

Growth responses and changes of active components as influenced by elevations and orchid mycorrhizae on *Anoectochilus formosanus* Hayata

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ABSTRACT. Two micropropagated lines, B and R, of *Anoectochilus formosanus* Hayata, were separately inoculated with orchid mycorrhizal fungi (OMF), *Rhizoctonia* sp. R02 [a bi-nucleated isolate of *Rhizoctonia* sp. (*Ceratobasidium* sp. AG-A)] and R04 [a multi-nuclei isolate of *Rhizoctonia solani*, AG-6], and grown by plastic bag cultivation method (PBCM) at three elevations above sea level, including National Taiwan University (NTU, 10 m elevation), Xindian (500 m elevation) and Wufong (1,000 m elevation) for seven months. Results showed that the survival rates for *ex vitro* growth were more than 80%, and plant production was significantly increased and there was no need to apply any agrichemical. If this orchid was not grown by PBCM, then after 3-4 months of cultivation, all plants would die if pesticide or fungicide were not sprayed for every one or two weeks in greenhouse, and the cultivation period was shortened to 1-2 months compared with traditional cultured method. Plants grown in Wufong achieved the best growth performance among three elevations, the fresh weight of mycorrhizal *A. formosanus* was significantly higher than the non-mycorrhizal (NM) control. In Wufong, R04 showed better growth-enhanced effect on line B, while R02 stimulated growth enhancement for line R. For both lines of *A. formosanus* cultivated at National Taiwan University (NTU), R02 inoculated plants contained higher level of phenolic compounds and hepatoprotective agent AFEE (*A. formosanus* extraction with ethyl acetate) compared with the non-mycorrhizal (NM) control. Analyses and measurements of antioxidant capacity by Trolox Equivalent Antioxidant Capacity (TEAC) showed that most of the antioxidant index of mycorrhizal plants were significantly higher than the non-mycorrhizal control. PBCM was proven to be a very labour saving, i.e. this cultural method can save all the human cost of irrigation and fertilization during the rest of cultivation period, and this is an effective method for mass production of agrichemical free *A. formosanus* plants. Inoculation of orchid mycorrhizal fungi such as R02 or R04 can significantly increase the production of this orchid with higher antioxidant capacity and hepatoprotective agent content for medicinal use.

Keywords: *Anoectochilus formosanus* Hayata; Antioxidation capacity; Hepatoprotective agent; Orchid mycorrhizal fungi (OMF); Phenolic compounds; Plastic bag cultivation method (PBCM).

Abbreviations: ACP, acid phosphatase; AFEE, *A. formosanus* extraction with ethyl acetate; CCl₄, carbon tetrachloride; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, Ferric reducing antioxidant power; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; MDA, malondialdehyde; NM, non-mycorrhizal; NTU, National Taiwan University; OMF, orchid mycorrhizal fungi; PBCM, plastic bag cultivation method; PPF, photosynthetic photon flux; R02, *Rhizoctonia* sp. (*Ceratobasidium* sp. AG-A); R04, *Rhizoctonia solani* AG-6; AG-6, Anastomosis group 6.; SOD, superoxide dismutase; TEAC, Trolox Equivalent Antioxidant Capacity.

INTRODUCTION

Anoectochilus formosanus Hayata is a terrestrial orchid species from wild forests in Taiwan. It is an ornamental plant with medicinal value, and is widely used for treating high blood pressure and hyperliposis, lowering blood

sugar level, and improving control of diabetes, liver diseases, heart diseases and lung diseases (Yen et al., 1996). It also displays anticancer and antiviral effects (Lin, 2007; Wu, 2007). Due to its multitudinous medicinal effects, the number of *A. formosanus* in the wild has been greatly reduced by intentional picking and uprooting. In recent years, with the advance of tissue culture techniques, large-scale propagation can be achieved by using seedlings and micropropagated plantlets. Future prospects for the

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development and application of these techniques to *A. formosanus* are therefore very bright. However, traditional intensive culture has several drawbacks. Survival rates of transplanted tissue-cultured plantlets are low, and plants tend to grow slowly and cultivation period is long. Additionally, the occurrence of diseases such as root and stem rots caused by *Fusarium oxysporum* and *Pythium ultimum*, bacterial soft rot disease, and pest infections by red spider mites and scale insects often result in the continuous usage of pesticides and fungicides during cultivation (Yen et al., 1996; Chang, 1999). How to raise the survival rate of the seedlings or plantlets for *ex vitro* growth and control cultivating environment for shortening growth period as well as reducing the chances of harmful infection are the major concern for the cultivation of *A. formosanus*.

