出國報告（出國類別：國際會議）

第 1 屆熱帶及溫帶園藝學國際研討會

1st International Symposia on Tropical and Temperate Horticulture

服務機關：行政院農業委員會高雄區農業改良場
姓名職稱：黃柄龍 副研究員
派赴國家：澳洲
出國期間：**105** 年 **11** 月 **18-26** 日
報告日期：**106** 年 **01** 月 **11** 日

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第 1 屆熱帶及溫帶園藝學國際研討會

壹、摘要

第 1 屆熱帶及溫帶園藝學國際研討會 (1st International Symposia on Tropical and Temperate Horticulture)於 2016.11.20-25 在澳洲/凱恩斯(Cairns)舉行，由 ISHS (International Society for Horticultural Science)主辦，計有論文發表 200 篇，其中 143 篇口頭報告，57 篇海報報告，臺灣共有 3 位學者專家參與論文發表。研討會分組討論共分成 9 個領域，包括熱帶園藝、設施栽培、桃金孃科作物、熱帶種植作物、園藝和消除隱藏飢餓、熱帶都市景觀、熱帶作物育種及基因組、熱帶作物營養及微體繁殖、飲料作物。本次研討會議題涵蓋廣泛，不僅涵蓋各類傳統園藝學領域，更涉及貧窮、隱藏飢餓等人道關懷。而此研討會集合園藝栽培、育種、景觀及植物組織培養等方面的專家或具有產業背景的專業人士，針對各類議題分享最新的研究進展，使來自世界各地的參與者能有機會共聚一堂，針對園藝科學進行互動和討論，對擴展視野及激發新的研究方向具有相當的助益。

貳、前言

第 1 屆熱帶及溫帶園藝學國際研討會議題共包含 9 個領域，第 1 個領域是有關熱帶園藝(Tropical Horticulture)，聚焦世界上人口增長最快的城市大多位於熱帶地區，而這些地區存在嚴重的能源、土地和水的競爭壓力，但熱帶地區也擁有許多高價值和高營養的熱帶物種，有很大的發展潛力，因此也是熱帶園藝學者的新市場和新挑戰的機會；第 2 是設施栽培(Protected Cultivation)，議題說明設施栽培可提高產量、延長生長季節、控制病蟲害、減少農藥使用、更高的水分及營養物的利用率和回收率，但開發低成本的結構及良好的溫度維持系統卻仍充滿挑戰；第 3 為有關桃金孃科作物(Myrtaceae)，除了介紹最新的成果，並對番石榴和其它桃金孃科作物所面臨的一些挑戰進行研討；第 4 為重要的熱帶種植作物(Tropical Plantation Crops)，包括椰子、油棕櫚、腰果、可可、茶、咖啡和橡膠等等，希望藉由學、研、業界的互動交流，以開發更高的垂直加工利用及提升作物的附加價值；第 5 是有關園藝和消除隱藏飢餓的部分(Poverty, Hidden Hunger and Horticulture)，探討新鮮的水果和蔬菜不僅可改善個人的微量營養素攝取量，更可促進低收入國家的社會和經濟發展及有助於保護和豐富生物多樣性，同時能於生產、加工、銷售和消費的關係上形成“可持續發展園藝”的概念；第 6 是熱帶都市景觀部分(Urban Landscapes in Tropical Cities)，藉由學術和科學的研究及尋找與歷史和藝術等特殊條件的關聯，以提升熱帶城市的花園和景觀設計樣貌；第 7 是熱帶作物育種及基因組(Tropical Plant Breeding and Genomes)，研討常規育種、分子標記輔助育種及利用誘變技術與基因表現研究以創造更多的變異性，讓處於不同地方的研究人員都能從事熱帶作物育種，此外，比較基因組序列的差異作為

研究植物基因組結構的演變，可更能改善作物的育種結果，獲得更有生產力及抗病蟲害的新品種；第 8 是熱帶作物營養及微體繁殖(Vegetative Propagation and In Vitro Culture of Tropical Plants)，隨著研究方法和設備的進步，越來越多的組織培養技術被開發來生產和保存高價值的無病原體種苗，並且克服了許多不易利用組織培養繁殖的作物的限制，讓一些經由育種改良的新品種，透過營養及微體繁殖技術的量產施行以賦於它更大的推廣意義；第 9 是飲料作物(Beverage Crops)，製作飲料的主要作物包括咖啡、茶、可可、水果、蔬菜、穀物、豆類、草本植物和啤酒花等，而飲料和飲料作物對就業、收入、營養和生活方式等產生巨大影響，它們更為許多開發中國家的農村提供了重要的經濟來源，是為一個相當重要的主題。

參、目的

第 1 屆熱帶及溫帶園藝學國際研討會是由 ISHS 主辦，為一個多元化的園藝學研討會，並以熱帶園藝為主要議題，明訂此次研討會的主題為「現在是熱帶園藝的時代」。此次會議除研討傳統的園藝栽培、生產、收穫、育種、繁殖等議題外，並與與全球園藝倡議合作，探討有關貧困、隱性飢餓等問題。而在營養及微體繁殖部分，針對扦插、生體外培養及育種、微體繁殖、植株再生、轉殖技術、植物荷爾蒙及超低溫冷凍技術等議題進行報告與探討。為了更了解目前植物組織培養新技術的發展與應用因而參與本次國際會議，會議中也發表「Effects of *p*-chlorophenoxyisobutyric acid and 2,3,5-triiodobenzoic acid on *in vitro* micropropagation of *Aglaonema* 'Lady Valentine'」，藉由探討不同劑量的 *p*-chlorophenoxyisobutyric acid (PCIB)及 2,3,5-triiodobenzoic acid (TIBA)對 A. 'Lady Valentine'側芽和再生不定芽誘導及芽體伸長之影響，以克服 *Aglaonema* 微體繁殖過程中新生芽體生長困難之現況。此外，也希望藉由本次研習能多方面蒐集植物微體繁殖及育種應用的最新國際發展趨勢及資訊，並與國際間學、業界進行經驗交流與分享，對我國未來在育種及種苗方面的發展規劃上將有極大的幫助。

肆、研習行程及內容

一、研習人員

黃柄龍 副研究員　行政院農業委員會高雄區農業改良場

Ping-Lung Huang, Associate Researcher, Kaohsiung District Agricultural Research and Extension Station, COA

二、行程概要

日期	行程	住宿地點	差勤類別

11/18 (五)	高雄國際機場 — 香港國際機場	飛機	國外出差
11/19 (六)	香港國際機場 — 澳洲/凱恩斯	凱恩斯 (Cairns)	
11/20 (日)	參加「第 1 屆熱帶及溫帶園藝學國際研討會 1st International Symposia on Tropical and Temperate Horticulture」及參觀 Industry Exhibition		
11/21 (一)			
11/22 (二)			
11/23 (三)			
11/24 (四)			
11/25 (五)			
11/26 (六)	澳洲/凱恩斯 — 香港國際機場 — 高雄國際機場		

三、研習行程及重要內容

抵達澳洲/凱恩斯(Cairns)

由高雄國際機場搭乘港龍航空班機抵達香港後，旋即轉機國泰航空直飛澳洲/凱恩斯，並利用大眾捷運及計程車等交通工具前往下榻飯店及往返 Cairns Convention Centre 會議會場。

