold water and mineral for plants (Nelson, 1991; Kuankumchom, 1999).

The results indicated the proper growing media for the first three months of K.Bep to be peat moss. After that the plants should be transferred to mixed soil, sand and coirdust (2:2:1) (T_2). Mixed soil, sand and compased manure (T_1) were found to be another choice of growing media of K.Bep because the growth was acceptable and only 2 plants were died after 9 months of the experiment.

Experiment 3. Morphology:

Morphology of the 7th generation of mature K. Bep were observed as follows: stem rhizome, psuedostem purplish red, young plant white, thick leaves, ovate leaf, leaf apex acuminate, leaf margin entire, leaf base obtuse with wing, short petiole, leaf arrangement spiral (clockwise) with unequal lamina, pinnately parallel venation, rolled immature leaf, leaf colour dark green (Fig.2).

Morphology of plant was found not to be different from the mutant one as reported by Silayoi (2000). K. Bep was therefore proved to be a solid mutant of K. Khai with no flower and good as ornamental plant.

Summary

1. The most suitable culture media for K. Bep is MS with 5 ppmBA

2. The most suitable growing media for K. Bep is mixed soil, sand and coirdust at the rate of 2:2:1

3. Morphology of the 7th generation of K. Bep appears to be the same as the plant, reported by Silayoi (2000), therefore it is a positively solid mutant of K. Khai.

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Received date : 12/10/01
Accepted date : 28/12/01