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Full length Research Paper

Ferric chelate reductase activity under iron deficiency stress in Azalea

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Evergreen azaleas grow optimally in acidic soil (pH 4.5 - 6.0) with adequate iron (Fe) levels. To combat Fe deficiency chlorosis when cultivated in alkaline soil under Fe shortage, Fe-efficient Strategy I azalea plants exploit the response mechanism of Ferric Chelate Reductase (FCR) activity. In this study, the ferric (Fe^{3+}) reduction capacity of two azaleas (*Rhododendron* x *pulchrum* 'Sen-e-oomurasaki' and *Rhododendron obtusum* 'Kirin') was evaluated during 10 days of cultivation in control (pH 6) and Fe-deficient (pH 9) nutrient solutions. At day 6, Fe deficiency tolerant and sensitive genotypes were distinguishable based on FCR activity. The survey of FCR response is suggested for rapid screening before visual symptoms occur in Fe deficiency tolerant azaleas.

Keywords: Alkalinity, chlorosis, hydroponics, iron efficient, *Rhododendron*, sodium hydrogen carbonate.

INTRODUCTION

Iron (Fe) is an essential micronutrient for plant growth and development (Marschner, 1995). While Fe is generally present in high quantities in cultivated soil, its bio-availability is often hampered by high bicarbonate concentration and above neutral pH levels (Marschner et al., 1986). Consequently, Fe deficiency is a worldwide problem that affects the production of many plants in calcareous soils (Marschner, 1995; Hansen et al., 2007). Inadequate Fe nutrition commonly inhibits root growth and causes interveinal chlorosis in new leaves (Schmidt, 1999). Higher plants have developed two strategies to cope with low iron availability in soil (Marschner et al., 1986). Strategy I plants (all dicots and monocots, except *Gramineae*) use several means to enhance Fe acquisition: chelating organic compound secretion, proton extrusion, ferric (Fe^{3+}) reduction, ferrous (Fe^{2+}) transportation, and changes in root morphology and cytology, while Strategy II plants (*Gramineae*) enhance extrusion of chelating non-proteinogenic amino acids (phytosiderophores) and related uptake systems (Schmidt, 1999).

Root Ferric Chelate Reductase (FCR) activity has been widely used to predict Fe deficiency tolerance in several fruit trees and vegetables (De la Guardia and Alcántara, 2002; Gogorcena et al., 2005), but rarely has it been employed in ornamentals (Albano and Miller, 1996; Valdez-Aguilar and Reed, 2006). Evergreen azaleas (family *Ericaceae*, genus *Rhododendron*, subgenus *Tsutsusi*), classified as Strategy I plants, are among the top selling ornamental pot plants worldwide (AIPH and Union Fleur, 2013). They require an acidic growing medium with a pH range between 4.5 and 6.0 for optimal development (Galle, 1987). In alkaline substrates, Fe chlorosis affects their production (Kofranek and Lunt, 1975; Wallace and Wallace, 1986). Scariot and Kobayashi (2008) reported evidence of azalea alkaline pH adaptability (up to 8.0) in Japanese wild habitats.

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Additional studies screened some Japanese genotypes for Fe deficiency tolerance in hydroponics to identify morphological changes (Demasi et al., 2015; Scariot et al., 2013). Azalea root FCR activity has never been characterized.

In this study we examined the FCR activity time course of a tolerant (*Rhododendron* x *pulchrum* 'Sen-e-oomurasaki') and a sensitive (*Rhododendron obtusum* 'Kirin') azalea in both control and Fe-deficient growing solutions. Fe deficiency was induced by the addition of sodium hydrogen carbonate ($NaHCO_3$), an effective medium buffer to screen for iron efficient plants (Campbell and Nishio, 2000).

MATERIALS AND METHODS

Three year old *R.* x *pulchrum* 'Sen-e-oomurasaki' and *R. obtusum* 'Kirin' plants were cultivated hydroponically in two different nutrient solutions for 10 days. The control nutrient solution (pH 6) was made with deionised water and 0.5 $g \cdot L^{-1}$ of soluble fertiliser containing 20% N, 20% P, 20% K, 0.02% B, 0.01% Mo, 0.7% Mg, 1.5% S, 0.015% Cu-EDTA, 0.12% Fe-DTPA, 0.06% Mn-EDTA and 0.015% Zn-EDTA (Peters Professional®, Scotts Company LLC, Dublin, OH, USA). The Fe-deficient nutrient solution (pH 9) was prepared by the addition of 1 $g \cdot L^{-1}$of sodium hydrogen carbonate ($NaHCO_3$) to the control solution. Growing media were renewed every two days. Plants were kept in a growth chamber at 24°C under 16-h photoperiod with a photosynthetically active radiation (PAR) of 160 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy top. There was one plant per 1.5 L glass pot and 15 plants per treatment and genotype for a total of 60 plants. The glass pots were distributed in a completely randomized design and the experiment was conducted in duplicate.