研討會記要

1.開幕式

第 1 屆熱帶及溫帶園藝學國際研討會 (1st International Symposia on Tropical and Temperate Horticulture)於澳洲/凱恩斯 Cairns Convention Centre 舉行，由 ISHS (International Society for Horticultural Science)主辦。本次會議計有專題演講，論文發表計 200 篇，其中 143 篇口頭報告，57 篇海報報告。

開幕式由 ISHS 主席 Professor Rod Drew 揭開序幕，介紹 ISHS 的相關會務及發展現況，接著由澳洲園藝產業研究所(Australlian Institute of Horticultures Inc.，AIH)總裁及凱恩斯市長致歡迎詞。

圖1.研討會舉辦場館Cairns Convention Centre

圖2. ISHS主席Professor Rod Drew

圖3.澳洲園藝產業研究所(AIH)總裁

圖4. Cairns市長

大會的第一場專題演講於開幕式後由 Dr. Dyno Keatinge (Tropical Agricultural Development Advisory Services，TADAS)發表「Horticulture for Sustainable Development: Evidence of Impact of International Vegetable R&D」，他提出“可持續發展園藝 - H4SD”的概念，說明在消除隱藏的飢餓/微量養分缺乏和貧困的爭論中，營養攝取的重要性經常被低估；而新鮮的水果和蔬菜不僅可改善個人的微量營養素攝取，更可促進低收入國家的社會和經濟發展及有助於保護和豐富生物多樣性。然而，儘管園藝學有助於實現這些好處，但目前仍存在諸多限制因子來阻止不同的行為者充分利用園藝產業價值鏈上的潛力，值得消費者及農企業省思。

開幕式後，緊接著進行合影留念及分組研討、海報論文發表。

<table>
<tr><td>  </td><td>  </td></tr>
<tr><td>圖5. Dr. Dyno Keatinge發表專題演講</td><td>圖6.與日本學者Astha Tuladhar合影</td></tr>
<tr><td>  </td><td>  </td></tr>
<tr><td>圖7.與ISHS主席Professor Rod Drew合影</td><td>圖8.與農試所李文立主任、剛果共和國學者Elisha合影</td></tr>
</table>

2.分組研討

本次研討會為期 5 天，會議涵蓋議題廣泛且發表的內容眾多，僅節錄簡述部分營養和微體繁殖議題如下：

1. *IN VITRO* CULTURE OF TROPICAL TREE SPECIES

由 Prof. Dr. Roderick A. Drew 主講，說明幾種重要的熱帶果樹營養繁殖方法，例如葡萄(Grape，*Vitis* sp.)，以 52℃，60 min 的溫湯處理休眠的插穗可以減少 Agrobacterium 的傳播，提高發根率；芒果(Mango，*Mangifera indica* L.)，幼年性插穗以 5,000 ppm IBA 預處理可促進發根率達 100%；番石榴(Guava，*Psidium guajava* L.)，具有 2 莖節、4 葉片的插穗以 3,000 ppm IBA 預處理，則發根率可達 80%；荔枝(Litchi，*Litchi chinensis* Sonn.)，不易以一般的無性繁殖法來繁殖，需要先以 ethrel 預處理才能促進發根率；木瓜(Papaya，*Carica papaya* L.)，具有 4-5 葉片、長 50-150 mm 的插穗浸泡於 BA、CPPU 藥劑，並噴灑 GA3、GA4+7，可於 3 週內發根良好，並形成具有活力的新一株種苗。

2. MASSPROPAGATION OF PHYTOPLASMA-FREE PLANTING MATERIALS IN SUGARCANE (*SACCHARUM* SPP.) USING TEMPORARY IMMERSION

BIOREACTOR

由來自泰國的 Karsedis Distabanjong 博士主講，說明組織培養技術可用來篩選甘蔗的原生質體，而短暫浸漬系統可用以量產乾淨的甘蔗種植材料，提高甘蔗的產量。

3. STRATEGIES FOR MAINTAINING AND INCREASING THROUGHPUT OF *IN VITRO* CULTURES OF SUGARCANE

由來自南非的 Sandy Snyman 博士主講，說明其在甘蔗上的研究進展，包括微體繁殖、去病毒、遺傳工程、誘變育種及原生質體保存等，並藉由修正實驗室的生產流程來節省微體繁殖的時間。

4. THE USE OF SUCROSE AND INDOLE-3-BUTYRIC ACID FOR INCREASING QUANTITY OF ROOT AND ACCLIMATIZATION OF ANT PLANT (*MYRMECODIA PENDANS*)

由來自印尼的 Innaka Ageng Rineksane 博士主講，說明該植物具有多種活性成分，如 Flavonoid、Tannin、Polyphenols 等，為一種高價值植物，每公斤售價可達 80 美元。其研究策略是提高發根量及提升種苗馴化的存活率，結果顯示高濃度的蔗糖(40 mg/L) + 0.5 mg/L IBA 可促進組培苗形成最大的根數，每一株組培苗達 7.2 條根，且於此一培養基培養的組培苗的馴化存活率最高。

3.海報報告

本次研討會海報報告部分共計 57 篇，內容涵蓋採後處理、園產品加工、病毒檢測、設施栽培、耐旱及抗性育種、灌溉、光合作用、儲運技術、原生質體保存、微體繁殖及 DNA 分析等方面。筆者亦發表「Effects of *p*-chlorophenoxyisobutyric acid and 2,3,5-triiodobenzoic acid on *in vitro* micropropagation of *Aglaonema* 'Lady Valentine'」，藉由探討不同劑量的 *p*-chlorophenoxyisobutyric acid (PCIB)及 2,3,5-triiodobenzoic acid (TIBA)對 *A.* 'Lady Valentine'側芽和再生不定芽誘導及芽體伸長之影響，用以克服 *Aglaonema* 微體繁殖過程中新生芽體生長困難之現況，詳細發表全文請參閱附錄。

