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臺灣白及〔*Bletilla formosana* (Hayata) Schlechter〕之組織培養 (二) 培養基組成分對未成熟種子萌芽與小苗發育之影響

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摘要： 臺灣白及 [*Bletilla formosana* (Hayata) Schlechter] 發育至類似魚雷前期之未成熟種子，培養於全量或半量MS基本鹽類添加1~3 mg/l BA (benzyladenine)、0.2 mg/l NAA (naphthaleneacetic acid)、2~4 g/l peptone、3%蔗糖及0.8% Difco agar之培養基中，在2,000 lux光強度、每天16小時光照及25±1°C之恆溫下培養，可獲得近100%之萌芽率。MS基本鹽類及蔗糖是臺灣白及芽球發育成小苗之必要營養成分，而光照、peptone及NAA則可促進小苗之發育。

關鍵語： 台灣白及、組織培養、無菌播種。

Tissue culture of *Bletilla formosana* (Hayata) Schlechter II. The influence of medium components on immature seed germination and seedling development

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Abstract. Nearly 100% germination rate was obtained, when seeds in early-torpedo-like shape stage of *Bletilla formosana* (Hayata) Schlechter were cultured on a medium containing full or half-strength MS basic salts supplemented with 1~3 mg/l BA (benzyladenine), 0.2 mg/l NAA (naphthaleneacetic acid), 2~4 g/l peptone, 3% sucrose and 0.8% Difco agar. The cultures were maintained at 25±1°C with a photoperiod regime of 16-h light (2,000 lux fluorecent light) and 8-h dark cycle. Light, peptone and NAA promoted seedling growth.

Key words: *Bletilla formosana*, Tissue culture, Aspetic seed germination.

前　　言

白及著錄於神農本草經，列爲草部下品，以後歷代諸家本草皆有收載與增著(Lee 1593, Tang 1249)，並延用迄今之重要藥材之一；本經云白及主癰腫惡瘡、敗疽傷陰死肌、胃中邪氣、賊風鬼擊、痱緩不收。實驗藥理學研究更顯示，白及具有止血、抗炎與抗肝腫瘤之作用(Na et al. 1978)，主治肺炎肺結核及胃、十二指腸潰瘍等疾病。

臺灣白及(*Bletilla formosana* (Hayata) Schlechter)係臺灣固有蘭科白及屬(*Bletilla*)之多年生草本植物，與白及(*Bletilla striata*)爲同屬植物，亦可採其塊莖乾燥後，視爲白及藥材(*Bletillae Radix*)使用。因產量不豐，復因屢被採摘爲園藝觀賞用，野生今已不多見，故罕被入藥用。白及傳統上皆賴塊莖分割法繁殖，速度極爲緩慢，致產量不足，無法大量生產以供藥用，故白及藥材自來皆仰賴進口以供市場所需。

本研究前報(Lin et al. 1994)報導臺灣白及各種成熟度之果莢，以發育至類似魚雷前期之種子萌芽率最佳，在2,000 lux光照下，3%雙氧水預措處理種子5分鐘，可使種子提早萌芽並顯著提高萌芽率，但小苗發育速度仍嫌緩慢，此可能與培養基之養分供給有關。本研究之目的爲探討培養基組成，對臺灣白及種子萌芽能力與小苗發育速度之影響，祈能建立臺灣白及種子無菌播種與小苗發育之最佳培養基。

材料與方法

一、果莢之消毒與接種

無菌播種所選用之材料爲臺灣白及經人工自花授粉後，剪取一側已變褐色其胚發育已至類似魚雷前期之果莢(Lin et al. 1994)，將果莢以清水洗淨，先以70%酒精消毒3分鐘，再用0.5%次氯酸鈉溶液，配合手振盪消毒10分鐘以加強效果後，移至無菌接種箱中，以無菌水清洗三次，再持刀片剖開果莢，挑取種子實施無菌播種。

