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Structural changes in roots of peach rootstock cultivars grown in soil with high zinc content

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ABSTRACT

The increase in zinc (Zn) content in orchard soils caused by successive applications of fertilizers and fungicides to control foliar diseases can modify the structure of the roots of young peach trees. The study aimed to evaluate the effect of the application of Zn doses in soil on the morphological and anatomical structure of the roots and on the growth of young peach plants grafted on three clonal rootstock cultivars. Samples of a Typic Hapludalf were collected, air dried, sieved and subjected to the application of 0, 60 and 120 mg Zn kg^{-1}. After the incubation period, one plant of each rootstock cultivar (Flordaguard, Rigitano and Tsukuba-1) was transplanted into rhizoboxes and grown for 65 days. In the shoots, we determined dry matter production and Zn concentration in tissue. In the roots, we analyzed root morphology using light microscopy. The increase in the availability of Zn in the soil stimulated the production and accumulation of phenolic compounds in the cortex and vascular cylinder of the roots, in addition to increasing the concentration of Zn in plant tissue to values above those considered normal. Rigitano and Tsukuba-1 rootstock cultivars exhibited cell rupture in the cortex, resulting in the formation of intercellular spaces. Flordaguard rootstock cultivar was the least sensitive to morphological and anatomical changes in the root apex and, therefore, may be the most recommended for cultivation in soils with high Zn contents.

1. Introduction

Orchards of peach [*Prunus persica* (L.) Batsch] are subjected to pre-planting fertilization with organic fertilizer sources when the need for nutrients such as nitrogen (N), phosphorus (P) and potassium (K) is diagnosed (Brunetto et al., 2007). However, these fertilizers also contain heavy metals such as zinc (Zn). In addition, peach trees are annually subjected to successive applications of fungicides for the preventive control of fungal diseases in leaves and fruits (Pavanello et al., 2016). As some fungicides have heavy metals (e.g., Zn) in their composition and, because of the unidirectionality of the applications, the flow of Zn from the shoots to the soil, as well as the deposition of senescent leaves and pruned branches (with Zn) on the soil along the cycles, there is an increase in Zn content in these soils (Mackie et al., 2012; Tiecher et al., 2016).

Zn is an essential micronutrient to plants and it is associated with carbohydrate metabolism, gene regulation and expression, ribosome structural integrity, phosphate metabolism and synthesis of several enzymes (Kabata-Pendias, 2011). In the soil, contents of available Zn below 0.2 mg dm^{-3} may be deficient to plants, but contents above 0.5 mg dm^{-3} may be considered high or excessive (CQFS-RS/SC, 2016), and higher contents of this element may cause toxicity fruit trees (Tiecher et al., 2017). Excess Zn concentration in plants may cause morphological and anatomical disturbances at the root apex (Stolárikova et al., 2012;

Mousavi Kouhi et al., 2016), causing an increase in mean root diameter (Ambrosini et al., 2015; Bochicchio et al., 2015), premature endodermal differentiation and lignification of cortical tissues (Arduini et al., 1994), as well as changes in the cell division process (Potters et al., 2007). Furthermore, Zn toxicity in plants may also increase the

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production and accumulation of secondary metabolites, such as phenolic compounds in roots (Michalak, 2006; Bouazizi et al., 2010).

Structural and metabolic changes in the root system due to high contents of heavy metals in the soil may reduce the uptake of water and nutrients and the photosynthetic rate, which decreases plant growth and development (Cambrollé et al., 2015). However, the effect of excess soil Zn on the root system of peach rootstock cultivars is not well known, nor is the rooting potential of most peach cultivars and their morphological and phenological characters, although these are typically selected by the high percentage of rooting and high rates of root formation (Tomaz et al., 2014). The aim of this study was to evaluate the effect of the application of Zn doses in soil on the morphological and anatomical structure of the roots and on the plant growth of three peach rootstock cultivars.

2. Material and methods

2.1. Description of the experiment

The soil is a Typic Hapludalf (Soil Survey Staff, 2006), generally used in peach production in southern Brazil. The soil was collected at 0.00–0.10 m in the experimental area of the Universidade Federal de Santa Maria (UFSM), located in the city of Santa Maria, state of Rio Grande do Sul, southern Brazil (29°45′S, 53°42′W and 95 m altitude). The soil was air dried, broken down and passed through a 2 mm mesh sieve. A portion of the soil was submitted to analysis of chemical and physical attributes (Table 1).

The remaining soil was divided into 2.5 L^{-1} portions, in which the doses of 0 (control/natural content of Zn), 60 and 120 mg Zn L^{-1} were applied as a solution of $ZnSO_4.7H_2O$. The soil was manually stirred, placed into plastic bags and incubated for 60 days in a greenhouse. Soil moisture was monitored by weighing every two days. Distilled water was applied when necessary to maintain the maximum water holding capacity (MWHC) at 60%. After incubation, the soil was air dried, sieved with a 2 mm mesh and transferred to rhizoboxes. Each rhizobox had dimensions of 20 cm in length, 32 cm in height and 4 cm in width, built on wooden frame and the internal faces covered with acrylic (Fig. 1a). The faces of the rhizobox were covered with aluminum foil to avoid the incidence of light within the soil mass. The rhizoboxes were placed onto wooden supports at a 45° slope.

