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The effectiveness of grafting to improve alkalinity tolerance in watermelon

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ABSTRACT

The aim of the present study was to determine whether grafting could improve alkalinity tolerance of watermelon, and to study the changes induced by the rootstock in the shoot growth at agronomical, physiological, and biochemical levels. Two greenhouse experiments were carried out to determine growth, net photosynthetic rate, electrolyte leakage, root Fe(III)-chelate reductase (FCR) activity, mineral composition and assimilate partitioning (experiment 1, 2007), and organic acid concentration in root exudates (experiment 2, 2008), of watermelon plants [*Citrullus lanatus* (Thumb.) Matsum. and Nakai cv. 'Ingrid'] either ungrafted or grafted onto the four commercial rootstocks: 'Macis', 'Argentario'[*Lagenaria siceraria* (Mol.) Standl.] and 'P360', 'PS1313' (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) grown in a closed-loop system. Plants were supplied with nutrient solutions having two levels of pH (6.0 or 8.1). The high pH nutrient solution had the same basic composition plus an additional of 10 mM $NaHCO_3$ and 0.5 g L^{-1} $CaCO_3$. Significant depression of shoot, root biomass production, and leaf macro- (N, P, K, and Mg) and microelements (Fe, Mn, Zn, and Cu) under high pH level was observed in both grafted and ungrafted plants. Increasing the concentration of $NaHCO_3$ from 0 to 10 mM in the nutrient solution significantly enhanced FCR activity of root tips to 2.3 times in high pH treatment in comparison to the control. At high pH level, the percentage of shoot biomass weight reduction was significantly lower in plants grafted onto pumpkins rootstocks in comparison to those grafted onto the bottle gourd rootstocks and the ungrafted plants. Moreover, at high pH level, the highest percentage of root biomass weight reduction was recorded in both grafting combinations 'Ingrid/P360' and 'Ingrid/Macis'. The high pH-related reduction in net assimilation was more severe in ungrafted plants in comparison with the grafted ones. The Fe concentration in leaves was significantly higher in plants grafted onto pumpkin rootstocks (avg. 109.5 μg g^{-1}) in comparison to that of bottle gourd rootstocks and ungrafted plants (avg. 86.7 μg g^{-1}). For plants grafted onto bottle gourd rootstocks and ungrafted plants the high pH level (8.1) in the nutrient solution caused significant decrease in macronutrient leaf concentration especially for P and Mg compared to plants grafted onto pumpkin rootstocks. Increasing the nutrient solution pH from 6.0 to 8.1 increased exudation of organic acids (citric, malic, tartaric and succinic acids). Watermelon plants grafted onto pumpkin rootstocks exuded more citric and malic acids than those grafted onto bottle gourd rootstocks and ungrafted plants especially under bicarbonate-enriched conditions. These results support the hypothesis that uptake of nutrients (e.g. P, Mg, and Fe) from the nutrient solution by pumpkin rootstocks was facilitated by exudation of organic acids from roots.

1. Introduction

In the Mediterranean area, alkaline soils are widely represented throughout the agricultural regions, particularly those with semi-arid climates (Troeh and Thompson, 2005). Alkaline soils are generally characterized by low bioavailability of plant nutrients, high concentrations of $CaCO_3$ and soil solution HCO_3^-, high pH and almost no exchangeable H^+ (Marschner, 1995; Misra and Tyler, 1999). Bicarbonate ions interfere with the uptake of macro elements, in particular P, K and Mg (Pissaloux et al., 1995). For instance in alkaline soils, P is largely unavailable to plants due to the formation of metal complexes (e.g. Ca–P and Mg–P), rendering P only sparingly soluble. Moreover, the concentration of HCO_3^- interacts strongly with the availability of several micro ions, especially Fe^{2+}, and it is often considered to be the primary factor responsible for chlorosis of plants on calcareous soils (Mengel, 1994; Nikolic and Kastori, 2000) leading to serious yield and quality losses. Reduction in iron availability is due to the incapacity of sensitive plants to acquire and to transport iron towards shoots (Reynier, 1997). Iron deficiency reflects upon the physiology and biochemistry of the

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whole plant, as iron is an important co-factor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls (Marschner, 1995). Thus, under iron deficiency conditions, the reduction in leaf iron concentration is often accompanied by a marked reduction of chlorophyll levels (Gogorcena et al., 2004), by a significant, although less intense, decrease in the chlorophyll fluorescence (Morales et al., 1991; Nedunchezhian et al., 1997) and by a reduction in photosynthesis (Marschner, 1995).

