科技部補助專題研究計畫成果報告

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銅在葡萄生態系統之時空變異模擬

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- 計畫主持人: 陳柏青
- 共同主持人: 莊愷瑋
- 計畫參與人員: 碩士班研究生-兼任助理:羅郁淨 碩士班研究生-兼任助理:李盈融 講師級-兼任助理:陳姿璇 講師級-兼任助理:廖珮彤 講師級-兼任助理:高瑩芬 助教-兼任助理:蔡佳靜
- 報告 附件: 出席國際學術會議心得報告

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中文摘要: 本研究建立水耕實驗以探討葡萄組培苗暴露於15 µM銅處理濃度之下 持續1, 2及3天之銅累積以及氧化緊迫現象。研究結果顯示,暴露於 銅將抑制根部生長;而添加鎂則會降低葡萄根部對銅之累積,進而 降低其毒性。當暴露於銅狀況下,葡萄植株中銅濃度會增加,且主 要累積於根部,而鈣、鎂濃度則降低。鉀濃度則不會受銅及鎂之添 加而影響。經過3天的銅處理後,鎂的添加顯著降低根部的SOD及 CAT活性,而增加葉部之SOD活性。在活性氧族群部分,銅暴露會增 加根部之MDA及H2O2。銅暴露處理下,SOD、CAT及APX在葉部及根部 之比例隨鎂濃度增加而降低。此外,淨光合作用速率及氣孔導度會 因暴露於銅而降低。由本研究結果可知,過量銅將造成葡萄組培苗 根部生長速度降低,並對其葉部與根部造成氧化緊迫現象。

中文關鍵詞:銅;葡萄;微量營養元素;氧化緊迫;毒性

- 英文摘要: In the present study, a hydroponic experiment was conducted to investigate the oxidative stress and the Cu accumulation in grapevines exposed to level of 15 µM for one, two, and three days. The results showed that the root elongation was inhibited under Cu exposure. The addition of Mg alleviated Cu toxicity through the decrease of the amount of Cu entering grapevine roots. The Cu accumulation in the grapevines increased under Cu treatments; however, micronutrient elements (Ca, Mg, K) accumulation in grapevine was not affected by Cu addition. Most of the Cu taken up by the grapevines was accumulated in the roots. After three days of treatment, the Mg-addition significantly decreased the SOD and CAT activity in the roots, yet increased the SOD activity in the leaves. For the reactive oxygen species, the MDA and H2O2 increased in the roots under Cu exposure. Under Cu exposure, the ratio of SOD, CAT, and APX in the leave to the root was decreased with increasing Mg levels. Additionally, the photosynthesis parameters, net photosynthesis rate (A) and stomatal conductance (gsw), were decreased significantly under stress condition. In conclusion, the present results indicated that excess Cu results in a reduction of the root growth and leads to oxidative stress for the grapevine leaves and roots.
- 英文關鍵詞: copper; grapevine; micronutrients; oxidative stress; toxicity

本研究建立水耕實驗以探討葡萄組培苗暴露於 15 µM 銅處理濃度之下持續 1, 2 及 3 天之銅累積以及氧化緊迫現象。研究結果顯示,暴露於銅將抑制根部生長; 而添加鎂則會降低葡萄根部對銅之累積,進而降低其毒性。當暴露於銅狀況下, 葡萄植株中銅濃度會增加,且主要累積於根部,而鈣、鎂濃度則降低。鉀濃度則 不會受銅及鎂之添加而影響。經過 3 天的銅處理後,鎂的添加顯著降低根部的 SOD 及 CAT 活性,而增加葉部之 SOD 活性。在活性氧族群部分,銅暴露會增 m 根部之 MDA 及 H₂O₂。銅暴露處理下, SOD、CAT 及 APX 在葉部及根部之比 例隨鎂濃度增加而降低。此外,淨光合作用速率及氣孔導度會因暴露於銅而降低。 由本研究結果可知,過量銅將造成葡萄組培苗根部生長速度降低,並對其葉部與 根部造成氧化緊迫現象。

