Scientia Horticulturae 238 (2018) 400–407

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Soil application of P can mitigate the copper toxicity in grapevine: physiological implications

E. Baldi[a,*], A. Miotto[b], C.A. Ceretta[c], G. Brunetto[c], E. Muzzi[a], G. Sorrenti[a], M. Quartieri[a], M. Toselli[a]

[a] Department of Agriculture and Food Sciences Bologna, University of Bologna, Italy
[b] Instituto Federal de Ciência e Tecnologia de Santa Catarina, Câmpus São Miguel do Oeste, Santa Catarina, Brazil
[c] Departamento de Solos, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil

ARTICLE INFO

Keywords:
Net photosynthesis
Plant biomass
Nutritional status
Copper toxicity
Phosphorus

ABSTRACT

The continuous use of copper-based fungicides in viticulture to contrast fungal diseases may result into an accumulation of Cu in the topsoil, reaching toxicity levels for plants and soil biota. A possible strategy to mitigate the negative effects of excess of Cu could involve the formation of insoluble molecules by the application of P to the soil. The aim of this study was to evaluate the effectiveness P soil application as a strategy to alleviate the toxic effect of excess of Cu on potted grapevines plants, with particular emphasis on the physiological response of plants. Micropropagated plants of 1103 Paulsen rootstock were grown according to a factorial experimental design with two factors: Cu (4 levels: 0, 100, 200, 300 mg kg^{-1} dw) and P (2 levels: 0 and 100 mg kg^{-1} dw) and three replicates. Net photosynthesis, efficiency of PSII photochemistry and linear electron transport rate decreased as soil Cu concentration increased. The addition of P increased net photosynthesis, improved the efficiency of PSII photochemistry and linear electron transport rate, but decreased stomatal conductance. As a consequence, shoot and root biomass declined with the increase of Cu concentration in soil, while the increment of P tackled this decline and improved nutritional status. CuEDTA extractable fraction in soil was increased by both the supply of Cu and P; this because the addition of P in combination with the highest Cu soil concentration determined a decrease of soil pH. In conclusion, the addition of P to the soil mitigated Cu excess toxicity symptoms in grape through a preservation of photosynthetic apparatus efficiency and an improvement of nutrient uptake.

1. Introduction

Copper (Cu) based fungicides, such as Bordeaux mixture, have been used intensively in Europe since the end of the 19th century to control vine fungal diseases (Brun et al., 1998; Chaignon et al., 2003). The consequent accumulation of Cu into the soil has pushed toward the substitution of traditional high rate of Cu sulfate ($CuSO_4$) with the low rate and more active Cu hydroxide and Cu oxychloride. Nonetheless, the European Union legislation has restricted the use of only 6 kg Cu ha^{-1} y^{-1} in order to reduce the Cu related pollution impact (European Commission, 2002). However, the intensive and long-term application of this fungicide has led to the accumulation of Cu in soils that have been found to contain 100 to 1500 mg Cu kg^{-1}, exceeding the background values (5–30 mg kg^{-1}) by up to 300 times (Brun et al., 1998, 2001; Chaignon et al., 2003), with a negative influence on soil flora and fauna and on human health. The accumulation of Cu in the topsoil of vineyard can lead to phytotoxicity, yield losses and impairment of wine quality (Ninkov et al., 2012) becoming a primary public concern all over the world (Garcia-Esparza et al., 2006; Lai et al., 2010).

In general, the main Cu phytotoxic symptoms on plants include stunted growth, chlorosis, senescence of leaves (Kopittke and Menzies 2006; Michaud et al., 2008; Wei et al., 2008; Mourato et al., 2009), reduction of root growth and elongation (Kopittke and Menzies 2006; Chopin et al., 2008; Juang et al., 2011).