In this study, we applied the plastic bag cultivation method (PBCM), which was developed in our laboratory (Chang et al., 2007), and combined with the inoculation of *Rhizoctonia* spp. of orchid mycorrhizal fungi (OMF) (R02 and R04) for the cultivation of *A. formosanus*. The PBCM offered several advantages such as timesaving, labour saving, and pesticide or fungicide free. Appropriate ecological conditions are important determinants for the successful artificial culture of *A. formosanus*. Low temperature has been known to favor the growth of *A. formosanus* and lower pathogen occurrence (Lee, 2001; Chang and Chou, 2007). However, many people tried to grow this orchid in low elevations for practical reasons. Therefore, we decided to conduct the experiments in three locations with different elevations and growth temperature including NTU campus (10 m elevation above sea level), Shihzhihtou Mountain in Xindian (500 m elevation) and Wufong (1,000 m elevation) to determine the most suitable site for the cultivation of *A. formosanus*.

The tissue cultured *A. formosanus* plants are considered to be less medicinally effective than the wild plants, which generally have 3-5 fold higher prices on the market. Previous studies demonstrated that *A. formosanus* inoculated with OMF exhibits higher level of superoxide dismutase (SOD), acid phosphatase (ACP) and alkaline phosphatase (AKP) activities, and higher ascorbic acid, phenolic compounds, flavonoids, polysaccharides and phosphoric acid contents, and as a result they are more effective as medicinal source (Chou, 2004; Chang and Chou, 2007). In this study, we would like to achieve three goals. (1) To evaluate the possibility for using the PBCM for the mass production of *A. formosanus*. (2) To understand the interactions between the cultivating lines and the environments such as elevation and temperature. (3) To analyze and determine the antioxidant capacity and hepatoprotective activity among the mycorrhizal and non-mycorrhizal *A. formosanus*.

MATERIALS AND METHODS

Mycorrhizal inocula

In this experiment, two strains of *Rhizoctonia* spp.

including R02 (*Rhizoctonia* sp.; *Ceratobasidium* sp.; AG-A) and R04 (*Rhizoctonia solani*; AG-6) were isolated and obtained stable research results in our laboratory for more than ten years. Their pathogenicity was tested in our laboratory for many times and was indicated by Chang (2008), and all proved to be nonpathogenic. They were cultured on the medium which is prepared by mixing peat with 20% V8 juice to reach field capacity (Chang and Chou, 2007). All *A. formosanus* lines in this experiment were inoculated with single fungal strain. Each isolate is evenly inoculated under the roots of per plant in an amount of 0.1-0.2 g of inoculum. R02 was identified as a bi-nucleated isolate of *Rhizoctonia* sp. (*Ceratobasidium* sp.; AG-A, accession NO. DQ102413, 99% match, unpublished data), while R04 was a multi-nuclei isolate of *Rhizoctonia solani*; AG-6 (Lee, 2001).

Plant materials

The micropropagated plantlets of *A. formosanus* were purchased from a tissue culture company Puli, Taiwan. Two lines of *A. formosanus*, line B (wild type) and line R (hybrid of the wild type and red stem type), were used in the experiment. The parental plants for both lines were collected from the wild-grown *A. formosanus*. The cutting segments of *A. formosanus* were cultivated on the agar medium for eight to ten months until root growth and bud burst, and then the plantlets over 6 cm height were selected. The tissue culture vessels were placed in the greenhouse for a week before transplanting for acclimation.