關鍵詞:銅;葡萄;微量營養元素;氧化緊迫;毒性

Abstract

In the present study, a hydroponic experiment was conducted to investigate the oxidative stress and the Cu accumulation in grapevines exposed to level of 15 µM for one, two, and three days. The results showed that the root elongation was inhibited under Cu exposure. The addition of Mg alleviated Cu toxicity through the decrease of the amount of Cu entering grapevine roots. The Cu accumulation in the grapevines increased under Cu treatments; however, micronutrient elements (Ca, Mg, K) accumulation in grapevine was not affected by Cu addition. Most of the Cu taken up by the grapevines was accumulated in the roots. After three days of treatment, the Mg-addition significantly decreased the SOD and CAT activity in the roots, yet increased the SOD activity in the leaves. For the reactive oxygen species, the MDA and H_2O_2 increased in the roots under Cu exposure. Under Cu exposure, the ratio of SOD, CAT, and APX in the leave to the root was decreased with increasing Mg levels. Additionally, the photosynthesis parameters, net photosynthesis rate (A) and stomatal conductance (g_{sw}) , were decreased significantly under stress condition. In conclusion, the present results indicated that excess Cu results in a reduction of the root growth and leads to oxidative stress for the grapevine leaves and roots.

Keywords: copper; grapevine; micronutrients; oxidative stress; toxicity

(二) 前言、研究目的及文獻探討

Copper (Cu) is a micronutrient to plants; however, excess accumulation of this metal may cause plant toxicity by affecting negatively biochemical and physiological processes (Tiecher et al., 2016; Ambrosini et al., 2018; Ferreira et al., 2018). The background Cu level in natural topsoil is reported to as about 5.0 mg kg^{-1} . In vine-growing areas of the world, however, Cu-based fungicides (e.g., Bordeaux mixture) have been frequently applied for preventing foliar diseases, thus leads to increased Cu level in vineyard soils (Tiecher et al., 2017; Ferreira et al., 2018). For example, it has been reported that the vineyard soils can contain as high as 3200 mg kg-1 of Cu in southern Brazil, which surpasses the background level by 640 times (Mirlean et al., 2007). At the individual level, high Cu concentration in grapevine may result in growth inhibition, reduction of root elongation, increased root diameter, darkening and thickening of the roots, root-tip swelling and abnormal branching, and leaf chlorosis (Juang et al., 2012; Tiecher et al., 2016, 2018; Ambrosini et al., 2018). At the cellular level, the excess of Cu may cause damage to the cell membranes, nutritional imbalance, and oxidative stress of grapevines (Miotto et al., 2014; Tiecher et al., 2016, 2018). The bioavailability of metals in soils may be influenced by several geochemistry parameters including soil pH, texture, water content, dissolved organic matter, and cations and anions concentrations in soils. In our published studies, Cu accumulation and translocation in grapevines was significantly affected by the coexisting cations such as magnesium (Mg^{2+}) (Juang et al., 2014) and calcium (Ca^{2+}) (Chen et al., 2013). The competition of these cations with Cu on biotic ligands in roots was reported to alleviate the phytotoxicity of Cu to grapevines. For a better understanding of ecotoxicological effects of Cu, therefore, it is necessary to investigate not only the fate and transport of Cu, but also the geochemistry parameters in soil-grapevine ecosystems.

Owing to direct contact with soils, plant roots have generally been recognized as the bioindicator of metal phytotoxicity. Several previous studies thus employed root elongation as endpoints while constructing the dose-response relationship of grapevines exposed to Cu (Juang et al., 2011, 2012; Cambrolle et al., 2015). In practice, however, a direct observation of root elongation is quite difficult. In recent years, some studies tried to relate the excess formation of reactive oxygen species (ROS) in leaves, such as superoxide (O_2) and hydrogen peroxide (H_2O_2) , to the oxidative stress of Cu in grapevines (Tiecher et al., 2017, 2018; Ferreira et al., 2018). An increase of ROS in plants may result in the activation of the antioxidant enzyme system; therefore, the production of protective enzymes, such as superoxide dismutase (SOD) and catalase, was also utilized as biomarkers for the Cu toxicity of grapevines.

