台灣農業研究
JOURNAL OF TAIWAN AGRICULTURAL RESEARCH
Vol.66 No.4 2017

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ISSN 0022-4847
Published by Taiwan Agricultural Research Institute, Council of Agriculture

台灣農業研究 JOURNAL OF TAIWAN AGRICULTURAL RESEARCH Vol.66 No.4 2017

ISSN 0022-4847
DOI:10.6156/JTAR

台灣農業研究
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行政院農業委員會農業試驗所出版

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台灣農業研究

第六十六卷　第四期　　中華民國一〇六年十二月三十一日

出版者　行政院農業委員會農業試驗所
發行人　陳駿季
總編輯　楊純明
地址　41362 台中市霧峰區萬豐里中正路 189 號
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電話　(04)2331-7236
E-mail　jtar@tari.gov.tw
線上投審稿系統　http://aspers.airiti.com/JTAR

編印　Airiti Press Inc.
地址　23452 新北市永和區成功路一段 80 號 18 樓
電話　(02)2926-6006
E-mail　press@airiti.com

定價　新台幣一七五元
展售門市　1. 國家書店／ 10485 台北市松江路 209 號 1 樓／電話 : (02) 2518-0207
國家網路書店 http://www.govbooks.com.tw
2. 五南文化廣場／ 40042 台中市中山路 6 號／電話 : (04) 2226-0330

台灣農業研究 (J. Taiwan Agric. Res.) 66(4):267–275 (2017)
DOI: 10.6156/JTAR/2017.06604.01

Research Article

Evaluation of Flower Thinning Applied to Yardlong Bean Production

Chen-Yu Lin[1], Ya-Suie Hwang[2], Hsiang-I Lee[1], Hui-Ling Lo[3], and San-Tai Wang[4,*]

Abstract

Lin, C. Y., Y. S. Hwang, H. I. Lee, H. L. Lo, and S. T. Wang. 2017. Evaluation of flower thinning applied to yardlong bean production. J. Taiwan Agric. Res. 66(4):267–275.

Due to fungal infection, using fungicide on yardlong bean during the harvest period is unavoidable. Application of fungicide caused fungicide residue on mature pods, as a result, the pods could not meet the recommended standards for edible period. In this study, we used flower thinning technique by spraying fungicide at juvenile pod stage instead of mature pod stage. The influence of flower thinning on yield of yardlong bean was evaluated. The results demonstrated that accumulative yield of 7-d interval of flower thinning treatment was significantly higher than 10-d interval. Hand thinning stimulated flower formation. The weekly yield of flower thinning treatment showed no significant difference compared to no thinning treatment. The trial attempted to improve hand flower thinning practice for saving labor cost. In consideration of food safety, organic materials were used as flower thinning agent in this study. The flowers dropped or delayed growth without leaves burning after treating organic material B. Phosphorus acid and potassium hydroxide mixture could effectively substitute hand flower thinning in yardlong bean production. However, the efficacy of flower thinning was affected by climatic interference in the field such as rain. The results indicated that the better ratio of phosphorus acid to potassium hydroxide was 1 : 0.7 and the mixture had a potential to use as flower thinning agent.

Key words: Yardlong bean, Flower thinning, Yield, Phosphorus acid, Potassium hydroxide.

INTRODUCTION

Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) is a climbing crop and harvest daily in the field. In recent years, the public concern about food safety and chemical residue in yardlong beans. Continuous harvest encounters a great difficulty in control of fungicide residue on mature pods during harvest period. Because of a continuous harvest, application of fungicide damages food safety when yardlong bean approaching to maturity. Based on the current guideline, the shortest harvest period for a safe product is 3 d after fungicide spraying. However, yardlong bean forms flower and pods daily, obeying this guideline means no harvest and no income for farmers during the 3-d period. Moreover, previous

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literature indicated that yield varied in different productive seasons and disease pressure (Coker *et al*. 2007). The aim of this technique is to create a spraying time in the juvenile pod stage. By this technique, farmer could avoid these unsafe periods until pods reach maturity. Therefore, we interrupted pod generation daily by flower thinning technique and evaluated the impact on the yield of yardlong bean.

Flower thinning techniques have been applied to several fruit crops such as loquat, blueberry and apples (Razeto *et al*. 2003; Williamson & NeSmit 2007; Hehnen *et al*. 2012). Previous reports demonstrated that flower thinning increased the quality and yield in kiwifruit (Pescie & Strik 2004) and peach (Sauerteig & Cline 2013). Most flower thinning techniques were used on fruit crops. For continuous harvest crop, hand thinning was not economical for farmer's practice. However, hand thinning of flowers provided useful information on yield evaluation of yardlong bean in this study. Several chemical thinning agents have been identified to decrease fruit number such as ammonium thiosulfate, potassium thiosulfate, hydrogen cyanamide, and naphthalene acetic acid (Razeto *et al*. 2003; Milić *et al*. 2011; Kacal & Koyuncu 2012). The appropriate application time and concentration of chemical thinning agents are important factors. These skills determined fruit growth and quality (Razeto *et al*. 2003; Pescie & Strik 2004; Fukuda *et al*. 2015). However, yardlong bean growth characteristic was significant different with fruit crops. The proper interval of flower thinning treatment needs to be modified during continuous harvest periods. On the other hand, the chemical thinning residue was unfavorable and inedible. Therefore, this trial sought for safe flower thinning material as an alternate strategy.

The flower drop phenomenon occurred after rain in the Meinong district of Kaohsiung City in Taiwan. This possibility of flower drop caused by acid rain in Kaohsiung area inspired us to have the idea of thinning flowers with acid agent. Phosphorus acid was chosen in this trial in consideration of organic cultivation. Neutralized phosphorus acid has been used in organic cultivation and protects plants from pathogens by inducing plant systemic resistance (Guest 1984; Panicker & Gangadharan 1999; McDonald *et al*. 2001; Choudhary *et al*. 2007; Keinath & DuBose 2012). Related reports demonstrated the efficiency of phosphate salts by foliar sprays on foliar diseases (Reuveni *et al*. 1996; Walker 1998). However, there is no literature on using phosphorus acid as flower thinning agent in yardlong bean production. This innovative study attempted to evaluate the feasibility of flower thinning by hand and phosphorus acid. The objectives of this study are (1) to assess a better interval of hand flower thinning time, (2) to investigate the effect of row spacing on the yield of hand flower thinning treatment, and (3) to evaluate the efficacy and yield by using organic flower thinning agent.

MATERIALS AND METHODS

Three trials were conducted in the field at Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung, during the fall of 2012 to 2014, respectively. Seeds of yardlong bean cv. 'San-Tse-Chin-Pi' used in this study were obtained from farmer of Ligang Township. Seeds were immersed with 42℃ water for 2 h to improve germination rate before sowing in 50-well pots (4.5 cm in diameter and filledll with peatmoss). Seedlings were transplanted to field 14 d after sowing. The effect of flower thinning spraying interval on yield was assessed in trial 1. The targets of flower thinning were blooming flowers and thinned by hand on the same day. Application of two flower thinning intervals (7- and 10-d intervals) were compared with a non-treated control. Yardlong beans planted along rows with 40 cm between plants and the density was 8 plants per 10 m^2. Three treatments were compared in this trial, including (1) no flower thinning

(control check), (2) flower thinning at 7-d interval, and (3) flower thinning at 10-d interval. Trial was randomized completely block design (RCBD) with eight plants in each replicate and three replicates for each treatment.

To evaluate the influence of flower thinning under different planting densities, yield of two rows with spacing 40 cm and 80 cm were investigated in trial 2. Yardlong bean planted in a single row for each of densities, 8 plants per 10 m^2 and 4 plants per 10 m^2, respectively. Four treatments were compared in this trial, including (1) no flower thinning with 40 cm between plants, (2) no flower thinning with 80 cm between plants, (3) flower thinning with 40 cm between plants, and (4) flower thinning with 80 cm between plants. The trial was randomized completely block design (RCBD) with 10 m^2 area of each replicate and three replicates in each treatment. This trial concerned about the production loss caused by the elimination of flower. Flower thinning treatments were carried out by hands and calculated the number of eliminated flowers weekly.

Development of labor-saving strategies for improving flower thinning was the main objective for trial 3. We used phosphorus acid and potassium hydroxide in different ratios to evaluate the efficiency of flower thinning on yield. Two ratios of phosphorus acid and potassium hydroxide were used as organic material A and organic material B. The ratios of organic material A to B were 1 : 0.6 and 1 : 0.7, and pH were 2.3 and 4.3, respectively. Four treatments were compared in this trial, including (1) no flower thinning as a control, (2) flower thinning by hand, (3) organic material A, and (4) organic material B. A period of three days after spraying fungicide was recommended for conventional harvest period in yardlong bean. Therefore, this trial simulated safe yardlong bean production and assessed harvest pods weekly. Seeds of yardlong beans were planted in double rows with 40 cm between plants and the density was 16 plants per 10 m^2. The trial was randomized completely block design (RCBD) with 10 m^2 area of each replicate and three replicates for each treatment.

RESULTS AND DISCUSSION

Continuous harvest crops like yardlong bean are harvested almost daily. It means that fungicide will remain on the mature yardlong bean. In this study, we attempted to create a fungicide spraying opportunity in juvenile pod stage after flower thinning. Several days after spraying, these juvenile pods become mature and could avoid from fungicide residue. However, the method is highly questioned and concerned about yield loss. We focus on the influence of yield by flower thinning.

Days required from flowering to pod maturity of yardlong bean cv. ‘San-Tse-Chin-Pi’ are between 7 to 10 d during spring to summer in Kaohsiung area. According to pods forming period, we evaluated the influence of flower thinning interval on accumulated yield in trial 1. The result showed a better accumulated yield at 7-d interval compared to10-d interval in the previous five weeks (Fig. 1). The accumulated yield of flower thinning at 7-d interval treatment was also better than no thinning control during the third to fifth week. According to this result, 7-d interval of flower thinning was performed in subsequent trials.

Farmers concerned about the yield drop after eliminating the flowers by flower thinning. Previous report indicated that mechanical and hand blossom thinning decreased fruit set (Sauerteig & Cline 2013), but increased marketable yield (Pescie & Strik 2004). Another literature demonstrated that one or two flower thinnings performed well in fruit trees (Hehnen *et al*. 2012). Different from fruit trees, yardlong bean belongs to continuous harvest crop having successive flowering and pod formation almost daily. This study investigated the influence of several times of flower thinning on yield. In trial 2, flower thinning was conducted in two row spacings, 40 cm and 80 cm. Flower number, elimination amount and yield were calculated and compared weekly.

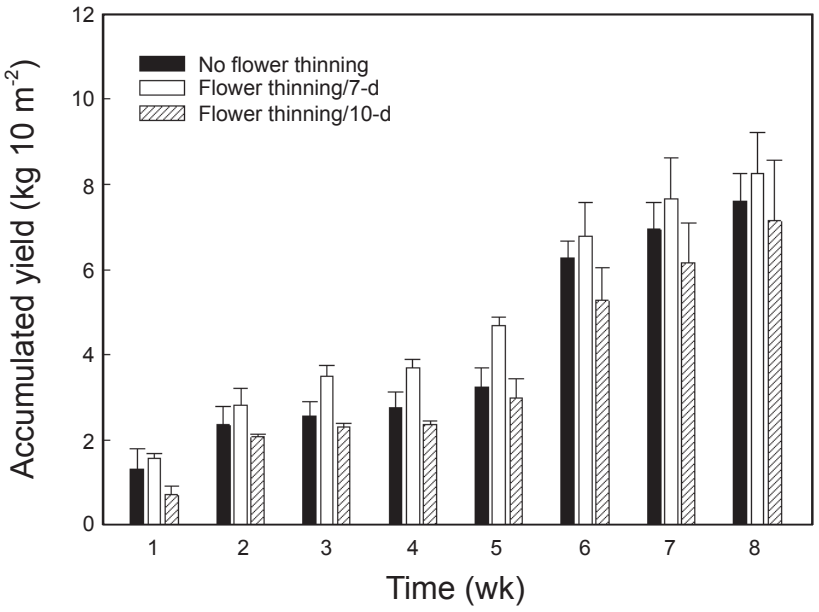


Fig. 1. Influence of different flower thinning intervals on the accumulated yield of yardlong bean. Error bars show the standard error of the mean (SE).

The flower thinning treatments showed a better flower formation weekly (Figs. 2A–2B). The total number of flowers of thinning treatment was significantly higher compared to no flower thinning treatment during the fourth to sixth week in 40 cm spacing. On the other hand, the weekly yield of flower thinning treatments showed no significant difference except the fourth to fifth week in 40 cm spacing compared with no thinning treatment (Fig. 3A). Moreover, compared to the accumulated yield in 40 cm spacing, flower thinning treatment was significantly higher than no flower thinning during the fourth to eighth week (Fig. 3B). Fukuda *et al*. (2015) indicated that flower thinning increased fruit number and quality in peaches. This study showed the relationship between flower number and yield. In general, the accumulated yield of flower thinning in plant spacing of 40 cm treatment was significantly higher than that of 80 cm treatment. We speculate that such a result was caused by the double plant density and the competition of growing space. Results of this trial suggest that flower thinning technique could be performed in row spacing of 40 cm. Flower thinning at 7-d interval obviously increased flower amounts and total yield.

Flower thinning technique was performed by hands in the previous trails. However, this technique required more labor and could not meet farmers' demand. Use of flower thinning agents may be a better choice to reduce the labor cost. Previous report indicated that application of gibberellic acid with a suitable concentration increased fruit weight and yield (González-Rossia *et al*. 2006), and reduced the number of flowering buds (Giovanaz *et al*. 2016). In consideration of continuous harvest and food safety, we were looking forward to seeking a flower thinning method with similar production, but without thinning, to reduce labor cost. In trial 3, we sought a convenient method to eliminate flowers and save the labor cost. The mixture of phosphorus acid and potassium hydroxide with different ratios were evaluated to eliminate flowers in this study. The accumulated yield showed significant difference after treated for four weeks. Flower thinning treatments either by hand or organic

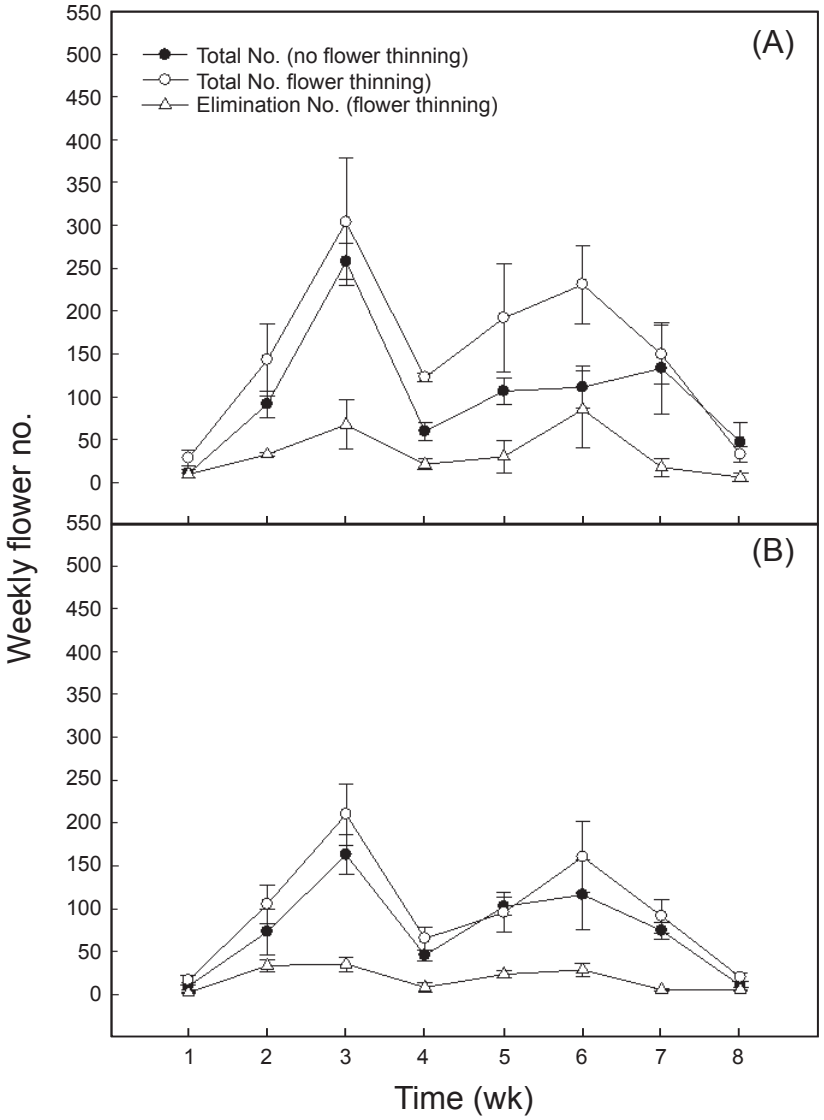


Fig. 2. Number of weekly flower formation in row spacing of 40 cm (A) and 80 cm (B). Error bars show the standard error of the mean (SE).

materials showed a better accumulated yield than no flower thinning treatment (Fig. 4A). The definition of safe and edible pods are pods harvested 3 d after spraying fungicide. In such a way, pods can be harvested four times a week. Simulation of weekly safe yield can be deducted from unsafe harvest period (Fig. 4B). The efficacy of organic materials on flower thinning was similar to hand flower thinning. Compared to weekly safe yield, although there were significantly different in most weeks except the fourth week, organic material treatments showed a better trend than no flower thinning treatment, implying more income for farmers. According to this trial, two organic materials A and B showed slightly different in eliminating flower patterns. After spraying organic material A, flowers dropped and leaves burned. The results were similar to ammonium thiosulfate or potassium thiosulfate. Both

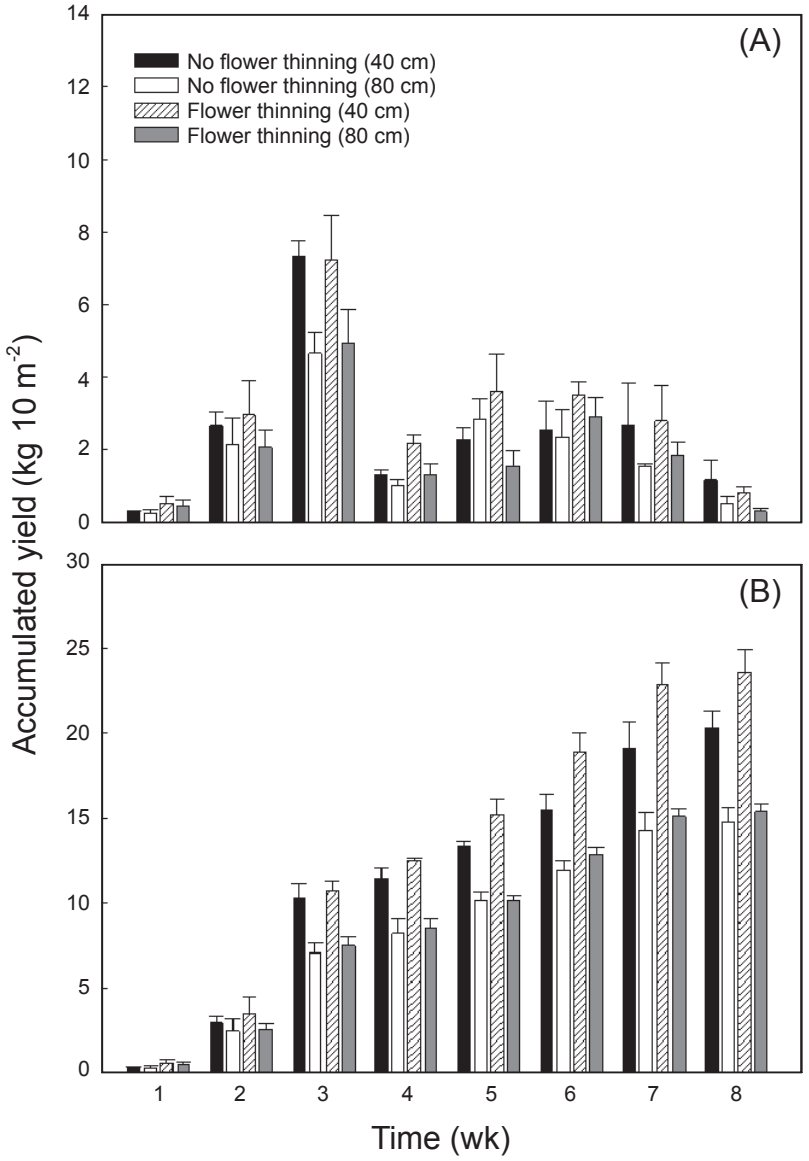


Fig. 3. Effect of hand flower thinning on weekly yield (A) and accumulated yield (B) in different row spacings. Error bars show the standard error of the mean (SE).

have been used as commercial flower thinning agents (Milić *et al*. 2011). On the other hand, the organic material B treatment showed flower drop and delay of partial flower development. This phenomenon was similar to the delay of flower bud differentiation by gibberellin sprays (García-Pallas *et al*. 2001). Results of this trial suggest that organic materials B was a better labor saving choice although the mechanism of flower thinning was indefinite. We surmise that acid materials induce flowers drop or inhibit flower development after spraying on plant. Moreover, the efficacy may be related to climatic factor such as rain and the suitable concentration of flower thinning agent needs an adjustment.

Overall, the results revealed that flower thinning in yardlong bean would maintain ac-

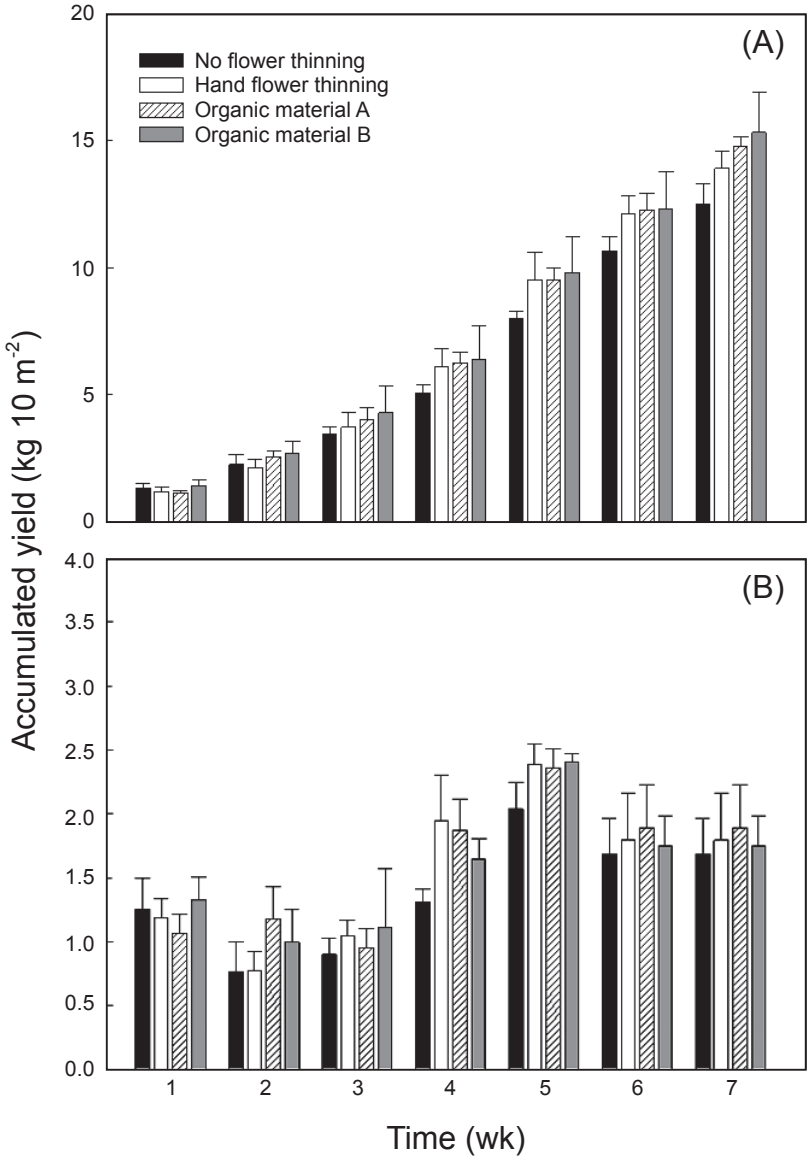


Fig. 4. Effect of different flower thinning methods on accumulated yield (A) and weekly safe yield (B). Error bars show the standard error of the mean (SE).

cumulated yield and stimulate the formation of flowers. These trials solved the farmer's doubt about yield reduction. This study demonstrated that 7-d was the best flower thinning interval under row spacing of 40 cm. Hand flower thinning showed a better flower formation. The mixture of phosphorus acid and potassium hydroxide saved the labor cost of flower thinning and may be a potential technique in future.

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疏花在長豇豆生產應用之評估

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摘要

林楨祐、黃雅穗、李香誼、羅惠齡、王三太。2017。疏花在長豇豆生產應用之評估。台灣農業研究 66(4):267–275。

長豇豆在收穫期間無可避免因罹病而需要噴施殺菌劑，當此成熟豆莢尚未達到農藥推薦使用之安全期標準而予以採收，極易有藥劑殘留於豆莢的情形。本研究中藉由疏花操作來創造幼莢階段，以提供噴施殺菌劑的機會，並評估疏花對長豇豆產量的影響。結果顯示，間隔 7 d 疏花一次，其產量顯著較間隔 10 d 疏花一次為高；在花朵量與產量方面，每週產量在相同種植株距中，人工疏花處理與不處理者幾乎無顯著的差異，但經由人工疏花可刺激更多花量的生成，且其總累積產量則有較佳的趨勢。此試驗中亦試圖改善因人工疏花導致勞力成本耗費的情形，在考量食品安全的前提下利用有機資材作為疏花劑，經處理有機資材 B 後，花朵呈現掉落或延遲生長，且無葉部燒傷現象，此亞磷酸和氫氧化鉀的混合物可有效地替代人工疏花。然而，疏花的有效性易受下雨等天候的干擾，該混合物在混合比率 1：0.7 的情形下具有當作長豇豆疏花劑的潛力。

關鍵詞：長豇豆、疏花、產量、亞磷酸、氫氧化鉀。

投稿日期：2016 年 11 月 5 日；接受日期：2016 年 12 月 22 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):276–285 (2017)
DOI: 10.6156/JTAR/2017.06604.02

Research Article

A Method for the Specific Detection of *Phomopsis destruens* in Sweet Potato by PCR

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Abstract

Lin, C. Y., C. W. Huang, H. R. Yang, S. Y. Lai, and H. F. Ni. 2017. A method for the specific detection of *Phomopsis destruens* in sweet potato by PCR. J. Taiwan Agric. Res. 66(4):276–285.

Foot rot caused by *Phomopsis destruens* (Harter) Boerema is a major concern for sweet potato production in Taiwan. The conventional methods for the identification of *P. destruens* are based on culture isolation and morphological analyses, but they are time-consuming and often inconclusive. To assist in a rapid and specific detection of the infection, a polymerase chain reaction (PCR) assay was developed to detect the pathogen from DNA extracted from fungi and plant tissues, respectively. Internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) from *P. destruens* isolates were used for the specific primers design. The amplification of expected 298 bp PCR products was obtained in all *P. destruens* isolates, but not for other fungal isolates from sweet potato storage roots including *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-3) and *Fusarium* sp. (Fu245). The PCR assay with the primers could detect 1 ng of DNA template. To enhance the sensitivity of detection, a nested PCR was performed and the detection limit was raised up to 10 pg. In addition, the primers proved efficient in detection of the pathogen in stems and storage roots of infected sweet potato. Positive detection was observed in 93% of the naturally infected stems of sweet potato, a rate which constituted a significant improvement in the identification of *P. destruens* in sweet potato as compared to conventional methods. These results indicated that the new PCR assay provided a rapid, specific and reliable diagnosis tool for the detection of sweet potato foot rot pathogen.

Key words: *Ipomoea batatas*, *Phomopsis destruens*, Foot rot, PCR assays.

INTRODUCTION

Sweet potato (*Ipomotea batatas* L.) grows in tropical and subtropical regions and belongs to the family Convolvulaceae. It ranks as the world's seventh most important food crop after wheat, rice, maize, potato, barley and cassava (Food and Agriculture Organization of the United Nations 2011). Sweet potato is an important crop in Taiwan, accounting for a total planted area of 9,819 ha in 2015, and is mainly cultivated in Yunlin County and Changhua County (http://www.afa.gov.tw/Public/Grain-Statistics/2016526104225334.pdf). The most popular sweet potato cultivars for tube produc-

Received: October 19, 2016; Accepted: February 16, 2017.
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tion are 'Tainung 57' ('TNG 57') and 'Tainung 66' ('TNG 66'), while the sweet potato cultivars of 'Tainung 71' ('TNG 71') and 'Taoyuan 2' ('TAY 2') are consumed as a leafy vegetable (Hsing *et al.* 2000; Lai *et al.* 2000).

Sweet potato is susceptible to a wide range of pests and pathogens, the effects of which can cause substantial economic loss. Some of these pests and pathogens have been reported in Taiwan, including bacterial wilt (*Ralstonia solanacearum*), scab (*Elsinoe batatas*), stem rot (*Fusarium oxysporum* f.sp. *batatas*), soft rot (*Rhizopus stolonifer*), Java black rot (*Lasiodiplodia theobromae*), foot rot (*Phomopsis destruens*), witches' broom (a mycoplasma-like organism), and virus diseases (Hsu *et al.* 2002; Chen *et al.* 2012; Huang *et al.* 2012; Shen *et al.* 2013; Huang *et al.* 2016).

Foot rot of sweet potato was first observed in the vicinity of the Dismal Swamp region of Virginia of U.S.A. in 1912, with almost 95% of the sweet potato plants in the area having been infected (Harter 1913a, 1913b; Harter & Weimer 1929). In Brazil, foot rot has been a destructive disease of sweet potato, with yield losses from the disease reaching as high as 80% (Lopes *et al.* 1994). Although the disease has been observed elsewhere in the world for more than ten decades, it was first observed in Taiwan from 2008 through 2011 (Huang *et al.* 2012), during which time it became a major concern of sweet potato producers. In those years, the disease incidence reached as high as 64% and resulted in considerable economic losses in Taiwan (Huang *et al.* 2012).

The symptoms of the disease on an infected sweet potato include black stem lesions at the soil level, vine wilting, storage root rot and, finally, plant death due to girdling of the base of the stem. Several plant diseases such as fusarium wilt and fusarium root rot show similar symptoms. The conventional methods used to identify pathogens often rely on visual symptoms, culturing isolation and morphological characteristics, and these methods are time-consuming and often inaccurate. In addition, *P. destruens* is a slow-growing fungus that may take at least 21 d to produce a noticeable colony with a diameter of 8.5 cm on a culture medium at 25℃ (Huang *et al.* 2012), and often resulting in a lack of clarity in identification due to the simultaneous growth of other nonpathogenic fungi that cover the colony of *P. destruens*. Therefore, it is necessary to develop a rapid and specific detection method for *P. destruens* that can facilitate the screening of sweet potato samples.

A rapid and specific detection and identification method is required for plant pathogen diagnosis and disease management. Molecular techniques based on polymerase chain reaction (PCR) amplification offer a specific and faster means of pathogen diagnosis (Ferrer *et al.* 2001; McCartney *et al.* 2003; Rickerts *et al.* 2007), and PCR-based methods for identifying *Phomopsis* spp. from plant tissues have previously been reported (Shishido *et al.* 2010; Jayaramaiah *et al.* 2013; Shirahatti *et al.* 2015). However, none of those methods are available for *P. destruens*. As such, the objective of this study was to develop a rapid and specific PCR-based identification method for detecting the presence of *P. destruens* within sweet potato tissue.

MATERIALS AND METHODS

Plants and fungal materials

Six fungi commonly found in sweet potato were selected to test the new set of specific primers. All the fungi including three isolates of *P. destruens* (SPPD-1, 5, and 7, which were isolated from Chiayi County, Changhua County and Nantou County in Taiwan, respectively), *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were lab collected and cultured separately on potato dextrose agar (PDA) mediun. The sweet potato plants naturally infected with *P. destruens* were obtained from a field in Chiayi County, Taiwan.

DNA extraction

The mycelia of *P. destruens* (SPPD-1, 5, 7), *L. theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *A. rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were grown in potato dextrose broth (PDB) at 25℃ for 7 d, respectively. The mycelia of the different types of fungi were then harvested by centrifugation (2,500× *g* for 5 min). The genomic DNA was extracted from these samples using a commercial MasterPure yeast DNA purification kit (Epicentre, Madison, WI) according to manufacturer's instructions. The extracted DNA from the fungal cultures was then quantified spectrophotometrically at OD260/280 nm with ratios between 1.7 to 1.8, and the sensitivity of the specific primers was evaluated by PCR amplification with serial diluted concentrations (100 ng–10 fg) of purified genomic DNA isolated from the fungal samples as described previously.

A NaOH-based method was used for plant DNA extraction. Basically, the NaOH extraction followed the protocol of Wang *et al.* (1993) with some modifications. The diseased tissue (0.2 cm × 1 cm) of sweet potato was homogenized using a sterilized plastic pestle in a microcentrifuge tube with 150 µl 0.5 N NaOH. The samples were centrifuged at 16,200× *g* for 5 min and the supernatant was diluted 1:10 in 100 mM Tris-HCl (pH 8).

Primer design

The 18S rRNA gene sequences of *P. destruens* (accession no. JN848791, JX421687 and JX421688), and two other pathogens of sweet potato including *L. theobrome* (B2225) (accession no. KX345935) and *A. rolfsii* (Sc-03) (accession no. KX186998), and one fungus commonly isolated from sweet potato: *Fusarium* sp. (Fu245) (accession no. KX345936) was obtained from the NCBI GenBank database and aligned to determine the pattern of sequences. The specific primers for foot rot of sweet potato were designed based on the regions that were conserved in the *P. destruens* isolates, but not conserved with other fungi. The specific primers for *P. destruens* were SPPD-3F: 5'-TCTCTGCTGAGGCCCCCGGAGA-3' and SPPD-3R: 5'-AAGGCAGTGCCCCATCACCAAGCCAG-3'. The fungal ribosomal 18S rRNA gene and internal transcribed spacer (ITS) region with primer binding locations are shown in Figure 1.

PCR amplification

The PCR reaction was performed in 25 µL volumes containing 1 µL of the DNA, 5 µL 5× PCR reaction buffer, 1.25 mM dNTPs, 7.5 mM $MgCl_2$, 5 pmol of each primer (SPPD-3F/SPPD-3R) and 0.75 U Tag DNA polymerase (Protech, Taiwan). PCR amplification was carried out in a thermal cycler (SensoQuest LabCycler 011-101, Göttingen, Germany) with initial denaturation at 94℃ for 4 min, followed by 30 cycles of denaturation at 94℃ for 20 s, annealing at 60℃ for 30 s and extension at 72℃ for 40 s. The thermal cycles were terminated by a final extension at 72℃ for 7 min. Ten microliters of PCR products were separated by electrophoresis in 2% agarose gel in 0.5 TBE buffer. Gels were photographed under UV light. For nested PCR, the universal primers ITS1/ITS4 (White *et al.* 1990) were used in a first-round of fungus-specific amplification. The resulting PCR products were diluted 1/100 with 1× Tris EDTA (TE) buffer and subsequently used as template DNA in a second PCR amplification using the specific primers SPPD-3F/SPPD-3R.

A comparison of methods for the diagnosis and identification of *P. destruens*

The sweet potato plants showed the symptom of vine blackening around the soil level, and the black pycnidia produced near the surface of the naturally infected vines were collected as samples for the identification of the presence of *P. destruens* by developed PCR-based method and the traditional method of using the medium to isolate fungi from the diseased tissue. The PCR-based method was performed as described previously. For the traditional method, the fresh diseased vines



Fig. 1. Schematic of the fungal ribosomal 18S rRNA gene and ITS regions. (A) The positions of primers and the sizes of amplified fragments are indicated. ITS, internal transcribed spacer region; (B) Sequence alignment of *Phomopsis destruens* and other three sweet potato fungi [*Lasiodiplodia theobrome* (B2225, accession no. KX345935), *Fusarium* sp. (Fu245, accession no. KX345936) and *Athelia rolfsii* (Sc-03, accession no. KX186998)]. The positions and sequences of the primers are indicated as boxed and bold capital letters.

were surface-sterilized with 75% ethanol and cut with a sterile scalpel into approximately 3 mm pieces. Surface-sterilized vine cuttings were then transferred onto the PDA medium containing 0.25% lactic acid and incubated at 25℃ until fungal hyphae emerged from the plant tissue. Based on morphological differences (such as difference in the color and shape of mycelia), fungal cultures were picked and cultured on PDA plates.

RESULTS

Primer design

After alignment of the conserved regions of *P. destruens* and the other three sweet potato fungi [*L. theobrome* (B2225), *Fusarium* sp. (Fu245) and *A. rolfsii* (Sc-03)], the specific primers for *P. destruens* SPPD-3F (location: nt 103–125 of *P. destruens* accession no. JN848791) and SPPD-3R (location: nt 375–400 of *P. destruens* accession no. JN848791) were selected and used (Fig. 1B). The SPPD-3F/SPPD-3R primer pair was predicted to yield a PCR product of 298 bp (Fig. 1A)

PCR specificity evaluation

To determine whether cross-amplification would occur, DNA samples from other fungi of sweet potato including *L. theobrome* (B2225),

Phomopsis sp. (Ph735), *Rhizopus* sp., *A. rolfsii* (Sc-03) and *Fusarium* sp. (Fu245) were tested for the primer set. The results indicated that the 298 bp fragment was amplified with the primers SPPD-3F/SPPD-3R from all three isolates of *P. destruens*. No amplification fragment was observed with the genomic DNA isolated from the other five fungi (Fig. 2). The specificity of the 298 bp fragments produced was verified by direct DNA sequencing, and the fragment was matched with the *P. destruens* DNA sequences (data not shown).