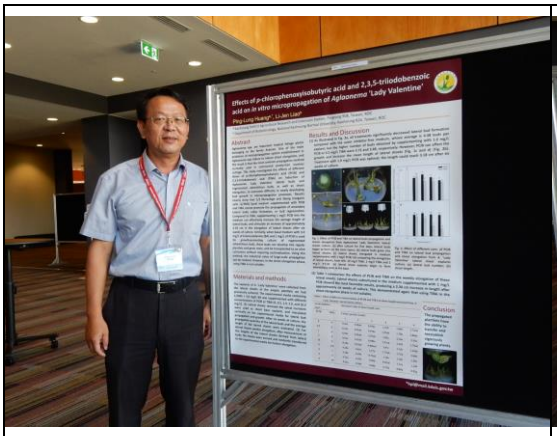

圖9.筆者與發表的海報報告合影

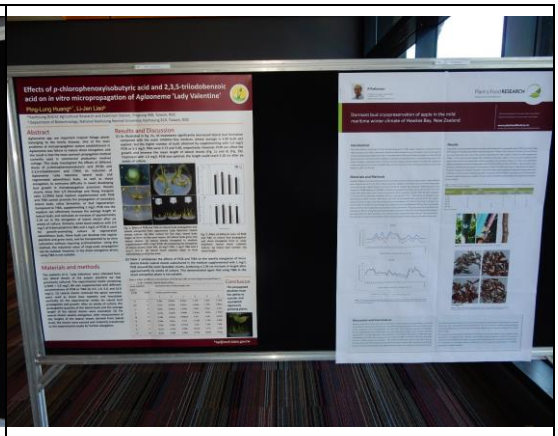

圖10.海報報告發表一隅

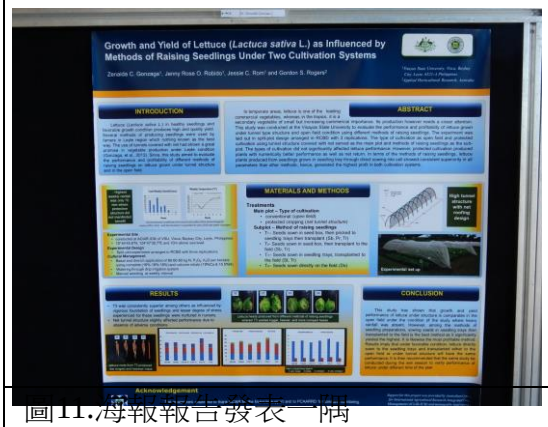

圖11.海報報告發表一隅

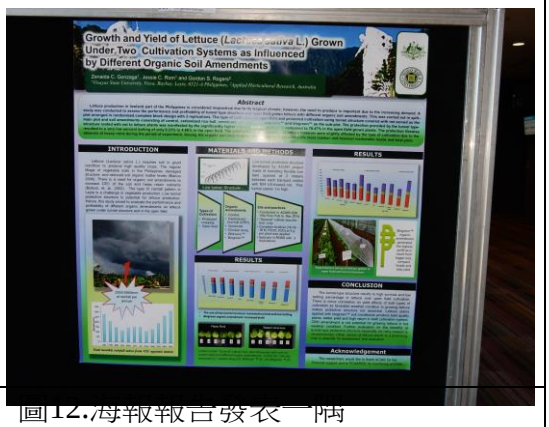

圖12.海報報告發表一隅

4.園藝產品展示

本次研討會，主辦單位亦規劃部分的園藝產業進行參展，介紹澳洲當地的熱帶水果、花卉等及一些應用於設施栽培的土壤、環境監控設備，而我國的臺灣大學園藝系也在陳右人教授的帶領下，以高山茶為主題進行多種臺灣茶葉的展示及試飲，吸引相當多的與會學者前來參觀，為一次成功的臺灣茶宣傳活動。

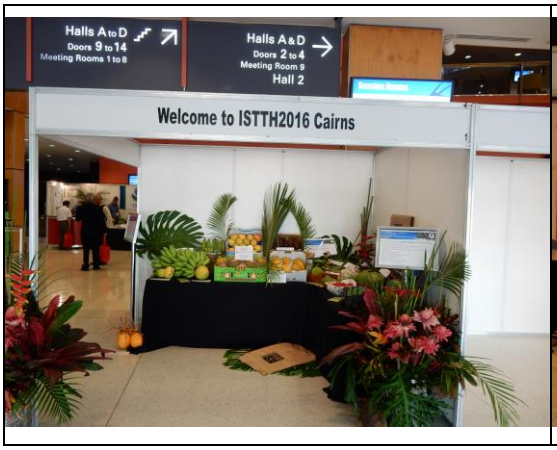

圖13.園藝產業參展攤位，介紹澳洲當地熱帶水果、花卉	圖14.園藝產業參展攤位，介紹設施栽培監控設備
	
圖15.澳洲園藝產業研究所展示攤位	圖16.我國臺灣大學園藝系的高山茶展示攤位

伍、心得與建議

1.現在是熱帶園藝的時代：據統計，2040 年世界人口將超過 90 億，到 2050 年將有一半的人口居住在熱帶地區，而熱帶地區擁有許多高價值和高營養的作物種原亟待開發。因此，各國的園藝學者需共同努力，發揮熱帶園藝作物的最大潛力，以滿足全球的需求。

2.擴大熱帶植物種原蒐集：熱帶地區植物種原豐富，宜設置種原蒐集據點，並請求當地相關人士有計畫地協助引種或種苗採購，惟應透過正式管道進行，並取得正式的檢疫證明文件，對後續防疫工作較能有效控管及避免特定病蟲害擴展的危機。

3.借鏡經驗改善問題：臺灣在園藝品種育成及栽培技術具有相當的水準，但小農制及農村人力老化問題常導致生產成本過高或生產力不足，而本次研討會的應用性質的議題很多，也有很多民間公司的代表參與。因此，我國的園藝產業發展可以多借鏡他人的經驗，朝向低勞力投入、省工及設施栽培的技術方向開發。

4.鼓勵研究人員積極參與國際研討會：此次研討會除了獲得最新的園藝新知外，並得以認識各領域的學者，有助於建立聯繫交流管道， 而若能再結合公部門與民間企業的研發量能，對未來相關產業的發展將有極大的幫助。因此，建議應提供更多元的管道，鼓勵研究人員從事國外短期研究、參與國際活動，這樣不僅能增進研發人員的國際視野及國際觀，也能提升國內的研究水準。

陸、附錄

Effects of *p*-chlorophenoxyisobutyric acid and 2,3,5-triiodobenzoic acid on *in vitro* micropropagation of *Aglaonema* 'Lady Valentine'

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Abstract

This study investigated the effects of different doses of *p*-chlorophenoxyisobutyric acid (PCIB) and 2,3,5-triiodobenzoic acid (TIBA) on induction of *Aglaonema* 'Lady Valentine' lateral buds and regenerated adventitious buds, as well as shoot elongation, to overcome difficulty in newly developing bud growth in micropropagation processes. Results clearly show that 1/3 Murashige and Skoog inorganic salts (1/3MS) basal medium supplemented with PCIB and TIBA cannot promote the propagation of secondary lateral buds, callus formation, or bud regeneration. Compared to TIBA, supplementing 1 mg l^{-1} PCIB into the medium can effectively increase the average length of lateral buds, and stimulate an increase of approximately 2.34 cm in the elongation of lateral shoots after six weeks of culture. Similarly, when basal medium with 3.0 mg l^{-1} of 6-benzyladenine (BA) and 1 mg l^{-1} of PCIB is used for growth-promoting culture of regenerated adventitious buds, these buds can develop into regular plantlets and grow roots, and be transplanted to *ex vitro* cultivation without requiring acclimatization. Using this method, the industrial value of large-scale propagation can be realized. However, in the shoot elongation phase, using TIBA is not suitable.

1. Introduction

To build a pathogen-free tissue culture micropropagation system of *Aglaonema* is not easy, because severe endophyte contamination in the vascular bundle tissue. Litz and Conover (1977) indicated that some Araceae plants have a long growth lag phase, which may also limit the micropropagation of *Aglaonema*. Chen (2006) attempted to use 0.5 mg l^{-1} gibberellic acid (GA_3) to promote shoot elongation of *Aglaonema*. However, supplemented GA_3 reduces the number of differentiated buds. Additionally, some plants cultured from buds cultured in GA_3-containing medium easily produce albino or weak plantlets or other abnormal shoots (Moshkov et al.,

2008; Reuveni et al., 1990). *Aglaonema* shoot differentiation and elongation are also affected by the type of cytokinin used. Low-concentration thidiazuron (1-phenyl-3-(1,2,3-thiadiazol-5-*yl*) urea, TDZ) can propagate more buds as compared to 6-benzyladenine (BA), but easily causes buds to form rosettes and be incapable of elongation growth (Chen and Yeh, 2007). Currently, we can successfully induce *Aglaonema* 'Lady Valentine' lateral shoot propagation and adventitious buds regenerated from calli. However, the regeneration of obtained buds still results in a growth lag and difficulty in shoot elongation.

