Scientia Horticulturae 214 (2017) 41–50

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Interactive effects of nitrate-ammonium ratios and temperatures on growth, photosynthesis, and nitrogen metabolism of tomato seedlings

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ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 26 August 2016
Accepted 7 September 2016
Available online 22 November 2016

Keywords:
Nitrate-ammonium ratio
Nutrient solution
Chilling temperature
Tomato

ABSTRACT

Growth of many species of plants is optimal when the two major forms of N that are assimilated by plants are supplied at a particular ratio. This ratio is affected by both species and the environment. We assessed the effects of different ratios of nitrate to ammonium (N-A ratio) supplied to hydroponically grown chilling stressed and non-stressed tomato seedlings on several parameters. When the plants were grown in normal temperature (14–30 °C), growth parameters, photosynthetic rate, chlorophyll concentration, soluble protein in roots, and leaf nitrates were greatest when the N-A ratio was 75:25. The activities of glutamine synthetase (GS) and NADH-dependent glutamate synthetase (NADH-GOGAT) in leaves were maximal when the N-A ratio was 50:50, while NADH-GOGAT in roots was maximal when the ratio was 25:75. Soluble protein in leaves and NO_3^- content in roots and nitrate reductase activity were positively correlated with N-A ratio, while free amino acids, total N, and NH_4^+ content were negatively with this ratio. Under chilling temperature (5–18 °C), growth parameters, photosynthetic rate, soluble protein in roots, leaf nitrate, and GS activity in roots also had an optimal N-A ratio of 50:50, while GS activity in leaves and NADH-GOGAT activity were the highest when the N-A ratio was 25:75. Increased NH_4^+, resulting in an N-A ratio of 75:25, improved NR activity, and NO_3^- in roots in CT. The leaf chlorophyll content was not affected by the N-A ratio. Plant growth parameters, N content in roots, NO_3^- and NH_4^+ concentrations, photosynthetic rate, leaf chlorophyll content and key enzymes activities of nitrogen metabolism were influenced by the N-A ratio, while the concentrations of soluble protein, free amino acids, and total leaf N did not appear to be influenced by the interaction of temperature and N supply. The optimal N-A ratio of tomato seedling appears to be 75:25 for unstressed plants and 50:50 for plants grown in chilling temperatures.

1. Introduction

Nitrogen is an essential nutrient for plants and, when limiting, can reduce growth and crop productivity (Glass, 2005; Piwpuan et al., 2013). Plants assimilate both ammonium (NH_4^+) and nitrate (NO_3^-) from the soil solution and these two inorganic forms are their most important nitrogen sources (Miller and Cramer, 2005; Luo et al., 2013). Uptake and assimilation of ammonium by plants requires less energy than uptake and assimilation of nitrate, leading to the proposal that, of the two, ammonium may be the more efficient and advantageous nitrogen source. However, it has been shown that the growth and development of crop plants cultured without soil may be more dependent on nitrate than ammonium. It is also clear that the relative availability of the two nitrogen forms differs between environments (Britto et al., 2001a,b), making the nitrogen source an important consideration in determining optimal growth conditions. In higher plants, uptake of NH_4^+ follows its electrochemical gradient, utilizing a uniport carrier system across the plasma membrane, while NO_3^- uptake is active, via a proton-anion carrier employing co-transport of two protons (Tischner, 2006; Lambers et al., 2008). Nitrate must be reduced to ammonium in cells by two consecutive reductions. First, nitrate is reduced to nitrite by nitrate reductase (NR), followed by reduction of the nitrite to ammonium by nitrite reductase. These reactions employ the reducing power of NADH or NADPH from respiration or photosynthesis (Piwpuan et al., 2013). The ammonium can be incorporated into carbon skeletons to produce a variety of amino acids (Miller

Abbreviations: CT, chilling temperature; NR, nitrate reductase; GS, glutamine synthetase; GOGAT, glutamate synthase; Pn, net photosynthetic rate; NO_3^-, nitrate; NH_4^+, ammonium; SP, soluble protein; FAA, free amino acids.

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http://dx.doi.org/10.1016/j.scienta.2016.09.006
0304-4238/

and Gramer, 2005). These complex processes may account for the higher energy costs associated with nitrate as a nitrogen source compared to ammonium (Britto and Kronzucker, 2002; Guo et al., 2007). In support of this suggestion, the addition of NH_4^+ to the nutrient solution increased the growth of several plant species (Sonneveld, 2002).

Despite the apparent preference of plants for one of the two nitrogen sources, it would appear that most plants perform best at a particular nitrate-ammonium ratio (N-A ratio). This ratio appears to regulate distribution of assimilated N between shoots and roots. It may also differ between species (Britto et al., 2001a,b; Britto and Kronzucker, 2002; Sonneveld, 2002), as Chinese cabbage exhibited maximal growth when the N-A ratio was 75:25 (Chen et al., 2005), while spinach grew maximally when it was 50:50 (Mills et al., 1976). The optimal ratio may also depend on environmental conditions such as pH (Borgognone et al., 2013), light intensity (Tabatabaei et al., 2008), and the temperature of the root zone (Sattelmacher et al., 1993). Of particular interest to us is the relationship between species' optimal N-A ratio and temperature. Little is known about this issue, although it has been shown that nitrate uptake is more temperature sensitive than ammonium uptake: nitrate uptake predominates at warm temperatures while ammonium predominates at cool temperatures (Warren, 2009).