PCR sensitivity evaluation

The sensitivity was evaluated using two PCR-based methods. The genomic DNA was extracted from fungal cultures and diluted subsequently. For PCR amplification with the primers SPPD-3F/SPPD-3R, the sensitivity was found to range from 1 ng to 100 pg (Fig. 3A). For nested PCR, the universal primers ITS1/ITS4 were used in the first PCR amplification and followed by the specific primers SPPD-3F/SPPD-3R of *P. destruens*. The result indicated that the sensitivity was thereby improved to 10 pg (Fig. 3B).

Detection of *P. destruens* in sweet potato by PCR

DNA was extracted from infected root tubers and vines of sweet potato using a NaOH-based method and then used as the template for PCR with the specific primers SPPD-3F/SPPD-3R. The 298 bp PCR fragment was observed in all the tested infected samples (Fig. 4).

A comparison of methods for diagnosis and identification of *P. destruens*

Two methods, one of which consisted of a PCR-based detection method with the specific primers SPPD-3F/SPPD-3R and the other of which was a conventional method consisting of fungi culture isolation, were utilized to evaluate the efficiency of diagnosis and identification of *P. destruens*. In each experiment, 18 or 19 samples out of 20 tested plant tissues (Fig. 5A) showed a positive reaction in PCR detection with the primers SPPD-3F/SPPD-3R. On average, the presence of *P. destruens* was detected in 18.6 of 20 plant tissues (93%) (Table 1). For the conventional method of using the medium to isolate fungi from the plant tissues, the orange-yellow color hyphae of *P. destruens* emerged for 4 to 11 out of the 20 tested plant tissues (Fig. 5B), and the fungal cultures were isolated and cultured on PDA plates (Fig. 5D). On average, *P. destruens* isolates were isolated from 7.3 of 20 plant tissues (38%) (Table 1). Colonies of non-target fungi such as sapro-

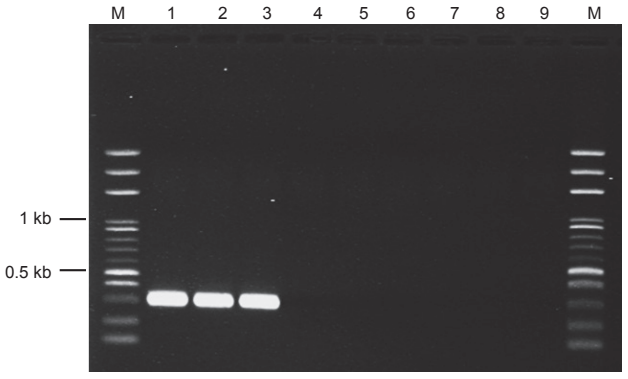


Fig. 2. Specificity of PCR amplification by primers SPPD-3F/SPPD-3R from various samples of genomic DNA. Genomic DNA was extracted from the fungal mycelia of sweet potato fungi and amplified with specific primers for foot rot of sweet potato. Lanes 1–3, *Phomopsis destruens* SPPD-1, 5, and 7; lanes 4–8, *Lasiodiplodia theobrome* (B2225), *Phomopsis* sp. (Ph735), *Rhizopus* sp., *Athelia rolfsii* (Sc-3) and *Fusarium* sp. (Fu245); lane 9, negative control (without genomic DNA); M, molecular weight markers.

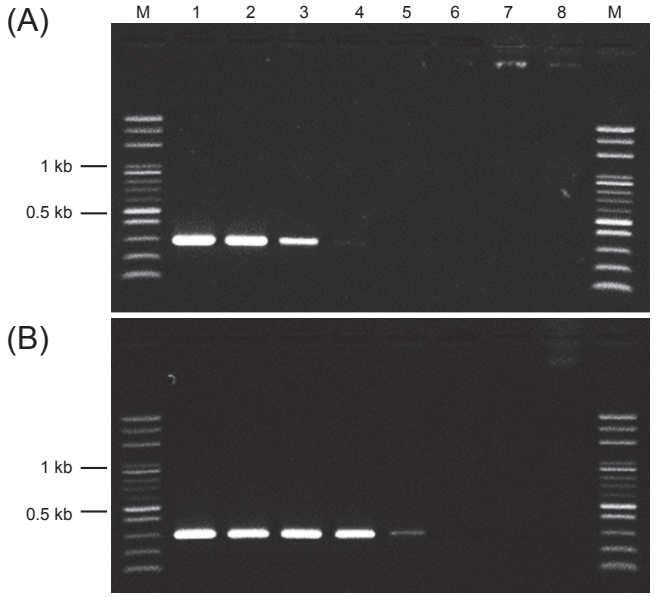


Fig. 3. Sensitivity of PCR amplification at the DNA level using 10-fold dilutions of *Phomopsis destruens* DNA. (A) Genomic DNA was extracted from the mycelia of *Phomopsis destruens* and amplified with the specific primers SPPD-3F/SPPD-3R; (B) Nested PCR using the ITS universal primers ITS1/ITS4 was followed by the specific primers SPPD-3F/SPPD-3R. Lane 1: 100 ng; lane 2: 10 ng; lane 3: 1 ng; lane 4: 100 pg; lane 5: 10 pg; lane 6: 1 pg; Lane 7: 100 fg; lane 8: 10 fg; M, molecular weight markers.

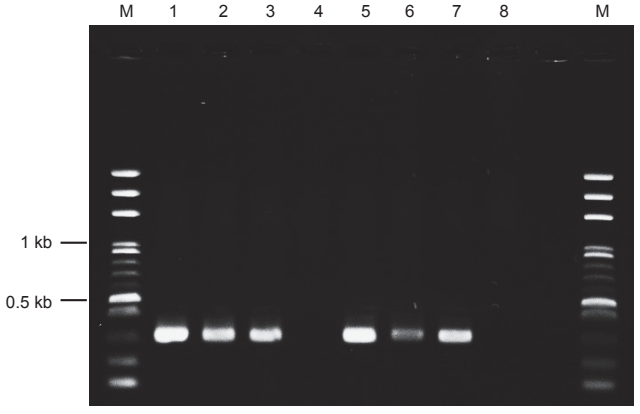


Fig. 4. Detection of *Phomopsis destruens* in sweet potato samples using the specific primers SPPD-3F/SPPD-3R. The DNA from the root tubers or vines of infected sweet potato plants was extracted with NaOH, and then used as the template for PCR. Lanes 1–3, the DNA from the infected sweet potato storage roots; lane 4, the DNA from healthy sweet potato storage roots; lanes 5–7, the DNA from the infected sweet potato stems; lane 8, healthy sweet potato stem; M, molecular weight markers.

phytic fungi were also observed on the residue of the culture plates (Fig. 5C).

DISCUSSION

In this study, the specific primers SPPD-3F/SPPD-3R were designed according to the ITS region sequences of *P. destruens* and were evaluated to develop a rapid and specific PCR method for detection of *P. destruens*. The results indicated that the developed PCR method could specifically and effectively detect the

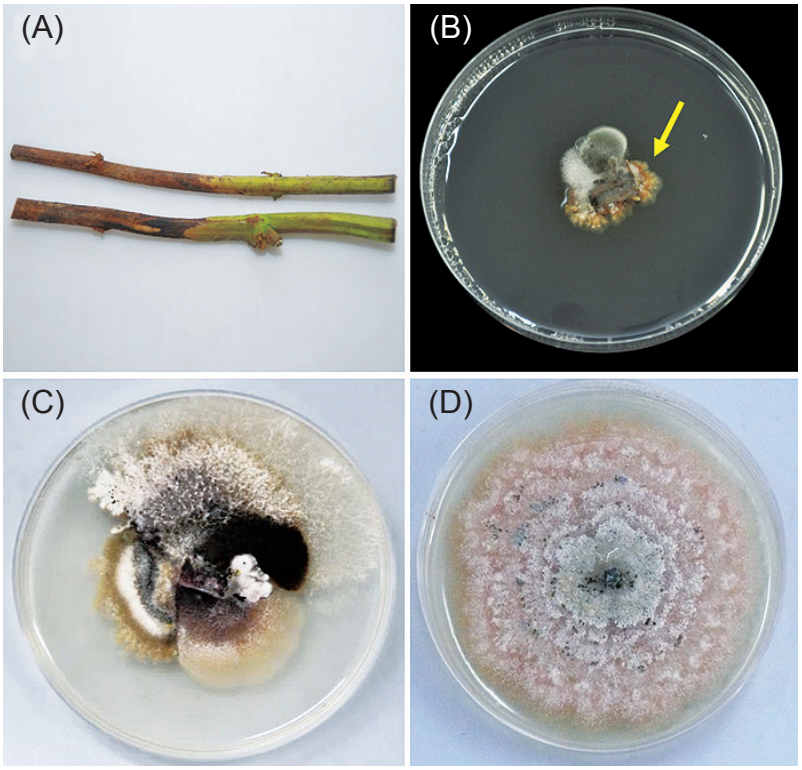


Fig. 5. Isolation of *Phomopsis destruens* from sweet potato with conventional method. (A) Sweet potato with foot rot symptoms; (B) *Phomopsis destruens* isolation on a lactic acid PDA (APDA) medium, with the arrow indicating a fungal colony of *Phomopsis destruens*; (C) *Phomopsis destruens* isolation on an APDA medium, but with the colony covered with non-pathogen fungus; (D) Pure culture of *Phomopsis destruens* on a PDA medium.

Table 1. Detection of *Phomopsis destruens* in sweet potato plants using different methods.

		No. of detected samples	
Experiment No.	*N*[z]	PCR method[y]	Conventional method[x]
1	20	18.0	10.0
2	20	18.0	7.0
3	20	19.0	11.0
4	20	19.0	6.0
5	20	19.0	4.0
avg.	20	18.6 (93%)	7.6 (38%)

[z] *N*, the number of sweet potato test samples with symptoms.
[y] Positive reaction in PCR amplification with specific primers SPPD-3F/ SPPD-3R.
[x] *Phomopsis destruens* was culture-isolated on PDA mediums.

presence of *P. destruens* from pure culture as well as from symptomatic sweet potato tissues and could provide a rapid and reliable diagnosis tool for sweet potato foot rot pathogen.

For PCR-based methods of species-specific detection, ribosomal DNA (rDNA) sequences such as the translation elongation factor 1-α (EF1-α) gene (Shirahatti *et al.* 2015) and ITS region sequences have been used for fungal species-specific detection, and the ITS se-

quences are widely utilized due to their characterization to the species level (Terashima *et al.* 2002; Zhao *et al.* 2007; Shishido *et al.* 2010; Santos *et al.* 2011). Therefore, the ITS regions of *P. destruens* was utilized to construct the SPPD-3F/SPPD-3R pair of PCR primers in this study. The primers successfully amplified the target DNA sequences of three isolates of *P. destruens* from different areas in Taiwan and were able to discriminate *P. destruens* from five species of plant pathogens of sweet potato including one other *Phomopsis* species (Fig. 2). These results indicated the specificity of the primers and confirmed the use of the primers in diagnostic applications.

The primers SPPD-3F/SPPD-3R were evaluated for their potential at low concentrations of the template and the results showed that the PCR products could be amplified from 1 ng to 100 pg of *P. destruens* DNA. The sensitivity of the PCR was promising when a nested PCR was performed even when the presence of pathogen DNA was at a concentration as low as 10 pg. The first PCR reaction used the universal primers ITS1/ITS4 (White *et al.* 1990) to enrich the fungal DNA template, thereby increasing the amplification efficiency of the *P. destruens* DNA at low concentrations.

The primers were used to detect the pathogen in plant tissues consisting of the stems and storage roots of sweet potato exhibiting typical symptoms, and the presence of the pathogen could be detected in both stems and roots. In addition, a simple, rapid and inexpensive NaOH-based method (Wang *et al.* 1993) for plant DNA extraction was used in this study, and only a small segment of sweet potato plant tissue (0.2 cm × 1 cm) was needed for such plant DNA extraction. The entire procedure could be completed in 4 h : 1.5 h for DNA extraction, 2 h for PCR amplification and 0.5 h for electrophoresis. This specific and rapid detection method for *P. destruens* had great practical importance for disease management as this method could also be used to identify disease-free seedlings in commercial nurseries.

One of the most common conventional methods for the diagnosis of fungi is culture isolation from a sample of diseased plant tissue. In this study, *P. destruens* was identified in only 38% of the tested infected vines using this method, and the method required at least 14 d for final identification, because of the slow growth of the *P. destruens* on culture medium (Huang *et al.* 2012). Some of non-pathogenic or saprophytic fungi that grew faster than *P. destruens* on medium affected the diagnosis and resulted in inaccurate identification (Fig. 5D). Relatedly, a selective medium for *P. destruens* is not available at present. These reasons may explain the low diagnosis efficiency of culture isolation in identifying *P. destruens*. While using the PCR method developed in this study, positive detection was observed for 93% of the tested infected vines. These results demonstrated the high specificity and sensitivity of the PCR method compared with the conventional methods.In summary, the results of the present study have demonstrated that the primer pair designed is effective in the detection and identification of *P. destruens*. Compared with conventional culture isolation and morphological methods, the developed method provides a specific and reliable diagnosis tool with significantly increased accuracy in identifying sweet potato foot rot infections, as well as required significantly less time to complete than those conventional methods.

ACKNOWLEDGMENTS

We wish to express our appreciation to Sui-Li Hsu and the staff of the Department of Plant Protection for their technical assistance.

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甘藷基腐病菌專一性 PCR 檢測技術之研發

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摘要

林靜宜、黃巧雯、楊宏仁、賴素玉、倪蕙芳。2017。甘藷基腐病菌專一性 PCR 檢測技術之研發。台灣農業研究 66(4):276–285。

甘藷基腐病 (foot rot) 是由 *Phomopsis destruens* 所引起，為近年來台灣甘藷生產的主要限制因子之一。目前 *P. destruens* 之鑑定仍依賴傳統方法，以病原菌之組織分離及形態特徵為主，不但耗時費力且成功分離出病原菌之比率不高。本研究依據 *P. destruens* 之去氧核醣核酸的內轉錄區 (internal transcribed spacer; ITS) 序列設計專一性引子對，並利用聚合酶連鎖反應 (polymerase chain reaction; PCR) 建立甘藷基腐病菌之檢測技術。結果顯示，本研究開發之檢測方法可專一性檢測 *P. destruens*，且對經常可於甘藷塊根上分離之 *Lasiodiplodia theobrome* (B2225)、*Phomopsis* sp. (Ph735)、*Rhizopus* sp.、*Athelia rolfsii* (Sc-3) 及 *Fusarium* sp. (Fu245) 等其他真菌並無誤檢反應。此法之靈敏度可達 1 ng 之菌絲 DNA，若利用巢式 PCR (nested PCR) 則靈敏度可提高至 10 pg 之菌絲 DNA。此外，本檢測方法亦可運用於分析受感染之甘藷莖部與塊根組織。而此技術於感染植物組織之檢測成效良好，93% 之受感染植物樣本可檢測出病原菌，相較於傳統鑑定法之 38%，可大幅提升其檢測成功率。此專一性 PCR 檢測法具有快速、專一性及靈敏度高之優點，未來可將此技術應用於檢測甘藷是否感染基腐病。

關鍵詞：甘藷、甘藷基腐病菌、甘藷基腐病、PCR 分析。

投稿日期：2016 年 10 月 19 日；接受日期：2017 年 02 月 16 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):286–297 (2017)
DOI: 10.6156/JTAR/2017.06604.03

研究報告

表面消毒、培植體及植物生長調節劑對建立孤挺花組織培養小鱗莖繁殖之影響

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摘要

陳威臣、丁一、吳姿穎、曹進義、夏奇鈱。2017。表面消毒、培植體及植物生長調節劑對建立孤挺花組織培養小鱗莖繁殖之影響。台灣農業研究 66(4):286–297。

本研究以孤挺花 (*Hippeastrum hybridum* Hort.)「紅獅」(‘Red Lion’)、「千禧之星」(‘Blossom Peacock’) 及「台農 1 號-紅粉佳人」(‘Tainung No.1-Pink Lady’) 等 3 個品種之鱗莖為材料，探討消毒方法、培植體部位及生長調節劑組合對消毒效率與小鱗莖增殖之影響。結果顯示，「紅獅」短縮莖培植體 (2 cm × 1 cm × 1 cm) 以 1.2% NaOCl 處理 15 min 之消毒效果較佳，且以靜置隔夜處理相對於直接處理的汙染率較低。比較「台農 1 號」上半部與下半部短縮莖培植體 (6 mm × 6 mm × 2 mm) 之消毒結果顯示，上半部培植體不僅汙染率低且小鱗莖誘導率較高。利用「千禧之星」小鱗莖 (周徑約 2.5 cm) 之 1/2 縱切或 1/4 縱切鱗莖作為培植體進行試驗，1/4 鱗莖處理之每個鱗莖可形成 5.2 個小鱗莖，顯著高於 1/2 鱗莖處理之 2.0 個小鱗莖。「紅獅」1/4 鱗莖培養於 1–4 mg L^{-1} 苯甲基腺嘌呤 (benzylaminopurine; BA) 或 0.1–0.4 mg L^{-1} 賽本隆 (thidiazuron; TDZ) 配合 0.2 mg L^{-1} 奈乙酸 (α-naphthalene acetic acid; NAA) 之 MS 培養基，結果顯示 0.1 mg L^{-1} TDZ 處理可獲得較高之小鱗莖增殖率，經 8 wk 培養後共獲得 11.6 個小鱗莖。本研究結果可提供新品種孤挺花在種苗量化參考。

關鍵詞：朱頂紅、消毒、離體繁殖、賽本隆。

前言

孤挺花 (*Hippeastrum hybridum* Hort.) 為石蒜科 (Amaryllidaceae)、孤挺花屬 (Hippeastrum) 多年生球根花卉，又名朱頂紅、華胄蘭、柱頂紅及喇叭花，其花色鮮豔、花朵碩大，具多樣花色與花型變化而深受消費者喜愛，廣泛應用於盆花、切花及花壇 (Chu *et al*. 2001; Ilczuk *et al*. 2005; Liu 2005; Lou *et al*. 2009; Shen & Yu 2011; Aslam *et al*. 2012; Amani *et al*. 2015)。孤挺花之園藝品種繁多，一般以雙鱗片法繁殖種苗供應市場需求 (Yanagawa & Osaki 1996; De Bruyn 1997; Liu 2005; Zhu *et al*. 2005; Lou *et al*. 2009; Aslam *et al*. 2012; Amani *et al*. 2015)。台灣近年來在各試驗改良場與業者努力下，已陸續推出許多優良品種，然卻面臨種苗量產不易而無法推廣的窘境。

孤挺花繁殖可利用分球、種子、雙鱗片或組織培養等方式，分球法最為簡單快速，經 1–2 年栽培後即可開花，但因子球數量有限而繁殖率低。種子播種後需 2–3 年可發育成開花球，雖然種苗數量較多，卻無法保有母本優良特性，通常僅育種者採用 (De Bruyn 1997; Smith *et al*. 1999; Aslam *et al*. 2012; Amani *et al*. 2015)。雙鱗片繁殖是目前量產種苗的主要方式，但存有遭受病原污染以致品種弱化

投稿日期：2016 年 06 月 14 日；接受日期：2016 年 12 月 28 日。
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的風險，且開花球養成也需 2–3 年 (De Bruyn 1997; Chu *et al*. 2001; Liu 2005; Zhu *et al*. 2005)。此外，本研究先期試驗結果顯示，雙鱗片繁殖僅適用於較大周徑鱗莖 (> 25 cm)，而較小周徑鱗莖 (約 10 cm) 的效率往往不佳 (資料未列)。組織培養具有繁殖倍率大、週期短、週年生產等優點，是種苗量產的主要技術之一，尤其對於初期種苗數量稀少的新品種而言，組織培養無疑是快速量產種苗的有效方法 (Yanagawa & Osaki 1996; De Bruyn 1997; Chang & Wang 1999; Zhang *et al*. 2002, 2006; Ilczuk *et al*. 2005; Liu *et al*. 2007; Shao & Shi 2008; Lou *et al*. 2009; Shen & Yu 2011; Aslam *et al*. 2012; Amani *et al*. 2015)。

孤挺花組織培養量產種苗的影響因子主要有：基因型、培植體種類，以及建立初代培養之消毒方式與培養基組成等 (Shen & Yu 2011)。雖然孤挺花組培技術自 1970 年代已被著手研究 (Hussey 1975; Seabrook & Cumming 1977)，然而時至今日仍有許多報告持續發表，顯然新品種、新藥劑或新方法均可被用以探討其種苗繁殖效率 (Aslam *et al*. 2012; Amani *et al*. 2015)。本研究以孤挺花鱗莖為材料，針對建立初代無菌培養之消毒方法、不同部位培植體及生長調節劑組成等因子進行測試，期能建立孤挺花組培小鱗莖增殖的方法，提供產業界在種球量化與新品種推展時之用。

材料與方法

材料來源、培養基配製及培養條件

本研究利用採自行政院農業委員會農業試驗所花卉研究中心試驗田之孤挺花單瓣品種「紅獅」('Red Lion') 與重瓣品種「千禧之星」('Blossom Peacock') 鱗莖 (周徑約 25 cm，2011 年 11 月採收)，以及農業試驗所育成之單瓣品種「台農 1 號-紅粉佳人」('Tainung No.1-Pink Lady') 鱗莖 (周徑約 12 cm，2012 年 6 月採收) 進行試驗。培植體培養於含有 3% 蔗糖、不同濃度之苯甲基腺嘌呤 (6-benzylaminopurine, Sigma B-3408, USA) 或賽本隆 (thidiazuron, Sigma P-6186, USA)，配合 0.1 或 0.2 mg L^{-1} 奈乙酸 (α-naphthalene acetic acid, Sigma N-0640, USA) 之 MS (Murashige & Skoog 1962) 培養基，培養基加入 9 g L^{-1} 洋菜 (Difco Bacto-agar) 前先以 0.1–1 N NaOH 或 HCl 將 pH 值調為 5.7 ± 0.1，再利用 121℃、1.05 kg cm^{-2} 滅菌 15 min 後冷卻備用。培植體接種於內含 20 mL 培養基之 125-mL 三角瓶 (Erlenmeyer flask) 後置於 26℃ ± 2℃、光照 14 h、光強度 38 μmol m^{-2} s^{-1} [T29R85 (FL40D-EX/38), China Electric, Taiwan] 的環境培養。

消毒方式對孤挺花「紅獅」成熟鱗莖建立初代培養之影響

將「紅獅」之成熟鱗莖去除葉片、基盤、鱗皮及兩層鱗片 (圖 1A、1B)，先縱切成 1/2 鱗莖後再縱切為 1/4 鱗莖 (圖 1C、1D)，接著切除上半部鱗片後留下帶有鱗片基部與短縮莖之 1/4 鱗莖 (圖 1D)，而後橫切使每個培植體 (2 cm × 1 cm × 1 cm) 均帶有短縮莖 (圖 1E)，最後接種於 125-mL 三角瓶 (圖 1F)。培植體分立即處理與隔日處理兩種方式，隔日處理係將 1/4 縱切鱗莖靜置於濾紙上過夜，次日進行消毒處理與接種。消毒方式係利用 75% 酒精浸漬 15 s，或以 0.6%、1.2% 次氯酸鈉 (NaOCl，稀釋自 Clorox®, USA) 處理 15 或 30 min，再以無菌水洗淨後接種於含有 1 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基，培養 8 wk 後調查培植體汙染情形。試驗採用 4 重複，每重複接種 12 個培植體。

培植體部位對孤挺花「台農 1 號-紅粉佳人」鱗莖建立初代培養之影響

利用周徑約 12 cm 之孤挺花「台農 1 號-紅粉佳人」鱗莖為材料，依上述方式進行前處理，將 1/4 鱗莖靜置過夜後，於隔日進行消毒處理與接種。將帶有鱗片基部之短縮莖橫切分為上、下兩部分，再橫切塊作為培植體 (6 mm × 6 mm × 2 mm)；利用 1.2% NaOCl 處理 15 min，再以無菌水洗淨後接種於含有 1 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基，培養 8 wk 後調查培植體汙染率，以及小鱗莖與根部形成情形。試驗採用 4 重複，每重複接種 15 個培植體。

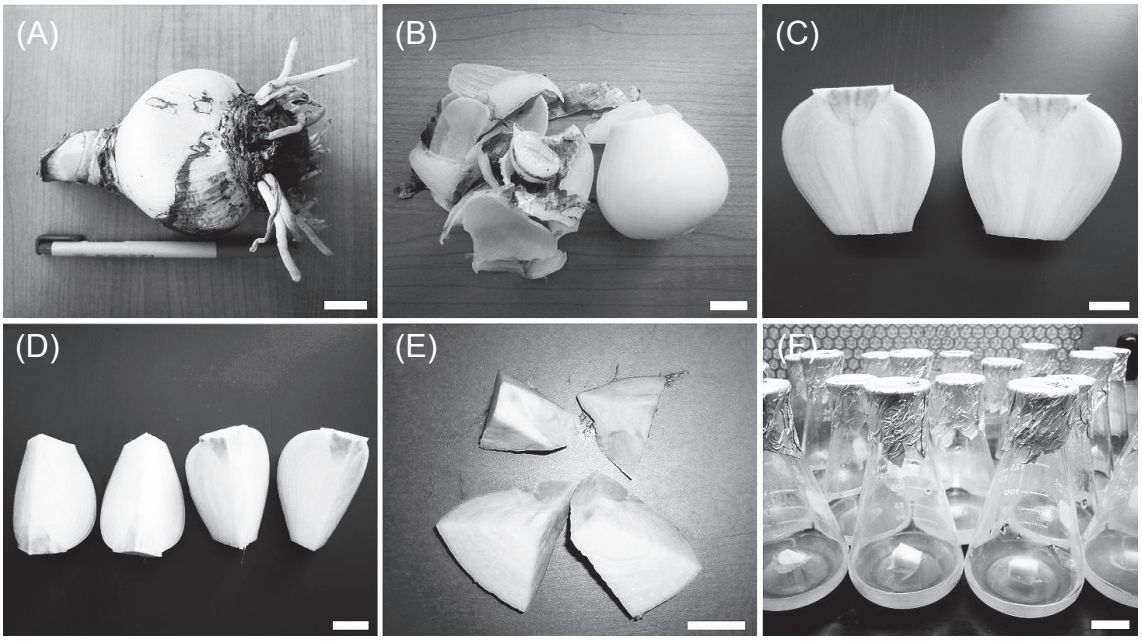


圖 1. 孤挺花「紅獅」成熟鱗莖建立組織培養無菌化之培植體取得情形。採自田間之成熟鱗莖 (A)；切除葉片、莖盤、鱗皮及兩層鱗片之鱗莖 (B)；1/2 鱗莖 (C)；1/4 鱗莖 (D)；帶有短縮莖與鱗片基部之 1/4 鱗莖 (E)；接種於三角瓶之短縮莖培植體 (F)。Bar = 2 cm。

Fig. 1. Explant obtained from field-grown bulb for establishment *in vitro* culture of *Hippeastrum hybridum* 'Red Lion'. Field-grown matured bulb (A), the bulb had been removed leaf, stem disc, tunic, and two leaf scales (B), half-bulb (C), quarter-bulb (D), quarter-bulb with stem and basal scale (E), and stem explant (2 cm × 1 cm × 1 cm) inoculated in 125-mL Erlenmeyer flask (F). Bar = 2 cm.

BA 對孤挺花「紅獅」與「千禧之星」小鱗莖增殖之影響

將切除根與葉之「紅獅」與「千禧之星」瓶內鱗莖 (周徑約 2.5 cm) 作為材料，以完整鱗莖與 1/2 縱切鱗莖 (1/2 鱗莖) 為培植體進行試驗。每處理 4 重複，亦即 4 個鱗莖 (1 鱗莖/重複)；因此，完整鱗莖處理係每瓶接種 1 個鱗莖，1/2 鱗莖處理則是每瓶接種 2 個 1/2 鱗莖。培植體培養於含有 0、1、2、4 mg L^{-1} BA 配合 0.2 mg L^{-1} NAA 之 MS 培養基進行試驗，培養 8 wk 後調查小鱗莖與根部形成之情形。

培植體切法對孤挺花「千禧之星」小鱗莖增殖之影響

將切除根與葉之「千禧之星」瓶內鱗莖 (周徑約 2.5 cm) 作為材料，以 1/2 鱗莖與 1/4 鱗莖 (quarter-bulb) 為培植體進行試驗。每處理 4 重複，亦即 4 個鱗莖 (1 鱗莖/重複)；因此，1/2 鱗莖處理係每瓶接種 2 個 1/2 鱗莖，1/4 鱗莖處理則每瓶接種 4 個 1/4 鱗莖。培植體培養於含有 0.2 mg L^{-1} TDZ 與 0.2 mg L^{-1} NAA 之 MS 培養基，經 4、8 及 12 wk 培養後調查小鱗莖與根部形成之情形。

BA 與 TDZ 對孤挺花「紅獅」小鱗莖增殖之影響

將切除根與葉之「紅獅」瓶內鱗莖 (周徑約 2.5 cm) 作為材料，以 1/4 鱗莖為培植體進行試驗。每處理採用 4 重複，每瓶接種 4 個 1/4 鱗莖，即每重複為 1 個鱗莖。培植體培養於含有 0.2 mg L^{-1} NAA 配合 TDZ (0.1、0.2、0.4 mg L^{-1}) 或 BA (1、2、4 mg L^{-1}) 之 MS 培養基，經 8 wk 培養後調查小鱗莖與根部形成之情形。

試驗設計和統計分析

本研究採用完全逢機設計 (completely

randomized design; CRD) 進行試驗。試驗所得資料經 SAS 8.2 (SAS Institute Inc. 2001) 套裝統計分析軟體進行變方分析 (analysis of variance; ANOVA)，若處理間差異顯著 ($P < 0.05$)，則利用最小顯著差異性測驗 (least significant difference test; LSD) 比較各處理平均值間之差異。

結果

消毒方式對孤挺花「紅獅」成熟鱗莖建立初代培養之影響

利用「紅獅」進行消毒試驗結果顯示，75% 酒精處理 15 s 無法達到消毒效果，培植體全數汙染。比較立即處理與隔日處理的汙染率結果顯示，1.2% NaOCl 處理之汙染率明顯較低於 0.6% NaOCl 處理；而 1.2% NaOCl 處理 15 min 的汙染率較低於 30 min 處理 (表 1)。因此，經過前處理之 1/4 鱗莖於濾紙上靜置過夜後，短縮莖培植體在隔天再以 1.2% NaOCl 處理 15 min 的消毒效果最佳，汙染率僅 16.7%。此外，培植體培養於含有 1 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基 8 wk 後，每個培植體可獲得 1–2 個小鱗莖 (資料未列)。

培植體部位對孤挺花「台農 1 號-紅粉佳人」鱗莖建立初代培養之影響

比較「台農 1 號-紅粉佳人」上、下部位短縮莖培植體之消毒試驗結果顯示，上半部培植體汙染率僅 3.3%，顯著優於下半部培植體之 36.7%。上、下兩部位培植體於 1 mg L^{-1} BA 配合 0.1 mg L^{-1} NAA 之 MS 培養基中經培養 8 wk 後，培植體的生長分化呈現 4 種型態，分別為有根小鱗莖、無根小鱗莖、未分化培植體及僅長根的培植體 (圖 2)。其中就發根小鱗莖的比率而言，取自上半部培植體之 57.3% 誘導率顯著高於下半部的 37.4%，但兩部位之每個培植體均可形成約 1.2 個小鱗莖；培植體的平均根數則以取自上半部培植體之 4.0 條根，顯著高於取自下半部之 2.7 條根 (表 2)。無根小鱗莖與只長根之培植體的比率，皆以取自上半部培植體顯著高於下半部培植體。

本研究利用上述方法已分別建立「紅獅」、「千禧之星」及「台農 1 號」之無菌瓶苗，並利用瓶內小鱗莖做為後續增殖試驗的培植體來源。

BA 對孤挺花「紅獅」與「千禧之星」小鱗莖增殖之影響

本研究在先期試驗中利用完整鱗莖或 1/2

表 1. 消毒方法對孤挺花「紅獅」成熟鱗莖建立無菌培植體之影響。

Table 1. Effect of surface disinfection methods on aseptic *in vitro* culture using mature bulb of *Hippeastrum hybridum* 'Red Lion'[z].

Explant treatment	Disinfection method			Contamination (%)
	75% Ethanol	0.6% NaOCl	1.2% NaOCl	
Disinfected immediately	15 s			100.0 a
		15 min		83.3 ± 8.35 ab[y]
		30 min		66.7 ± 11.80 ab
			15 min	41.6 ± 10.50 b
			30 min	58.3 ± 8.35 ab
Disinfected after left on filter paper for overnight	15 s			100.0 a
		15 min		83.3 ± 4.80 ab
		30 min		75.0 ± 12.50 ab
			15 min	16.7 ± 4.80 c
			30 min	33.3 ± 6.80 bc

[z] Explants cultured on MS medium containing 1 mg L^{-1} BA and 0.1 mg L^{-1} NAA for 8 wk. Twelve explants were used in each treatment.

[y] Means in each column followed by different letter(s) are significantly different at the 5% level by LSD test.

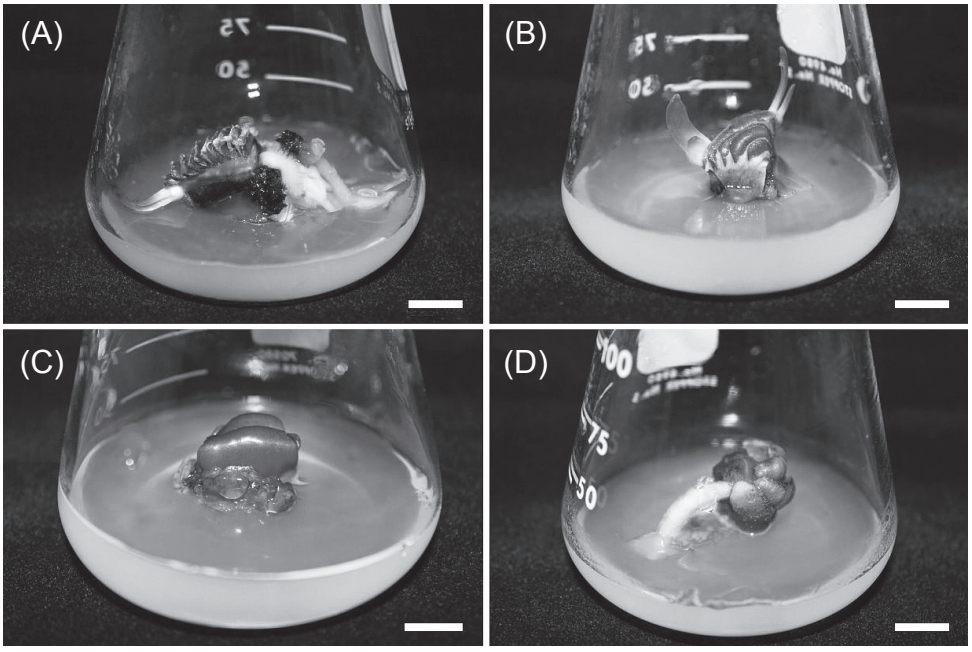


圖 2. 孤挺花「台農 1 號-紅粉佳人」鱗莖培植體培養於含有 1 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基 8 wk 時培植體的反應。發根小鱗莖 (A)、無根小鱗莖 (B)、未分化之培植體 (C) 及僅長根之培植體 (D)。Bar = 1 cm。

Fig. 2. Growth conditions of *in vitro* cultured of *Hippeastrum hybridum* 'Tainung No.1-Pink Lady'. Stem explants were cultured on MS medium containing 1 mg L^{-1} BA and 0.1 mg L^{-1} NAA after 8 wk of culture. Rooting bulblet (A), bulblet without root (B), explant without bulblet and root (C), and explant with only root (D). Bar = 1 cm.

表 2. 孤挺花「台農 1 號」鱗莖不同部位短縮莖培植體對消毒效率與小鱗莖形成之影響。

Table 2. Position of stem explant on disinfection efficiency and bulblet formation of *Hippeastrum hybridum* 'Tainung No.1-Pink Lady'[z].

Explant position	Contamination (%)	Bulbing explant (%)		Rooting explant without bulbing (%)	No response (%)	Bulblets/explant (No.)	Roots/explant (No.)
		With roots	Without root				
Upper part	3.3 ± 1.92 b[y]	57.3 ± 6.29 a	12.1 ± 5.17 a	6.8 ± 2.72 a	23.8 ± 6.14 b	1.2 ± 0.07 a	4.0 ± 0.52 a
Lower part	36.7 ± 6.38 a	37.4 ± 10.43 b	6.3 ± 3.72 b	0.0 ± 0.00 b	56.2 ± 12.40 a	1.2 ± 0.10 a	2.7 ± 0.46 b

[z] Explants cultured on MS medium containing 1 mg L^{-1} BA and 0.1 mg L^{-1} NAA for 8 wk. Each treatment had 4 replications, and 15 explants per replication.
[y] Means in each column followed by different letter(s) are significantly different at 5% level by LSD test.

鱗莖作為培植體，培養於含有適量 BA 與 NAA 之 MS 培養基中，若以每鱗莖可產出的小鱗莖為計算基準，則 1/2 鱗莖處理可形成 2–3 個小鱗莖苗，而完整鱗莖處理僅形成 1 個小鱗莖苗 (資料未列)；因此，本試驗以 1/2 鱗莖作為培植體，且為使小鱗莖形成後能發根良好成為小鱗莖苗，於是以不同濃度 BA 搭配 0.2 mg L^{-1} NAA 進行試驗。

利用「紅獅」與「千禧之星」1/2 鱗莖培養於含有不同濃度 BA 配合 0.2 mg L^{-1} NAA 之 MS 培養基進行試驗，表 3 結果顯示，就「紅獅」而言，1–4 mg L^{-1} BA 處理之小鱗莖增殖效果相當，3 種 BA 濃度間並無顯著差異為 1.1–1.2 個/培植體，但顯著高於對照組，其中以 2 mg L^{-1} BA 處理較佳；1 與 2 mg L^{-1} BA 兩處理在小鱗莖發根表現並無顯著差異，分別為 3.4 與 2.9

表 3. BA 對孤挺花「紅獅」與「千禧之星」1/2 鱗莖培植體形成小鱗莖之影響。

Table 3. Effect of BA on *in vitro* bulblet formation by using half-bulb explant of *Hippeastrum hybridum* 'Red Lion' and 'Blossom Peacock'[z].