p-Chlorophenoxyisobutyric acid (PCIB) has been discovered to increase the rate of root growth and root hair elongation and stimulate internode elongation in *Avena sativa* seedlings (Almestrand, 1957; Burstrom, 1951; Devlin and Jackson, 1961; Ng and Audus, 1964; Poljakoff-Mayber et al., 1959). They suggested that PCIB be termed a weak auxin instead of an antiauxin. The addition of 20 μM PCIB in media for *Brassica juncea* microspore could enhance embryogenesis (Agarwal et al., 2006) and 15.8 mg l^{-1} abscisic acid (ABA) + 5.0 mg l^{-1} PCIB most effectively promoted the maturation of cotyledon-staged somatic embryos of *Larix leptolepis* (Kim and Moon, 2009). Oono et al. (2003) reported that PCIB reduced auxin-induced accumulation of *Aux*/*IAA* gene transcripts, and impaired auxin-signaling pathways by regulating Aux/IAA protein stability, thereby affecting the auxin-regulated *Arabidopsis* root physiology. Genetic characterization of *Arabidopsis* mutants for roots resistant to PCIB also indicated that PCIB can facilitate the identification of factors involved in auxin or auxin-related signaling (Biswas et al., 2007).

2,3,5-Triiodobenzoic acid (TIBA) has been used in media for the induction of direct organogenesis in *Pisum sativum* (Tetu et al., 1990), induction of somatic embryogenesis in rhizomatous irises (Laublin et al., 1991), and *Oncidium* (Chen and Chang, 2004). 2,3,5-Triiodobenzoic acid has also been used to inhibit somatic embryogenesis in sweet potatoes (Chee and Cantliffe, 1989), carrots (Nissen and Minocha, 1993), geraniums (Hutchinson et al., 1996) and ginseng (Choi et al., 1997, 2001); and to form calli in coffee plants (Sreenath et al., 1995).

This study examined the effects of auxin receptor antagonist compound PCIB and auxin polar transport inhibitor TIBA on lateral buds induction, adventitious buds regeneration from calli and shoot elongation.

2. Materials and methods

2.1. Plant material and culture conditions

The explants of *A.* 'Lady Valentine' were collected from the lateral shoots of the aseptic plantlets we had previously cultured. The basal medium comprised 1/3 Murashige and Skoog inorganic salts (1/3MS) (Murashige and Skoog, 1962),

supplemented with 0.4 mg l^{-1} thiamine·HCl, 0.5 mg l^{-1} pyridoxine·HCl, 0.5 mg l^{-1} nicotinic acid, 2.0 mg l^{-1} glycine, 100 mg l^{-1} myo-inositol, 30 g l^{-1} sucrose, and 3.0 mg l^{-1} BA and was solidified with 8 g l^{-1} agar (Merck). The experimental media containing the basal medium described above was supplemented with different concentrations of PCIB or TIBA (0, 0.5, 1.0, 5.0, and 10.0 mg l^{-1}). The pH of the medium was adjusted to 5.7-5.8 prior to autoclaving at 121℃ and 1.2 kg cm^{-2} for 20 min.

2.2. Lateral bud propagation and shoot growth

Lateral shoots removed the apical meristem were used as shoot base explants and inoculated vertically on the experimental media for lateral bud propagation and growth. After six weeks of culture, the propagation quantity of the lateral buds and the average length of the lateral shoots were evaluated.

2.3. Lateral shoots weekly elongation

Extension of aforementioned experiment: after measurement of the lengths of the lateral shoots derived from lateral buds, the shoots were excised and randomly transferred to the experimental media for further elongation. Following culture, the increase in length of the lateral shoots (excluding adventitious roots) were calculated at 1-week intervals within six weeks of culture.

2.4. Adventitious root formation

Lateral shoots with a length of 2-3 cm were collected and randomly transferred to the experimental media. Rooting response was evaluated in terms of the number and length of adventitious roots per explant every week for four weeks after a 3-week culture.

2.5. Callus induction and adventitious bud regeneration

The shoot base (excluding apical meristem) were horizontally cut into 1-2 mm-thick as stem section explants and inoculated on the same experimental media for callus induction and further regeneration. After four weeks of culture, the induction rate of the organogenic callus and regenerated adventitious buds per explant or non-organogenic callus were calculated.

3. Results

3.1. The effects of PCIB and TIBA on lateral bud propagation and shoot elongation

After the *Aglaonema* lateral shoot base explants that were removed the apical meristem had been cultured in media with various concentrations of auxin inhibitors

for five days, lateral buds began to form on the different nodes of stem in each treatment (Fig. 1a). These buds gradually grew into lateral shoots (Fig. 1b). As illustrated in Fig. 2a, all treatments significantly decreased lateral bud formation compared with the auxin inhibitor-free medium, whose average is 4.48 buds per explant; but added PCIB or TIBA to the basal medium during the lateral bud induction stage also caused a significant difference in the induction quantity of the lateral buds. Media supplemented with a high concentration of PCIB or TIBA (10 mg l^{-1}) produced an inhibition of bud formation. The mean number of induced lateral buds per explant increased when decreasing the auxin inhibitor concentration. A higher number of buds obtained by supplementing with 1.0 mg l^{-1} PCIB or 0.5 mg l^{-1} TIBA were 3.72 and 3.48, respectively.

However, PCIB can affect the growth and increase the mean length of lateral shoots (Fig. 1c and d) (Fig. 2b). Treatment with 1.0 mg l^{-1} PCIB was optimal; the length could reach 3.18 cm after six weeks of culture, which was a significant difference as compared to treatment without auxin inhibitors. Furthermore, media with a low concentration of TIBA did not trigger shoot growth at all, and even decreased as TIBA concentration increased; these lateral shoot lengths reduced from 2.70 to 1.03 cm when cultured on media containing 0.5 to 10 mg l^{-1} TIBA

3.2. The effects of PCIB and TIBA on the weekly elongation of lateral shoots

Cut the propagated lateral shoots of above experiments, and transplanted on same experimental media for further growth. Table 1 summarizes the effects of PCIB and TIBA on the weekly elongation of these lateral shoots. Within a 6-week culture period, PCIB may promote lateral shoot elongation significantly. Lateral shoots subcultured in the medium supplemented with 1 mg l^{-1} PCIB showed the most favorable results, producing a 2.34 cm increase in length after approximately six weeks of culture. This result was significantly different from those produced by other treatments. Regarding the results of TIBA treatment, the medium with a low concentration of 0.5 mg l^{-1} produced a 2.04 cm increase in length. However, growth was stunted following the subculture of the lateral shoots in media supplemented with a TIBA concentration of 1 mg l^{-1} and higher; the effects became poorer as the concentration increased. This demonstrated again that using TIBA in the shoot elongation phase is not suitable.

3.3. Adventitious root formation of lateral shoots

Table 2 shows that the lateral shoots can begin to form adventitious roots at the base as early as the third week (Fig. 1e). In particular, treatment with 0.5 mg l^{-1} of PCIB produced the highest number of adventitious roots per week, demonstrating that

different concentrations of auxin inhibitors can affect the formation of the adventitious roots of lateral shoots.

However, high concentrations of TIBA were discovered to limit the formation and elongation of adventitious roots. In media with 5-10 mg l^{-1} TIBA, after six weeks of culture, some of the shoots had not developed roots and the mean length was only 0.22-0.24 cm, compared to other treatments which could produce root lengths of 0.62 cm and longer.