From the microscopic point of view, the cytotoxic effects of Cu on plant roots

have also been researched in several previous studies. Observed previously for grass plants exposed to excessive Cu, the main histological changes in root tissues include strong cell vacuolization, damage in epidermal cells, plasmolysis (a phenomenon whereby the cells among the cortex were obviously enlarged), and cell rupturing of the rhizodermis and outer cortex (Liu and Kottke, 2004; Kopittke et al., 2008; Rossini Oliva et al., 2010). In our published work (Juang et al., 2012), histological changes in the grapevine rhizodermal cells were also found when exposed to excessive Cu. For a better understanding of intracellular fate and the transport of Cu in grapevine cells, however, it is necessary to add ultrastructural study and micro-morphometric analysis in the evaluation of the phytotoxic effects of Cu, especially in regions of the grapevine not showing any visible symptoms.

Considering these factors, the aim of this study was to conduct a hydroponic experiment to investigate the root growth, copper accumulation, and oxidative stress in tissue-cultured grapevine seedlings exposed to Cu level of 15 µM for one, two, and three days. The photosynthesis parameters, net photosynthesis rate (A) and stomatal conductance (g_{sw}) , were also examined under stress condition.

(三) 研究方法

The annual shoots of Kyoho grapevines (Vitis vinifera L.) were collected from vine-growing areas in central Taiwan and transferred to the laboratory. Each shoot was divided into several cuttings so that each cutting contained three nodes and spurs. One end of each grapevine cutting was placed in distilled water for 30 days until the spurs had enough leaves, and the axillary buds were utilized as tissue cultures. When the axillary bud explants are rooting and shooting in vivo, they can be transplanted into potting mixes and acclimated in vitro for 30 days.

The seedlings with three new leaves were used for the experiment. Grapevine roots were pruned to 5 centimeters and transplanted into a 0.7-L plastic cup filled with 10% modified Hoagland solution for two days. The seedlings were exposed to three Cu levels $(0, 5, \text{ and } 15 \mu M)$ for one, two, and three days. The treatment solution was renewed every day. One seedling was placed in a cup containing the treatment solution. Each experimental unit consisted of six plants, totaling two replicates per treatment. The experiment was conducted in growth chambers with fixed temperatures and relative humidity. The light cycle was 16:8 light:dark. The test media were aerated throughout the experiment.

The roots of seedlings from each test-solution-set were photographed after the experiment and transferred into electronic files. The roots, stems, and leaves of the grapevine seedlings were separately harvested and thoroughly washed with distilled water. The tissue samples were oven-dried at 75ºC for 72 hours, and the dry-weights

of the plant tissues were recorded between 0.01 g and 0.2 g. Plants were ground and digested with $HNO₃/HClO₄$ (4:1 v/v), and the Ca, Mg, K, and Cu concentrations both in the plants and in the hydroponic medium were then determined with a flame atomic absorption spectrophotometer (Thermo scientific iCE 3000). All chemical analyses were performed in duplicates.

For the extraction of antioxidant enzymes (i.e. SOD, CAT, APX, and H_2O_2), 0.05 g of grapevine root and leaf samples from the hydroponic experiment were homogenized with 3 mL of a 50 mM sodium phosphate (Na₂-PO₄) buffer (pH 7.0) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 rpm for 30 minutes at 4° C to collect supernatant for the estimation of SOD, CAT, and H_2O_2 .

 The SOD (EC. 1.15.1.1) activity was assayed according to Beauchamp et al. (1971). The assay mixture consisted of a total volume of 0.75 mL, containing 0.1 M potassium phosphate, a 7.8 pH, 1 mM Na2-EDTA, 130 mM methionine, 0.63 mM nitroblue tetrazolium (NBT), 7.5μM riboflavin, and a sample. The illumination of the reaction mixtures caused the formation of the blue formazan which increased absorbance at 560 nm. One unit of SOD is defined as the amount of enzyme that inhibited the reduction of NBT by 50% at 560 nm.

 Total CAT (EC 1.11.1.6) activity was determined spectrophotometrically by following the decline in A_{240} as H_2O_2 (extinction coefficient 40 mM⁻¹ cm⁻¹) catabolized as described by Beers and Sizer (1952). The 2-mL reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM DTT, and 5 mM H_2O_2 . The activities of all the antioxidant enzymes were expressed as units per minute per gram from weight.

Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min in 2 mL of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA-Na₂, 0.5 mM ascorbic acid, 0.1 mM H_2O_2 and 50 mL of crude enzyme extract (Nakano and Asada, 1981). The activity was calculated using the extinction coefficient $(2.8 \text{ mM}^{-1} \text{ cm}^{-1})$ for the ascorbate.

