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ORIGINAL PAPER

Growth and physiological responses of grape (*Vitis vinifera* "Combier") to excess zinc

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Abstract Cuttings of *Vitis vinifera* (cultivar Combier) were exposed to seven different zinc (Zn) concentrations (control, 3.5, 7.0, 14.0, 21.0, 28.0, and 35.0 mM) to investigate growth and physiological responses to excess amount of zinc (Zn). The apparent plant growth, as indicated by daily height growth, daily stem diameter variation, and biomass accumulation, was increased by 3.5–7.0 mM surplus Zn addition. Coupled with the increase in plant growth, grape retained low level of leaf Zn concentration, and also retained high level of leaf iron concentration due to increasing translocation of iron (Fe) from root and shoots to leaves. Leaf N and K were increased or found at a constant high level, paralleling with low oxidative pressure and enhanced catalase (CAT) activity. Moreover, plant growth was depressed under high Zn levels (>14.0 mM). Generally excess Zn was stored in the non-sensitive plant parts (roots and shoots), and it caused significant reductions of P, Fe, Mn, Cu in different parts of plant. At the same time, excess Zn caused a pronounced increase in abscisic acid concentration. Our results showed that cultivar Combier is a highly Zn-tolerant grape cultivar and could be used as pioneer plants in metalliferous site and in acidic soil of the tropical and subtropical area.

Keywords *Vitis vinifera* · Zinc · Tolerance · Iron · Nutrition balance · Antioxidative enzyme · Abscisic acid

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Introduction

Zinc (Zn) is an essential trace element for plants, it plays catalytic and/or structural roles in many cell physiological processes (Vallee and Auld 1990). However, for most plant species, the range of beneficial concentration of Zn is often very narrow and generally maintaining at 15–100 ppm (Clemens 2006), but Zn is phytotoxic at high concentration. Recently, Zn toxicity in plants has become of one special concern since Zn contamination is a widespread problem in soil. Human activities (such as the disposal of municipal wastes, use of irrigation water containing industrial effluents, and residues from metalliferous mining) have contaminated large areas of cultivated land with Zn, leading to the increase of Zn concentrations in contaminated soils (Mateos-Naranjo et al. 2008). This metal ion is easily assimilated by plants from such contaminated soil, exceeding the required amount for plant nutrition. In addition, Zn level in soil is highly related to pH, with rapid increase by decrease in soil pH. In acid soils in tropical and subtropical region, Zn availability is generally high (Chaney 1993), and always exhibits toxicity to plants.

Plants affected by excess Zn may show symptoms similar to those found in other heavy metal toxicities. Some of the symptoms are inhibition of root penetration and growth, generation of reactive oxygen species, induction of

chlorosis in young leaves, changing the P concentrations in plant tissues, as well as resulting in deficiency of other essential elements (e.g., Fe or Mg) by interference with the uptake, translocation, and utilization (Marschner 1995; Kramer et al. 2007). However, the mechanisms controlling Zn homeostasis in plants are still not fully known.

For the biochemical influences of toxic levels of heavy metal in higher plants, one of the major consequences is the induced oxidative stress by generation of superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and singlet oxygen ($^1O_2$), collectively termed ROS (Devi and Prasad 1998; Zhang et al. 2007). ROS can attack all types of biomolecules and lead to irreparable metabolic dysfunction. The non-quenched ROS would result in lipid peroxidation expressed by increase of malondialdehyde (MDA). ROS would damage cell membrane and cause enhancement of polyphenol oxidase (PPO) activity, which functions in hydroxylation and oxidation of polyphenolic compounds and is thought as an oxidation indicator in plant cell (Mayer 2006). It is well known that superoxide dismutases (SOD) quench $O_2^{\cdot-}$ to H_2O_2, and H_2O_2 could be further reduced to H_2O by catalase (CAT) or peroxidases (POD) (Dat et al. 2000). Therefore, the induction of antioxidant enzymes including SOD, CAT, and POD is an important protective mechanism to minimize oxidative damage under heavy metal stress. On the other hand, circumstantial evidence validated that abscisic acid (ABA) may induce a number of genes and proteins involved in stress defenses, including SOD, glutathione peroxidase, ascorbate peroxidase, and pathogenesis-related proteins (Jiang and Zhang 2001), thus ABA play an important role in plant tolerance to heavy metal.