3.4. The effects of PCIB and TIBA on callus induction and adventitious bud regeneration

After being cultured on the experimental media, the stem section explants from *A.* 'Lady Valentine' lateral shoots effectively formed transparent granular callus (Fig. 3a) or loose-textured non-organogenic callus (Fig. 3b) at the cut surface. However, the callus induction rate shows extreme differences corresponding to different concentrations of auxin inhibitors (Fig. 4a). Using a treatment with PCIB produces a higher induction rate, and the rate increases with an increase in concentration. Treatment with 5 mg l^{-1} PCIB can achieve an induction rate of 80%. However, a majority of calli can only maintain continuous cell division and are incapable of regenerating somatic embryos or organs. Additionally, excess calli growth can easily cover existing organs (Fig. 3c) and prevent the growth of differentiated buds.

The induction of organogenic callus from stem section explants is opposite to those of non-organogenic callus. TIBA is significantly better than PCIB on organogenic callus induction. The induction rate of 1.0 mg l^{-1} TIBA treatment, which can stimulate 64% of explants to produce organogenic calli, is the highest. However, compared to the control (72%), this result shows that supplemented auxin inhibitors in media inhibit the formation of organogenic calli (Fig. 4b). Furthermore, the highest induction rate of regenerated bud primordia is derived from the organogenic calli which cultured without any auxin inhibitors: a single callus can produce five regenerated bud primordia on average, and 15 regenerated bud primordia at maximum (data not shown). This is significantly different from the treatments with auxin inhibitors, reaching only 0-1.8 regenerated bud primordia on average (Fig. 4c).

The regenerated bud primodia (Fig. 3d) cultured on 1/3MS basal medium with 3.0 mg l^{-1} BA for four weeks, regenerated adventitious buds can be cut into individuals and transplanted to a medium with 1.0 mg l^{-1} PCIB to promote growth (Fig. 3e). Shoots developed into vigorous plantlets following eight weeks of culture (Fig. 3f) can be transferred to natural environment and grown normally *ex vitro*.

4. Discussion

Our previous experimental results show that the proliferation rate of shoots of *A.* 'Lady Valentine' lateral bud explants treated with various concentrations of BA is low. However, treatment with a low concentration of BA can produce more adventitious roots (unpublished). This is significantly different from the principle, which indicates that a high cytokinin/auxin ratio allows for adventitious buds regeneration easily, and a low ratio allows for easy adventitious root formation (Skoog and Miller, 1957). Therefore, we infer that this variety may have possessed a high quantity of endogenous auxin to cause the effects of cytokinin to be insignificant. Regarding using auxin and cytokinin to regulate cell division (Van Staden et al., 2008), excessive endogenous auxin can affect the function of cytokinin on mitosis, thereby influencing bud growth. It is possible that this is the reason for the growth lag of *Aglaonema* spp. regenerated buds.

PCIB is known as a putative antiauxin and is widely used to inhibit auxin action by interfering with upstream auxin signaling events. A previous study determined that PCIB stimulates internode elongation in *Avena sativa* seedlings (Ng and Audus, 1964). They suggested that PCIB be termed a weak auxin instead of an antiauxin. This study also found that low concentrations of PCIB can increase the mean length of *Aglaonema* lateral shoots, seems to have similar results as Ng and Audus (1964). Fischer and Neuhaus (1996) believed that PCIB affects cell division, cell shape, and even stimulates cell elongation. Perhaps for these reasons, PCIB was found to promote the growth of *Aglaonema* plantlets in this study. These results are similar to those reported by Liao et al. (2008), who found that the application of 1 mg l^{-1} PCIB produced more elongated shoots of *Pinus elliottii* Engelm.

However, TIBA is an auxin efflux inhibitor that blocks polar transport by preventing auxin efflux and inhibits auxin transport in part by competing with auxin at the efflux carrier site (Noh et al., 2001). In this study, TIBA may have inhibited the polar transport of auxin synthesized from apical meristem in *Aglaonema*, causing insufficiency in the quantity of endogenous auxin in the lateral shoots, thereby inhibiting the growth of the shoots (Liu et al., 1993).

Machakova et al. (2008) indicated that auxin could stimulate differentiation of the vascular bundle and part of the shoot and root. Therefore, adding PCIB or TIBA to the medium reduces the differentiation rate of the lateral buds, although different results could be produced by different concentration and application period (Choi et al., 1997, 2001; Find et al., 2002). In our experiments on induction of lateral buds, treatment without PCIB or TIBA induced the highest number of buds (4.48), and supplemented with PCIB or TIBA reduced the induction quantity of lateral buds, demonstrating that a higher quantity of auxin is required in the differentiation stage of

lateral buds. Additionally, using low concentrations of PCIB or TIBA could induce a higher number of buds, results that were also similar to that of Liao et al. (2008).

p-Chlorophenoxyisobutyric acid has also been discovered to increase the rate of root growth and root hair elongation (Almestrand, 1957; Burstrom, 1951; Devlin and Jackson, 1961; Poljakoff-Mayber et al., 1959); however, Oono et al. (2003) indicated that PCIB inhibits lateral root production, gravitropic response of roots, and growth of primary roots in *Arabidopsis*. Twenty μM (4.3 mg l^{-1}) of PCIB can effectively inhibit GUS activity in the root elongation zone of the *BA*::*GUS* transgenic *Arabidopsis*; however, this inhibition can be overcome by increasing the concentration of indole-3-acetic acid (IAA). This study determined that the number and root length of the PCIB-induced adventitious roots were not significantly different from those of the auxin inhibitor-free control. Therefore, we infer that whether PCIB is termed a weak auxin or antiauxin, the quantity of auxin of *Aglaonema* explants that have been treated with PCIB is sufficient to induce adventitious roots. However, high concentrations (5-10 mg l^{-1}) of TIBA inhibit the growth of adventitious roots of *Aglaonema* lateral shoots. This result is similar to those reported by Fujita and Syōno (1996), who found that 0.5-5 mg l^{-1} of TIBA could clearly inhibit the growth of the roots of *Arabidopsis thaliana* seedlings. This inhibitive effect is enhanced as TIBA concentration increases.

Regarding callus induction in this study, although the induction rate of organogenic calli resulting from treatment without auxin inhibitors or with a low concentration of TIBA was higher, auxin inhibitor-free treatment produced the highest number of regenerated bud primordia of *Aglaonema*. Zhang et al. (2004) believed that regarding callus induction and regeneration, TIBA served to promote cell division and organogenesis, and could promote regeneration of sugar beet buds. However, without exogenous auxin, both PCIB and TIBA can reduce the proliferation ability of calli (Find et al., 2002) and limit the differentiation of the embryos or shoots of monocots (Fischer and Neuhaus, 1996). Additionally, the internal concentration gradient of auxin must be constructed through polar transport to stimulate plant cells to differentiate into organs (Choi et al., 1997, 2001; Liu et al., 1993). From this process it can be suggested that although the induction effects of a low concentration of TIBA on the organogenic calli of *Aglaonema* stem section explants were not extremely different from those of the control, PCIB and TIBA could inhibit the subsequent proliferation of the organogenic calli and the regeneration of buds, causing significant reduction in the number of regenerated buds.

References

Agarwal, P.K., Agarwal, P., Custers, J.B.M., Liu, C.M., Bhojwani, S.S., 2006. PCIB an antiauxin enhances microspore embryogenesis in microspore culture of *Brassica juncea*. Plant Cell Tiss. Organ Cult. 86, 201-210.