BA (mg L^{-1})	NAA (mg L^{-1})	Red Lion		Blossom Peacock	
		Bulblets/explant	Roots/explant	Bulblets/explant	Roots/explant
0	0.2	1.0 ± 0.00 b[y]	9.6 ± 0.39 a	1.1 ± 0.07 b	6.3 ± 0.27 a
1	0.2	1.1 ± 0.07 ab	3.4 ± 0.78 b	1.4 ± 0.19 a	3.9 ± 0.30 b
2	0.2	1.2 ± 0.06 a	2.9 ± 0.53 b	1.6 ± 0.16 a	1.3 ± 0.28 c
4	0.2	1.1 ± 0.07 ab	1.3 ± 0.27 c	1.0 ± 0.00 b	1.3 ± 0.00 c

[z] Explants were cultured on MS medium containing various concentrations of BA in combination with 0.2 mg L^{-1} NAA for 8 wk of culture. Each treatment had 4 replications, and 4 explants were used in each replication.
[y] Means in each column followed by different letter(s) are significantly different at the 5% level by LSD test.

根/培植體，但只含 0.2 mg L^{-1} NAA 的對照組，發根效果最佳達 9.6 根/培植體，4 mg L^{-1} BA 處理之發根效果最差，每個培植體僅形成 1.3 條根。就「千禧之星」而言，1 與 2 mg L^{-1} BA 處理之小鱗莖增殖效果約為 1.4 與 1.6 個/培植體，兩處理間並無顯著差異，而 0 與 4 mg L^{-1} BA 兩處理間亦無顯著差異，分別為 1.1 與 1.0 個/培植體；小鱗莖發根效率以對照組最佳，每個培植體可達 6.3 條根，其次為 1 mg L^{-1} BA 處理之 3.9 根/培植體，而 2 與 4 mg L^{-1} BA 處理發根效果最差，每個培植體均僅形成 1.3 條根。

培植體切法對孤挺花「千禧之星」小鱗莖增殖之影響

利用「千禧之星」1/2 鱗莖與 1/4 鱗莖作為培植體進行試驗，培養 4 wk 的結果顯示，1/2 鱗莖處理之小鱗莖形成率達 87.5%，顯著高於 1/4 鱗莖處理的 56.3%；但兩處理形成之小鱗莖數分別為 0.9 與 0.6 個小鱗莖/培植體，兩處理間並無顯著差異；但若以每瓶為計算基準時，則 1/4 鱗莖處理形成 2.4 個小鱗莖顯著高於 1/2 鱗莖處理之 1.8 個小鱗莖；此外，每個培植體之根數則以 1/2 鱗莖處理之 4.3 條根顯著高於 1/4 鱗莖處理的 1.9 條根 (表 4、圖 3A、圖 3C)。當培養達 8 wk 時，兩個處理的小鱗莖誘導率均可達到 100%，但是每個培植體形成之小鱗莖數也無顯著差異；同樣若是以每瓶為計算基準時，則 1/4 鱗莖處理可形成 4.4 個小鱗莖顯著高於 1/2 鱗莖處理之 2.0 個小鱗

表 4. 不同鱗莖切法對孤挺花「千禧之星」小鱗莖形成之影響。

Table 4. Effect of bulb cutting method on *in vitro* bulblet formation of *Hippeastrum hybridum* 'Blossom Peacock'[z].

Explant treatment	Bulbing explant (%)	Bulblets/explant (No.)	Roots/explant (No.)	Bulblets/original bulb (No.)
4 wk after inoculated				
Half-bulb	87.5 ± 12.50 a[y]	0.9 ± 0.18 a	4.3 ± 0.35 a	1.8 ± 0.4 b
Quarter-bulb	56.3 ± 11.95 b	0.6 ± 0.26 a	1.9 ± 0.48 b	2.4 ± 1.0 a
8 wk after inoculated				
Half-bulb	100.0 a	1.0 ± 0.00 a	5.1 ± 0.50 a	2.0 ± 0.0 b
Quarter-bulb	100.0 a	1.1 ± 0.17 a	3.1 ± 0.43 b	4.4 ± 0.7 a
12 wk after inoculated				
Half-bulb	100.0 a	1.0 ± 0.00 a	6.4 ± 0.53 a	2.0 ± 0.0 b
Quarter-bulb	100.0 a	1.3 ± 0.30 a	4.8 ± 0.74 b	5.2 ± 1.2 a

[z] Explants cultured on MS medium containing 0.2 mg L^{-1} TDZ and 0.2 mg L^{-1} NAA for 4, 8 and 12 wk. Each treatment had 4 bulblets in a treatment, and half-bulb treatment with 2 explants and quarter-bulb treatment with 4 explants in each Erlenmeyer flask.
[y] Means in each column followed by different letter(s) are significantly different at the 5% level by LSD test.

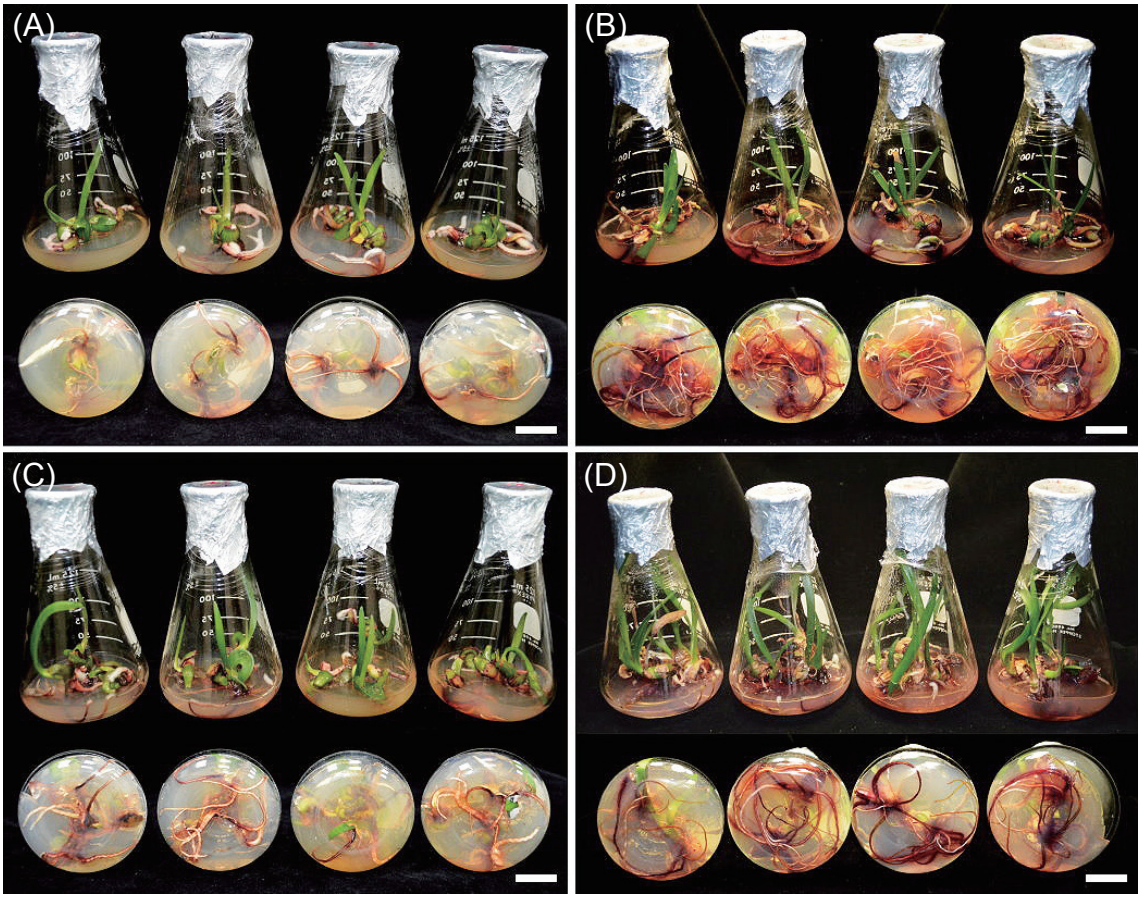


圖 3. 孤挺花「千禧之星」以 1/2 鱗莖 (A)、(B) 與 1/4 鱗莖 (C)、(D)；培植體培養於含有 0.2 mg L^{-1} TDZ 與 0.2 mg L^{-1} NAA 之 MS 培養基 4 wk (A)、(C) 與 12 wk (B)、(D) 之小鱗莖增殖、生長與發根情形。Bar = 2 cm。

Fig. 3. Bulblet growth and rooting condition of *in vitro* cultured *Hippeastrum hybridum* 'Blossom Peacock' using half-bulb explants (A), (B) or using quarter-bulb explants (C), (D). Explants were cultured on MS medium containing 0.2 mg L^{-1} TDZ in combination with 0.2 mg L^{-1} NAA after 4 wk (A), (C), and 12 wk (B), (D). Bar = 2 cm.

莖；此外，每個培植體根數以 1/2 鱗莖處理之 5.1 條根顯著高於 1/4 鱗莖處理的 3.1 條根 (表 4)。延長培養時間至 12 wk 時，則 1/4 鱗莖處理可形成 5.2 個小鱗莖/瓶，顯著高於 1/2 鱗莖處理之 2 個小鱗莖/瓶；根的形成方面仍以 1/2 鱗莖處理之 6.4 條根/培植體，顯著高於 1/4 鱗莖處理的 4.8 條根/培植體 (表 4、圖 3B、圖 3D)。上述結果顯示，「千禧之星」1/4 鱗莖培養於含 0.2 mg L^{-1} TDZ 配合 0.2 mg L^{-1} NAA 之 MS 培養基的小鱗莖增殖效果較佳，每個周徑約 2.5 cm 的小鱗莖經 12 wk 培養後可獲得約 5.2 個小鱗莖。

BA 與 TDZ 對孤挺花「紅獅」小鱗莖增殖之影響

利用「紅獅」1/4 鱗莖作為培植體，比較 BA 與 TDZ 對小鱗莖增殖之影響，結果顯示培養 8 wk 後兩處理之小鱗莖增殖效率並無顯著差異，其中以 0.1 mg L^{-1} TDZ 處理較佳，每個培植體可誘得 2.9 個小鱗莖，而對照組較差僅得 1.8 個小鱗莖，其餘處理可得 2.1–2.2 個小鱗莖。若以每瓶為計算基準，則 0.1 mg L^{-1} TDZ 處理可誘得約 11.6 個小鱗莖的效果較佳。就每個培植體形成根數而言，以 1 mg L^{-1} BA 與 0.1 mg L^{-1} TDZ 配合 0.2 mg L^{-1} NAA 之處

理較佳，每個培植體分別可得 11.6 與 10.4 條根，較高於其餘處理之 7.4–9.3 條根，所有處理中以 4 mg L^{-1} BA 配合 0.2 mg L^{-1} NAA 之處理顯著最差，僅得 7.4 條根/培植體 (表 5)。綜合上述結果顯示，「紅獅」1/4 鱗莖培養於含有 0.1 mg L^{-1} TDZ 與 0.2 mg L^{-1} NAA 之 MS 培養基，小鱗莖的增殖效果較佳，每個周徑約 2.5 cm 的鱗莖經 8 wk 培養可獲得約 11.6 個小鱗莖。

討論

在組織培養的無菌化過程中，若培植體取自於土壤中較易受微生物汙染，因而嚴重影響消毒效率，孤挺花鱗莖的初代培養就是典型例子 (Seabrook & Cumming 1977; De Bruyn *et al*. 1992; Chang & Wang 1999; Smith *et al*. 1999; Aslam *et al*. 2012)。前人研究為獲得孤挺花無菌苗進行量化繁殖，往往採取許多費時且複雜的操作步驟，例如利用熱水浸泡 (De Bruyn *et al*. 1992; De Bruyn 1997; Chang & Wang 1999; Smith *et al*. 1999; Shao & Shi 2008; Amani *et al*. 2015)、氯氣燻蒸 (Smith *et al*. 1999) 或是植物保護混合劑 (plant preservative mixature; PPM) (Smith *et al*. 1999; Aslam *et al*. 2012) 進行前處理，而後再將培植體表面消毒與接種；消毒大多採用酒精、次氯酸鈉或次氯酸鈣 [Calcium hypochlorite, $Ca(ClO)_2$] 溶液浸泡處理 (Hussey 1975; Seabrook & Cumming 1977; De Bruyn *et al*. 1992; Yanagawa & Osaki 1996; Chang & Wang 1999; Smith *et al*. 1999; Ilczuk *et al*. 2005; Shao & Shi 2008; Lou *et al*. 2009; Shen & Yu 2011; Aslam *et al*. 2012; Amani *et al*. 2015)，少數利用氯化亞汞 (mercuric chloride; $HgCl_2$) 溶液浸泡消毒 (Zhang *et al*. 2002, 2006; Lou *et al*. 2009; Shen & Yu 2011)。然而任何消毒方式均需利用合適濃度配合適當時間，才能達到較佳的消毒效果。本研究結果顯示，將洗淨且去除根與 2–3 層鱗片之 1/4 縱切鱗莖靜置隔夜，次日再以 1.2% NaOCl 處理 15 min 後可得最佳的消毒效果 (表 1)。推測鱗莖在縱切後立即進行消毒處理，培植體容易因為富含黏液而影響藥劑效果，當培植體在靜置過夜後，可能由於黏液量減少而有較佳的消毒效果。

Yanagawa & Osaki (1996) 與 Zhang *et al*. (2002) 的研究結果顯示，培植體越接近於基盤 (extreme base)，其不定芽誘導效率越高。Lou *et al*. (2009) 於孤挺花「蘋果」('Apple') 與「蘇州雜交 18 號」('Filial generation No. 18') 的報告也顯示，不同鱗莖部位的小鱗莖誘導率明顯不同；比較上、中、下部鱗莖培植體 (均帶有莖盤) 的結果顯示，下部誘導率最高，中部次之，上部則無法誘得小鱗莖。本研究結果顯示，短縮莖之上半部培植體的汙染率顯著低於下半部培植體，且上半部培植體之小鱗莖誘導

表 5. BA 與 TDZ 對孤挺花「紅獅」1/4 鱗莖培植體形成小鱗莖之影響。

Table 5. Effect of BA and TDZ concentration on *in vitro* bulblet formation by using quarter-bulb explant of *Hippeastrum hybridum* var. 'Red Lion'[z].

BA (mg L^{-1})	TDZ (mg L^{-1})	NAA (mg L^{-1})	Bulblets/explant (No.)	Roots/explant (No.)	Bulblets/original bulb (No.)
		0.2	1.8 ± 0.2 b[y]	9.3 ± 0.8 bc	7.2 ± 0.8 b
1		0.2	2.1 ± 0.4 ab	11.6 ± 0.4 a	8.4 ± 1.6 ab
2		0.2	2.2 ± 0.3 ab	9.1 ± 0.4 bc	8.8 ± 1.2 ab
4		0.2	2.1 ± 0.3 ab	7.4 ± 0.8 d	8.4 ± 1.2 ab
	0.1	0.2	2.9 ± 0.3 a	10.4 ± 0.2 ab	11.6 ± 1.2 a
	0.2	0.2	2.2 ± 0.4 ab	9.2 ± 0.4 bc	8.8 ± 1.6 ab
	0.4	0.2	2.1 ± 0.4 ab	7.9 ± 0.5 cd	8.4 ± 1.4 ab

[z] Explants were cultured on MS medium containing various concentrations of BA and TDZ in combination with 0.2 mg L^{-1} NAA for 8 wk of culture. Each treatment had 4 replications, and 4 explants were used in each replication.
[y] Means in each column followed by different letter(s) are significantly different at the 5% level by LSD test.

率顯著高於下半部培植體，但是每個培植體形成小鱗莖數則無顯著差異 (表 2)。就汙染率而言，可能因為上半部培植體距離根部較遠造成汙染率較低的結果；另就小鱗莖誘導率而言，本研究結果與上述研究有所不同，推測其原因可能係因為鱗莖大小不同、下半部培植體汙染率過高，以及對於上、中、下部培植體之定義不同所導致。

De Bruyn *et al*. (1992) 與 De Bruyn (1997) 的研究結果均顯示，含有 5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基為誘導南非孤挺花 (*Amaryllis belladonna*) 小鱗莖形成的最佳處理。Chang & Wang (1999) 於「紅獅」初代培養結果顯示，4 mg L^{-1} BA 處理雙鱗片雖可誘得最多小鱗莖，但其生長受抑制且發育異常，建議利用較低濃度處理 (0.5–2 mg L^{-1} BA) 的效果較佳。「紅獅」與「橙王」('Orange Sovereign') 的研究結果顯示，1–4 mg L^{-1} BA 處理之鱗莖誘導率極低，但以 2 mg L^{-1} BA 搭配 1–2 mg L^{-1} NAA 則有較佳效果 (Zhang *et al*. 2006)。本研究之初代培養結果顯示，短縮莖培植體於 1 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基培養 8 wk 後，每個培植體平均可得 1–2 個小鱗莖；因此，本研究依上述方法已分別建立「紅獅」、「千禧之星」及「台農 1 號」之無菌苗，所得小鱗莖可做為後續試驗的培植體來源。

南非孤挺花 (De Bruyn *et al*. 1992) 與「橙王」(Shao & Shi 2008) 的研究結果均顯示，鱗莖增殖率以四分切的效率為最高，其次為二分切，完全未切之效果最差。Shen & Yu (2011) 的評論也有類似結果。此外，Chu *et al*. (2001) 以不同切割方式誘導「紅獅」鱗莖子球形成的研究結果顯示，其切割數較少者所形成之子球數亦較少，但其形成的子球則較大。Zhu *et al*. (2005) 於孤挺花「雙紀錄」('Double Record') 的研究也顯示相似的結果。本研究利用「千禧之星」1/2 鱗莖與 1/4 鱗莖進行試驗之結果與上述結果一致，就每個培植體形成的小鱗莖數而言，兩種切割處理並無顯著差異，但若以每瓶小鱗莖數進行比較，則 1/4 鱗莖處理增殖率顯著高於 1/2 鱗莖處理；此外，本研究追蹤培養期間至 12 wk 的結果顯示，1/2 鱗莖處理於 4 wk 後之小鱗莖數即不再增加，但 1/4 鱗莖處理仍持續形成小鱗莖，因此若以培養 4 wk 的數據來看，可能誤判 1/2 鱗莖處理與 1/4 鱗莖處理之繁殖率相當 (表 4)。因此推論，較小培植體之再生鱗莖可能需要較長時間的生長發育；此外，在單一培植體繁殖率相當時，若每個鱗莖縱切越多培植體，則每個鱗莖的繁殖總量會因而增加，然是否會因培植體太小而無法誘得鱗莖，則需要進一步試驗證實。

孤挺花「紅孔雀」研究結果顯示，1–2 mg L^{-1} BA 搭配 0.1 mg L^{-1} NAA 是鱗莖增殖的最佳處理 (Liu *et al*. 2007)。「蘋果」與「蘇州雜交 18 號」的鱗莖增殖率以 2 mg L^{-1} BA 配合 0.2 mg L^{-1} NAA 的效果較好 (Lou *et al*. 2009)。Shen & Yu (2011) 於評論報告指出，孤挺花組織培養苗增殖多以 2–8 mg L^{-1} BA 或 0.5 mg L^{-1} TDZ 搭配 0.1–2 mg L^{-1} NAA，其最佳組合則因品種不同而異。本研究以「紅獅」與「千禧之星」1/2 鱗莖進行試驗結果顯示，兩品種誘導率均以 2 mg L^{-1} BA 配合 0.2 mg L^{-1} NAA 之處理效果較佳，較低與較高濃度 (1 或 4 mg L^{-1} BA) 的處理效率不佳 (表 3)，與前人研究結果相類似。Lou *et al*. (2009) 於「蘋果」與「蘇州雜交 18 號」的研究結果顯示，TDZ 對鱗莖增殖較 BA 效率為佳，最佳增殖培養基為 0.5 mg L^{-1} TDZ 搭配 0.2 mg L^{-1} NAA 之組合。本研究以「千禧之星」1/2 鱗莖進行試驗結果顯示，每個鱗莖培養於 2 mg L^{-1} BA 處理 8 wk 後誘得 3.2 個小鱗莖 (表 3)；然而 0.2 mg L^{-1} TDZ 處理僅得到 2 個小鱗莖 (表 4)；顯示 0.2 mg L^{-1} TDZ 處理效果較 2 mg L^{-1} BA 處理為低；若提高 TDZ 濃度是否具有較佳增殖效果，尚需進一步試驗證實。但是「紅獅」1/4 鱗莖的試驗結果顯示，1–4 mg L^{-1} BA 與 0.1–0.4 mg L^{-1} TDZ 對增殖均有良好效果，其中以 0.1 mg L^{-1} TDZ 搭配 0.2 mg L^{-1} NAA 的處理較佳 (表 5)。

Ilczuk *et al*. (2005) 利用孤挺花 (*Hippeastrum* × *chmielii* Chm.) 1/4 鱗莖 (周徑約 1.8 cm) 培養於含有 0.5 mg L^{-1} BA 與 0.1 mg L^{-1} NAA 之 MS 培養基的結果顯示，每個鱗莖在 4 wk 後可得 3.9 個小鱗莖。本研究「千禧之星」1/4

鱗莖 (周徑約 2.5 cm) 於 0.2 mg L^{-1} TDZ 配合 0.2 mg L^{-1} NAA 處理 4 wk 時可形成 2.4 個小鱗莖，當培養至 8 wk 與 12 wk 時分別達 4.4 與 5.2 個小鱗莖 (表 4)；顯示培養 4 wk 後仍有小鱗莖持續形成。此外，「紅獅」1/4 鱗莖培養於 TDZ 配合 NAA 處理之每個鱗莖在 8 wk 後可得 8.4–11.6 個小鱗莖 (表 5)。因此顯示，「紅獅」小鱗莖增殖效果較「千禧之星」為高。Lou *et al*. (2009) 於「蘋果」與「蘇州雜交 18 號」研究結果顯示，在相同培植體與培養基組成情況下，「蘋果」較「蘇州雜交 18 號」具有較高的小鱗莖誘導率。上述結果亦印證前人研究結果，Shen & Yu (2011) 評論指出，孤挺花小鱗莖增殖效率在品種間具有極大差異的觀點。此外，本研究結果也顯示，培養調查時間也是影響小鱗莖增殖率高低的因子之一。

Zhang *et al*. (2002) 的研究結果顯示，孤挺花不定芽培養於 MS 培養基即可達到 100% 發根率。Shen & Yu (2011) 評論指出，小鱗莖培養於含有 0.1–0.5 mg L^{-1} NAA 之培養基均具有良好發根效果。此外，孤挺花之單鱗片或雙鱗片於 1–2 mg L^{-1} BA 處理下也會有良好發根率，作者推論係因 BA 誘導小鱗莖形成後而變得容易發根 (Amani *et al*. 2015)。本研究結果顯示，小鱗莖於 0.2 mg L^{-1} NAA 處理之根系最佳，較低濃度之 BA (1–2 mg L^{-1}) 或 TDZ (0.1–0.2 mg L^{-1}) 處理亦發根良好，但 4 mg L^{-1} BA 或 0.4 mg L^{-1} TDZ 之較高濃度處理則會抑制根的生長發育 (表 5)。由此可知，當孤挺花新生鱗莖形成後即可伴隨發根，因此其組織培養繁殖體系可能無需再進行誘導發根。

本研究利用孤挺花「紅獅」、「千禧之星」及「台農 1 號-紅粉佳人」之田間鱗莖，經過清洗、切割並靜置隔夜後，以 1.2% NaOCl 進行 15 min 處理的消毒效果最佳，其中又以短縮莖上半部培植體的污染率最低，本研究以上述方法獲得 3 品種無菌鱗莖進行試驗。「紅獅」1/4 鱗莖培養於含有 0.1 mg L^{-1} TDZ 與 0.2 mg L^{-1} NAA 之 MS 培養基，每個周徑約 2.5 cm 鱗莖培養 8 wk 後，可獲得 11.6 株發根良好的小鱗莖苗，對於加速新品種繁殖具有相當大的效益。

誌謝

本文稿承農業試驗所花卉研究中心蔡嫻婷博士協助審閱，特此申謝。

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Effect of Surface Disinfection, Explant Type, and Plant Growth Regulators on Establishment of *In Vitro* Bulblet Proliferation of Amaryllis

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Abstract

Chen, U. C., I. Din, T. Y. Wu, J. Y. Tsao, and C. N. Hsia. 2017. Effect of disinfection method, explant type, and plant growth regulator on establishment *in vitro* bulblet proliferation of Amaryllis. J. Taiwan Agric. Res. 66(4):286–297.

Field-grown bulbs of Amaryllis (*Hippeastrum hybridum*) 'Red Lion' (about 25 cm circumference), 'Blossom Peacock', and 'Tainung No. 1-Pink Lady' (about 12 cm circumference) were used as culture materials. Effects of surface disinfection, explant type, and plant growth regulators combinations on establishment *in vitro* bulblet proliferation were investigated. Stem explants (2 cm × 1 cm × 1 cm) of 'Red Lion' left on a filter paper overnight before using 1.2% sodium hypochlorite solution for 15 min resulted in the least contamination. Stem explants (6 mm × 6 mm × 2 mm) cut from the upper or lower part of 'Tainung No. 1-Pink Lady' were compared. Although explants derived from different positions of the stem had the same proliferation rates of 1.2 bulblets per explant, the upper stem explants had a significant lower contamination rate of 3% than that of 37% from the lower part explants. Comparison of proliferation efficiencies was conducted using half- and quarter-bulb explants of 'Blossom Peacock' derived from an *in vitro* bulb with 2.5 cm circumference in size, and no significant difference was found on bulblet formation between both treatments. However, a higher proliferation rate of 5.2 bulblets per original bulb was calculated by using quarter-bulb explants than that of 2.0 bulblets from the half-bulb explants. The highest bulblet proliferation rate with 11.6 bulblets from per 2.5 cm circumference in 'Red Lion' *in vitro* bulb was obtained by culturing quarter-bulb explants on the MS medium supplemented with 0.1 mg L^{-1} thidiazuron (TDZ) and 0.2 mg L^{-1} α-naphthalene acetic acid (NAA) for 8 wk. Results of this study could provide useful information on bulblet proliferation for new varieties of Amaryllis.

Key words: *Hippeastrum hybridum*, Disinfection, *In vitro* propagation, Thidiazuron.

Received: June 14, 2016; Accepted: December 28, 2016.
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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):298–306 (2017)
DOI: 10.6156/JTAR/2017.06604.04

研究報告

白花三葉草 (*Trifolium repens* L.) 作為覆蓋植物應用於蟲媒南瓜病毒病害防治

蔡濰安 [1,*]　翁崧夏 [2]　邱智迦 [3]

摘要

蔡濰安、翁崧夏、邱智迦。2017。白花三葉草 (*Trifolium repens* L.) 作為覆蓋植物應用於蟲媒南瓜病毒病害防治。台灣農業研究 66(4):298–306。

葫蘆科 (Cucurbitaceae) 作物為台灣重要蔬果，但病毒病害長期為葫蘆科作物生產體系中之限制因子，作物罹病後無任何可供防治之藥劑，因此如何有效防治田間病媒昆蟲成為當今重要的議題。銀葉粉蝨 (*Bemisia argentifolii* Bellows and Perring) 是葫蘆科作物中重要的害蟲之一，已知可專一性傳播豆類金黃病毒屬 (*Begomovirus*) 的病毒，近年來南瓜捲葉菲律賓病毒 (*Squash leaf curl Philippine virus*; SLCPHV) 最為常見。蚜蟲也是葫蘆科作物中重要的病媒昆蟲，已知至少可以傳播 4 種常見的植物病毒病害，危害葫蘆科作物甚大。許多研究顯示，無論是生物性覆蓋物 (living mulches) 或人造資材覆蓋物 (synthetic mulches) 皆具有防治銀葉粉蝨或蚜蟲的效果。本研究利用白花三葉草 (*Trifolium repens* L.) 作為覆蓋植物應用於南瓜栽培，結果發現可以有效降低銀葉粉蝨田間的族群，且 SLCPHV 的感染率為 55.2%，顯著低於銀黑塑膠布處理的 91.3% 和銀黑塑膠布搭配寡聚半乳糖醛酸噴施處理組的 95.2%。田間病徵發生比例最終至採收期僅達約 13%。本研究認為白花三葉草於田間栽培具有防治銀葉粉蝨的功能，未來可應用來建構適合有機南瓜栽培的環境。

關鍵詞：覆蓋植物、葫蘆科作物、蟲媒病毒、銀葉粉蝨。

前言

葫蘆科 (Cucurbitaceae) 作物為台灣重要蔬果作物，生產面積達 20,000 ha 以上且病蟲害種類繁多，當中又以病毒病害最嚴重。病毒病害可藉由種子、媒介昆蟲及機械傳播，且罹病後無任何可供防治之藥劑，因此已成為瓜類作物生產體系中之限制因子。在自然環境下可感染瓜類作物的病毒種類超過 50 種，台灣現有紀錄感染瓜類的病毒有 16 種 (Deng 2011)。

銀葉粉蝨 (*Bemisia argentifolii* Bellows and Perring) 是葫蘆科作物中重要的害蟲之一，且經常取食造成植物生理性失調，如南瓜產生銀葉 (squash silverleaf disorder; SSL) 的症狀 (Yokomi *et al*. 1990; Schuster *et al*. 1991)。銀葉粉蝨也是葫蘆科作物病毒重要的媒介昆蟲，已知可專一性傳播豆類金黃病毒屬 (*Begomovirus*) 的病毒 (Brown *et al*. 1995)，其中瓜類退綠黃化病毒 (*Cucurbit chlorotic yellows virus*; CCYV)、南瓜捲葉菲律賓病毒 (*Squash leaf curl Philippine virus*; SLCPHV)、番茄捲葉新德里病毒 (*Tomato leaf curl New Delhi virus*; ToLCNDV) 是近期為害台灣葫蘆科作物重要的病毒病害 (Deng 2011)。

蚜蟲是植物病毒中最重要的病媒昆蟲，已知可傳播 275 種植物病毒病害 (Nault 1997)。

投稿日期：2016 年 8 月 27 日；接受日期：2017 年 1 月 15 日。
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[2] 農委會花蓮區農業改良場作物環境課研發替代役。台灣 花蓮縣。
[3] 農委會花蓮區農業改良場作物環境課研究助理。台灣 花蓮縣。

其中多種可感染葫蘆科作物，如矮南瓜黃化嵌紋病毒 (*Zucchini yellow mosaic virus*; ZYMV)、西瓜嵌紋病毒-2 (*Watermelon mosaic virus*-2, WMV-2)、胡瓜嵌紋病毒 (*Cucumber mosaic virus*; CMV) 和木瓜輪點病毒 - 西瓜系統 *(Papaya ringspot virus*-watermelon strain; PRSV-W) (Provvidenti *et al*. 1984; Purcifull *et al*. 1988)。除了傳播病毒造成為害之外，病媒昆蟲產生的蜜露容易衍生煤煙病影響光合作用。近年來殺蟲劑仍是主要防治病媒昆蟲的方法，但往往會增加生產成本及潛在抗藥性族群的發展。

許多研究顯示，無論是生物性覆蓋物 (living mulches) 或人造資材覆蓋物 (synthetic mulches) 皆具有防治銀葉粉蝨或蚜蟲的效果，此技術可用來控制蟲媒病毒病害或是延緩病毒病害的散播和發生 (Stapleton & Summers 2002; Hooks & Fereres 2006; Abd-Rabou & Simmons 2012)。覆蓋植物 (cover plant) 和生物性覆蓋物對作物生產有許多益處。一般來說，覆蓋作物作為綠肥增加土壤氮源的效益，然而其更強化的效用在於：防止土壤沖蝕、提高土壤水分，以及抑制雜草的生長；生物性覆蓋物指的是覆蓋在土壤上的保護層，幫助土壤保持濕潤、減少侵蝕、提供養分、抑制雜草的生長。覆蓋作物和生物性覆蓋物有別於雜草，與作物具有相輔相成的效果。其功能有：(1) 增加土地的肥力；(2) 改善土壤理化性；(3) 翻埋可分解土壤中的養分供作物吸收利用；(4) 防止雜草生長；(5) 保持土中水分 (Kuo *et al*. 1995; Wyland *et al*. 1996; Reicosky & Forcella 1998; Unger & Vigil 1998)。

研究顯示，利用白花三葉草 (*Trifolium repens* L.) 種植於矮南瓜行株之間當作生物性覆蓋作物，可以有效降低矮南瓜葉子上的蚜蟲和銀葉粉蝨族群數量，並明顯降低銀葉粉蝨造成之典型病徵的嚴重度 (Hooks & Wrights 2008; Manadhar *et al*. 2009)。因此，應用白花三葉草於田間來抑制蟲媒病毒病害的可行性，乃本研究欲探討的問題。近年來葫蘆科作物病毒病害猖獗，卻缺乏有效抑制蟲媒傳播的防治技術。目前可以用來預防瓜類病毒病發生的市售產品是寡聚糖，具有調控生長、發育、繁殖、防病及抗病的效果 (Albersheim & Darvill 1985)；研究顯示，寡聚糖可降低感染植株體內的病毒濃度，並抑制病毒長距離的移動 (Kuo *et al*. 1995)。本研究目標以白花三葉草作為生物性覆蓋應用於有機南瓜栽培，評估防治 SLCPHV 專一性媒介昆蟲-銀葉粉蝨的能力，以及是否會影響銀葉粉蝨對於南瓜的偏好性，並建構適合有機南瓜栽培的環境且抑止田間 SLCPHV 的危害度上升。

材料與方法

銀葉粉蝨族群動態調查

供試作物自農友種苗購買之「東英」南瓜品種，溫室育苗 2 wk 於 2016 年 3 月 14 日達 2–3 真葉期並定植於田間，採露天匍地方式進行栽培，株距為 0.8 m，於畦面側邊採用單行植栽種，每 1 株往畦面另一側等距留 2 子蔓，每 1 子蔓留 1–2 果。試驗田區設計採完全逢機設計，共 3 處理，每處理各 3 重複，每重複各 2 小區，1 小區各栽種單行共 13 株 (圖 1A)，白花三葉草植株全貌可見圖 1B。各試驗處理如下：(1) 以白花三葉草作為生物性覆蓋。(2) 以銀黑塑膠布作為非生物性覆蓋並於定植後以解毒 747 處理 (解毒 747 為市售半乳糖醛酸，由高等植物細胞初生壁經降解酵素提取的物質，聚合度在 2–20 之間的溶液)。(3) 僅以銀黑塑膠布作為非生物性覆蓋為對照組。自 2016 年 4 月 8 日開始於每處理 2 小區間懸掛 1 張綠色黏蟲紙 (綠色黏紙為高冠牌誘蟲黏紙，長 0.215 m，寬 0.15 m 調查銀葉粉蝨成蟲數量，每張距離 10 m，以降低試驗處理間之誤差，每次調查懸掛時間約 1 wk。調查數據採用 SAS 套裝軟體 (SAS Institue, Cary, NC) 中之 PROC ANOVA (analysis of variance procedure) 進行變方分析 ($\alpha = 0.05$)，以 Fisher's LSD (least significant difference) test 檢測處理間平均值之差異顯著性。

田間南瓜病毒病害進程觀察及檢測

自 2016 年 4 月 15 日子蔓始生長時，調查田間病毒病害發生進程，調查項目如下：(1)

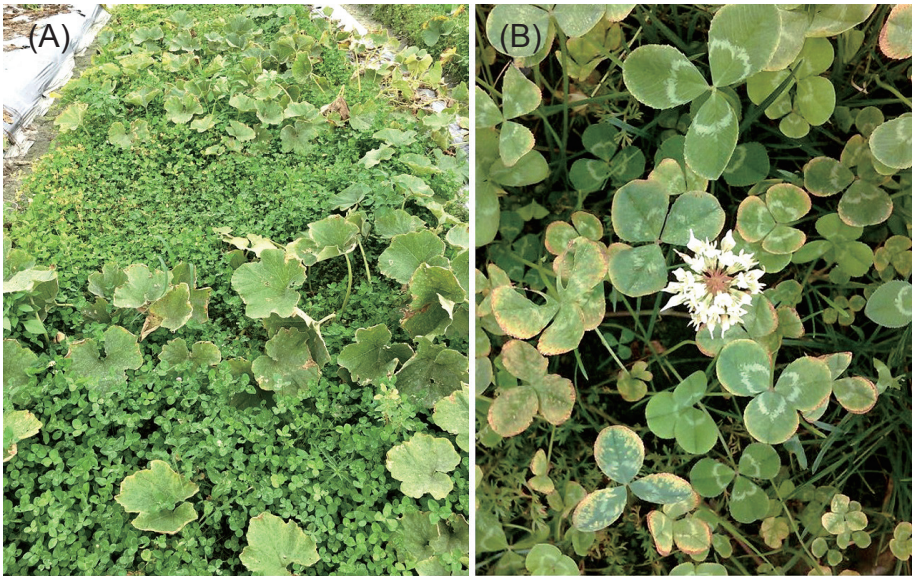


圖 1. 南瓜試驗田間以白花三葉草覆蓋栽培 (A)；白花三葉草植株近貌 (B)。
Fig. 1. The demonstration of using white clover as cover plants in pumpkin field (A); Full view of white clover (B).