In recent years, grapes (*Vits vinifera* or *Vitis vinifera* × *Vitis labrusca*) have been introduced and widely cultivated in the tropical and subtropical area in south China. However, there exists little information concerning the tolerance of grape to excess amount of Zn, as well as the mechanisms of Zn uptake, translocation, and detoxification in plants of grape. In our previous study, several grape cultivars were used to compare their performances to Mn stress. The results showed that the cultivar Combier is a highly tolerant one to Mn (Mou et al. 2011). In this study, we examined the effects of high concentrations, in comparison with adequate doses, of Zn on *V. vinifera* Linn. (cultivar Combier) as responses in growth parameters, distribution of Zn, plant nutrition, and some biochemical indices. The specific aims of the study are (i) to evaluate the tolerance of cultivar Combier to excess Zn and to testify whether this Mn-tolerant cultivar is also a Zn-tolerant one, and (ii) to achieve a better understanding of the influence by excess zinc on nutrient balance and the possible adaptive response of grape.

Materials and methods

Plant materials and experimental setup

Vitis vinifera, cultivar Combier, was used as plant material in the current experiment. This cultivar was extensively grown in China, and has been demonstrated as a highly tolerant cultivar to excess Mn stress in our previous study (Mou et al. 2011). During dormancy, cuttings (middle parts of 1 year shoot, with 20–25 cm length and three nodes) were rooted in humid sand crates and placed in a controlled greenhouse room. In spring, healthy cuttings of approximately unique size were selected and planted in 5-l plastic pots filled with homogenized soil (acid yellow soil type), two cuttings per pots. The plants were then grown under semi-controlled environmental conditions in a greenhouse naturally lit with sunlight, with a temperature range of 17.0–28.0°C and relative humidity range of 45–90%, and supplied with 800 ml Hoagland's solution every other day. After culturing for 30 days, the cuttings with the length about 30–33 cm (keeping only one main shoot as shown in Fig. 1a and being removed other shoots) were exposed to basic Hoagland's solution supplemented with excess Zn addition. Totally there were seven treatments for surplus Zn addition: 0 (control), 3.5, 7.0, 14.0, 21.0, 28.0, and 35.0 mM Zn^{2+}, as used by $ZnSO_4{\cdot}7H_2O$. Each treatment included three replications and four plants per replication. Eighty-four plants were randomly allocated to different treatments and cultured for 60 days.

Measurements of plant morphological symptom

All plants were harvested at the end of the experiment and divided into three parts: leaves, shoots (including stem and branches but not leaves as showed in Fig. 1a), and roots. All the plant parts were dried at 80°C for 24 h to constant weight in an oven, and weighed, respectively. Final plant biomass and root/aerial part ratio were then determined. The plant height (length of main shoot in each plant) and the diameter of the basal section of the plant (stem diameter) were determined by measuring at the beginning and the end of the experiment. Thereafter we calculated the daily height growth (DHG) and daily stem diameter variation (DSDV) according to the formulas: $DHG = (H_2 - H_1)/t$, and $DSDV = (D_2 - D_1)/t$, where H_2 and H_1 are the plant height at harvest time and at the initial of experiment, D_2 and D_2 are the stem diameter at harvest time and at the initial of experiment, t is the duration of experimental time.

Quantification of leaf photosynthetic pigments content

During harvest time, the third–fifth leaves of main shoot were plucked off for analysis of photosynthetic pigments.

