Alleviation effects of magnesium on copper toxicity and accumulation in grapevine roots evaluated with biotic ligand models

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Abstract Copper toxicity and accumulation in plants are affected by physicochemical characteristics of soil solutions such as the concentrations of coexistent cations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+, and H^+). The biotic ligand model (BLM) approach has been proposed to predict metal phyto-toxicity and -accumulation by taking into account the effects of coexistent cations, given the assumption of the partition equilibrium of metal ions between soil solution and solid phase. The alleviation effects of Mg on Cu toxicity and accumulation in grapevine roots were the main concerns in this study and were investigated by using a hydroponic experiment of grapevine cuttings. The BLM approach, which incorporated competition of Mg^{2+} with Cu^{2+} to occupy the biotic ligands on root surfaces, was developed to predict Cu rhizotoxicity and accumulation by grapevine roots. In the results, the effective activity of Cu, $\{Cu^{2+}\}$, resulting in a 50 % reduction of root elongation (EA_{50}), linearly increased with increments of Mg activity, $\{Mg^{2+}\}$. In addition, the Cu concentration in root, Cu_{root}, was retarded by an increase of $\{Mg^{2+}\}$. The linear model was significantly fitted to the relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Mg^{2+}\}$. According to the concept of BLM, the present results revealed that the amelioration effects of Mg on Cu toxicity and accumulation in roots could arise from competition between Mg^{2+} and Cu^{2+} on the binding sites (i.e., the biotic ligands). Then, the developed Cu-BLMs incorporating the Mg^{2+} competition effectiveness were validated provide accurate predictions of Cu toxicity and accumulation in grapevine roots. To our knowledge this is the first report of the successful development of BLMs for a woody plant. This BLM approach shows promise of being widely applicable for various terrestrial plants.

Keywords Terrestrial biotic ligand model (t-BLM) · Rhizotoxicity · Bioavailability · Competition absorption · Kyoho grapevine

Introduction

In agroecosystems, Cu-based fungicides, such as copper hydroxide and copper sulfate, are widely used to control fungi, bacteria, invertebrates, and algae (Van Zwieten et al. 2004). Copper also is added to soils with manure and sludge composts and application (Lai et al. 2010). The intensive and long-term applications of these fungicides and composts in the field have led to accumulation of Cu in some agricultural soils throughout the world (Mirlean et al. 2007), thus posing problems of Cu toxicity to the organisms therein (Komárek et al. 2009). There are however extensive evidences that both total and soluble Cu concentrations are not good predictors of Cu toxicity and accumulation in plants. Copper toxicity to some model plants has been shown to be dependent on a variety of physicochemical characteristics of soil solutions (Luo et al. 2008; Kopittke et al. 2011) such as the concentrations of coexistent cations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+, and H^+). Copper uptake (or availability) affected by Ca^{2+}, Mg^{2+}, and H^+ was also discussed in several studies (Wu 2007; Wang et al. 2011a). Given the assumption of

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the partition equilibrium of metal ions between soil solid and solution phases, the biotic ligand model (BLM) approach has been proposed to predict the metal toxicity to plants while taking the effects of coexistent cations into account (Thakali et al. 2006a, b; Lock et al. 2007a, b). Although initially developed for aquatic systems, the BLM has been promisingly used in terrestrial environments, including plants (Antunes et al. 2006). The main assumption of BLM is that the alleviation effects of metal toxicity are referred to competition between coexistent cations and toxic metals at binding sites (called biotic ligands) on the plasma membrane (De Schamphelaere and Janssen 2002). Recently, the scientific and regulatory communities have become interested in the BLM and have incorporated it into regulations (USEPA 2007; Van Sprang et al. 2007).

Until present, terrestrial BLMs were mainly used to predict Cu toxicity to herbal plants (Luo et al. 2008; Wang et al. 2009; Antunes et al. 2012). There have not been any studies yet to examine whether the BLM approach is applicable for woody plants as well as herbal plants. Due to the elevated Cu concentrations in vineyard soils, it is necessary to study the phytotoxicity and accumulation of Cu in grapevine plants for assessing the eco-toxicological risks and food safety (Chaignon et al. 2003; Juang et al. 2012a). In the previous studies (Lock et al. 2007c; Wang et al. 2012b), Mg^{2+} was reported to be more effective than other cations in alleviation of Cu rhizotoxicity. The alleviation effects of Mg on Cu toxicity and accumulation in grapevine plants are thus our priority in this study.

Rhizo-toxicity and -accumulation of metals in soil involve the two main consecutive steps: release of the metals from soil solid phases into solution and absorption from soil solution onto root surfaces. According to the terrestrial BLM proposed by Thakali et al. (2006a), it is necessary to consider the equilibrium partitioning of metals between soil solid and solution phases, as given competitive sorption of coexistent cations onto solid phases approaching to be equilibrium. Thus based on the assumption of equilibrium partitioning, one can use metal speciation and coexistent cations in solution as the starting point to model the toxicity and uptake of metals with the BLM approach (Mertens et al. 2007). The hydroponic study is thus regarded as the first step in evaluating the feasibility of BLM for modeling Cu toxicity and accumulation in grapevine plants. The objectives of the present study are (1) to assess the alleviation effects of Mg on Cu rhizotoxicity and accumulation by grapevine roots and (2) to model the Cu toxicity and accumulation in grapevines with the derived BLMs, which take into account Mg^{2+} competing with Cu^{2+} to occupy the biotic ligands on root surfaces.

Materials and methods

Preparation of grapevine cuttings and experimental design

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 L polypropylene pots filled with 10 % modified Hoagland solution (0.5 mM KCl, 0.5 mM CaCl$_2$, 0.1 mM KH$_2$PO$_4$, 0.2 mM MgSO$_4$, 0.01 mM Fe–EDTA, and 1.5 mM NH$_4$NO$_3$) for acclimation for about 30 days (Juang et al. 2012a).