田間病毒病害發生比率，肉眼觀察病徵統計結果。(2) 每小區採樣植株上位第 2–3 片葉子，參考 Weng *et al*. (2015) 植物核酸 (DNA) 粗萃取方法，每片葉子取 0.1 g 經均質研磨後加入 ELISA grinding buffer (1.59 g L^{-1} Na_2CO_3, 2.93 g L^{-1} $NaHCO_3$, pH 9.6 containing 2% PVP-40, 0.2% bovine serum albumin, 0.05% Tween 20 and 1% $N_2S_2O_3$) 2 mL 混合均勻後，吸取 2 μL 加入 25 μL GES buffer (0.1 M Glycine, 0.05 M NaCl, 1 mM EDTA, containing 0.5% Triton X-100) 混合均勻，反應 95℃/10 min，吸取 1 μL 粗萃取之 DNA sample 以農試所提供之引子對，SLCVup (5'-GTGGGCCCTCRACCA WTGAAATTC-3') 及 SLCVdw (5'-GAACYAG AATRAGATC GGRACA-3') 進行聚合酶連鎖反應 (polymerase chain reaction, PCR)。在 0.5 mL PCR 離心管中加入 12.5 μL GoTaq® (Taq DNA polymerase, dNTP, $MgCl_2$, reaction buffer)、9.5 μL 無菌水、1 μL 10 μM SLCVup、1 μL 10 μM SLCVdw 及 1 μL DNA sample，總反應體積為 25 μL。溫度循環條件為：Denaturation：94℃/2 min；擴增反應：30 循環 (94℃/1 min→52℃/1 min→72℃/2 min)；elongation：72℃/6 min。取 5 μL PCR 反應產物，進行洋菜膠體電泳反應並統計 SLCPHV 之罹病率。(3) 每小區每株採樣植株以市售專一性單株抗體 (DSMZ, Germany) 進行夾層酵素免疫測定 (Double Antibody Sandwich-Enzyme-linked immunosorbent assay, DAS-ELISA) 檢測田間其他瓜類病毒病害如 CMV、ZYMV、胡瓜綠斑嵌紋病毒 (CGMMV)、CCYV。CMV 和 ZYMV 皆可由蚜蟲傳播，CCYV 由銀葉粉蝨所傳播。此外，本研究額外檢測 CGMMV 的主要目的為屏除種子傳播和機械傳播的疑慮。

銀葉粉蝨降落偏好性和產卵偏好性檢定 (landing assay)

本試驗參照 Omondi *et al*. (2005) 種植 2–3 真葉期的南瓜苗於長型花盆 [長 (L) × 寬 (W) × 高 (H) = 65 cm × 25 cm × 20 cm]，每長型花盆種植 2 株瓜苗，並置放 2 盆於帳籠 [長 (L) × 寬 (W) × 高 (H) = 75 cm × 75 cm × 115 cm] 內，分別為無覆蓋和白花三葉草覆蓋處理。吸取約 200 隻銀葉粉蝨成蟲於傍晚 6 點至 6 點半間釋放至帳籠裡，並於 12、24、36、48、60 h 觀察每株由下數來 3 片瓜葉葉背成蟲的數量並以編號貼紙標記確認每次觀察為同一葉片，

待成蟲釋放後 3 d 觀察葉背產卵數量。本試驗進行 3 次重複試驗，並利用成對樣本 *t* 檢定 (Paired-Samples *t*-test) 比較兩處理間 3 次重複試驗的平均數據的差異。

有機南瓜採收期果實性狀及產量調查

5 月上旬開始為採收期並調查果高、果徑及單果重。調查數據採用 SAS 套裝軟體 (SAS Institue, Cary, NC) 中之 PROC ANOVA (analysis of variance procedure) 進行變方分析 ($\alpha = 0.05$)，以 Fisher's LSD (least significant difference) test 檢測處理間平均值之差異顯著性。

結果

銀葉粉蝨族群動態調查

試驗結果顯示南瓜於栽培中期時，各處理間的銀葉粉蝨成蟲數無顯著差異且平均每張黏紙誘集的數量皆不超過 5 隻，直至採收期之前 (4 月下旬)。銀黑塑膠布處理組和銀黑塑膠布搭配解毒 747 處理組的平均誘集成蟲數量，分別上升至 32.0 和 29.3 隻，白花三葉草覆蓋處理的成蟲數僅平均 1.3 隻，顯著低於其他試驗處理 (圖 2)。一週後銀黑塑膠布處理組和銀黑塑膠布搭配解毒 747 處理組，平均誘集成蟲數量分別降至 9.7 和 5.0 隻，但仍顯著高於白花三葉草覆蓋處理組。此試驗結果顯示，銀葉粉蝨於南瓜生長後期停駐於白花三葉草的偏好性降低，雖然以往文獻顯示銀黑塑膠布所反射的 UV 光亦有抑制銀葉粉蝨停駐作物的偏好性，但本試驗結果顯示效果以白花三葉草覆蓋處理較佳。

田間病毒病害發生進程

本病害於子蔓開始生長時 (2016 年 4 月 15 日) 開始調查田間南瓜病毒病發生比率，調查起前兩週各處理區的病毒病發生比率皆低於 0.1，僅銀黑塑膠布處理組達 0.13，各處理間無顯著差異。待至第 3 週 (5 月上旬) 起病徵觀察統計結果顯示，銀黑塑膠布處理組和銀黑塑膠布搭配解毒 747 處理組的病毒病發生比率，分別上升至 0.39 和 0.33，白花三葉草處理區僅達 0.13，發生比率顯著較低 (圖 3)。

採收期各植株經 PCR 分子檢測後，發現白花三葉草處理區內的 SLCPHV 感染率達 55.2%，顯著較低於銀黑塑膠布處理組的 91.3% 和銀黑塑膠布搭配解毒 747 處理組的 95.5%。其他瓜類病毒，如 ZYMV、CMV、CGMMV 和 CCYV 的感染率，經 ELISA 檢測後發現無明顯差異，且多為 CMV 和 SLCPHV 複合感染 (表 1)。此外，CGMMV 感染率偏低，最高僅達 1.7%，屏除種子傳播和機械傳播病

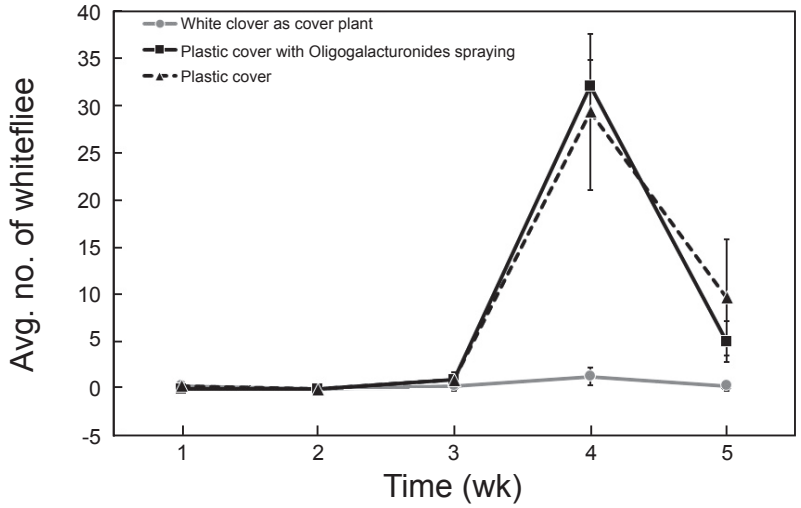


圖 2. 不同覆蓋南瓜田銀葉粉蝨數量動態比較。

Fig. 2. Comparison on the population dynamics of whiteflies in pumpkin field with different cover materials.

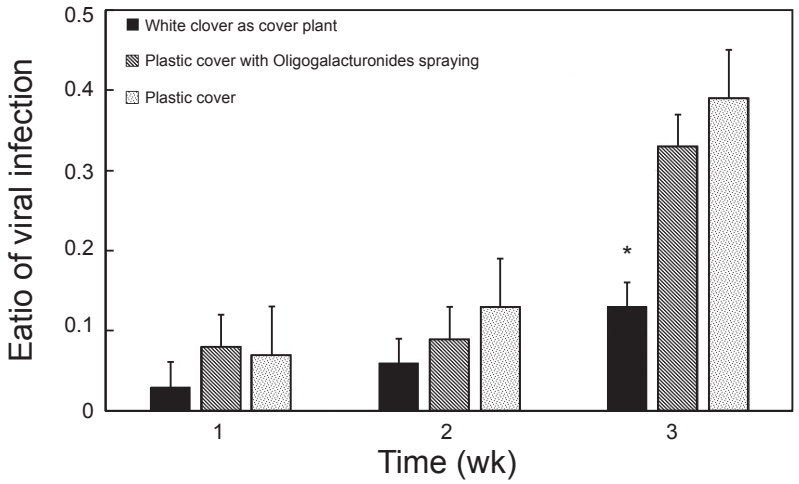


圖 3. 不同覆蓋南瓜田病毒病發生率比較。

Fig. 3. Comparison on the infection ratio of viral disease in pumpkin field with different cover materials. Asterisk indicates significant difference as determined by a Student *t*-test ($^{*}P < 0.05$).

表 1. 南瓜病毒病害感染率比較。

Table 1. The infection rates of viral diseases occurred on organic pumpkin.

Treatment	SLCPHV[z]	CMV	ZYMV	CGMMV	CCYV	No. of Samples
White clover	55.2 ± 5.71% a[y]	89.7 ± 3.96% a	5.2 ± 0.14% a	1.7%	1.7%	58
Plastic cover	91.3 ± 3.24% b	91.3 ± 3.24% a	6.5 ± 0.19% b	0.0%	0.0%	46
Plastic cover with Oligogalacturonides spraying	95.5 ± 3.33% b	75.0 ± 15.45% a	6.8 ± 0.59% b	0.0%	4.5%	44

[z] Infection rate of plant virus.
[y] Mean separation within columns by the least significant difference (LSD) at $P < 0.05$.

毒病害的疑慮。

有機南瓜採收期果實性狀及產量調查

南瓜採收期平均果重調查顯示，銀黑塑膠布處理組、銀黑塑膠布搭配解毒 747 處理組和白花三葉草覆蓋處理組分別為 1.13 kg、1.13 kg 和 1.12 kg，經統計分析兩兩比較後並無顯著差異。本試驗由於南瓜植株皆雙子蔓整蔓處理，因此果實大小形狀較為一致，經果實性狀調查果實高度和果實直徑亦無顯著差異 (表 2)。此外，白花三葉草處理區的缺株比率僅 0.28 較低於其他處理區。

銀葉粉蝨降落偏好性和產卵偏好性檢定 (landing assay)

銀葉粉蝨降落偏好性試驗結果顯示，白花

表 2. 南瓜採收期果實性狀及產量調查。

Table 2. Comparison on the characteristics and yield of pumpkin.

Treatment	Fruit weight (kg)	Fruit diameter (cm)	Fruit height (cm)	No. of fruit per 80 m^2	The rate of missing plant
White clover	1.12 ± 0.12 a[z]	14.6 ± 1.5 a	10.9 ± 1.0 a	55	0.28 ± 0.08
Plastic cover	1.13 ± 0.06 a	14.5 ± 1.8 a	11.2 ± 1.0 a	41	0.40 ± 0.09
Plastic cover with Oligogalacturonides spraying	1.13 ± 0.13 a	14.7 ± 1.8 a	10.8 ± 1.0 a	53	0.41 ± 0.14

[z] Mean separation within columns by the least significant difference (LSD) at $P < 0.05$.

三葉草覆蓋處理組平均每次試驗調查的成蟲數量自 10 隻上升至 14.8 隻；相較之下，對照組的成蟲數量自 8.7 隻上升至 12.2 隻，兩處理組的 3 次試驗平均成蟲數量並無顯著差異 (圖 4)。白花三葉草敷蓋處理者和對照組的平均蟲數皆隨著接種後時間越長，數量隨之增加，顯示銀葉粉蝨對於處理組和對照組無明顯的偏好性差別。另外，於接種後 60 h，本試驗觀察葉片上產卵量的結果顯示，銀葉粉蝨平均每次試驗於葉背分別產下 57.7 粒卵及 45.8 粒卵，經統計比較分析並無顯著差異 (表 3)。

討論

經田間調查結果顯示，白花三葉草作為覆蓋植物的南瓜苗具有降低銀葉粉蝨族群大量發生的效果，也間接導致田間銀葉粉蝨所傳播的 SLCPHV 發生率相較於無覆蓋植物者和覆蓋銀黑塑膠布者顯著較低。在採收期以 PCR 和 ELISA 檢測病毒感染的結果顯示，田間多為 CMV 和 SLCPHV 複合感染，其中田間病徵如黃化 (yellowing)、嵌紋 (mosaic)、捲葉 (leaf curl) 於第 3 次調查結果顯示，上述病徵發生植株比例於白花三葉草處理區內顯著較少，對應 PCR 檢測結果顯示相符合。田間病徵觀察統計病毒病發生率較低，推測應為病毒潛伏感染未顯現病徵所導致。此試驗結果印證，銀葉粉蝨的數量減少確實有助於降低田間 SLCPHV 的發生率，但是經 ELISA 檢測結果顯示，每處理區內受到 CMV 的感染比例皆高達 75.0% 以上，處理區間無顯著差異。據推測應為白花三葉草作為覆蓋植物並無防治蚜蟲的功能，導致田間 CMV 普遍發生。然而本研究溫室銀葉粉蝨生物檢定顯示，白花三葉草生物性覆蓋無顯著地降低銀葉粉蝨降落及產卵偏好性，因此白花三葉草生物性覆蓋處理對於瓜類作物病毒病發生是否有幫助仍待進一步證實。Frank & Liburd (2009) 研究證實白花三葉草並無抑制有翅型蚜蟲於矮南瓜田間發生；相反地，

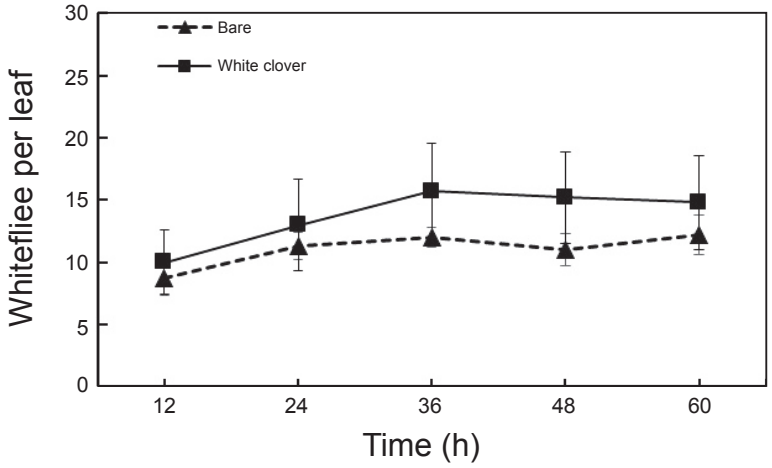


圖 4. 銀葉粉蝨降落偏好性檢定。
Fig. 4. Landing assay of whiteflies.

表 3. 每片葉子平均產卵數量。
Table 3. The average number of eggs per leaf.

Treatment	Avg. no. of eggs per leaf
White clover	57.7 ± 13.3 a[z]
Bare (control)	45.8 ± 13.8 a

[z] Mean separation within columns by the least significant difference (LSD) at $P < 0.05$.

有研究卻顯示白花三葉草處理可以降低蚜蟲傳播的病毒病害，如 CMV、ZYMV、WMV-2 和 PRSV-W 的病徵嚴重度 (Hooks & Wright 2008)。為模擬白花三葉草覆蓋及無植物覆蓋是否會影響銀葉粉蝨的生物行為，本研究利用南瓜苗種植於長盆模擬田間白花三葉草覆蓋處理，於溫室進行銀葉粉蝨降落偏好性和產卵偏好性檢定，結果顯示處理組和對照組並無顯著差異。前人研究結果顯示，田間白花三葉草覆蓋處理區的矮南瓜苗於定植後 21 d 方可見銀葉粉蝨成蟲數量顯著較無敷蓋處理區低，但產卵量並無顯著差異 (Hooks & Wright 2008)，此結果和本研究結果些許不同，推論原因有：(1) 供試植物不同產生行為的差異。(2) 銀葉粉蝨對南瓜苗種植於不同敷蓋植物的降落偏好性並無顯著差異，南瓜種植至苗期後才會造成偏好性差異。(3) 田間的銀葉粉蝨乃依靠自然環境衍生的族群和發生的位置，易受氣候和鄰田的影響；相對地，溫室無氣候環境和鄰田的因子干擾。未來本研究會進一步調查田間葉片上的銀葉粉蝨成蟲數量，避免黏紙與實際處理區內的蟲數產生落差。本研究無針對銀葉粉蝨與蚜蟲體內是否帶有 SLCPHV、ZYMV 和 CMV 作探討，未來研究將額外取樣蟲體檢測其體內是否帶有病毒，方可佐證田間瓜類病毒病發生率與傳播蟲媒的直接關係。SLCPHV 和銀葉粉蝨之間的流行病學研究並無許多文獻著墨，僅 Cohen *et al*. (1983) 指出 20 隻成蟲給予獲毒時間 (acquisition access period; AAP) 30 min 和接種時間 (inoculation access period; IAP) 6 h 即可達到 100% 的傳播效率，此外此研究保毒時間 (Retention time) 試驗結果指出，給予銀葉粉蝨雌性成蟲 4 h AAP，保毒時間最高可達 17 d 仍保有傳毒能力。未來研究盼能結合 SLCPHV 和銀葉粉蝨之間的傳播生物學基礎，評估白花三葉草是否具有影響銀葉粉蝨 AAP、IAP 和保毒時間等的效果，藉此佐證白花三葉草於田間防治 SLCPHV 擴散的機制。本研究無法直接證明，白花三葉草具有忌避銀葉粉蝨的功能，但田間試驗結果顯示，銀葉粉蝨的族群數量於白花三葉草覆蓋處理區內顯著較少，溫室降落偏好性和產卵偏好性檢定結果，反而顯示白花三葉草覆蓋處理組和無覆蓋對照組無顯著差異。據前人研究指出，銀葉粉蝨帶病毒成蟲與無帶病毒成蟲的取食行為有差異性 (Moreno-Delafuente *et al*. 2013)，因此本研究推測此為導致田間試驗結果與溫室試驗結果相互矛盾的原因，未來將探討帶病毒成蟲與無帶病毒成蟲生存於不同覆蓋處理區的南瓜植株上，取食行為和產卵行為有無差異性。

產量調查結果顯示，3 個處理區內的平均果重和果型無顯著差異，但栽培過程中發現白花三葉草的南瓜成熟時間較其他 2 區延遲約 1 wk，推測白花三葉草和南瓜生育期重疊時可能會與南瓜競爭養分。另外，本研究發現白花三葉草處理區內的南瓜於生育後期較能抵抗高溫造成植株萎凋的情形，經分離鑑定多數為蔓枯病引起。經田間觀察推論，南瓜栽種於銀黑塑膠布覆蓋處理者在氣溫較高時容易有萎凋的情形發生。Lay (2003) 指出白花三葉草可增加果園土壤有機物質含量，土壤總體密度檢測結果更顯示，白花三葉草具有有降低土壤壓實之效果，因此本研究推測經白花三葉草覆蓋處理後，土壤溫度較其他處理區低，水分平衡性和通氣性較好，可以幫助南瓜於生育後期度過高溫不良環境和防止蔓枯病感染。

由本研究結果證實，白花三葉草作為覆蓋植物能夠防止 SLCPHV 的大量發生，雖然產量無顯著增加，但可以間接減少除草劑施用成本和銀黑塑膠布資材的浪費。未來值得探討的是，白花三葉草對於下一期作的土壤是否具有涵養肥份的功能，節省下一期作基肥的施用。近年來白花三葉草有推廣利用於果園栽培的例子，屬多年生綠肥作物，並證實具有抑制雜草滋生、減少除草劑使用和降低生產成本的功能，更可降低土壤密度使土壤疏鬆，有助果樹根系群伸展 (Lay 2003)。未來應用於有機蔬果栽培，仍需要更多研究評估白花三葉草的功能性及經濟效益產生。

誌謝

本研究係執行行政院農委會 105 農科-10.5.3-花-V1 計畫之成果。本試驗期間承本課室同仁林瑋祥先生協助作物栽培管理及試驗相關工作，在此致謝。

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Using Cover Plants, *Trifolium Repens* L., to Enhance the Control of Insect-Borne Viral Disease in Pumpkin Field

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Abstract

Tsai, W. A., S. H. Weng, and C. J. Chiou. 2017. Using cover plants (*Trifolium repens* L.) to enhance the control of insect-borne viral disease. J. Taiwan Agric. Res. 66(4):298–306.

Cucurbitaceae fruit and vegetables are important crops in Taiwan. Plant viral disease usually cause the loss of crop production. There is no pesticide which can effectively control this viral disease. However, control of insect vectors become an important issue to date. Whitefly (*Bemisia argentifolii* Bellows and Perring) is one of the important cucurbit pests known to specifically transmit Begomoviruses, and *Squash leaf curl virus Philippines* (SLCPHV) has become widespreading in Taiwan in recent years. Aphids are also important insect vectors, known to spread at least four common plant viral diseases that damage Cucurbitaceae crops commonly. Living and synthetic mulches were evaluated for control of the *Bemisia argentifolii* Bellows and Perring and aphids. This study focused on the application of white clover (*Trifolium repens* L.) as cover plant in pumpkin cultivation. It showed that whitefly population in white clover treatment significantly reduced, and infection rate of SLCPHV was 55.2%, significantly lower than those of synthetic (reflective) mulches with 91.3% and oligomeric galacturonic acid spray treatment with 95.2%. The proportion of viral infection symptoms occurrence was about 13% at the end of the experiment. This study suggests that white clover would control the population of whiteflies in pumpkin field efficiently. The practice has the potential to be applied to construct organic pumpkin cultivation environment.

Key words: Cover plant, Cucurbitaceae, Insect-borne virus, *Bemisia argentifolii* Bellows and Perring.

Received: August 27, 2016; Accepted: January 15, 2017.

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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):307–317 (2017)
DOI: 10.6156/JTAR/2017.06604.05

研究報告

快速、精確地使用影像分析來測量水稻瘤野螟 (縱捲葉蟲) 取食葉面積

劉弘一 [1]　黃守宏 [2,*]　蔣國司 [3,**]

摘要

劉弘一、黃守宏、蔣國司。2017。快速、精確地使用影像分析來測量水稻瘤野螟 (縱捲葉蟲) 取食葉面積。台灣農業研究 66(4):307–317。

瘤野螟 [Rice leaf folder, *Cnaphalocrocis medinalis* (Guenée)] 是水稻主要害蟲之一，其幼蟲取食水稻葉片致使減少葉片的光合作用，進而造成水稻極大的產量損失。傳統對於瘤野螟幼蟲取食葉片面積採用方格紙描繪測量，此人工方法需耗費相對多的時間及人力，本研究證實利用影像分析 (ASSESS 2.0) 來進行瘤野螟取食葉面積的測量，其結果與黃金標準 (人工描繪測量) 顯示具有極高的一致性 (ρ_c 高於 0.983)，而且經變方分析驗證此兩測量方法並無顯著差異；此外，影像分析可以減少 29% 以上的測量時間，故推薦影像分析可作為瘤野螟取食稻葉面積測量的工具。

關鍵詞：水稻、瘤野螟、取食面積、測量、影像分析。

前言

水稻 (*Oryza sativa* L.) 是世界上最重要的糧食作物之一，超過 95 個國家種植及生產水稻，而且全球有一半以上人口以稻米作為每日所需熱量的主要來源 [Counce *et al*. 2000; International Rice Research Institute (IRRI) 2002; Coats 2003]。瘤野螟 [Rice leaf folder, *Cnaphalocrocis medinalis* (Guenée)] 是水稻主要害蟲之一，尤其當高產水稻品種被廣泛種植，以及密植與增施氮肥等耕作方式改變後日益嚴重 (Dale 1994; Nathan *et al*. 2004, 2005)。瘤野螟幼蟲會刮食水稻葉片的葉肉組織，影響光合作用的進行，因此造成產量降低。Padmavathi *et al*. (2013) 的研究更詳細說明了瘤野螟幼蟲對於水稻劍葉取食，造成葉綠素損失、減少光合作用及相對水含量，是造成水稻產量損失的主要原因。在台灣之研究指出，若無防治或防治不當，可以影響稻穀產量達 18–24% (Yen 1981)；而在印度的坦米爾納德邦之研究，顯示瘤野螟是可以造成 11.18% 的產量損失 (Shanmugam *et al*. 2006)。

在其他相關研究，例如評估瘤野螟危害所造成的水稻產量損失影響，或是對瘤野螟抗性品種改良之檢定評估，多需透過調查取食面積而作進一步分析及研究 (Selvaraju *et al*. 2007; IRRI 2013; Padmavathi *et al*. 2013; Manikandan *et al*. 2016)，因此調查方法能否夠精確、快速測量瘤野螟取食葉面積將是一個重要課題。以往，對於瘤野螟取食葉面積測量多是利用在方格紙上描繪被取食區域，再利用人工計數方格數後估計面積 (Bautista *et al*. 1984; Cheng 1987; Dangar 2008; Mohapatra *et al*. 2009)，這樣的測量方法也同時被運用於

投稿日期：2016 年 12 月 7 日；接受日期：2017 年 3 月 14 日。
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棉鈴蟲 (*Helicoverpa armigera* Hübner) (Selvapandiyan *et al*. 1998) 及其他昆蟲 (Lowman & Heatwole 1992) 之危害測量上。在植物病害測量上，影像分析 (image analysis; IA) 早已被論證可以可靠且正確的估計植物病害面積 (Lindow & Webb 1983; Martin & Rybicki 1998)。而近年，影像分析軟體 ASSESS 2.0：image analysis software for plant disease quantification (American Phytopathological Society, St. Paul, MN) 則相對廣泛被運用在植物病害面積測量上 (Bock *et al*. 2008, 2009a, 2009b, 2009c; Xie *et al*. 2012; Yadav *et al*. 2013)，其中 Bock *et al*. (2008, 2009c) 論證使用 ASSESS 2.0 分析之結果較具準確度 (accuracy) 及精密度 (precision)，並將影像分析結果視為真實病害嚴重程度以進行後續之分析，其餘報告則多承襲這個結論，直接採用影像分析結果為真實病害嚴重程度。目前亦有利用 ASSESS 2.0 運用於蟲害面積測量上之研究 (Mirik *et al*. 2006; Perović *et al*. 2008; Prior & Hellmann 2010)，但前述在蟲害上的研究皆直接採用 ASSESS 2.0 作為測量危害面積之工具，但並未對其測量結果的精密度及準確度做進一步的探討，因此本研究以實際的數據來驗證影像分析方法之精密度及準確度。

從之前研究可知，以方格紙描繪及測量植物葉片受損面積，在蟲害研究上已廣泛被使用 (Bautista *et al*. 1984; Lowman & Heatwole 1992; Selvapandiyan *et al*. 1998; Dangar 2008; Mohapatra *et al*. 2009)，其測量結果有相當的代表性，然而這樣的方法可能相對較為費時及耗費人力。鑑於前述，ASSESS 2.0 軟體既然多被推薦運用於植物病害測量上，且亦有少數蟲害取食葉面積的測量亦使用此軟體，因此本研究首次將 ASSESS 2.0 運用於瘤野螟取食水稻葉面積測量之探究，評估測量瘤野螟取食葉面積的精密度及準確度，並比較人工方式 (方格紙描繪及測量) 與影像分析之耗時差異。

材料及方法

測量的水稻葉片

本研究分別使用粳稻品種「台農 67 號」('TNG67') 以及秈稻品種「台中在來 1 號」('TN1') 植株提供瘤野螟 1 至 5 齡幼蟲取食，供試水稻的肥料供應乃依據作物施肥手冊 (http://www.afa.gov.tw/publish_tree.aspx?catid=70)，取樣之葉片為水稻分蘗期 (30 DAT；移植後 30 d)，被取食葉片取下後攤平於白色紙張上，並以玻璃片壓置後，旁邊置放 1 元硬幣作為尺度參考 (圖 1)，於翻拍架下拍照。其中，除了 'TN1' 供五齡幼蟲取食僅 4 重複外，其餘皆為 5 重複。

取食面積之測量

各被取食葉片影像經編號後，於每一次評估以隨機方式重新排序供人工方式 (manual approach; MA) 及影像分析分別判讀取食面積，其評估方法的程序說明如圖 2，每次評估間隔 1 wk 以上，藉以排除評估者的記憶效應。

人工方式以方格紙進行測量時，分別描繪及計數「1 元硬幣直徑之方格數」及「取食區域之方格數」，並以 1 元硬幣直徑所佔方格數推算被取食葉面積，此外同時也記錄每一個影像測量所耗費的時間 (單純測量耗時)，即自開始描繪方格至計數取食區域所佔方格數之秒數，其中亦包含 1 元硬幣直徑的測量。由於人工方式測量無須額外程序，故單純測量耗時亦為完整測量程序耗時 (圖 2)。

在進行樣本葉片影像分析前，先利用免費的繪圖軟體 PhotoCap 4.0 (http://www.photocap.com.tw) 將各個影像進行去背處理，以



圖 1. 瘤野螟取食水稻葉片之影像。

Fig. 1. The image of damaged leaf caused by rice leaf folder.

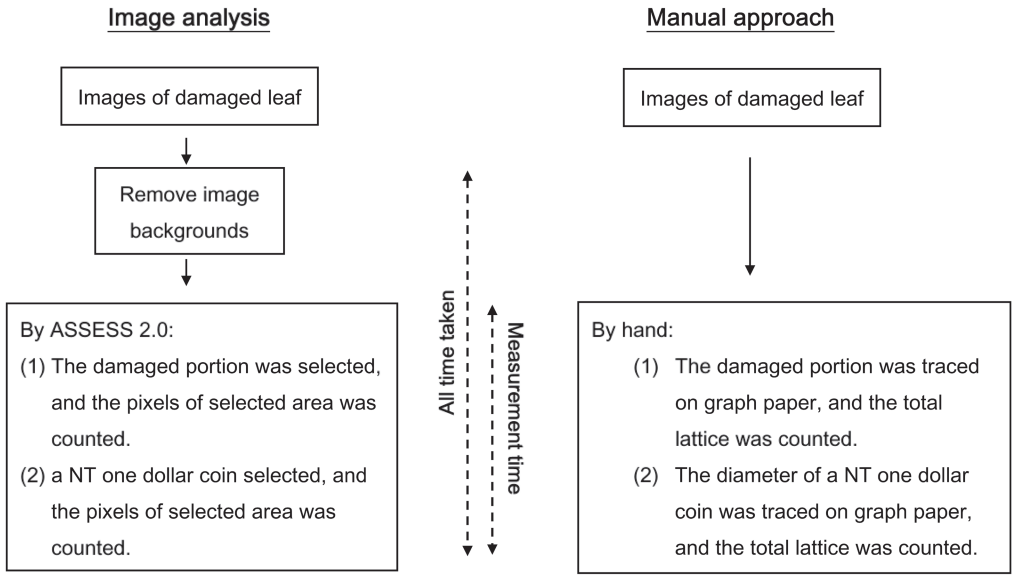


圖 2. 不同評估方法的程序說明。

Fig. 2. Procedures of different assessment methods.

便使 ASSESS 2.0 可以輕易辨識照片中物件 (1 元硬幣或葉片) 區域，再依照隨機順序逐一利用 ASSESS 2.0 將樣本葉片影像開啟，並透過其 HIS (Hue-Saturation-Intensity color model) 或 RGB (Red-Green-Blue color model) 功能產生像素的色調、飽和度、強度或顏色分布直方圖，並調整設定最佳門檻值用以圈選被取食部分及 1 元硬幣區域，單一目標圈選完成後即可計算該區域之像素 (pixel)，隨後再由 1 元硬幣及被取食葉片部分之像素比例推算被取食葉面積。此外，試驗進行時亦記錄每片葉片每次進行去背及影像分析所耗費之時間，去背及影像分析總耗時即為完整測量程序耗時，而影像分析耗時為單純測量耗時 (圖 2)。

統計分析

為了檢測測量方法的優劣，本試驗研究採用 Lin (1989) 提出的一致性相關係數 (Lin's concordance correlation coefficient; LCC) 作為衡量標準，其與傳統利用迴歸分析的結果相比較，顯示對於試驗結果之準確度及一致性 (agreement)，提供一個無偏且可量化的檢測方法 (Bock *et al*. 2010)。其中，所謂的一致性是指測量結果之間的接近程度，因此它是一個同時包含準確度及精密度的用詞 (Lin 2008)。

用來衡量兩個方法或是兩次評估一致性的 LCC (ρ_c) 可被表示為：

$$\rho_c = r \times C_b \quad (1)$$

r 是兩數對相關係數，用來測量精密度，對於重複測量數對之相關係數，亦用做測量方法內可靠性 (intra-rater reliability) 或稱為再現性 (reproducibility)；而 C_b 是偏差校正係數，用來度量最佳配適線與 45 度角直線的差異，因此被用來評估準確度。ρ_c 用於評估觀測值數對落於 45 度角直線 (斜率為 1、截距為 0) 的程度，ρ_c 值會介於 -1 至 1 之間，當 ρ_c 為 1 時表示兩變數具完美的一致性，ρ_c 為 0 時則表示完全缺乏一致性，而 ρ_c 小於 0 呈現出兩變數趨勢相反，C_b 又可被表示為：

$$Cb = 2/(\upsilon + 1/\upsilon + u^2) \quad (2)$$

這裡 $\upsilon = \sigma_x/\sigma_y$ 以及 $u = (\mu_x - \mu_y)/(\sigma_x \times \sigma_y)^{1/2}$，而 μ_x 和 μ_y 分別為 x 和 y 的平均，σ_x 及 σ_y 分別是 x 和 y 的標準差 (standard deviation)。υ 被稱為尺度偏差 (scale bias) 或是斜率偏移，其

值為 1 時表示 x 和 y 之變異數完全相同；而 u 被稱為位置偏差 (location bias)，當其值為 0 時表示 x 和 y 之平均值完全相同。

對於不同方法測量取食面積之耗時差異，本研究藉由簡單線性迴歸來分析耗費時間與取食面積大小之關係。研究中並利用變方分析，進一步瞭解不同因子 (測量方法、水稻品種及瘤野螟齡期) 對測量結果及耗時的效應，模式表示為：

$$y_{ijk} = \mu + m_i + v_j + a_k + \varepsilon_{ijk} \quad (3)$$

這裡 y_{ijkl} 表示某一片葉片測量到的取食面積或耗費的時間，m_i 為測量方法的效應 (i = 1, 2)，v_j 為水稻品種效應 (j = 1, 2)，a_k 為瘤野螟齡期效應 (k = 1–5)，而 ε_{ijkl} 是服從獨立且具有相同分布 (independent and identically distributed; i.i.d.) 的試驗誤差。進一步利用最小顯著差異檢定法 (least significant difference test; LSD)，針對測量方法、水稻品種及瘤野螟齡期因子進行檢定，來探討對於取食面積或耗費時間的差異；本研究中相關統計分析利用 SAS V9.4 版及 R 統計軟體 3.2.3 版進行。

結果

表 1 呈現測量結果之初步敘述統計，就取食面積而言，測量之最大、最小值在人工方式 (0.02–21.89 cm^2) 與影像分析 (0.03–22.01 cm^2) 間差異不大，但平均值及標準差則顯示影像分析皆有稍大的現象。就測量所耗費的時間而言，人工方式顯示需要耗費較長的時間，最大需要 1,106 s；而各葉片測量所費的時間之標準差，人工方式亦較影像分析高約 3–9 倍。以兩次測量之平均耗時進行比較，顯示在完整測量程序耗時比較下，人工方式每葉平均需增加 62.5 s；而單純測量耗時比較下，則需增加 144 s。由於在完整測量程序情況下，因為影像分析額外考慮了進行去背處理所需耗費時間，故使得兩個測量方法在完整測量程序上耗時有較小的差異。整體平均而言，兩方法所測量之取食面積上差異不大 (僅相差約 0.31 cm^2)，而耗費時間上則顯示影像分析相對於人工方式約可省時 29–67% (表 1)。

將人工方式測量取食面積的兩次結果，繪製成散布圖並計算 LCC 相關之統計量 (圖 3)，顯示散布點皆非常貼近 45 度角之直線，而 r、C_b 及 ρ_c 分別為 0.997、0.996 及 0.999，

表 1. 影像分析及人工方法對 49 片水稻葉片進行兩次取食面積、耗時量測量結果之平均、最大、最小值及標準差。

Table 1. The minimum, maximum, mean and standard deviation of estimates of damaged leaf area, all time taken and measurement time taken for 49 rice leaves measured by image analysis and manual approach on two separate occasions.

Item	Measurement method	Min.	Max.	Mean	Std Dev
Damaged leaf area (cm^2)	Manual approach #1	0.02	19.48	3.20	4.70
	Manual approach #2	0.02	21.89	3.22	4.86
	Image analysis #1	0.03	22.01	3.50	5.19
	Image analysis #2	0.03	21.61	3.53	5.33
All time taken (s)	Manual approach #1	40	1,106	212	193
	Manual approach #2	34	984	215	193
	Image analysis #1	50	307	153	56
	Image analysis #2	64	333	149	61
Measurement time taken (s)	Manual approach #1	40	1,106	212	193
	Manual approach #2	34	984	215	193
	Image analysis #1	35	151	71	21
	Image analysis #2	25	188	68	30

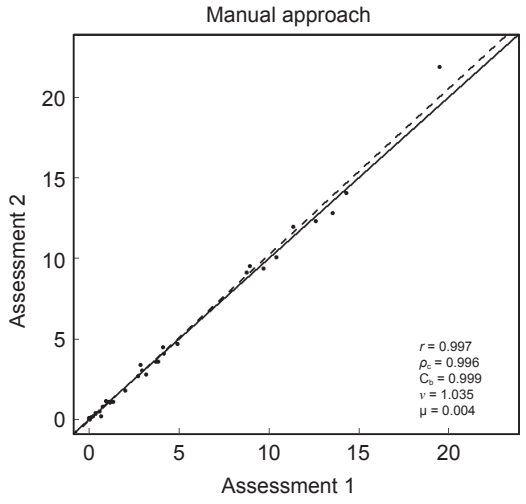


圖 3. 以 LCC 度量人工方法兩次評估的方法內可靠性。實體直線表示 45° 角之一致線，而虛線為兩次評估一致性的配適線。

Fig. 3. Intra-rater reliabilities of two separate assessment dates by manual approach were measured using Lin's concordance correlation (LCC). The concordance line is indicated by the solid line, and the dashed line shows the actual relationship between the two assessments.