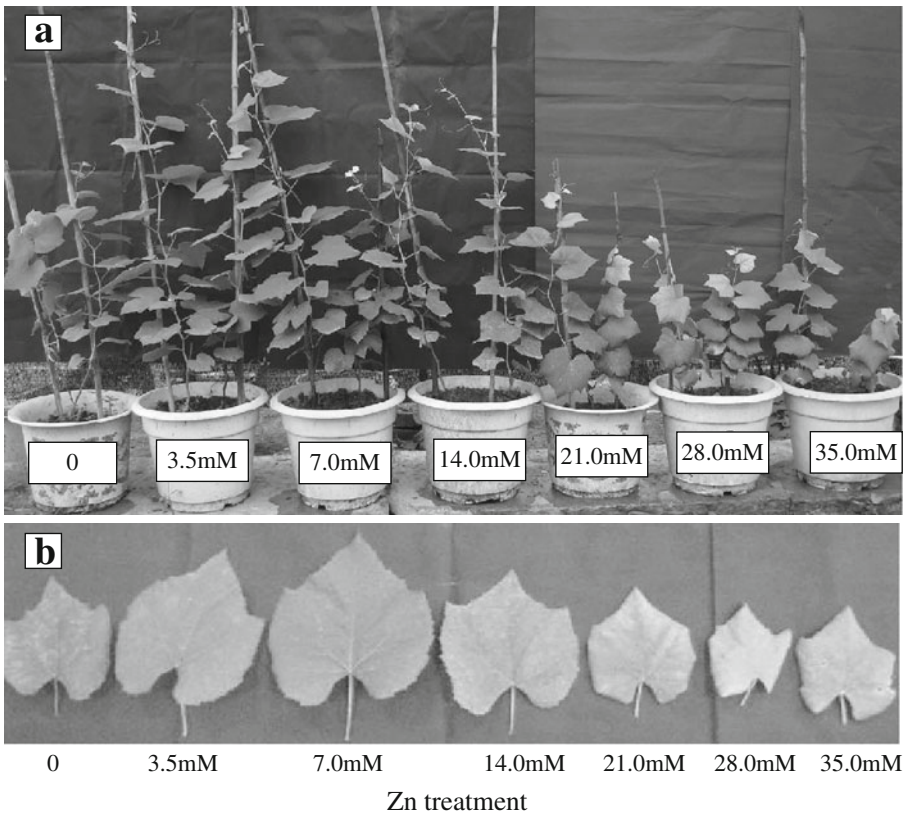

Fig. 1 The state of plant individuals and single leaf of grape under Zn treatment. **a** Plant individuals; **b** Single leaf

The leaves were ground in 80% acetone at room temperature with a small amount of $MgCO_3$. The chlorophyll (Chl) contents (Chla, Chlb, and Chla + b) were determined with the spectrophotometry (Unicam UV-330, USA) at 470, 646, and 663 nm and were calculated according to Wellburn (1994). The content of the pigments was calculated on a fresh weight basis.

Determinations of antioxidative enzymes activity

The third–fifth leaves of main shoots were used for measurement of antioxidative enzyme activity. The superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) as described by Giannopolitis and Ries (1977). One unit of SOD activity (EU g^{-1} FW) was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. The catalase (CAT, EC 1.11.1.6) activity was measured according to Cakmak et al. (1993). The reaction mixture (1.5 ml) consisted of 100 mmol l^{-1} phosphate buffer (pH 7.0), 0.1 mmol l^{-1} EDTA, 20 mmol l^{-1} H_2O_2, and 50 µl enzyme extract. The reaction was started by addition of the extract. The decrease in absorbance of H_2O_2 within 1 min at 240 nm was recorded, and one unit of CAT enzyme was defined as the amount of enzyme which degraded 1 µmol H_2O_2 per min for 1 g fresh leaf (EU g^{-1} min^{-1} FW). The peroxidase (POD, EC 1.11.1.7) activity was determined at 25°C with guaiacol (Thongsook and Barrett 2005). In the presence of H_2O_2, POD catalyzes the transformation of guaiacol to tetraguaiacol (brown product). The oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. The reaction mixture contained 50 µl of 20 mM guaiacol, 2.8 ml of 10 mM phosphate buffer (pH 7.0), and 0.1 ml enzyme extract. The reaction was started with 20 µl of 40 mM H_2O_2. POD activity was expressed as the increase in absorbance at 470 nm per minute for enzyme solution per fresh leaf gram extract (EU g^{-1} FW min^{-1}). For polyphenol oxidase (PPO, EC 1.10.3.2) extraction, 1 g fresh leaves were homogenized in 15 mM β-mercaptoethanol, 20 mM Tris–HCl (pH 7.8), 20% glycerol, 1 mM phenylmethyl sulfonyl fluoride (PMSF), and 1% (v/v) Triton X-100. PPO activity was measured using 30 mM catechol in sodium acetate buffer (pH 4.5) and the reaction was initiated by the addition of the enzyme extract containing 50 mM phosphate buffer (pH 7.0) at 420 nm, and assessed by the enzymatic oxidation of catechol (Sánchez-Ferrer et al. 1988). One unit of PPO activity was expressed as a change of absorbance of 0.01 per minute for enzyme solution of per fresh leaf gram extract (EU g^{-1} FW min^{-1}).