For the clonal propagation of the rootstocks, herbaceous cuttings of 15 cm in length were prepared from branches harvested in November 2012 in three-year-old parent plants kept in the "*Prunus* Rootstock Collection" of Embrapa Clima Temperado, in Pelotas, Rio Grande do Sul, Brazil. The rootstock cultivars used in this study were Flordaguard ('Chico-11′ x *Prunus davidiana*), Rigitano (*Prunus mume*) and Tsukuba-1 (*Prunus persica*). The adventitious rooting of the herbaceous cuttings was carried out in masonry benches filled with fine vermiculite, under an intermittent mist chamber. After the rooting period, the cuttings were transplanted into plastic bags (30 cm × 18 cm) containing commercial substrates based on bark (30%) and peat (70%), and then transported to a greenhouse with a shading screen for initial acclimatization. The clonal rootstocks were conducted on a single stem and grafted with cv. Jade (*Prunus persica*) by patch budding at the beginning of 2014. In July 2014, the seedlings were removed from the plastic bags containing substrate and the roots were washed with distilled water. Then, one seedling was transplanted into each rhizobox containing one of the Zn doses. The experimental design was completely randomized with 5 replicates of each rootstock cultivar (Flordaguard, Rigitano and Tsukuba-1) at each dose of Zn (0, 60 and 120 mg Zn L^{-1}), totaling 45 sample units.

The experiment was conducted in greenhouse with average temperature of 25 °C and average air humidity of 60%. The rootstock cultivars were grown for 65 days (August to October 2014). Every two days throughout the cultivation period, each rhizobox was weighed and distilled water was added when necessary to maintain the MWHC at 60%. At 30 days after transplanting (DAT), we applied 100 mL of nutrient solution containing 50 mg N kg^{-1} soil (urea), 50 mg P kg^{-1} soil (P_2O_5) and 62 mg K kg^{-1} soil (KH_2PO_4).

Table 1
Chemical and physical properties of a Typic Hapludalf at 0.0–0.10 m prior to the installation of the experiment.

Properties	Value
Clay (Pipette method) (g kg^{-1})[a]	250
Organic matter (Organic carbon method) (g kg^{-1})[a]	13.7
pH in water (1:1 ratio)[b]	5.8
Exchangeable Al (extracted by KCl 1 mol L^{-1}) ($cmol_c$ dm^{-3})[b]	0.5
Exchangeable Ca (extracted by KCl 1 mol L^{-1}) ($cmol_c$ dm^{-3})[b]	2.42
Exchangeable Mg (extracted by KCl 1 mol L^{-1}) ($cmol_c$ dm^{-3})[b]	2.32
Available P (extracted by Mehlich-1) (mg dm^{-3})[b]	50.9
Available K (extracted by Mehlich-1) (mg dm^{-3})[b]	18.3
Available Zn (extracted by EDTA) (mg dm^{-3})[b]	16.18
Cation exchange capacity ($CEC_{pH7.0}$) ($cmol_c$ dm^{-3})[c]	12.0
Al saturation (%)[b]	15.5
Base Saturation (%)[c]	40.0

[a] Embrapa (1997).
[b] Tedesco et al. (1995).
[c] Calculated according to CQFS-RS/SC (2016).

2.2. Dry matter production and total Zn content in tissues

At 65 DAT, the plant shoots were cut at soil level with a pruning shear, and then separated into leaves, stem and branches. Afterwards, the soil surrounding the roots was removed with a brush to minimize damage to external root tissue. The surface of the rhizobox was divided into quadrants for the standardization of the root collection site. The roots, at least 3.0 cm in length (Fig. 1b) measured from the root apex, were collected with a disposable stainless steel blade (Fig. 1c). The roots were immediately fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer for morphological analysis (Fig. 1d). The remaining roots were separated from the soil by hand, washed in running water, distilled water and then reserved.

The reserved leaves, branches, stem and roots were dried in an oven with forced air at 65 °C until constant dry matter. Then, dry matter production was determined using a precision scale. After drying, the samples were ground in a Wiley-type mill and the total concentrations of Zn in shoot organs and roots were determined after digestion of 0.1 g of tissue in 3.0 mL of HNO_3 and 1 mL of $HClO_4$ (Embrapa, 1997). The total concentrations of Zn in samples were determined in an atomic absorption spectrophotometer (AAS) (AAnalyst 200, PerkinElmer, United States) (Embrapa, 1997).

2.3. Zn available in soil

A soil sample was collected from each rhizobox, and then it was air dried, passed in a 2 mm mesh sieve and reserved. After drying, the soil was weighed on a precision scale and Zn was extracted by EDTA (Chaignon and Hinsinger, 2003). We determined the available Zn content in the extract in atomic absorption spectrophotometer (AAS) (AAnalyst 200, PerkinElmer, United States). The available Zn contents in soil after cultivation of the plants were 16.2, 55.1 and 94.1 mg Zn kg^{-1} in the control soil and the 60 and 120 mg Zn kg^{-1} treatments, respectively.

2.4. Morphological analysis of the roots

The fixed roots were washed in 0.1 mol L sodium phosphate buffer solution, followed by washing in distilled water. A sonicator (Maxiclean 1400, Brazil) was used to remove remaining soil particles in the roots. The roots were dehydrated in ethanol series (10%, 30%, 50% and 70%) and were kept in 70% ethyl alcohol until the time of evaluation