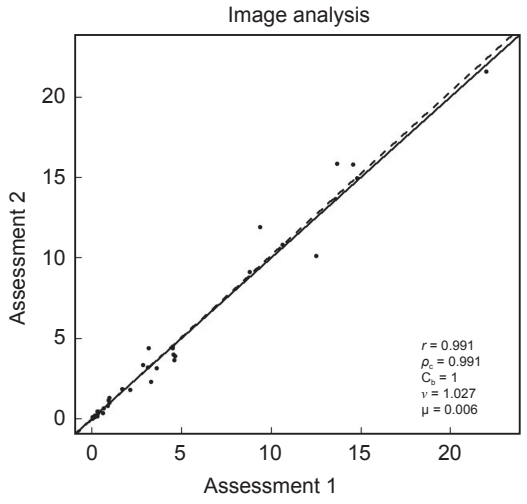


圖 4. 以 LCC 度量影像分析兩次評估的方法內可靠性。實體直線表示 45° 角之一致線，而虛線為兩次評估一致性的配適線。

Fig. 4. Intra-rater reliabilities of two separate assessment dates by image analysis were measured using Lin's concordance correlation (LCC). The concordance line is indicated by the solid line, and the dashed line shows the actual relationship between the two assessments.

表示人工方式測量結果具有非常高的方法內可靠性，且兩次測量結果亦具高度準確度及一致性。由於人工方式進行測量瘤野螟取食葉面積是此蟲害測量的常用方法，加上本研究調查結果人工方式測量具有高度的方法內可靠性，故可以合理將人工方式測量結果視為黃金標準 (或真實值) (Lin 1989; Bock *et al*. 2010)，並以此為標準，進而評估影像分析運用於瘤野螟取食面積測量之可行性。

在影像分析的取食面積測量結果，我們也進行類似的可靠性分析 (圖 4)，兩次測量結果亦具相當高的一致性 (ρ_c = 0.991)，所以影像分析測量結果也具有高度的方法內可靠性。

將人工方式測量結果視為黃金標準，進一步透過計算 LCC 相關統計量，評估影像分析測量對於黃金標準之一致性。圖 5 分別呈現兩次測量的方法間一致性的比較，影像分析測量結果對於黃金標準顯示具極高的準確度 (C_b 分別為 0.993、0.994) 及精密度 (r 分別為 0.992、0.989)，整體一致性亦相當高 (ρ_c 分別為 0.986、0.983)，因此可以斷定影像分析測量結果與黃金標準幾乎沒有差異。

為了進一步探究兩個測量方法耗時與瘤野螟取食葉面積 (黃金標準) 的關係，前述數據皆採用兩次評估的平均值進行分析，圖 6 及圖 7 分別顯示完整測量程序耗時、單純測量耗時相對差異 [(MA − IA)/IA × 100%] 對取食面積的關係，以及所配適的簡單迴歸關係式。觀察圖 6 及圖 7 的散布點可發現取食葉面積超過 3 cm^2 後，所有的散布點皆落於縱軸大於 0 的位置，意味此時人工方式測量耗時皆大於影像分析，且呈現隨取食葉面積增加而增加之趨勢。由配適關係式亦可看出人工方式測量較為耗時的關係，完整測量程序與單純測量所估之斜率估值分別為 14.71 (圖 6) 及 52.28 (圖 7)，所以可以斷定在測量較大範圍的取食面積時，影像分析占有時間上之優勢；另外，是否考慮影像去背所耗費的時間，將影響約 3.5 倍影像分析在時間效率上的優勢。

為探究測量方法 (method)、水稻品種 (va-

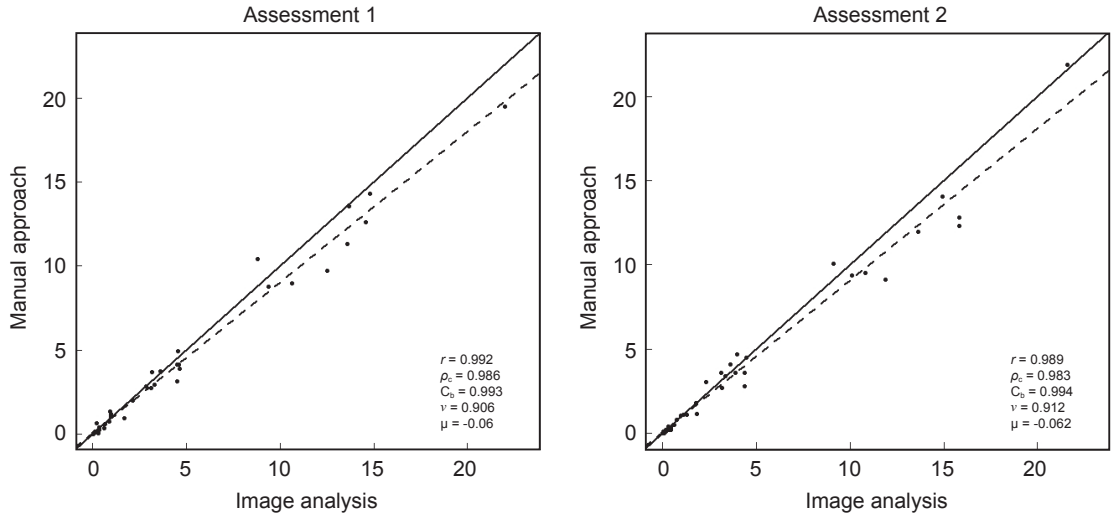


圖 5. 以人工方法為標準，測量兩次影像分析個別準確度及精密度。實體直線表示 45° 角之一致線，而虛線為兩次影像分析評估之一致線。

Fig. 5. The accuracy and precision of damaged leaf area measurements on two separate assessment dates were evaluated by image analysis when measurements of manual approach were regarded as true values. The concordance line is indicated by the solid line, and the dashed line shows the actual relationship between the two assessment methods.

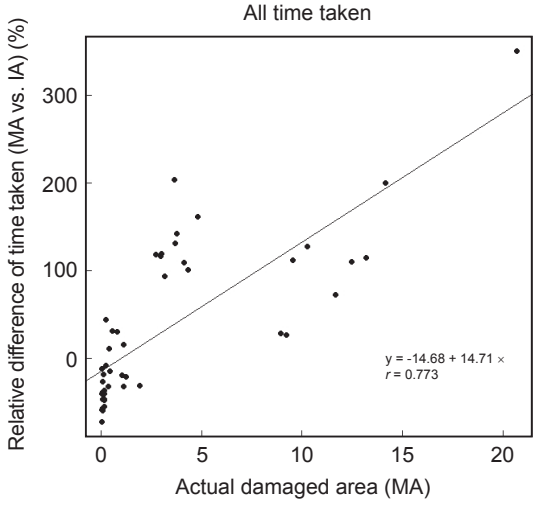


圖 6. 人工測量與影像分析在完整測量程序耗時的關係圖。實體直線表示最佳配適線。

Fig. 6. Relationship between manual approach and image analysis for all time taken during measurement processing. The solid line is the best fitting line.

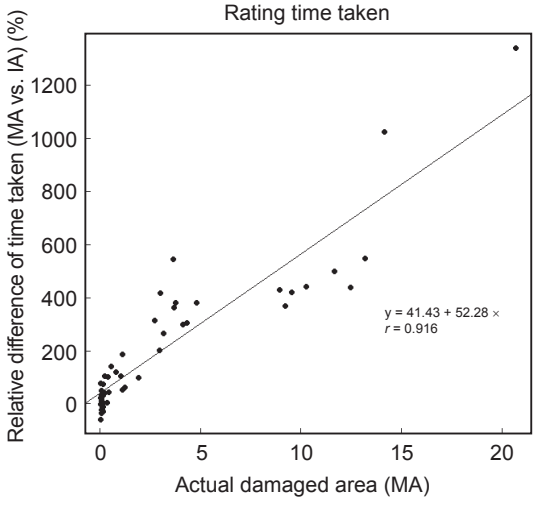


圖 7. 人工測量與影像分析在單純測量耗時的關係圖。實體直線表示最佳配適線。

Fig. 7. Relationship between manual approach and image analysis for measurement time taken for damaged leaf area. The solid line is the best fitting line.

riety) 及瘤野螟齡期 (instar) 等因子，對取食面積測量結果及耗時的效應之影響，各變方分析結果一併呈現於表 2。在取食面積的測量部分，測量方法間並無顯著差異，而水稻品種、瘤野螟齡期則分別呈現顯著差異 ($P < 0.05$) 及極顯著差異 ($P < 0.01$)。而在測量所耗費的時間上，無論是完整測量程序或是單純測量，人工方式與影像分析則具有極顯著差異，此外，

表 2. 取食面積、完整測量程序耗時及單純測量耗時之變方分析結果。

Table 2. Variance analysis for damaged leaf area, all time taken and measurement time taken.

		Mean Square		
Source	DF	Damaged leaf area	All time taken	Measurement time taken
Variety	1	12.9*	4,396.4	1,960.0
Instar	4	1,081.6**	462,126.2**	333,734.1**
Method	1	4.6	191,468.8**	1,011,317.6**
Error	189	2.6	10,908.0	12,225.5

*,** denote significances at 5% and 1% levels, respectively.

瘤野螟齡期也是具有極顯著差異。因此，基於變方分析的結果，顯示人工方式與影像分析對取食面積的測量並無統計上的差異性，而耗時多寡則具有差異。

進一步由 LSD 檢定各因子對取食面積測量結果及耗時的差異。就測量方法而言，影像分析的平均取食面積雖然稍微高於人工方式的測量結果，但差異未達 LSD 差異臨界值 (α = 0.05)，顯示兩個測量方法在取食面積測量並無統計差異 (表 3)；而在耗時的效應上，人工方式在完整測量程序耗時、單純測量耗時之平均值分別較影像分析高出 41.5% (多 62.5 s) 及 206.3% (多 143.7 s) (表 3)。就水稻品種而言，'TNG67' 的平均被取食面積高出 31.1% (較 'TN1' 多 0.9 cm^2) (表 4)；而在耗時的效應上則無顯著差異。此外，就瘤野螟齡期而言，在取食面積、完整測量程序或單純測量耗時皆有一些差異 (表 5)，主要隨蟲齡增加而增加取食面積，因此測量耗時也是隨之增加；幼蟲在粳稻 'TNG 67' 及秈稻 'TN 1' 水稻品種的平均取食面積分別為 19.01 及 16.29 cm^2 (表 6)；4–5 齡幼蟲的取食面積分別佔總取食量的 92.16% 及 94.90%。

討論

隨著近年來高產水稻品種的選育及化學肥料大量施用，促使瘤野螟嚴重發生並大量取食水稻葉片組織，危害水稻產量至鉅 (Sogawa *et al*. 2003)，諸多研究也訂立適當經濟危害基準

表 3. 不同測量方法對於取食面積、完整測量程序耗時及單純測量耗時之 LSD 檢定結果。

Table 3. Least significant difference test for damaged leaf area, all time taken and measurement time taken of different measurement methods.

Method	Damaged leaf area	All time taken	Measurement time taken
Manual approach	3.2 A[z]	213.3 a	213.3 a
Image analysis	3.5 A	150.8 b	69.6 b
LSD (α = 0.05)	0.5	29.4	31.2

[z] Means within each column by the different letters are significantly different.

表 4. 不同水稻品種對於取食面積、完整測量程序耗時及單純測量耗時之 LSD 檢定結果。

Table 4. Least significant difference test for damaged leaf area, all time taken and measurement time taken of different rice varieties.

Variety	Damaged leaf area	All time taken	Measurement time taken
TN1	2.9 B[z]	183.2 a	141.8 a
TNG67	3.8 A	180.9 a	141.2 a
LSD (α = 0.05)	0.5	29.4	31.2

[z] Means within each column by the different letters are significantly different.

表 5. 不同瘤野螟齡期對於取食面積、完整測量程序耗時及單純測量耗時之 LSD 檢定結果。

Table 5. Least significant difference test for damaged leaf area, all time taken and measurement time taken of different instars.

Instar	Damaged leaf area	All time taken	Measurement time taken
5	12.9 A[z]	357.3 a	282.0 a
4	3.7 B	232.7 b	199.2 b
3	0.9 C	141.7 c	104.4 c
2	0.2 D	107.0 cd	70.3 c
1	0.1 D	89.1 d	65.5 c
LSD (α = 0.05)	0.7	46.6	49.3

[z] Means within each column by the different letters are significantly different.

表 6. 不同瘤野螟齡期對粳稻 'TNG 67' 及秈稻 'TN 1' 水稻品種的平均取食面積。

Table 6. Means of damaged leaf area of different instars for 'TNG67' and 'TN1'.

	Damaged leaf area (cm^2)	
Instar	TN1	TNG67
1	0.06	0.05
2	0.16	0.21
3	0.61	1.23
4	3.57	3.83
5	11.89	13.69
Total	16.29	19.01

提供採取防治措施之參考，且其經濟危害研究之基準也多以取食面積為基礎 (Bautista *et al*. 1984; Cheng 1987)。往昔研究判定瘤野螟幼蟲取食危害面積，多以方格紙 (Cheng 1987) 或將被害葉片經標示後再經葉面積儀進行判讀 (Heong 1990)，操作程序相對耗時費力，且依據其研究之結果，瘤野螟在粳稻 ('TNG 67', 30 DAT) 上之平均取食面積為 21.4 cm^2 (17.0–26.9 cm^2)，與本研究 ('TNG 67', 30 DAT) 之結果相近 (19.01 cm^2) (表 6)；而秈稻 ('MR-84', 40 DAT) 上之取食面積 (24.3 cm^2) 則與本研究 ('TN1', 30 DAT) 之結果 (16.29 cm^2) (表 6) 有些差距，此結果可能是受到水稻品種、水稻生育期、栽培管理 (肥料施用量) 及測量方法等因素之影響 (Cheng 1987; Heong 1990)。

傳統以方格紙描繪瘤野螟幼蟲取食葉片處，進而計數葉片受損面積，本研究指出需耗費相對多的時間及人力，而以 ASSESS 2.0 軟體進行影像分析方法，可以減少 29% 以上的測量時間 (表 3)，此外影像分析測量的結果精密度及準確度與傳統人工方式一致，因此以 ASSESS 2.0 軟體進行影像分析，相當值得推薦做為瘤野螟取食稻葉面積測量的工具。ASSESS 2.0 雖然已被運用於進行蟲害葉面積測量，然而研究皆直接運用測量結果於進一步的分析討論中，並未對測量結果的精密度及準確度做進一步討論 (Mirik *et al*. 2006; Perović *et al*. 2008; Prior & Hellmann 2010)。本研究利用 ASSESS 2.0 來進行瘤野螟取食葉面積的測量，並論證此方法具有相當高的精密度及準確度，此將提供後續運用相當充足的證據，此外相信同樣也能提供給其它蟲害研究做為參考。

人工方式測量在方法內可靠性相當的高，而影像分析的方法內可靠性亦有極佳的表現，這也顯示具有豐富經驗或訓練有素的昆蟲學家，透過方格紙描繪測量取食面積，結果將會具有相當高的再現性，而透過影像分析將可以大大輔助測量的穩定性，例如本研究的影像分

析操作人員，其僅具基本瘤野螟取食葉片區域辨識認知，並無豐富相關判讀經驗，亦能透過 ASSESS 2.0 軟體進行影像分析，獲得高度可靠性之取食面積測量結果，這將也是影像分析另一項被推薦的優點。

影像分析方法可以提高測量的時間效率，分析中額外區別出影像去背的時間耗費，主要是在凸顯去背處理將是進一步可以改進時間效率的主要因素；而提高去背處理的時間效率上，除了考慮藉由更為自動化的影像處理軟體的協助外，在取得受危害葉片影像時，考慮背景顏色的單一性及可區別性，也是可以考慮的方向。係因為一般影像編輯軟體，主要以顏色為基準，可自動選取欲去除之背景區域，若背景顏色與受危害葉片顏色相似，或因拍照環境光線影響，使背景顏色不易區別，則需進一步透過手動選取欲去除之背景，因此將大為增加去背的時間耗費；而且當無法獲得最佳化的去背影像，無論如何多精密度及準確度的測量方法，都將無法獲得正確的測量結果，而此也正是此試驗進行拍攝時，將被害葉片置於白色紙張上之原因。

瘤野螟啃食水稻葉片表皮組織，留下白色薄膜，形成可供人工及影像判讀分析之基礎，惟在水稻葉片表面造成白色葉片組織之情況，不僅僅只有瘤野螟，其他蟲害 (如鐵甲蟲、負泥蟲)、病害 (白葉枯病)，甚至物理傷害等，均可能造成影像分析之誤判，分析前宜仔細辨認、去除雜訊，是影像分析上需要加強及注意之處。

誌謝

本試驗進行期間承蒙蕭繡琴、楊雪鳳及賴錦雲等小姐的協助，使試驗得以順利完成，在此一併誌謝。

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A Rapid, Accurate, and Precise Image Analysis to Measure the Damaged Leaf Area Infested with Rice Leaf Folder

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Abstract

Liu, H. I., S. H. Huang, and K. S. Chiang. 2017. A rapid, accurate, and precise image analysis to measure the damaged leaf area infested with rice leaf folder. J. Taiwan Agric. Res. 66(4):307–317.

Rice leaf folder [*Cnaphalocrocis medinalis* (Guenée)] is one of the major pests of rice. Injury to flag leaf caused by rice leaf folder larva will reduce the efficiency of photosynthesis process, thereby leading to serious yield loss. Traditionally, the damaged leaf area of rice leaf folder has been measured using manual approach. However, the traditional method takes relatively more time and manpower. In this study, the image analysis via ASSESS 2.0 was used to measure the damaged leaf area. The result showed that measurement and analysis of image was highly consistent with that of the manual approach, which is regarded as the gold standard (ρ_c higher than 0.983). Moreover, the variance analysis showed that the two methods had no significant difference. In addition, the image analysis can reduce at least 29% measurement time. Thus, image analysis can be recommended as a tool for the damaged leaf area measurement infested with rice leaf folder.

Key words: Rice, *Oryza sativa* L., Rice leaf folder, *Cnaphalocrocis medinalis* (Guenée), Measurement, Image analysis.

Received: December 7, 2016; Accepted: March 14, 2017.
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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):318–325 (2017)
DOI: 10.6156/JTAR/2017.06604.06

研究報告

咖啡果小蠹於咖啡果實內之齡期組成與防治藥劑之室內藥效評估

朱宜芬[1] 楊滿霞[2] 張淑芬[3] 陳柏宏[4] 王泰權[4,*]

摘要

朱宜芬、楊滿霞、張淑芬、陳柏宏、王泰權。2017。咖啡果小蠹於咖啡果實內之齡期組成與防治藥劑之室內藥效評估。台灣農業研究 66(4):318–325。

咖啡果小蠹 [*Hypothenemus hampei* (Ferrari)] 是危害全球咖啡生產最嚴重的害蟲，近年來入侵台灣造成咖啡生產嚴重損害，本研究進行咖啡果小蠹於果實內之齡期發生結構調查及藥劑篩選試驗。咖啡果小蠹於果實內，可以同時發現卵、1 齡幼蟲、2 齡幼蟲、前蛹、蛹及雌雄成蟲，雌雄成蟲比率非常懸殊，為 13 : 1。藥劑直接噴灑蟲體及果實浸藥試驗顯示，藥劑直接噴灑試驗之藥劑效果並不理想；各種藥劑造成的死亡率並不明顯，蟲體經藥劑處理 24 h 及 48 h 後，以加保利、陶斯松、益達胺及第滅寧較佳，累積死亡率為 11.1–26.7% 及 11.1–28.9%。經 72 h 後，以加保利、陶斯松、益達胺及賜諾特較佳，累積死亡率為 15.6–28.9%。果實浸藥試驗以陶斯松及培丹處理死亡率最佳，於藥劑處理果實後接種咖啡果小蠹 24 h 後之死亡率可達 86.7% 及 73.3%。比較供試果實入侵率與入侵孔數，培丹處理組 (6.7% 及 1.7 個) 比起陶斯松處理組為低 (40.0% 及 4.0 個)，顯示培丹為最佳防治藥劑。

關鍵詞：咖啡果小蠹、殺蟲劑藥效、齡期。

前言

咖啡果小蠹 (*Hypothenemus hampei*) 為咖啡果實重要的害蟲 (Damon 2000; Jaramillo *et al*. 2006; Vega 2008; Vega *et al*. 2009; Vega *et al*. 2015)，首次發現於非洲中部地區。由於咖啡果小蠹可以存活於水分含量低的咖啡豆中，隨著全球咖啡的國際貿易，咖啡果小蠹已經擴散至世界各地 (Vega *et al*. 2015)，目前台灣亦為咖啡果小蠹的入侵地區 (Lin *et al*. 2010)。咖啡果小蠹主要造成未成熟的咖啡果實落果與收穫果實的品質不良，對世界主要的咖啡產區造成極大損失，該蟲在烏干達、哥倫比亞、牙買加、坦尚尼亞、馬來西亞和墨西哥等地的危害率高達 50–90%，每年所造成咖啡果實的損失估計超過 5 億美元 (Vega *et al*. 2002b; Vega 2008)。

咖啡果小蠹雌蟲於咖啡果實內完成交配後，會離開果實尋找入侵下一顆咖啡果實 (Vega 2008)。雌蟲會於果實上直接鑽孔深入胚乳 (endosperm) 取食與產卵，幼蟲孵化後直接取食胚乳。受害的咖啡種仁可能因咖啡果小蠹鑽孔時帶入病原菌，更造成採收的果實內部發黴，失去經濟價值 (Pérez *et al*. 2003; Carrión & Bonet 2004)。

咖啡果小蠹蟲的生活史為卵、1 齡幼蟲、2 齡幼蟲、前蛹、蛹，最後發育為成蟲 (Gómez *et al*. 2015)。於 25℃卵發育至成蟲為 26.6 d，

投稿日期：2016 年 9 月 26 日；接受日期：2017 年 3 月 15 日。
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各階段分別為，卵期 5.3 d、1 齡幼蟲 2.8 d、2 齡幼蟲 5.8 d、前蛹期 6 d、蛹 6.3 d。以有效發育溫度來進行推估年發生世代，於哥倫比亞為 2.95–4.30 代，坦尚尼亞為 2.39–4.71 代，肯亞為 2.03–3.31 代 (Jaramillo *et al*. 2009)。

目前於台灣並無登記咖啡果小蠹的防治藥劑，生產者僅使用誘殺器內置入誘引劑 (甲醇與乙醇以 1 : 1 的混合液) 誘殺雌蟲，降低果園中的危害蟲源。但以每分地吊掛 20 個咖啡果小蠹誘殺器的田間試驗，僅可以降低咖啡果小蠹危害率約 19.1% (Lin *et al*. 2010)，仍無法有效地防治咖啡果小蠹。因此，本研究探討咖啡果小蠹於咖啡果實內齡期組成，以瞭解田間實際發生情況，並以藥劑直接噴灑及果實浸藥法測試殺蟲劑的藥效，以提供作為咖啡果小蠹登記防治藥劑的參考。

材料與方法

供試昆蟲

本研究中之 2 種藥劑試驗方式所使用之蟲源，來自 2016 年 1 月嘉義縣中埔鄉同仁村咖啡樹上受害果實內的咖啡果小蠹人工飼育的後代，以 Brun (1993) 所建立使用於大量繁殖咖啡果小蠹之人工飼料配方，將使用於抑制黴菌之丙酸鈉 (sodium propionate) 與維他命 C (ascorbic acid) 換成苯丙酸鈉 (sodium benzoate) 與山梨酸 (sorbic acid)，於試管中放入咖啡果小蠹雌蟲，於實驗室內進行大量飼養。

咖啡果小蠹於咖啡果實內的發生生態

本次試驗以 2016 年 1 月 8 日於嘉義縣中埔鄉同仁村採集受到咖啡果小蠹危害的咖啡果實，攜回實驗室切開，共檢視 40 顆受害的咖啡果實。依照 Gómez *et al*. (2015) 所描述咖啡果小蠹的齡期特徵，計算果實內的卵數、各齡期幼蟲數、蛹數、雌雄成蟲數，進行果實內各齡期之分布比例與數量計算，果實中的咖啡果小蠹雌雄比則以果實內所有發現雌蟲與雄蟲數量來計算。

供試藥劑

供試藥劑以市售之成品農藥配置，包括 85% 加保利可濕性粉劑 (WP，日農科技股份有限公司) 稀釋 850×、40.8% 陶斯松乳劑 (EC，雅飛股份有限公司) 稀釋 1000×、2.4% 第滅寧水懸劑 (SC，台灣拜耳股份有限公司) 稀釋 1000×、18.2% 益達胺水懸劑 (SC，世大農化工廠股份有限公司) 稀釋 2000×、2% 阿巴汀乳劑 (EC，國豐股份有限公司) 稀釋 2000×、11.6% 賜諾殺水懸劑 (SC，台灣道禮股份有限公司) 稀釋 3000×、50% 培丹可溶性粉劑 (SP，龍燈生物科技有限公司) 稀釋 1000×、18.4% 剋安勃水懸劑 (SC，台灣杜邦股份有限公司) 稀釋 2500×、11.7% 賜諾特水懸劑 (SC，台灣道禮股份有限公司) 稀釋 2000× 及水 (對照組)，藥劑均以逆滲透水進行配製後，分別進行咖啡果小蠹之藥劑直接噴灑測試及果實浸藥法試驗。

藥劑直接噴灑試驗

藥劑直接噴灑試驗，挑選 15 隻咖啡果小蠹雌成蟲，將蟲置於 6 cm 培養皿上，底部鋪濾紙。以噴藥塔 (Burkard Manufacturing Co. Ltd., UK)，以噴藥壓力 13 lb in^{-2} 將 2 mL 藥液直接噴灑於蟲體上。每次噴施後即更換培養皿底部濾紙，供試藥劑更換時，培養皿亦進行更換。噴施後之蟲體放置於 12 mL 之平口取樣瓶內，內置面積約 1 cm^2、厚度 0.3 cm 之人工飼料，瓶口以棉花封緊。每取樣瓶為 1 重複，共 3 重複。試驗完成，將瓶口取樣瓶置於恆溫生長箱中 (25℃，光週期為 12 D : 12 L)，每日記錄蟲體死亡數，死亡率判定以害蟲無法爬行移動者視為死亡，於藥劑處理後 24、48 及 72 h 進行觀察。

果實浸藥試驗

果實浸藥試驗，使用與藥劑直接噴灑試驗所用相同之供試藥劑種類與濃度，每個藥劑處理以未轉色的綠色咖啡果實各 10 顆進行。浸藥約 10 s 後取出，於室內放置約 2 h 陰乾，再放入飢餓 24 h 的咖啡果小蠹共 10 隻，置於塑膠布丁杯 (7.5 cm × 6 cm × 4.8 cm) 中。於杯中底部放一片濾紙，每個藥劑處理共 3 重複，依照藥劑直接噴灑所敘方式，放入咖啡果小蠹於 24 h 後觀察死亡率、危害果數及果實蛀孔

數，以進行藥劑效果評估分析。

統計分析

本研究所進行之藥劑直接噴灑試驗所獲得之累積死亡率，經常態分布檢定後為非常態分布，原始資料經角度轉換後，再經常態分布布檢定仍非常態分布，故採用無母數統計方法 Kruskal-Wallis rank sum test (Kruskal & Wallis 1952)，以顯著水準為 0.05 來檢定供試藥劑之殺蟲效果。倘 Kruskal-Wallis rank sum test 顯著後，以 Dunn's test 進行兩兩比較分析。果實浸藥試驗，經常態分布檢定後為常態分布，以單因子變方分析，在顯著水準為 0.05 下檢定。倘變方分析顯著後，以最小顯著差異性測驗法 (Fisher's least significant difference test; LSD test) 進行兩兩比較分析。

結果

咖啡果小蠹於果實內之各齡期分布比

於嘉義縣中埔鄉田間所採集到之咖啡果實經剖開後，各齡期於每顆果實內平均帶卵率為 70.0%，6.1 顆；1 齡幼蟲為 62.5%，1.9 隻；2 齡幼蟲為 70.0%，2.4 隻；前蛹期為 65.0%，2.0 隻；蛹為 50.0%，1.3 隻；雄成蟲為 35%，0.4 隻；雌成蟲 90.0%，5.0 隻 (表 1)。於 40 顆果實內之雌雄比分為 13：1。經過各齡期的計算之後，25% 咖啡豆同時存在咖啡果小蠹各齡期，且雌蟲數量遠高於雄蟲。此外，亦發現果實內無雌成蟲，卻存在有其他發育期，占總數比率為 10%。

表 1. 由田間收集受到咖啡果小蠹入侵的果實內咖啡果小蠹各齡期分布與數量。

Table 1. The composition of different instars of coffee berry borer in infested coffee berries collected from field.

	The composition of different developed stage in coffee berries (n = 40)	
Stage	Percentage (%)	Number
Egg	70.0	6.1
1st larva	62.5	1.9
2nd larva	70.0	2.4
Prepupa	65.0	2.0
Pupa	50.0	1.3
Female adult	35.0	0.4
Male adult	90.0	5.0

咖啡果小蠹藥劑直接噴灑試驗

藥劑直接噴灑蟲體試驗顯示，咖啡果小蠹經過處理藥劑後的死亡率並不顯著，處理 24–48 h 後的造成的死亡率 4 種較佳的藥劑依序為加保利、陶斯松、益達胺及第滅寧，24 h 死亡率分別為 26.7、15.6、15.6 及 11.1%。48 h 累積死亡率僅加保利略增為 28.9%，另 3 個藥劑並無改變；其餘藥劑造成之死亡率介於 0–6.7%。72 h 累積死亡率較佳藥劑依序為加保利、陶斯松、益達胺及賜諾特，分別為 28.9、15.6、15.6 及 17.8%，其餘藥劑累積死亡率介於 4.4–11.1% (表 2)。

咖啡果小蠹果實浸藥試驗

果實浸藥試驗結果顯示，咖啡果小蠹放入經藥劑處理果實 24 h 後死亡率以陶斯松及培丹處理效果最佳，死亡率分別為 86.7% 與 73.3%；其次為剋安勃、第滅寧、加保利及賜諾特，死亡率為 53.3、53.3、40.0 及 40.0%。其餘藥劑處理之死亡率介於 13.3–23.3%。比較供試果實受害率及平均入侵孔數經統計分析結果顯示，防治效果最佳的藥劑依序為培丹 (6.7%，0.7 個)、第滅寧 (10.0%，1.0 個)、加保利 (13.3%，1.3 個)、剋安勃 (16.7%，1.7 個)、阿巴汀 (33.3%，3.3 個)、陶斯松 (40.0%，4.0 個)、賜諾特 (43.3%，4.3 個)、賜諾殺 (46.7%，5.3 個)、益達胺 (50%，5.7 個) 及對照組 (70.0%，7.7 個) (表 3)。根據比較死亡率、果實受害率及平均入侵孔數，以培丹的防治效果最佳。

討論

本試驗果實中所發現咖啡果小蠹，每個生活史階段皆可於咖啡豆內發現，因此生活史皆於咖啡豆內完成 (表 1)。有些咖啡豆內並無發現成蟲，但仍有其他齡期，這個現象可能是由於咖啡豆內的生長環境不佳，造成雌成蟲離去的現象 (Baker *et al*. 1992)。以咖啡豆內雌雄

分比為 13：1，顯示雌蟲的數量遠比雄蟲為多，與以往文獻所述咖啡果小蠹的雌雄約為 10：1 現象相近。近來研究發現，咖啡果小蠹內帶有可以造成性比偏差的共生菌 (Wobachia) (Vega *et al*. 2002a; Werren *et al*. 2008)，是否為本種共生菌的作用而使雌雄的性比偏差增大，仍待後續的研究。

本試驗利用 2 種不同的藥劑試驗方式，去探討不同作用機制殺蟲劑對於咖啡果小蠹的藥效，發現以不同的施藥方式下藥劑對於咖啡果小蠹的藥效亦有不同 (表 2、表 3)，以藥劑直接噴灑的方式進行的試驗死亡率皆偏低，可能

表 2. 咖啡果小蠹以不同藥劑直接噴灑蟲體後 24、48 及 72 h 的累積死亡率。

Table 2. The cumulative mortality of coffee berry borer by direct spraying with different insecticides after 24, 48 and 72 h.

Chemical	Dilution factor	*n*	The cumulative mortality (%) of each insecticides treatments by direct spraying		
			24 h	48 h	72 h
80% Carbaryl WP	850	45	26.7 ± 3.9 a[z]	28.9 ± 5.9 a	28.9 ± 5.9 a
40.8% Chlorpyrifos EC	1000	45	15.6 ± 2.2 ab	15.6 ± 2.2 ab	15.6 ± 2.2 ab
2.4% Deltamethrin SC	1000	45	11.1 ± 2.2 ab	11.1 ± 2.2 abc	11.1 ± 2.2 bc
18.2% Imidacloprid SC	2000	45	15.6 ± 4.4 ab	15.6 ± 4.4 abc	15.6 ± 4.4 ab
2% Abamectin EC	2000	45	4.4 ± 2.2 bc	6.7 ± 3.9 bcd	8.9 ± 2.2 bcd
11.6% Spinosad SC	3000	45	0.0 ± 0.0 c	0.0 ± 0.0 d	8.9 ± 2.2 bcd
50% Cartap hydrochloride SP	1000	45	4.4 ± 2.2 bc	4.4 ± 2.2 cd	4.4 ± 2.2 cd
18.4% Chlorantraniliprole SC	2500	45	6.7 ± 3.9 bc	6.7 ± 3.9 bcd	11.1 ± 2.2 bc
11.7% Spinetoram SC	2000	45	0.0 ± 0.0 c	6.7 ± 3.9 bcd	17.8 ± 5.9 ab
H_2O, Control	-	45	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d

[z] Means within each column followed by the same letter(s) are not significantly different at 5% level by Dunn multiple comparison test.

表 3. 果實以不同藥劑浸藥處理，於 24 h 後咖啡果小蠹之死亡率、果實上的危害率及平均入侵孔數。

Table 3. Mortality, averaged damaged fruit and infestation hole of coffee berry borer by fruit dipping treatments with different insecticides after 24 h.

Chemical	Dilution factor	Coffee berry borer exposed with fruit dipping with different insecticides treatment after 24 h		
		Mortality (%)	Damaged fruit (%)	Infestation hole
80% Carbaryl WP	850	40.0 ± 5.8 bc[z]	13.3 ± 3.3 a[z]	1.3 ± 0.3 a[z]
40.8% Chlorpyrifos EC	1000	86.7 ± 6.7 a	40.0 ± 5.8 bc	4.0 ± 0.6 cd
2.4% Deltamethrin SC	1000	53.3 ± 3.3 b	10.0 ± 5.8 a	1.0 ± 0.6 a
18.2% Imidacloprid SC	2000	13.3 ± 3.3 de	50.0 ± 5.8 c	5.7 ± 0.3 d
2% Abamectin EC	2000	23.3 ± 8.8 cd	33.3 ± 8.8 b	3.3 ± 0.9 bc
11.6% Spinosad SC	3000	16.7 ± 3.3 de	46.7 ± 3.3 bc	5.3 ± 0.9 d
50% Cartap hydrochloride SP	1000	73.3 ± 13.3 a	6.7 ± 3.3 a	0.7 ± 0.3 a
18.4% Chlorantraniliprole SC	2500	53.3 ± 3.3 b	16.7 ± 6.7 a	1.7 ± 0.7 ab
11.7% Spinetoram SC	2000	40.0 ± 5.8 bc	43.3 ± 3.3 bc	4.3 ± 0.3 cd
H_2O, Control	-	0.0 ± 0.0 e	70.0 ± 5.8 d	7.7 ± 0.7 e

[z] Means within each column followed by the same letter(s) are not significantly different at 5 % level by Fisher's least significant difference (LSD) test.