Root exudation has been suggested to play a central role in some nutrient acquisition mechanisms operating in alkaline soils in particular Fe and P which are the primary limitations to growth in this environment (Ström, 1997; Jones, 1998; Ström et al., 2005). Once exuded, organic acids may undergo complexation reactions with target metals (e.g. Fe, Mn, Zn and Cu or Ca, which enhances Ca–P mineral dissolution) or non-target metals (e.g. Al, which does not mobilize much P; Jones and Darrah, 1994) and induces the dissolution of unavailable insoluble nutrients like ferric oxyhydroxides (Jones et al., 1996).

One way to avoid or reduce losses in production caused by alkalinity in high-yielding genotypes would be to graft them onto rootstocks capable of reducing the detrimental effect of external pH on the shoot. In the past, grafting was used widely with vegetable crops to limit the effects of soil pathogens (Lee, 1994), but the reasons for grafting as well as the kinds of vegetables grafted have increased dramatically over the years. Grafts were used to induce resistance against low and high temperatures (Rivero et al., 2003; Venema et al., 2008), to enhance nutrient uptake (Ruiz et al., 1997), increase synthesis of endogenous hormones (Proebsting et al., 1992), improve water use efficiency (Rouphael et al., 2008a), reduce uptake of persistent organic pollutants from agricultural soils (Otani and Seike, 2006, 2007), raise salt and flooding tolerance (Colla et al., 2006a,b; Yetisir et al., 2006; Martinez-Rodriguez et al., 2008), and limit the negative effect of boron, copper and cadmium toxicity (Edelstein et al., 2005; Rouphael et al., 2008b; Arao et al., 2008). Nevertheless, no published data is available concerning the effects of high pH in the rooting medium on agronomical, physiological and biochemical responses of grafted watermelon. Our hypothesis is that grafting may raise alkalinity tolerance of watermelon by enhancing the uptake and translocation of mineral elements in particular iron and phosphorus.

To verify, the current hypothesis, two greenhouse experiments were carried out to compare the growth, net photosynthetic rate, electrolyte leakage, the root Fe(III)-chelate reductase activity, mineral composition and assimilate partitioning, and the organic acid concentration in root exudates of grafted and ungrafted watermelon plants grown in a closed-loop system with a nutrient solution having a pH of 6.0 or 8.1.