Culture medium and method for the cultivation

The culture medium for *A. formosanus* used is a compost of peat and coconut fiber blended in a ratio of 7:4 (v:v). The plants for each line including non-mycorrhizal (NM) control and mycorrhizal treatment and the *ex vitro* plants were cultivated by PBCM (Chang et al., 2007), to culture 10 plants of *A. formosanus* in 5 inch plastic pot, and every two of the 5 in. pots were covered with an OPP transparency plastic bags (35.5×55 cm). In preliminary experiments, all cultivars would die if pesticide or fungicide were not sprayed every one or two weeks after three or four months of cultivation, thus PBCM was applied in this experiment. One gram Hyponex No. 5 re-suspended in one liter water was added into the growth medium and field capacity of water was attained. Totally three experimental sites were included: 1) Greenhouse in the campus of National Taiwan University [10 m elevation above sea level, an average photosynthetic photon flux (PPF) at $58.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (maximum $63.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at noon)], 2) Shihzhihtou Mountain in Xindian [500 m elevation, an average PPF at $53.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (maximum $57.7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at noon)], and 3) Wufong [(1,000 m elevation, an average PPF at $55.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (maximum $60.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at noon)]. Each treatment contained 400 plants for evaluating the practical use of mass production, and treatments were arranged by a completely randomized design (CRD). The plants cultured by PBCM were

transported to the Xindian and Wufong on April 14-15, 2005 respectively. All plants were shipped back to NTU, than random sampling (5 plants per pot, total of 40 pots, i.e. 200 plants per treatment) for statistical analyses on Nov. 14-16, 2005.

Analysis of the antioxidant capacity

Two methods were used to examine the antioxidant capacity. The Folin-Ciocalteu method was used to examine the total phenolic contents (Kujala et al., 2000). And Trolox Equivalent Antioxidant Capacity (TEAC) was measured according to the method reported by Arnao et al. (1996). Spectrophotometer was used to determine the absorbance at 734 nm. Regression equation of standard curve was obtained based on the relation of absorbance and concentration of trolox. The absorbance was substituted into the equation to attain the TEAC value of the sample.

Analysis of the hepatoprotective agent

Analysis of the concentration of hepatoprotective agent was performed according to the method reported by Wu et al. (Wu et al., 2007). The weight of a dry *A. formosanus* plant was precisely measured and suitable quantity of water was added. The sample was extracted for three times using ultrasonic treatment and then processed by gravity filtration. The filtrate was diluted, the target ingredient AFEE was determined quantitatively by high-performance liquid chromatography (HPLC). Test samples and reference standard were processed following the procedures described above and then examined by HPLC. After obtaining the standard calibration curve, the values of test samples were substituted into the regression equation of the standard reference to calculate their concentrations. The analyses were performed by Shih Hwa Biotech Co.

RESULTS AND DISCUSSION

Plastic bag cultivation method (PBCM)

PBCM has been applied for the cultivation of *A. formosanus* (Figure 1). This method offers several advantages. No more watering or fertilization is needed during the whole cultivation period, and the potential threat of pest attack and labor need for the in cultivation management are greatly reduced (Chang and Chou, 2007). In this study, PBCM was applied to large-scale cultivation of *A. formosanus* in three sites with different elevations, and more than 80% survival rate was reached. Cultivation of *A. formosanus* in greenhouse, chemical spray program is intensively needed and regularly performed. Most of the plants would be attacked, if pesticide, fungicide and mites-killing chemical are not regularly sprayed. By PBCM, survival rate of the *ex vitro* growth of plantlets and thus plant production are greatly increased without applying any pesticide, fungicide or any other agrichemicals and the growth period is also shortened. As a result of our

