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Effect of two nutrient solution temperatures on nitrate uptake, nitrate reductase activity, NH_4^+ concentration and chlorophyll *a* fluorescence in rose plants

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The effect of two nutrient solution temperatures (cold (10 °C) and warm (22 °C)) during two flowering events of rose plants (*Rosa* × *hybrida* cv. Grand Gala) were examined by measuring chlorophyll (Chl) *a* fluorescence, ammonium (NH_4^+) content and nitrate reductase (NR) activity in four different leaf types, that is, external and internal leaves of bent shoots and lower and upper leaves of flowering stems. Besides, nitrate (NO_3^-) uptake and water absorption, total nitrogen (N) concentration in the plant, dry biomass, and the ratios of shoot/root and thin-white roots/suberized-brown roots were determined. Generally, cold solution increased NO_3^- uptake and thin-white roots production but decreased water uptake, so plants grown at cold solution had to improve their NO_3^- uptake mechanisms to obtain a higher amount of nutrient with less water absorption than plants grown at warm solution. The higher NO_3^- uptake can be related to an increase in NR activity, NH_4^+ content and total N concentration at cold solution. Nutrient solution temperature also had an effect on the photosynthetic apparatus. In general terms, the effective quantum yield (ϕ_{PSII}) and the fraction of open PSII reaction centres (q_L) were higher in rose plants grown at cold solution. These effects can be associated to a higher NO_3^- uptake and total N concentration in the plants and were modulated by irradiance throughout all the experiment. Plants could adapt to cold solution by enhancing their metabolism without a decrease in total dry biomass. Nevertheless, the effect of nutrient solution temperature is not simple and also affected by climatic factors.

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1. Introduction

Soilless culture techniques are used for commercial production of several high value ornamental plants in the Mediterranean area. In this region, in winter, night air temperature can get down below the rose plant biological minimum of 14–16 °C (Tesi, 1969) in an unheated greenhouse. The temperature of the nutrient solution can frequently get to 7–9 °C considered critical for root functions (Mortesen and Gislerød, 1996). In hydroponics, root temperature can be controlled by warming or cooling the nutrient solution (Moss and Dalgleish, 1984) providing the energy requirements for optimum plant development. Sometimes an excessive energy input is spent to protect the crop from climatic constraining conditions due to poorly established guidelines (Willits and Peet, 2001). In order to reduce energy costs in greenhouse production it is necessary to know the range of temperatures that permits plant growth without negative effects on yield.

Root temperature has been shown to have pronounced effects on shoot growth of a number of plant species (Bowen, 1991), including roses, but optimal values can vary among them (Barr and Pellet, 1972). The research about root temperature in rose plants is contradictory and can depend on cultivars, climatic conditions, the combination root–air temperatures, and other factors. Some studies indicate that 18 °C root-zone temperature is the optimal for shoot growth of "Better Times" and "Sonia" roses grafted on the rootstock of *Rosa indica* (Shanks and Laurie, 1949; Zeroni and Gale, 1982). No effects of root temperature increases from 18 °C to 25 °C, on stem length and flower production were reported (Kohl et al., 1949; Zeroni and Gale, 1987). Other studies show that soil heating is beneficial for roses (Brown and Ormrod, 1980; Zeroni and Gale, 1982) and if root temperature is lowered from 18 °C to 10–12 °C, shoot growth is reduced (Moss and Dalgleish, 1984; Mortesen and Gislerød, 1996).

Main root functions are water and nutrient uptake and synthesis of plant hormones (Dielman et al., 1998). Soil temperature affects water and nutrient uptake, metabolic processes and root and shoot growth (Dong et al., 2001) and, among them, nutrient uptake is one of the processes more sensitive to temperature (Xu and Huang, 2006). Dong et al. (2001) have shown that low soil temperature

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(8 °C) reduced absorption of ^{15}N by roots of apple trees. In contrast, the whole root system of soybean plants absorbed NO_3^- similarly at both cool (14 °C) and warm temperatures (22 °C) (Osmond et al., 1982).

Nitrogen absorption is directly related to the reduction rate of nitrate nitrogen ($N–NO_3^-$) to nitrite. This reduction is the first step of N assimilation and involves enzyme nitrate reductase (NR) (Toselli et al., 1999) which is sensitive to high temperature (Lauri and Stewart, 1993). Younis et al. (1965) found that an increase in temperature from 30 °C to 35 °C caused a 60 to 70% decrease in NR activity in young corn plants. Increase of NR activity after low temperature treatment has been reported in wheat (Yaneva et al., 2002) and in oil-seed rape (Macduff and Trim, 1986) too. As mentioned above, root temperature also influences water uptake. This is due to the fact that, at low temperature, the viscosity of water increases causing a decrease in water flow to the root, and root permeability and metabolic activity also decrease (Pavel and Fereres, 1998).