On treatment day 1 and on days 4, 6, 8, and 10, plant morphological characteristics were recorded and root FCR activity was assessed for three plants per treatment and genotype, according to Pinton et al. (1999). The root system of each intact azalea plant was placed in deionised water for 30 min; it was then transferred to an aerated assay solution, in which it was kept dark at 24°C for 1 h. The assay solution contained 0.25 mM Fe(III)–EDTA, 0.5 mM $CaSO_4$, 0.6 mM bathophenanthroline-disulfonate (BPDS) and 10 mM Mes buffer at pH 5.5, adjusted by 1 M KOH. After 1h, the assay solution absorbance was measured by a spectrophotometer (Ultrospec 2100 Pro, GE Healthcare, USA) at 535 nm. Ferric (Fe^{3+}) reduction was determined by the concentration of Fe^{2+}–$BPDS_3$ complex formed in the solution, using an extinction coefficient of 22.1 $mM \cdot cm^{-1}$ and the root fresh weight.

The data was analysed statistically by ANOVA tests ($P \leq 0.05$) with SPSS Statistics Software (version 21.0, SPSS, Chicago, USA). Significant differences between samples were detected with the Ryan-Einot-Gabriel-Welsch's multiple step-down F (REGW-F) post-hoc test.

RESULTS AND DISCUSSION

Limited information on the capability of azalea to dissolve Fe is problematic because these ornamental shrubs are sensitive to Fe deficiency. Morphological screening to select Fe-efficient genotypes requires several weeks due to slow azalea plant growth (Demasi et al., 2015; Kobayashi and Scariot, 2008; Scariot et al., 2013). In this study, we investigated an enzymatic method to try to shorten the evaluation time. To this end we compared the root FCR activity of tolerant (*R.* x *pulchrum* 'Sen-e-oomurasaki') and sensitive (*R. obtusum* 'Kirin') azalea during 10 days of hydroponic cultivation in (pH 6) and Fe-deficient (pH 9) growing solutions (Figure 1).

In the control treatment, *R.* x *pulchrum* 'Sen-e-oomurasaki' and *R. obtusum* 'Kirin' exhibited the same root enzymatic activity trend, ranging from 0.81 to 1.01 $mmol \cdot g^{-1}$ $FW \cdot h^{-1}$ and from 0.64 to 1.09 $mmol \cdot g^{-1}$ $FW \cdot h^{-1}$, respectively. Under Fe shortage, the Fe deficiency-sensitive genotype appeared as healthy as the deficiency-tolerant one. The two genotypes showed no symptoms of Fe chlorosis or morphological differences (data not shown). Most likely, longer periods are needed to observe Fe chlorosis symptoms in slow growing plants, such as *Rhododendron* (Chaanin and Preil, 1994; Preil and Ebbinghaus, 1994).

Differences were, however, observed in the root enzymatic activity of the two genotypes. When grown in a Fe-deficient nutrient solution for 10 days, azalea FCR responded to Fe deficiency in the typical fashion: higher and increased activity in the tolerant plant compared to the sensitive one (Marschner et al., 1986). In *R. obtusum* 'Kirin', FCR activity remained stable throughout the experiment. Conversely, *R.* X *pulchrum* 'Sen-e-oomurasaki' increased its enzymatic activity at day 6 of cultivation with a Fe^{3+} reducing capacity 1.5-fold higher than *R. obtusum* 'Kirin' (1.16 and 0.78 $mmol \cdot g^{-1}$ $FW \cdot h^{-1}$, respectively; Figure 2). This finding agrees with work by Pestana et al. (2012) that recorded enzymatic activity variations in asymptomatic carob plants.

The relationship between root FCR activity and Fe deficiency tolerance has been highlighted in many vege tables (De la Guardia and Alcántara, 2002) and fruit trees (Gogorcena et al., 2005, Martínez-Cuenca et al., 2013). This study confirmed that increased root Fe^{3+} reduction capacity is also involved in Fe deficiency tolerance in azalea. In Fe-efficient kiwifruit (Rombolà et al., 2002) and citrus rootstocks (Martínez-Cuenca et al., 2013; Wulandari et al., 2014), the FCR activity peaked after 14-15 days of Fe starvation. In azalea, the Fe-efficient genotype (*R.* x *pulchrum* 'Sen-e-oomurasaki') increased its Fe^{3+} reduction capacity at day 6, which is similar to the