The H_2O_2 content of the grapevine seedlings was determined according to Jana and Choudhuri (1981). The reaction mixture contained 0.1 % (v/v) titanium chloride dissolving 20% (v/v) H_2SO_4 and the supernatant at a proportion of 1:2 at 1000 rpm for 15 minutes to mix homogeneity. The content was evaluated by comparing its absorbance at 410 nm with a standard calibration curve.

 The level of lipid peroxidation products was estimated following the method of Heath and Packer (1968) by measuring the concentration of malondialdehyde (MDA) as an end-product of lipid peroxidation through reaction with thiobarbituric acid (TBA). Root and leaf samples were homogenized with 2 mL of 5% (w/v) trichloroacetic acid (TCA) in a prechilled mortar and pestle and centrifuged at 10,000

rpm for 5 minutes at 4ºC. One mL of the supernatant and 2 mL of 20% TCA containing 0.5% TBA were added. The mixture was heated at 95ºC for 30 minutes and quickly cooled in an ice bath for 15 minutes. After centrifugation at 3000 rpm for 10 minutes, the absorbance of the supernatant was recorded at 532 nm and corrected by measurement at 410 nm and 600 nm.

 The leaf discs were randomly mixed and rinsed with distilled water and then wiped dry. In situ localization of O_2^- and H_2O_2 was performed as described by Liu et al. (2014). Compared to the data for three days after treatment, the leaf discs were infiltrated using 0.5 mg/mL nitroblue tetrazolium (NBT) with 10 mM NaN₃ in a 50 mM HEPES-NaOH buffer (pH 7.6) for O_2 ⁻ histochemical localization or in a 0.5 mg/mL diaminobenzidine (DAB)-phosphate buffer (50 mM, pH 5.8) for the histochemical detection of H_2O_2 . Infiltration was conducted under vacuum for 30 minutes and centrifuged at 3000 rpm until the discs were below the solution. The leaf discs were then held at room temperature until a blue or brown color became visible. Chlorophylls in leaves stained with a blue $(O_2^-$ -NBT formazan precipitates) or deep-brown $(H_2O_2-DAB$ polymerization) color were removed using a 95% ethanol solution 2-3 times. We used stereo-zoom microscopes (Motic, SMZ-171 series) to observe the stained leaf discs and took images with a moticam connected to a computer.

(四) 結果與討論

 The results showed that the root elongation was inhibited under Cu exposure. The addition of Mg alleviated Cu toxicity through the decrease of the amount of Cu entering grapevine roots (Fig. 1, 2).

 The phytotoxic effects of excess Cu on grapevines have been widely studied in recent years. Briefly, high Cu level in the root growth environment may cause auxin homeostasis disorder, thus result in the reduction of lateral root-hair numbers and root elongation (Tiecher et al., 2017, 2018; Ambrosini et al., 2018). These morphological alterations may further affect water and nutrient uptake and eventually stunt the growth of grapevines. The symptoms observed in the present study are similar to previously published works. On the other hand, the threshold of Cu level that causes toxic effects may be varied under different situations. Kopittke et al. (2010) indicated that the median toxic concentration of Cu to plants mainly ranged from 0.9 to 20 μM. Based on our results, the concentration that inhibited the root growth of the grapevines (15 μM) fell well within the range proposed by Kopittke et al. (2010). However, Cambrolle et al. (2015) studied the toxic effect of Cu on wild grapevine and reported that the median toxic concentration was higher than 23mM. These variations may mainly be attributed to the difference of plant species and geochemical

properties.

 In the current findings, increases in Cu-levels and the duration of treatment led to the increase of the Cu-concentration in grapevine seedlings. As shown in Fig. 2, the mean Cu-concentrations were much higher in roots than those in leaves and stems, revealing that the Cu absorbed by grapevines remain mainly in the roots. The highest Cu-concentration was $1,807\pm131$ mg kg⁻¹ in the roots at the Mg exposure level of 2 μM after one-days' exposure (Table 1); however, the Cu-concentration was only 78±4 and 13 ± 0.7 mg kg⁻¹, respectively, for stems and leaves under the same exposure concentration and duration (Table 2, 3). On the contrary, the addition of Cu did not affect cations (K, Mg and Ca) accumulation compared to the control (Fig. 3-5). As shown in Fig. 3, increases in Mg levels resulted in the reduction of Ca accumulation in roots. On the other hand, the Ca and Mg accumulation in the plant parts were decreased in this order: leaf>stem≈root (Fig. 3, 4); still, the K-level remained relatively constant in different grapevine parts (Fig. 5). Generally, the translocation of Ca, Mg, and K from the roots to above-ground parts was far higher than that of Cu.