The cuttings were exposed separately to 0 (control), 1, 5, 10, 15, and 25 μM of Cu^{2+} as CuSO$_4$, while keeping the Mg^{2+} concentrations at 0.2, 2, 4, and 8 mM, respectively. The testing solution sets were shown in Table 1. Three cuttings as the experimental replicates were placed in one pot containing a testing solution. For each solution, pH was maintained at 5.6–5.9 with 2-(*N*-morpholino) ethane sulfonic acid (MES) buffers. The pot experiment was conducted in growth chambers with the temperature and relative humidity of 25 ± 5 °C and 75 ± 5 %, respectively, during the 16:8 h of light to dark cycles. Testing solutions were aerated throughout the experiment. The solutions were renewed every five days. After the exposure period for 15 days' growth, the cuttings were harvested for measurements of root elongation and Cu concentration in root.

Copper toxicity test with the root elongation of cuttings

In order to assess the growth inhibition of Cu on grapevine roots, the root images of cuttings for each testing solution set were photographed before and after the experiment and transferred into draft files. The total root length was then calculated by DIGIROOT V. 2.5 software (Stefanelli et al. 2009) and then the root elongation of each cutting was obtained with the deviation of total root lengths before and after the experiment. The relative root elongation (*RRE*, %) of grapevines with respect to control can be calculated by

$$RRE = \frac{RE_t}{RE_c} \times 100\% \tag{1}$$

where RE_t is the root elongation in the testing solution and RE_c is the root elongation in the control. For each testing solution set given a specific Mg^{2+} activity, $\{Mg^{2+}\}$, the dose–response relationship between Cu^{2+} activity, $\{Cu^{2+}\}$, as shown in Table 1 and *RRE* was fitted to an exponential

Table 1 Overview of the Mg and Cu concentrations ($[Cu^{2+}]$ and $[Mg^{2+}]$) compositions of the testing solution sets and their activities ($\{Cu^{2+}\}$ and $\{Mg^{2+}\}$) shown within the parentheses and the relative root elongations (RRE) and Cu concentrations in root (Cu_{root}) and leaf (Cu_{leaf}) (mean ± stand error ($n = 3$)) of the grapevine cuttings grown

Testing solution sets		RRE (%)	Cu_{root} (mg kg^{-1})	Cu_{leaf} (mg kg^{-1})
$[Mg^{2+}]$ ($\{Mg^{2+}\}$) (mM)	$[Cu^{2+}]$ ($\{Cu^{2+}\}$) (μM)			
0.2 (0.1532)	0 (0.0000)	100.00 ± 5.43[a†]	133.38 ± 2.85[d]	18.46 ± 0.94[a]
0.2 (0.1532)	1 (0.7271)	122.01 ± 15.60[a]	161.00 ± 19.61[d]	15.37 ± 1.71[a]
0.2 (0.1531)	5 (3.6320)	27.02 ± 14.52[b]	1,128.74 ± 27.40[c]	19.30 ± 2.82[a]
0.2 (0.1529)	10 (7.2556)	4.10 ± 1.78[b]	2,072.66 ± 12.86[b]	17.64 ± 3.23[a]
0.2 (0.1527)	15 (10.871)	0.17 ± 0.17[b]	2,212.66 ± 237.29[b]	21.35 ± 4.99[a]
0.2 (0.1524)	25 (18.075)	0.00 ± 0.00[b]	6,152.14 ± 58.36[a]	19.49 ± 2.02[a]
2.0 (1.1799)	0 (0.0000)	100.00 ± 11.44[a]	69.91 ± 1.84[d]	15.25 ± 0.71[bc]
2.0 (1.1798)	1 (0.5525)	91.83 ± 12.38[a]	239.40 ± 31.52[d]	13.92 ± 1.09[c]
2.0 (1.1794)	5 (2.7614)	17.80 ± 2.89[b]	1,006.25 ± 78.82[c]	12.88 ± 0.30[c]
2.0 (1.1788)	10 (5.5199)	14.14 ± 2.32[b]	1,471.10 ± 211.27[bc]	14.61 ± 1.46[c]
2.0 (1.1782)	15 (8.2754)	6.87 ± 4.21[b]	1,806.18 ± 134.85[b]	23.19 ± 5.53[ab]
2.0 (1.1771)	25 (13.788)	3.25 ± 1.26[b]	5,084.32 ± 177.41[a]	26.34 ± 4.99[a]
4.0 (2.0073)	0 (0.0000)	100.00 ± 30.57[a]	141.07 ± 49.06[f]	15.94 ± 3.23[a]
4.0 (2.0072)	1 (0.4663)	NA[‡]	441.65 ± 43.06[e]	14.70 ± 2.22[a]
4.0 (2.0067)	5 (2.3308)	62.99 ± 14.19[b]	717.04 ± 92.78[d]	18.68 ± 3.19[a]
4.0 (2.0061)	10 (4.6600)	32.57 ± 4.08[b]	2,081.49 ± 14.89[b]	14.17 ± 2.07[a]
4.0 (2.0054)	15 (6.9876)	3.59 ± 1.93[c]	1,203.60 ± 106.07[c]	21.08 ± 2.55[a]
4.0 (2.0042)	25 (11.638)	2.85 ± 2.18[c]	2,620.36 ± 138.81[a]	15.59 ± 6.58[a]
8.0 (3.2821)	0 (0.0000)	100.00 ± 22.12[b]	171.25 ± 26.11[d]	14.35 ± 1.96[a]
8.0 (3.2819)	1 (0.3778)	187.78 ± 10.07[a]	209.10 ± 30.67[d]	19.95 ± 5.90[a]
8.0 (3.2815)	5 (1.8887)	98.53 ± 11.37[b]	849.46 ± 49.57[c]	13.18 ± 3.19[a]
8.0 (3.2808)	10 (3.7766)	10.70 ± 6.08[c]	2,042.92 ± 33.85[b]	14.63 ± 1.66[a]
8.0 (3.2802)	15 (5.6636)	6.59 ± 3.42[c]	2,703.92 ± 72.03[a]	16.82 ± 2.14[a]
8.0 (3.2790)	25 (9.4354)	23.45 ± 7.45[c]	2,729.65 ± 46.30[a]	22.01 ± 6.27[a]