Several options to remediate Cu toxicity have been developed including immobilization through pH alterations, organic matter addition, Cu removal, sequestration, active mixing and plant uptake or phytoextraction (Pietrzak and Uren, 2011; Mackie et al., 2012). Phosphate has been proven to immobilize heavy metals in contaminated soils mainly due to the P ability in creating stable metal forms (Cao et al., 2004; Miretzky and Fernandez-Cirelli 2008). The use of phosphoric acid (Yang and Mosby 2006), natural phosphate rocks (Ma et al., 1995) and synthesized apatites (Ryan et al., 2001) were found able to

* Corresponding author.
E-mail address: elena.baldi7@unibo.it (E. Baldi).

https://doi.org/10.1016/j.scienta.2018.04.070
Received 18 September 2017; Received in revised form 19 February 2018; Accepted 29 April 2018
Available online 11 May 2018

reduce lead mobility in polluted soils. In addition phosphate-containing compounds were successfully applied to stabilize cadmium (Raicevic et al., 2005), zinc (Zn) (Wang et al., 2001; Chen et al., 2006) and Cu (Wang et al. 2001; Cao et al., 2004) in contaminated soils.

Beside a direct effect of phosphate in immobilizing Cu in soil; P plays a fundamental role on plant physiology being fundamental for energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling and carbohydrate metabolism (Marschner, 1995; Taiz and Zeiger, 2006). Therefore, the supply of P could help, in different ways, plants to better manage the Cu excess in soil.

The aim of the present experiment was to evaluate the effectiveness of P soil addition on plant physiology to mitigate the toxic effect of excess of Cu on grapevine on sandy non-calcareous soil. The present study is the continuity of a previous one (Baldi et al., 2018) which stressed the response of root morphology and physiology to P application under high Cu soil concentration.

2. Material and method

2.1. Plant material and experimental conditions

The study was carried out at the Department of Agricultural Sciences of the University of Bologna in the same experimental condition of an analogous experiment already published (Baldi et al., 2018). Similarly to the former trial micro-propagated plants of Paulsen 1103 (*Vitisberlandier*i X *V. rupestris* L.) were transplanted in 5 L pots with 4 kg of soil and grown in the greenhouse for 100 days. Four levels of Cu (0, 100, 200 e 300 mg kg^{-1} dry soil) and two levels of P (0 and 100 mg kg^{-1}dry soil) were combined to set up a factorial experimental design with two factors and 3 replicates. The non-calcareous soil used in this experiment (Table 1) was collected in the dolomitic area of Laimburg, South Tirol (46°23′04,55″N; 11°16′56, 95″E). To increase the porosity, before the addition of Cu and P the soil was mixed with a 30% silica not calcareous sand (Accornero s.r.l., Viarigi, Asti, Italy) and then sieved at 2 mm. Copper was added to the soil as a concentrated solution of $CuSO_4$.(5.H_2O; 30 days later, the levels of P were established by adding a grounded triple superphosphate.

2.2. Soil analysis

Soil samples were collected at transplanting of plants and air dried until constant weight. pH was determined in a soil:water 1:1 (v/v) proportion suspension in a after 30 min of equilibration (Tedesco et al., 1995). The fraction of EDTA-exctractable Cu (CuEDTA) was determined according to Chaignon et al. (2009). Briefly, a sample of 0.25 g dry soil was added to a 15 mL capacity centrifuge tube and agitated for 2 h with 10 mL of solution containing disodium EDTA 0.01 mol L^{-1} + NH_4CH_3COO 1.0 mol L^{-1}, with the pH corrected to 7.0. After agitation, the suspension was centrifuged and filtered. The fraction of Mehlich-exctractable P (PMel) was determined according to Tedesco et al. (1995) by extracting 10 g of soil with 100 mL of Mehlich solution (0.05 mol L^{-1}HCl + 0.0125 mol L^{-1} H_2SO_4) for 16 h. For the determination of total Cu and P, soil was mineralized according to US EPA Method 3052 (Kingston, 1988) by treating 0.5 g of dry soil with 6 mL of hydrochloric acid (37%), 2 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) at 180 °C in an Ethos TC microwave labstation (Milestone, Bergamo, Italy). All the extracts were analyzed by atomic absorption spectrophotometry (VarianAA200, Mulgrave, Victoria, Australia).