Determinations of root activity, ABA, and MDA

Root activity was determined using the triphenyl tetrazolium chloride (TTC) method as described by Clemensson-Lindell (1994). For detail, 0.5 g fresh root was dipped into the mixture of 0.5 ml buffer and 0.4% 2,3,5-triphenyltetrazolium chloride (TTC) and kept at 37°C for 2 h in dark, then 2 ml H_2SO_4 (1 M) were added to stop the reaction, and the root was picked up and then milled in 3 ml acetic ester, and the red triphenylformazan (TPF) extraction were harvested and the absorbance of it was measured at 485 nm, the TPF were calculated according standard curve. The root activity was defined as the product of TPF per hour and per gram fresh weight (FW) of the root. The third–fifth leaves of main shoot were also picked and used to measure ABA content. The samples were weighed immediately after harvest in the morning, then frozen in liquid nitrogen and stored at −80°C until analyzed. Leaf samples (0.5 g) were homogenized and extracted twice in a total volume of 4 mL of 80% methanol at 4°C for 2 h. The extract was purified through Sep-Pak C18 cartridges (Waters, Milford, MA, USA). The extracted solution was dried in vacuo at 30°C. The dried residues were dissolved in 1 mL of the extracted solution, and approximately 100 μL was analyzed for ABA by ELISA using assay kits (made by China Agricultural University), according to the manufacturer's instructions and that described by Zacarias et al. (1995). Lipid peroxidation was measured by equivalents of MDA contents. About 0.5 g leaf segments were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12,000*g* for 10 min. After that, 2 ml 0.6% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10,000*g* for 10 min, the absorbance of the supernatant at 450, 532, and 600 nm was determined. The MDA content was calculated according to Hodges et al. (1999).

Assay for mineral element

The dried tissue of different plant parts was ground, weighed, and then digested using a microwave sample preparation system. One gram sample powder was digested with a solution containing 4 ml HNO_3 (71% w/w) and 1 ml of HCl (32% w/w), and then the digested sample was made up to 10 ml and analyzed by a Perkin Elmer Analyst 800 AAS. The mineral element (K, Zn, Fe, Mn, and Cu) concentration of different plant parts were measured by atomic absorption spectroscopy according to Rashed (1995). The EC10, the Zn concentration of nutrient solution in corresponding to a 10% plant biomass reduction, were used to determine the Zn threshold concentration causing plant toxicity (Odlare and Pell 2009), and EC10 was calculated by regression analysis. Biological transfer coefficient (BTC), the ratio of aboveground Zn content to root Zn content, was also calculated. The contents of nitrogen (N) and phosphorus (P) in the leaves were determined on ground subsamples of oven-dried plant material after digestion in a mixture of concentrated H_2SO_4 and H_2O_2. N was measured by the micro-Kjeldahl procedure and P by the vanadomolybdate method (Page 1982).

Statistical analyses

Statistical analyses were conducted using SPSS 11.5 for Windows. One-way ANOVA was used and pairwise comparisons between different Zn levels were conducted using Tukey's test at $P < 0.05$ levels.

Results

Effects of Zn treatment on morphological traits and total biomass accumulation

As shown in Fig. 1, the growth of grape was significantly enhanced with increasing Zn concentrations range from 0 to 7.0 mM, but a sharp decrease in the growth pattern was observed when the Zn concentration was further increased. The leaf showed the similar performance to Zn treatment, leaf size increased with increasing Zn levels below 7.0 mM. However, significant toxic symptoms could be observed as yellowing, brown spot, and necrosis under higher Zn levels. The total plant biomass was also increased by Zn range from 0 to 7.0 mM but decreased after that, the EC10 was 8.35 mM with the Zn surplus ranging from 7 to 35 mM. The root/aerial part ratio fluctuated with Zn treatment (Fig. 2), exhibiting lower values

