J Plant Growth Regul (2013) 32:831–842
DOI 10.1007/s00344-013-9348-2

Role of Grafting in Resistance to Water Stress in Tomato Plants: Ammonia Production and Assimilation

Eva Sánchez-Rodríguez · Luis Romero · Juan Manuel Ruiz

Received: 5 September 2012 / Accepted: 23 April 2013 / Published online: 28 June 2013

Abstract In general, drought depresses nutrient uptake by the root and transport to the shoot due to a restricted transpiration rate, which may contribute to growth limitation under water deprivation. Moreover, water stress may also restrict the ability of plants to reduce and assimilate nitrogen through the inhibition of enzymes implicated in nitrogen metabolism. The assimilation of nitrogen has marked effects on plant productivity, biomass, and crop yield, and nitrogen deficiency leads to a decrease in structural components. Plants produce significant quantities of NH_4^+ through the reduction of NO_3^- and photorespiration, which must be rapidly assimilated into nontoxic organic nitrogen compounds. The aim of the present work was to determine the response of reciprocal grafts made between one tomato tolerant cultivar (*Lycopersicon esculentum*), Zarina, and a more sensitive cultivar, Josefina, to nitrogen reduction and ammonium assimilation under water stress conditions. Our results show that when cv. Zarina (tolerant cultivar) was used as rootstock grafted with cv. Josefina (*ZarxJos*), these plants showed an improved N uptake and NO_3^- assimilation, triggering a favorable physiological and growth response to water stress. On the other hand, when Zarina was used as the scion (*JosxZar*), these grafted plants showed an increase in the photorespiration cycle, which may generate amino acids and proteins and could explain their better growth under stress conditions. In conclusion, grafting improves N uptake or photorespiration, and increases leaf NO_3^- photoassimilation in water stress experiments in tomato plants.

E. Sánchez-Rodríguez (✉) · L. Romero · J. M. Ruiz
Department of Plant Physiology, Faculty of Sciences, University of Granada, 18071 Granada, Spain
e-mail: evasr@ugr.es

Keywords Ammonium assimilation · *Lycopersicon esculentum* Mill · Nitrate reduction · GS/GOGAT cycle · Photorespiration · Grafting

Introduction

Water is crucial for plant growth (Boyer 1982), and, as such, an increase in the prevalence of drought will have an important negative impact on the productivity of agriculture (Passioura 2007). In general, drought depresses nutrient uptake by the root and transport to the shoot due to a restricted transpiration rate affecting active transport and membrane permeability (Kramer and Boyer 1995). Drought-dependent nitrogen deficiency may contribute to growth limitation under water deprivation (Heckathorn and others 1997). Many researchers have shown a directly proportional relationship between nitrate (NO_3^-) and yield, and also between yield and foliar nitrogen (N) content (Kim and others 2011; Li and Lascano 2011). For this reason, crops that maintain a high N content and productivity under water stress are indispensable.

Nitrate is the main nitrogen source in agricultural soils. However, the reduced nitrogen form available to plants for assimilation into amino acids and proteins is ammonium (NH_4^+) (Miflin and Habash 2002). The reduction of NO_3^- to NO_2^- catalyzed by nitrate reductase (NR) is considered the limiting step in N assimilation (Fig. 1). Drought affects different steps of nitrogen metabolism, namely, ion uptake, nitrogen assimilation, and amino acid and protein synthesis. Water stress may also restrict the ability of plants to reduce and assimilate nitrogen through the inhibition of enzymes implicated in nitrogen metabolism, such as NR. Therefore, NR, the first enzyme in the pathway of nitrate assimilation, has proved to be one of the enzymes that

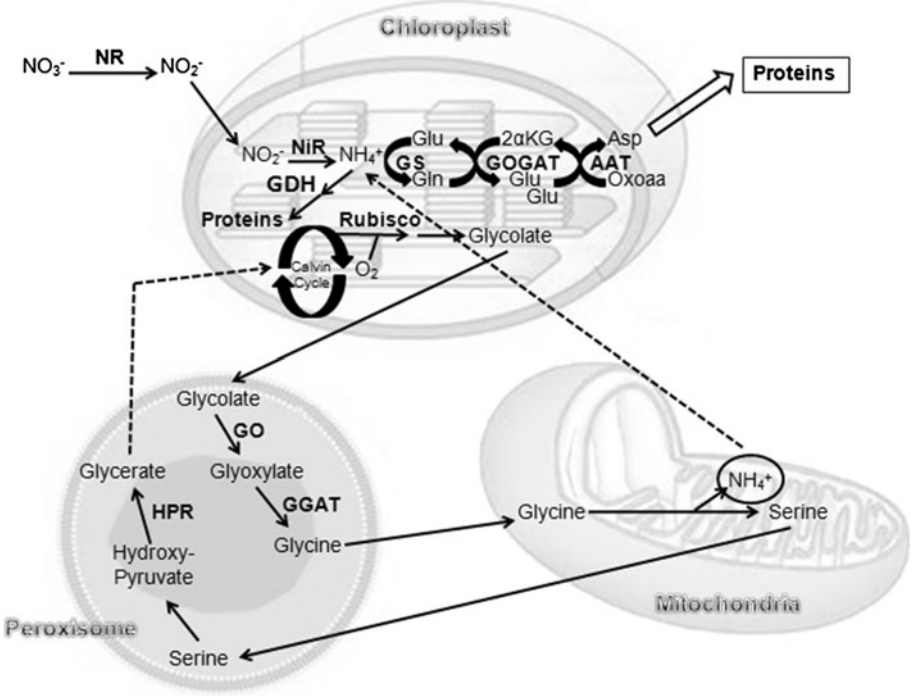

Fig. 1 A schematic representation of nitrate reduction, the photorespiratory cycle, and ammonium assimilation. Photorespiration takes place in the chloroplasts, peroxisomes, and mitochondria, and is a consequence of oxygenation of ribulose-1,5-biphosphate (RuBP) catalyzed by RuBP carboxylase/oxygenase (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 the peroxisome and oxidized 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 the peroxisome, where it is transformed by serine:glyoxylate 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. NH_4^+ assimilation occurred via glutamine synthetase (GS) and glutamate synthase (GOGAT). Nitrogen is incorporated into aspartate and other amides and amino acids by aspartate aminotransferase (AAT)

exhibit declining activity in water-stressed leaves of several species (Fresneau and others 2007; Robredo and others 2011). As such, an increase in the NR activity leads to a corresponding increase in the potential for nitrate reduction and confers a greater capacity for general amino acid synthesis, protein synthesis, or total nitrogen (Singh and Usha 2003).

Another known effect of water stress in plants is a closure of stomata to avoid further loss of water through transpiration (Lawlor 1995). This could cause photoinhibition by diminishing the use of electrons by photosynthesis (Roland and others 2006). As protection of the photosynthetic apparatus against such damage, this excess of photons can be used by photorespiration in C3 plants. Thus, this process has been viewed as a wasteful process, a vestige of the high CO_2 atmosphere under which plants evolved (Wingler and others 2000). At best, according to current thought, photorespiration may mitigate photoinhibition under high light and drought stress (Wingler and others 2000), or it may generate amino acids such as glycine for other metabolic pathways (Noctor and others 1999).