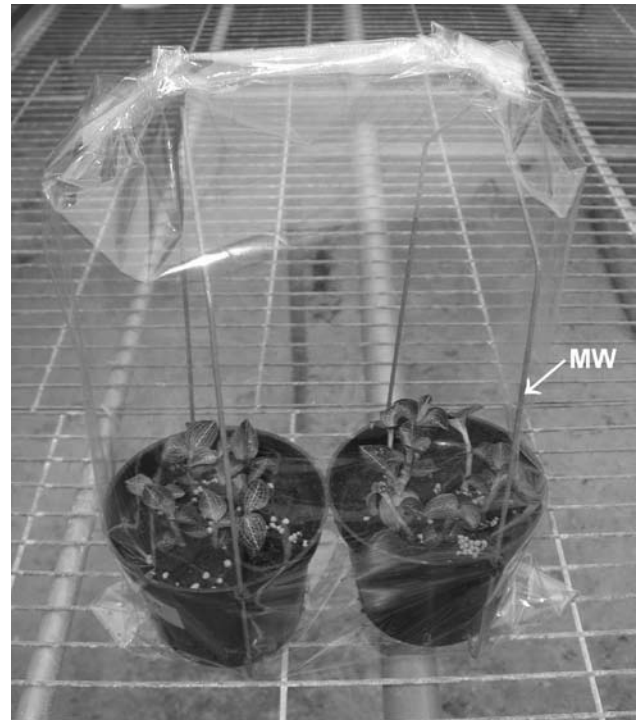


Figure 1. Plastic bag cultivation method (PBCM) for *Anoectochilus formosanus*. The metal wire (MW) was to prevent the collapse of plastic bag in the wildness.

studies, PBCM has now been promoted in the Wufong area and applied to large-scale and year-round commercial cultivation for more than 100,000 plants, and the survival rates were more than 90%.

Inoculation of OMF for the cultivation of *A. formosanus*

PBCM was adopted for the vessel cultivation of line B and line R of *A. formosanus* for both mycorrhizal and non-mycorrhizal control plants. After seven months of growth in three locations, the best plant growth was achieved in Wufong (1,000 m elevation). The number of leaves, fresh weight and chlorophyll meter reading of the plants in this location were all higher than those placed in other sites (Figures 2, 3 and 4). In addition, among the plants in Wufong, those inoculated with OMF (either R02 or R04) attained better growth than the non-mycorrhizal control. Based on the fresh weight, R04 showed better growth-promoting effect on line B, while R02 stimulated better growth for line R (Figures 2 and 3). Therefore, different lines of *A. formosanus* plants appeared to react differently to different OMF, and OMF inoculation does influence the growth of plants. Compared to the non-mycorrhizal control, OMF inoculation obviously promoted the plant growth and development. The fresh weight of mycorrhizal plants was consistently higher in all three elevations (Figures 2 and 3). We also found that fresh weight of plants in Wufong was the heaviest in all three treatments even though taller plants were observed in two lower elevations such as NTU and Xindian (Figures 2 and 3). Further exam-

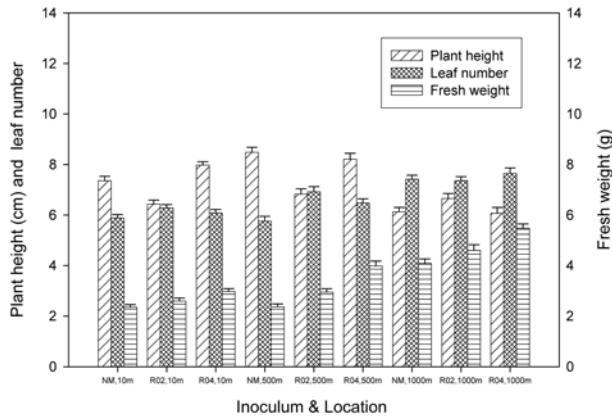


Figure 2. Growth of orchid mycorrhizal fungi (*Rhizoctonia* sp. R02 and R04) inoculated and non-mycorrhizal (NM) plants of *Anoectochilus formosanus* (line B) with plastic bag cultivation method (PBCM) at National Taiwan University (NTU, 10 m of elevation), and in Xindian (500 m) and Wufong (1,000 m) for seven months.