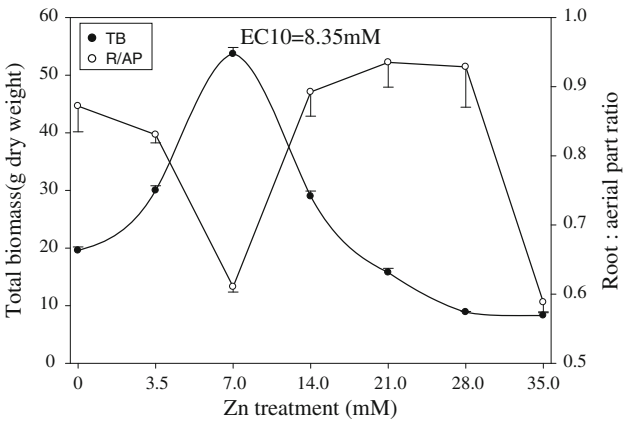

Fig. 2 The effects of Zn treatment on biomass accumulation and root/aerial part ratio in grape. Values shown are mean ± SE. *TB* total biomass accumulation, *R/AP* root/aerial part ratio

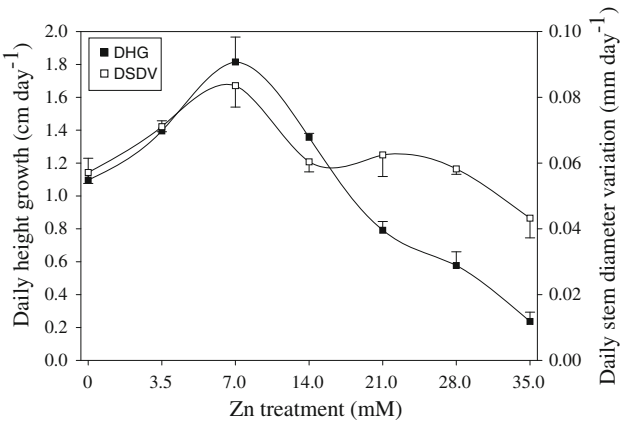

Fig. 3 The effects of Zn treatment on plant daily height growth and daily stem diameter variation in grape. Values shown are mean ± SE. *DHG* daily height growth, *DSDV* daily stem diameter variation

at 7.0 and 35.0 mM Zn concentration. Plant DHG and DSDV also showed the similar change as that of apparent growth trends, with pronounced increase under lower Zn levels but suppressed under higher Zn levels (Fig. 3).

Effects of Zn treatment on leaf chlorophyll

Leaf chlorophyll a, chlorophyll b, and total chlorophyll content was at constantly high level when treated with Zn, at concentrations up to 7.0 mM. Further chlorophyll contents decreased with increasing Zn levels beyond 7.0 mM (Table 1).

Nutrition balance under Zn treatments

In our study, the N, P, and K concentrations were determined only in leaf tissues (Fig. 4). Generally leaf N concentration was decreased with increasing Zn level, but there existed little differences on leaf N concentration among 0–7.0 mM Zn concentration. P concentration was decreased with the increasing of Zn concentration. On the other hand, leaf K concentration exhibited a curved change, it was peaked in 3.5 mM and then decreased.

For all the plant parts [leaves, shoot (stem and branches), roots], Zn concentration was increased with increasing Zn level (Table 2). However, shoots stored highest level of Zn, and leaf kept at lowest tissue level, with 270–600 μg/g below 14.0 mM Zn treatment. The BTC value of Zn was beyond 1.0 at middle Zn levels (7.0–14.0 mM) (Fig. 5). On the other hand, Fe concentration in root kept a high level under 0–14 mM and was significantly decreased at higher Zn level (21.0–35.0 mM), while Fe concentration in shoot and leaf exhibited a contrary trend, with significant reduction in shoot and pronounced increase in leaf under excess Zn levels. Mn and Cu concentrations in different parts (root, shoot, leaf) were decreased with increasing Zn level, and then increased at higher Zn levels (28.0–35.0 mM) (Table 2).

Effects of Zn treatment on antioxidant enzyme activity, root activity, ABA, and MDA

The effects of Zn treatment on antioxidant enzyme activity, root activity, ABA, and MDA were shown in Tables 1 and 3. The SOD activity was little affected by excess Zn, but the POD activity was decreased by Zn^{2+} stress. The CAT and PPO activities were increased with increasing Zn levels, peaked in 14.0 mM, and then decreased beyond that dose. Root activity was suppressed only under higher Zn levels as 28.0 and 35.0 mM (Table 1). ABA was profoundly enhanced at excess Zn levels ($P < 0.001$). The MDA content was observed to be constant at low content range of surplus Zn (0–7.0 mM), but significantly enhanced beyond 7.0 mM Zn levels.