Plants produce significant quantities of NH_4^+ through the reduction of NO_3^- and photorespiration in the step from glycine to serine (Fig. 1). In fact, photorespiration 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 C3 plants (Hirel and Lea 2001). NH_4^+ is toxic to plants, causing proton extrusion associated with ammonium uptake, cytosolic pH disturbances, uncoupling of photophosphorylation, and so on (Kronzucker and others 2001). Therefore, it must be rapidly assimilated into nontoxic organic nitrogen compounds. This assimilation occurs via glutamine synthetase (GS) and glutamate synthase (GOGAT) (Fig. 1). Nitrogen is incorporated into aspartate and other amides and amino acids by aspartate aminotransferase (AAT). Alternatively, glutamate dehydrogenase (GDH) can also catalyze NH_4^+ incorporation into glutamate by reductive amination of 2-oxoglutarate (Cammaerts and Jacobs 1985). The function of the alternative GDH pathway remains unclear; it is proposed to play a complementary role under adverse environmental conditions (Lu and others 2005). Wang and others (2007)

reported that the GS/GOGAT cycle does not play a major role in NH_4^+ assimilation under salinity stress in wheat plants. In cucumber plants under nitrate stress, the GS/GOGAT cycle decreased, possibly due to low water potential and NH_3 toxicity (Yang and others 2010). Other authors have shown that resistance to water stress was increased to improve the activity of N metabolism in key enzymes (Xu and Zhou 2006; Sánchez-Rodríguez and others 2011a).

Grafting is a horticultural technique, practiced for many years and in many parts of the world, used to overcome many abiotic stresses (Estañ and others 2005; Venema and others 2008; Abdelmageed and Gruda 2009). Grafted plants usually show increased uptake of water and minerals compared with self-rooted plants as a consequence of the vigorous root system used as rootstock (Ruiz and others 2006). Ruiz and others (1997) concluded that N content was influenced more by the rootstock genotype than by the scion in melon plants. The utilization of certain rootstocks has been found to stimulate NR activity and nitrogen metabolism in roses, melon, and tobacco plants (Pulgar and others 2000; Ruiz and others 2006). The characteristics of the rootstocks could result in increased absorption, upward transport, and accumulation of NO_3^- in the scion, thereby stimulating NR and NO_3^- assimilation (Martínez-Ballesta and others 2010). However, little is known about the effect of grafting on the activity of enzymes involved in NH_4^+ assimilation.

The practical and horticultural aspects of grafting technology have been described in several reviews (Lee and Oda 2003; Martinez-Ballesta and others 2010), but less has been compiled about the physiological implications of rootstock–scion interactions as a barrier for the translocation of water and nutrients, or the effect of the rootstock–scion connection on N metabolism of the grafted plants. In a previous work, we selected the most drought tolerant (cv. Zarina) and sensitive (cv. Josefina) from among five commercial tomato cultivars (*Lycopersicon esculentum*) (Sánchez-Rodríguez and others 2010) and observed that cv. Zarina presented an improvement in N metabolism under water stress conditions (Sánchez-Rodríguez and others 2011a). Therefore, the aim of the present work was to examine the ways in which the grafting affects enzymes involved in N metabolism in response to moderate water stress associated with photorespiration as a mechanism to generate NH_4^+, to determine the involvement of grafting in this process under stress conditions. We studied the response to moderate water stress with different combinations of grafted scion–rootstocks and self-grafted and ungrafted tomato plants using cv. Zarina and cv. Josefina to test the viability and efficiency of this grafting technique in terms of N metabolism.

Materials and Methods

Plant Material and Treatments

Two tomato (*L. esculentum* Mill) cultivars, Zarina and Josefina, were used as scion and rootstock (Fig. 2). The seeds of these cultivars were germinated and grown for 30 days in a tray with wells (each well was 3 cm × 3 cm × 10 cm) in the nursery Semillero Saliplant S.L. (Carchuna, Granada). Grafting was performed when seedlings had developed three to four true leaves. In the vermiculite trays used for germination, the seedlings were cut over the cotyledons, using the shoot as scion and the remaining plant part as rootstock. Grafts were made immediately after cutting the plants, and grafting clips were used to hold the graft union. Self-grafted plants were included as controls. After grafting, seedlings were covered with a transparent plastic lid to maintain a high humidity level and to facilitate graft formation and were left in the shade for 24 h. The lid was opened slightly every day to allow reduction in relative humidity; it was removed 6 days after grafting. Afterward, ungrafted and grafted plants were transferred to a cultivation chamber at the Plant Physiology Department of the University of Granada under controlled conditions, with relative humidity of 50 ± 10 %, 25 °C/15 °C (day/night), 16-h/8-h photoperiod, and a PPFD (photosynthetic photon flux density) of 350 μmol m^{-2} s^{-1} (measured with an SB quantum 190 sensor, LI-COR Inc., Lincoln, NE, USA). Under these conditions, the plants grew in individual 8-L pots (25-cm upper diameter, 17-cm lower diameter, and 25 cm in height) filled with a 1:1 perlite:vermiculite mixture. Throughout the experiment, the plants were grown in a complete nutrient solution (Sánchez-Rodríguez and others 2010). The water stress treatments began 45 days after germination and were maintained for 22 days. The control treatment received 100 % field capacity (FC) irrigation, whereas moderate water stress corresponded to 50 % field capacity. The experimental design was a randomized complete block with 12 treatments (Zarina ungrafted, Josefina ungrafted, Zarina self-grafted, Josefina self-grafted, *JosxZar* and *ZarxJos* well-watered 100 % FC and water stress 50 % FC) (Fig. 2) arranged in individual pots with six plants per treatment (one plant per pot) and three replications each.

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 % nonionic detergent and then blotted on filter paper. A part of the plant material was used for the assay of fresh weight (FW), amino

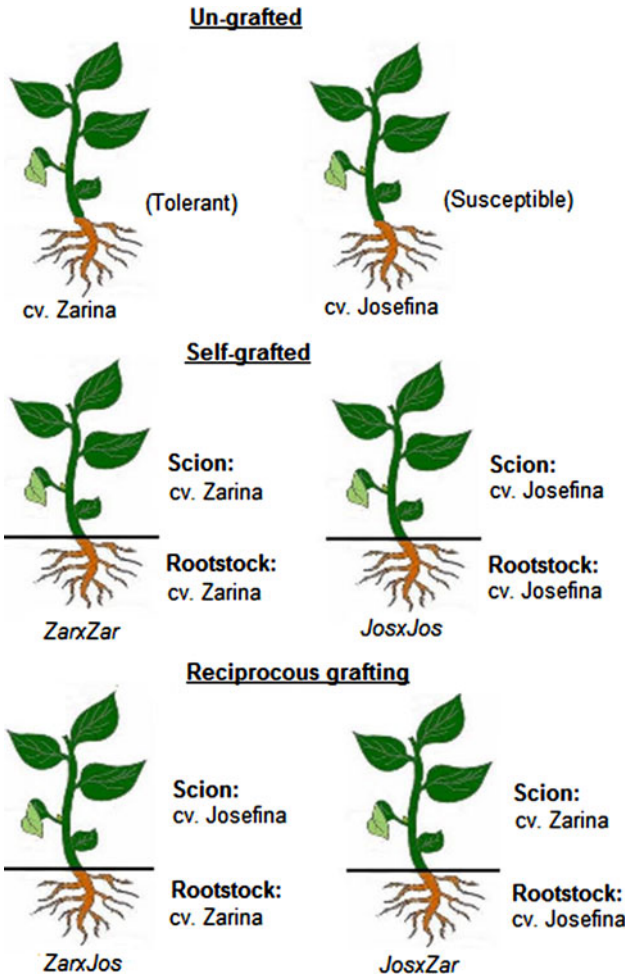

Fig. 2 Outline of the grafting design

acids, and proteins, and of NR, nitrite reductase (NiR), glutamine synthase (GS), glutamate synthetase (GOGAT), aspartate aminotransferase (AAT), Rubisco, glyoxylate oxidase (GO), glutamate:glyoxylate aminotransferase (GGAT), hydroxypyruvate reductase (HR), and glutamate dehydrogenase (GDH) enzymatic activities. The rest of the plant material was lyophilized and used to determine NO_3^-, NH_4^+, and organic and total reduced 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 sulfuric acid at 96 %, and the NO_3^- concentration was measured by spectrophotometry as performed by Cataldo and others (1975). NH_4^+ was analyzed from an aqueous extraction and was determined by using the colorimetric method described by Krom (1980).