[†] Values followed by the same letter, within each column for the same Mg concentration setting, are not significantly different at the 0.05 level according to the Duncan multiple range test

[‡] Data not available due to the root photographs before the experiment was missing

decay curve and the median effective activity (EA_{50}) of Cu^{2+} for RRE was then determined corresponding to $\{Mg^{2+}\}$. All activity calculations of Mg^{2+} and Cu^{2+} were conducted using the chemical equilibrium model Visual MINTEQ (Gustafsson 2007). Input data included temperature, pH, and the ion concentrations of Cu^{2+}, Mg^{2+}, Ca^{2+}, Na^+, K^+, Cl^-, and SO_4^{2-}. The data of pH and ion concentrations input for activity calculations were given the pH and ion composition of 10 % modified Hoagland solution. Inorganic carbon was assumed to be in equilibrium with atmospheric CO_2 (Luo et al. 2008).

Determination of copper contents in the roots of cuttings

The harvested grapevine roots were thoroughly washed with deionized water. The root samples were oven dried at 65 °C for 72 h, and the dry weight of each sample was recorded. The Cu concentrations of root samples following a $HNO_3/HClO_4$ (v:v = 87:13) digestion procedure were then determined with a flame atomic absorption spectrophotometer (FAAS) (Thermo Scientific iCE 3000). All chemical analyses were performed in two duplicates. Analytical quality control was achieved by digesting and analyzing identical amounts of certificate reference material (CRM). Recovery could then be determined. The quality of the analyses in our laboratory was controlled, and the deviations from the standard value were <5 % (Juang et al. 2012a).

Description of the biotic ligand models for copper toxicity and accumulation

To evoke a biotic ligand model for Cu toxicity and accumulation, we assume that metal toxicity and uptake in plants are via the binding sites on root surfaces. Metal binding on root surfaces can be characterized with a competition concept of metals for binding sites, which has been illustrated as the metal and biotic ligand complex

(MBL) formation reaction with metal cation (M^{n+}) and negative charged biotic ligand (BL^-) on root surfaces (Thakali et al. 2006a):

$$M^{n+} + BL^- \leftrightarrow MBL. \qquad (2)$$

And the reaction equilibrium of metals and biotic ligands can be denoted as

$$K_M = \frac{[MBL]}{\{M^{n+}\}[BL^-]} \qquad (3)$$

where $\{M^{n+}\}$ is the activity of free metal cations in solution, $[BL^-]$ is the density of free biotic ligand on root surfaces, and $[MBL]$ is the density of the MBL complex on root surfaces. The value of K_M is the formation constant of the MBL complex. According to the formation reaction of MBL complexes, the total density of biotic ligands (TBL) can be expressed as

$$TBL = [BL^-] + \sum_{i=1}^{k} [M_i BL] \qquad (4)$$

where $[M_i BL]$ indicates the density of biotic ligands bound by different metal M_i cations. Considering the competition of Mg^{2+} with Cu^{2+} to bind on the biotic sites of grapevine root surfaces, one can put Eq. (3) into Eq. (4) and yield the relation:

$$TBL = [BL^-](1 + K_{Mg}\{Mg^{2+}\} + K_{Cu}\{Cu^{2+}\}). \qquad (5)$$

Then, the fraction (f) of the biotic ligands bound by Cu^{2+} can be derived from Eq. (5) and expressed as

$$f = \frac{[CuBL]}{TBL} = \frac{K_{Cu}\{Cu^{2+}\}}{1 + K_{Mg}\{Mg^{2+}\} + K_{Cu}\{Cu^{2+}\}}. \qquad (6)$$

For modeling the Cu toxicity with consideration of Mg effects by using a biotic ligand model, the value of f is taken up to be related with toxicity symptoms. A logistic dose–response relationship for testing Cu toxicity is stated as

$$Logit(100 - R) = \beta (f - f_{50}) \qquad (7)$$

where R is growth response as % of the control, f_{50} is the fraction of the biotic ligands bound by Cu resulting in a 50 % response, and β is the shape parameter (Thakali et al. 2006a; Wang et al. 2012b). $Logit(100 - R)$ is defined as the natural logarithmic value of the ratio of $100 - R$ to R (i.e., $Ln[(100 - R)/R]$). While putting Eq. (6) into Eq. (7), one can re-express $Logit(100 - R)$ as

$$
\begin{aligned}
&Logit(100 - R) \\
&= \beta \left(\frac{K_{Cu}^T\{Cu^{2+}\}}{1 + K_{Mg}^T\{Mg^{2+}\} + K_{Cu}^T\{Cu^{2+}\}} - f_{50} \right)
\end{aligned} \qquad (8)
$$

where K_{Mg}^T and K_{Cu}^T are the specific notations for K_{Mg} and K_{Cu}, respectively, in modeling Cu toxicity. Equation 8 forms the mathematical basis of a BLM with consideration

of the competition between Mg^{2+} and Cu^{2+} on root surface binding sites.

In order to model the Cu accumulation with consideration of Mg effects by the BLM approach, the value of f is taken up to be related with the Cu concentration in root (Cu_{root}). A Michaelis–Menten kinetic type absorption model is stated as

$$Cu_{root} = \frac{Cu_{max}f}{\kappa + f} \qquad (9)$$

where Cu_{max} is the maximum Cu concentration in root and κ is the f value that is causing a half of Cu_{max}. While the Cu concentration in the testing solution is very low, the density of biotic ligands occupied by Cu^{2+} is also very low. That is,

$$[BL^-] + [MgBL] >> [CuBL]. \qquad (10)$$

Thus, one can assume $f \ll \kappa$ to simplify Eq. (9) as

$$Cu_{root} = nf \qquad (11)$$

where $n = Cu_{max}/\kappa$. While putting Eq. (6) into Eq. (11), given the consideration of Eq. (10), Cu_{root} can be rewritten as

$$Cu_{root} = \frac{nK_{Cu}^A\{Cu^{2+}\}}{1 + K_{Mg}^A\{Mg^{2+}\}} \qquad (12)$$

where K_{Mg}^A and K_{Cu}^A are the specific notations for K_{Mg} and K_{Cu}, respectively, in modeling Cu concentration in root (i.e., accumulation). Equation 12 thus forms the mathematical basis of a BLM with consideration of the competition between Mg^{2+} and Cu^{2+} on root surface binding sites.

