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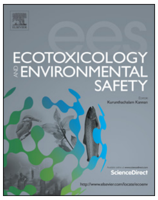

Influence of magnesium on copper phytotoxicity to and accumulation and translocation in grapevines

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ABSTRACT

The phytotoxic effects of excess copper (Cu) on grapevines (*Vitis vinifera* L. var. Kyoho) were examined, both from macroscopic and microscopic perspectives, by using a fifteen-day hydroponic experiments. The influence of magnesium (Mg) on Cu phytotoxicity to, and accumulation and translocation in grapevines was also observed. For phytotoxicity effect, results showed that a relative low median growth inhibition level of Cu was found for grapevine roots (0.809–3.671 μM). Moreover, Cu toxicity was significantly alleviated by Mg treatment at Mg^{2+} activity between 0.15 and 2.01 mM. For accumulation and translocation effects, results indicated that competition for binding sites between Cu and Mg occurred for roots; however, Mg and Cu levels in stems and leaves were not affected by solution metals concentration. At Cu concentration less than 1 μM, the translocation of Cu was decreased significantly for the highest Mg treatment; at Cu concentrations greater than 5 μM, no obvious change was observed in leaf *TF* value between Mg treatments, while an increasing trend of stem *TF* value was observed with increasing Mg. These results suggest that the toxic effect resulted from metals depend not only on the competition of coexistent cations for plasma membrane surface, but also on the transport and distribution of toxic metals in physiological active sites in plants.

1. Introduction

Copper (Cu) is widespread in the environment as a consequence of various anthropogenic (e.g., agricultural, mining and industrial activities) and natural (e.g., geochemical change) processes (Zheng et al., 2007). This transition metal is essential to plants, animals, and humans as a constituent of several enzymes and as a redox catalyst in a variety of metabolic pathways (Devez et al., 2005). It is well recognized, however, that elevated concentrations of Cu are toxic to organisms. The toxic effects of excessive Cu are primarily exerted through the disturbance of the biochemical and physiological processes such as photosynthesis, enzyme activity, pigment and protein synthesis, and cell division (Khellaf and Zerdaoui, 2010). The main phytotoxic symptoms of an excess of Cu in plants include stunted growth, delay in flowering and fruiting, chlorosis and senescence of leaves, and cracking of the root cell membranes (Kopittke and Menzies, 2006; Rusjan et al., 2007; Michaud et al., 2008; Wei et al., 2008; Mourato et al., 2009).

In agro-ecosystems, intensive and long-term application of cupric fungicides in the field has led to the accumulation of Cu on the surface of some agricultural soils around the world. In viniculture, a mixture of Cu sulfate and lime (i.e., Bordeaux mixture) has been extensively applied to control vine downy mildew for more than a century (Rusjan et al., 2007; Mackie et al., 2012). Therefore, relatively higher Cu levels have been found in vineyard soils than its background value. For example, previous studies indicated that vineyard soils can contain as much as 100–1500 mg kg^{-1} of Cu, which surpasses the background value (5–30 mg kg^{-1}) by up to 300 times (Brun et al. 1998, 2001; Chaignon et al., 2003). In our recent work, the maximum total Cu concentration in topsoils was found to be 100 mg kg^{-1} in vine-growing areas in central Taiwan (Lai et al., 2010). Furthermore, Mirlean et al. (2007) conducted a field investigation in southern Brazil and reported that the maximum Cu concentration in vineyard soils was as much as 3200 mg kg^{-1}. Consequently, Cu contamination in vineyard soils and its toxicity has become a growing public concern worldwide.

In terrestrial ecosystems, plant root is the first target of rhizosphere metals owing to direct contact with the growth medium. Root growth is generally recognized as a sensitive endpoint while assessing phytotoxicity of metals to plants (Wei et al., 2008;

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Mourato et al., 2009; Le et al., 2012). Therefore, several studies dealing with the toxic effects of plants exposed to heavy metals have been performed on the inhibition of root elongation (e.g., Wang et al., 2009; Li et al., 2009; Wu and Hendershot, 2010; Juang et al., 2011). On the other hand, root anatomy has also been adopted as an alternative endpoint of rhizotoxicity by some recent researches because of the difficulty of direct observation of root growth (Kopittke and Menzies, 2006; Madejon et al., 2009; Juang et al., 2012).

It has been generally recognized that the level of metal accumulated in the root increases with increasing metal concentration in the rhizosphere. However, some physicochemical parameters such as pH, dissolved organic matter, and cation exchange capacity may also influence the phytoavailability of metals (Antunes et al., 2006; Luo et al., 2008; Wang et al., 2011a). For characterizing plant bioaccumulation of metals, the free metal ion activity is regarded as a better predictor than total metal concentration (Lock et al., 2007). Based on this concept, free ion activity model (FIAM) and biotic ligand model (BLM) have been successfully applied by a number of studies to account for the metal phytoavailability and phytotoxicity both in aquatic and terrestrial environment.

Some cations such as magnesium (Mg^{2+}), calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}), and hydrogen (H^{+}) may alleviative the rhizotoxic effects of heavy metals on plants. Lock et al. (2007) employed the BLM to demonstrate the interaction between Cu and competing cations at binding sites on barley roots. Kinraide (1998, 1999), Kinraide et al. (2004), Kinraide and Hagermann (2010) conducted a series of researches to quantify the multiple toxic and alleviative effects of cations on plants' root based on the principle of the electrical potential of cations at the cell membrane surface. Furthermore, Wang et al. (2010, 2011b, 2012) developed an electrostatic toxicity model to evaluate alleviative effects of cations on rhizotoxicity caused by some heavy metals. Although the above-mentioned methodologies have been successfully applied in assessing the alleviative effects of cations on metals toxicity to lettuce, wheat, pea, and other grass species, to the best of the authors' knowledge, no literature regarding the feasibility of applying these models to woody plants is available.