Results of the present study showed that Cu accumulation in grapevines occurred mainly in the roots, with low translocation to the above-ground parts. This result aligned with many previous studies regarding Cu distribution in grapevines (Juang et al., 2012; Cambrolle et al., 2015; Tiecher et al., 2017). Recently, Ambrosini et al., (2018) studied Cu translocation in 'Red Niagara' (Vitis labrusca), an important grape varieties in Brazil, and indicated that grapevines cope with Cu-stress by accumulation of Cu in apoplast and reducing its translocation to the shoots. However, leaf Cu level may increase when grapevine was exposed to extremely high Cu content (Cambrolle et al., 2015). In addition, it generally has been recognized that the Cu levels of between 20 to 100 mg kg^{-1} in leaves may cause toxic effects to plants (Cambrolle et al., 2015). In this study, mean Cu concentration in grapevine leaf was 43.46 mg kg⁻¹ after Cu treatment of 15 μM for 3 day, thus leading to toxic effects that resulted in growth inhibition. For Mg, Ca, and K, the present result aligned with some previous findings which indicated that the maintenance of adequate micronutrients levels in leaves is critical for grapevines (Perez-de-los-Reyes et al., 2013; Cambrolle et al., 2015; Ambrosini et al., 2018). Furthermore, these micronutrients concentrations in grapevine seedlings were reduced at the exposure Cu level of 15 μM. This may be due to the impairment of nutrient uptake, or the alteration of membrane permeability when exposing grapevines to higher Cu level (Ambrosini et al., 2018).

The antioxidant enzymes are important components in preventing oxidative stress in plants (Thounaojam et al., 2012). In this study, however, the increase in the SOD activity within the leaves was only significant at Cu exposure concentration of 50 μM for one day (Fig. 6). On the contrary, CAT activity within the roots and leaves

was stimulated when exposed to Cu (Fig. 7). No obvious dose-effect relationship was found between the APX activity in the grapevine organs and the Cu exposure concentration for all other treatments (Fig. 8). After three days of treatment, the Mg-addition significantly decreased the SOD and CAT activity in the roots, yet increased the SOD activity in the leaves. Under Cu exposure, the ratio of SOD, CAT, and APX in the leave to the root was decreased with increasing Mg levels (Fig. 11, 12).

 It was generally recognized that SOD constitutes the first line-of-defense against ROS. Induction of SOD was associated with a strategy to overcome Cu-induced stress. In the present study, a Cu-induced increase in SOD activities was observed for grapevine leaves when exposed to 15 μM Cu after three days. This result aligned with several previous studies (Thounaojam et al., 2012; 2014). Under severe oxidative stress, however, a decline of SOD activity may happen in preventing cellular damage (Mostsofa et al., 2014). The decline of Cu-induced SOD activity after long-term exposure to higher levels of Cu was also observed for rice seedlings (Thounaojam et al., 2014).

In the case of heavy metal stress, the production of H_2O_2 and MDA generally increases in plants (Gill and Tuteja, 2010). In the present results, the addition of Cu significantly increased H_2O_2 content in roots after a three-day treatment; however, compared with the control, no obvious increase was observed for H_2O_2 content in the leaves. In addition, the Mg addition had no significant effect on the production of $H₂O₂$ content (Fig. 9). On the other hand, both in the grapevine leaves and roots, the MDA content treated with Cu was not significantly difference compared with the control after one-day and three-day treatment (Fig. 10).