Almestrand, A., 1957. Growth and metabolism of isolated cereal roots. Physiol. Plant. 10, 521-620.

Biswas, K.K., Ooura, C., Higuchi, K., Miyazaki, Y., Nguyen, V.V., Rahman, A., Uchimiya, H., Kiyosue, T., Koshiba, T., Tanaka, A., Narumi, I., Oono, Y., 2007. Genetic characterization of mutants resistant to the antiauxin *p*-chlorophenoxyisobutyric acid reveals that *AAR3*, a gene encoding a DCN1-like protein, regulates responses to the synthetic auxin 2,4-dichlorophenoxyacetic acid in Arabidopsis roots. Plant Physiol. 145, 773–785.

Burstrom, H., 1951. Studies on growth and metabolism of roots. VI. The relative growth action of different isobutyric acid derivatives. Physiol. Plant. 4, 470-485.

Chee, R.P., Cantliffe, D.J., 1989. Inhibition of somatic embryogenesis in response to 2,3,5-triiodobenzoic acid and 2,4-dichlorophenoxyacetic acid in *Ipomoea batatas* (L.) Lam. cultured *in vitro*. J. Plant Physiol. 135, 398-403.

Chen, J.T., Chang, W.C., 2004. TIBA affects the induction of direct somatic embryogenesis from leaf explants of *Oncidium*. Plant Cell Tiss. Organ Cult. 79, 315–320.

Chen, W.L., 2006. *In vitro* shoot multiplication and *ex vitro* physiology of *Aglaonema*. Dissertation. University of Taiwan, Taipei, 2006.

Chen, W.L., Yeh, D.M., 2007. Elimination of *in vitro* contamination, shoot multiplication, and *ex vitro* rooting of *Aglaonema*. HortScience 42, 629-632.

Choi, Y.E., Kim, H.S., Soh, W.Y., Yang, D.C., 1997. Developmental and structural aspects of somatic embryos formed on medium containing 2,3,5-triiodobenzoic acid. Plant Cell Rep. 16, 738-744.

Choi, Y.E., Katsumi, M., Sano, H., 2001. Triiodobenzoic acid, an auxin polar transport inhibitor, suppresses somatic embryo formation and postembryonic shoot/root development in *Eleutherococcus senticosus*. Plant Sci. 160, 1183-1190.

Devlin, R.M., Jackson, W.T., 1961. Effect of *p*-Chlorophenoxyisobutyric acid on rate of elongation of root hairs of *Agrostis alba* L. Physiol. Plant. 14, 40-48.

Find, J., Grace, L., Krogstrup, P., 2002. Effect of anti-auxins on maturation of embryogenic tissue cultures of Nordmanns fir (*Abies nordmanniana*). Physiol. Plant. 116, 231-237.

Fischer, C., Neuhaus, G., 1996. Influence of auxin on the establishment of bilateral symmetry in monocots. Plant J. 9, 659-669.

Fujita, H., Syōno, K., 1996. Genetic analysis of the effects of polar auxin transport inhibitors on root growth in *Arabidopsis thaliana*. Plant Cell Physiol. 37, 1094–1101.

Hutchinson, M.J., Murch, S.J., Saxena, P.K., 1996. Morphoregulatory role of thidiazuron: Evidence of the involvement of endogenous auxin in thidiazuron-induced somatic embryogenesis of geranium (*Pelargonium* × *hortorum* Bailey). J. Plant Physiol. 149, 573-579.

Kim, Y.W., Moon, H.K., 2009. Effect of TIBA, PCIB and phloroglucinol on somatic embryo maturation and germination in Japanese larch (*Larix leptolepis*). Korean J. Plant Biotechnol. 36, 230-235.

Laublin, G., Saini, H.S., Cappadocia, M., 1991. *In vitro* plant regeneration via somatic embryogenesis from root culture of some rhizomatous irises. Plant Cell Tiss. Organ Cult. 27, 15-21.

Liao, Y.K., Jhuang, C.T., Wu, Y.H., Zhan, H.C., 2008. Improved shoot initiation and elongation by ABA, TIBA or PCIB as *in vitro* adventitious shoot propagation was applied in *Pinus elliottii* Engelm. Quart. J. Chin. Forest. 41, 29-42.

Litz, R.E., Conover, R.A., 1977. Tissue culture propagation of some foliage plants. Proc. Fla. State Hort. Soc. 90, 301-303.

Liu, C.M., Xu, Z.H., Chua, N.H., 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5, 621-630.

Machakova, I., Zazimalova, E., George, E.F., 2008. Plant growth regulators I: Introduction; auxins, their analogues and inhibitors. In: George, E.F., Hall, M.A., De Klerk. G.J. (Eds.), Plant Propagation by Tissue Culture, 3rd ed. Volume 1. The background. Springer, New York, pp. 175-204.

Moshkov, I.E., Novikova, G.V., Hall, M.A., George, E.F., 2008. Plant growth regulators III: Gibberellins, ethylene, abscisic acid, their analogues and inhibitors; miscellaneous compounds. In: George, E.F., Hall, M.A., De Klerk. G.J. (Eds.), Plant Propagation by Tissue Culture, 3rd ed. Volume 1. The background. Springer, New York, pp. 227-281.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio-assays with tobacco tissue culture. Physiol. Plant. 15, 473–497.

Ng, E.K., Audus, L.J., 1964. Growth-regulator interactions in the growth of the shoot system of *Avena sativa* seedlings I. The growth of the first internode. J. Exp. Bot. 15, 67-95.

Nissen, P., Minocha, S.C., 1993. Inhibition by 2,4-D of somatic embryogenesis in carrot as explored by its reversal by difluoromethylornithine. Physiol. Plant. 89, 673-680.

Noh, B., Murphy, A.S., Spalding, E.P., 2001. Multidrug resistance-like genes of Arabidopsis required for auxin transport and auxin-mediated development. Plant Cell 13, 2441-2454.

Oono, Y., Ooura, C., Rahman, A., Aspuria, E.T., Hayashi, K.I., Tanaka, A., Uchimiya, H., 2003. *p*-Chlorophenoxyisobutyric acid impairs auxin response in Arabidopsis root. Plant Physiol. 133, 1135–1147.

Poljakoff-Mayber, A., Mayer, A.M., Zacks, S., 1959. The effect of PCIB on the germination and growth of lettuce in the presence of thiourea. Ann. Bot. 23, 361-363.

Reuveni, O., Shlesinger, D.R., Lavi, U., 1990. *In vitro* clonal propagation of dioecious *Carica papaya*. Plant Cell Tiss. Organ Cult. 20, 41-46.

Skoog, F., Miller, C.O., 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symp. Soc. Exp. Biol. 11, 118-131.

Sreenath, H.L., Shanta, H.M., Babu, K.H., Naidu, M.M., 1995. Somatic embryogenesis from integument (perisperm) cultures of coffee. Plant Cell Rep. 14, 670-673.

Tetu, T., Sangwan, R.S., Sangwan-Norreel, B.S., 1990. Direct somatic embryogenesis and organogenesis in cultured immature zygotic embryos of *Pisum sativum* L. J. Plant Physiol. 137, 102-109.

Van Staden, J., Zazimalova, E., George, E.F., 2008. Plant growth regulators II: Cytokinins, their analogues and antagonists. In: George, E.F., Hall, M.A., De Klerk. G.J. (Eds.), Plant Propagation by Tissue Culture, 3rd ed. Volume 1. The background. Springer, New York, pp. 205-226.