Determination of the parameters in biotic ligand models

According to the results of the Cu toxicity and uptake experiments for grapevine cuttings, the model parameters including β, f_{50}, K_{Mg}^T, and K_{Cu}^T in Eq. (8) and n, K_{Mg}^A, and K_{Cu}^A in Eq. (12) can be determined by using the numerical analyzing procedures. To obtain the values of β, f_{50}, K_{Mg}^T, and K_{Cu}^T, the effective activity of Cu resulting in a 50 % reduction of root elongation (i.e., EA_{50}) can be included into the relationship of Eq. (6), and then is given by

$$EA_{50} = \frac{f_{50}}{(1 - f_{50})K_{Cu}^T} + \frac{f_{50}K_{Mg}^T}{(1 - f_{50})K_{Cu}^T}\{Mg^{2+}\}. \qquad (13)$$

If Cu toxicity to grapevine root elongations observed in the experiment reliably follows the concept of biotic ligand models, a linear relationship between EA_{50} and $\{Mg^{2+}\}$ will be consistent with Eq. (13) (De Schamphelaere and Janssen 2002). Linear regression analysis of EA_{50} versus

$\{Mg^{2+}\}$ gives an intercept (I_{Mg}^T) and a slope (S_{Mg}^T), which can be expressed as

$$I_{Mg}^T = \frac{f_{50}}{(1-f_{50})K_{Cu}^T} \tag{14}$$

and

$$S_{Mg}^T = \frac{f_{50}K_{Mg}^T}{(1-f_{50})K_{Cu}^T}. \tag{15}$$

The value of K_{Mg}^T can thus be estimated by the ratio of S_{Mg}^T to I_{Mg}^T:

$$K_{Mg}^T = \frac{S_{Mg}^T}{I_{Mg}^T}. \tag{16}$$

Next, in order to obtain a reliable approximation of K_{Cu}^T to fit the responses of grapevine root elongation observed in the experiment, a simulation procedure to vary K_{Cu}^T in Eq. (6) and generate f values with the data of responses of grapevine root elongation and the determination of K_{Mg}^T has been proposed. A K_{Cu}^T can be chosen that as a result of the best fit of the linear relationship between $Logit(100 - R)$ and f [Eq. (7)]. Finally, the best-fit parameters of β and f_{50} in Eq. (7) can be obtained with the choice of K_{Cu}^T (Lock et al. 2007a).

In addition, to estimate the values of n, K_{Mg}^A, and K_{Cu}^A, Eq. (12) can be rewritten as

$$\frac{\{Cu^{2+}\}}{Cu_{root}} = \frac{1}{nK_{Cu}^A} + \frac{K_{Mg}^A}{nK_{Cu}^A}\{Mg^{2+}\}. \tag{17}$$

If Cu accumulation by grapevine roots observed in the experiment confirmedly follows the concept of BLM, a linear relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Mg^{2+}\}$ will be consistent with Eq. (17) (Cheng and Allen 2001). Linear regression analysis of $\{Cu^{2+}\}/Cu_{root}$ versus $\{Mg^{2+}\}$ gives an intercept (I_{Mg}^A) and a slope (S_{Mg}^A), which are expressed as

$$I_{Mg}^A = \frac{1}{nK_{Cu}^A} \tag{18}$$

and

$$S_{Mg}^A = \frac{K_{Mg}^A}{nK_{Cu}^A}. \tag{19}$$

The determination of K_{Mg}^A can thus be given by the ratio of S_{Mg}^A to I_{Mg}^A:

$$K_{Mg}^A = \frac{S_{Mg}^A}{I_{Mg}^A}. \tag{20}$$

Next, in order to obtain a reliable approximation of K_{Cu}^A to fit the Cu uptakes by root observed in the experiment, one can use a simulation procedure to vary K_{Cu}^A in Eq. (6) and

generate f values with the data of root Cu concentrations and the determination of K_{Mg}^A. A K_{Cu}^A can be chosen that is the result of the best fit of the linear relationship between Cu_{root} and f [Eq. (11)]. Then, the best-fit parameters of n can be obtained with the choice of K_{Cu}^A.

Results and discussion

Magnesium effects on copper rhizotoxicity to grapevine cuttings

The responses of RRE to the exposure sets of $[Cu^{2+}]$ with different coexistent $[Mg^{2+}]$ were shown in Table 1. The values of RRE seemingly decrease with an increase of $[Cu^{2+}]$. Compared with the control ($[Cu^{2+}] = 0$ μM), the lowest-observed-adverse-effect (LOAE) levels of $[Cu^{2+}]$ were 5 μM while $[Mg^{2+}]$ was set as 0.2, 2, or 4 mM and 10 μM while $[Mg^{2+}]$ was set as 8 mM. Thus, for grapevine cuttings, a significant reduction of root elongation would result from an exposure of 5 μM Cu or higher. And according to the relationship between $\{Cu^{2+}\}$ and RRE, given a goodness-of-fit determination, the values of EA_{50} obtained were 1.06, 2.13, 2.31, and 2.65 μM, respectively, for $\{Mg^{2+}\}$ being 0.1532, 1.1799, 2.0073, and 3.2821 mM. In several previous studies (Thakali et al. 2006b; Lock et al. 2007c; Luo et al. 2008; Wang et al. 2009), the Cu toxicity to root elongation of barley and wheat resulted in EA_{50} commonly lower than 1 μM. The present results indicated that grapevine cuttings had a superior tolerance to Cu compared with barley and wheat seedlings.

