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Grafting of cucumber as a means to minimize copper toxicity

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Abstract

The aim of the current work was to determine whether grafting could improve copper (Cu) tolerance of cucumber, and to study the changes induced by the rootstock in the shoot growth at agronomical and physiological levels. A greenhouse experiment was carried out to determine yield, growth, fruit quality, leaf gas exchange, chlorophyll and carotenoids contents, electrolyte leakage, mineral composition and assimilate partitioning of cucumber plants (*Cucumis sativus* L. cv. 'Akito'), either ungrafted or grafted onto the commercial rootstock 'Shintoza' (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) and cultured in nutrient film technique (NFT). Plants were supplied with nutrient solutions having three levels of Cu concentration [0.3 (control), 47, or 94 μM]. Significant depression of yield, shoot and root biomass production, and fruit quality (low fruit pH, and high Cu content) in Cu treated cucumber plants was observed, and this effect varied as a function of Cu concentration in NFT solution. The relative yield of cucumber decreased by ≈3.4% for each unit of increase in leaf tissue Cu concentrations above the threshold value (7.8 $\mu g\, g^{-1}$). At the two higher Cu concentrations (47 and 94 μM Cu), the percentages of yield, shoot and root biomass weight reductions were significantly lower in grafted plants in comparison to those of the ungrafted plants. Excessive Cu, especially at 94 μM Cu, inhibited photosynthesis, pigment synthesis, and membrane integrity. The Cu-related reductions in net assimilation, stomatal conductance, chlorophyll and carotenoid content were more severe in ungrafted plants in comparison with those grafted on 'Shintoza'. The percentage of electrolyte leakage was significantly higher in ungrafted plants especially those with severe Cu toxicity (94 μM Cu). The accumulation of Cu in leaf tissue at 47 and 94 μM Cu, with respect to control, were significantly lower in grafted plants (138 and 181%, respectively) in comparison to that of ungrafted plants (about 235 and 392%, respectively). Significant reduction of macro- (N, K, Ca and Mg) and micro-elements (Fe, Mn and Zn) in cucumber leaf tissue was found under moderate and severe Cu stresses especially on ungrafted plants. The improved crop performance of grafted plants was attributed to their strong capacity to inhibit Cu accumulation in the aerial parts and to maintain a better plant nutritional status.

Keywords: Cu; *Cucumis sativus*; Gas exchange; Grafting; Mineral composition

1. Introduction

Heavy metal contamination in soil and water is currently one of the most troublesome environmental problems faced by mankind nowadays. Copper (Cu) in particular, poses serious problems due to its widespread industrial and agricultural use (Fernandes and Henriques, 1991). In agriculture, elevated concentrations of Cu may develop with time in soils due to prolonged use of organic fertilizers composed of sewage sludge and poultry manure (Marschner, 1995) and to the frequent use of electrolytically generated Cu (Zheng et al., 2004), cupric sulphate (Alva et al., 1999; Hill et al., 2000), or some other Cu containing fungicides and bactericides (Kaplan, 1999) to control diseases.

Copper is an essential micronutrient for normal plant growth and development. In plants, Cu plays a vital role in photosynthesis and respiratory electron transport where it functions as a cofactor for a variety of enzymes such as superoxide dismutase, cytochrome *c*, oxidase and plastocyanin (Clemens, 2001). However, excessive levels of Cu can cause a range of morphological and physiological disorders (Fernandes and Henriques, 1991), such as reduction of growth (Zheng et al., 2005), photosynthetic activity (Burzynski and Klobus, 2004), and uptake of mineral nutrients (Monni et al., 2000; Wang et al., 2004). Moreover, it may result in chlorosis, inhibition of root growth, and damage to plasma membrane permeability that leads to ion leakage (De Vos et al., 1991; Ouzounidou et al., 1992). For most crop species, the normal Cu concentration in plant tissues is 5–20 $\mu g\, g^{-1}$,

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and above this upper limit, toxicity effects are likely to occur (Kabata-Pendias and Pendias, 2001).