For total reduced-N determination, a sample of 0.1 g DW was digested with sulfuric 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 the 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 which was then centrifuged 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 $NAD(P)^+$]. The extracts were boiled for 6 min, cooled on ice, and centrifuged at 12,000*g* for 6 min. Samples were adjusted to pH 8.0 with HCl or NaOH and 100 mM bicine (pH 8.0). Nucleotides were quantified by the enzyme-cycling method (Matsumura and Miyachi 1980) with some modification (Gibon and Larher 1997).

Enzyme Extractions and Assays

Leaves were ground in a mortar at 0 °C in 50 mM KH_2PO_4 buffer (pH 7.5) containing 2 mM EDTA, 1.5 % (w/v) soluble casein, 2 mM dithiothreitol (DTT), and 1 % (w/v) insoluble polyvinylpolypyrrolidone. The homogenate was filtered and then centrifuged at 30,000 *g* for 20 min. The resulting extract (cytosol and organelle fractions) was used to measure the enzyme activity of NR, NiR, GOGAT, and GDH. The extraction medium was optimized for these enzyme activities so that they could be extracted together according to the same method (Singh and Srivastava 1986).

The NR assay followed the methodology of Kaiser and Lewis (1984). The NO_2^- formed was colorimetrically determined at 540 nm after azocoupling with sulfanilamide and naphthylethylenediamine dihydrochloride according to the method of Hageman and Hucklesby (1971).

NiR activity was defined by the disappearance of NO_2^- from the reaction medium (Lillo 1984). After incubation at 30 °C for 30 min, the NO_2^- content was determined colorimetrically as above.

GOGAT activity was assayed spectrophotometrically at 30 °C by monitoring the oxidation of NADH at 340 nm, essentially as indicated by Singh and Srivastava (1986), always within 2 h of extraction. Two controls, without ketoglutarate and glutamine, respectively, were used to correct for endogenous NADH oxidation. The decrease in absorbance was recorded for 5 min.

GDH activity was assayed by monitoring the oxidation of NADH at 340 nm, essentially as indicated by Singh and Srivastava (1986). The reaction mixture consisted of 50 mM KH_2PO_4 buffer (pH 7.5) with 200 mM NH_4^+ sulfate, 0.15 mM NADH, 2.5 mM 2-oxoglutarate, and enzyme extract. Two controls, without ketoglutarate and NH_4^+ sulfate, respectively, were used to correct for endogenous NADH oxidation. The decrease in absorbance was recorded for 3 min.

GS was determined by an adaptation of the hydroxamate synthetase assay published by Kaiser and Lewis (1984). Leaves were ground in a mortar at 0 °C in 50 mL of maleic acid–KOH buffer (pH 6.8), containing 100 mM sucrose, 2 % (v/v) β-mercaptoethanol, and 20 % (v/v) ethylene glycol. The homogenate was centrifuged at 30,000*g* for 20 min. The resulting extract was used to measure the enzyme activity of GS. The reaction mixture used in the GS assay was composed of 100 mM KH_2PO_4 buffer (pH 7.5), with 4 mM EDTA, 100 mM L-sodium glutamate, 450 mM $MgSO_4 \cdot 7H_2O$, 300 mM hydroxylamine, 100 mM ATP, and enzyme extract. Two controls were prepared, one without glutamine and the other without hydroxylamine. After incubation at 28 °C for 30 min, the formation of glutamylhydroxamate was colorimetrically determined at 540 nm after complexing with acidified ferric chloride.

AAT activity was assayed spectrophotometrically at 340 nm using the method published by Gonzalez and others (1995). AAT enzyme was extracted in conditions identical to those for GS. The reaction mixture consisted of 50 mM Tris–HCl buffer (pH 8), 4 mM $MgCl_2$, 10 mM aspartic acid, and enzyme extract. The decrease in absorbance was recorded for 3 min.

Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25 °C according to Nakano and others (2000). The total activity was assayed after the crude extract was activated in a 0.1-mL activation mixture containing 33 mM Tris–HCl (pH 7.5), 0.67 mM EDTA, 33 mM $MgCl_2$, and 10 mM $NaHCO_3$ for 15 min. Initial Rubisco activity measurements were carried out in a 0.1-mL reaction medium containing 5 mM Hepes–NaOH (pH 8.0), 1 mM $NaHCO_3$, 2 mM $MgCl_2$, 0.25 mM DTT, 0.1 mM EDTA, 1 U glyceraldehyde 3-phosphate dehydrogenase, 0.5 mM ATP, 0.015 mM NADH, 0.5 mM phosphocreatine, 0.06 mM RuBP, and 10 µL of extract. The change in absorbance at 340 nm was monitored.

For the GO determination, fresh leaf tissue (0.25 g) was ground in a chilled mortar with PVPP and 1 mL of 50 mM Tris–HCl buffer (pH 7.8) with 0.01 % Triton X-100 and 5 mmol 1,4-dithioerythritol (DTT). The homogenate was centrifuged at 30,000*g* for 20 min. The supernatant was decanted and immediately used for the enzyme assay. GO was assayed as described by Feierabend and Beevers (1972) with modifications. A volume of assay mixture containing 50 mM Tris–HCl buffer (pH 7.8), 0.009 % Triton X-100, 3.3 mM phenylhydrazine HCl (pH 6.8), 50 µL of plant extract, and 5 mM glycolic acid (neutralized to pH 7 with KOH) was used to start the reaction. GO activity was determined by following the formation of glyoxylate phenylhydrazone at 324 nm for 2 min after an initial lag phase of 1 min.

For determination of GGAT and HR, leaves were ground in a chilled mortar in 100 mM Tris–HCl buffer (pH 7.3) containing 0.1 % (v/v) Triton X-100 and 10 mM DTT. The homogenate was centrifuged at 20,000*g* for 10 min. The resulting extract was used to measure enzyme activity. The extraction medium was optimized for the enzyme activities such that they could be extracted together using the same method (Hoder and Rej 1983).

GGAT activity was measured by coupling the reduction of 2-oxoglutarate by NADH in a reaction catalyzed by GDH. The reaction was assayed in a mixture containing 100 mM Tris–HCl (pH 7.3), 20 mM glutamate, 1 mM glyoxylate, 0.18 mM NADH, 0.11 mM pyridoxal-5-phosphate, 83 mM NH_4Cl, and 0.3 U GDH in a final volume of 0.6 mL (Igarashi and others 2006).

HR assay was performed with 100 mM Tris–HCl (pH 7.3), 5 mM hydroxypyruvate, and 0.18 mM NADH. Activity was assayed spectrophotometrically by monitoring NADH oxidation at 340 nm (Hoder and Rej 1983).

The protein concentration of the extracts was determined according to the method of Bradford (1976) using bovine-serum albumin as the standard.

Statistical Analysis

Data were subjected to a simple analysis of variance (ANOVA) at 95 % confidence using the Statgraphics 6.1 program (Statpoint Technologies, Warrenton, VA, USA). Means were compared using Fisher's least-significant differences (LSD).

Results

NH_4^+ Production: NO_3^- Reduction and Photorespiration

Nitrate levels showed a significant increase in ungrafted Zarina under water stress conditions; however, in cv.

