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Research article

Effects of calcium on rhizotoxicity and the accumulation and translocation of copper by grapevines

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ABSTRACT

We assessed the effects of background concentrations of calcium (Ca) in solution on rhizotoxicity of copper (Cu) in and the accumulation and translocation of Cu by the grapevine, *Vitis vinifera* L. var. Kyoho. Grapevine cuttings in a hydroponic system were exposed to Cu-spiked solutions (0, 1, 2.5, 5, 10, and 25 μM) with two Ca backgrounds (0.5 and 5 mM) for 15 days. We found that when Cu exposure exceeded 5 μM, no new white roots were generated from the cuttings. When exposed to a Cu concentration of 25 μM, the lateral roots were sparse, appeared dark and exhibited malformed terminal swellings. The morphological phenomena of root response to an increase in Cu levels were relatively pronounced at a background concentration of 5 mM Ca; epidermal cell walls thickened, cortical cells remained intact and root terminal swelling was enhanced with Cu exposure. A 5 mM Ca background concentration enhanced the reduction in relative root elongation, but alleviated the reduction in relative root dry weight with increased Cu exposure. Moreover, there was a prominent increase in root Cu concentrations with increased Cu exposure, but the increases in leaf Cu concentrations were much less. The Cu profile of Cu exposure in a 5 mM Ca background concentration appeared higher in root, but lower in leaf than the Cu profile in a 0.5 mM Ca background; therefore, increase of Ca background concentrations would enhance Cu to be accumulated by root, but not translocated into the leaf.

1. Introduction

Copper (Cu) in soil is becoming increasingly elevated as a result of intensive agriculture (i.e., manure applications, compost, sewage sludge, and Cu-based fungicides) [1]. Some industrial activities, such as mining and waste deposition, also contribute to the enrichment of Cu in soil [2]. Although Cu is an essential element for animals and plants, adverse effects of excess Cu in soil on agroecosystems are a concern [3]. Long-term accumulation of Cu in soil could result in the elimination of earthworm populations [4] and in reduced microbial activity [5]. Copper excess commonly inhibits plant growth with a range of morphological and physiological disorders, such as reductions in cell elongation, photosynthetic activity and uptake of mineral nutrients [6,7]. With high Cu toxicity, chlorosis in leaf, malformation in root and damage to plasma membrane permeability are likely [8]. As little as 55 mg kg^{-1} total Cu in soil could induce negative effects on organisms; however, directly measuring bioavailable Cu is difficult in a wide range of soils [1]. Plant Cu concentrations exceeding 20 mg kg^{-1} are considered to be the threshold for the delineation of Cu phytotoxicity [9].

In viticulture, application of Cu-based fungicides for combating downy mildew can result in plant stress as a result of Cu accumulation in the topsoil [10]. Mackie et al. [1] documented that plant response to Cu toxicity correlated with rooting depth in vineyards. Grasses and shallow-rooting plants were more sensitive to Cu than were grapevines. Excess Cu in the soil, however, is detrimental to the development of new vine shoots [11]. Compared to other plants (e.g., wheat, maize and sorghum) [12–14], the grapevine has a high lowest observed adverse effect level (LOAEL) for Cu toxicity [15]. Consequently, there has been limited concern for the mechanisms responsible for Cu toxicity in and accumulation and translocation by the grapevine [16].

Hydroponic experiments are frequently used to investigate the toxicity, absorption and translocation processes involved in metal accumulation by plants [17]; they are easy to conduct, require less time and space and have low environmental variability. The media used in solution culture can be easily prepared to represent soil solution characteristics and associated metal activity that will govern plant metal toxicity and absorption. Thus, a hydroponic

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study thus could be regarded as the first step for investigating phytotoxicity and phytoabsorption of metals in soil. There is currently a better understanding of Cu toxicity and distribution in plants (e.g., oregano, wheat, barley, cucumber, Ethiopian mustard, sunflower, white lupin) [8,9,18–21] other than grapevine. Additionally, Marschner [22] suggested that roots would show the first signs of Cu stress in soil and could be used as an indicator of Cu availability. Copper tends to be accumulated in root tissues with little translocation to shoots [22]; thus symptoms of Cu toxicity on the aerial parts will not be apparent. In order to assess the impacts of Cu excess in soil on the grapevine, more information is needed about the Cu rhizotoxic phenomena—accumulation by root and translocation to aerial parts in the grapevine.

