

***In vitro* Root Initiation of ‘Champi Sirindhorn’ (*Magnolia sirindhorniae* Noot. & Chalermglin)**

Somnuk Chaidaroon, Ittirit Ungvichian, and Kalaya Ratanathavornkiti

**Thailand Institute of Scientific and Technological Research
Technopolis, Khlong Luang, Pathum Thani, Thailand**

Abstract

*A study of root initiation of ‘Champi Sirindhorn’ (*Magnolia sirindhorniae* Noot. & Chalermglin), a newly discovered plant in Thailand since 1999, was made by using modified USK II medium supplemented with varying IBA levels from 0 to 14 mg l⁻¹. Root initiation was observed after 17 days of culture, in contrast to MS medium, in which, no root formation was observed even after 60 days. Treatment without IBA gave the lowest yield of 5 - 10% of root initiation while the highest yield was up to 90% at 4 mg l⁻¹ IBA after 24 days of culture and at the end of the experiment, 32 days of culture, the averages of root number and root length were 4.89 roots/explant and 26.15 mm/root, respectively. The maximum averages of 9.19 roots/explant and a total root length of 172.75 mm/explant were obtained from the treatment with 12 mg l⁻¹. The control treatment yielded the longest root of 34.25 mm, the lowest average shoot length/explant, and the lowest increased in shoot growth at the end of the experiment.*

Keywords: ‘Champi Sirindhorn’, *Magnolia sirindhorniae* Noot. & Chalermglin, tissue culture, root initiation, USK II medium, MS medium, IBA, explant.

Introduction

‘Champi Sirindhorn’ (*Magnolia sirindhorniae* Noot. & Chalermglin), a newly discovered plant in Thailand, belonging to the family Magnoliaceae, is endemic to Thailand, and was found growing only in a freshwater bog in Lop Buri Province, in the central part of the Kingdom. It is an evergreen tree, up to 25 m high and 70 cm diameter. Plant hairy at least in youngest parts. Twigs when young are 2-5 mm thick, hairy at least when young, appressed puberulous, glabrescent, brown. Leaves evenly distributed, elliptic, 14-20 x 7-10 cm, base rounded to cuneate, margin not recurved, apex rounded, shortly hairy above when young, at least on midrib and base. Flowers appearing together with or after the leaves, greenish white, bisexual; petals 12-15, sub-similar. Fruits consist of free carpels, which dehisce along the dorsal suture, short cylindrical, 4-6 by 2.5-3.5 cm. Seeds 4-6 mm long (Chalermglin *et al.*, 2002; Nooteboom and Chalermglin 2000).

In an earlier review, the plants in Magnoliaceae had difficulty in root formation (Henselova 2002). Following the report of Kamanicks and Takats (1997), inclusion of 4 mg l⁻¹ IAA on media containing 3 mg l⁻¹ of activate charcoal was the best treatment for root initiation of *M. x soulangiana*. Furthermore, Kamenicka (1996) found that the highest performance of root initiation of *M. x soulangiana* was at 4 mg l⁻¹ IBA, while the longest rooting occurred at 1 mg l⁻¹ IBA. A preliminary study by the Thailand Institute of Scientific and Technological Research (TISTR) revealed that *M. sirindhorniae* established on MS medium without growth regulator for at least 60 days without sub-culturing hardly formed any roots. On the other hand, added with high amounts of IBA of 40 mg l⁻¹, some root initiation could take place.

Consequently, the present experiment was conducted to investigate the components of modified media to encourage rapid and high efficiency in root initiation of ‘Champi Sirindhorn’.

Materials and Methods

The lateral buds of a two-year old 'Champi Sirindhorn' plant grown at TISTR (Bangkok) were used as the explants of this experiment. After the multiplication program and maintained in shoot-inducing medium for proliferation, the explants were individually cut at about 1.5-2.0 cm below the shoot tip. They normally consisted of 2-3 axillary buds. They were established on USK II medium containing 30 g l⁻¹ of sucrose and added with IBA of various concentrations: 0, 2, 4, 6, 8, 10, 12, and 14 mg l⁻¹. Before sterilization, the pH of the media was adjusted to 6.00 and solidified with 6 g l⁻¹ agar. Twenty replications were employed for each treatment. Each sample was cultured in 4-oz, wide-mouthed vessel, containing 30 ml of media.

Table 1. The components of USK II medium

Components	Concentration (mg l ⁻¹)
(CaNO ₃) ₂ .4H ₂ O	833.77
CoCl ₂ .6H ₂ O	0.025
CuSO ₄ .5H ₂ O	0.025
FeSO ₄ .7H ₂ O	27.8
Glycine	2
H ₃ BO ₃	6.2
KH ₂ PO ₄	16.9
KI	0.83
KNO ₃	1800
MgSO ₄ .7H ₂ O	175.79
Myo-inositol	100
Na ₂ -EDTA	37.3
NaMoO ₄ .2H ₂ O	0.25
NH ₄ NO ₃	400
Nicotinic acid	0.5
Pyridoxin-HCl	0.5
Thiamine-HCl	0.1
ZnSO ₄ .7H ₂ O	8.6

The cultures were maintained in the culture room at TISTR, Bangkok at 25±2°C under fluorescent lighting with a light regime of 16 hrs of light and 8 hrs of darkness.