二、培養基成分與培養環境

無菌播種之培養基以MS (Murashige and Skoog 1962)基本鹽類，添加不同濃度之sucrose、BA (benzyladenine)、NAA (naphthaleneacetic acid)、coconut milk、peptone、adenine sulfate、活性碳及0.8% Difco agar；培養基於加入agar前，先用1 N NaOH或HCl將pH值調至5.7±0.1，再以121 °C、15 lb/in^2 (1.05 kg/cm^2)之高溫高壓滅菌15分鐘。每支試管(25×120

mm)內含有10 ml培養基，擺成斜面放冷備用；用接種環將種子以散佈方式平均播種。每試管內約有100~150個種子，每一處理均有10根試管以上之重複數；將接種後之材料置於25±1 °C之恆溫，每日光照16小時（光強度2,000 lux）下培養。爲方便調查多種處理對種子萌芽之效果，將白及種子之早期發育時期區分爲如表一所示的七個時期；各處理間之比較，係以Duncan's拔多變域測定法(Multiple Range Test)測定其差異性。

Table 1. Developmental stages during seed germination and seedling growth of *B. formosana*

Code	Developmental stages
0	Seed germination
	a. Embryo swelling inside the seed coat and turning green.
	b. Papilla formed and extruded out of seed coat.
1	Embryo burst out of the seed coat and formed a protocorm with a pointed vegetative apex.
2	Protocorm with 1st leaf initiation and stomata formed under apex. Apex length is between 0.2~0.5 mm.
3	Protocorm with 1st leaf. Apex length is between 0.5~2.0 mm.
4	Protocorm with 1st leaf. Apex length is more than 2.0 mm.
5	Protocorm with 2nd leaf formed. Leaf length is smaller than 0.5 mm.
6	Protocorm with 2nd leaf formed. Leaf length is larger than 0.5 mm.

結果與討論

一、光照強度、MS基本鹽類與蔗糖濃度對種子萌芽率與小苗發育之影響

表二結果顯示，在充足營養供應下，暗培養40天之種子，僅有54.8%之萌芽率，小苗發育不健康，葉片細長而不開展，尖端有黃化現象。若每日供給2,000 lux，16小時之照光，則可獲得99.2%萌芽率，且小苗十分健壯，葉片開展而翠綠，顯然光照對臺灣白及種子萌芽

Table 2. Influence of light intensity on seed germination and seedling development of *B. formosana**

Light intensity (lux)	Germination rate (%)	% of seedling at different developmental stages**				
		1	2	3	4	5
Dark	54.8c***	30.6	24.2	0.0	0.0	0.0
100	71.1a	23.7	43.2	4.2	0.0	0.0
2,000	99.2b	0.0	0.0	0.0	70.4	28.8

*Basal medium: MS salts with 1% sucrose,20% coconut milk, 2 g/l peptone, 40 mg/l adenine sulfate, 3 mg/l BA, 0.2 mg/l NAA and 0.8 % agar.

**Seeds in early-torpedo shape stage were pretreated by 3% H_2O_2 for 5 min before inoculation. Culture duration: 40 days. Developmental stages as shown in Table 1.

***Means with the same letter are not signiticantly different at 5% level by Duncan's multiple range test.

與小苗發育極爲重要。雖然小苗亦可藉光照進行光合作用製造養分，以供給本身發育，但表三與表四之結果卻顯示雖有2,000 lux之光照，如缺乏MS基本鹽類或蔗糖，則不僅種子萌芽率低且小苗發育停滯。比較表三與表四結果，可知缺乏蔗糖對發芽率之影響較缺乏MS基本鹽類爲大，顯見組織培養中由植物組織進行光合作用所製造之養分有限。許多學者指出光照影響種子萌芽率與小苗之發育。Anderson(1976)發現增加光照強度可縮短豌豆在組織培養中之休眠時間。Chung et al. (1981)在石斛蘭種子萌芽試驗中指出，24小時連續光照所獲得之萌芽率與小苗發育狀況均比暗處理佳，並因此推論不同的光照可能會改變植物的生化反應與營養吸收，進而改變植物之生長形態。