Discussion

In our study, the growth of the grape, expressed by dry weight, plant DHG, DSDV, and morphological symptoms (Figs. 1, 2, 3), was increased by 3.5–7.0 mM surplus Zn concentration, but repressed by higher level of Zn. Previous studies also documented that plant growth in some

Table 1 The effects of Zn treatment on leaf photosynthetic pigments, root activity, and leaf ABA concentration in grape

Zn^{2+} (mM)	Chla (mg g^{-1} FW)	Chlb (mg g^{-1} FW)	Chla + b (mg g^{-1} FW)	ABA (μg g^{-1})	Root activity (TPF g h^{-1} g^{-1} FW)
0	1.731a	1.107a	2.838a	0.50e	48.06b
3.5	1.700a	0.995a	2.696a	19.40d	61.74a
7.0	1.647a	0.999a	2.647a	38.22c	53.82b
14.0	1.347b	0.671b	2.018b	42.75b	46.28b
21.0	1.263b	0.615b	1.878b	54.84a	46.64b
28.0	0.946c	0.509b	1.454c	44.51b	38.50c
35.0	0.546c	0.317c	0.864d	43.62b	30.61d

Values shown are means of at least three replicates. Values within a column followed by the same letter do not differ significantly at $P < 0.05$

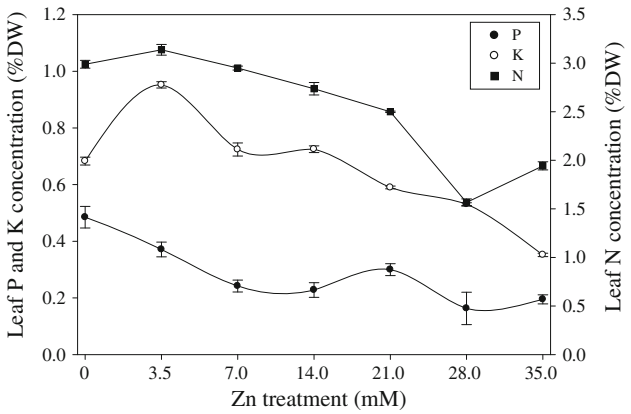

Fig. 4 The effects of Zn treatment on leaf N, P, and K in grape. Values shown are mean ± SE

Table 2 The effects of Zn treatment on some element content in leaf, shoot (stem + branch), and root of grape

Zn^{2+} (mM)	Zn (μg g^{-1})	Fe (μg g^{-1})	Mn (μg g^{-1})	Cu (μg g^{-1})
Root				
0	227.96e	309.20a	305.06ab	63.16b
3.5	2,039.16d	294.31a	279.25ab	35.31c
7.0	2,137.10d	324.60a	211.03c	46.49bc
14.0	3,225.75c	297.23a	239.99bc	45.65bc
21.0	6,984.64b	207.98b	219.53c	52.48bc
28.0	7,532.17a	249.24b	224.48c	42.27bc
35.0	7,553.35a	208.71b	318.89a	101.60a
Shoot				
0	220.50f	134.96a	274.49b	34.85b
3.5	3,219.33e	60.64c	173.00d	15.10d
7.0	5,786.47d	66.53c	84.31e	18.13 cd
14.0	6,880.56c	77.89bc	51.88f	24.46c
21.0	8,217.48b	61.12c	161.29d	32.19b
28.0	8,840.28a	73.86c	239.89c	35.04b
35.0	7,224.84c	93.60b	328.87a	36.89a
Leaf				
0	270.70e	93.36d	249.32c	108.49b
3.5	423.85de	155.14a	176.99de	114.69b
7.0	582.42d	146.05ab	184.90d	72.40d
14.0	1,007.54c	135.89b	155.11e	81.52c
21.0	1,088.60c	139.75b	203.76d	81.35c
28.0	1,604.85b	139.13b	287.55b	118.06b
35.0	2,982.97a	112.20c	460.29a	159.00a

