Journal of Plant Physiology 168 (2011) 816–823

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Ammonia production and assimilation: Its importance as a tolerance mechanism during moderate water deficit in tomato plants

Eva Sánchez-Rodríguez*, María del Mar Rubio-Wilhelmi, Juan José Ríos, Begoña Blasco, Miguel Ángel Rosales, Rubén Melgarejo, Luis Romero, Juan Manuel Ruiz

Department of Plant Physiology, Faculty of Science, University of Granada, 18071 Granada, Spain

ARTICLE INFO

Article history:
Received 8 September 2010
Received in revised form
11 November 2010
Accepted 12 November 2010

Keywords:
Ammonia
GS–GOGAT cycle
Nitrate reductase
Nitrogen assimilation
Photorespiration
Solanum lycopersicum L.
Water stress

ABSTRACT

Nitrate assimilation diminishes under water stress. This can augment the photorespiratory rate as a protection mechanism, increasing the ammonium concentration, which must be rapidly assimilated. We therefore examined the effect of moderate water stress in photorespiration and N assimilation, as possible tolerance mechanisms in cherry tomato. Five cherry tomato cultivars with different degrees of water stress tolerance were submitted to two water treatments: well-watered (100% FC) and water stress (50% FC). In the susceptible cultivars, nitrate assimilation declined but without stimulating photorespiration. Zarina, a stress-tolerant cultivar, showed increased activity of the main enzymes involved in photorespiration, together with greater assimilation of nitrates and of the resulting ammonium. This translates as higher concentrations of N as well as amino acids and proteins. We characterize these mechanisms in the cv. Zarina (tolerant) as essential to water stress tolerance, acting on N metabolism as well as helping to maintain or augment biomass.

Introduction

The growing frequency of dry periods in many regions of the world is imposing drought in cultivated areas. Generally, drought depresses nutrient uptake by the root and transport to the shoot due to a restricted transpiration rate, affecting active transport and membrane permeability. The overall result is lower uptake by the plant (Hsiao, 1973; Kramer and Boyer, 1995). Nitrogen (N), required in great quantities by plants, is essential in the biochemistry of non-enzyme compounds such as coenzymes, photosynthetic pigments, secondary metabolites, and polyamines (Maathuis, 2009). Under water stress conditions, N uptake diminishes in soybean and rice (Tanguilig et al., 1987), wheat (Hu et al., 2006) and bean (Zayed and Zeid, 1997). The loss in N uptake can be attributed to a lower transpiration rate and N transport from the root to the shoot (Alam, 1999). Although there are several forms of soil N, nitrate (NO_3^-) is the most bioavailable and best assimilated by plants (Lea and Azevedo, 2006). The reduction of NO_3^- to NO_2^- catalyzed by nitrate reductase (NR) is considered the limiting step in N assimilation. In turn, NO_2^- is reduced by nitrite reductase (NiR) to form NH_4^+. Under water deficit, foliar NR activity reportedly diminishes in *Leymus chinensis* plants and wheat (Xu and Zhou, 2006; Fresneau et al., 2007), with this being attributed to a decline in the internal CO_2 concentration in the leaf and/or a fall in the nitrate supply, although in other studies with tomato undergoing low environmental moisture, this inhibition was not detected (Brewitz et al., 1996).

Another known consequence of moderate water stress is photosynthesis limitation, primarily from restriction of the intercellular CO_2 concentration due to stomatal closure (Cornic and Briantais, 1991; Quick et al., 1992). This could provoke photoinhibition by diminishing the use of electrons by photosynthesis (Roland et al., 2006). As protection of the photosynthetic apparatus against such damage, this excess of photons can be used by photorespiration in C_3 plants. Photorespiration takes place in the chloroplasts, peroxisomes, and mitochondria, a consequence of the oxygenation of ribulose-1,5-biphosphate (RuBP) catalyzed by RuBP carboxylase/oxygenase (Rubisco), which generates one molecule of glycerate-3-phosphate (3-PGA) and one of glycolate-2-phosphate (2-PG). This 2-PG is hydrolyzed by phosphoglycolate phosphatase to glycolate, which is transported to peroxisome and oxidized