Moreover, to investigate the alleviation effects of Mg on Cu toxicity, the relationship between EA_{50} and $\{Mg^{2+}\}$ was then shown in Fig. 1a. The linear model significantly fitted to observations of EA_{50} versus $\{Mg^{2+}\}$ showed that EA_{50} increases with increments of $\{Mg^{2+}\}$ and seemingly followed the statement of BLM in Eq. (13). Thus, the amelioration effectiveness of Mg for grapevine cuttings could arise from competition between Mg^{2+} and Cu^{2+} on the toxicity binding sites (i.e., the biotic ligands). It thus shows promise of the prediction of Cu toxicity to grapevines by using the BLM approach. Nevertheless, the deviant effects of high concentrations of coexistent cations, such as that the curvature or downward tendency occurs (Luo et al. 2008), should be of concern for adopting an appropriate range of $\{Mg^{2+}\}$ with mitigating effects on Cu toxicity. In the previous studies, the positively linear relationships between EA_{50} versus $\{Mg^{2+}\}$ were observed within the ranges of $\{Mg^{2+}\}$ up to 2.5 mM for barley (Lock et al. 2007c) and up to 1.5 mM for wheat (Luo et al. 2008). In this study there was a positively linear relationship between EA_{50} versus $\{Mg^{2+}\}$ for grapevines within a
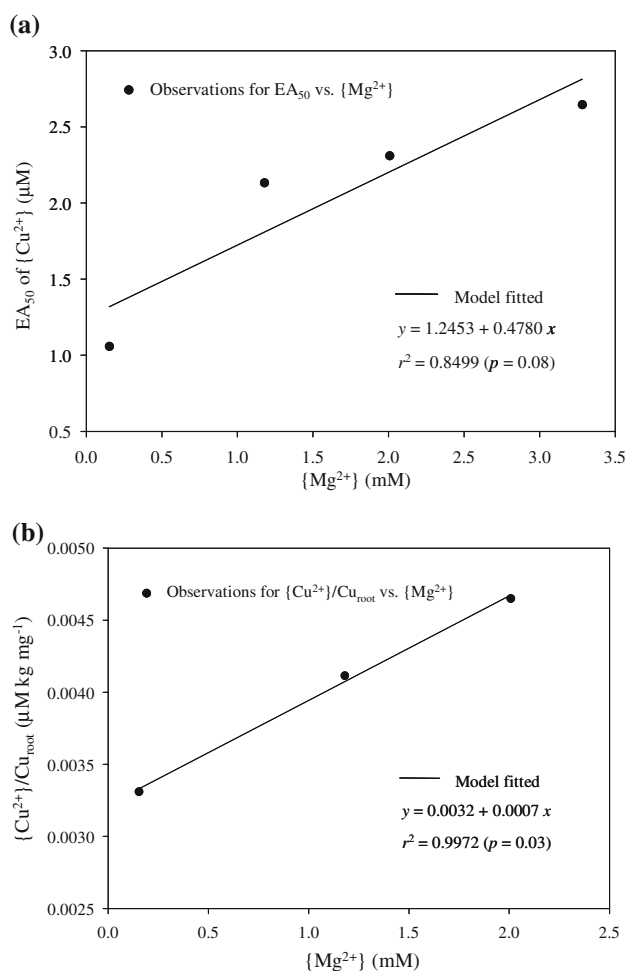
(a)

(b)

Fig. 1 a 15-d EA_{50} of Cu and **b** the ratio of Cu activity to Cu concentration in root, $\{Cu^{2+}\}/Cu_{root}$, as a function of the Mg activity, $\{Mg^{2+}\}$, respectively, for grapevine cuttings

relatively wide range of $\{Mg^{2+}\}$ up to about 4.0 mM. However, 4.0 mM $\{Mg^{2+}\}$ will be rarely observed in soil solutions, except for just after application of Mg.

Magnesium effects on copper accumulation in grapevine roots

Copper concentrations in roots (Cu_{root}) absorbed from the exposure sets of $[Cu^{2+}]$ with different coexistent $[Mg^{2+}]$ were significantly increased by raising the exposure levels of $[Cu^{2+}]$ (Table 1). Compared with the control ($[Cu^{2+}] = 0$ μM), there were significant increases of Cu_{root} under the exposure level of 5 μM $[Cu^{2+}]$ while $[Mg^{2+}]$ was set as 0.2, 2, or 8 mM and under the exposure level of 1 μM $[Cu^{2+}]$ while $[Mg^{2+}]$ was set as 4 mM. There was a steep gradient of Cu_{root} during the range of exposure levels from 1 to 10 μM $[Cu^{2+}]$. The present result was consistent with the previous study (Juang et al. 2012a) showing that Cu phytotoxicity was stimulated between

1 and 10 mM Cu in solution. The critical range of Cu exposures resulting in high accumulations of Cu in roots overlapped that resulting in dramatic decreases of *RRE*. However, Cu concentrations in leaf (Cu_{leaf}) shown in Table 1 presented much lower than Cu_{root} under the same Cu exposure levels. While $[Mg^{2+}]$ was set as 0.2, 4, and 8 mM, there was not significant difference among the values of Cu_{leaf} for different Cu exposure levels. The moderately significant differences between Cu_{leaf} were only observed in the testing solution set at 2.0 mM $[Mg^{2+}]$. The findings indicate that grapevines may take the exclusion strategy to avoid Cu translocation from roots to aerial parts when exposed to excessive Cu (Juang et al. 2012a).