Josefina a decrease of 40 % was observed (Table 1). In self-grafting, *ZarxZar* and *JosxJos*, no significant differences were observed between well-watered and water stress. In reciprocal grafting, only *ZarxJos* showed a stronger increase in nitrate concentration under stress conditions, whereas in *JosxZar* no significant differences were observed (Table 1). The results of NR assays reflected significant differences in cv. Zarina and its self-graft, in which we observed an increase under water stress conditions (Table 1). Besides, Josefina ungrafted and *JosxJos* showed decreased NR activity over well-watered conditions (Table 1). In reciprocal grafting, NR activity increased only in *ZarxJos*. Moreover, for NiR activity, no significant differences were observed in any cultivars (Table 1). In the case of NH_4^+ concentration, ungrafted Zarina showed a decrease over control conditions (Table 1). In the reciprocal grafting, no significant differences in NH_4^+ content were observed under stress conditions (Table 1).

With regard to the photorespiration process, only the initial activity and the total Rubisco showed significant differences in ungrafted Zarina and *JosxZar*, which presented an increase in Rubisco activity under water stress conditions (Fig. 3). The activity of enzymes that complete the cycle of photorespiration, that is, GO, GGAT, and HPR, showed a general increase under water stress conditions in ungrafted Zarina, *ZarxZar*, and *JosxZar* (Table 2). However, for cv. Josefina self-graft and *ZarxJos*, a general decrease was observed with respect to the well-watered condition (Table 2). With respect to the different forms of pyridine dinucleotides, our results showed a decrease in NADH in Josefina ungrafted, *JosxJos*, and *ZarxJos* under water stress (Table 3), whereas *JosxZar* presented an increase of 32 % in the NADH concentration under water stress with respect to well-watered plants. Zarina ungrafted and *JosxZar* showed an increase in $NADP^+$ under water stress (Table 3). For the NADH/NAD ratio, only *JosxZar* showed a significant increase of 43 % under water stress (Table 3).

NH_4^+ Incorporation and Assimilation Products

The enzymes of the GS/GOGAT cycle increased under water stress conditions in Zarina ungrafted and in reciprocal grafting (*JosxZar* and *ZarxJos*) (Table 4). However, in cv. Josefina no significant differences were observed. Self-grafting showed a decrease in GS and GOGAT activities under water stress conditions (Table 4). With regard to AAT activity, cv. Zarina, *JosxZar*, and *ZarxJos* showed an increase of 47, 22, and 67 %, respectively. No

Table 1 Influence of moderate water stress on response of NO_3^- reduction and NH_4^+ concentration in ungrafted, grafted, and self-grafted tomato plants

Cultivar/water treatment	NO_3^-	NR	NiR	NH_4^+
Zar ungrafted				
Well-watered	10.89 ± 0.09	1.01 ± 0.15	6.57 ± 0.65	1.12 ± 0.01
Water stress	19.09 ± 0.09*	2.29 ± 0.30*	5.71 ± 1.33	0.44 ± 0.01*
$LSD_{0.05}$	4.18	0.72	2.16	0.02
ZarxZar				
Well-watered	10.09 ± 0.14	0.88 ± 0.04	5.72 ± 0.79	0.49 ± 0.00
Water stress	9.75 ± 0.11	1.89 ± 0.13*	4.22 ± 0.49	0.72 ± 0.01*
$LSD_{0.05}$	2.38	0.31	1.99	0.04
Jos ungrafted				
Well-watered	30.62 ± 0.14	4.28 ± 0.46	3.32 ± 0.37	0.34 ± 0.01
Water stress	20.50 ± 0.14*	2.87 ± 0.14*	4.20 ± 0.91	0.67 ± 0.01*
$LSD_{0.05}$	5.11	1.04	2.08	0.02
JosxJos				
Well-watered	20.81 ± 0.09	2.00 ± 0.07	2.71 ± 0.51	0.42 ± 0.01
Water stress	21.01 ± 0.09	1.17 ± 0.22*	3.12 ± 0.54	0.86 ± 0.01*
$LSD_{0.05}$	6.28	0.50	1.59	0.03
JosxZar				
Well-watered	20.73 ± 0.09	2.59 ± 0.14	3.42 ± 0.34	0.67 ± 0.01
Water stress	20.75 ± 0.18	1.93 ± 0.36	4.26 ± 0.78	0.66 ± 0.01
$LSD_{0.05}$	4.13	0.82	1.82	0.02
ZarxJos				
Well-watered	20.60 ± 0.13	2.01 ± 0.22	5.46 ± 0.73	0.61 ± 0.02
Water stress	29.93 ± 0.06*	3.14 ± 0.27*	4.69 ± 0.49	0.63 ± 0.02
$LSD_{0.05}$	6.22	0.75	1.87	0.06

NO_3^- and NH_4^+ were expressed as mg g^{-1} DW; nitrate reductase (NR) was expressed as mM NO_2^- h^{-1} mg^{-1} prot; nitrite reductase (NiR) was expressed as mM NO_2^- h^{-1} mg^{-1} prot

* Significant difference with controls groups (well-watered)

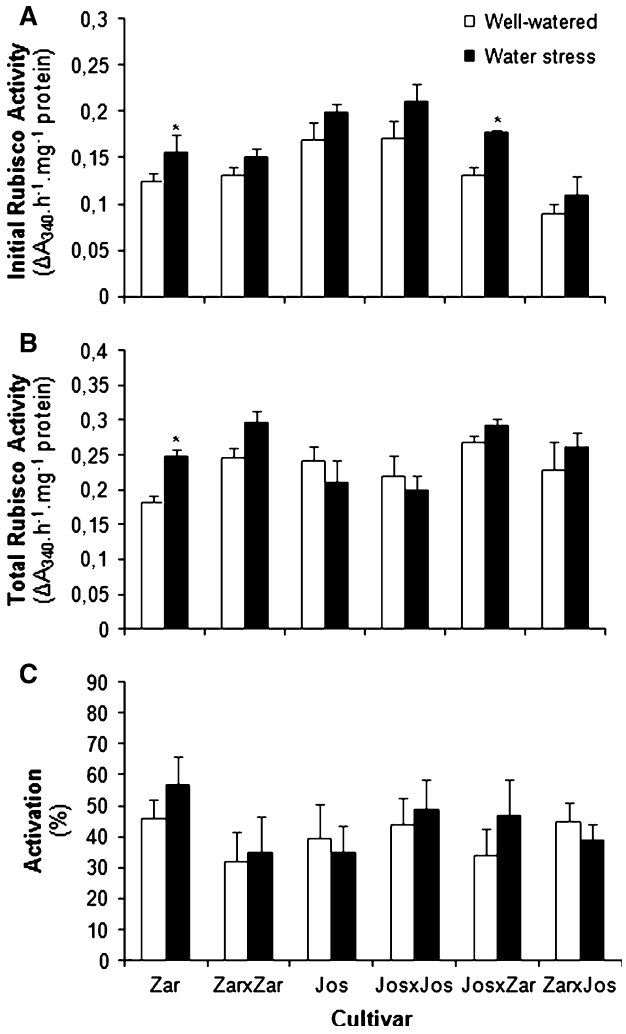

Fig. 3 Response of Rubisco activity in ungrafted, grafted, and self-grafted tomato plants well-watered and subjected to moderate water stress. Columns are mean ± SE ($n = 9$), and differences between means were compared using Fisher's least-significant difference test (LSD; $p = 0.05$). *Asterisk* (*) indicates significant difference with control groups

Table 2 Influence of moderate water stress on some photorespiration enzymes in ungrafted, grafted, and self-grafted tomato plants