為噴藥撒布塔進行藥劑噴灑布方式時接觸咖啡果小蠹體表的藥劑較少的關係，以及藥劑不易進入咖啡果小蠹體內。雖然藥劑直接撒布試驗蟲體的試驗死亡率低，藥劑處理後 24–48 d 的累積死亡率最高依序為加保利、陶斯松、益達胺、第滅寧，72 h 後為加保利、賜諾特、陶斯松、益達胺。上述這些藥劑除了益達胺之外，於果實浸藥處理試驗中亦具有相當程度的防治效果，藥效最佳為陶斯松，其次為第滅寧、賜諾特及加保利 (表 3)。培丹及剋安勃雖於藥劑直接撒布處理下，對於咖啡果小蠹造成死亡率並不高，累積死亡率僅為 4.4% 及 6.7–11.1%，但是此兩個藥劑於咖啡果小蠹經果實浸藥處理後卻有防治效果，死亡率可達 73.3% 及 53.3%，可能為這兩個藥劑的取食毒性高於接觸毒性所致。

本研究果實浸藥試驗除了觀察死亡率，亦觀察咖啡果小蠹對於果實的危害比率及危害孔數來做為藥劑效果的參考指標 (表 3)，每種藥劑處理後的果實危害比率與入侵孔數趨勢相同，危害果率與危害孔數最少的藥劑依序為培丹、第滅寧、加保利及剋安勃。培丹對於果實浸藥處理造成的咖啡果小蠹接觸果實 24 h 後，死亡率極高，且造成的果實危害率與危害孔數最低，而造成果實的危害最小。而果實浸藥試驗中，陶斯松比起其他藥劑對於咖啡果小蠹具有高度的致死率 (86.7%)，但是其果實受害率仍高達 40% (表 3)，顯示培丹的防治效果仍優於陶斯松。根據 Lin & Chen (2015) 報告中指出，以這兩個藥劑於本試驗同濃度下對咖啡果小蠹直接進行噴灑藥劑於蟲體後 24 h 後，亦具有 100% 的死亡率，儘管於本研究中以藥劑直接撒布造成處理後 24 h 的死亡率明顯偏低 (15.6%)，可能為本試驗噴藥後給予人工飼料而降低了死亡率。雖然無任何研究推薦培丹於防治咖啡果小蠹的研究，但是於國外於田間已推薦使用陶斯松來進行咖啡果小蠹化學防治 (Baker *et al*. 2002; Rutherford & Phiri 2006)，但是陶斯松目前可用於防治咖啡介殼蟲，於未來可進一步來評估本藥劑用於防治咖啡果小蠹的防治適期。

果實浸藥研究中，加保利、第滅寧、剋安勃及賜諾特於放入咖啡果小蠹 24 h 處理後，死亡率介於 40.0–53.3%。這些藥劑除了賜諾特之外，藥劑處理後明顯減少了果實的受害率 (表 3)。雖無研究報告指出加保利可防治咖啡果小蠹，但許多試驗指出加保利對於果小蠹的毒效並不佳 (Brun *et al*. 1994; Lin & Chen 2015)，但這些試驗皆僅限直接噴灑藥劑於咖啡果小蠹蟲體上所獲得之數據，並無實際評估於本藥劑於果實上之藥效。第滅寧已推薦於咖啡上防治東方果實蠅，本試驗所使用的濃度 (1000×)，高於對於東方果實蠅的推薦用量 (1500×)。如依照第滅寧於咖啡東方果實蠅上的藥劑用量來防治咖啡果小蠹，可能所造成之死亡率應該更低，因此本藥劑之最佳防治稀釋倍數仍需進行更深入的探討。剋安勃目前於台灣用於防治鱗翅目害蟲，國外仍有研究指出剋安勃於田間對咖啡果小蠹具防治效果 (de Souza *et al*. 2013)。因此，這些藥劑於田間之實際防治效果仍待後續更深入的研究。

根據咖啡果小蠹的發生生態，如要在田間進行咖啡果小蠹綜合防治，耕作防治的實施是非常重要的。由於咖啡果小蠹於果實內繁衍，不易受到殺蟲劑的噴灑而死亡 (Lin & Chen 2015)，且許多藥劑對於咖啡果小蠹並無良好防治效果，於國外應用整合害蟲防治咖啡果小蠹清除田間受害落果減少危害蟲源，為非常重要的防治措施 (Aristizábal *et al*. 2012)。咖啡果小蠹於台灣目前尚無推薦藥劑，根據本研究所進行之藥劑直接噴灑及果實浸藥試驗結果顯示，未來於可評估推薦使用培丹於田間防治咖啡果小蠹。

誌謝

本研究感謝行政院農業委員會農業試驗所嘉義農業試驗分所植物保護系倪蕙芳系主任對於本研究的推動，黃守宏副研究員協助稿件的校閱，周紹宸先生及黃鈞愉小姐協助進行試驗，園藝系沈秀芳小姐、胡採滿小姐、林雅芬小姐協助收集試驗材料。

附錄 40 顆受害果實內咖啡果小蠹各齡期分布情況。

Appendix The composition of life stages of coffee berry borer in the 40 infested coffee berries.

Number	Egg	First larval instar	Second larval instar	Prepupa	Pupa	Male adult	Female adult
1	0	0	5	0	1	1	6
2	3	0	1	1	4	0	5
3	6	1	2	0	0	1	0
4	0	1	2	2	1	0	0
5	16	3	12	8	0	2	6
6	0	0	0	0	0	0	5
7	5	4	6	4	0	1	1
8	1	5	2	1	2	1	12
9	7	1	1	1	0	0	6
10	2	3	4	1	1	0	1
11	8	2	6	4	3	0	3
12	2	0	1	2	3	0	8
13	3	4	1	3	2	0	1
14	12	0	0	6	0	1	8
15	0	0	0	0	0	0	1
16	2	0	1	1	2	1	17
17	0	2	3	0	0	1	8
18	4	2	5	6	1	0	3
19	15	1	0	1	0	0	1
20	0	0	0	1	3	0	3
21	0	1	2	0	6	0	11
22	6	4	7	5	0	0	4
23	6	5	0	0	0	0	0
24	21	2	3	3	0	1	4
25	7	10	0	0	0	0	1
26	16	0	1	3	2	0	2
27	23	2	1	0	0	0	2
28	0	0	3	2	2	1	8
29	0	0	1	1	2	1	12
30	2	4	0	0	0	0	3
31	0	0	0	1	5	0	31
32	0	0	0	1	2	0	10
33	11	1	3	0	0	0	2
34	7	6	2	4	0	0	2
35	14	4	3	2	2	1	1
36	0	3	6	0	0	0	0
37	2	0	0	0	0	0	4
38	20	0	0	0	0	1	4
39	5	3	4	7	3	0	1
40	19	3	6	8	1	1	2
Ave.	6.125	1.925	2.35	1.975	1.2	0.375	4.975

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The Composition of Life Stages of Coffee Berry Borer (Scolytidae: *Hypothenemus hampei*) in the Coffee Berry and the Control Efficacy of Different Insecticides for the Pest

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Abstract

Chu, Y. F., M. H. Yang, S. F. Chang, P. H. Chen, and T. C. Wang. 2017. The composition of life stages of coffee berry borer (Scolytidae: *Hypothenemus hampei*) in the coffee berry and the control efficacy of different insecticides for the pest. J. Taiwan Agric. Res. 66(4):318–325.

Coffee berry borer (Scolytidae: *Hypothenemus hampei*) is the most destructive pest in coffee industry worldwide. Recently the pest was as an in Taiwan and caused severe economic loss in planted areas. In this study, the composition of different development stages of the pest in coffee berries and control efficacy with different insecticides were investigated by direct spraying and fruit dipping methods. The all stages of coffee berry borer were found in the same infested berry and the female to male sex ratio was 13 : 1. The insecticides control efficacy by direct spraying showed the low morality in every treatment. The insecticides with the best control efficacy were carbaryl, chlorpyrifos, imidacloprid, and deltamethrin treatments after 24 h and 48 h, causing the morality at 11.1–26.7% and 11.1–28.9% separately. However, the insecticides with the best control efficacy were carbaryl, chlorpyrifos, imidacloprid, and spinotram treatments after 72 h treatments, causing the morality at 15.6–28.9%. Fruit dipping method showed chlorpyrifos and cartap hydrochloride treatments caused the highest morality at 86.7% and 73.3% after inoculating the coffee berry borer. However, comparing the infested fruit rate and infestation hole, cartap hydrochloride treatment had the lowest numbers at 6.7% and 1.7, while chlorpyrifos at 40.0% and 4.0, indicating cartap hydrochloride was the best insecticide to control coffee berry borer.

Key words: Coffee berry borer, Insecticides efficacy, Instars.

Received: September 26, 2016; Accepted: March 15, 2017.

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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):326–332 (2017)
DOI: 10.6156/JTAR/2017.06604.07

研究報告

芒果小黃薊馬之田間族群變動及其藥劑防治

郝秀花 [1,*]

摘要

郝秀花。2017。芒果小黃薊馬之田間族群變動及其藥劑防治。台灣農業研究 66(4):326–332。

自 2011 年 1 月至 2012 年 12 月調查芒果薊馬類之發生，其中發生密度最高的種類為小黃薊馬 (*Scirtothrips dorsalis* Hood)，全年均有發生；花薊馬 [*Thrips hawaiiensis* (Morgan)] 次之，僅發生於開花期。芒果園中小黃薊馬之發生，自 1 月芒果正值開花期，其密度逐漸升高，至 3 月時達高峰；在 6、8 及 10 月，正值芒果抽新梢，其密度亦升高，在 10 月達最高峰。芒果園中小黃薊馬田間的族群變動受相對濕度 (Relative humidity; RH) 顯著影響，呈現負相關 (r = -0.85840, P = 0.0015)。以 8 種藥劑進行防治試驗，經 2 年試驗結果，以 9.6% 益達胺溶液 (SL)、50% 滅賜克可濕性粉劑 (WP)、15% 脫芬瑞水懸劑 (SC) 及 10% 克凡派 (SC) 的防治效果較好，防治率均達 90% 以上。

關鍵詞：小黃薊馬、族群變動、藥劑試驗、芒果。

前言

台灣芒果園出現的薊馬種類至少有 6 種 (Shih *et al*. 2013)，其中以小黃薊馬 (*Scirtothrips dorsalis* Hood)、花薊馬 [*Thrips hawaiiensis* (Morgan)] 及腹鉤薊馬 (*Rhipiphorothrips cruentatus* Hood) 發生最為普遍，三種薊馬之為害時期及部位均不相同。其中，小黃薊馬發生最多，幾乎全年均有出現，無論在生育期、花期或結果期，均可見其蹤跡；腹鉤薊馬發生於生育期，結果期較少；花薊馬僅發生於花期 (Lee & Wen 1982)。

小黃薊馬原分布於亞洲，但陸續蔓延至南非、美國、墨西哥及澳洲等地區發生為害 (Centre for Agriculture and Biosciences International 2005; Nietschke *et al*. 2008)，屬多食性，其寄主植物達 40 科 112 種以上，包括辣椒、蔥、豆類、玉米、棉花、瓜類、花生、芒果、葡萄、茶、煙草、花卉、柑橘、香蕉、草莓、番茄及馬鈴薯等重要經濟作物 (Mound & Palmer 1981; Umeya *et al*. 1988; Wang 2002; Seal *et al*. 2006)，近幾年陸續嚴重為害台灣的釋迦、番石榴、印度棗、楊桃、蓮霧及芒果等果樹，為台灣經濟作物之重要害蟲 (Chiu *et al*. 2010a)。近年來氣候變遷，溫度略升，有利於各種害蟲的發育與繁殖，而小黃薊馬因體小，善躲特性，不易被發現，而農民通常都在作物受害出現徵狀時才會進行防治，因而錯過防治的適當時期。本研究調查田間小黃薊馬之族群變動與氣象因子的關係，提供農民芒果生長期間小黃薊馬的週年族群密度變動，另篩選優良藥劑，以提供農民能適時用藥防治參考。

材料與方法

芒果薊馬類之田間族群變動調查

2011 年 1 月至 2012 年 12 月於設於高雄市之行政院農業委員會農業試驗所鳳山熱帶園藝試驗分所內芒果園進行，芒果品種為「海頓」，為栽培 20 年以上樹齡之植株，每株間距 3 m，每行 5 株，計有 12 行，共 60 株芒果。定期

投稿日期：2017 年 3 月 21 日；接受日期：2017 年 4 月 13 日。
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於芒果園逢機懸掛黃色黏紙 25 張 (215 mm × 150 mm，高冠牌)，懸掛高度 1.5 m，每星期更換 1 次，並將黃色黏紙攜回實驗室。以解剖顯微鏡檢查，記錄黏紙上捕捉到的薊馬種類及數量，並記錄田間氣象因子 (溫度、雨量及相對濕度)，再將每月薊馬族群累計數量與每月氣象因子 (平均溫度、累積雨量及平均相對濕度) 以 SAS EG 4.1 統計軟體進行相關分析。

藥劑防治試驗

在 2011 年 8 月及 2012 年 9 月在鳳山熱帶園藝試驗分所芒果園區各進行 1 次，每次於芒果修剪後的抽梢期，薊馬發生時開始進行田間藥劑防治試驗。參試藥劑選用《植物保護手冊》中推薦在防治芒果薊馬類之 8 種藥劑 (Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture 2010)：包括 9.6% 益達胺 (imidacloprid) SL (拜耳作物科學股份有限公司) 2000×、2.5% 賜諾殺 (spinosad) SC (台灣道理股份有限公司) 1000×、48.34% 丁基加保扶 (carbosulfan) 乳劑 EC (國際技術社股份有限公司) 1000×、11.7% 賜諾特 (spinetoram) SC (台灣道理股份有限公司) 4000×、18.2% 益達胺 (imidacloprid) SC (拜耳作物科學股份有限公司) 3000×、50% 滅賜克 (metiocarb) WP (興農股份有限公司) 800×、15% 脫芬瑞 (tolfenpyrad) SC (日佳農藥股份有限公司) 1500×、10% 克凡派 (chlorfenapy) SC (台灣日產股份有限公司) 2000× 等，與無施藥對照處理共 9 處理。

藥劑試驗採逢機區集設計 (RCBD)，每小區 2 株，每處理 3 重複，共 54 株。薊馬發生時開始噴藥，隔 7 d 後再噴 1 次，共 2 次，於每次噴藥前及第二次噴藥後 7 d 調查 1 次。調查時每小區逢機採取 20 片新葉，將葉片迅速置入封口塑膠袋，置於冰箱內冷藏，隔日計算其上之蟲數，再以 Henderson & Tilton's 公式計算防治率 (Henderson & Tilton 1955)。

$$\text{防治率 (\%)} = \left(1 - \frac{\text{處理區施藥後蟲數} \times \text{對照區處理前蟲數}}{\text{處理區施藥前蟲數} \times \text{對照區處理後蟲數}}\right) \times 100$$

統計分析方法：蟲數經 $(x + 0.5)^{1/2}$ 轉換後，以 SAS EG 4.1 統計軟體進行變方分析，若不同處理間具顯著差異 ($P < 0.05$)，則以 Fisher's LSD 進行事後檢定比較各處理之差異。

結果

芒果薊馬類之田間族群變動調查

田間調查結果，在芒果園內發生的薊馬種類以小黃薊馬 (*S. dorsalis*)、花薊馬 (*T. hawaiiensis*)、台灣花薊馬 (*Frankliniella intonsa*) 及菊花薊馬 (*Microcephalothrips abdominalis*) 較多。其中以小黃薊馬發生密度較高，全年都有發生；花薊馬只發生在芒果開花時期，其餘時期零星發生 (圖 1)。

自 2011 年 1 月至 2012 年 12 月進行園區薊馬發生消長調查，結果 2 年的資料顯示自 1 月芒果開始抽花穗至盛花期間，小黃薊馬與花薊馬的田間密度均略升，而花薊馬僅只發生在芒果開花期。小黃薊馬在 2011 年的田間密度受雨量的影響，有顯著的高低起伏，族群的高峰分別在 2–3 月的開花期、6 月的採果前及 9–10 月採果後，因為此期間均未施藥，有利薊馬生存。2012 年 4–5 月梅雨期雨量較多，期間不利小黃薊馬的生存，因而族群密度較低，惟於 7 月採果前及 9–10 月採果後雨量漸緩才稍有升高。芒果植株一般在採果後，會進行枝條修剪，修剪完後芒果抽出新梢，田間薊馬密度則漸漸升高，芒果陸續抽梢，至 9–10 月小黃薊馬的族群密度達高峰。以 2012 年 9–10 月高峰期平均每張黏紙有 96.3 隻，但不如 2011 年 9–10 月的每張黏紙 370.8 隻。可能是因為 2012 年的雨量高於 2011 年 (圖 1)。

將每月小黃薊馬田間族群累計數量與每月氣象資料 (平均溫度、累積雨量及平均相對濕度) 進行相關分析，結果顯示芒果園中小黃薊馬的田間族群變動受園區相度對濕度 (RH) 影響，呈現顯著負相關 (r = -0.85840, P = 0.0015)，並與雨量成負相關 (r = -0.38050, P = 0.2781)，但與溫度成正相關 (r = 0.49507, P = 0.1457) (表 1)。

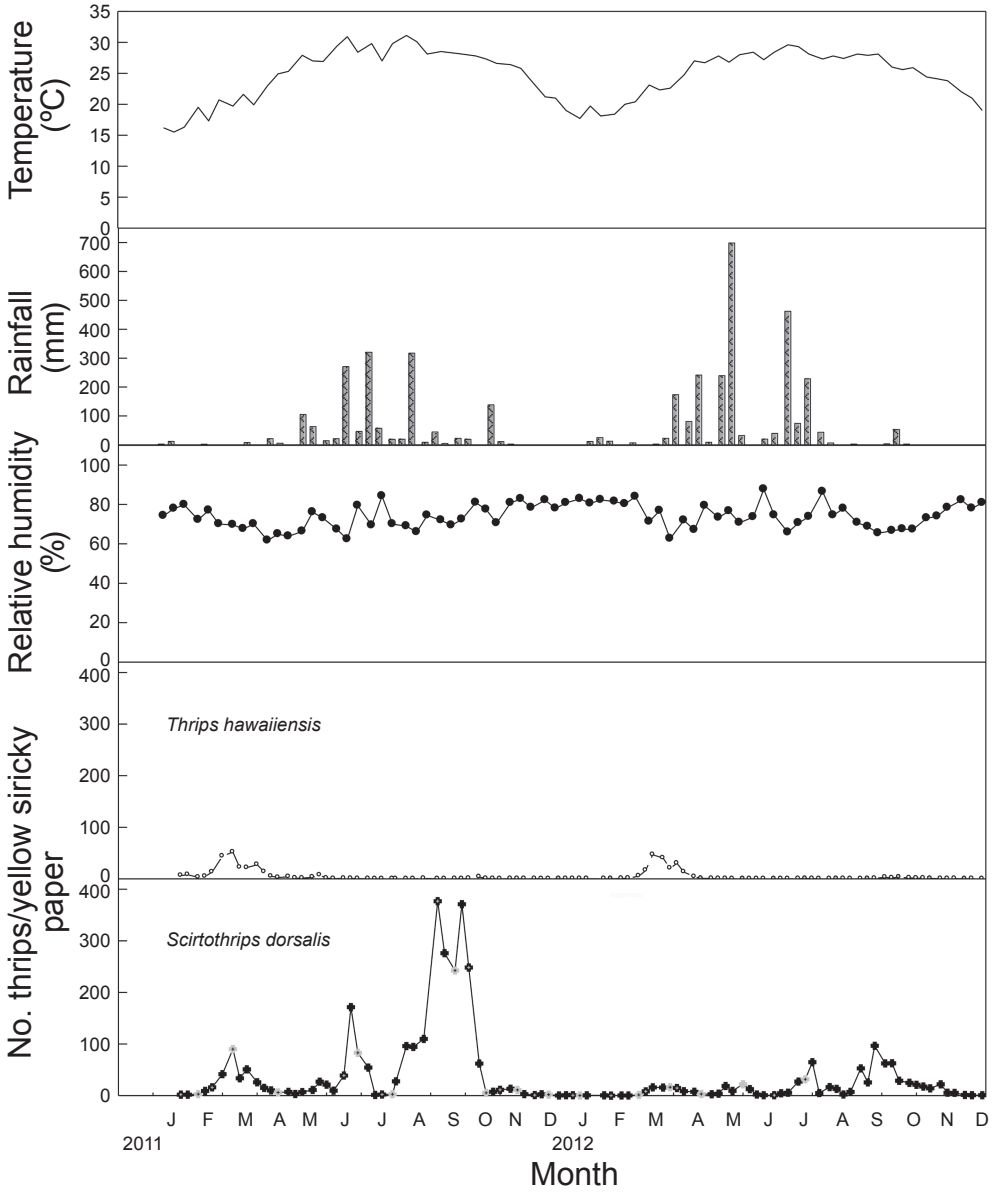


圖 1. 鳳山地區芒果花薊馬與小黃薊馬之週年族群變動 (2011–2012)。

Fig. 1. The population dynamics of *Thrips hawaiiensis* and *Scirtothrips dorsalis* in mango orchard at Fengshan, Kaohsiung City, Taiwan from 2011 through 2012.

藥劑防治試驗

芒果小黃薊馬之藥劑防治試驗分別在 2011 年 8 月及 2012 年 9 月在鳳山熱帶園藝試驗分所芒果園進行 1 次，2011 年之試驗在第一次噴藥後 7 d 的調查結果，除了 2.5% 賜諾殺 SC 與 15% 脫芬瑞 SC 防治率較低，僅有 35.4% 及 65.5%，其他 6 個藥劑防治率均達 78% 以上。第二次噴藥後 7 d 的調查結果，則以 9.6% 益達胺 SL、50% 滅賜克 WP、15% 脫芬瑞 SC 及 10% 克凡派 SC 的防治效果較好，防治率可達 90% 以上，2.5% 賜諾殺 SC 防治率最低，僅 61.8% (表 2)。

表 1. 鳳山地區薊馬族群與氣象因子之相關係數。

Table 1. Correlation coefficient of the population of thrips with meteorological factors, in Fengshan District, Kaoshiung City.

Parameter	*Scirtothrips dorsalis*	*Thrips hawaiiensis*
Temperature		
r	0.49507	-0.23383
P	0.14570	0.51550
Rainfall		
r	-0.38050	-0.28507
P	0.27810	0.42470
Relative humidity		
r	-0.85840	-0.30708
P	0.00150	0.38810

表 2. 2011 年以不同藥劑防治芒果小黃薊馬的效果。

Table 2. Efficacy of pesticides for the control of *Scirtothrips dorsalis* on mango in 2011.

		7 d after treatment No. thrips/20 leaves (% control)	
Treatment	Pre-treatment ± SD	1st	2nd
9.6% imidacloprid SL 2000×	119.3 ± 7.7 a[z]	15.7 ± 18.3 a (86.1)	6.7 ± 5.0 a (95.0)
2.5% spinosad SC 1000×	112.7 ± 9.0 a	67.7 ± 26.0 b (35.4)	47.0 ± 17.3 b (61.8)
48.34% carbosulfan EC 1000×	133.3 ± 12.7 a	20.0 ± 15.0 a (86.3)	41.0 ± 15.0 b (71.9)
11.7% spinetoram SC 4000×	124.7 ± 5.7 a	15.7 ± 5.7 a (86.3)	28.7 ± 18.0 b (79.0)
18.2% imidacloprid SC 3000×	129.7 ± 4.3 a	33.7 ± 22.0 ab 72.1)	25.7 ± 12.0 b (82.0)
50% methiocarb WP 800×	110.7 ± 9.0 a	13.7 ± 8.7 a (86.9)	8.0 ± 7.0 a (93.3)
15% tolfenpyrad SC 1500×	121.3 ± 7.3 a	39.0 ± 24.0 ab (65.5)	7.7 ± 2.7 a (94.3)
10% Chlorfenapy SC 2000×	133.0 ± 14.0 a	26.7 ± 15.3 a (78.6)	7.3 ± 3.0 a (91.8)
CK	134.3 ± 15.7 a	125.3 ± 22.0 c	147.0 ± 20.0 c

[z] Means within a column followed by the same letter(s) are not significantly different by LSD test at 5% level.

2012 年之試驗經第一次噴藥後 7 d 調查，除了 2.5%賜諾殺 SC 防治率僅 66.3%較低外，其他 7 種藥劑防治率均達 84.0%以上。第二次噴藥後 7 d 的調查結果，則以 9.6%益達胺 SL、50%滅賜克 WP、15%脫芬瑞 SC 及 10%克凡派 SC 的防治效果較好，防治率達 90%以上，仍以 2.5%賜諾殺 SC 防治率最低，防治率為 62.8% (表 3)。

討論

Lee & Wen (1982) 調查為害台灣芒果的薊馬種類至少有 5 種，其中以小黃薊馬、花薊馬及腹鉤薊馬發生最為普遍。三種薊馬之為害時期及部位均不相同，其中小黃薊馬發生最多，幾乎全年均有出現，無論在芒果生育期 (7–12 月)、開花期 (1–3 月) 或結果期 (3–6 月)，均可見其蹤跡；腹鉤薊馬發生於生育期，結果期較少；花薊馬僅發生於花期。而本試驗調查中，以小黃薊馬與花薊馬出現的數量較多，與上述危害時期及發生部位吻合；腹鉤薊馬的發生數量則較少，對芒果危害較小。

經二年的調查結果，發現小黃薊馬喜群聚於芒果的新葉、花蕾及幼果，尤有新梢抽出時薊馬數量會急劇升高，所以在芒果植株抽新梢時，如能及時噴藥防治，當可大幅降低小黃薊馬密度。Chiu *et al*. (1991) 在台南、屏東地區調查芸香科上小黃薊馬的族群消長，發現 8–12 月間密度最高，期間正逢植株的嫩葉及幼果發

表 3. 2012 年以不同藥劑防治芒果小黃薊馬的效果。

Table 3. Efficacy of pesticides for the control of *Scirtothrips dorsalis* on mango in 2012.

Treatment	Pre-treatment ± SD	7 d after treatmemt No. thrips/20 leaves (% control)	
		1st	2nd
9.6% imidacloprid SL 2000×	231.3 ± 11.0 b[z]	46.3 ± 24.0 a (88.1)	41.3 ± 30.0 a (91.1)
2.5% spinosad SC 1000×	221.0 ± 7.3 b	125.7 ± 19.3 b (66.3)	164.7 ± 35.0 b (62.8)
48.34% carbosulfan EC 1000×	251.0 ± 18.7 b	47.3 ± 16.7 a (88.8)	59.3 ± 24.0 a (88.2)
11.7% spinetoram SC 4000×	47.0 ± 16.7 b	46.7 ± 18.3 a (88.9)	88.0 ± 29.7 a (82.2)
18.2% imidacloprid SC 3000×	169.3 ± 12.3 a	38.0 ± 22.0 a (86.6)	47.7 ± 22.3 a (86.1)
50% methiocarb WP 800×	197.7 ± 12.3 a	46.0 ± 23.7 a (86.1)	34.7 ± 30.0 a (91.4)
15% tolfenpyrad SC 1500×	212.7 ± 20.0 ab	50.3 ± 23.7 a (85.9)	39.7 ± 21.0 a (90.8)
10% Chlorfenapy SC 2000×	226.3 ± 13.0 b	60.7 ± 32.0 a (84.2)	37.3 ± 31.3.0 a (91.8)
CK	211.7 ± 22.0 ab	354.3 ± 31.0 c	421.0 ± 45.7 c

[z] Means within a column followed by the same letter(s) are not significantly different by LSD test at 5% level.

育期，至 1 月時溫度下降，小黃薊馬之族群密度減少，故其發生密度與植物生長及溫度均有密切關係，此與本調查結果亦同。另由 Lin *et al*. (2010) 年在台南玉井對愛文芒果小黃薊馬監測結果顯示，其族群在 1 月時驟增後維持在高密度狀態，至 6 月密度大幅下降。台南地區每年月平均氣溫於 1–2 月最低，為 15–18℃，但均高於小黃薊馬的發育臨界低溫 (9.6℃) (Chen *et al*. 2013)，顯示小黃薊馬在台南地區芒果上全年均可發育繁殖。而高雄地區的最低氣溫也在 1–2 月，平均高於 15℃，因此小黃薊馬在高雄地區也是全年均可發育繁殖。

Chen *et al*. (2013) 研究指出在台灣小黃薊馬全年發生約 40 個世代，在日本年發生 8 代 (Tatara 1994)，而在美國佛羅里達州其族群年發生達 18 代 (Nietschke *et al*. 2008)，顯示台灣的氣候條件較日本及美國更適合小黃薊馬發育與繁殖，亦印證近年來小黃薊馬對台灣農作物危害嚴重之情況。

由圖 1 可看出，小黃薊馬的族群在 1–2 月時密度最低，此時也是這二年田間溫度最低時，所以田間小黃薊馬之族群受溫度之影響很大，成正相關 (r = 0.49507, P = 0.1457) (表 1)。Li (2010) 調查小黃薊馬於印度棗園之族群變動，顯示溫度因子對小黃薊馬發生量之影響大於雨量因子，而本調查結果則是雨量因子的影響大於溫度因子；另 Li (2010) 在小黃薊馬內在增殖率之研究中，由 20–30℃間之世代增殖率，由 7.4 倍增至 10.5 倍，世代時間由 34.4 d 縮短為 17.8 d，證實溫度影響其甚大。本試驗調查芒果上的小黃薊馬族群密度，會因園區內的相對濕度 (RH) 升高，呈現顯著負相關 (r = -0.85840, P = 0.0015)，故建議可在園區內噴水提高濕度，以降低小黃薊馬族群密度，如此也可減少用藥量。

小黃薊馬之田間藥劑試驗經二年調查結果，以 9.6% 益達胺 SL、50% 滅賜克 WP、15% 脫芬瑞 SC 及 10% 克凡派 SC 的防治效果較好，防治率可達 90% 以上。Wang *et al*. (1999) 篩選蓮花小黃薊馬的藥劑試驗結果指出，以 9.6% 益達胺 SL、20% 亞滅培 SP、50% 馬拉松 EC 及 2.8% 賽洛寧 EC 的處理，16 hr 後，對小黃薊馬成蟲的致死率達 89.4–100%，與本試驗中 9.6% 益達胺對小黃薊馬的防治效果有相同結果。Chiu *et al*. (2010b) 之室內藥劑試驗結果顯示，40% 滅大松 EC、50% 芬殺松 EC、40.64% 加保扶 SC、48.34% 丁基加保扶 EC、40% 納乃得 SG、50% 滅賜克 WP 及 2.5% 賜諾殺 SC 等 7 種殺蟲劑對芒果小黃薊馬的效果較佳，與本試驗中 9.6% 益達胺 SL 及 50% 滅賜克 WP 對小黃薊馬的防治效果有相同結果，而 2.5% 賜諾殺則有不同的結果，其原因有待進一步的探討。

本試驗結果除了提供農民芒果小黃薊馬田

間族群變動的資料，適時防治以降低田間密度外，所篩選的藥劑也可提供農民參考。

誌謝

本試驗期間承本研究室同仁謝淑婷、葉孟婷小姐及胡登淵先生協助試驗調查及噴藥等工作，在此一併致謝。

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Population Dynamics and Evaluation of Insecticides for Control of *Scirtothrips dorsalis* Hood on Mango

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Abstract

Hao, H. H. 2017. Population dynamics and evaluation of insecticides for control of *Scirtothrips dorsalis* Hood on mango. J. Taiwan Agric. Res. 66(4):326–332.

Study on the occurrence of mango thrips was carried out in southern Taiwan from 2011 to 2012. Result indicated the chilli thrips (*Scirtothrips dorsalis* Hood), occurred all the year round in mango orchards and with the highest population density, followed by the flower thrips [*Thrips hawaiiensis* (Morgan)], which only occurred in blooming stage. The population of chilli thrips built up during mango early blossom in January, and with a peak in March. Its population increase rapidly during mango developing new leaves between July and September, and the first peak appeared from September to October. The population abundance of chili thrips decreased with high relative humidity in mango orchard (r = -0.85840, P = 0.0015). Eight insecticides were tested in the field against chili thrips. Results indicated that imidacloprid 9.6% SL, methiocarb 50% WP, tolfenpyrad 15% SC, and chlorfenapy 10% EC showed more effective in controlling this thrips.

Key words: *Scirtothrips sorsalis* Hood, Population-dynamics, Evaluation of insecticides, Mango.

Received: March 21, 2017; Accepted: April 13, 2017.
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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):333–342 (2017)
DOI: 10.6156/JTAR/2017.06604.08

研究報告

大南薑根莖抑制黃嘌呤氧化酶之活性

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摘要

洪子桓、賴瑞聲、林素月、楊婕、李雅琳。2017。大南薑根莖抑制黃嘌呤氧化酶之活性。台灣農業研究 66(4):333–342。

高尿酸血症易引發痛風、糖尿病等多項代謝異常疾病。抑制黃嘌呤氧化酶 (xanthine oxidase; XO) 可抑制體內尿酸合成，緩和高尿酸血症。本研究選擇 24 種本土青草藥材料，以其酒精及水萃物測試抑制 XO 之活性，結果顯示大南薑 (*Alpinia galanga* Willd.) 之酒精萃取物具有最佳活性，仙草 (*Mesona procumbens* Hemsl.) 之「台 E」品系亦稍具有活性。大南薑根莖酒精萃取物中的活性物質經氣相層析質譜儀 (gas chromatography-mass spectrum; GC-MS) 分析，顯示其抑制 XO 活性的主要成分可能是 (1'*S*)-1'-acetoxychavicol acetate 及 *p*-coumaryl diacetate。過去文獻證實這兩種化合物與目前治療高尿酸血症的藥物 allopurinol 具有相仿之 XO 抑制活性，顯示大南薑具有開發為調節尿酸產品或新藥之潛力。

關鍵詞：高尿酸血症、黃嘌呤氧化酶、抑制活性、大南薑。

前言

尿酸是人體分解食物中的嘌呤 (purine) 產生的代謝廢棄物，在血液中的濃度受到飲食、族群、性別、生活習慣等因素的影響。男性血液中尿酸的濃度比女性稍高，一般而言，當男性血液中的尿酸濃度超過 6.5 mg dL^{-1}，女性超過 6.0 mg dL^{-1} 時，便認為尿酸的含量過高，被稱為高尿酸血症 (hyperuricemia) (Johnson *et al*. 2003)。高尿酸血症的患者，若尿酸結晶沉積於關節處，誘發關節炎而產生疼痛時，即為俗稱的痛風。除痛風之外，高尿酸血症也易引發腎結石、高血壓、心血管疾病以及糖尿病等疾病 (Quinones Galvan *et al*. 1995; Johnson *et al*. 2003; Chien *et al*. 2008)。衛生署於 2005–2008 年進行調查顯示，台灣男性罹患高尿酸血症比率為 21.6%，大於 65 歲的老年男性罹患率升高至 25.8%；女性罹患高尿酸血症的比率為 9.57%，較男性低，但老年女性罹患率卻高達 33.6% (Chuang *et al*. 2011)。此外，台灣原住民與紐西蘭毛利人 (Māori) 同為全球罹患高尿酸血症及痛風比例最高的族群 (Chang *et al*. 1997; Chou & Lai 1998)。高尿酸血症及其併發症已屬於我國長期醫療支出的重要項目。

黃嘌呤氧化酶 (xanthine oxidase; XO) 是人體合成尿酸的關鍵酵素，其代謝途徑是先催化次黃嘌呤 (hypoxanthine)、合成黃嘌呤 (xanthine)，再進一步催化黃嘌呤產生尿酸 (Ramallo *et al*. 2006)。當 XO 被抑制時，尿酸的合成受阻，因此可降低體內尿酸的濃度。Allopurinol 為目前市面上最廣泛使用於治療高尿酸血症的藥物，其主要機制是抑制 XO 的活性、減少尿酸生成，另一機制是次黃嘌呤因為 XO 被抑制而無法被代謝、濃度上升，進而回饋抑制體內嘌呤的合成，即是減少合成尿酸

投稿日期：2017 年 1 月 5 日；接受日期 : 2017 年 4 月 14 日。
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的前驅物產生，所以抑制 XO 活性可以直接及間接地降低血液中的尿酸濃度 (Cameron *et al*. 1993)。

Allopurinol 的結構為一種嘌呤類似物，服用後 2 h 內會被 XO 催化轉變為 oxypurinol，oxypurinol 再與 XO 發生共價結合造成自殺抑制反應 (suicide inhibition) (Massey *et al*. 1970)。不幸的是，高達 20% 的患者曾在使用 Allopurinol 時產生不良副作用，症狀包括發燒、皮疹、腎功能降低、肝炎等 (Stamp *et al*. 2007)。另外，約有數萬分之一的機率，會引起嚴重的藥物過敏反應，如「史帝文 - 強生症候群」(Stevens-Johnson Syndrome) 及毒性表皮溶解症，致死率可達 20–25% (Mockenhaupt 2011; Kim *et al*. 2012)。因此，開發有效且副作用小的相關新藥，仍在醫藥界持續進行中。

從天然物尋找具有機能性功效，且具有較低副作用的醫藥原料，成為現代生命科學及醫學的顯學 (Sweeney *et al*. 2001)。青草藥通常泛指非中醫常用藥材，使用於民俗療法，是民間具有藥用歷史及價值的植物。青草藥植物通常適合當地氣候與土宜，若有科學證據支持具有保健功效，則相當適合作為國產特色機能性作物開發。本研究挑選 24 種在民間用於利尿、治療痛風，或是風濕性關節炎的本土青草藥材料，包括綠莧草 (*Alternanthera paronychioides* St. Hil.)、野莧 (*Amaranthus viridis* L.)、芹菜 (*Apium graveolens* L.)、艾草 (*Artemisia indica* Willd.)、紫背草 (*Emilia sonchifolia* DC.)、向日葵 (*Helianthus annuus* L.)、兔兒菜 (*Ixeris chinensis* Nakai)、黃鵪菜 (*Youngia japonica* DC.)、山葵 (*Eutrema japonica* Matsum.) 品種「真妻」(Mazuma) 及「台農 70-1-1 號」(Tainung 70-1-1)、化石草 (*Orthosiphon aristatus* Miq.)、仙草 (*Mesona procumbens* Hemsl.) 品系「桃園 2 號」(Taoyuan No. 2)、「農試 1 號」(TARI No. 1) 及「台 E」(Tai E)、丹蔘 (*Salvia miltiorrhiza* Bunge)、水丁香 (*Ludwigia octovalvis* Raven)、車前草 (*Plantago asiatica* L.)、通天草 (*Setaria viridis* P.Beauv.)、甜珠草 (*Scoparia dulcis* L.)、葉用枸杞 (*Lycium chinense* Mill.)、苦蘵 (*Physalis angulata* L.)、大南薑 (*Alpinia galanga* Willd.)、春鬱金 (*Curcuma aromatica* Salisb.) 及薑黃 (*Curcuma longa* L.)，以其酒精萃取物及水萃取物測試體外抑制 XO 活性之效果，其中發現大南薑及仙草之酒精萃取物具有較好之抑制效果。大南薑為薑科月桃屬植物，原產於東南亞，在民俗療法中常被用於治療過敏及支氣管相關疾病 (Seo *et al*. 2013)，亦可治療類風濕性關節炎 (Latha *et al*. 2009; Weerakkody *et al*. 2011)，顯示有消炎效果。然而過去並無明確文獻記載大南薑可用於緩解痛風性關節炎，或是調節尿酸之效果。本研究進一步探討大南薑具有抑制 XO 活性的有效成分，以提供未來產業利用、開發為調節尿酸產品之基礎資料。

材料與方法

試驗材料與萃取

青草藥試驗材料列於表 1，所有材料皆於 2014–2016 年由台灣各地採集。材料採回洗淨後，以 55℃烘箱熱風乾燥 2 d 以上至完全乾燥。所有乾燥材料使用桌上型磨粉機 (群策，台灣) 磨碎，再經過 6 mm 孔徑的篩網過篩收集粉末，之後進行萃取或冷藏於 4℃備用。萃取方法為索氏萃取法 (Soxhlet extraction)，使用 B-811 多功能萃取裝置 (BÜCHI，瑞典)，取 10–30 g 材料粉末放置於索氏萃取裝置中，加入 100 mL 溶劑，迴流 5 h 後收集萃取液。所有萃取液在進行後續試驗前皆以 0.45 μm PVDF 濾膜 (Merck，德國) 過濾。萃取液濃度之定義為：萃取液經減壓濃縮並徹底冷凍乾燥後之乾燥物乾重除以萃取液之體積。

體外 XO 抑制活性分析

XO 可將黃嘌呤氧化為尿酸，萃取物對 XO 的抑制率可藉由偵測尿酸合成降低的比率計算 (Nguyen *et al*. 2004)。試驗方法如下：240 μL 反應混合物，包括 100 μL 50 mM KH_2PO_4、120 μL 0.15 mM 黃嘌呤、12 μL 0.147 U mL^{-1} XO 以及 8 μL 待分析樣品。所有藥品皆購自 Sigma-Aldrich (美國)。每次試驗均同時分析正控制組 0.33、0.67、1.33 μg mL^{-1} Allopurinol (溶於 50 mM KH_2PO_4) 及空白組

表 **1.** 24 種青草藥抑制 XO 活性之分析。

Table 1. Analysis of XO inhibitory activity of 24 herbal medicinal plants.