In addition, alleviation effects of Mg on Cu accumulation by root were also found from the relation between $\{Cu^{2+}\}/Cu_{root}$ versus $\{Mg^{2+}\}$, shown in Fig. 1b. However, as the testing solution was set with 8 mM of $[Mg^{2+}]$, its $\{Mg^{2+}\}$ was extremely high, which is rare in soil solutions. And a positively liner relationship between $\{Cu^{2+}\}/Cu_{root}$ versus $\{Mg^{2+}\}$ was only satisfied within a range of $\{Mg^{2+}\}$ up to about 2.0 mM. Thus the testing solution sets with 0.2, 2, and 4 mM $[Mg^{2+}]$ were primarily regarded in modeling the relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Mg^{2+}\}$. In Fig. 1b, the linear model was significantly fitted to the relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Mg^{2+}\}$ and thus the present result was consistent with the statement of Eq. (17). Then, the reduction of Cu accumulation in root due to the increase of Mg also could be illustrated by using the BLM approach. Moreover, this finding was in agreement with the findings by Cheng and Allen (2001), who suggested that the presence of competing ions (e.g., Ca^{2+}) in nutrient solutions could reduce Cu content in lettuce roots and then proposed a linear relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Ca^{2+}\}$. In the culture solution of this study, $[Ca^{2+}]$ was kept at 0.5 mM and we only considered the effects of changing $[Mg^{2+}]$. Nevertheless, there will be further studies to consider the effects of $[Ca^{2+}]$ and to develop the relationship between $\{Cu^{2+}\}/Cu_{root}$ and $\{Ca^{2+}\}$ for grapevine plant.

Development of a Cu-BLM to predict copper rhizotoxicity to grapevine cuttings

According to the BLM approach derived by De Schamphelaere and Janssen (2002), BLM parameters can be estimated from toxicity data alone for different coexistent cations respectively. Only the effects of Mg were taken into account in this study, so the 15-d EA_{50} of Cu for *RRE* of grapevine cuttings was thus expressed as the linear function of $\{Mg^{2+}\}$ shown as Fig. 1a. Then the BLM parameter of K_{Mg}^{T} was obtained by Eq. (16) and its logarithmic value of 2.58 was obtained. Next, other parameters, K_{Cu}^{T}, f_{50}, and b, were obtained and incorporated into
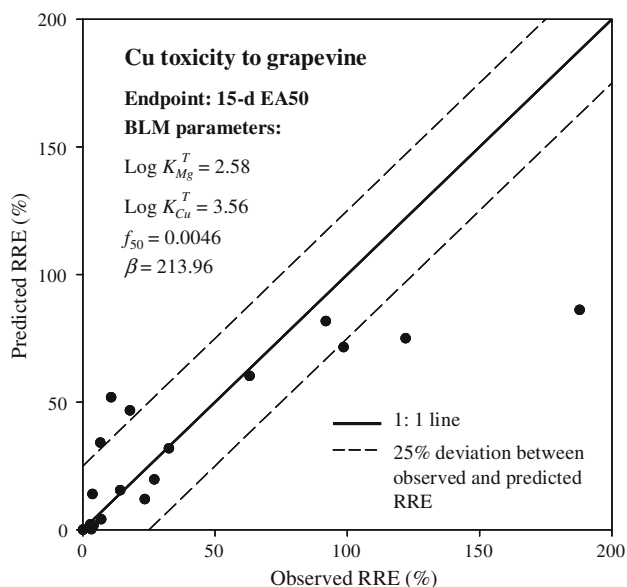
Fig. 2 Comparison of predicted relative root elongations (*RRE*) using the biotic ligand model and observed *RRE* of grapevine cuttings

the highest linear correlation between the generated *f* and the *Logit*(100 − R) [Eq. (7)]. Finally, the logarithmic value of K_{Cu}^{T}(3.56) and the associated f_{50} and β (0.0046 and 213.96) were then used to build up the BLM of Cu toxicity for grapevine [Eq. (8)]. Eventually, one can predict *RRE* to assess the root growth responses to Cu toxicity with consideration of Mg alleviation effects. In an attempt to examine the predictive capacity of the developed Cu-BLM of toxicity, an auto-validation was carried out, as shown in Fig. 2. The predicted values of *RRE* were plotted against the observed values, clustering near the 1:1 line indicating a reliable match between the predicted and observed values of *RRE*. The dash lines indicating a deviation of 25 % *RRE* between the predicted and observed were plotted also. Most of the deviations between the predicted and observed values of *RRE* were <25 %, suggesting that the model is well-calibrated to the BLM-development dataset. However, there were several predicted values against observed values with high deviations. The deviant phenomenon was referred to the extreme observations of *RRE* (e.g., 122.01 and 187.08), which in the pot experiment presented much higher growth than the control (Table 1). On the other hand, there were several low-valued *RRE* with prediction deviation higher than 25 %. This was referred to the low-valued observations of *RRE* (e.g., 17.80, 10.70, and 6.59), which in the pot experiment presented a dramatic reduction of growth with a moderate increase of [Cu^{2+}] (Table 1). This revealed that the predictive capacity of the developed Cu-BLM of toxicity for grapevines would be violated by some extreme outcomes.
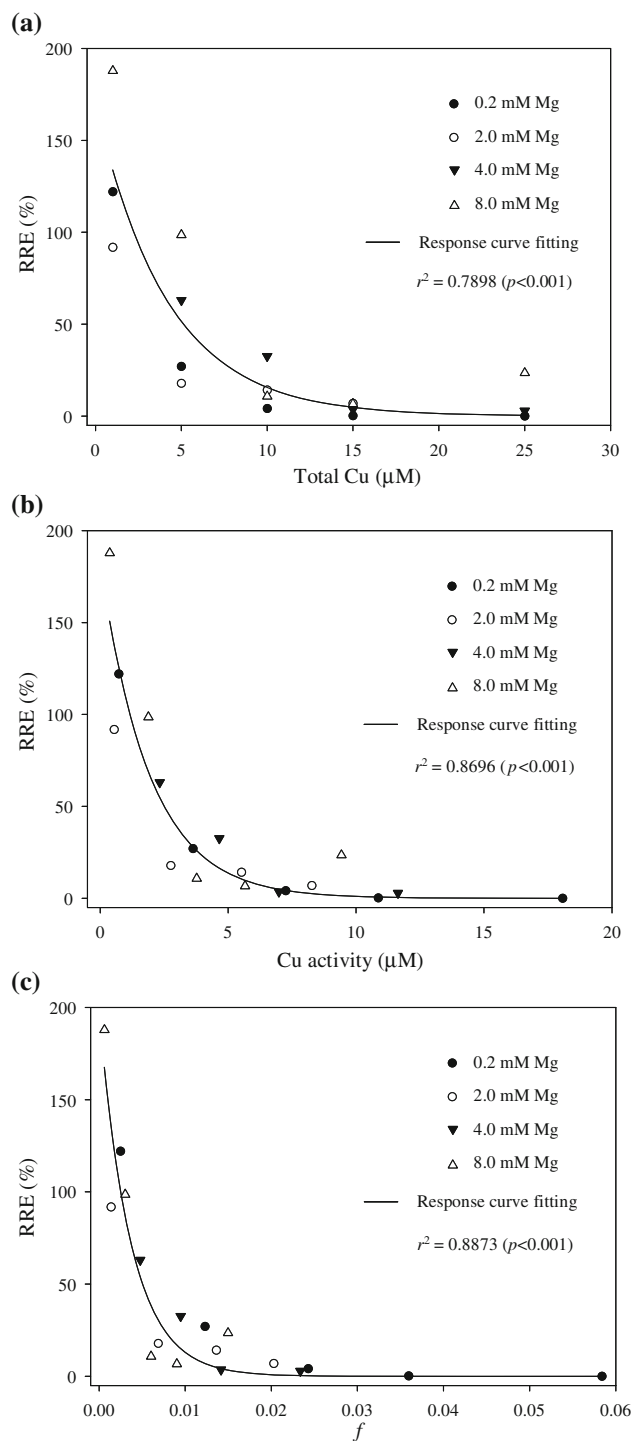