表三結果顯示在僅含3%蔗糖及0.8% agar之培養基中，白及種子雖有84.1%之萌芽率，但僅短暫發育至原球體(protocorm)時期即死亡（約在播種30天後）。若培養基中添加1/4濃度之MS基本鹽類，則不僅提高萌芽率至96.1%且對小苗早期發育之促進效果極爲顯著。Knudson (1925)證實蘭花種子只須外界供應充足養分，於適當環境下，即使不需共生菌之共

Table 3. Influence of MS salts strength on seed germination and seedling development of *B. formosana* under light condition*

MS salts strength	Germination rate (%)	% of seedling at different developmental stages**					
		0	1	2	3	4	5
0 x	84.1c***	36.4	34.1	13.6	0.0	0.0	0.0
1/4 x	96.1b	8.7	13.6	73.8	0.0	0.0	0.0
1/2 x	98.6a	0.0	3.4	8.2	12.6	70.0	4.6
1 x	98.6a	0.0	0.0	2.6	12.7	80.2	3.2
2 x	98.3a	0.0	0.0	6.8	9.8	78.7	2.9

* Basal medium: 3% sucrose and 0.8% agar. **, *** Same as Table 2

Table 4. Influence of sucrose concentration on seed germination and seedling development of *B. formosana* under light condition*

Sucrose conc. (%)	Germination rate (%)	% of seedling at different developmental stages**				
		1	2	3	4	5
0	56.0c***	0.8	1.4	23.6	30.2	0.0
1	98.0a	0.0	10.2	17.4	70.6	0.0
3	98.2a	0.0	2.6	12.7	80.2	3.2
5	86.4b	3.4	36.5	29.1	11.4	0.0

* Basal medium: MS salts with 8% agar. **, ** Same as Table 2.

生亦可發芽生長。Arditt (1967)認爲剛發芽未長葉之蘭苗，無法完成維生素之生合成，因此對維生素的需求特別殷切。菸鹼酸(nicotinic acid)是唯一被普遍認定爲蘭花種子發芽及小苗生長所需之維生素(Arditti 1967, Flamee 1978, Fonnesbech 1972, Mead and Bulard 1979)。Arditti (1967)和Cooper et al. (1982)也指出蘭花由種子發芽後形成芽球時，尚無能力將tryptophan轉化成niacin，待葉片形成後，方具此能力。故於播種培養基中加入MS基本鹽類（內含nicotinic acid），可促進芽球之發育及小苗生長。又因MS基本鹽類之總鹽類濃度高達93.41 mM，而Ichihashi (1979)認爲總鹽類濃度在60 mM時，較有利B. striata地上部之生長。Lu and Lee (1990)亦採用半量MS基本鹽類之培養基播種鳳蘭種子。Wu and Lee (1991)更報導1/4 MS基本鹽類可降低紅花鶴頂蘭褐化與促進小苗發育；而臺灣白及小苗於半量或全量MS基本鹽類中生長較佳，但2倍量MS基本鹽類之處理於培養末期，可能因鹽類濃度或因滲透壓過高，呈現鹽類濃度過高之害，小苗皺縮變形且抑制小苗生長。