Abbreviations: AAT, aspartate aminotransferase; GDH, glutamate dehydrogenase; GGAT, glutamate: glyoxylate aminotransferase; GO, glyoxylate oxidase; GOGAT, glutamate synthase; GS, glutamine synthetase; HR, hydroxypyruvate reductase; LRWC, leaf relative water content; N, nitrogen; NH_4^+, ammonia; NO_2^-, nitrite; NO_3^-, nitrate; NR, nitrate reductase; NiR, nitrite reductase; 2-PG, glycolate-2-phosphate; 3-PG, glycerate-3-phosphate; RGR, relative growth rate; ROS, reactive oxygen species; RUBP, ribulose-1,5-biphosphate; Rubisco, RUBP carboxylase/oxigenase; SGAT, serine: glyoxylate aminotransferase.

* Corresponding author. Tel.: +34 958 243255; fax: +34 958 248995.
E-mail address: evasr@ugr.es (E. Sánchez-Rodríguez).

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doi:10.1016/j.jplph.2010.11.018

to glyoxylate by glyoxylate oxidase (GO). Glyoxylate is transaminated to glycine by the reaction catalyzed by glutamate: glyoxylate aminotransferase (GGAT), and is transported to the mitochondria. Subsequently, glycine is transformed into serine by the action of the enzymes glycine decarboxylase and hydromethyltransferase. The serine formed in the mitochondria is transported to peroxisome, where it is transformed by serine: glioxylate aminotransferase (SGAT) to hydroxypyruvate, which is reduced to glycerate by hydroxypyruvate reductase (HR). Finally, glycerate moves to the chloroplast, where it is phosphorylated by glycerate kinase, giving rise to a molecule of 3-PGA, which enters the Calvin cycle (Wingler et al., 2000).

Osmond and Björkman (1972) proposed that photorespiration could be an important photoprotective mechanism, although there is great controversy in this regard. Some researchers have demonstrated that this process can protect the photosynthetic apparatus against photoinhibition (Park et al., 1996; Guan et al., 2004; Bai et al., 2008) and bolster protection against different types of stress (Shi-Wei et al., 2007). Other authors suggest that photorespiration plays no significant part in protection against photoinhibition (Meng et al., 1999; Nogués and Alegre, 2002). However, a suppression of photorespiration can harm plants, slowing the assimilation rate of CO_2 as well as growth, and altering chloroplast structure (Shi-Wei et al., 2007). Under water stress, CO_2 assimilation diminishes, resulting in a lower level of electron use by photosynthesis. This decline in CO_2 assimilation is caused mainly by stomal closure (Wingler et al., 2000).

Plants produce significant quantities of ammonium (NH_4^+) through the reduction of NO_3^- and photorespiration in the step from glycine to serine. In fact, this process can produce 20-fold more NH_4^+ than that generated by NO_3^- reduction and is considered the largest source of this cation, especially in C_3 plants (Hirel and Lea, 2001). At high concentrations, NH_4^+ is toxic for plant cells and should be rapidly assimilated to organic compounds (Linka and Weber, 2005). Here, other key enzymes associated with N metabolism intervene: glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH). The GS/GOGAT cycle incorporates photorespiratory and non-respiratory NH_4^+, providing N for transport and for maintenance of the nitrogen status in the plant (Suzuki and Knaff, 2005), and it can thus be used as a marker of the N status (Kichey et al., 2006). With regard to GDH, a possible role is to be an adaptive enzyme susceptible to environmental variables (Stitt et al., 2002). The activity of these enzymes can be inhibited by moisture deficit (Sibout and Guerrier, 1998; Xu and Zhou, 2006).

To date, the effect of water stress on the enzymes related to N metabolism is poorly known. Our research group has designed an experimental model of five commercial cherry tomatoes which display different degrees of tolerance to moderate water stress (Sánchez-Rodríguez et al., 2010a) and which present genotypic differences in N uptake (Sánchez-Rodríguez et al., 2010b). The aim of the present work was to examine the ways in which the enzymes involved in N metabolism respond to moderate water stress associated with photorespiration as a mechanism to generate NH_4^+, in order to determine the involvement of this process under stress conditions.