Values shown are means of at least three replicates. Values within a column followed by the same letter do not differ significantly at $P < 0.05$. The concentration (μg g^{-1}) could be also expressed by ppm

species could be stimulated by Zn concentration that is far exceeding the toxic threshold for most other plant species. Those reported species are involved in Zn hyperaccumulator such as *Thlaspi caerulescens* (Tolrà and Poschenrieder 1996), *Sedum alfredii* Hance (Yang et al. 2004), *Arabis paniculata* (Tang et al. 2009), and Zn non-hyperaccumulator, e.g., *Silene vulgari* (Ernst 1998) and Geyer willow (Shanahan et al. 2007). We also observed that *V. vinifera* cultivar could be increased by 12–35 mM Mn treatment under sand culture (Mou et al. 2011). The mechanism for the stimulation effects is not clearly clarified, and many authors ascribed it to two reason: one is that those species may have a much higher requirement of Zn than normal plants; the other is the hormesis effects (Calabrese et al. 1999). Considering the stimulation effects on plant growth by 3.5–7.0 mM Zn and exceptional EC10 value that was rarely reported so high (Rout and Das 2009), we conclude that cultivar Combier is highly tolerant to Zn toxicity, and it has an exceptional ability on co-tolerance to Zn and Mn. Our discovery thus provides a new material to study the underlying mechanisms of stimulation effect and co-tolerance of multiple heavy metals. On the other hand, plant root exhibited much higher tolerance than aerial part, keeping constant or moderately increased level of root activity and accompanying with extremely high Zn concentration (3000–7000 ppm) in the roots (Tables 1, 2).

Chlorophyll is very sensitive to heavy metal and has been suggested as an indicator of metal toxicity (Clairmont et al. 1986). In agreement with the variations of plant growth, photosynthetic pigments (Chla, Chlb, and Chla + b) kept in high constant level among 0–7.0 mM Zn levels (Table 1), but were significantly decreased with higher surplus Zn supply, and chlorophyll loss resulted in leaf chlorosis under 21–35 mM Zn treatment (Fig. 1). The chlorophyll reductions were well documented in many previous studies due to a peroxidation of chloroplast membranes or the inhibition of chlorophyll biosynthesis (Gallego et al. 1996; Rau et al. 2007).

Under 0–7.0 mM of surplus Zn treatment, leaf Zn concentration kept at 270–600 ppm (Table 2), which could be considered to be in an adequate range of leaf Zn concentration for optimum growth in grape, although the Zn concentration in roots and shoots increased by tenfold. Previous studies documented that leaf Zn concentrations of 20–100 ppm are considered to be sufficient for normal plant growth (Marschner 1995), however, our study indicated that grape leaf could tolerate as high as 600 ppm with no toxic symptom. It was also observed that from increase to suppression in plant growth at 7.0–14.0 mM Zn treatments, leaf Zn concentration was doubled. Therefore, it is important to keep at relatively lower Zn concentration for Zn-sensitive photosynthetic organ (leaves), and store majority of excess Zn in non-sensitive organs (shoots and roots). This pattern of Zn distribution could improve grape tolerance to excess Zn. The limited transfer of excessive Zn to the leaves was also observed in sugarcane beet and red clover (Jain et al. 2010; Chen et al. 2003).

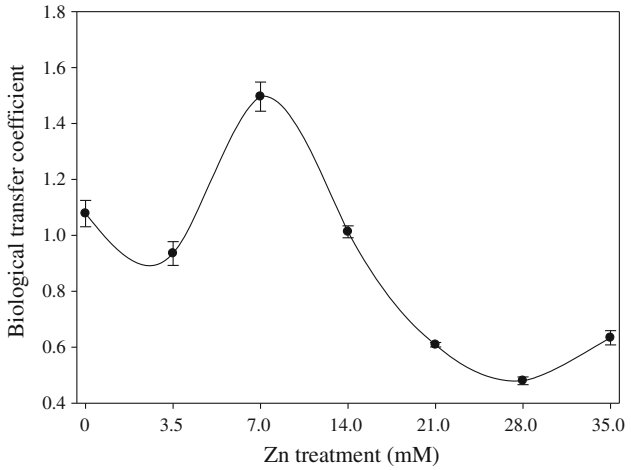

Fig. 5 The effects of Zn treatment on biological transfer coefficient for Zn in grape. Values shown are mean ± SE