Low root temperature can also affect photosynthesis. Chlorophyll *a* fluorescence, an indicator of the fate of excitation energy in the photosynthetic apparatus, has been used as early indication of many types of plant stress (Calatayud et al., 2004), as reflected in non-radiative energy dissipation (Schreiber et al., 1994). We propose the use of Chl *a* fluorescence imaging technique as a tool to detect the possible stress in rose plant under low root temperature. It has been shown in potato (Greaves and Wilsion, 1987), tomato (Willits and Peet, 2001), lettuce (He and Lee, 2004), cucumber (Ahn et al., 1999), maize (Fracheboud et al., 1999) or roses (Hakan et al., 2000) that sensitivity to low temperature may be verified by measured Chl *a* fluorescence.

Temperature studies are difficult to understand because temperature affects in a different way depending on the physiological process and on the plant organ that is studied (Theodorides and Pearson, 1982). But it is necessary to comprehend the underlying mechanism to the response of the plant to low temperature in order to be able to optimise greenhouse climate and reduce energy cost in winter.

The objective of this study was to test root chilling tolerance of rose plants (*Rosa* × *hybrida* cv. Grand Gala) during two flower cycles under winter–spring greenhouse conditions. To reach this objective, Chl *a* fluorescence, NR activity and NH_4^+ concentration were measured in different types of leaves, as well as NO_3^- and water absorption by the roots, total biomass produced and total nitrogen concentration in roots and leaves under two root-zone temperature conditions, a level limiting root activity (10 °C) and a non-limiting level for root processes (22 °C).

2. Materials and methods

2.1. Plant management and greenhouse conditions

A three-year-old rose crop (*Rosa* × *hybrida* cv. Grand Gala), was grown in a polycarbonate greenhouse, equipped with convective heating (minimum 16 °C), high pressure fogging and roof ventilation. Two units of recirculating aeroponic growing system were used. For more information see Martínez et al. (2004) and Calatayud et al. (2007).

Thirty plants were grown in each aeroponic unit at two different nutrient solution temperatures while their aerial parts were subjected to the same climate conditions, i.e. radiation, air temperature and relative humidity. In the cold solution treatment, a heat exchanger placed in the solution tank and connected to a cooling equipment, cooled the solution down to 9 °C, automated by means of a thermostat. The average values and standard deviation of nutrient solution temperatures along the whole experiment were 10.5 ± 1.02 °C in the cold solution treatment and 21.72 ± 2.22 °C in the warm one, which was the control treatment. The climate variables inside the greenhouse, temperatures of the air and nutrient solution, air humidity and vapour pressure deficit of air (VPD), were recorded. Solar radiation integration per period ($MJ\,m^{-2}\,period^{-1}$) (see periods in Table 1) and the average radiation ($W\,m^{-2}$) when the physiological measurements were done (11:00 to 13:00), defined here as growth radiation, are shown in Table 1. They increased as the experiment progressed and were higher in the second flowering event.

Plants were grown following the bending technique as it is commonly done by local growers (see Calatayud et al., 2007).

The flowering shoots of 60 plants (30 plants of each aeroponic system), were pruned down to two nodes from their base on the 25th of November and then, solution temperature treatments were applied. The experiment was finished at the beginning of April, after two complete flowering cycles. All physiological parameters (see below) were measured at the same physiological stages independently of the date:

T0: Plants with flowering bud (in the middle of January).
T1: Flower stems in commercial harvesting stage (at the beginning of February) (end of the first floral cycle).
T2: Plants with flowering bud (at the beginning of March).
T3: Flower stems in commercial harvesting stage (at the beginning of April) (end of the second floral cycle).

All measurements done in the aerial part of the plant were carried out in fully developed leaves. Samples were taken from four different positions within the plant: external and internal bent shoots, and basal (second leaf from the base) and upper leaf (below the flower or bud) of flower shoots (Calatayud et al., 2007).

At the end of each flowering cycle (T1 and T3), five plants from each treatment were taken for destructive measurements. Fresh and dry weight (FW and DW, respectively), and total N concentration of roots and leaves, which was done by using a C/N analyser (NC 2500, Eager 300 software®, CE instruments, ThermoQuest Italia, Rodano, Italy), were measured.

2.2. Water and nitrate absorption by plants

Following previously described methodology (Roca et al., 2003) each aeroponic unit was provided with a precision weighting scale (±0.1 g resolution) connected to a data logging system where the water uptake by 30 plants was recorded every 5 s. Daily water uptake, expressed as $L\,plant^{-1}\,day^{-1}$, was calculated by volume difference in the system between two consecutive days. The system was watertight, so all volume losses were attributed to water and nutrient uptake.

Each day (from the 28th of November until the end of the experiment) at noon, 40 mL of nutrient solution were collected from the tanks of both treatments, i.e. cold and warm solution and their volume was registered. These samples represent the root zone solution since the system inertia is assumed as nil. Their NO_3^- concentration was measured using Flow Injection Analyzer (FIASTAR 5000, Foss Analytical, Höganäs, Sweden). Daily NO_3^- uptake, expressed as $mmol\,NO_3^-\,plant^{-1}\,day^{-1}$, is calculated using equation (Roca et al., 2005):

$$\text{Nitrate uptake rate} = (V_1 \times C_1) - (V_2 \times C_2) \quad (1)$$

where V1 and C1 are the volume of the system and the NO_3^- concentration on day 1, and V2 and C2 are the volume of the system and the NO_3^- concentration on day 2.

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