In this study, Cu-stress increased the production of H_2O_2 in grapevine roots after a three-day treatment with 15 μM of Cu. The increase of ROS production such as $H₂O₂$ can disturb metabolic pathways through oxidative damage to the cells. MDA is a product of lipid peroxidation and generally increases with the increase of ROS contents (Mostofa et al., 2014; Thounaojam et al., 2014). A higher MDA level in plants indicates severe cell-membrane damage. The present results show that Cu-exposure causes oxidative stress to grapevine roots. However, the reason for the decline of H_2O_2 content in leaves after long-term exposure needs further investigation.

Plant roots are generally recognized as the most sensitive target for toxic effect. Thus, many previous studies have recommended that root elongation can be used as an ideal indicator for the metal toxicity of plants. In field application, however, it seems difficult to directly measure the total root length of plants. In the present study, the photosynthesis parameters, net photosynthesis rate (A) and stomatal conductance (g_{sw}) , were decreased significantly under Cu stress. In practice, therefore, it is more

appropriate to employ the photosynthesis parameters as indicative of the early phytotoxicity of grapevine exposed to excessive Cu. Furthermore, an early warning of the phytotoxicity of grapevines due to Cu is also important when it comes preventing potential human health risks through the consumption of grapes and/or grape productions. Based on the present results, therefore, it is possible to employ the photosynthesis changes in leaves as a biomarker for monitoring the phytotoxicity of Cu in grapevines.

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表 1、銅暴露處理下之 10% Hoagland 背景溶液中,添加不同鎂處理濃度 0、2、4和 8 mM Mg 一天及三天,葡萄組培苗根部銅、鈣、 鎂和鉀濃度之變化

*在相同銅暴露濃度及天數下,不同鎂處理濃度中相同英文字母表示兩者間沒有顯著差異(LSD test, p<0.05)。 *表中 Mg 0、Mg 2、Mg 4 及 Mg 8 分別代表鎂處理濃度 0 mM Mg、2 mM Mg、4 mM Mg 及 8 mM Mg。

表 2、銅暴露處理下之10% Hoagland 背景溶液中,添加不同鎂處理濃度 0、2、4和 8 mM Mg 一天及三天,葡萄組培苗莖部銅、鈣、 鎂和鉀濃度之變化

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表 3、銅暴露處理下之10% Hoagland 背景溶液中,添加不同鎂處理濃度 0、2、4和 8 mM Mg 一天及三天,葡萄組培苗葉部銅、鈣、 鎂和鉀濃度之變化

*在相同銅暴露濃度及天數下,不同鎂處理濃度中相同英文字母表示兩者間沒有顯著差異(LSD test, p<0.05)。 *表中 Mg 0、Mg 2、Mg 4 及 Mg 8 分別代表鎂處理濃度 0 mM Mg、2 mM Mg、4 mM Mg 及 8 mM Mg。

圖1、無銅(a)和銅暴露處理15 μM Cu (b)之10% Hoagland背景溶液中,添加不同鎂處理 濃度連續三天,計算每日根的總伸長量(LSD test,p<0.05)。

圖2、無銅(a)、(c)、(e)和銅暴露處理15 μM Cu (b)、(d)、(f)之10% Hoagland背景溶液中,添加 不同鎂處理濃度一天及三天,分別比較葡萄組 培苗根部(a)、(b)和莖部(c)、(d)以及葉部(e)、(f) 中銅濃度之差異(LSD test,p<0.05)。

圖3、無銅(a)、(c)、(e)和銅暴露處理15 μM Cu (b)、(d)、(f)之10% Hoagland背景溶液中,添加 不同鎂處理濃度一天及三天,分別比較葡萄組 培苗根部(a)、(b)和莖部(c)、(d)以及葉部(e)、(f) 中鈣濃度之差異(LSD test,p<0.05)。

圖4、無銅(a)、(c)、(e)和銅暴露處理15 μM Cu (b)、(d)、(f)之10% Hoagland背景溶液中,添加 **不同鎂處理濃度一天及三天,分別比較葡萄組** 培苗根部(a)、(b)和
**蓝根部(a)、(b)和
草线濃度之差異(LSD test,p<0.05)。**

圖5、無銅(a)、(c)、(e)和銅暴露處理15 μM Cu (b)、(d)、(f)之10% Hoagland背景溶液中,添加 不同鎂處理濃度一天及三天,分別比較葡萄組 培苗根部(a)、(b)和堇部(c)、(d)以及葉部(e)、(f) 中鉀濃度之差異(LSD test,p<0.05)。