Fig. 3 Relative root elongations (*RRE*) of grapevine as the functions of **a** total Cu concentration, **b** Cu activity, and **c** fraction of the total biotic ligands bound by Cu

In addition, a comparison of the total concentration model (TCM), free ion activity model (FIAM), and BLM for prediction of Cu toxicity to grapevines was carried out. The dose–response relationships for Cu toxicity to grapevines were illustrated as *RRE* against [Cu^{2+}], {Cu^{2+}}, and

f, respectively, in Fig. 3. In contrast, root growth responses were much better correlated with $\{Cu^{2+}\}$ and f than $[Cu^{2+}]$. Thus, the fraction of the biotic ligands bound by Cu would be superior to the total Cu concentration and the free Cu ion activity to express the Cu toxicity to grapevines. The coefficient of determination (r^2) improved from 0.8696 for $\{Cu^{2+}\}$ to 0.8873 for f. This suggested that compared with using FIAM, using BLM with consideration of the competition mechanism between Mg^{2+} and Cu^{2+} at the toxicity biotic ligands would be better for prediction of Cu toxicity to grapevines.

Development of a Cu-BLM to predict copper accumulation in grapevine roots

According to the linear relationship between $\{Cu^{2+}\}/Cu_{root}$ versus $\{Mg^{2+}\}$ (shown in Fig. 1b), the parameter of K_{Mg}^A was determined by Eq. (20) and its logarithmic value was 2.35. Both parameters K_{Cu}^A and n were obtained given the highest linear correlation between the calculated f and the Cu_{root} [Eq. (11)]. The logarithmic value of K_{Cu}^A (4.29) and the associated n (16,005.86) were then obtained to build up the BLM of Cu accumulation by grapevine roots [Eq. (12)]. Eventually, one can predict the Cu concentration in grapevine roots with consideration of Mg alleviation effects. An auto-validation as shown in Fig. 4 was also carried out to examine the reliability of the prediction of Cu accumulation by grapevine roots with the developed Cu-BLM. The predicted values of Cu_{root} were plotted against the observed values, clustering near the 1:1, line indicating a reliab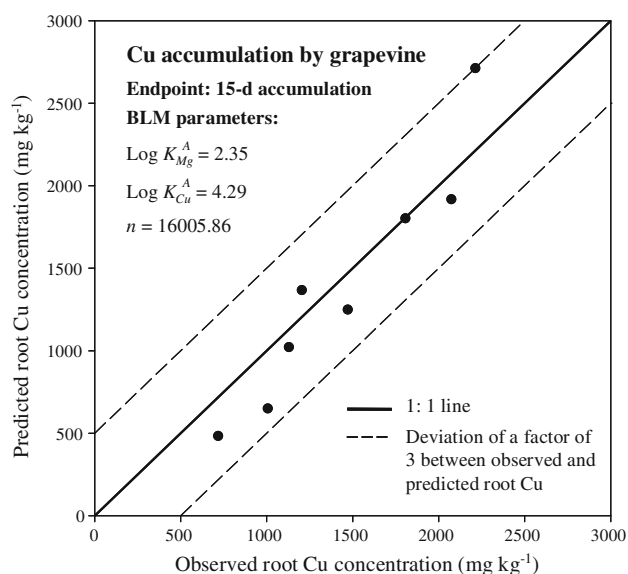le match between the predicted and observed values of Cu_{root}. The dash lines indicating a deviation of a factor of 3 between the predicted and observed values were plotted also. The predicted Cu_{root} differed by a factor of <3 from the observed Cu_{root}. It should be recognized, however, that the model is well-calibrated to the BLM-development dataset.