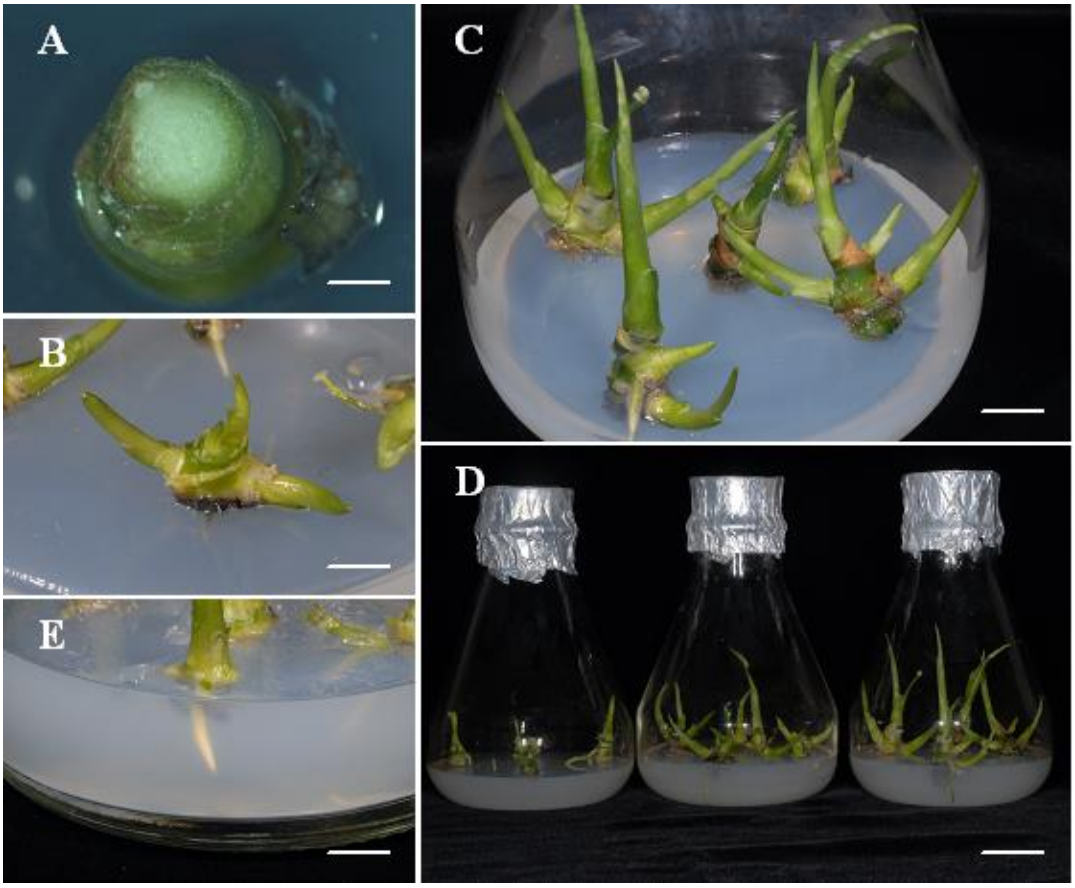

Fig. 1. Effect of *p*-chlorophenoxyisobutyric acid (PCIB) and 2,3,5-triiodobenzoic acid (TIBA) on lateral buds propagation and shoots elongation from *Aglaonema* 'Lady Valentine' lateral shoots culture. (a) after culture for five days, lateral buds began to form on the stem layers (bar = 3 mm); (b) lateral buds grew into lateral shoots (bar = 6 mm); (c) lateral shoots elongated in medium supplemented with 1 mg/l PCIB (bar = 10 mm); (d) comparing the elongation of lateral shoots, from left: 10 mg/l TIBA, 1 mg/l TIBA and 1 mg/l PCIB (bar = 27 mm); (e) lateral shoot explants begin to form adventitious roots at the base (bar = 8 mm).

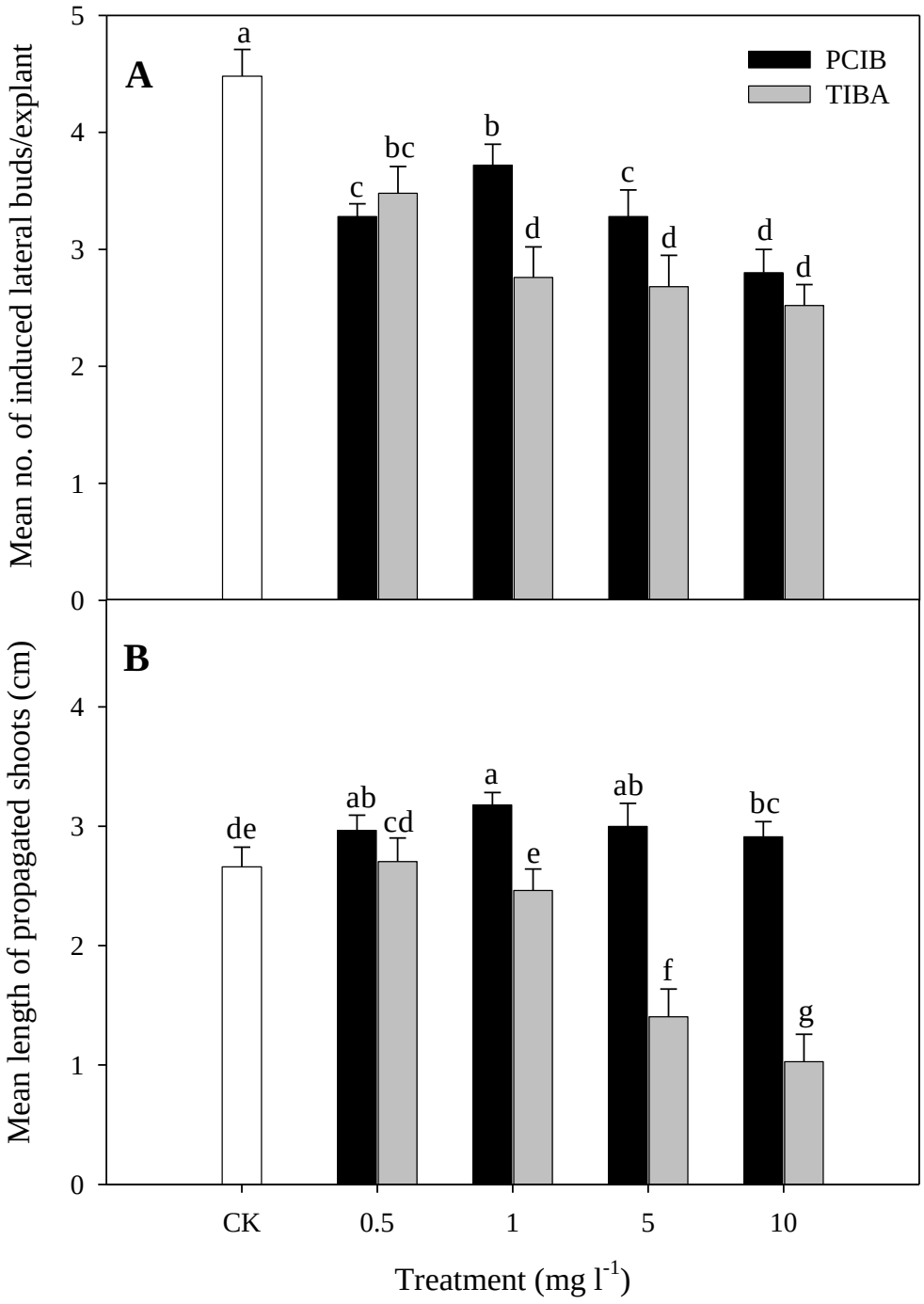

Fig. 2. Effect of different concentrations of PCIB and TIBA on lateral bud propagation and shoot elongation from *A.* 'Lady Valentine' lateral shoot explants culture. (a) lateral bud number; (b) shoot length.

