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Root respiratory components of *Prunus* spp. rootstocks under low oxygen: Regulation of growth, maintenance, and ion uptake respiration

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ABSTRACT

Hypoxia in the root zone activates several mechanisms to cope with the energetic imbalance that triggers hypoxia. *Prunus* is a hypoxia-sensitive species, and there is not information about the relationship between components of the root respiration and the hypoxia tolerance. We investigated whether adjusting root respiratory components plays a role in the hypoxia tolerance in *Prunus* rootstocks genotypes. Three *Prunus* rootstocks genotypes, 'Mariana 2624' (tolerant), 'CAB6P' (moderately sensitive) and 'Mazzard F12/1' (sensitive), were exposed to normoxia and hypoxia in a hydroponic system for 30 days. During the first 10 days of the experiment, the hypoxia-tolerant genotype under hypoxia exhibited a higher root relative growth rate, maintained net nitrogen uptake rate, and had lower protein turnover and electrolyte leakage, as well as increased root porosity (aerenchyma formation from 0.3 to 6.0 cm from the root tip at 30 days of experiment) compared to hypoxia-sensitive genotypes. 'Mariana 2624' roots exhibited tolerance to hypoxia by reducing maintenance respiration by 50% compared to normoxia, while growth respiration increased. Control of root respiratory components, early response of ion uptake favors root relative growth rate, and change of the root anatomy are associated with the ability to tolerate hypoxia by reducing maintenance costs.

1. Introduction

Low O_2 availability (hypoxia) in the rhizosphere alters plant metabolism causing a dramatic reductions in growth in a wide range of plant species (Greenway and Gibbs, 2003). One of the most important factors that control O_2 diffusion into the rhizosphere is the water level in the soil. This is because in water, O_2 diffusion is around 10.000-fold less than in the air (Armstrong and Drew, 2002). In many regions of the world, high rainfall and/or poor soil drainage induce hypoxia in plants and may have negative effects on crop production (Bailey-Serres and Colmer, 2014). The response to hypoxia varies greatly among species, from tolerant that are normally adapted to wetland conditions (Nakamura and Nakamura, 2016) to sensitive species that are adapted to dry conditions where hypoxia may reduce the plant growth (Amador et al., 2012; Liu et al., 2015; Pimentel et al., 2014).

Hypoxia has a negative impact on the whole plant behavior, but the roots can be the most affected organs because low oxygen impacts directly on the root respiration (Bailey-Serres and Colmer, 2014). For proper functioning, roots require a suitable supply of O_2 otherwise anaerobic respiration will occur, inducing an 'energy crisis' in the plants because of a decreased ATP supply (Greenway and Gibbs, 2003). In roots, the energy generated by root respiration can theoretically be separated into three major components: growth, maintenance and ion uptake (Lambers et al., 2008; Poorter et al., 1991). Briefly, growth respiration is the component that produces energy for new biomass synthesis (Penning de Vries et al., 1974). Maintenance respiration involves energy for processes related to structural and cellular maintenance (Penning de Vries, 1975), and ion uptake respiration supplies energy for nutrient uptake, which is principally nitrate (NO_3^-) (Veen, 1980). The studies on respiration components have been done in different species under different environmental conditions (Bouma et al., 1996; Laureano et al., 2013; Nakamura and Nakamura, 2016; Poorter et al., 1991; Rachmilevitch et al., 2006; Thongo M'Bou et al., 2010; van der Werf et al., 1988). However, there are no previous reports about of the root respiratory components in woody fruit trees under hypoxic stress. A recent study in *Carex* species shows that the respiratory components could be a key factor in the O_2 allocation in roots under hypoxic conditions, as well as in the relative growth rate (RGR) and nitrogen net uptake rate (NNUR) of roots (Nakamura and Nakamura, 2016).