			XO inhibitory activity (%)						Extraction rate (%)	
			8.3 μg mL^{-1}		16.7 μg mL^{-1}		33.3 μg mL^{-1}			
Family	Species	Tissue	E[z]	W[y]	E	W	E	W	E	W
Amaranthaceae	*Alternanthera paronychioides* St. Hil.	Whole grass	2.3	1.7	2.0	0.3	1.5	0.3	15.3	21.2
Amaranthaceae	*Amaranthus viridis* L.	Whole grass	-1.4	0.1	1.3	0.5	1.4	-1.0	12.8	5.9
Apiaceae	*Apium graveolens* L.	Seeds	-0.6	-6.7	0.5	-4.5	-3.4	0.8	11.1	13.3
Asteraceae	*Artemisia indica* Willd.	Leaves	-1.5	-0.2	-1.7	-3.3	-4.9	-6.1	22.8	28.8
Asteraceae	*Emilia sonchifolia* DC.	Leaves	-3.4	0.4	-2.3	-1.4	-1.7	0.8	18.7	23.3
Asteraceae	*Helianthus annuus* L.	Leaves	1.2	-7.5	0.1	-2.2	-2.1	-0.6	21.9	15.2
		Seeds	-4.0	-6.5	-5.1	-1.2	-2.6	5.1	26.4	8.0
		Roots	-2.6	-0.2	-3.6	0.8	-2.6	2.9	9.3	11.9
Asteraceae	*Ixeris chinensis* Nakai	Leaves	-2.5	2.0	-2.3	-2.9	-2.1	-2.9	38.0	14.7
Asteraceae	*Youngia japonica* DC.	Leaves	-3.8	-1.6	-1.9	-2.0	-2.5	-5.1	20.7	13.2
Brassicaceae	*Eutrema japonica* Matsum. 'Mazuma'	Leaves	-2.3	-3.4	0.4	-3.1	0.8	-0.8	2.8	2.9
Brassicaceae	*Eutrema japonica* Matsum. 'Tainung 70-1-1'	Leaves	0.9	2.1	1.2	0.5	-1.1	0.9	1.7	1.9
Lamiaceae	*Orthosiphon aristatus* Miq.	Whole grass	-10.0	3.9	-22.7	2.8	-22.4	4.4	17.4	25.8
Lamiaceae	*Mesona procumbens* Hemsl. 'Taoyuan No. 2'	Whole grass	-13.9	-7.3	-4.2	-7.7	3.6	-6.1	21.9	6.7
Lamiaceae	*Mesona procumbens* Hemsl. 'Tai E'	Whole grass	1.2	2.5	-0.9	2.5	**19.2**[x]	3.9	7.3	19.5
Lamiaceae	*Mesona procumbens* Hemsl. 'TARI No. 1'	Whole grass	3.3	1.3	1.9	0.3	6.3	2.2	9.7	19.3
Lamiaceae	*Salvia miltiorrhiza* Bunge	Whole grass	3.2	3.0	4.3	-0.6	-0.2	-0.8	8.1	4.1
Onagraceae	*Ludwigia octovalvis* Raven	Whole grass	3.2	3.9	1.8	5.9	2.9	6.0	5.6	9.3
Plantaginaceae	*Plantago asiatica* L.	Whole grass	-13.4	-11.3	-18.2	-16.7	-24.4	-25.9	19.7	12.0
Poaceae	*Setaria viridis* P. Beauv.	Roots	-22.9	-20.9	-21.8	-22.7	-22.4	-25.2	9.3	6.2
Scrophulariaceae	*Scoparia dulcis* L.	Whole grass	-1.1	1.8	-0.6	3.4	2.9	6.3	15.9	13.7
Solanaceae	*Lycium chinense* Mill.	Leaves	0.6	0.3	2.7	0.6	0.6	-1.9	13.6	7.4
		Stems	0.4	-1.4	2.9	-2.8	-5.6	-0.2	22.3	8.9
Solanaceae	*Physalis angulata* L.	Whole grass	0.1	0.8	-1.2	2.1	-0.2	3.1	7.8	14.4
Zingiberaceae	*Alpinia galanga* Willd.	Rhizomes	2.3	-0.8	**27.9**	0.4	**54.3**	2.2	10.4	6.9
		Leaves	-5.5	-5.3	-4.2	-9.0	4.6	-9.8	13.9	20.1
Zingiberaceae	*Curcuma aromatica* Salisb.	Rhizomes	-6.6	-6.0	-4.6	-6.0	-0.3	2.0	17.4	4.6
Zingiberaceae	*Curcuma longa* L.	Rhizomes	4.2	-5.6	1.2	4.2	2.7	-1.6	7.2	5.3
Standards			0.33 μg mL^{-1}		0.67 μg mL^{-1}		1.33 μg mL^{-1}			
Allopurinol			**29.1**		**48.9**		**94.1**			
Bisdemethoxycurcumin			-1.0		-0.9		0.2			

[z] 95% Ethanol extracts by using Soxhlet method.
[y] Hot water extracts by using Soxhlet method.
[x] Bold words indicate the XO inhibitory activity > 15.

50 mM KH_2PO_4，另外同時測試薑科植物中含量豐富之類薑黃素 bisdemethoxycurcumin (BDMC) 之活性。試劑混和後於 25℃下反應 30 min，以分光光度計偵測波長 290 nm 吸收值。XO 抑制率計算方法如下：

抑制率
= (1 − 樣品吸光值/對照組吸光值) × 100%

純化萃取物中抑制 XO 之活性成分

使用高效液相層析儀 (high performance liquid chromatography; HPLC) 分離大南薑根莖之酒精萃取物，機型為 Waters 600 (Waters，美國)，選用製備級管柱 Kromasil 100-5-C18 column 250 mm × 21.2 mm (AkzoNobel，瑞典)，管柱溫度 40℃，連接 Waters 2745 偵測器 (Waters，美國)，偵測吸收波長 220 nm，注射樣品 500 µL。流動相為二次水及甲醇 (methanol)，梯度條件一開始為 20% 甲醇，之後線性升高甲醇比例，至第 10 分鐘達 35% 甲醇，第 24 分鐘達到 100% 甲醇，最後保持 100% 甲醇到第 30 分鐘。流速 16 mL min^{-1}，每 8 mL 收集成一管，共分離為 60 個區分物，分別命名為 G01、G02 至 G60。上述區分物依體外 XO 抑制活性分析方法進行分析，選出抑制活性最高者，再進行後續活性成分之探討。

GC-MS 分析萃取物中抑制 XO 之活性成分

使用氣相層析儀-質譜儀 (gas chromatography-mass spectrum; GC-MS) 分析大南薑抑制 XO 活性最高之 HPLC 區分物，機型為 GCxGC_TOFMS (LECO，美國)。管柱使用 30 m × 0.25 mm × 0.25 µm Rtx-5MS (Fisher Scientific，美國)，流動相為氦氣，速率 1 mL min^{-1}。注射器溫度 250℃，GC 的起始溫度 40℃ 持續 1 min，之後以每分鐘 10℃的速度升高至 310℃，之後保持 8 min。Transfer line 溫度 300℃，數據擷取速率 10 spectra s^{-1}。偵測質譜範圍 m/z 50−600，離子來源溫度 200℃，偵測器電壓 1650 V。

結果

青草藥萃取物之抑制 XO 活性

本研究挑選 24 種 (品種) 本土青草藥，共製備 28 種材料樣品 (包含不同組織部位之相同植物)，進行 95% 酒精萃取及熱水萃取，再分別以 3 種不同萃取物濃度進行體外 XO 抑制活性分析 (表 1)。在 28 種樣品的萃取物中，使用濃度 33.3 µg mL^{-1} 試驗時，有 2 種青草藥萃取物的 XO 活性抑制率超過 15%，分別是大南薑根莖之酒精萃取物 (54.3%)，以及仙草「台 E」品系之酒精萃取物 (19.2%)。使用濃度為 16.7 µg mL^{-1} 時，超過 15% XO 活性抑制率者，只有大南薑根莖之酒精萃取物 (27.9%)。於濃度 8.3 µg mL^{-1} 時，所有萃取物之 XO 抑制率皆低於 15%。正控制組 allopurinol 之標準品在濃度為 1.33 µg mL^{-1} 時有 94.1% XO 抑制率。類薑黃素 BDMC 之標準品在濃度為 1.33 µg mL^{-1} 時，對 XO 的抑制率為 0.2% (表 1)。

大南薑不同溶劑萃取物之抑制 XO 活性

將大南薑根莖以 55℃熱風乾燥後，磨碎的粉末使用 95% 酒精、正己烷 (n-hexane)、甲醇及水 4 種溶劑，以索氏萃取法 (多功能萃取裝置) 進行萃取，其萃取率及萃取物之 XO 抑制活性詳見表 2。大南薑根莖使用甲醇溶劑之萃取率最高 (12.1%)，其次依序為酒精 (10.4%)、水 (6.9%) 及正己烷 (1.8%)。對 XO 的抑制活性，以酒精萃取物最高，在濃度為 33.3 µg mL^{-1} 時，對 XO 的抑制活性為 54.3%。其他溶劑萃取物對 XO 的抑制效果，依序為甲醇萃取物 (22.7%)、正己烷萃取物 (18.2%) 及水萃取物 (2.2%)。

大南薑 HPLC 區分物抑制 XO 之活性

大南薑酒精萃取物以 HPLC 分離產生之 60 個區分物，進行 XO 抑制活性分析，結果顯示大南薑酒精萃取物之區分物中，活性最高者為 G47，在濃度為 1.33 µg mL^{-1} 時之 XO 抑制率可達 67.3%，其他區分物之抑制活性皆低於 10% (圖 1)。

表 2. 大南薑之 4 種不同溶劑萃取物抑制 XO 活性。

Table 2. XO inhibitory activity of four solvent extracts from *Alpinia galanga* rhizomes.

XO inhibitory activity (%)															
8.3 μg mL^{-1}				16.7 μg mL^{-1}				33.3 μg mL^{-1}				Extraction rate (%)			
E[z]	H[y]	M[x]	W[w]	E	H	M	W	E	H	M	W	E	H	M	W
2.3	1.5	1.1	-0.8	27.9	0.8	-0.1	0.4	54.3	18.2	22.7	2.2	10.4	1.8	12.1	6.9

[z] 95% Ethanol extracts by using Soxhlet method.
[y] n-Hexane extracts by using Soxhlet method.
[x] Methanol extracts by using Soxhlet method.
[w] Hot water extracts by using Soxhlet method.

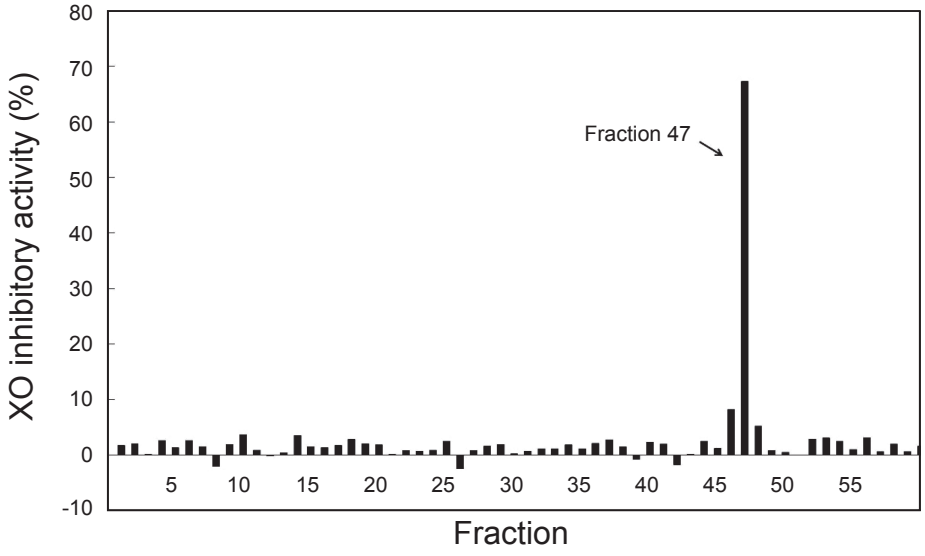


圖 1. 大南薑根莖酒精萃取物 HPLC 區分物 (G01–G60) 之抑制 XO 活性分析。試驗濃度為 1.3 μg mL^{-1}。

Fig. 1. XO inhibitory activity of HPLC fractions (G01–G60) separated from ethanol extracts of *Alpinia galanga* rhizomes. The activity was determined with samples at concentration of 1.3 μg mL^{-1}.

抑制 XO 活性成分之分子結構鑑定

以 GC-MS 分析大南薑 G47 區分物，結果如下：G47 在 16.6 min (Peak 1) 及 18.8 min (Peak 2) 各有一個波峰 (圖 2)，Peak 1 的質譜數據：m/z 55 (24)、77 (40)、103 (27)、105 (28)、121 (30)、131 (72)、132 (100)、133 (47)、149 (20)、150 (73)、174 (4)、192 (33)、234 (1)；Peak 2 的質譜數據：m/z 51 (31)、55 (23)、65 (28)、77 (65)、78 (27)、91 (30)、94 (55)、103 (44)、105 (38)、107 (47)、121 (70)、131 (69)、132 (44)、133 (77)、149 (100)、150 (69)、175 (3)、192 (85)、193 (11)、194 (2)、234 (16)、235 (2)。

Peak 1 之質譜符合 (1'*S*)-1'-acetoxychavicol acetate (ACA) 之質譜描述 (Noro *et al*. 1988; Yang & Eilerman 1999; Lin *et al*. 2016)，Peak 2 之質譜則符合 *p*-coumaryl diacetate (CDA) 之質譜描述 (Noro *et al*. 1988; Yang & Eilerman 1999)。

討論

我國中央健保署使用治療痛風的第一線降尿酸藥物「allopurinol」，其機制是抑制 XO 活性，因有造成嚴重藥物過敏反應「史帝芬-強生症候群」的風險，使醫師用藥有所顧忌，因此自 103 年 3 月 1 日起，將原第二線藥物

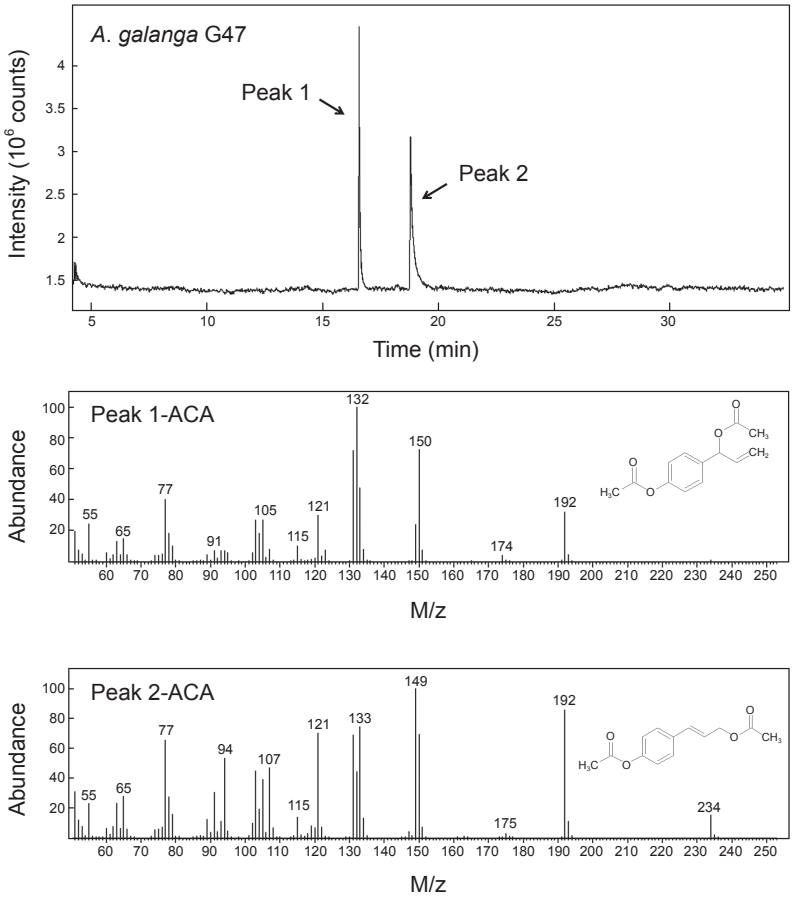


圖 2. 大南薑根莖酒精萃取物 HPLC 區分物 G47 之 GC-MS 層析圖以及質譜圖。

Fig. 2. GC-MS chromatograms and mass spectrums of HPLC fractions G47 separated from ethanol extracts of *Alpinia galanga* rhizomes.

febuxostat (價格較高) 開放給有尿酸性尿路結石或尿酸性腎結石之慢性痛風病人可於第一線使用。然而，包含 febuxostat 在內，其他治療痛風的藥物如 benzbromarone、probenecid、sulfinpyrazone 等，亦都有各式不同的副作用 (Boger & Strickland 1955; Kaegi *et al*. 1974; Love *et al*. 2010)，同時這些藥物均僅適用於已有慢性痛風症狀之病患，用以維持痛風不發作，無法預防高尿酸血症之發生。尋找適當天然可日常食用的食材，調降尿酸、降低痛風發生的機率，就成為一個極佳的保健策略。同時，XO 是超氧化物的產生酵素 (superoxide-producing enzyme)，高活性時容易升高體內的氧化壓力，所以適度的抑制其活性有利於人體的整體健康 (Liu *et al*. 2012)。

許多研究報告同樣是針對抑制 XO 酵素的活性，於各地的民俗青草藥中尋找可以治療痛風的植物，這些研究來自中國 (Kong *et al*. 2000)、澳洲 (Sweeney *et al*. 2001)、越南 (Nguyen *et al*. 2004) 及巴拿馬 (Gonzalez *et al*. 1995) 等國家。台灣融合華人及南島文化，民間利用的民俗青草藥種類繁多，但尚無一研究進行較大規模之抑制 XO 活性篩選。本研究從 24 種台灣民俗青草藥材料製備的 28 種萃取物中，發現大南薑根莖及仙草「台 E」的酒精萃取物具有最佳之 XO 抑制活性 (表 1)。

檢索文獻發現前人已經證實大南薑根莖之酒精萃取物具有抑制 XO 之活性 (Noro *et al*.

1988；Yumita *et al*. 2013)，本研究則進一步試驗不同溶劑的效應，顯示大南薑根莖之甲醇及正己烷萃取物亦具有抑制 XO 之活性，水萃取物則無 XO 抑制活性 (表 2)。大南薑莖葉之水及酒精萃取物皆無抑制 XO 活性 (表 1)，顯示大南薑之活性成份主要儲存於根莖。

以 GC-MS 分析大南薑酒精萃取物抑制 XO 活性最高的區分物，發現包含 2 個主要化合物：ACA 及 CDA (圖 2)。ACA 及 CDA 為大南薑根莖中之已知成分 (Yang & Eilerman 1999)，且已經證實兩者均為良好的 XO 抑制劑， 其 IC_{50} 與 Allopurinol 相 近 (Noro *et al*. 1988)，據此推測 ACA 及 CDA 很可能為本研究中，大南薑抑制 XO 活性之主要成分。ACA 的功效被研究得相當廣泛，目前已知具有護腦 (Kojima-Yuasa *et al*. 2016)、抗癌 (Moffatt *et al*. 2000; Ito *et al*. 2005; Yaku *et al*. 2011)、抗氣喘 (Seo *et al*. 2013)、抗過敏 (Matsuda *et al*. 2003)、抗肥胖 (Ohnishi *et al*. 2012) 等諸多功效。相較於 ACA，CDA 的研究較少，目前僅知可能具有抗菌的效果 (Aziz *et al*. 2013)。然而不論 ACA 或 CDA，迄今都尚無動物或人體研究報告驗證是否具有治療痛風或高尿酸血症之功效。大南薑根莖酒精萃取物幾無急毒性及慢性毒性 (Qureshi *et al*. 1992)，其活性成分 ACA 也被認為不影響正常細胞生長 (In *et al*. 2012)，若未來於動物及人體實驗驗證其具有調節尿酸之功效，大南薑根莖酒精萃取物將有潛力開發為預防高尿酸血症及痛風之保健產品或新藥。

除 ACA 及 CDA 之外，前人曾在大南薑根莖氯仿萃取物中發現另外 3 種化合物具有抑制 XO 的活性，包括 coniferyl diacetate、(1'*S*)-1'-acetoxyeugenol acetate 及 4-hydroxybenzaldehyde (Noro *et al*. 1988)。然而在 G47 的質譜中，並無發現這 3 種化合物的特徵片段 (圖 2)，推測使用不同的萃取溶劑 (該研究使用氯仿，本研究使用 95% 酒精)，有效成分的組成可能因此不同。大南薑根莖富含類薑黃素 BDMC (Lo *et al*. 2013)，此分子具有抗癌、抗氧化活性 (Huang *et al*. 1995; Sandur *et al*. 2007; Pei *et al*. 2016)，然而本研究證實 BDMC 並無抑制 XO 的活性 (表 1)，G47 的質譜亦沒有發現 BDMC 的特徵片段，顯示確實不是大南薑根莖抑制 XO 的活性成分。

本研究發現仙草的酒精萃取物也具有些許抑制 XO 的活性。仙草在民間被認為有利尿的功效 (Lai *et al*. 2001) ，亦有報導仙草酒精萃取物可減輕大鼠高尿酸血症 (Jhang *et al*. 2016)，所以仙草可能也具有開發作為調節尿酸產品之潛力。值得注意的是，不同仙草品種的 XO 抑制效果差異極大，本研究所使用的 3 種仙草品系中，「台 E」的酒精萃取物具有較強的抑制 XO 活性，其他 2 種品系「桃園 2 號」及「農試 1 號」的酒精萃取物則幾乎無抑制活性 (表 1)。仙草含有高量的酚類及類黃酮 (Jhang *et al*. 2016)，而許多酚類及類黃酮被認為有抑制 XO 的效果 (Bindoli *et al*. 1985; Pauff & Hille 2009)。但是 Jhang *et al*. (2016) 認為仙草抑制 XO 之活性成分可能並非酚類及類黃酮，仙草抑制 XO 之活性成分仍需進一步研究。

誌謝

本研究承蒙中央研究院植物暨微生物學研究所謝明勳副研究員、吳玉菁小姐，以及中央研究院農業生物科技研究中心代謝體實驗室林芝毓博士協助進行 GC-MS 分析，謹致謝忱。

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Inhibitory Activity of *Alpinia galanga* Rhizomes on Xanthine Oxidase

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Abstract

Hung, T. H., J. S. Lai, S. Y. Lin, C. Yang, and Y. L. Lee. 2017. Inhibitory activity of *Alpinia galanga* rhizomes on xanthine oxidase. J. Taiwan Agric. Res. 66(4):333–342.

Hyperuricemia is a risk factor for causing gout, diabetes and some metabolic diseases. The reduction of uric-acid biosynthesis and alleviating the symptom of hyperuricemia may be intervened by inhibiting the xanthine oxidase (XO) activity. In this study, the XO inhibitory activity of ethanol or water extracts of 24 indigenous medical plants were evaluated. Results showed that the ethanol extract of *Alpinia galanga* rhizomes possessed the best XO inhibitory activity. The ethanol extract of *Mesona procumbens* line 'Tai E' showed mild activity as well. The active XO inhibitors in the *A*. *galanga* rhizomes were suggested to be (1'*S*)-1'-acetoxychavicol acetate and *p*-coumaryl diacetate based on the analytical results of gas chromatography-mass spectrum (GC-MS). Both compounds were reported exhibiting similar XO inhibitory activities as allopurinol, a common medicine for hyperuricemia treatment. These results suggest that *A*. *galanga* is a potential herb for developing functional food or therapeutic agent to regulate the biosynthesis of uric acid.

Key words: Hyperuricemia, Xanthine oxidase, Inhibitory activity, *Alpinia galangal*.

Received: January 5, 2017; Accepted: April 14, 2017.
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台灣農業研究 (J. Taiwan Agric. Res.) 66(4):343–355 (2017)
DOI: 10.6156/JTAR/2017.06604.09

研究報告

超級雜交稻在台灣的產量潛力分析

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摘要

卓緯玄、陳宗禮、李長沛、賴明信、吳東鴻、顏信沐。2017。超級雜交稻在台灣的產量潛力分析。台灣農業研究 66(4):343–355。

水稻為台灣最主要生產與消費的糧食作物，開發高產品種一直是水稻育種重要目標之一，其中雜種優勢運用是一可行的方向。本研究以大陸地區引進之「錢優 0508」('Qianyou 0508'； 'QY 0508')、「II 優 7954」('II You 7954'; 'II Y 7954')、「Y 兩優 689」('Y Liangyou 689'; 'YLY 689')、「Y 兩優 302」('Y Liangyou 302'; 'YLY 302')、「皖稻 153」('Wandao 153'; 'WD 153')、「五優 308」('Wuyou 308'; 'WY 308')、「株兩優 168」('Zhuliangyou 168'; 'ZLY 168') 等 7 個秈型超級雜交稻組合為材料，對照品種為台灣秈稻純系品種「台中秈 17 號」('Taichung Sen 17'; 'TCS 17')，在台灣地區進行一年兩期作生育特性及產量潛力評估試驗。變方分析結果顯示，參試水稻品種的農藝性狀及產量構成要素在不同插植株數及不同品種間具有顯著差異。參試品種的產量、產量構成要素及農藝性狀的表現普遍比對照品種 'TCS 17' 優異，而且一期作的增產效果明顯比二期作高。超級雜交稻高產的主要原因為一期作單株總穎花數 (積儲容積) 較高所致。一期作多本植的超級雜交稻平均產量較對照品種 'TCS 17' 高 20%，兩本植則高 5%。二期作多本植的超級雜交稻平均產量較對照品種 'TCS 17' 高 7%，兩本植則高 10%。不同插植株數間之比較，以兩本植產量較低，主因為兩本植之穗數不足所致。本研究顯示超級雜交稻在台灣栽培，必須保證其單株穎花數及維持一定的單位面積穗數，才能保證獲致高產。

關鍵詞：超級雜交稻、產量構成要素。

前言

近年來全球氣候變遷導致糧食生產的不穩定性增加，全世界正面臨著潛在的糧食短缺問題 (Long *et al*. 2015)，如何提高水稻單位面積產量，突破產量高原障礙，是水稻育種者追求的目標。國際上水稻產量潛力的提昇主要歷經了兩個重要階段，第一階段是國際水稻研究所 (International Rice Research Institute; IRRI) 將來自台灣品種的半矮性基因 *DGWG–sd1* 導入印尼傳統高大植株品種 'Peta'，於 1966 年育成改良型半矮性品種 'IR 8'，將熱帶地區水稻產量潛力由 6 Mg ha^{-1} 提高到 10 Mg ha^{-1} (Khush *et al*. 2001)；第二個階段為 1976 年，中國大陸以雜種優勢為基礎所發展的雜交稻成功應用於生產，將中國大陸的水稻產量提高了 20% 左右 (Yuan 2006)。台灣於 1935 年即開始以秈粳雜交 (japonica/indica cross) 方式增加遺傳歧異度，選育高產品種；2004 年育成之粳稻「台南 11 號」，兼具高產及優良米粒外觀與食味品質，單位面積產量高達 9.5 Mg ha^{-1} (Lin 2004)，截至 2015 年已佔全台灣水稻栽培面積 60.73%，為目前全台領先栽培品種。中國大陸於 1996 年訂定了超級稻育種計畫，其產量指標分為 4 期：第 1 期 (1996–2000 年) 目標較 1996 年增產 20% (10.5 Mg ha^{-1})，第 2

投稿日期：2017 年 4 月 5 日；接受日期：2017 年 5 月 22 日。
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期 (2001–2005 年) 目標增產 40% 以上 (12 Mg ha^{-1})，第 3 期 (2006–2015 年) 目標為 13.5 Mg ha^{-1}，第 4 期 (2016–2020 年) 目標為 15 Mg ha^{-1} (Yuan 2006, 2017)，並以北方粳型超級稻與南方秈型超級雜交稻同時發展。

作物會利用表現型的可塑性來調節器官的生長與發育，以適應環境的變化 (Schlichting 2002)。水稻產量由積儲大小和供源能力間的平衡決定 (Ohsumi *et al*. 2011)。在合適環境生長下，積儲容量大小被認為是穀粒產量的決定因素 (Sheehy *et al*. 2001; Yoshida *et al*. 2006)。積儲容量大小可透過提高單位面積穗數或一穗穎花數來增加，但是因為兩者具有很高的補償機制，使得我們很難同時提高這兩種性狀，因此想要提高產量通常是以增加單位面積的穗數 (Katsura *et al*. 2007; Badshah *et al*. 2014; Liang *et al*. 2014) 或是提高一穗穎花數 (Sheehy *et al*. 2001; Katsura *et al*. 2008) 來著手。Lai *et al*. (2016) 將 *Gn1a* 多粒數基因回交導入水稻「台農 71 號」中，提升了 26% 的一穗穎花數，但增產幅度僅 4.2%，其主因係稔實率由 78.8% 下降到 64.7%。因此在增加積儲容積時，仍要注意需有足夠的供源，才能獲得較好的增產效果 (Ohsumi *et al*. 2011; Yoshinaga *et al*. 2013)。超級雜交稻品種的開發是以理想株型為基礎，理想株型的主要特徵是大穗、少分蘖 (Peng *et al*. 2008)；單位面積穗數可透過早期增施氮肥、改變插植密度來改善，或在幼穗分化期施用氮肥提高一穗穎花數 (Yoshida *et al*. 2006)。然而，高產品種除了挑戰遺傳和生理產量上限之外，還必須具備良好的環境適應性，以便在氣候變遷、密植及高投施等風險挑戰下能維持產量的穩定性 (Rötter *et al*. 2015)。因此，育種者除了針對超級雜交稻的廣適應性進行研究外，仍須就不同栽種環境建立適合的超高產栽培模式。

台灣以往曾進行三系雜交水稻之研究，當時的研究認為雜種品系對環境較為敏感，產量穩定性較差 (Tseng & Huang 1987)。單交雜種品系的特殊組合力，因環境有很大的差異 (Huang *et al*. 1988)。雜交稻產量一般可較對照品種增產 10.1–24.9%，適當調整種植時期並利用其感光性，可使其發揮高產潛力 (Chen *et al*. 1991)。後因台灣推動良質米政策及雜交稻米質提升困難，因而中斷研究。由於中國大陸對雜交稻研究長期持續進行，近來在超級雜交稻研究中屢有佳績，2011 年超級雜交稻「Y 兩優 2 號」產量已達 13.9 Mg ha^{-1}，「Y 兩優 900」於 2013 年在湖南省隆回縣與 2014 年在湖南省徐浦縣示範田中分別達到 14.8 Mg ha^{-1} 和 15.4 Mg ha^{-1} 的產量表現，2015 年「超優 1000」品種在雲南省箇舊市示範田中已成功將單位面積產量提升到 16.0 Mg ha^{-1} (Yuan 2017)。水稻的產量會因地區、環境氣候、栽培技術等因素而有不同的表現 (Katsura *et al*. 2007; Yang *et al*. 2008)。這些具有超高產特性的超級雜交稻品種從未在台灣環境中進行產量表現的研究，因此本研究自中國大陸地區引進 7 個超級雜交稻組合為材料，並以台灣高產秈稻純系品種「台中秈 17 號」為對照品種進行產量比較試驗，以評估雜交稻在台灣環境下的生產潛力，期能提供台灣超高產水稻栽培及育種改良工作之參考。

材料與方法

試驗材料

本研究以行政院農業委員會農業試驗所 (以下簡稱農試所) 自中國大陸地區引進之「錢優 0508」('Qianyou 0508'; 'QY 0508')、「II 優 7954」('IIYou 7954'; 'IIY 7954')、「Y 兩優 689」('Y Liangyou 689'; 'YLY 689')、「Y 兩優 302」('Y Liangyou 302'; 'YLY 302')、「皖稻 153」('Wandao 153'; 'WD 153')、「五優 308」('Wuyou 308'; 'WY 308')、「株兩優 168」('Zhuliang-you 168'; 'ZLY 168') 等 7 個超級雜交稻品種為材料，其中有 3 個品種為三系法雜交水稻，4 個品種為兩系法雜交水稻；比較試驗以台灣目前高產純系秈稻栽培品種「台中秈 17 號」('Taichung sen 17'; 'TCS 17') 為對照 (表 1)；雜交稻組合種子由中國大陸「中國水稻研究所 (China Rice Research Institute; CRRI)」提供，「台中秈 17 號」種子則由農試所稻作研究室自行繁殖，所有參試品種 (系) 均屬秈稻 (*indica* rice)。

本試驗中 'ZLY 168' 屬早稻品種；'QY 0508'、

表 1. 本研究超級雜交稻與「台中秈 17 號」對照品種的特性[z]。

Table 1. Characteristics of super hybrid rice varieties and check variety 'Taichung sen 17' in this study[z].

Variety	Breeding method	Cross combination	Regional yield trial (kg ha^{-1})[y]	Growth duration (d)	Plant height (cm)	Spikelets per panicle	1000-grain weight (g)	Disease/pest resistance[x]
Qianyou 0508 (QY 0508)	Three-line hybrid	Qianjiang 1A/ Zhehui 0508	8,527	119.7	108.1	153.3	26.40	HS to BL.
II You 7954 (II Y 7954)	Three-line hybrid	II-32A/Zhehui 7954	8,516	136.3	118.9	174.1	27.30	S to BL, MS to BBL, HS to BPH.
Y Liangyou 689 (YLY 689)	Two-line hybrid	Y58S/Wenhui 689	8,767	138.3	122.3	164.5	26.50	HS to BL, S to BBL, HS to BPH.
Y Liangyou 302 (YLY 302)	Two-line hybrid	Y58S/F302	9,064	136.5	131.9	178.1	26.50	HS to BL, S to BBL, HS to BPH.
Wandao 153 (WD 153)	Two-line hybrid	1892S/RH003	8,750	134.3	113.4	176.0	23.54	HS to BL, S to BBL.
Wuyou 308 (WY 308)	Three-line hybrid	Wufeng A/ Guanghui 308	7,568	112.2	99.6	157.3	23.60	MS to BL, S to BBL, MS to BPH.
Zhuliangyou 168 (ZLY 168)	Two-line hybrid	Zhu 1S/R168	7,772	108.0	85.0	111.5	26.10	S to BL, MS to BBL.
Taichung sen 17 (TCS 17)	Inbred line	–	7,508 (I) 6,864 (II)	127.0 (I) 106.0 (II)	103.0 (I) 105.0 (II)	134.0 (I) 140.0 (II)	34.00 (I) 33.00 (II)	MR to BL, MS to BBL, R to BPH

[z] The data compiled in this study. Hybrid rice data from Continental National Rice Data Center (http://www.ricedata.cn/index.htm).
[y] (I): first crop; (II): second crop.
[x] R: resistance; MR: moderate resistance; MS: moderate sensitive; S: sensitive; HS: high sensitive; BBL: bacterial blight; BL: blast; BPH: brown plant hopper.