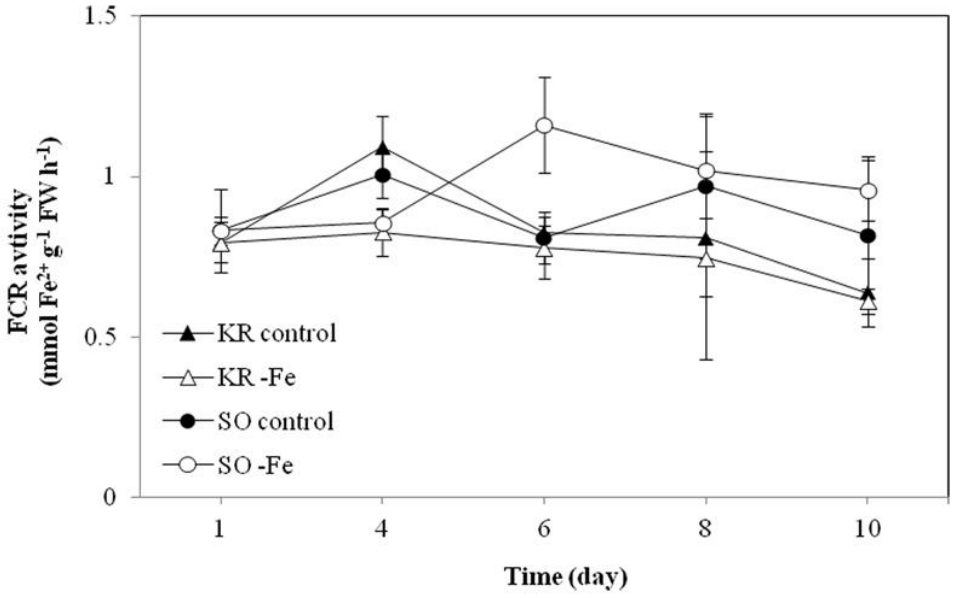

Figure 1. Time course of root FCR activity in *R.* x *pulchrum* 'Sen-e-oomurasaki' (SO) and *R. obtusum* 'Kirin' (KR) during 10 days of cultivation in control (pH 6) and Fe-deficient solutions (-Fe; pH 9). Data are mean ± SD of three replications.

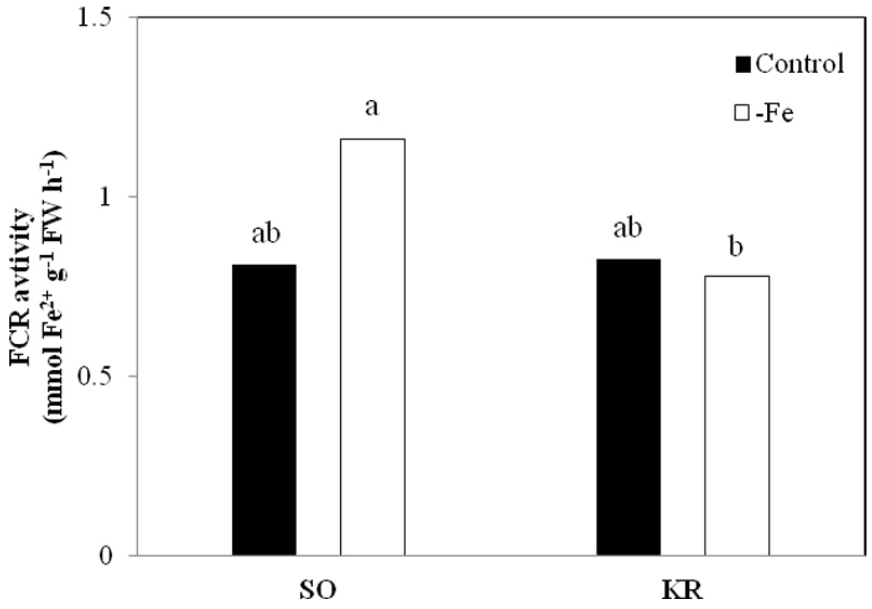

Figure 2. Root FCR activity at day 6 of cultivation in R. x pulchrum 'Sen-e-oomurasaki' (SO) and R. obtusum 'Kirin' (KR) in control (pH 6) and Fe-deficient solutions (-Fe; pH 9). Significant differences are indicated by different letters at $P < 0.05$ (REGW-F post hoc test).

activity trend in tomato seedlings (day 4; Zuchi et al., 2009).

The selection of iron deficiency tolerant genotypes is considered the most convenient approach for safeguarding plant production in calcareous soil (Rombolà and Tagliavini, 2007). The survey of FCR response has been suggested for rapid screening before visual symptoms occur in Fe deficiency tolerant genotypes

(Jolley et al., 1996). The FCR activity analysis performed in this study was able to discriminate a tolerant azalea genotype in six days.

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