Cultivar/water treatment	GO	GGAT	HR
Zar ungrafted			
Well-watered	1.96 ± 0.12	0.20 ± 0.07	2.45 ± 0.11
Water stress	4.59 ± 0.75*	0.32 ± 0.08*	3.26 ± 0.22*
$LSD_{0.05}$	1.62	0.10	0.43
ZarxZar			
Well-watered	1.28 ± 0.22	0.29 ± 0.04	0.63 ± 0.16
Water stress	3.79 ± 0.27*	0.59 ± 0.15*	0.98 ± 0.05*
$LSD_{0.05}$	0.74	0.14	0.28
Jos ungrafted			
Well-watered	7.77 ± 0.70	1.10 ± 0.57	3.24 ± 0.22
Water stress	2.52 ± 0.03*	0.17 ± 0.03*	0.68 ± 0.01*
$LSD_{0.05}$	1.48	0.22	0.46
JosxJos			
Well-watered	7.68 ± 0.97	0.37 ± 0.09	1.98 ± 0.44
Water stress	6.13 ± 0.36	0.06 ± 0.01*	0.13 ± 0.01*
$LSD_{0.05}$	2.21	0.19	0.94
JosxZar			
Well-watered	1.67 ± 0.17	0.18 ± 0.04	1.72 ± 0.06
Water stress	6.71 ± 0.40*	0.59 ± 0.19*	1.68 ± 0.17
$LSD_{0.05}$	0.93	0.11	0.39
ZarxJos			
Well-watered	4.52 ± 0.40	0.64 ± 0.12	3.00 ± 0.26
Water stress	0.93 ± 0.12*	0.14 ± 0.03*	2.95 ± 0.14
$LSD_{0.05}$	0.89	0.26	0.64

Glycolate oxidase (GO), glutamate:glyoxylate aminotransferase (GGAT), and hydroxypyruvate reductase (HR) activities are expressed as $\Delta A\ h^{-1}\ mg^{-1}$ prot

* Significant difference with controls groups (well-watered)

significant differences were observed in other graft combinations (Table 4). Finally, by contrast, the GDH activity increased significantly only in cv. Josefina, *JosxJos*, and *ZarxZar*, whereas no significant differences were observed in the other cases (Fig. 4).

Reduced N was increased in Zarina ungrafted and *ZarxZar* under water stress conditions (Fig. 5a). However, a significant decrease was observed in cv. Josefina and its self-graft. There was no significant difference with respect to well-watered conditions in the reciprocal grafts (Fig. 5a). With regard to total N, only cv. Zarina and *ZarxJos* showed an increase under stress conditions (Fig. 5b). For soluble amino acids, no significant differences were observed in different grafting combinations (Fig. 5c). Also, soluble proteins increased in the cv. Zarina and the reciprocal grafts, whereas in the rest of the cases the values were not affected or decreased after water stress (Fig. 5d).

Discussion

NH_4^+ Production: NO_3^- Reduction and Photorespiration

In general, drought can depress nutrient uptake by the root and transport to the shoot as a result of a restricted transpiration rate (Kramer and Boyer 1995). However, water and nutrient uptake could be increased in grafted plants as a result of the enhancement of vigor by the rootstock's root system and its effects on plant yield (Ruiz and others 1997). Indeed, in our previous work we studied the effects of grafts in uptake fluxes and found that the use of cv.

Table 3 Influence of moderate water stress on pyridine dinucleotides concentration in ungrafted, grafted, and self-grafted tomato plants

Cultivar/water treatment	NAD^+	NADH	$NADP^+$	NADPH	NADH/NAD
Zar ungrafted					
Well-watered	2.22 ± 0.12	3.54 ± 0.25	1.62 ± 0.08	2.24 ± 0.15	1.57 ± 0.09
Water stress	1.95 ± 0.11	4.01 ± 0.26	2.32 ± 0.10*	2.62 ± 0.11	2.01 ± 0.14
$LSD_{0.05}$	0.32	0.52	0.24	0.54	0.55
ZarxZar					
Well-watered	2.00 ± 0.12	2.44 ± 0.14	1.86 ± 0.18	1.85 ± 0.12	1.26 ± 0.04
Water stress	1.85 ± 0.11	2.01 ± 0.05	2.06 ± 0.17	1.84 ± 0.06	1.11 ± 0.02
$LSD_{0.05}$	0.42	0.62	0.43	0.48	0.36
Jos ungrafted					
Well-watered	0.79 ± 0.11	2.49 ± 0.15	2.54 ± 0.21	1.98 ± 0.11	3.14 ± 0.19
Water stress	1.58 ± 0.14*	1.21 ± 0.13*	2.15 ± 0.31	2.15 ± 0.22	0.89 ± 0.12*
$LSD_{0.05}$	0.58	0.45	0.55	0.46	0.41
JosxJos					
Well-watered	1.01 ± 0.14	2.65 ± 0.09	2.16 ± 0.15	1.78 ± 0.08	2.64 ± 0.14
Water stress	1.68 ± 0.11*	1.59 ± 0.10*	2.11 ± 0.10	1.98 ± 0.15	0.99 ± 0.08*
$LSD_{0.05}$	0.34	0.54	0.26	0.35	0.26
JosxZar					
Well-watered	1.98 ± 0.08	3.01 ± 0.11	2.15 ± 0.14	1.86 ± 0.10	1.50 ± 0.09
Water stress	1.89 ± 0.05	3.98 ± 0.12*	2.62 ± 0.20*	1.97 ± 0.20	2.15 ± 0.11*
$LSD_{0.05}$	0.25	0.61	0.40	0.26	0.28
ZarxJos					
Well-watered	1.95 ± 0.14	2.54 ± 0.14	1.99 ± 0.11	1.97 ± 0.11	1.34 ± 0.15
Water stress	2.04 ± 0.11	2.01 ± 0.09*	2.09 ± 0.08	1.98 ± 0.09	0.97 ± 0.04*
$LSD_{0.05}$	0.24	0.34	0.22	0.29	0.24

$NAD(P)^+$ and NAD(P)H are expressed as μM g^{-1} DW

* Significant difference with controls groups (well-watered)

Zarina as rootstock (*ZarxJos*) improves the NO_3^- uptake flux under stress conditions (Sánchez-Rodríguez and others 2011b). According to these data, our results showed an increase in NO_3^- concentration and NR activity under water stress only in cv. Zarina ungrafted and in *ZarxJos* (Zarina used as rootstock) (Table 1). The characteristics of the rootstocks could result in increased absorption, upward transport, and accumulation of NO_3^- in the scion, thereby stimulating NR and NO_3^- assimilation. Similar results were obtained by Ruiz and Romero (1999) in melon plants; NR activity and NO_3^- accumulation were conditioned significantly by the scion–rootstock interaction and by rootstock genotype, whereas the scion genotype did not show any such effect. Many researchers have shown a directly proportional relationship between NO_3^- and yield (Kim and others 2011; Li and Lascano 2011). Also, Ruiz and others (1997, 2006) have shown the essential role of NO_3^- assimilation in the yield increase. Our result showed that cv. Zarina, *JosxZar*, and *ZarxJos* had greater biomass and a relative growth rate (RGR) associated with high leaf relative water content (LRWC) under water deficit conditions, indicating that these cultivars are more tolerant to this growth situation (Sánchez-Rodríguez and others 2011c).