Cold temperatures can severely restrict plant growth, development, and result in physiological disorders (Theocharis et al., 2012) such as decreased chlorophyll biosynthesis, net photosynthetic rate (Pn), carbohydrate metabolism, and crop yield and quality (Tewari and Tripathy, 1998; Beck et al., 2004). Chilling can also damage membrane lipids, proteins, nucleic acids, and disrupt cellular homeostasis (Kang and Saltveit, 2002; Feng et al., 2003; Theocharis et al., 2012). In agriculture areas subjected to chilling temperatures (CT), including high latitude regions such as northern China (Qin et al., 2011; Liu et al., 2013), production of chilling sensitive plants may be compromised during cold seasons, even when they are grown in a greenhouse (Miao et al., 2009). Tomato (*Lycopersicon esculentum* Mill.), of tropical origin, is sensitive to temperatures less than 10 °C (Lurie and Klein, 1991). This is an important agricultural issue, as tomatoes are often grown in unheated greenhouses in the winter, where they experience low temperatures. Night temperatures of 6 or 9 °C led to an irreversible reduction in Pn, stomatal limitation of CO_2 supply, and decreased activity of ribulose-bisphosphate carboxylase in tomato (Liu et al., 2012).

Uptake and assimilation of nitrogen may be affected by chilling temperatures and, specifically, the optimal N-A ratio in a particular species may be altered by CT. This experiment was undertaken to assess the effect on various parameters of different N-A ratios on tomato plants subjected to CT. Specifically, we examined growth, Pn, and nitrogen metabolism characteristics in tomato seedlings.

2. Materials and methods

2.1. Plant material and growth conditions

Tomato seeds (*Lycopersicon esculentum* Mill. cv. Xi Nong 2011) were sown in hole trays in a seedling substrate (pH 5.5–6.5) with an organic mass fraction ≥50% and a humic acid mass fraction ≥ 20%. When the third leaves were fully expanded, seedlings were transplanted into rectangular hydroponic containers and supplied with half-strength modified ShanQi's nutrient solution containing 0.75 mM $Ca(NO_3)_2$, 2 mM KNO_3, 0.5 mM $(NH_4)_2HPO_4$, 0.5 mM $MgSO_4{\cdot}7H_2O$, 25 μM EDTA-Fe, 1.25 μM H_3BO_3, 1.0 μM $MnSO_4{\cdot}4H_2O$, 1.0 μM $ZnSO_4{\cdot}7H_2O$, 0.25 μM $CuSO_4{\cdot}5H_2O$, and 0.25 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$. The containers were supplied with air bubblers to ensure adequate oxygen supply. The plants were placed in a greenhouse with an average temperature of 25 °C during the day and 15 °C during the night. Plants received 16 h of light (800 mmol·photons m^{-2} s^{-1}) and 8 h of dark. The relative humidity was 60%–90%.

Table 1
Nutrient solution composition (mmol/L).

Nutrient source	Nitrate-ammonium ratios in the nutrient solutions				
	100/0	75/25	50/50	25/75	0/100
KNO_3	3	1			
$Ca(NO_3)_2$	2.5	2.5	2	1	
KH_2PO_4	1	1	1	1	1
KCl		2	3	3	3
$MgSO_4$	1	1	1	1	1
$CaCl_2$			0.5	1.5	2.5
NH_4Cl		2	4	6	8

2.2. Chilling temperature and NO_3^--N/NH_4^+-N ratio treatments

Five days after transplanting to the hydroponic containers, tomato seedlings were divided into two groups and randomly placed in two identical environmentally controlled multi-span greenhouses. Temperature was maintained by regulating the radiator heating system, a wet-pad cooling system, and opening and closing a skylight. The CTs ranged from 5 °C to 18 °C and the normal temperatures ranged from 14 °C to 30 °C. The greenhouse temperatures were monitored and recorded with automated sensors. The highest and lowest temperatures are presented in Fig. 1.

Five N-A ratios were selected and supplied in Modified ShanQi's solution. These ratios were: 100:0, 75:25, 50:50, 25:75, and 0:100. The details are shown in Table 1. The final concentrations of the macroelements were 8 mM N, 4 mM K, 2.5 mM Ca, 1 mM P, and 1 mM Mg. The final concentration of the microelements were 2.5 μM B, 2.0 μM Mn, 2.0 μM Zn, 0.5 μM Cu, and 0.5 μM Mo. Iron was supplied as EDTA-Fe for a final concentration of 50 μM and 7 μM dicyandiamide was added in all nutrient solutions to prevent the conversion of ammonium into nitrate. The nutrient solution was replaced every 5 days. The changes in N-A ratios were balanced by varying the Cl^- concentration to maintain constant N, P, K, Ca, and Mg concentrations in all treatments. The electrical conductivity was 2.5–2.6 dS m^{-1}. The pH was adjusted daily within the range of 6.0–6.1.

2.3. Plant growth analysis

At the start of the experiment and 20 days later, five seedlings from each treatment were selected randomly to measure the fresh and dry weights of roots and shoots. Plant material was weighed and then dried in an air-forced oven at 105 °C for 20 min and then at 80 °C 48 h, at which time the material had reached constant weight. The data were used to calculate relative growth rate (RGR). The RGR in d^{-1} = (ln W2–ln W1)/(t2–t1), where W1 and W2 were the initial and final biomass and t1 and t2 were the initial and final time in days, respectively (Kingsbury et al., 1984).

2.4. Photosynthetic rate and chlorophyll analysis

Net photosynthesis and the chlorophyll content were measured in the third youngest fully expanded leaf of five plants. Photosynthesis was measured over a 2-h period early in the photoperiod with a portable photosynthetic system, Li-6400 (LI-COR, USA). Chlorophyll was analyzed according to the method described by Harmutk (1987).