Materials and methods

Plant material and treatments

Five cherry tomato cultivars were used: Kosaco, Josefina, Katalina, Salomé, and Zarina [*Solanum lycopersicum* L. (*Lycopersicon esculentum* Mill.)]. The seeds of these cultivars were germinated and grown for 30 days in a tray with wells (each well 3 cm × 3 cm × 10 cm) in the nursery Semillero Sali-plant S.L. (Carchuna, Granada). Afterwards, the seedlings were transferred to a cultivation chamber at the Plant Physiology Department of the University of Granada under controlled conditions with relative humidity 50 ± 10%, at 25 °C/15 °C (day/night), and a 16 h/8 h photoperiod with a PPFD (photosynthetic photon-flux density) of 350 μmol^{-2} s^{-1} (measured with an SB quantum sensor, LI—COR Inc., Lincoln, NE, USA). Under these conditions, the plants grew in individual pots (25 cm upper diameter, 17 cm lower diameter, and 25 cm high) of 8 L in volume and filled with a 1:1 perlite:vermiculite mixture. Throughout the experiment, the plants were grown in a complete nutrient solution (Sánchez-Rodríguez et al., 2010a). The water stress treatments began 45 days after germination and were maintained for 22 days. The control treatment received 100% field capacity irrigation, whereas moderate water stress corresponded to 50% field capacity (FC). Independent of the procedure for watering (100 or 50% FC), plants received the same quantity of nutrients. We used a randomized complete block design with 2 treatments, arranged in individual pots with six plants per treatment and three replications each. The experiment was repeated three times under the same conditions.

Plant sampling

All plants were at the late vegetative stage when harvested. Leaves (excluding petioles) were sampled on day 67 after germination. The plant material was rinsed three times in distilled water after disinfection with 1% non-ionic detergent and then blotted on filter paper. A part of the plant material was used for the assay of fresh weight (FW), amino acids, proteins, and of NR, NiR, GS, GOGAT, aspartate aminotransferase (AAT), Rubisco, GO, GGAT, HR and GDH enzymatic activities. The rest of the plant material was lyophilized and used to determine NO_3^-, NH_4^+ and organic and total reduce N and total N.

Analysis of N forms, soluble protein and free amino acid concentration

NO_3^- was analyzed from an aqueous extraction of 0.2 g of DW in 10 mL of Millipore-filtered water. A 100 μL aliquot was taken for NO_3^- determination and added to 10% (w/v) salicylic acid in sulphuric acid at 96%, measuring the NO_3^- concentration by spectrophotometry, as performed by Cataldo et al. (1975). NH_4^+ was analyzed from an aqueous extraction and was determined by using the colorimetric method described by Krom (1980).

For the total reduced N determination, a sample of 0.1 g DW was digested with sulphuric acid and H_2O_2 (Wolf, 1982). After dilution with deionized water, a 1 mL aliquot of the digest was added to the reaction medium containing buffer (5% potassium sodium tartrate, 100 μM sodium phosphate and 5.4% (w/v) sodium hydroxide), 15%/0.03% (w/v) sodium silicate/sodium nitroprusside and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37 °C for 15 min, and total reduced N was measured by spectrophotometry according to the method of Baethgen and Alley (1989). Total N concentration was assumed to represent the sum of total reduced N and NO_3^-.

Amino acids and proteins were determined by homogenization of 0.5 g FW in 50 mM cold KH_2PO_4 buffer at pH 7 and centrifugation at 12,000 × *g* for 15 min. The resulting supernatant was used for the determination of total amino acids by the ninhydrin method (Yemm and Cocking, 1955). Soluble proteins were measured with Bradford G-250 reagent (Bradford, 1976).

Nucleotide analysis

Pyridine nucleotides were extracted from liquid N-frozen leaf material in 1 mL of 100 mM NaOH (for NAD(P)H) or 5% TCA (for