圖6、無銅(a)、(c)和銅暴露處理15 μM Cu (b)、(d)之10% Hoagland背景溶液中,添加不同鎂處 理濃度一天及三天,分別比較葡萄組培苗根部(a)、(b)和葉部(c)、(d)中SOD活性之差異(LSD test, $p<0.05$) \circ

圖7、無銅(a)、(c)和銅暴露處理15 μM Cu (b)、(d)之10% Hoagland背景溶液中,添加不同鎂處 理濃度一天及三天,分別比較葡萄組培苗根部(a)、(b)和葉部(c)、(d)中CAT活性之差異(LSD test, $p<0.05$) \circ

*圖中未顯示部分表示偵測不到。

圖8、無銅(a)、(c)和銅暴露處理15 μM Cu (b)、(d)之10% Hoagland背景溶液中,添加不同鎂處 理濃度一天及三天,分別比較葡萄組培苗根部(a)、(b)和葉部(c)、(d)中APX活性之差異(LSD test, $p<0.05$) \circ

圖9、無銅(a)、(c)和銅暴露處理15 μM Cu (b)、(d)之10% Hoagland背景溶液中,添加不同鎂處 理濃度一天及三天,分別比較葡萄組培苗根部(a)、(b)和葉部(c)、(d)中H2O2含量之差異(LSD test, $p<0.05$) ·

圖10、無銅(a)、(c)和銅暴露處理15 μM Cu (b)、(d)之10% Hoagland背景溶液中,添加不同鎂 處理濃度一天及三天,分別比較葡萄組培苗根部(a)、(b)和葉部(c)、(d)中MDA含量之差異 (LSD test, p<0.05) .

圖11、無銅和銅暴露處理15 µM Cu 之10% Hoagland背景 溶液中,添加不同鎂處理濃 度一天(a)、(c)、(e)及三天(b)、 (d)、(f)後,比較葡萄組培苗 葉部和根部SOD、CAT和APX 活性比值之差異(LSD test, $p<0.05$) \circ

圖12、無銅和銅暴露處理15 µM Cu 之10% Hoagland背景 溶液中,添加不同鎂處理濃 度一天(a)、(c)及三天(b)、(d) 後,比較葡萄組培苗葉部和 根部H2O2及MDA含量比值之 差異(LSD test,p<0.05)。

圖13、無銅(a)、(c)和銅暴露處理15 μM Cu(b)、(d)之10% Hoagland背景溶液中,添加不同鎂處理濃 度連續三天,分別比較葡萄組培苗上位葉中光合速率(A)及氣孔導度(gsw)之差異。

科技部補助專題研究計畫出席國際學術會議心得報告

日期:107 年 5 月 30 日

一、 參加會議經過

本人此次獲科技部補助參加於日本大阪市舉辦之第四屆國際公共衛生、流行 病學及營養研討會(4 th World Congress on Public Health, Epidemiology & Nutrition),會議自民國107年5月24日至5月25日,為期兩天。

國際公共衛生、流行病學及營養研討會為Conference Series LLC Ltd所舉辦的 研討會之一,此研討會每年在世界不同地區舉辦之形式,為常態性會議。此次為 第四屆會議,於大阪市君悅酒店舉辦,下屆會議則將於明年五月在泰國普吉島舉 行。筆者於5月23日下午,由桃園機場搭機於5月23日傍晚抵達日本關西機場,於 5月24日上午參加於大阪君悅酒店Hamony Hall由主辦單位所舉行之開幕式,並報 到註冊,隨後聆聽Peton博士之主題演講: Understanding primary care healthcare disparities at the community, regional and state level through visulization。5月24-25

日為正式會議議程,共有來自歐、美及亞洲地區包括我國、中國大陸、東南亞及 澳紐各國30餘位學者齊聚一堂,共襄盛舉。本次會議論文發表分為論文宣讀及論 文海報展示兩種方式,共有近50篇,大會主要議題為Promoting Solutions to Global Health,所涵蓋主題包括Epidemiology & Diseases control、Global Environmental Health、Healthcare & Hospital Management、Public Health Nursing、Public Health Nutrition、General Practice & Primary Healthcare、Nutritional Epidemiology、Obesity & Public Health、Community Health、Clinical Epidemiology、Occupational Health & Safety、Patient Safety & Quality Healthcare、Biomedical & Health Informatics、 Personalized Medicine、Healthcare & Technologies、Healthcare Associated Diseases 等。