Table 1
Main soil properties at the beginning of the trial before sand addition.

Soil characteristics	Value
Clay (g kg^{-1})	320
Sand (g kg^{-1})	260
Organic matter (g kg^{-1})	23
pH in H_2O	6.0
P (mg kg^{-1})	42
Cu (mg kg^{-1})	8.0
Exchangeable K^+ (mg kg^{-1})	112
Exchangeable Ca^{2+} (cmol$_c$ kg^{-1})	3.2
Exchangeable Mg^{2+} (cmol$_c$ kg^{-1})	1.7
Exchangeable Al^{3+} (cmol$_c$ kg^{-1})	0
CEC (cmol$_c$ kg^{-1})	5.2

2.3. Plant measurements

Leaf net photosynthesis (Pn), stomatal conductance (gs), efficiency of excitation capture by open PSII reaction centers(Fv'/Fm'), efficiency of PSII photochemistry (PSII) and linear electron transport rate (ETR) were determined using an open circuit infra-red gas exchange system (Li-COR 6400, LI-COR, Lincoln, Nebraska, USA) equipped with a fluorimeter on the first two mature leaves from the shoot apex at 60 and 100 days after transplant (DAT).

At 60 and 100 DAT 24 plants (3 for each treatment) were removed from the pots, divided into leaves, shoot and roots and washed with distilled water. Samples were oven dried (65 °C) to constant weight, weighted, milled and mineralized according to US EPA Method 3052 (Kingston, 1988) by treating 0.5 g of dry matter with 8 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (30%) at 180 °C in an Ethos TC microwave labstation (Milestone, Bergamo, Italy). Calcium (Ca), potassium (K), magnesium (Mg), Cu, iron (Fe), manganese (Mn) and Zn concentration were determined by atomic absorption spectrophotometry (SpectrAA-200, Varian, Australia). Phosphorous content was determined as follows (Saunders and Williams 1955): the extracts were mineralized with 96% (v/v) sulphuric acid and 35% (v/v) oxygen peroxide, neutralized with 0.1 M sodium hydroxide and enriched with 0.1 M ascorbic acid, 32 mM ammonium molybdate, 2.5 M sulphuric acid and 3 mM potassium antimonyl tartrate to develop a phosphomolybdic blue colour; P was spectrophotometrically quantified at 700 nm.

Transfer factor (TF) index was calculated as the ratio of metal concentration in shoots (TFS) and leaves (TFL) to roots and was used to measure the effectiveness of plant in translocating Cu from roots to shoots and leaves (Sun et al., 2009; Cambrollé et al., 2013).

2.4. Statistical analysis

All the data were statistically analyzed using the software SAS 9.0 (SAS Institute Inc., Cary, North Carolina, USA), as in a factorial experimental design with Cu addition (4 levels: Cu0, Cu100, Cu200 and Cu300), P addition (2 levels: P0 and P100) and time (60 and 100 DAT) as main factors. When analysis of variance showed an effect of treatment statistically significant ($P \leq 0.05$), means were separated by Student Newman-Keuls (SNK) test; when interaction between factors was significant, 2 times standard error of means (SEM) was used as the minimum difference between two means statistically different for $P \leq 0.05$. Since sampling time did not have any effect, data of organ biomass, P and Cu concentration in leaves and roots were reported only at 100 DAT.

In addition, Pearson correlation analysis was performed to evaluate the linear relationship between all the parameters measured; in the paper only the most interesting data were presented.

Procrustes analysis was performed with the R statistical software to evaluate the different distribution of data between 60 and 100 DAT for macro and micronutrient leaves concentration, plant mineral nutrient content and photosynthesis. In this analysis differences between the mean scores of individuals are examined in the translation terms of the analysis and in the between groups sum-of-squares. After eliminating