'WY 308' 等屬晚稻品種；'II Y 7954'、'YLY 689'、'YLY 302'、'WD 153' 等屬中稻品種。參試雜交稻品種依據中國大陸國家水稻數據中心資料整理顯示，其區域試驗產量表現約在 7,568–9,064 kg ha^{-1} 範圍，台灣栽培品種 'TCS 17' 區域試驗產量則為 7,508 kg ha^{-1} (一期作) 與 6,864 kg ha^{-1} (二期作)，所有參試品種的病蟲害抗性及特性列於表 1。

試驗方法

除比較超級雜交稻在台灣的產量潛力外，本試驗亦同時探討雜交稻在不同插植株數的產量表現，因此試驗採裂區設計 (split plot design)，不同插植株數為主區，品種為副區，重複 3 次，插植株數處理分為多本植及兩本植，於農試所農場試驗田進行。供試品種於 2012 年第一期作與第二期作，經育苗後分別於 2012 年 2 月 10 日及 2012 年 8 月 9 日移植，每小區種植 5 行 × 20 株，行株距為 28 cm × 16 cm (小區面積 4.48 m^2)；肥料施用量為 N : P2O5 : K_2O = 132 : 72 : 48 kg ha^{-1}，分為基肥 (台肥 39 號複合肥料 200 kg ha^{-1}) 、兩次追肥 (每次硫酸銨 200 kg ha^{-1}) 及穗肥 (台肥 39 號複合肥料 200 kg ha^{-1}) 施用，其他病蟲害防治及灌排水依一般慣行栽培法進行管理。栽培期間調查抽穗期、株高，成熟期記錄收穫時間，每小區收穫 4 叢，剪下稻穗後混合置於精密烘箱 (ULE-600, Memmert) 以 40℃烘乾至水份含量約 13–14% 時進行考種，調查其穗數、穗長、穗重、稔實粒重、不稔實粒重、稔實粒數及不稔實粒數，並計算全生育期 (growth duration)、每株穗數 (panicle number per hill)、單株總穎花數 (spikelet number per hill)、單穗長 (panicle length)、單穗重 (panicle weight)、一穗穎花數 (spikelet number per panicle)、稔實率 (fertility)、千粒重 (1000-grain weight) 及單株產量 (yield per hill)。試驗小區全部採收烘乾至約 13–14% 時進行風選後稱重，並以容量式水分計 (PM-600, KETT) 測量水分含量、容重 (volume weight) 等，小區產量校正為水分含量 13% 之重量後計算單位面積產量 (yield per hectare) 及日產量 (daily yield)。

統計分析

調查性狀使用 SAS 9.2 版本軟體 (Systat-Software, Inc. 2006) 進行變方分析 (Analysis of variance; ANOVA) 及最小差異顯著性測驗 (Fisher's protected least significant difference test; LSD test)，分開一、二期作下探討各參試品種在不同插植株數間之表現差異，以瞭解超級雜交稻在台灣環境栽種的生產潛力。

結果

一期作產量潛力分析

一期作下單株產量的插植株數與品種之交感效應不顯著，單株產量在不同插植株數間不顯著但品種間具極顯著差異，顯示單株產量的品種間差異性不會因插植株數而有明顯影響；單位面積產量及日產量插植株數與品種之交感效應顯著或極顯著差異，表示參試品種的單位面積產量及日產量會隨著插植株數不同而異 (表 2)。由表 3 結果發現，單株產量在多本植時以 'WY 308'、'II Y 7954' 及 'YLY 302' 顯著較對照品種 'TCS 17' 高產，兩本植時所有參試品種均與 'TCS 17' 無顯著差異。7 個參試品種的單位面積產量在多本植時均顯著較 'TCS 17' 增產，兩本植時則僅有 'II Y 7954'、'WD 153' 兩品種顯著高於 'TCS 17'；不同插植株數間則以 'QY 0508'、'YLY 689'、'YLY 302' 及 'WY 308' 等品種在兩本植時單位面積產量均顯著低於多本植。日產量以 'II Y 7954' 的日產量最高可達 85.8 kg ha^{-1}，其次為 'ZLY 168'；在多本植時 7 個參試品種的日產量均顯著高於 'TCS 17'，兩本植時則僅有 5 個品種顯著高於 'TCS 17'；比較不同插植株數間的日產量，以多本植之日產量有較高之趨勢，其中又以 'II Y 7954' 的日產量表現最佳；'ZLY 168' 之單株及單位面積的產量雖較差但日產量則有較佳成績，顯示該品種能以較短生育日數達成一定的產量。綜合以上結果，顯示參試超級雜交稻品種在多本植時的產量均較台灣對照品種 'TCS 17' 為高，尤其日產量表現更為明顯，其中又以 'II Y 7954' 的表現最佳。

一期作產量構成要素之變方分析顯示，插植株數與品種之交感效應，除稔實率沒有顯著差異外，每株穗數、一穗穎花數及千粒重均具顯著差異，顯示參試品種在該 3 個產量構成要素之表現會隨著插植株數不同而異 (表 2)。由表 3 結果發現，每株穗數在多本植時以 'WY 308' 與 'II Y 7954' 兩品種顯著高於對照品種 'TCS 17'；兩本植時除 'ZLY 168' 與 'QY 0508' 兩品種之每株穗數與 'TCS 17' 無顯著差異外，其他參試品種之每株穗數均顯著較 'TCS 17' 少；比較不同插植株數處理之間，多本植之每株穗數有較多之趨勢，其中 'II Y 7954' 與 'WY 308' 兩品種在多本植與兩本植間的每株穗數差異極為懸殊，顯示這兩個品種的分蘗能力最差。一穗穎花數在多本植與兩本植時，除 'ZLY 168' 品種外，其他參試品種均顯著高於對照品種 'TCS 17'；比較不同插植株數處理之間，兩本植的平均一穗穎花數比多本植多，顯示在兩本植時參試品種會明顯提高一穗穎花數來因應，尤其以 'II Y 7954'、'WD 153' 及 'ZLY 168' 最為明顯。一期作稔實率除 'ZLY 168' 品種在多本植時顯著低於 'TCS 17' 外，其他參試品種均與 'TCS 17' 沒有顯著差異；兩本植時所有參試品種的稔實率則與 'TCS 17' 無顯著差異。在千粒重方面，不同插植株數均以對照品種 'TCS 17' 的穀粒顯著較大；所有參試品種在不同插植株數間的千粒重差異則均無顯著。

一期作農藝性狀之變方分析結果，除穗長外，其餘性狀之插植株數與品種間交感效應均顯著或極顯著，顯示參試品種在幾乎所有農藝性狀之表現會隨著插植株數不同而異 (表 2)。由表 4 結果發現，一期作生育日數以 'YLY 689'、'YLY 302' 及 'TCS 17' 的生育日數顯著較其他參試品種長，以 'ZLY 168' 為最早熟的品種；兩本植植株的生育日數略多於多本植，其中以 'QY 0508'、'WD 153'、'WY 308' 及 'TCS 17' 品種顯著較長。株高部分，除 'ZLY 168' 外，其餘參試品種均顯著比 'TCS 17' 品種高；比較不同插植株數處理間的株高，顯示不同品種在不同插植株數的株高表現有所不同。穗長在多本植有略短於兩本植之趨勢，其中 'QY 0508'、'II Y 7954'、'YLY 689' 及 'ZLY 168' 等

表 2. 2012 年一期作不同水稻品種的產量、產量構成要素及農藝性狀之變方分析。

Table 2. ANOVA of yield, yield components and agronomic traits for different rice varieties grown in the first cropping season of 2012.

Source of variation	df	Mean square												
		Yield per hill	Yield per hectare	Daily yield	Panicle number per hill	Spikelet number per panicle	Fertility	1000-grain weight	Growth duration	Plant height	Panicle length	Panicle weight	Spikelet number per hill	Volume weight
Block	2	8.25	357,519	30.8*	1.36	0.7	9.37	0.29	0.52	3.42	0.60	0.01	26,094	69.6
Seedling number (SN)	1	41.20	2,350,436**	217.1**	70.06**	2,655.6**	0.60	0.14	13.02**	11.12*	8.38*	1.99**	152,439*	5.6
Error (a)	2	8.34	61,404	4.4	0.78	17.5	2.25	0.05	0.27**	6.09	2.21	0.00	13,918	223.0
Variety (V)	7	61.58**	3,268,237**	313.0**	8.54**	2,057.7**	23.53*	22.34**	452.45**	458.59**	16.32**	0.81**	246,790**	2,528.7**
SN × V	7	23.15	487,945**	29.0*	10.90**	514.8**	8.70	3.20**	1.40*	8.84**	1.47	0.31**	133,676**	283.0**
Error (b)	28	11.82	142,916	8.9	1.19	51.2	8.38	0.33	0.59	1.88	1.71	0.03	23,440	48.9

*,** Significant at 5% and 1% probability levels, respectively.

表 3. 2012 年一期作不同水稻品種在不同插植株數下產量及產量構成要素之平均值比較。

Table 3. Mean comparison of yield and yield components for different rice varieties under different seedling numbers in first cropping season of 2012.

Character / Seedling number / Variety	Yield per hill (g hill^{-1})		Yield per hectare (kg ha^{-1})		Daily yield (kg ha^{-1})		Panicle number per hill		Spikelet number per panicle		Fertility (%)		1000-grain weight (g)	
	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two
QY 0508	39.8 Abc^{z}	40.4 Aab	9,972 Aab	9,152 Bbc	81.5 Aab	73.0 Bcd	13.9 Ad	13.8 Aab	122.6 Ab	126.3 Ac	87.9 Aab	87.8 Aab	26.7 Ac	26.4 Acd
Ⅱ Y 7954	45.6 Aa	43.7 Aa	10,524 Aa	10,688 Aa	85.8 Aa	86.4 Aa	17.1 Aab	11.0 Bd	108.3 Bc	167.8 Aa	86.0 Aabc	85.7 Aab	28.6 Ab	27.6 Ab
YLY 689	38.4 Abc	32.8 Ac	9,316 Acd	8,168 Be	71.7 Ac	62.8 Bf	13.9 Ad	10.8 Bd	128.6 Aab	135.7 Abc	83.7 Abc	82.8 Ab	25.8 Acd	27.0 Abc
YLY 302	41.7 Aab	39.2 Aabc	10,093 Aa	9,200 Bbc	78.4 Ab	71.1 Bde	13.4 Ad	12.9 Abc	138.8 Aa	142.7 Ab	88.3 Aa	84.6 Aab	25.3 Ade	25.2 Ae
WD 153	36.8 Abc	39.7 Aabc	9,980 Aab	9,504 Ab	81.4 Aab	76.8 Bbc	13.3 Ad	12.1 Acd	129.8 Bab	146.6 Ab	86.3 Aabc	89.4 Aa	24.7 Ae	25.0 Ae
WY 308	46.4 Aa	37.4 Aabc	9,418 Abc	8,915 Bbcd	79.1 Ab	74.3 Bcd	18.6 Aa	11.7 Bcd	131.1 Aab	141.0 Ab	83.4 Abc	87.5 Aab	23.0 Af	25.9 Ade
ZLY168	36.3 Ac	35.8 Abc	8,751 Ad	8,299 Ade	84.4 Aa	80.1 Ab	16.3 Abc	15.2 Ba	95.9 Bd	102.1 Ad	82.7 Ac	82.8 Ab	28.0 Ab	27.8 Ab
TCS 17	35.5 Ac	36.9 Aabc	8,115 Be	8,703 Acde	62.6 Bd	66.3 Aef	14.8 Acd	14.6 Aa	88.1 Bd	100.0 Ad	87.6 Aab	87.1 Aab	31.2 Aa	29.1 Aa

^{z} Means within treatment of seedling number (in capital letter) and within variety (in small letter) for each character follow by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test.

表 4. 2012 年一期作不同水稻品種在不同插植株數下農藝性狀之平均值比較。

Table 4. Mean comparison of agronomic traits for different rice varieties under different seedling numbers in the first cropping season of 2012.

Character / Seedling number / Variety	Growth duration (d)		Plant height (cm)		Panicle length (cm)		Panicle weight (g)		Spikelet number per hill		Volume weight (g L^{-1})	
	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two
QY 0508	122.3 Bb[z]	125.3 Ac	93.4 Ad	93.5 Ae	20.6 Bbcde	22.1 Abcd	3.2 Aab	3.2 Ac	1,700 Abc	1,741 Aabc	540.7 Ac	528.2 Abc
Ⅱ Y 7954	122.7 Ab	123.7 Ad	102.5 Ab	102.5 Ab	21.0 Bbcd	22.5 Aabc	3.0 Bbc	4.3 Aa	1,854 Ab	1,848 Aa	554.7 Ab	561.8 Aa
YLY 689	130.0 Aa	130.0 Ab	102.1 Ab	99.9 Bc	22.3 Bab	24.6 Aa	3.2 Aab	3.4 Abc	1,785 Abc	1,464 Bcd	501.4 Af	509.5 Ae
YLY 302	128.7 Aa	129.3 Ab	113.8 Ba	117.4 Aa	23.4 Aa	24.0 Aab	3.4 Aa	3.4 Abc	1,867 Ab	1,838 Aa	532.4 Acd	513.0 Ade
WD 153	122.7 Bb	123.7 Ad	98.8 Ac	96.4 Bd	21.8 Aabc	20.9 Acde	3.0 Bbc	3.6 Ab	1,731 Abc	1,776 Aab	523.6 Ade	538.5 Ab
WY 308	119.0 Bc	120.0 Ae	90.5 Be	93.0 Ae	20.1 Acde	20.8 Acde	2.8 Bcd	3.5 Abc	2,422 Aa	1,650 Babcd	511.4 Aef	521.2 Acd
ZLY168	103.7 Ad	103.7 Af	86.6 Bf	89.1 Af	18.6 Be	19.8 Ade	2.4 Be	2.6 Ad	1,565 Ac	1,554 Abcd	514.9 Aef	526.9 Ac
TCS 17	129.7 Ba	131.3 Aa	90.4 Be	94.0 Ae	19.5 Ade	19.5 Ae	2.6 Bde	2.8 Ad	1,302 Bd	1,452 Ad	572.3 Aa	557.8 Aa

[z]Means within treatment of seedling number (in capital letter) and within variety (in small letter) for each character follow by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test.

4 個品種多本植的穗長顯著較兩本植短。穗重除 'WY 308' (多本植) 及 'ZLY 168' (多本植與兩本植) 外，其他參試品種均顯著比 'TCS 17' 高，顯示大部分參試雜交稻品種的穗明顯較 'TCS 17' 大；'Ⅱ Y 7954'、'WD 153'、'WY 308'、'ZLY 168' 及 'TCS 17' 等品種在兩本植的穗重顯著比多本植之表現為重。單株總穎花數在多本植時所有參試品種均顯著比 'TCS 17' 來得多；兩本植時則以 'Ⅱ Y 7954'、'YLY 302'、'WD 153' 及 QY 0508 的單株總穎花數顯著較 'TCS 17' 高；兩本植的單株總穎花數以 'YLY 689' 與 'WY 308' 顯著低於多本植。在穀粒容重部分，以 'Ⅱ Y 7954' 最高而 'YLY 689' 最低，但均低於 'TCS 17'。

二期作產量潛力分析

二期作單株產量、單位面積產量及日產量只有在不同參試品種間有顯著差異，顯示這些產量性狀在品種間差異性不因插植株數不同而異 (表 5)。由表 6 分析結果發現，不論多本植或兩本植，單株產量以 'QY 0508' 與 'WY 308' 顯著較對照品種 'TCS 17' 高，其他參試品種則與 'TCS 17' 無顯著差異。在單位面積產量方面，'YLY 689'、'YLY 302' 及 'WY 308' 等 3 個品種不論在多本植或兩本植時均顯著較 'TCS 17' 高產。參試品種以 'ZLY 168' 的日產量最高，多本植與兩本植可分別達 93.5 kg ha^{-1} 與 94.9 kg ha^{-1}，其次為 'WY 308'，對照品種 'TCS 17' 的日產量則僅約 60 kg ha^{-1}，顯著低於所有參試品種。

二期作產量構成要素之變方分析結果，每株穗數與千粒重之插植株數與品種間交感效應不顯著，顯示其在品種間差異性不因插植株數不同而異；一穗穎花數與稔實率在插植株數與品種間交感效應顯著或極顯著，顯示參試品種間在這兩個產量構成要素之表現會隨著插植株數不同而異 (表 5)。由表 6 分析結果發現，'QY 0508' 之每株穗數不論在多本植或兩本植均顯著高於對照品種 'TCS 17'，多本植時 'Ⅱ Y 7954'、'YLY 689' 及 'YLY 302' 等 3 個品種的每株穗數顯著較 'TCS 17' 少，兩本植時除 'QY 0508' 與 'WY 308' 外的其他參試品種均顯著較 'TCS 17' 來得少；多本植的每株穗數較兩

表 5. 2012 年二期作不同水稻品種的產量、產量構成要素及農藝性狀之變方分析。

Table 5. ANOVA of yield, yield components and agronomic traits for different rice varieties grown in the second cropping season of 2012.

Source of Variation	Df	Mean square												
		Yield per hill	Yield per hectare	Daily yield	Panicle number per hill	Spikelet number per panicle	Fertility	1000-grain weight	Growth duration	Plant height	Panicle length	Panicle weight	Spikelet number per hill	Volume weight
Block	2	19.17	178,753	11.6	2.85	349.4*	4.21	0.41	0.52	0.05	1.42	0.25*	56,649*	257.6*
Seedling number (SN)	1	0.00	183,460	12.6	9.63**	2,298.7**	4.07	0.46	0.33	11.21*	1.32	1.14**	14,352	51.0
Error (a)	2	5.12	100,037	8.8	1.76	59.8	3.23	0.08	0.27	7.65	0.56	0.06	23,221	17.7
Variety (V)	7	64.58**	1,073,018**	598.4**	25.67**	4,242.8**	119.53**	40.83**	592.13**	440.06**	26.53**	0.98**	533,111**	1,824.2**
SN × V	7	2.47	133,373	12.7	1.38	241.0*	29.77**	0.21	0.67	10.39**	0.52	0.13*	43,700*	23.2
Error (b)	28	6.67	116,082	9.7	1.01	76.6	3.60	0.14	0.80	2.28	1.14	0.05	15,788	50.1

*,** Significant at 5% and 1% probability levels, respectively.

表 6. 2012 年二期作不同水稻品種在不同插植株數下產量及產量構成要素之平均值比較。

Table 6. Mean comparison of yield and yield components for different rice varieties under different seedling numbers in the second cropping season of 2012.

Character / Seedling number / Variety	Yield per hill (g hill^{-1})		Yield per hectare (kg ha^{-1})		Daily yield (kg ha^{-1})		Panicle number per hill		Spikelet number per panicle		Fertility (%)		1000-grain weight (g)	
	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two
QY 0508	46.8 Aa^{z}	45.2 Aab	7,753 Abc	7,509 Ac	74.5 Ab	72.2 Acd	16.8 Aa	16.7 Aa	118.5 Ae	107.6 Be	86.5 Ba	91.5 Aa	27.2 Ab	27.5 Ab
Ⅱ Y 7954	37.2 Ad	37.7 Ac	7,956 Abc	7,896 Abc	74.3 Ab	74.3 Ac	11.2 Ae	9.4 Bd	145.7 Bbc	166.1 Abc	82.9 Bbc	87.6 Ab	27.6 Ab	27.5 Ab
YLY 689	38.9 Acd	40.8 Ac	8,363 Aab	8,336 Aab	73.6 Ab	73.8 Acd	12.0 Ae	12.6 Ac	153.9 Aab	167.8 Ab	82.8 Abc	77.0 Bd	25.6 Ac	25.3 Bc
YLY 302	39.6 Acd	40.4 Ac	8,366 Aab	8,427 Aab	75.1 Ab	75.7 Ac	12.8 Ade	11.8 Ac	155.8 Bab	189.7 Aa	81.8 Ac	74.8 Bd	24.4 Ad	24.3 Ad
WD 153	41.3 Abcd	39.5 Ac	8,126 Aabc	7,387 Bc	76.7 Ab	69.2 Bd	14.8 Abc	12.5 Bc	160.2 Ba	176.8 Aab	75.4 Ad	76.5 Ad	23.2 Ae	23.4 Ae
WY 308	46.0 Aab	46.2 Aa	8,610 Aa	8,767 Aa	88.5 Aa	89.8 Ab	16.0 Aab	15.3 Aab	138.5 Bcd	150.4 Acd	85.3 Aab	85.9 Abc	24.4 Ad	23.5 Be
ZLY168	42.3 Aabc	41.6 Abc	8,133 Aabc	8,285 Aab	93.5 Aa	94.9 Aa	14.1 Acd	12.4 Bc	127.0 Bde	144.2 Ad	86.2 Aa	85.9 Abc	27.5 Ab	27.1 Bb
TCS 17	37.7 Acd	38.5 Ac	7,617 Ac	7,329 Bc	63.5 Ac	61.8 Ae	14.8 Abc	14.7 Ab	94.6 Bf	102.3 Ae	85.5 Aab	82.6 Bc	31.4 Aa	31.1 Aa

^{z} Means within treatment of seedling number (in capital letter) and within variety (in small letter) for each character follow by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test.

本植為多，尤以 ‘ⅡY 7954’、‘WD 153’ 及 ‘ZLY 168’ 的差異較大，顯示這 3 個品種在二期作的分蘖能力較差。一穗穎花數除 ‘QY 0508’ (兩本植) 外，所有參試品種在多本植或兩本植均一致較 ‘TCS 17’ 顯著為多；除 ‘QY 0508’ 外，其餘參試品種在兩本植之一穗穎花數均與多本植略多或顯著增加，顯示在兩本植時參試品種通常會提高一穗穎花數來因應。稔實率以 ‘WD 153’ 與 ‘YLY 302’ 在多本植顯著較 ‘TCS 17’ 低，兩本植則以 ‘WD 153’、‘YLY 689’ 及 ‘YLY 302’ 顯著較 ‘TCS 17’ 低，‘QY 0508’ 及 ‘Ⅱ Y 7954’ 的稔實率則顯著較 ‘TCS 17’ 高；整體來看，部分參試品種在台灣二期作環境下栽培的稔實率有偏低之問題，尤其在插植株數較少時。千粒重則均以 ‘TCS 17’ 顯著較其他參試品種來得高。

二期作農藝性狀之變方分析結果，株高、穗重及單株總穎花數的插植株數與品種間交感效應顯著或極顯著，顯示參試品種間在這些性狀會隨不同插植株數而異；但生育日數、穗長及穀粒容重之交感不顯著，其品種間差異不因不同插植株數而有明顯差別 (表 5)。由表 7 分析結果發現，二期作生育日數以對照品種 ‘TCS 17’ 的生育日數顯著較其他參試品種長，其中以 ‘ZLY 168’ 為最早熟品種；多本植與兩本植處理間的生育日數沒有顯著差異。不論多本植或兩本植之株高均以 ‘YLY 302’ 顯著較其他參試品種高；不同插植株數間以 ‘YLY 302’、‘WY 308’、‘ZLY 168’ 及 ‘TCS 17’ 在兩本植時顯著較高，其他參試品種則沒有顯著差異。穗長在多本植時以 ‘YLY 689’、‘YLY 302’ 及 ‘II Y 7954’ 顯著較對照品種 ‘TCS 17’ 長；在兩本植時除 ‘WD 153’ 外，其他參試品種的穗長均顯著較 ‘TCS 17’ 長。穗重除 ‘QY 0508’ 及 ‘WY 308’ 外，其他參試品種均顯著較 ‘TCS 17’ 高，顯示大部分參試雜交稻品種的穗明顯較 ‘TCS 17’ 大；穗重在兩本植有重於多本植之趨勢。單株總穎花數除 ‘II Y 7954’ 在兩本植時較少外，所有參試品種均顯著較 ‘TCS 17’ 來得多，其中又以 ‘WD 153’ 與 ‘WY 308’ 表現最佳。穀粒容重在多本植時以 ‘II Y 7954’ 較高，兩本植時則以 ‘ZLY 168’ 與 ‘II Y 7954’ 較高，且與對照品種 ‘TCS 17’ 沒有顯著差異，其他參試品種之容重則均顯著低於 ‘TCS 17’，其中以 ‘YLY 689’ 容重最低。

討論

本試驗發現，參試超級雜交稻品種原生育日數與本試驗一期作調查結果極為相近，但不同品種表現略有增減 (表 1、表 4)，推測可能與參試超級雜交稻品種大部分來自中國大陸南方，對於溫度、日長的反應與台灣環境接近有關；二期作則生育日數有明顯減少的情形 (表 1、表 7)。試驗中，原生育日數較長的品種在台灣環境的生育日數也較長，但二期作則比一期作短約 17 d，且普遍比 ‘TCS 17’ 早熟，顯示這些參試品種可能對於溫度或日長反應較為敏感，主要可能為積溫需求已足夠，因此二期作超級雜交稻皆比 ‘TCS 17’ 早熟。

參試超級雜交稻品種以往從未在台灣進行產量比較試驗，本試驗結果發現，單位面積產量一期作以 ‘Ⅱ Y 7954’ 品種的 10,524 kg ha^{-1} (多本植) 與 10,688 kg ha^{-1} (兩本植) 最高，顯著高於對照品種 ‘TCS 17’ 的 8,115 kg ha^{-1} (多本植) 與 8,703 kg ha^{-1} (兩本植)，增產幅度約達 2,000 kg ha^{-1}。在二期作則以 ‘WY 308’ 品種的 8,610 kg ha^{-1} (多本植) 與 8,767kg ha^{-1} (兩本植) 最高，顯著高於對照品種 ‘TCS 17’ 的 7,617 kg ha^{-1} (多本植) 與 7,329 kg ha^{-1} (兩本植)，增產幅度約 990–1,438 kg (表 3、表 6)。但與超級雜交稻在中國大陸雲南省大於 15 Mg ha^{-1} 的產量表現 (Katsura *et al*. 2008; Yuan 2017) 相比，仍有極大差距。惟中國大陸大部分的高產報導均為示範栽培的資料，所使用的栽培管理模式各地區亦不儘相同，需以一般栽培管理方式進行生產比較，才符合商業生產模式。

中國大陸水稻區域試驗技術規範與本試驗之產量比較試驗方法較為接近，為評估本試驗參試品種的產量差異，本研究綜整各品種的區域試驗資料，發現以 ‘YLY 302’ 產量最高 (9,064 kg ha^{-1})，‘WY 308’ 品種產量最低 (7,568 kg ha^{-1})，但都較 ‘TCS 17’ 高 (表 1)。經本研究在台灣栽種的試驗調查結果，發現一期作各參試品種的單位面積產量明顯比各該品種原

表 7. 2012 年二期作不同水稻品種在不同插植株數下農藝性狀之平均值比較。

Table 7. Mean comparison of agronomic traits for different rice varieties under different seedling numbers in the second cropping season of 2012.

Character / Seedling number / Variety	Growth duration (d)		Plant height (cm)		Panicle length (cm)		Panicle weight (g)		Spikelet numberper hill		Volume weight (g L^{-1})	
	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two	Multiple	Two
QY 0508	104.0 Ae[z]	104.0 Ae	88.4 Ad	87.7 Ad	21.7 Abc	21.5 Ac	3.0 Acd	3.0 Ad	1,993 Ab	1,793 Bb	513.7 Acd	511.8 Abcd
II Y 7954	107.0 Ad	106.3 Ad	97.7 Ab	96.2 Ab	23.1 Aab	22.5 Ab	3.6 Ba	4.5 Aa	1,622 Ac	1,564 Acd	545.0 Aab	543.4 Aa
YLY 689	113.7 Ab	113.0 Ab	96.3 Ab	94.6 Ab	24.3 Ba	25.5 Aa	3.5 Aa	3.7 Ab	1,834 Bbc	2,097 Aa	504.2 Ad	504.3 Ad
YLY 302	111.3 Ac	111.3 Ac	108.1 Ba	111.9 Aa	25.5 Aab	25.5 Aa	3.3 Bab	3.8 Ab	1,982 Bb	2,228 Aa	521.8 Ac	521.3 Abc
WD 153	106.0 Ad	106.7 Ad	92.2 Ac	90.4 Ac	19.7 Bc	20.4 Ad	3.1 Abc	3.4 Abc	2,359 Aa	2,206 Aa	513.3 Acd	509.5 Acd
WY 308	97.3 Af	97.7 Af	84.9 Be	89.1 Acd	20.9 Bbc	21.4 Ac	3.0 Acd	3.1 Acd	2,213 Aa	2,294 Aa	535.0 Ab	525.3 Ab
ZLY168	87.0 Ag	87.3 Ag	81.3 Bf	83.8 Ae	20.6 Abc	21.0 Acd	3.2 Bbc	3.5 Abc	1,786 Abc	1,783 Abc	539.6 Ab	543.7 Aa
TCS 17	120.0 Aa	118.7 Ba	85.6 Be	88.5 Acd	19.7 Bc	20.4 Ad	2.7 Ad	2.8 Ad	1,404 Ad	1,504 Ad	553.5 Aa	550.2 Aa

[z]Means within treatment of seedling number (in capital letter) and within variety (in small letter) for each character follow by the same letter(s) are not significantly different at 5% level by Fisher's protected LSD test.

本區域試驗的產量來得高；二期作則除 'WY 308' 與 'ZLY 168' 兩品種外，單位面積產量均明顯比原本區域試驗產量低 (表 1、表 3、表 6)。由於水稻的產量會因地區、環境氣候、栽培技術等因素而產生變化 (Katsura *et al*. 2007; Yang *et al*. 2008)，經本試驗所有參試品種在相同栽培環境下比較結果，參試超級雜交稻品種的產量普遍比對照品種 'TCS 17' 高產，一期作的增產效果比二期作明顯，二期作產量明顯比一期作低，顯示超級雜交稻在台灣生產上有產量優勢，二期作適應之品種則須評估。

Chen *et al*. (1984) 認為積儲容積為台灣一期作之產量限制因子，而積儲容積大小可以單位面積穎花數表示。本研究發現一期作單株產量愈高的品種，其單株總穎花數也愈高；兩本植單株產量都比多本植低，因為其單株總穎花數亦較低 (表 3、表 4)。許多學者研究指出一些高產粳稻品種具有較大的積儲容量 (Yoshida *et al*. 2006; Kamiji *et al*. 2011)，且水稻高產的主要因素是具有較大的積儲容量 (Yoshinaga *et al*. 2013)。Huang *et al*. (2011) 研究發現超級雜交稻高產是因為單位面積穎花數之提高，與本研究所獲結果一致。而單株總穎花數為每株穗數與一穗穎花數的乘積，可做為積儲容積大小代表，經本研究一期作試驗結果發現，參試超級雜交稻中除 'ZLY 168' 品種外，單株總穎花數都很多。分析多本植比兩本植高產的主要因素為每株穗數較多，因而增加了單株總穎花數，亦即增加了積儲容積；而兩本植時，參試品種雖透過補償機制提高了一穗穎花數，但最終仍因每株穗數表現較低，導致積儲容積的限制而使產量較低 (表 3、表 4)。

二期作 'QY 0508' 與 'WY 308' 兩品種之單株產量不論在多本植或兩本植均為產量較高之品種，但 'QY 0508' 的一穗穎花數顯著比 'WY 308' 少，且兩品種之每株穗數皆較高，此為導致兩品種單株高產之主因 (表 6)。二期作 'YLY 689'、'YLY 302' 及 'WY 308' 等三品種單位面積產量較高，主要是每株穗數及一穗穎花數兩項特性相對較高所致。而 'WD 153' 的單株穎花數雖與 'WY 308' 沒有顯著差異，但單株產量卻顯著較低，稔實率較低是主因 (表

6、表 7)。試驗中亦發現二期作多本植與兩本植間的單株產量差異不像一期作那麼明顯，參試 7 個超級雜交稻品種在多本植時有 3 個品種的單株產量顯著比 'TCS 17' 高，在兩本植時則僅有 2 個品種顯著比 'TCS 17' 高 (表 6)，但是所有參試品種的單株總穎花數均顯著比 'TCS 17' 多 (表 7)，此為造成參試雜交稻二期作皆比對照品種 'TCS 17' 產量高的主要原因。

全球氣候變遷對於水稻栽培產生重大的影響，生育期長所面對的不確定風險亦高，但過度縮短生育期對產量會有負面影響，Huang *et al*. (2013) 研究顯示最高溫度每升高 1℃也會使水稻生育期縮短 3.2 d，Yoshida (1981) 報告則指出約 100 d 是獲得合理高產水稻的最短持續時間，生育期再縮短將對產量產生負面影響。因此，如何穩定生長期與產量是未來克服全球暖化對水稻潛在負面影響的重要課題。日產量為計算穀物產量除以生長持續時間，適當縮短生育期且維持較高的日產量，對於環境因素變化的風險自然降低，是一個很好的品種生產力評估指標。分析參試雜交稻品種的日產量發現，不論一期作或二期作，參試品種的日產量均顯著高於對照品種 'TCS 17'，其中 'II Y 7954' 為日產量最高的品種，但在二期作則沒有相同的表現。本試驗參試品種一期作生育期範圍約在 103.7–130 d，二期作生育期範圍約在 87–113.7 d，'WY 308' 二期作僅需 98 d，而 'ZLY 168' 更短，僅需 87 d 即可收穫。'WY 308' 為二期作參試品種中最高產，分別為 8,610 kg ha^{-1} (多本植) 與 8,767 kg ha^{-1} (兩本植)，兩者因產量高，加上生育期短，因此日產量為參試品種中最高，甚至比一期作還高，是適合台灣二期作栽培的超級稻品種。Yuan (1998) 認為超級雜交稻的日產量至少應維持在 100 kg ha^{-1}，且過長的生育期會增加風險。本研究的參試超級雜交品種均尚未達到前述標準，仍有待進一步調整在台灣環境生產的栽培技術。

超級雜交稻品種的開發以理想株型為基礎，理想株型的特徵是大穗、少分蘗 (Peng *et al*. 2008)；一期作兩本植時 'II Y 7954'、'YLY 689'、'YLY 302'、'WD 153' 及 'WY 308' 等品種的每株穗數均較少，顯示這些品種的分蘗能力較弱，但這些品種的一穗穎花數明顯較多，均符合超級雜交稻的一般概念。而 'QY 0508' 品種則屬於每株穗數與一穗穎花數兼俱的高產雜交稻品種。'ZLY 168' 品種則以每株穗數表現最佳，但終因其一穗穎花數偏低而造成產量的限制，惟因生育日數較短的優勢，使其在日產量上有優異表現。由於高產的超級雜交稻特徵為少分蘗、大穗，必須保證其單位面積穎花數，才能獲致高產，試驗中兩本植試驗的單位面積穎花數顯然無法達到滿足，歸納其原因主要為每株穗數的不足；試驗中多本植似乎可滿足其需求，惟雜交稻種子價格普遍較高，提高每叢插植株數，成本相對亦較高。單位面積穗數可透過早期增施氮肥或改變插植密度來改善；高氮肥可在初始生長階段刺激作物生長速率增加分蘗 (Yoshinaga *et al*. 2013)；在抽穗期有足夠的氮肥才能滿足穗對氮和碳水化合物的需求 (Zhang *et al*. 2007)。

整體而言，本研究針對參試超級雜交稻之品種間、期作間、插植株數間的產量差異進行分析，在台灣慣行多本植栽培法下所有參試超級雜交稻品種的產量都比對照品種 'TCS 17' 來得高，且產量表現比在中國大陸環境下的區域試驗資料更高，顯示超級雜交稻在台灣生產上有產量優勢，但仍需面對二期作低產的挑戰。單株總穎花數較高是一期作高產的主要原因，而二期作則為單株總穎花數與稔實率；亦即積儲容積較高是一期作超級雜交稻高產的主要原因；而二期作供源活性與積儲容積兩者均為超級雜交稻產量的限制因子；在兩個期作中，每株穗數是積儲容積 (單株總穎花數) 的主要影響因子。未來在超級雜交稻的栽培上可能需注意早期適度增施氮肥或重新調整株距方式，以發揮其高產特性。

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Yield Potential Analysis of Super Hybrid Rice Grown in Taiwan

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Abstract

Jwo, W. S., C. L. Chen, C. P. Li, M. H. Lai, D. H. Wu, and H. M. Yen. 2017. Yield potential analysis of super hybrid rice grown in Taiwan. J. Taiwan Agric. Res. 66(4):343–355.

Rice is the most widely grown and consumed staple crop in Taiwan. Increasing rice yield potential is eventually a major target in rice breeding programs and the use of heterosis is also a feasible strategy. The objective of this study was to evaluate growth characters and yield potential of the recently developed hybrid rice in Mainland China. Field trials were conducted for two cropping seasons using seven Chinese varieties including 'Qianyou 0508' ('QY 0508'), 'II You 7954' ('II Y 7954'), 'Y Liangyou 689' ('YLY 689'), 'Y Liangyou 302' ('YLY 302'), 'Wandao 153' ('WD 153'), 'Wuyou 308' ('WY 308'), 'Zhuliangyou 168' ('ZLY 168') and check variety 'Taichung Sen 17' ('TCS 17'). The results of ANOVA revealed that rice agronomic characters and yield-related traits were significantly affected by different seedling number and plant varieties. Grain yield, yield components and agronomic traits of all imported rice were superior than indica check variety 'TCS 17'. In addition, grain yield in the first cropping season of all tested varieties was higher than that in the second cropping season. The high yield of super hybrid rice is due to the increase in the total number of spikelet per plant (sink size). In the first cropping season, average grain yield of super hybrid rice was 20% and 5% higher than check variety 'TCS 17' in multiple and two-seedling treatment, respectively. In the second crop, average grain yield of super hybrid rice was 7% and 10% higher than check variety 'TCS 17' in multiple and two-seedling treatment, respectively. Comparison between two treatments of seedling number showed that yield performance of two plants per hill was inferior than multiple seedling mainly attributed to the lack of panicle number. These results indicate that increased spikelet number per panicle and maintained enough panicle number per meter square are a foundation to obtain higher yield potential for supper hybrid rice cultivated in Taiwan.

Key words: Super hybrid rice, Yield components.

Received: April 5, 2017; Accepted: May 22, 2017.
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JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol. 66 No. 4 December 31, 2017

Published by Taiwan Agricultural Research Institute, Council of Agriculture

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Editor-in-Chief	Chwen-Ming Yang
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Website	http://www.tari.gov.tw; E-mail: jtar@tari.gov.tw; Tel: +886-4-2331-7236
Submission	http://aspers.airiti.com/JTAR
Press	Airiti Press Inc.
Address	18F., No.80, Sec.1, Chenggong Rd., Yonghe Dist., New Taipei City 23452, Taiwan, ROC
Tel	+886-2-2926-6006
E-mail	press@airiti.com
Price	NT$ 175 (per copy)
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