筆者論文發表方式為海報展示,被分配於Community Health主題下,論文題 目為Risk Assessment and Management of Taiwan Residents Exposed to Arsenic Associated with Rice Consumption。本人於24日上午至會場設置海報,展示時間至 25日下午結束。於會議舉辦期間,筆者除於發表當日進行海報展示與解說外,並 於會場聆聽有興趣之主題演說及解說與觀摩海報。本次會議期間出席學者均熱烈 發言討論,收穫不少。由於本人於5月26日後另有私人行程,於是在會議結束後 搭火車至京都市,並於5月28日中午搭機返抵桃園國際機場,結束此次研討會行 程。

二、 與會心得

1. 筆者原擬參加於義大利羅馬舉辦之 SETAC 年會,然因校內校務評鑑之故,後 改參加本項會議。會議規模雖不大,但大家研究領域相近,在彼此討論下,常可 激發出更多火花。而此會議包含筆者的稿件,皆被收錄在國際期刊 Journal of Community Medicine and Health Education 中,則為意外的驚喜。

2. 由於食品安全問題日益受到重視,因此重金屬在環境的流佈及對於人體之健康 風險評估,也成為此次大會重點之一。除了筆者外,亦有多位學者所發表的內容 在於探討重金屬對生態及對人體健康之風險。本次筆者發表論文之內容,為研提 107 年度科技部計畫之前期研究成果,在此也希望計畫能順利通過,讓筆者可持 續進行後續研究。

3. 本次研討會中增加許多有關 nanoparticle 毒性及風險評估之文章,可見目前此 議題也受到重視,或可作為筆者日後研究方向的參考。

2

Title: Risk Assessment and Management of Taiwan Residents Exposed to Arsenic Associated with Rice Consumption

Abstract: Rice and rice products are staple foods in Asia. Rice grains may accumulate excess arsenic (As) when exposed to As-contaminated soil. Therefore, it is importance to assess potential human health risks through daily rice consumption. This study aims to perform dietary As risk assessment to estimate the probability of As from contaminated soils entering local residents. Field investigations were conducted in paddy rice fields in central Taiwan to determine the correlation between As levels in soil and in brown rice. The ingestion rate of rice of local residents was also investigated. A probabilistic risk assessment was then employed to estimate carcinogenic and non-carcinogenic risks of Taiwan residents via rice consumption. The result showed that the mean total As concentration in soil was 44.96 mg kg⁻¹, which was a little lower than the local risk-based limit of As for soil used for food crop production (60 mg kg^{-1}) . The total daily intakes of inorganic As from rice consumption were 0.0002 and 0.0011 mg kg^{-1} day⁻¹ for the 50th and 95th percentiles, respectively. The assessment results show that the predicted 50th and 95th percentile for target cancer risks (TRs) were respectively 0.0003 and 0.0016, both markedly higher than the acceptable target cancer risk of 10^{-4} - 10^{-6} . To manage the health risk of local residents due to the ingestion of inorganic As from rice, our results suggested that the regulation standard of As in farmland soil should be set below 15 mg kg^{-1} .

四、 建議

1.近年蒙科技部補助參加各項國際會議,發現對岸中國近年來積極參加各項國際 研討會項目,每年參與人數(包含教師及博士生)愈來愈多,且取得多項會議之主 導權,在國際學術地位日益提升,值得我國注意。

2. 此次研討會所有形式之論文,皆被收錄在國際期刊中,這對於吸引各國學者投 稿為一大誘因。未來國內辦理相關學術研討會宜思考結合研討會及國際期刊,以 吸引更多參與人員。

3. 此次與會承蒙科技部計畫補助提供相關經費,謹致謝忱。

五、攜回資料名稱及內容

此次攜回大會議程手冊及論文集一本。

六、其他

106年度專題研究計畫成果彙整表

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價 值(簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性)、是否適 合在學術期刊發表或申請專利、主要發現(簡要敘述成果是否具有政策應用參考 價值及具影響公共利益之重大發現)或其他有關價值等,作一綜合評估。