Calcium (Ca) is an essential element and is known to have a positive influence on plant growth and to ameliorate phytotoxicity of heavy metals. Vulkan et al. [18] showed that an increase of Ca levels in solution reduced Cu rhizotoxicity and absorption by wheat roots. Österås and Greger [23] also suggested Ca addition could decrease the accumulation and translocation of Cu in woody plants. And the biotic ligand model (BLM) has been used to illustrate the competition between Cu^{2+} and Ca^{2+} for adsorption on the root surfaces by Kopittke et al. to assess alleviation effects of Ca on Cu toxicity in cowpea [24]. In the early study, Brun et al. [25] have suggested that the Ca contents in vineyard soils ranged widely for the different soils with pH ranging from 4.5 to 8.5, and the total amounts of soil $CaCO_3$ from 1 to 322 g kg^{-1} were positively related to accumulation of Cu in soil but regressed against Cu assimilated by grapevine plants. However, to date there has been no documented evidence regarding Ca effects on Cu toxicity and grapevine uptake. In addition, soil pH plays an important role in Ca-uptake. In non-acidic soils (pH $>$ 5) the dominant cation at the soil cation exchange sites usually is Ca and thus Ca^{2+} in soil solution will be sufficient or high. On the other hand, at low soil pH (pH $<$ 5), aluminum and hydrogen ions are usually the dominant cation at the soil cation exchange sites. It interferes with Ca-uptake by reducing Ca binding to root cell walls. Under these conditions, plants may be very susceptible to Ca deficiency [26]. In grapevines, Ca deficiency symptoms start as a narrow necrotic border at the leaf margin that moves in steps towards the petiole attachment. Dark brown pimples may also appear up to 1 mm in diameter, on the primary bark of the inter node. The growing bunches can also dry up from the tip resembling severe stem necrosis [27]. Thus in order to prevent from Ca deficiency and Cu excess assimilation in grapevine plants, application of $CaCO_3$ (i.e., liming) should be adopted for viticulture in acidic soil regions.

The objectives of the present study are (1) to improve the knowledge base on the morphology and anatomy of grapevine roots given Cu stress and on the effect of Ca addition on Cu rhizotoxicity and (2) to investigate the influence of Ca addition on Cu accumulation and translocation in the grapevine.

2. Results

2.1. Morphological and anatomical observations of the grapevine root with Cu exposure

There was a prominent morphological difference in the roots of the grapevine cuttings treated with increasing Cu concentrations, as shown in Fig. 1. The roots of the cuttings showed increasing browning with an increasing concentration of Cu. No new white root emerged when Cu concentrations exceeded 5 μM. When the cutting was exposed to 25 μM Cu, its roots were sparse, appeared dark and exhibited malformed terminal swellings. At a background level of 0.5 mM Ca (Fig. 1A), the morphological phenomena of root response to increasing Cu concentrations were more pronounced compared to the root response at a background level of 5 mM Ca (Fig. 1B).

Fig. 2 shows cross- and vertical-sections of the grapevine roots at a distance of 0.5–1.0 cm from the tip when exposed to 25 μM Cu. At a background level of 0.5 mM Ca, the epidermal cells showed disintegration and some cortical cells were deformed and plasmolyzed (Fig. 2A and B, arrow). However, at a background level of 5 mM Ca, plasmolysis in the cortex due to exposure to 25 μM Cu appeared to diminish; the epidermal cells appeared intact with obvious flattened and thick cell walls (Fig. 2C and D). In addition, cortical cells were significantly enlarged in cross-section (Fig. 2C)

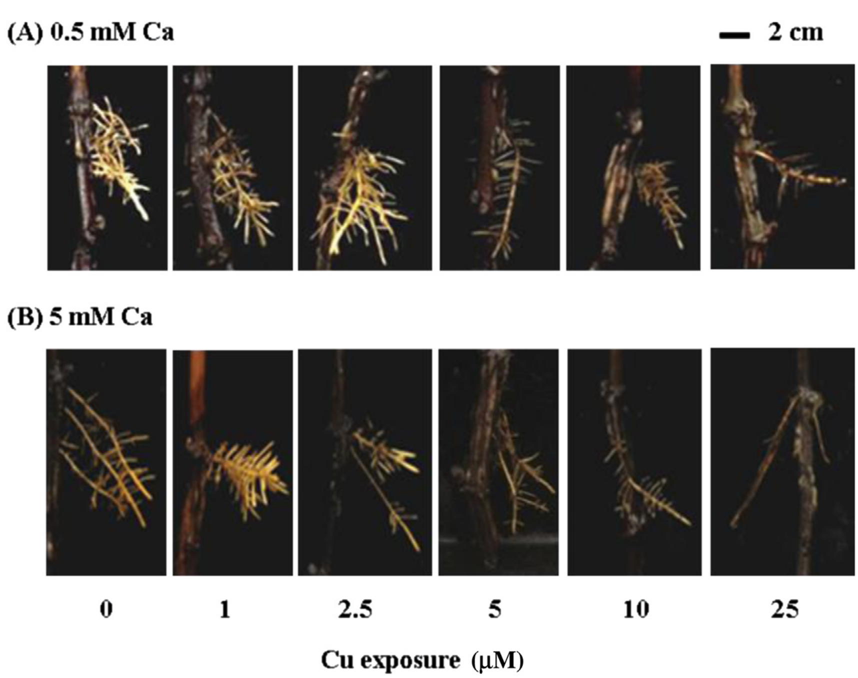

Fig. 1. Appearances of grapevine roots exposed on the solutions with different Cu spikes (0, 1, 2.5, 5, 10, and 25 μM), as given the Ca backgrounds, (A) 0.5 and (B) 5 mM, respectively.