ination found that the plants in Wufong displayed shorter internodes but thicker stems, while the plants at NTU generally showed the opposite features with longer internodes and thinner stems. We speculate that temperature probably was the cause of heavier fresh weight for the Wufong's plants. The average temperatures of the NTU and Xindian were about 30-23°C and 27-18°C respectively, while the Wufong site was around 25-15°C. When comparing *A. formosanus* plants cultivated in phytotron, those cultivated plants under controlled temperature at 20/15°C (day/night) had shorter internodes and thicker stem; samples cultivated in phytotron at 30/25°C (day/night) grew taller and had thinner stem (unpublished data). Based on our studies, we conclude that temperature plays a critical role on the growth of *A. formosanus*, and is the main reason for the higher fresh weight of the plants grown in Wufong. Therefore, the ideal growing location for *A. formosanus* is at high elevation which has lower temperature and greater day/night temperature difference. Previous studies reported that when line C and line T of *A. formosanus* was inoculated either with *Rhizoctonia* sp R02 or R04, growth-promoting effects are obviously observed (Chang et al., 2007). In this experiment, two other different lines, line B and line R, were chosen and also inoculated with R02 or R04, and similar growth-enhancing effects were also observed. The results indicated that OMF inoculation can promote the growth of *A. formosanus* both in the cultivation vessel and after transplantation. The absorbing areas of roots are increased as a result of OMF inoculation and therefore the plantlets can efficiently absorb nutrients and water. It was also reported that the quantity of chlorophyll as well as the efficiency of photosynthesis are increased due to OMF inoculation (Chu, 2000). In our experiments, the chlorophyll meter readings among mycorrhizal plants were generally higher than the controls (Figure 4). However, when fresh weight and chlorophyll meter readings were cross-referenced, positive correlation was not found.

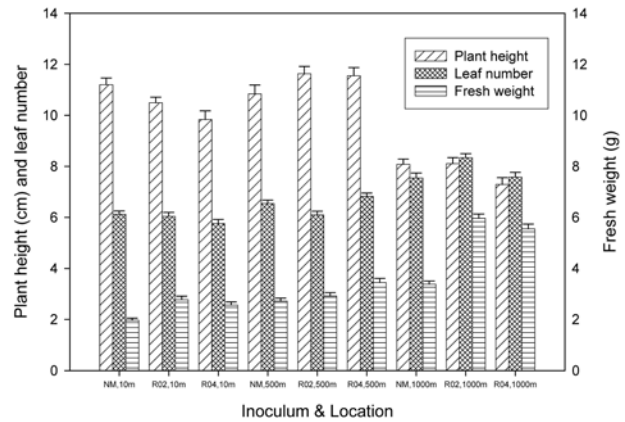


Figure 3. Growth of orchid mycorrhizal fungi (*Rhizoctonia* sp. R02 and R04) inoculated and non-mycorrhizal (NM) plants of *Anoectochilus formosanus* (line R) with plastic bag cultivation method (PBCM) at National Taiwan University (NTU, 10 m of elevation), and in Xindian (500 m) and Wufong (1,000 m) for seven months.

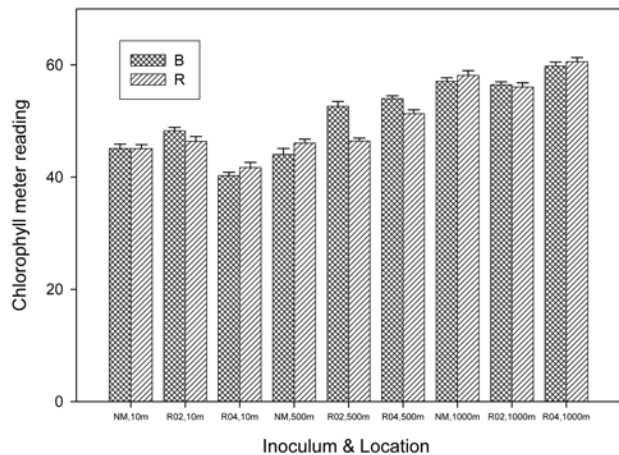


Figure 4. Chlorophyll meter reading value of orchid mycorrhizal fungi (*Rhizoctonia* sp. R02 and R04) inoculated and non-mycorrhizal (NM) plants of *Anoectochilus formosanus* (line B and line R) with plastic bag cultivation method (PBCM) at National Taiwan University (10 m of elevation), and in Xindian (500 m) and Wufong (1,000 m) for seven months.