It has been estimated that the production of NH_4^+ by photorespiration is much greater than the primary assimilation of NH_4^+ resulting from nitrate reduction (Wingler and others 2000). Our results showed that only cv. Zarina and *JosxZar* (cv. Zarina-like scion) had an increase in initial and total Rubisco activity under water stress (Fig. 3) and in the activities of the enzymes GO, GGAT, and HPR (Table 3). Thus far, the effects of drought on Rubisco activity were inconsistent in studies in which different plant species and stress durations were used (Parry and others 2002). Rubisco activity varies with plant species and cultivars that differ in drought tolerance; drought-tolerant plants typically exhibit higher Rubisco activity (Galmés and others 2011; Carmo-Silva and others 2012). These results agree with those of Ferreira-Silva and others (2010), who observed that the higher stability shown by Rubisco in cashew BRS/BRS grafted plants could indicate that this combination can be more resistant under salinity. Moreover, it was found that the grafted bitter melon seedlings had a higher Rubisco activity than ungrafted seedlings under flooding stress (Liao and Lin 1996). On the other hand, photorespiration serves as an important redox mechanism that increases the cytosolic NADH/NAD ratio (Lee and Oda 2003). In *JosxZar*, which increases the

Table 4 Influence of moderate water stress on enzymes responsible for NH_4^+ assimilation in ungrafted, grafted, and self-grafted tomato plants

Cultivar/water treatment	GS	GOGAT	AAT
Zar ungrafted			
Well-watered	0.17 ± 0.01	1.34 ± 0.26	1.87 ± 0.40
Water stress	0.25 ± 0.01*	2.18 ± 0.35*	2.75 ± 0.33*
$LSD_{0.05}$	0.03	0.62	0.80
ZarxZar			
Well-watered	0.22 ± 0.02	1.13 ± 0.29	7.52 ± 1.46
Water stress	0.17 ± 0.01*	0.37 ± 0.04*	5.17 ± 0.44
$LSD_{0.05}$	0.04	0.62	2.24
Jos ungrafted			
Well-watered	0.13 ± 0.01	0.89 ± 0.11	5.74 ± 0.37
Water stress	0.18 ± 0.02	0.85 ± 0.17	5.92 ± 0.56
$LSD_{0.05}$	0.05	0.14	1.42
JosxJos			
Well-watered	0.15 ± 0.01	1.32 ± 0.41	3.62 ± 1.10
Water stress	0.11 ± 0.01*	0.40 ± 0.07*	0.89 ± 0.05
$LSD_{0.05}$	0.02	0.88	2.34
JosxZar			
Well-watered	0.18 ± 0.01	1.18 ± 0.08	4.19 ± 0.61
Water stress	0.26 ± 0.02*	1.84 ± 0.52*	5.11 ± 0.53*
$LSD_{0.05}$	0.05	0.12	0.71
ZarxJos			
Well-watered	0.14 ± 0.01	0.91 ± 0.28	3.43 ± 0.53
Water stress	0.24 ± 0.01*	1.61 ± 0.21*	5.73 ± 0.82*
$LSD_{0.05}$	0.02	0.55	2.08

Glutamine synthetase (GS), glutamate synthase (GOGAT), and aspartate aminotransferase (AAT) activities are expressed as $\Delta A\ h^{-1}\ mg^{-1}$ prot

Values are mean ± SE ($n = 9$), and differences between means were compared using Fisher's least-significance test (LSD; $p = 0.05$)

* Significant difference with controls groups (well-watered)

photorespiration, we observed an increase in NADH concentration and the NADH/NAD ratio, whereas in Zarina ungrafted and *ZarxZar*, no significant difference was observed (Table 3). Because the first step of NO_3^- assimilation occurs in the cytosol and uses NADH, this may explain why we observed NO_3^- assimilation to be greater when photorespiration was highest in cv. Zarina ungrafted and its self-graft. However, in *JosxZar*, photorespiration is high although nitrate uptake is not enhanced by the root and NO_3^- assimilation is therefore lower.

NH_4^+ Incorporation and Assimilation Products

The reassimilation of NH_4^+ produced by the photorespiratory nitrogen cycle is essential for maintaining nitrogen status (Wingler and others 2000). In higher plants, NH_4^+ is assimilated mainly through the concerted action of GS and GOGAT. Several authors have shown that the decline in GS activity is correlated with water stress (Robredo and others 2011), and in our study, drought stress provoked a marked decrease in GS activity in cv. Josefina and the self-graft (Table 4). The GS/GOGAT cycle increased in cv. Zarina ungrafted, *JosxZar*, and *ZarxJos* only under stress conditions (Table 4). In *ZarxJos*, increased ammonium assimilation could result from increased NO_3^- assimilation, whereas in *JosxZar*, NH_4^+ in the water-stressed plant might result mainly from increased photorespiration. These results agree with those of Masclaux-Daubresse and others (2006) from their study of tobacco plants. They provide strong evidence that the GS/GOGAT cycle is the primary route of ammonium assimilation and that GDH plays a minor role. In fact, GDH activity showed an increase only in Josefina ungrafted, its self-graft, and *ZarxZar* under water stress (Fig. 4). In this sense, it has been demonstrated that NH_4^+ might be a signal responsible for the induction of GDH activity (Masclaux-Daubresse and others 2006). Thus, the increased NH_4^+ observed in Josefina ungrafted, its self-graft, and *ZarxZar* under water stress (Table 1) might be responsible for the higher GDH activity (Fig. 4), which has been previously shown in wheat seedlings exposed to salinity (Wang and others 2007). Moreover, NH_4^+ is toxic to plants which might result in decreased biomass in these cultivars (Sánchez-Rodríguez and others 2011c). Besides, it has been demonstrated that the combined action of GS and GOGAT is the principal pathway for assimilating ammonia, and the amination activity of GDH functions only when the GS/GOGAT cycle pathway is inhibited under stress conditions such as salinity or drought (Mena-Petite and others 2006). Finally, the glutamate and glutamine generated in the GS/GOGAT cycle are allocated to the synthesis of aspartate and asparagine,

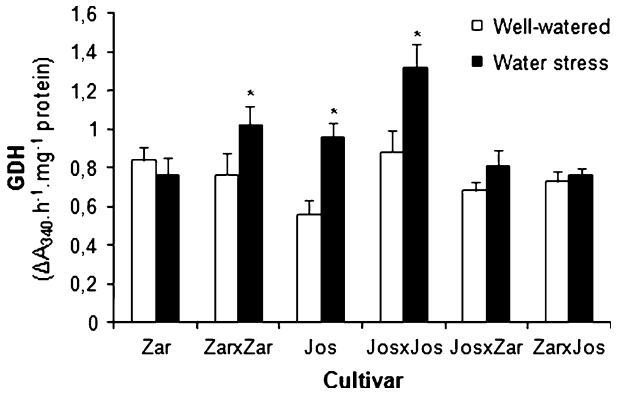

Fig. 4 Response of glutamate dehydrogenase (GDH) in ungrafted, grafted, and self-grafted tomato plants well-watered and subjected to moderate water stress. Columns are mean ± SE ($n = 9$), and differences between means were compared by Fisher's least-significant difference test (LSD; $p = 0.05$). *Asterisk* (*) indicates significant difference with control groups