The purpose of this study is to conduct a hydroponic experiment to investigate the influence of Mg on Cu phytotoxicity to grapevines. Magnesium is selected in the present study as a competing cation because it is a macronutrient for grapevine. A deficiency of Mg will result in interveinal chlorosis and marginal necrosis of grapevine leaves, thus reduce the photosynthetic rate and glucose production (Hermans and Verbruggen, 2005). Application of Mg fertilizers is therefore a habitually practice for optimum grapevine growth and yield. In this study, Cu toxicity to grapevine was modeled by FIAM. Root elongation as well as the microscopic changes in root tissue at the cell level was adopted as endpoints to assess the phytotoxic effects caused by Cu. The bioaccumulation and translocation of Cu and Mg were also examined to better understand the interaction and competition effects of these two metals within grapevines.

2. Materials and methods

2.1. Plant materials

The annual shoots of Kyoho grapevine (*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 two nodes and three spurs. One end of each of the grapevine cuttings was placed in deionized water until the cuttings were rooting. The rooting cuttings were then transplanted into 1.5-L polypropylene pots filled with 1.2 L 10 percent modified Hoagland solution (0.5 mM KCl, 0.5 mM $CaCl_2$, 0.1 mM KH_2PO_4, 0.2 mM $MgSO_4$, 0.01 mM Fe-EDTA, and 1.5 mM NH_4NO_3).

2.2. Experimental conditions

After 30 days of acclimation, three healthy and uniform cuttings were selected and transplanted to a 1.5-L polypropylene pot filled with 1 L 10 percent modified Hoagland solution. The experiment was performed for 15 days with seven levels of Cu treatments: 0 (control), 1, 5, 10, 15, 25, and 50 μM. Solution Cu^{2+} was supplemented as $CuSO_4 \cdot 5H_2O$. At each Cu exposure level, Mg^{2+} was supplied at three concentrations (0.2, 2, and 4 mM). For each solution, pH was maintained at 5.6–5.9 with 0.75 mg L^{-1} 2-(N-morpholino) ethane sulfonic acid (MES) buffers. The pot experiment was conducted in growth chambers with a mean temperature and relative humidity of 25 °C and 75 percent, respectively. The light cycle was 16:8 light:dark. Test media were aerated throughout the experiment. The solutions were renewed completely both with Hoagland solution and with fresh concentrations of Cu and Mg every five days. The pot experiment was carried out in triplicate.

2.3. Relative root elongation

Grapevine roots of each exposed group were photographed before and after exposure to waterborne Cu and transferred into draft files. The total root length was then calculated by DIGIROOT V. 2.5 software. The relative root elongation (*RRE*, percent) with respect to control was calculated by

$$RRE = \frac{RE_t}{RE_c} \times 100, \quad (1)$$

where RE_t is the root elongation in the test medium and RE_c is the root elongation in the control.

2.4. Histological analyses

For histological analyses, grapevine root samples were fixed with two percent paraformaldehyde and 25 percent glutaraldehyde in 0.1 M sodium phosphate buffer, pH 6.8, at 4 °C overnight. After three 15 min buffer rinses, the material was post-fixed with 1 percent OsO_4 in the same buffer for 4 h at room temperature and then rinsed in three 15 min changes of buffer.

Following fixation, the material was dehydrated in a graded EtOH series, and embedded in LR White resin (LR White, London Resin Company, Basingstoke, UK). Semi-thin sections 1 μm thick were cut using a glass knife on an ultramicrotome (Richert-Jung Ultracut S, Vienna, Austria). These sections were stained with toluidine blue O for general histological examination. The sections were examined under an optical microscope (Axioskop 2, Carl Zeiss).

2.5. Chemical measurements

The harvested grapevine roots, stems, and leaves were thoroughly washed with deionized water. The samples were oven dried at 65 °C for 72 h, and the dry weight of the sample of each exposed group was recorded. The plants' Cu and Mg concentrations following a HNO3/HClO4 (v:v=87:13) digestion procedure as well as Cu concentration in the hydroponic medium were determined with a flame atomic absorption spectrophotometer (FAAS) (PerkinElmer AAnalyst 200). All chemical analyses were performed in duplicate. Analytical quality control was achieved by digesting and analyzing identical amounts of Certificate Reference Material (CRM). The quality of the analyses in our laboratory was controlled, and the deviations from the standard value were less than 10 percent.

2.6. Modeling the effects of $\{Mg^{2+}\}$ on $\{Cu^{2+}\}$ toxicity

In the present study, the dose–response relationship between Cu treatments and root elongation of grapevine is expressed by the exponential decay equation, a nonlinear model that is commonly employed to characterize the biological effect under different exposure levels:

$$RRE = 100 \times \exp(-a \times C), \quad (2)$$

where a denotes the strength coefficient of toxicity (μM^{-1}) and C represents the free Cu activity $\{Cu^{2+}\}$ (μM).

The free ion activities of Mg^{2+} and Cu^{2+} were calculated using Visual MINTEQ based on the chemical equilibrium model (Gustafsson, 2007). Input data included temperature, pH, the total ion concentrations of Cu^{2+}, Mg^{2+}, and the ion composition of 10 percent modified Hoagland solution. Inorganic carbon was assumed to be in equilibrium with atmospheric CO_2 (Luo et al. 2008). With the dose–response data obtained from the hydroponic experiment, the best-fit values of the coefficients in Eq. (2) were calculated by nonlinear regression. The Cu activity that causes 50 percent growth inhibition of grapevine (EA50) was then determined.