蘭花種子發芽及幼苗早期生長，需依賴外界供給碳水化合物爲能源(Knudson 1924)。Fonnesbech (1972)認爲蔗糖比果糖、葡萄糖及麥芽糖更適宜蕙蘭生長，而濃度以3~4%爲佳。Chung et al. (1983)以*B. striata*種子播種，發現3%蔗糖對小苗高度及形成葉數促進作用最大。本研究表四結果發現不含蔗糖之MS鹽類配方中，播種40天後，已有56.0%之萌芽率，雖小苗發育緩慢，初期種子已可發育至第四期，此與Knudson (1924)認爲蘭花種子如無共生菌幫忙，則需仰賴外界供應蔗糖才能發芽之論點不符，然Nagashima (1982)卻指出*B. striata*經授粉發育之未成熟胚，已略具些微發芽能力，顯示可能因植物品種間差異所致。臺灣白及種子內可能含有微量碳水化合物可供種子萌發至芽球，惟因能量不足，添加1%蔗糖後，萌芽率大幅提高，屬第四期之小苗數大幅提高，但葉片仍發育不佳，展開遲緩；若添加3%蔗糖，則小苗發育快速且健康，小苗發育可達到第五期；超過5%蔗糖，可能因濃度過高致滲透壓太大，原球體發育受阻，且出現黃化及褐化現象，反不利小苗及葉片生長。

二、Coconut milk、peptone及adenine sulfate對種子萌芽率與小苗發育之影響

Coconut milk爲椰子種子之胚乳，養分豐富且複雜，可能提供芽球早期生長所需之物質，咸信爲cytokinins類之物質，且coconut milk也具緩衝pH值作用(Vacin and Went 1949)。Kerbauy and Handro (1981)發現15% coconut milk可使*Cattleya epindrum*之未成熟胚發育成芽球。Kusumoto (1980)報導10~15% coconut milk對促進芽球分化效果甚佳。Juang and Lee (1986)則指出10% coconut milk可促進臺灣一葉蘭早期芽球之發育及小苗鮮重，對小苗後期則影響不大，5%及20%則對小苗後期發育略有抑制現象。Kerbauy (1984)也報導15% coconut milk可促使由*Oncidium varicosum*根所誘導之癒合組織分生成芽球相似體。以上學者研究顯示，coconut milk對蘭花之生長，或促進或抑制，不一而論。本研究顯示，coconut milk添加於MS培養基中，無益於臺灣白及種子之萌芽，且隨著coconut milk濃度由5%上升至40%時，萌芽率呈下降趨勢，且芽球與小苗發育漸緩慢，顯示臺灣白及播種培養基中不適宜添加coconut milk（表五）。

Peptone爲蛋白質添加物。Chung et al. (1983)報導添加4 g/l peptone於播種培養基中，可顯著促進*B. striata*小苗早期發育。Kukulczanka et al. (1987)亦指出peptone與白麥精(pearl barley)配合可使蕙蘭之芽球、芽與根之生長較佳。本研究結果顯示，peptone添加於含20%coconut milk之MS培養基中，可抵消coconut milk對小苗發育之抑制作用，濃度由1 g/l上升至4 g/l時，小苗生長較快速（表六），其中以添加2~4g/l爲適宜濃度。本研究peptone之效果都是在和coconut milk配合下進行，是否在不含coconut milk之培養基，pepton仍具有促進小苗發育之效果，則需進一步試驗證實。

表七結果顯示，添加20 mg/l adenine sulfate於培養基中，小苗發育不均質，並無促進小苗發育之效果，40 mg/l之處理小苗發育雖逐漸快速，但葉片已略有肥厚現象，且濃度提高至80 mg/l時，小苗發育雖然快速，但葉片明顯肥厚寬大，不夠健康，產生類似Debergh et al. (1981)所描述玻璃質化(vitrification)之現象。而造成玻璃質化之原因甚多(Ku and Tsay 1994)，是否因adenine sulfate爲cytokinins之前趨物質，而導致玻璃質化，則需進一步研究

Table 5. Influence of coconut milk concentration on seed germination and seedling development of *B. formosana* under light condition*

Coconut milk (%)	Germination rate (%)	% of seedling at different developmental stages**		
		2	3	4
0	98.0a***	2.6	12.7	83.4
5	98.1a	6.3	39.7	52.1
10	97.4a	7.3	41.7	38.4
20	93.5b	22.4	57.6	3.5
40	87.4C	17.8	69.6	0.0

* Basal medium: MS salts with 3% sucrose and 0.8% agar. **, ** Same as Table 2.