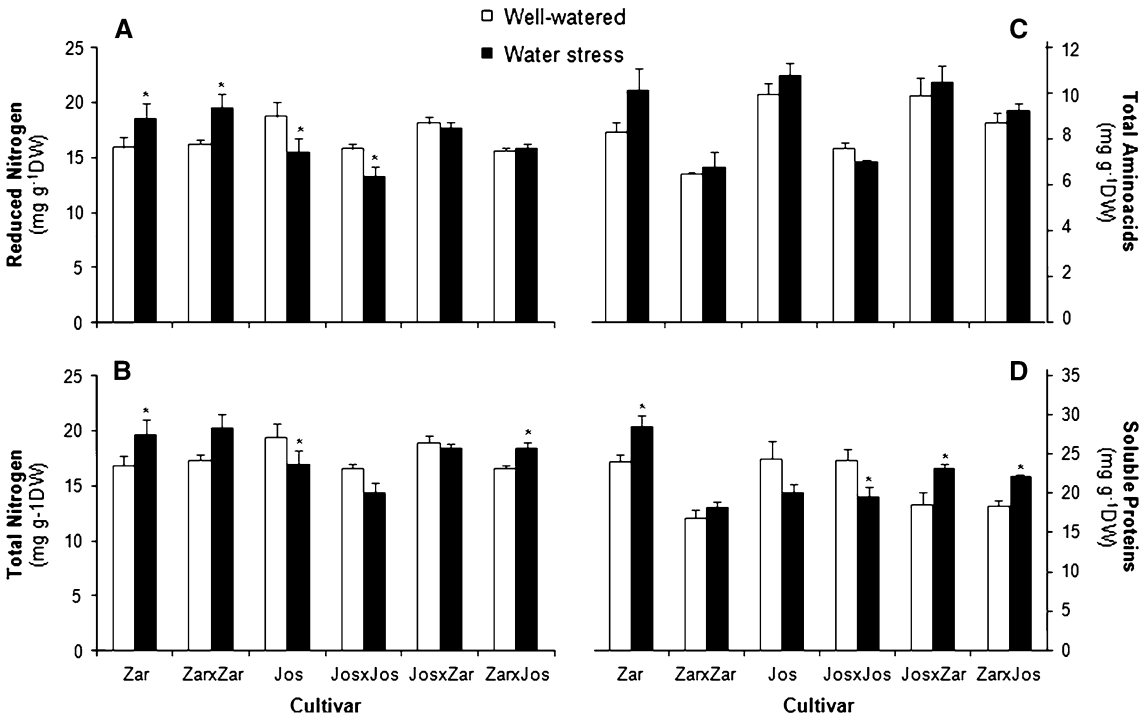

Fig. 5 Response in foliar concentration of organic (**a**), total N (**b**), total amino acids (**c**), and soluble proteins (**d**) in ungrafted, grafted, and self-grafted tomato plants well-watered and subjected to moderate water stress. Columns are mean ± SE ($n = 9$), and differences between means were compared using Fisher's least-significant difference test (LSD; $p = 0.05$). *Asterisk* (*) indicates significant difference with control groups

which are produced in the reactions catalyzed by AAT and asparagine synthetase (Hodges 2002). Our results showed an increase in AAT activity in cv. Zarina, *JosxZar*, and *ZarxJos* under water stress (Table 4). This could be related to the increase in the GS/GOGAT cycle in these cultivars and grafts (Table 4).

The result of the incorporation of NH_4^+ can be quantified by the analysis of reduced N, which is generally the product of N assimilation and is formed mainly by amino acids and proteins. The total N, the sum of the total reduced N and NO_3^-, is considered a critical parameter in the determination of the nutritional state of plants (Ruiz and Romero 1999). The greater efficiency in NO_3^- reduction and NH_4^+ reassimilation in Zarina ungrafted, *JosxZar*, and *ZarxJos* plants was confirmed by the results for protein and total N, which were higher under water stress (Fig. 5). Ruiz and others (2006) had similar results with grafted tobacco plants, observing higher amino acids, protein, and total N in all grafted plants with respect to nongrafted plants. These results confirm those previously found by other authors, who observed that an increase in the NR activity leads to a corresponding increase in the potential for NO_3^- reduction and confers a greater capacity for general amino acid synthesis, protein synthesis, or total N assimilation (Singh and Usha 2003).

The increase in N metabolism displayed by the *ZarxJos* and *JosxZar* combinations, which was associated with other physiological factors such as maintenance of leaf relative water content (LRWC) in *ZarxJos* or higher photorespiration in *JosxZar*, strongly suggests that these combinations are the most capable of coping with moderate drought stress. In addition, the results demonstrate that interactions between the rootstock and scion may exert a strong effect on the N metabolism responses of tomato plants under water stress. In this study, when Zarina (tolerant cultivar) was used as a rootstock grafted with Josefina (*ZarxJos*), these plants showed an improved N uptake and NO_3^- assimilation (Table 1). On the other hand, when Zarina was used as the scion (*JosxZar*), these grafted plants showed an increase in the photorespiration cycle (Table 2), which may generate amino acids and proteins (Fig. 5c, d).

Our study offers promising results that could improve the understanding of some physiological mechanisms involved in scion and rootstock interactions under water stress conditions. However, further studies are needed to better elucidate biochemical, molecular, and genetic traits that might exert control on N metabolism associated with drought resistance in grafted plants. Such traits could be utilized in plant breeding programs by means of the selection of improved genotypes of rootstocks and scions

using molecular marker-assisted techniques. In conclusion, grafting improves NO_3^- photoassimilation or photorespiration in water stress experiments on tomato. Consequently, complex interactions between photorespiratory metabolism and NO_3^- assimilation may be more important than previously recognized in plant leaves.

Acknowledgments This work was financed by the PAI program (Plan Andaluz de Investigación, Grupo de Investigación AGR161) and by a Grant from the FPU of the Ministerio de Educación y Ciencia awarded to ESR.

References

Abdelmageed AHA, Gruda N (2009) Influence of grafting on growth, development and some physiological parameters of tomatoes under controlled heat stress conditions. Eur J Hortic Sci 74:16–20

Baethgen WE, Alley MM (1989) A manual colorimetric procedure of measuring ammonium nitrogen in soil and plant. Commun Soil Sci Plant 20:961–969

Boyer JS (1982) Plant productivity and the environment. Science 218:443–448

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cammaerts D, Jacobs M (1985) A study of the role of glutamate dehydrogenase in the nitrogen metabolism of *Arabidopsis thaliana*. Planta 163:517–526

Carmo-Silva AE, Gore MA, Andrade-Sanchez P, French AN, Hunsaker DJ, Salvucci ME (2012) Decreased CO_2 availability and inactivation of Rubisco limit photosynthesis in cotton plants under heat and drought stress in the field. Environ Exp Bot 83:1–11

Cataldo DA, Haroon M, Schreader LE, Young VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Commun Soil Sci Plant 6:71–80

Estañ MT, Martinez-Rodriguez MM, Perez-Alfocea F, Flowers TJ, Bolarin MC (2005) Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. J Exp Bot 56:703–712

Feierabend J, Beevers H (1972) Developmental studies on microbodies in wheat leaves. I. Conditions influencing enzyme development. Plant Physiol 49:28–32

Ferreira-Silva SL, Silva EN, Carvalho FEL, de Lima CS, Alves FAL, Silveira JAG (2010) Physiological alterations modulated by rootstock and scion combination in cashew under salinity. Sci Hortic 127:39–45

Fresneau C, Ghashghaie J, Cornic G (2007) Drought effect on nitrate reductase and sucrose-phosphate synthases activities in wheat (*Triticum durum* L.): role of leaf internal CO_2. J Exp Bot 58:2983–2992

Galmés J, Ribas-Carbó M, Medrano H, Flexas J (2011) Rubisco activity in Mediterranean species is regulated by the chloroplastic CO_2 concentration under water stress. Environ Exp Bot 62:653–665

Gibon Y, Larher F (1997) Cycling assay for nicotinamide adenine dinucleotides: NaCl precipitation and ethanol solubilization of the reduced tetrazolium. Anal Biochem 251:153–157

Gonzalez EM, Gordon AJ, James CL, Arrese-Igor C (1995) The role of sucrose synthase in the response of soybean nodules to drought. J Exp Bot 26:1515–1523

Hageman RH, Hucklesby DP (1971) Nitrate reductase. Meth Enzymol 23:497–503

Heckathorn SA, de Lucia EH, Zielinski RE (1997) The contribution of drought related decreases in foliar nitrogen concentration to decrease in photosynthetic capacity during and after drought in prairie grasses. Physiol Plantarum 101:173–182