This may be due to the fact that plant growth is indeed influenced by various factors.

Analysis of the antioxidant capacity

1. Comparison and analysis of the phenolic compounds. Phenolic compounds are widely distributed in the plant kingdom. They are plant secondary metabolites which are often used as an index for antioxidant capacity (Castelluccio et al., 1995). Our analyses revealed that *A. formosanus* line B grown in Wufong contained higher level of phenolic compounds; however the levels of phenolic compounds of the NM control and mycorrhizal plants in this location were no significant difference (Figure 5A). The levels of phenolic compounds of mycorrhizal plants at

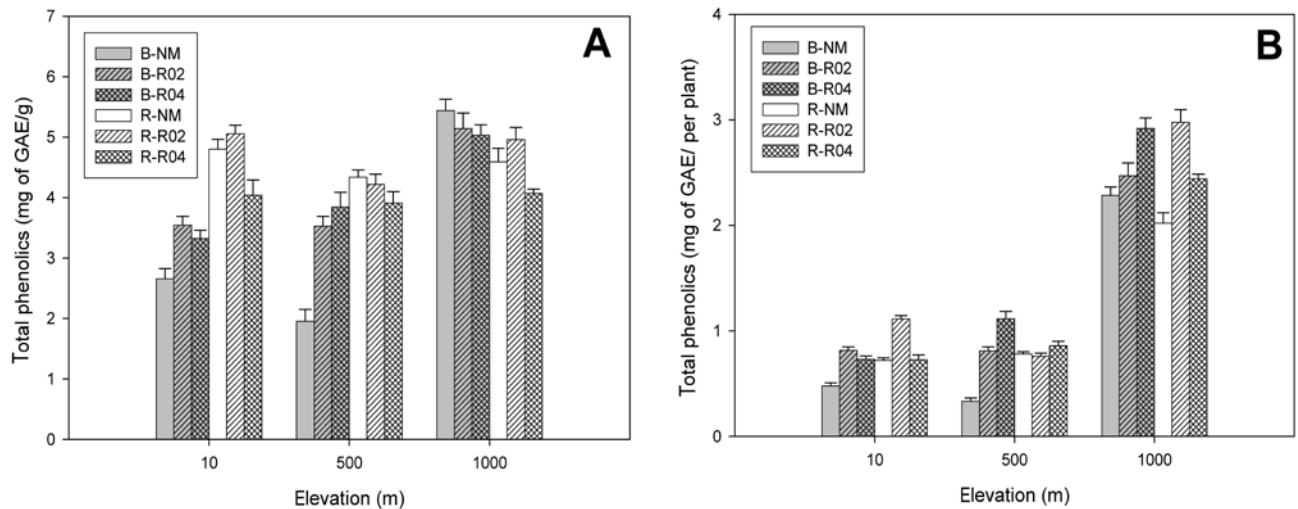


Figure 5. Changes of phenolic compounds level of two lines of *Anoectochilus formosanus* inoculated with *Rhizoctonia* sp. R02 and R04 in the bag culture at National Taiwan University (10 m of elevation), and in Xindian (500 m) and Wufong (1,000 m) for seven months. A, level of phenolic compounds per gram; B, level of phenolic compounds per plant.

NTU and in Xindian were higher than the non mycorrhizal control (Figure 5A). The levels of phenolic compounds among line R plants inoculated with R04 in three locations were similar (Figure 5A). For the NM and R02-inoculated, the levels of phenolic compounds were higher at NTU and in Wufong; the R02-inoculated group had higher phenolic compounds level, with 5.06 mg/g in the NTU plants and 4.96 mg/g in the Wufong ones. Considering the dilution effect of fast-growing plants, we converted the level of phenolic compounds into the whole plant basis as shown in Figure 5B. Results showed that the level of phenolic compounds was the highest among the plants grown in Wufong both for line B and line R, and the phenolic compound levels found in the OMF inoculated plants were higher than the NM plants (Figure 5B). Previous studies indicated that the cultivation environment affects the types and quantity of phenolic compounds in a plant (Dumas et al., 2003; Roussous et al., 2007). Our results demonstrate that cooler area such as Wufong was more suitable for the growth of *A. formosanus* and the level of phenolic compounds found obtained from these plants was higher.