Table 6. Influence of peptone concentration on seed germination and seedling development of *B. formosana* under light condition*

Peptone conc. (g/l)	Germination rate (%)	% of seedling at different developmental stages**			
		2	3	4	5
0	93.5c***	22.4	57.6	3.5	0.0
1	94.8b	0.0	26.4	59.3	9.1
2	98.8a	0.0	24.4	67.7	6.7
4	98.7a	0.0	12.6	71.7	14.3

* Basal medium: MS salts with 3% sucrose, 20% coconut milk and 0.8% agar. **, *** Same as Table 2.

Table 7. Influence of adenine on seed germination and seedling development of *B. formosana* under light condition*

Adenine sulfate conc. (mg/l)	Germination rate (%)	% of seedling at different developmental stages**		
		4	5	6
0	98.8a***	24.4	67.7	6.7
20	96.8b	90.4	3.4	3.0
40	95.4c	82.1	2.3	11.0
80	94.9c	53.2	5.4	36.3

* Basal medium: MS salts with 3% sucrose, 20% coconut milk, 2 g/l peptone and 0.8% agar. **, *** Same as Table 2

Table 8. Influence of cytokinins on seed germination and seedling development of *B. formosana* under light condition*

Basal medium with			Germination rate (%)	% of seedling at different developmental stages**			
BA	Kinetin (mg/l)	2ip		3	4	5	6
0	0	0	95.4d***	0.0	82.1	2.3	11.0
1	0	0	98.1b	0.0	77.4	2.1	18.6
3	0	0	99.8a	0.0	76.8	4.2	18.8
5	0	0	95.5d	4.0	91.4	0.0	0.0
7	0	0	90.8f	15.6	75.0	0.0	0.0
0	1	0	98.6b	0.0	91.5	1.0	6.1
0	3	0	98.6b	0.0	90.4	2.8	5.4
0	5	0	97.8b	0.8	95.6	1.4	0.0
0	7	0	96.7c	3.6	93.5	0.0	0.0
0	0	1	95.7d	2.7	80.6	2.6	9.8
0	0	3	92.8e	3.4	79.6	6.2	3.6
0	0	5	90.5f	4.5	80.5	4.8	0.8
0	0	7	83.8g	12.5	76.4	5.1	0.0

* Basal medium: MS salts with 3% sucrose, 20% coconut milk, 2 g/l peptone, 40 mg/l adenine sulfate and 0.8% agar. **, ***Same as Table 2.

，但就臺灣白及種子萌芽與小苗發育而言，添加adenine sulfate較不適宜。

三、植物生長調節劑對種子萌芽率與小苗發育之影響

Kim and Kako (1984)報導添加BA可顯著促進蕙蘭不定芽增殖。Hasegawa et al. (1985)更報導蕙蘭根尖必需培養於含BA之培養基才能誘導不定芽。許多學者發現BA具有促進細胞分裂、分化與生長的生理作用。本研究結果亦顯示，BA對臺灣白及種子萌芽與小苗發育有明顯促進效果，且未見有明顯玻璃質化現象，尤其添加1~3 mg/l BA可得到良好的小苗生長