2. Materials and methods

2.1. Plant material, treatments and growth conditions

Two experiments were conducted, one in 2007 (experiment 1) and one in 2008 (experiment 2) in a 300 m^2 polyethylene greenhouse situated on the Experimental Farm of Tuscia University, Central Italy (42°25′N; 12°08′E; 310 m a.s.l.). In both years, *Citrullus lanatus* (Thumb.) Matsum. and Nakai cv. 'Ingrid' (Takii Seeds, Japan) was grafted onto the following commercial rootstocks: 'Macis' [*Lagenaria siceraria* (Mol.) Standl., Nunhems Zaden, The Netherlands], 'Argentario' [*L. siceraria* (Mol.) Standl., Syngenta, Milano, Italy], 'P360' (*Cucurbita maxima* × *Cucurbita moschata*; Società Agricola Italiana Sementi, Cesena, Italy), and 'PS1313'(*Cucurbita maxima* × *Cucurbita moschata*; Peto Seeds, CA, USA) using the procedure of "cleft grafting" described by Lee (1994), while ungrafted cv. 'Ingrid' was used as a control. The two bottle gourds ('Macis' and 'Argentario') and the two pumpkins ('P360' and 'PS1313') were selected as the most representative commercial rootstocks used in Italy. Randomized complete block designs were used for both greenhouse experiments, with treatments replicated 3 times. Treatments were defined by a factorial combination two pH levels of the nutrient solution (6.0 or 8.1) and five grafting combinations (i.e., grafted or ungrafted plants). Each experimental unit consisted of ten plants. Daily temperatures were maintained between 20 and 30 °C, with night temperatures never lower than 15 °C, with day and night relative humidities of 55% and 85%, respectively. In experiment 1, grafted and ungrafted watermelons were transplanted at the two true-leaf-stages, on 3 October 2007 into pots (*d* 14 cm, h 12 cm) containing 1.5 L of perlite. The pots were placed on 16 cm wide and 5-m long troughs, with 30 cm between pots. In each trough, an independent tank was provided to supply plants with nutrient solution. The nutrient solution was pumped and delivered at a rate of 2 L min^{-1} at the top end of every bench and allowed to run slowly down the trough, whereas the excess solution was drained back to the tank for recirculation. In experiment 2, grafted and ungrafted watermelons were transplanted at the two true-leaf-stages, on 6 October 2008 in a floating system.

The basic nutrient solution used in both experiments was a modified Hoagland and Arnon formulation. All chemicals used were of analytical grade, and composition of the nutrient solution was: 13.0 mM $N–NO_3$, 1.0 mM $N–NH_4$, 1.5 mM S, 1.5 mM P, 5.0 mM K, 4.0 mM Ca, 1.5 mM Mg, 0.5 mM Na, 0.5 mM Cl, 20 μM Fe, 9 μM Mn, 0.3 μM Cu, 1.6 μM Zn, 20 μM B, and 0.3 μM Mo. The pH of the nutrient solution was 6.0. The high pH treatment had the same nutrient composition plus 10 mM $NaHCO_3$ and 0.5 g L^{-1} $CaCO_3$. The sodium bicarbonate and $CaCO_3$ were added to the basic nutrient solution to mimic the effects of alkalinity. The pH of the bicarbonate treatment was 8.1. The alkaline treatment was initialized 7 days after transplanting. In all treatments the nutrient solution was changed twice a week to ensure sufficient nutrient for plant growth and to keep pH level in solution close to the targeted level.

2.2. Foliar symptoms

Visible leaf injury in grafted and ungrafted watermelon plants grown under the two pH levels was checked and recorded at different stages of development: 20 and 40 days after transplanting.

2.3. Plant growth measurement

At final harvest 41 (13 November 2007) and 42 days after transplanting (17 November 2008), in experiments 1 and 2, respectively, six plants per plot were separated into stems, leaves, and roots, and their tissues were dried in a forced-air oven at 80 °C for 72 h for biomass determination. Shoot biomass was equal to the sum of aerial vegetative plant parts (leaves + stems). For experiment 1 only, root to shoot ratio was calculated by dividing root dry weight by the sum of leaf and stem dry weights. For experiment 1 only, leaf area (LA) was measured with an electronic area meter (Delta-T Devices Ltd., Cambridge, UK).

2.4. Net photosynthesis measurement

At the termination of the experiment 1, the net assimilation of CO_2 (A_{CO_2}, μmol CO_2 m^{-2} s^{-1}) was determined with a portable photosynthesis system (LI-6200; LI-COR Inc., Lincoln, NE, USA). This measurement was made on the most recent fully expanded leaves, using six replicate leaves per treatment. The LI-6200 was equipped with a well-stirred 2.5×10^{-5} m^3 leaf chamber with constant-area inserts (1.2×10^{-3} m^2) and fitted with a variable intensity red source (Model QB1205LI-670), Quantum Devices Inc. Barneveld, WI, USA) (Tennessen et al., 1994). Leaf temperature within the chamber was 30 ± 2 °C, vapour pressure difference between the leaf