The root RGR and NNUR are sensitive to O_2 deficiencies, but the hypoxia-tolerant species may have a continuous ion uptake in

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developing root tissues to maintain growth (Liu et al., 2015). According to van der Werf et al. (1988), lower costs related to the ion uptake processes could be associated with high O_2-use efficiency. Typically, the cost of growth and maintenance has received more attention (Laureano et al., 2013) than the costs of ion uptake (Bouma et al., 1996; Nakamura and Nakamura, 2016; van der Werf et al., 1988; Veen, 1980). The cost of ion uptake is importance because the mineral uptake by roots it is a major sink for respiratory energy (van der Werf et al., 1988; Veen, 1980). However, ion uptake respiration is dependent on the reduction state of nitrogen, and thus, NO_3^- has a higher costs of uptake than NH_4^+ (Lambers et al., 2008; Nakamura and Nakamura, 2016). To maintain the ion uptake from soil under hypoxia, is important to keep functioning the whole protein pool and avoid damage to cell membrane (Greenway and Gibbs, 2003). Maintaining protein turnover and the membrane stability is one of the most expensive processes related to maintenance respiration and both processes may account up to 80% of the root respiratory costs (Bouma et al., 1994). Through the control of protein turnover and membrane stability, it is possible to avoid an excess consumption of assimilates for the root maintenance respiration, which could be a significant factor that limits plant growth (Penning de Vries, 1975). Wetland species have many mechanisms to cope with low O_2, including anatomical changes such as the formation of root cortical aerenchyma and hypertrophied lenticels (Parent et al., 2011; Yamauchi et al., 2013). The aerenchymatous roots may allow O_2 diffusion from the shoot to the root (Yamauchi et al., 2013) to maintain and avoid inhibition of root respiration (Perata and Alpi, 1993; Shimamura et al., 2010). Strategies to generate aerenchyma in the cortex cells of the roots are widely known in wetlands species (Colmer, 2003; Kreuzwieser et al., 2004; Shimamura et al., 2010), but little information is known in woody fruit trees. In *Prunus*, for example, the aerenchyma formation was observed after 14 days of waterlogging in a hypoxia-tolerant species (Pimentel et al., 2014).

The *Prunus* genus has been classified as very sensitive to soil hypoxia (Amador et al., 2012; Pistelli et al., 2012). Normally, in commercial orchards of cherries, peaches and other stone fruits, the scion are grafted onto rootstocks, which can be obtained from the same or other *Prunus* species (Webster, 2005). Rootstocks have a direct influence on the scion and can determine the tolerance or sensitivity to hypoxia (Domingo et al., 2002). Most of the *Prunus* species used as rootstocks are classified as hypoxia-sensitive, although differences between genotypes in their ability to tolerate this stress have been reported (Amador et al., 2012; Pistelli et al., 2012; Ranney, 1994). For example, 'Mariana 2624' is classified as tolerant, 'CAB6P' as moderately sensitive, and 'Mazzard F12/1' as sensitive (Pimentel et al., 2014). However, the changes in the respiratory components of the different *Prunus* rootstock genotypes under hypoxia, as well as the cost of protein turnover and maintenance of cell membrane stability is still unknown. To investigate the relationship between maintenance respiration and tolerance to hypoxia, we therefore hypothesize that the hypoxia-tolerant genotype presents lower costs associated with the maintenance processes (maintenance respiration) than the hypoxia-sensitive genotype.