2. Test of Trolox Equivalent Antioxidant Capacity (TEAC). In previous experiments, antioxidant capacity of *A. formosanus* was tested by TEAC, FRAP (Ferric reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazyl) method. In this study, TEAC was chosen to determinate of the antioxidant capacity by its comparative stability and significant differences in data reading between the mycorrhizal and non-mycorrhizal plants.

Our results showed that the TEAC readings of the mycorrhizal line B at NTU and in Xindian were higher than the NM plants. In line R plants, the R02-inoculated ones at NTU and in Wufong had higher TEAC readings (Figure 6).

Previous studies showed the *A. formosanus* extract displays free radical scavenging activity (Wang et al., 2002).

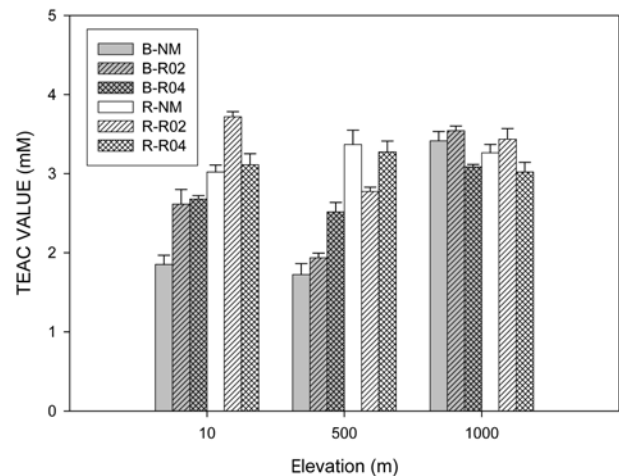


Figure 6. The analyses of trolox equivalent antioxidant capacity (TEAC) values for two lines of *Anoectochilus formosanus* inoculated with *Rhizoctonia* sp. R02 and R04 and cultivated at NTU (10 m of elevation), and in Xindian (500 m) and Wufong (1,000 m), for seven months.

Under the treatment of *A. formosanus*, HepG22 cell demonstrated stronger solvency over free radicals and hydrogen peroxide and maintain effective antioxidant capacity (Hsieh, 2001). *Anoectochilus formosanus* also contains various enzymes including superoxide dismutase (SOD), catalase, peroxidase and ascorbate peroxidase (Wang, 1999). In 2004, analyses of the enzyme activities and the plant components demonstrated that stronger antioxidant capacity was found in the mycorrhizal *A. formosanus* plants. Our results showed that mycorrhizal line B plants at NTU and in Xindian exhibited higher level of TEAC than the NM control plants. R02 inoculated line R plants cultivated at NTU and in Wufong had higher TEAC reading as well. The results demonstrated that the mycorrhizal

A. formosanus plants showed different antioxidant capacity under different environments.

Analysis of the hepatoprotective components

Previous studies revealed that *A. formosanus* contains hepatoprotective components and thus displays hepatoprotective effect (Huang, 2000). Ethyl estate (EtOAc) extract from *A. formosanus* can lower the GOT and GPT concentrations of liver cells, increase the quantity of glutathione, and decrease the quantity of malondialdehyde (MDA), a product of lipid peroxidation, and thus the hepatoprotective effect is achieved (Lin et al., 1991). Our studies demonstrated that OMF inoculation can increase the quantity of hepatoprotective agent AFEE, an ethyl acetate-philic fraction of the extracts of *A. formosanus*. The levels of AFEE were higher in either R02 or R04 inoculated line B and line R plants than those of the NM control plants at NTU. The level of AFEE found in the mycorrhizal line B plants was more than two folds higher than that of the NM control plants (Table 1). Therefore, our results showed that OMF inoculation can effectively promote the growth of *A. formosanus*, and also increase the levels of their medicinal components, their antioxidant capacity and the quantity of AFEE (Table 1).