Data on root initiation and root growth were recorded periodically at 17, 19, 21, 24, 26, 28, and 32 days. These included the percentage of root initiation (recorded as the number of samples in which rooting was observed), and, towards the end of experiment (32 days of incubation), the number and length of roots/explant, and the average length/root.

Results

The percentage of root initiation of 'Champi Sirindhorn' at various intervals of incubation on USK II medium supplemented with various concentrations of IBA is shown in Table 2. At 14 days after incubation, no root growth was observed. The control treatment (no IBA added) showed the least root initiation (5-10%) at every interval of incubation all the way to the end of the experiment (32 days). The highest yield was up to 90% at 4 mg l⁻¹ IBA after 24 days of culture.

The root growth of 'Champi Sirindhorn' grown on USK II medium supplemented with various concentrations of IBA at the end of experiment (32 days) is shown in Table 3. The data of root growth included average root number/explant, average total root length/explant, and average root length/root. The maximum average of 9.19 roots/explant and maximum average total root length/explant of 172.75 mm were obtained from the treatment with 12 mg l⁻¹. The control treatment yielded the longest root of 34.25 mm on the average.

The effect of different concentrations of IBA supplementing USK II medium on shoot growth of 'Champi Sirindhorn' is shown in Table 4. The control treatment yielded the lowest average shoot length/explant of 19.80 and 24.21 mm at 18 and 32 days of incubation, respectively, and the lowest increase in shoot growth of 4.41 mm at the end of the experiment.

Conclusion and Discussion

From the results obtained, it can be concluded that:

1. It took only 17 days for root initiation of 'Champi Sirindhorn' when USK II medium supplemented with IBA of various concentrations was employed (Table 2). This is in contrast to MS medium, in which, from preliminary investigation by TISTR, without growth regulator, no root formation was observed even after 60 days, while with the addition of IBA of 40 mg l⁻¹, some root initiation could take place. Thus, USK II is preferred for root initiation of 'Champi Sirindhorn'. The best concentration of supplemented IBA for root initiation was 4 mg l⁻¹, followed by 2 mg l⁻¹. They both exhibited highest percentage of root initiation as early as

17 days of incubation, all the way to the end of the experiment, 32 days of incubation. Of particular interest was the treatment with 4 mg l⁻¹, which exhibited 90% of root initiation after 24 days of incubation all the way to the end of the experiment (Table 2).

2. The addition of IBA of various concentrations to the USK II medium yielded proportionally higher root number/explant and total root length/explant, and obviously much higher than those of the control treatment (without IBA) at the end of the experiment (Table 3). The maximum concentration was 12 mg l⁻¹, which yielded 9.19 roots/explant and the total root length of 172.75 mm/explant.

Table 2. Percentage of root initiation of 'Champi Sirindhorn' at various intervals of incubation on USK II medium supplemented with various concentrations of IBA

IBA (mg l ⁻¹)	Percentage of root initiation at various intervals (days) of incubation							
	14	17	19	21	24	26	28	32
0	0	5.26	5.26	5.26	10.53	10.53	10.53	10.53
2	0	20.00	60.00	65.00	75.00	75.00	75.00	80.00
4	0	25.00	50.00	75.00	90.00	90.00	90.00	90.00
6	0	10.53	42.11	68.42	78.95	78.95	78.95	78.95
8	0	15.00	50.00	60.00	70.00	70.00	70.00	77.78
10	0	5.26	42.11	63.16	68.42	68.42	68.42	73.68
12	0	10.00	40.00	55.00	75.00	75.00	80.00	80.00
14	0	10.00	35.00	40.00	55.00	55.00	55.00	65.00

Table 3. Root growth of 'Champi Sirindhorn' grown on USK II medium supplemented with various concentrations of IBA at the end of experiment (32 days)

IBA (mg l ⁻¹)	Aver. root number/explant	Aver. total root length/explant (mm)	Aver. root length/root (mm)
0	1.50	47.00	34.25
2	4.44	105.00	22.49
4	4.89	127.67	26.15
6	6.20	122.13	20.92
8	7.14	152.21	21.87
10	6.14	126.71	20.60
12	9.19	172.75	19.91
14	6.31	112.92	16.91

Table 4. Effect of different concentrations of IBA supplementing USK II medium on shoot growth of 'Champi Sirindhorn'

IBA (mg l ⁻¹)	Aver. shoot height/ explant (mm)		Aver. increase in shoot growth at the end of experiment (mm)
	After 18 days of culture	After 32 days of culture	
0	19.80	24.21	4.41
2	20.55	27.55	7.00
4	20.00	28.30	8.30
6	20.15	24.89	4.74
8	20.10	28.17	8.07
10	19.85	27.42	7.57
12	22.25	30.00	7.75
14	20.80	26.05	5.25

3. On the other hand, the root length/root was shorter with increasing concentrations of IBA (Table 3). The control treatment gave the longest root.

4. Although not being the main objective of the present experiment, shoot growth has

been observed, as shown in Table 4. There was not much difference in the average shoot height/explant after 18 and 32 days of incubation, as well as the average increase in shoot growth at the end of the experiment.

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