，但當濃度提高至5 mg/l以上時，則又不利小苗生長。kinetin與2iP (isopentenyl adenine) 促進小苗發育之作用並不明顯。

將auxins類植物生長調節劑添加於培養基中，高濃度時可誘導癒合組織形成，低濃度則可促進不定芽發根。本研究結果顯示，NAA添加於含3 mg/l BA之培養基中，有明顯促進臺灣白及小苗發育之效果（表九）。播種30天後，含有0.4 mg/l NAA之培養基，原球體則略呈膨大，有少量吸收毛(papilla)形成，濃度提高至0.8 mg/l或1.0 mg/l時，原球體更形膨大，原球體表面形成非正常發育之瘤狀突起，更有大量吸收毛形成，第一心葉不開展而緊包圍第二原葉，隨著第一眞葉伸出後，小苗發育極爲快速，且瘤狀突起部分有分化不定芽現象，但小苗發育已呈畸型，可能NAA濃度提高將導致癒合組織形成，但因種子本身內生荷爾蒙具強勢長芽能力，且協同3 mg/l BA之作用下，NAA誘致癒合組織效果降低，僅能形成callus-like protocorm(CLP)之組織，並隨後分化出畸形不定芽，此與Kusumoto (1979)由卡多麗亞蘭花(cattleya)根尖與葉片表面所誘導之callus-like-bodies構造類似。故NAA雖可加速小苗發育，但過高濃度時有誘致變形之虞，因此仍以0.2 mg/l之NAA較適合；IAA (indoleacetic acid)之生理作用太弱，效果不明顯。2, 4-D (2,4-dichlorophenoxyacetic acid)雖具有較強誘導癒合組織能力，但因種子太幼嫩，易被2, 4-D抑制，故促進小苗發育效果不理想。

GA_3 (gibberellic acid)雖具有打破種子休眠及使種子提早發芽之作用，但Bose and

Table 9. Influence of auxins on seed germination and development of *B. formosana* under light condition*

Basal medium with			Germination	% of seedling at different developmental stages**		
NAA	IAA (mg/l)	2,4-D	rate (%)	4	5	6
0	0	0	99.8a***	76.8	4.2	18.8
0.1	0	0	99.1a	74.6	4.6	20.9
0.2	0	0	99.4a	70.4	9.6	19.8
0.4	0	0	99.2a	56.7	21.6	20.9
0.8	0	0	99.4a	4.3	30.3	64.8
1.0	0	0	99.6a	1.7	19.3	78.6
0	0.2	0	99.3a	75.7	3.4	20.2
0	0.4	0	98.5a	77.6	3.3	17.8
0	1.0	0	99.2a	76.4	3.1	19.7
0	0	0.1	98.4a	97.5	1.1	0.0
0	0	0.2	98.2a	97.3	0.9	0.0
0	0	0.4	92.8b	91.8	1.0	0.0
0	0	1.0	90.4b	90.2	0.2	0.0

* Basal medium: MS salts with 3% sucrose, 20% coconut milk, 2 g/l peptone, 40 mg/l adenine sulfate, 3 mg/l BA and 0.8% agar. **, *** Same as Table 2.

Table 10. Influence of GA3 on seed germination and seedling development of *B. formosana* under light condition*

GA_3 (mg/l)	Germination rate (%)	% of seedling at different developmental stages**		
		4	5	6
0	99.4[a]***	70.4	9.0	19.8
0.1	99.2[a]	81.7	9.6	7.9
0.5	99.1[a]	94.2	4.9	0.0
1	95.4[b]	95.4	0.0	0.0
5	92.6[c]	92.6	0.0	0.0
10	87.3[d]	86.9	0.0	0.0
20	80.3[e]	80.3	0.0	0.0

* Basal medium: MS salts with 3% sucrose, 20% coconut milk, 2 g/l peptone, 40 mg/l adenine sulfate, 3 mg/l BA, 0.2 mgle NAA and 0.8 % agar. **, *** Same as Table 2.

Mukherjee (1976)卻報導GA_3會導致蕙蘭小苗葉片纖細伸長，發育變形。本研究結果亦顯示未添加GA_3之培養基中，需長達12天後，種子才吸水膨大並變綠，而添加GA_3之培養基，僅需七天即可達成，雖GA_3對種子之發芽有加速作用，然隨著GA_3濃度由1 mg/l提高至20 mg/l時，小苗逐漸伸長，葉片面積顯著縮小，小苗顏色較淡，萌芽率並有下降趨勢，播種40天後，小苗發育反而有延遲情形（表十）。

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