Our studies demonstrated that PBCM is an effective mass production method for producing agrichemical free *A. formosanus* plants. Both NM and mycorrhizal plants grew well in Wufong, due to the higher elevation, lower temperature and greater temperature difference for day and night. Inoculation of OMF (R02 or R04) significantly promoted the growth of *A. formosanus*, but the effects might vary dependent upon the combination of OMF and orchid line and as well as the growth environment. Generally our results showed that the mycorrhizal plants contained higher amount of phenolic compounds, stronger antioxidant capacity, and more abundance of the hepatoprotective agent AFEE in comparing to NM plants of all locations, but varied with the different environments. We presume

Table 1. The analysis of hepatoprotective activity of kinsenoside (AEEE) derived from mycorrhizal and non-mycorrhizal *Anoectochilus formosanus* plants cultivated at NTU campus for seven months.

Cultivar	<i>Rhizoctonia</i> inoculum	AFEE (g/g)
B	NM	0.0174±0.0003 b
	R02	0.0417±0.0011 a
	R04	0.0400±0.0019 a
R	NM	0.0126±0.0004 c
	R02	0.0242±0.0029 a
	R04	0.0191±0.0025 b

NM: non-mycorrhizal control group.

Three replicates were conducted for each treatment.

Means in each column followed by different letters were significantly different (P=0.05) as determined by LSD test.

that the wild plants of *A. formosanus* may be all infected with some kind of OMF fungi and thus result in better medicinal effects than the tissue-cultured NM plants in greenhouse. Based on the results, we highly recommend PBCM for mass production of agrichemical free *A. formosanus* plants. One thousand meter of elevation is an ideal place for cultivating *A. formosanus* plants in Taiwan. The inoculation of *Rhizoctonia* sp, either R02 or R04, can significantly increase the production of *A. formosanus* containing higher antioxidant capacity and hepatoprotective agent for medicinal use.

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台灣金線連於不同海拔地區接種蘭菌後其生長發育及有效成分之變化

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於台大 (海拔 10 公尺)、新店 (海拔 500 公尺)、五峰 (海拔 1000 公尺) 等三個不同海拔地區以塑膠袋栽培法 (PBCM)，種植接種絲核菌屬蘭菌 R02 及 R04 之 B 及 R 等兩種不同品系的台灣金線連，栽培七個月，結果顯示以塑膠袋套袋栽培法栽培之所有臺灣金線連組培苗，於出瓶後其成活率均在 80% 以上，且可顯著提高植株產量，及不需施用任何化學農藥，假使台灣金線連不是利用塑膠袋培法 (PBCM) 栽種，則在溫室中栽培 3-4 個月後，每 1-2 個星期若不進行殺蟲劑與殺菌劑的噴施，則所有植株將會死亡，和傳統栽培方法相比較，則可縮短栽培期 1-2 個月。其中種植於五峰，且接種蘭菌之植株生長最佳，鮮重亦較對照組高，B 品種則以接種蘭菌 R04，R 品種以接種蘭菌 R02 可顯著促進生長。種植於台大之台灣金線連 B 及 R 兩品系均以接種 R02 之菌根植株有較高的總酚類及保肝活性成分 AFEE 之含量。以 TEAC 的方法分析測試其抗氧化能力，結果顯示大部分菌根植株較非菌根植株為高。本研究結果證實塑膠袋栽培法為一非常省工 (可省下栽培期間之所有灌溉與施肥之人力)，且不需噴施任何殺蟲劑及殺菌劑等化學藥劑即可大量生產臺灣金線連之有效方法，接種蘭菌 R02 及 R04 可顯著增加台灣金線連的產量，並提高抗氧化能力及保肝成分之含量。

關鍵詞：台灣金線連；抗氧化能力；保肝成分；蘭菌；總酚類含量；塑膠袋栽培法。