In addition, different changes in microelement, such as Fe, Mn, and Cu contents in the different plant parts (Table 2), indicated that excess Zn interfered not only with nutrient uptake but also with nutrient distribution into the different plant parts. Because of the similarities in ion radii of bivalent cations (like Mn, Fe, Cu), excess Zn can shift certain physiological equilibria by local competition at various sites (Tewaru et al. 2008), such as at the primary absorption site or at the loading site of the roots. Therefore, excess Zn decreased Mn, Fe, Cu content in different plant parts. Nevertheless, grape leaf retained high level of Fe concentration partly due to increased translocation of iron (Fe) from root and shoot to leaves, which is very important for keeping high level of photosynthesis and carbon assimilation. Tolrà and Poschenrieder (1996) also reported that the stimulation and hypertolerance to excess Zn in *Brassicaceae* was accompanied with Fe translocation from roots to leaves. Being consistent with the growth stimulation at 0–7.0 surplus Zn level, N and K concentrations were significantly increased or kept at constant level (Fig. 4), while excess Zn interfered with phosphorus uptake and decrease leaf P concentration in our results, as conformed to recent report by Jain et al. (2010). Marked reduction of leaf N, P, and K concentration under higher Zn levels (14–35.0 mM) would ultimately reduce plant biomass and growth.

In our study, the grape retained good balance between ROS production and quenching capacity of the antioxidative system under 0–7.0 mM surplus Zn treatments, so MDA and PPO activity kept at a constantly low level (Table 3), accompanying with pronounced increase of CAT activity. Although both CAT and POD could reduce H_2O_2 to H_2O, CAT played a central role due to significant reduction of POD activity under excess Zn stress (Table 3). We also observed the similar changes of CAT and POD activity of three grape cultivars under excess Mn condition (Mou et al. 2011). Under high level of Zn treatment (beyond 21.0 mM), both CAT and PPO activity were decreased, possibly due to damage of enzyme function. In agreement with observation on *Populus cathayana* by Lei et al. (2007) and our previous study on grape tolerance to excess manganese (Mou et al. 2011), SOD activity kept relatively constant in all Zn treatments. Previous reports and our present results clearly show that the activity of individual participants of antioxidative enzyme systems is rather different and is often species-dependent. Lei et al. (2007) reported that Mn-tolerant population of *P. cathayana* exhibited higher ABA enhancement in comparison with sensitive population under excess Mn treatment. Hsu and Kao (2003) also found Cd treatment increased ABA content rapidly in Cd-tolerant rice cultivar, but not in the Cd-sensitive cultivar. Over 40-fold enhancement of ABA concentration were observed in our study (Table 1) and exhibited an important role for Zn tolerance in the grapes.

In conclusion, our results showed that *V. vinifera* cultivar Combier is highly tolerant to excess Zn, which is expressed by plant growth stimulation under 3.5–7.0 mM surplus Zn treatment and by extremely high EC10 value. Coupled with the increase in plant growth, grape retained low level of leaf Zn concentration, and also retained high level of leaf iron concentration due to increasing translocation of iron (Fe) from root and shoots to leaves. The

Table 3 The effects of Zn treatment on the antioxidant enzyme activity and MDA of grape based on fresh weight

Zn^{2+} (mM)	SOD (U g^{-1} FW)	CAT (μmol g^{-1} FW min^{-1})	POD (U g^{-1} FW)	PPO (U g^{-1} FW min^{-1})	MDA (μmol g^{-1})
0	278.59a	11.94d	3.21a	32.51c	5.68d
3.5	244.10a	20.58c	2.25b	39.87c	5.37d
7.0	243.33a	35.14b	1.53c	43.97c	5.59d
14.0	220.22a	46.27a	1.27c	181.86a	6.79c
21.0	261.43a	10.94d	1.22c	170.60b	7.17bc
28.0	215.11a	5.05e	0.94c	39.15c	8.90b
35.0	212.16a	8.13de	0.93c	12.38d	13.87a

Values shown are means of at least three replicates. Values within a column followed by the same letter do not differ significantly at $P < 0.05$ level

levels of leaf N and K were increased or constant at high level, paralleling with low ROS level and enhanced CAT activity. Generally excess Zn were stored in the non-sensitive plant parts (roots and shoots), and caused significant reductions of P, Fe, Mn, Cu in different parts. Due to its large root system, high annual biomass and high tolerance to excess Zn and Mn, we suggested that cultivar Combier could be used as pioneer plants in metalliferous site and in acidic soil of the tropical and subtropical area.

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