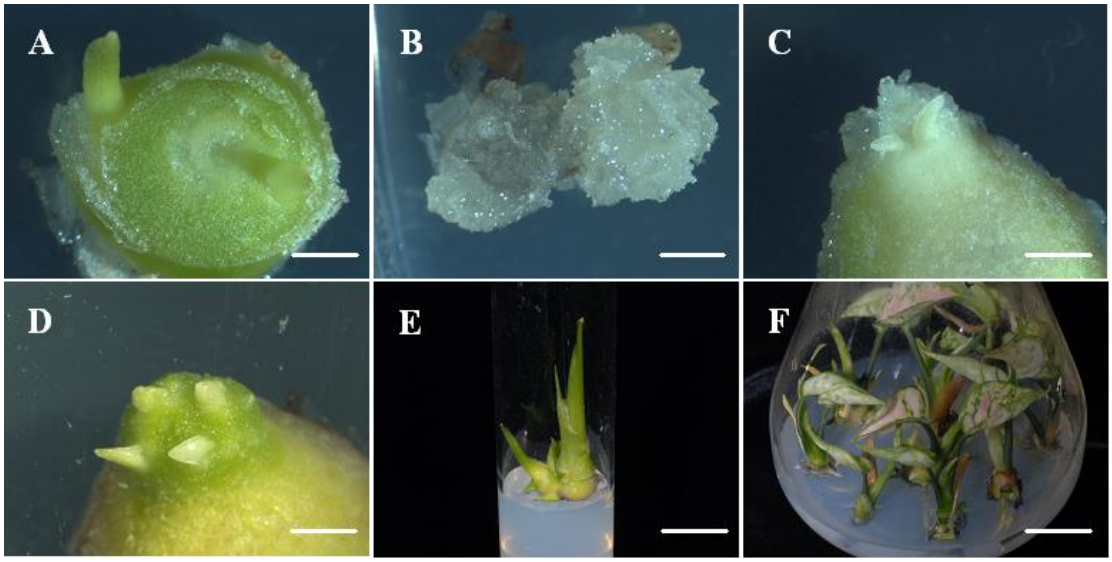

Fig. 3. Effect of PCIB and TIBA on callus induction and plantlet regeneration from *A.* 'Lady Valentine' stem section explants culture. (a and b) transparent granular cells and loose-textured non-organogenic calli dervied from stem discs after a 4-week culture on 1/3MS basal medium supplemented with 3.0 mg/l BA + 0.5 mg/l TIBA (bar = 3 mm); (c) excess non-organogenic calli grown on 1/3MS basal medium supplemented with 3.0 mg/l BA + 1.0 mg/l PCIB cover the existing organs (bar = 3 mm); (d) organogenic callus induction and bud primordia regeneration after culture in 1/3MS basal medium with 3.0 mg/l BA (bar = 3 mm); (e) adventitious shoot transplanted to 1/3MS basal medium with 3.0 mg/l BA + 1.0 mg/l PCIB to promote elongation (bar = 13 mm); (f) shoots developed into vigorous plantlets (bar = 20 mm).

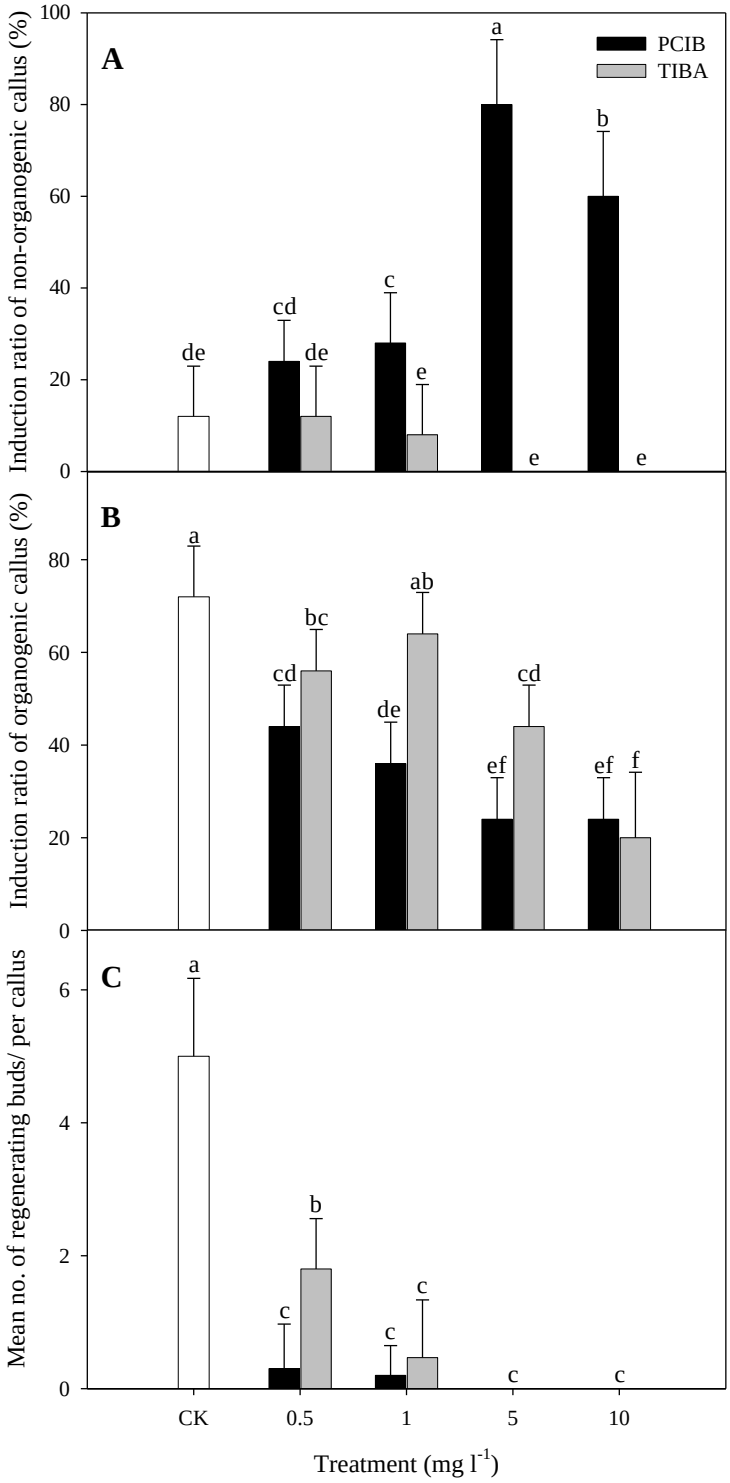

Fig. 4. Effect of different concentrations of PCIB and TIBA on callus induction and adventitious bud regeneration from *A.* 'Lady Valentine' stem section explants culture. (a) non-organogenic callus formation; (b) organogenic callus formation; (c) adventitious buds regeneration from callus.