There are, however, marked differences in Cu tolerance between plant species; these differences are often related to the copper content of shoots. The mechanisms of Cu tolerance in higher plants include two main strategies: (1) exclusion, whereby plants avoid excessive uptake and transport of metal ions, and (2) accumulation and sequestration, whereby plants detoxify free metals by compartmentation of metals in vacuoles, complexation of metal ions by organic ligands, such as organic acids, amino acids and metal-binding peptides (Clemens, 2001; Hall, 2002). One way to avoid or reduce losses in production caused by copper in high-yielding genotypes would be to graft them onto rootstocks capable of reducing the effect of external heavy metal 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 (Bulder et al., 1990) and high (Rivero et al., 2003) temperatures and against iron chlorosis in calcareous soils (Romera et al., 1991) and to enhance nutrient uptake (Ruiz et al., 1997), increase synthesis of endogenous hormones (Proebsting et al., 1992), improve water use (Cohen and Naor, 2002), reduce uptake of persistent organic pollutants from agricultural soils (Otani and Seike, 2006), raise salt tolerance (Estan et al., 2005), and limit the negative effect of boron toxicity (Edelstein et al., 2005). Nevertheless, no published data is available concerning the effects of copper concentration in the rooting medium on agronomical and physiological responses of grafted cucumber. Our hypothesis is that grafting may raise copper tolerance of cucumber by limiting the transport of copper to the shoot.

To verify the current hypothesis, grafted and ungrafted cucumber plants were grown in a recirculating nutrient solution system (NFT) with three levels of copper concentration. Grafted and ungrafted plants were compared in terms of yield, growth, fruit quality, leaf gas exchange, chlorophyll and carotenoids contents, electrolyte leakage, and mineral composition and assimilate partitioning.

2. Materials and methods

2.1. Plant material, treatments and growth conditions

The experiment was conducted in Spring–Summer 2005 in a 300 m^2 polyethylene greenhouse situated on the Experimental Farm of Tuscia University, Central Italy (42°25′N, 12°08′E). Plants were grown under natural light conditions. The greenhouse was maintained at daily temperatures between 18 and 33 °C, and day/night relative humidities of 55/85%. *Cucumis sativus* L. cv. Akito (Enza Zaden, Verona, Italy) was grafted onto the commercial ‘Shintoza’ rootstock (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne, Syngenta, Switzerland) using the ‘tongue approach grafting’ described by Lee (1994), whereas ungrafted ‘Akito’ was used as a control plant. ‘Akito’ was selected as a representative cucumber hybrid cultivated in Italy. At the two true-leaf stage (April 22), grafted and ungrafted plants were grown into rockwool cubes (7.5 cm × 7.5 cm × 6.5 cm), which were placed in 25 cm wide and 5.5-m-long nutrient film technique (NFT) channels, with 50 cm space between rockwool cubes and 120 cm space between NFT channels. These arrangement resulted in a plant density of 1.67 plants m^{-2}. Plants were trained vertically using plastic nets.

The experiment was designed as a factorial combination of three Cu concentrations [0.3 (control), 47, or 94 μM] and two grafting treatments (ungrafted Akito; or grafted Shintoza/Akito). Each experimental unit consisted of one NFT channel containing ten plants. The treatments were arranged in a randomized complete-block design with four replicates (four NFT channels) per treatment. The Cu treatments were initialized 7 days after the transplanting.

The basic nutrient solution used in this experiment was a modified Hoagland and Arnon formulation. All chemicals used were of analytical grade, and composition of the nutrient solution was: 14.4 mM NO_3–N, 0.6 mM NH_4–N, 1.8 mM S, 1.2 mM P, 6.0 mM K, 4.0 mM Ca, 2.3 mM Mg, 1.0 mM Na, 1.0 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 enriched-Cu treatments had the same nutrient composition plus 47, or 94 μM Cu supplied as $CuSO_4{\cdot}5H_2O$. The electrical conductivity (EC) and pH of the nutrient solutions were 2.0 dS m^{-1} and 6.0, respectively. The EC and pH were measured daily in all the nutrient solutions and if necessary adjustments were made to the initial value through the addition of respectively concentrated nutrient solution and acid mixture with the same anionic ratio of nutrient solution (0.83 NO_3–N, 0.07 H_2PO_4–P, 0.10 SO_4–S; values are given in terms of fraction of total macro-anion concentration on mM basis). The volume of solution in each tank was 120 L and was brought to its initial volume by daily addition of deionized water. In all treatments the nutrient solution was changed twice a week to ensure sufficient nutrient for plant growth and to keep Cu level in solution close to the targeted level. To prevent large fluctuations in pH and ionic concentrations in the intervals between the replacements of the nutrient solution, a relatively high volume of nutrient solution per plant (12 L) was recirculated in all treatments. In each NFT channel 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.

2.2. Foliar symptoms

Visible leaf injury in grafted and ungrafted cucumber plants grown under the three Cu levels was checked and recorded at different stages of development: 20, 40, and 60 days after transplanting.

2.3. Yield and biomass measurements

Fruits were harvested from May 16 to July 11, and number of fruits, mean fruit weight, and fruit yield were determined on six plants per plot. At final harvest (July 11, 80 days after transplanting), six plants per plot were separated into stems,