2. Materials and methods

2.1. Plant material and growth conditions

Self-rooted cloned 1-year-old plants of three *Prunus* rootstocks genotypes with contrasting tolerance to hypoxia were obtained from a commercial nursery (Agromillora Sur S.A., Talca, Chile). Healthy 'Mariana 2624' (*P. cerasifera* x *P. munsionana* W. Wight & Hedrick; tolerant), 'CAB6P' (*P. cerasus* L.; moderately tolerant) and 'Mazzard F12/1' (*P. avium*; sensitive) plants were selected and acclimated for 10 days in a greenhouse. All plants were virus-free and in the same vegetative developmental stage. The plants were grown hydroponically in a full-aerated 50% strength nutrient solution according to Hoagland and Arnon (1950) and adapted to *Prunus* by Jiménez et al. (2008). The nutrient solution was renewed weekly. The macronutrient composition (mM) was as follows: 2.5 $Ca(NO_3)_2$, 2.5 KNO_3, 1 $MgSO_4$, and 1 KH_2PO_4. Micronutrients concentrations (μM) were as follows: 46.2 H_3BO_3, 9.2 $MnCl_2$, 0.38 $CuSO_4$, 2.4 $ZnSO_4$, 0.12 $NaMoO_4$, 90 Fe(III)-EDTA and 2.5 M MES, and the pH was adjusted to 6.3 using KOH as needed. During the experiment, the night/day temperature was 17/30 °C, and the humidity fluctuated between 40–60%. In the greenhouse, the natural irradiation was supplemented with light from metal halide lamps, to have a photoperiod of 16/8 day/night and a maximum light intensity of 810 ± 25 μmol PAR m^{-2} s^{-1} on the top of the plants.

2.2. Normoxia and hypoxia treatments

Twenty-eight plants were grown under two O_2 treatments for 30 days. Normoxia was obtained by continuous air bubbling into the nutrient solution and maintained at 7.8 ± 0.4 mg O_2 L^{-1} (Supplementary Table 1). Hypoxia was obtained according to Wiengweera et al. (1997), by bubbling gaseous nitrogen (100 ml min^{-1}) overnight into 0.1% (w/v) agar nutrient solution, which was added to each pot and renewed weekly. For hypoxia treatment, the O_2 concentration was maintained at 0.5 ± 0.2 mg O_2 L^{-1} (Supplementary Table 1). The O_2 concentration was periodically checked by a Clark-type oxygen electrode sensor (Oxygraph, Hansatech Instruments, Norfolk, UK).

2.3. Determination of root growth, RGR and NNUR

The root dry matter was measured every 10 days and was determined (mg $plant^{-1}$) after drying in an oven at 70 °C for 72 h. To determine the root RGR, seven whole root systems per rootstock were sampled at each harvest. A polynomial function was fitted to ln-transformed root dry biomass *vs* time to calculate RGR (Poorter, 1989) using Eq. (1):

$$RGR = b + c \times X \quad (1)$$

where, RGR is the relative growth rate of roots (mg g^{-1}DW d^{-1}), b and c are the first and second coefficients derived from the polynomial function, respectively and X is the time (days).

The NNUR (μmol N g^{-1} DW d^{-1}) was calculated according to Garnier (1991) using Eq. (2), assuming that NO_3- is the principal nitrogen source and total anion taken up by plants is NO_3- (Veen, 1981):

$$NNUR = \frac{N2 - N1}{DW2 - DW1} \times RGR \quad (2)$$

where N2 and N1, are the elemental nitrogen content of the whole plant measured over a period of time. DW2 and DW1 are the root dry biomass measured in a period of time.

2.4. Determination of root respiration and its components

Root respiration measurements were performed in seven intact plants of each genotype every 10 days. The root systems from both treatments were transferred to an enclosed airtight dark flask containing 400 ml of air-saturated nutrient solution. The root respiration was measured as the capacity of roots to take up O_2 from the nutrient solution. Root respiration was measured using a Clark-type liquid-phase O_2 electrode (Hansatech Co. Ltd., Norfolk, UK) connected to a constant-temperature circulating water bath (Labtech, Singapore) at 25 °C, and the O_2 concentration was monitored over 10 min. The root respiration rate (mmol O_2 g^{-1} DW d^{-1}) was obtained from the slope of the linear regression of O_2 concentration over the time.

The three major components of root respiration—maintenance, growth and ion uptake—were determined using Eq. (3). This was derived from a multiple linear regression analysis between root respiration, RGR, and NNUR (Lambers et al., 1983; Poorter et al., 1991).

$$R_t = R_m + [C_g] \times RGR + [C_u] \times NNUR \quad (3$$