Fig. 4 Comparison of predicted Cu concentrations using the biotic ligand model and observed Cu concentrations in grapevine roots
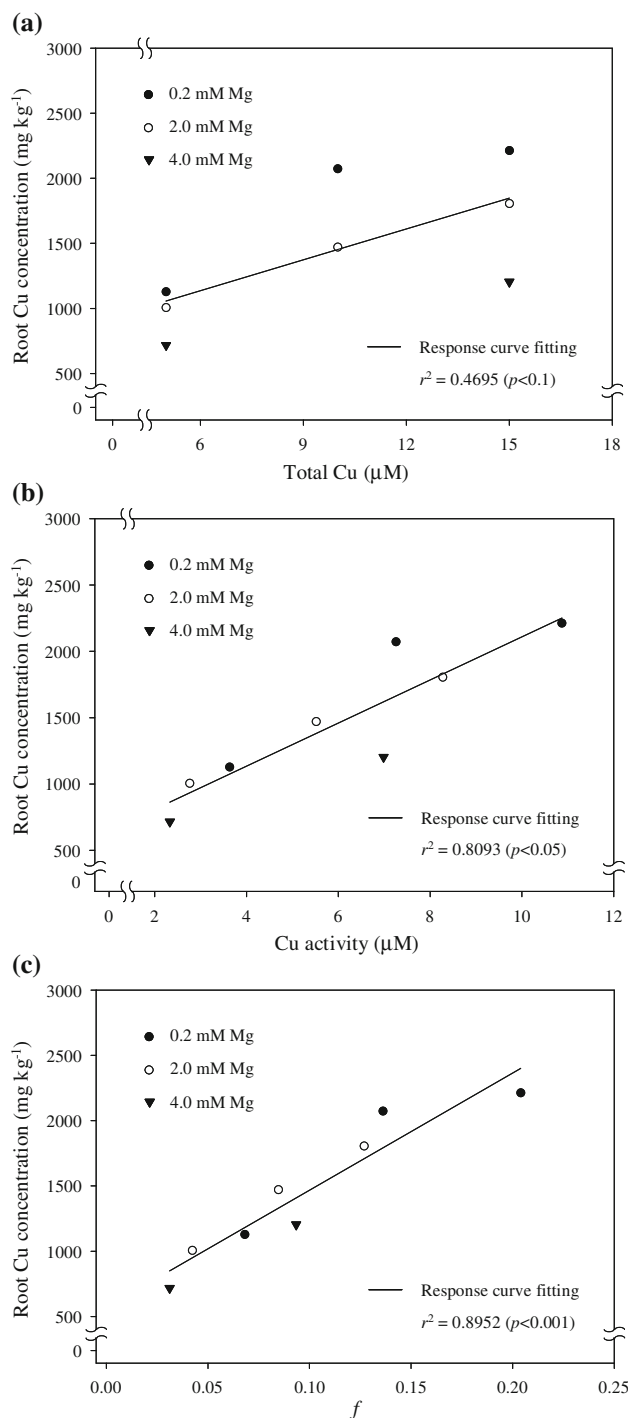

Fig. 5 Copper concentrations in grapevine roots as the functions of **a** total Cu concentration, **b** Cu activity, and **c** fraction of the total biotic ligands bound by Cu

A comparison of TCM, FIAM, and BLM for prediction of Cu accumulation by grapevine roots also was carried out. The dose–response relationships for Cu accumulation were illustrated as Cu_{root} against $[Cu^{2+}]$, $\{Cu^{2+}\}$, and f, respectively in Fig. 5. In contrast, Cu_{root} was much better linearly correlated with $\{Cu^{2+}\}$ and f than $[Cu^{2+}]$. The results evidenced that TCM could not predict Cu accumulation very well, as many previous studies have shown (Bell et al. 1991; Buckley 1994). In a comparison of FIAM and BLM, the coefficient of determination (r^2) improved from 0.8093 for $\{Cu^{2+}\}$ to 0.8952 for f. Thus, the fraction of the biotic ligands bound by Cu may be superior to the free Cu ion activity to express the Cu accumulation by grapevine roots. This clearly suggests that compared with using FIAM, using BLM with the assumption of the competition mechanism between Mg^{2+} and Cu^{2+} at the absorption biotic ligands would be better for prediction of Cu accumulation.

Conclusions

The results of the present study suggest that Cu toxicity to root elongation and Cu accumulation by grapevine roots were modulated by both Cu^{2+} activity in solution and competition between Mg^{2+} and Cu^{2+} at the biotic ligands. According to the concept of BLM, competition between Mg^{2+} and Cu^{2+} on the binding sites of plasma membranes (i.e., the biotic ligands) could result in the amelioration effectiveness of Mg on Cu toxicity and accumulation. Therefore, the alleviations of Mg on Cu toxicity and accumulation in grapevine roots were referred to the competition between Mg^{2+} and Cu^{2+} on the biotic ligands of root surfaces. The developed Cu-BLMs for grapevines were validated to provide accurate predictions of Cu toxicity and accumulation in roots. To our knowledge, this is the first report of the successful development of BLMs for a woody plant. This shows promise that the BLM approach may be widely applicable for various terrestrial plants. Recently, the alternative approach, called the electrostatic model (ESM), proposed to illustrate the alleviation of metal toxicity by coexistent cations, has gained increasing attention (Kopittke et al. 2011; Wang et al. 2011b). The concept of ESM states that the negativity of electrical potential of the plasma membrane is reduced by the addition of coexistent cations in bulk solution and then the metal activity on the plasma membrane diminishes. Wang et al. (2012a) have suggested that the possibility of the competition assumption by BLM could not be excluded from metal toxicity assessment, and then proposed the integration of BLM and ESM to predict metal toxicity thresholds (i.e., EA_{50}). For further studies in future, the results of our study could be the pioneering information for

integration of BLM and ESM to model metal toxicity in woody plants.

Nevertheless, different approaches are adopted for assessing metal toxicity and uptake in hydroponic and soil cultures (Zabłudowska et al. 2012; Tavakkoli et al. 2012). In hydroponic, metal toxicity and uptake are directly influenced by the intensity of metal in solution (i.e., metal concentration or activity) (Kopittke et al. 2010). However, in soil, the metal source of toxicity and uptake is not only from solution but also from the replenishment of soil solid. Metal toxicity and uptake are governed by the available quantity (or called availability) of metal in soil (i.e., soluble and replenished metals). Currently the contribution of replenishment of metal from soil solids to phyto-toxicity and –accumulation has been studied frequently for different metal species and soils (Udovic and Lestan 2009; Singh and Agrawal 2010; Juang et al. 2012b).

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