Hirel B, Lea P (2001) Ammonia assimilation. In: Lea PJ, Morot-Gaudry JF (eds), Plant nitrogen. Springer, Berlin, pp 79–100

Hoder M, Rej R (1983) Alanine aminotransferase. In: Bergmeyer HU, Bergmeyer J, Gral M (eds) Methods of enzymatic analysis, vol 3. Weinhein, Chemie, pp 444–456

Hodges M (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J Exp Bot 53:905–916

Igarashi D, Tsuchida H, Miyako M, Oshumi C (2006) Glutamate: glyoxylate aminotransferase modulates amino acid content during photorespiration. Plant Physiol 142:901–910

Kaiser JJ, Lewis OAH (1984) Nitrate reductase and glutamine synthetase activity in leaves and roots of nitrate fed *Helianthus annuus* L. Plant Soil 70:127–130

Kim HY, Lim SS, Kwak JH, Lee DS, Lee SM, Ro HM, Choi WJ (2011) Dry matter and nitrogen accumulation and partitioning in rice (*Oryza sativa* L.) exposed to experimental warming with elevated CO_2. Plant Soil 342:59–71

Kramer PJ, Boyer JS (1995) Water relations of plants and soils. Academic Press, San Diego, pp 495–524

Krom MD (1980) Spectrophotometric determination of ammonia: study of a modified Berthelot reaction using salicylate and dichloroisocianurate. Analyst 105:305–316

Kronzucker HJ, Britto DT, Davenport RJ, Tester M (2001) Ammonium toxicity and the real cost of transport. Trends Plant Sci 6:335–337

Lawlor DW (1995) Effects of water deficit on photosynthesis. In: Smirnoff N (ed) Environment and plant metabolism. Bios Scientific Publishers Ltd, Oxford, pp 129–160

Lee JM, Oda M (2003) Grafting of herbaceous vegetable and ornamental crops. Hortic Rev 28:61–124

Li H, Lascano RJ (2011) Deficit irrigation for enhancing sustainable water use: comparison of cotton nitrogen uptake and prediction of lint yield in a multivariate autoregressive state-space model. Environ Exp Bot 71:224–231

Liao CT, Lin CH (1996) Photosynthetic responses of grafted bitter melon seedlings to flood stress. Environ Exp Bot 32:167–172

Lillo C (1984) Diurnal variations of nitrite reductase, glutamine synthetase, glutamate synthase, alanine aminotransferase and aspartate aminotransferase in barley leaves. Physiol Plantarum 61:214–218

Lu B, Yuan Y, Zhang C, Ou J, Zhou W, Lin Q (2005) Modulation of key enzymes involved in ammonium assimilation and carbon metabolism by low temperature in rice (*Oryza sativa* L.) roots. Plant Sci 169:295–302

Martinez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, Carvajal M (2010) Physiological aspects of rootstock-scion interactions. Sci Hortic 127:112–118

Masclaux-Daubresse C, Reisdorf-Cren M, Pageau K, Lelandais M, Grandjean O, Kronenberger J, Valadier MH, Feraud M, Jouglet T, Suzuki A (2006) Glutamine synthetase-glutamate synthase pathway and glutamate dehydrogenase play distinct roles in the sink-source nitrogen cycle in tobacco. Plant Physiol 140:444–456

Matsumura H, Miyachi S (1980) Cycling assay for nicotinamide adenine dinucleotides. Methods Enzymol 69:465–470

Mena-Petite A, Lacuesta M, Muñoz-Rueda A (2006) Ammonium assimilation in *Pinus radiata* seedlings: effects of storage treatments, transplanting stress and water regimes after planting under simulated field conditions. Environ Exp Bot 55:1–14

Miflin BJ, Habash DZ (2002) The role of glutamine synthetase and glutamate dehydrogenase in nitrogen assimilation and possibilities for improvement in the nitrogen utilization of crops. J Exp Bot 53:979–987

Nakano H, Muramatsu S, Makino A, Mae T (2000) Relationship between the suppression of photosynthesis and starch accumulation in the pod-removed bean. Aust J Plant Physiol 27:167–173

Noctor G, Arisi AC, Jouanin L, Foyer CH (1999) Photorespiratory glycine enhances glutathione accumulation in both the chloroplastic and cytosolic compartments. J Exp Bot 50:1157–1167

Parry MAJ, Andralojc PJ, Khan S, Lea PJ, Keys AJ (2002) Rubisco activity: effects of drought stress. Ann Bot 89:833–839

Passioura J (2007) The drought environment: physical, biological and agricultural perspectives. J Exp Bot 58:13–117

Pulgar G, Villora G. Moreno DA, Romero L (2000) Improving the mineral nutrition in grafted melon plants: nitrogen metabolism. Biol Plantarum 43:607–609

Robredo A, Pérez-López U, Miranda-Apodaca J, Lacuesta M, Mena-Petite A, Muñoz-Rueda A (2011) Elevated CO_2 reduces the drought effect on nitrogen metabolism in barley plants during drought and subsequent recovery. Environ Exp Bot 71:399–408

Roland P, Ulrich S, Manfred J, Wilfried FW, Siegfried J (2006) Lateral diffusion of CO_2 from shaded to illuminated leaf parts affects photosynthesis inside homobaric leaves. New Phytol 169:779–788

Ruiz JM, Romero L (1999) Cucumber yield and nitrogen metabolism in response to nitrogen supply. Sci Hortic 82:309–316

Ruiz JM, Belakbir A, López-Cantarero I, Romero L (1997) Leaf-macronutrient content and yield in grafted melon plants: a model to evaluate the influence of rootstock genotype. Sci Hortic 71:227–234

Ruiz JM, Rivero RM, Cervilla LM, Castellano R, Romero L (2006) Grafting to improve nitrogen-use efficiency traits in tobacco plants. J Sci Food Agric 86:1014–1021

Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Cervilla LM, Blasco B, Rios JJ, Rosales MA, Romero L, Ruiz JM (2010) Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. Plant Sci 178:30–40

Sánchez-Rodríguez E, Rubio-Wilhelmi MM, Rios JJ, Blasco B, Rosales MA, Melgarejo R, Romero L, Ruiz JM (2011a) Ammonia production and assimilation: its importance as a tolerance mechanism during moderate water deficit in tomato plants. J Plant Physiol 168:816–823

Sánchez-Rodríguez E, Leyva R, Constant C, Romero L, Ruiz JM (2011b) How affect the grafting to ionome on cherry tomato plants under water stress? Sci Hortic (under review)

Sánchez-Rodríguez E, Ruiz JM, Federico F, Moreno DA (2011c) Phenolic metabolism in grafted versus nongrafted cherry tomatoes under the influence of water stress. J Agric Food Chem 59:8839–8846

Singh RP, Srivastava HS (1986) Increase in glutamate synthase activity in maize seedlings in response to nitrate and ammonium nitrogen. Physiol Plantarum 66:413–416

Singh B, Usha K (2003) Salicylic acid induced physiological and biochemical changes in wheat seedling under water stress. J Plant Growth Regul 39:137–141

Venema JH, Dijk BE, Bax JM, van Hasselt PR, Elzenga JTM (2008) Grafting tomato (*Solanum lycopersicum*) onto the rootstock of a high-altitude accession of *Solanum habrochaites* improves suboptimal-temperature tolerance. Environ Exp Bot 63:359–367

Wang ZQ, Yuan YZ, Ou JQ, Lin QH, Zhang CF (2007) Glutamine synthetase and glutamate dehydrogenase contribute differentially to proline accumulation in leaves of wheat (*Triticum aestivum*) seedlings exposed to different salinity. J Plant Physiol 164:695–701

Wingler A, Lea PJ, Quick WP, Leegood RC (2000) Photorespiration: metabolic pathways and their role in stress protection. Physiol Trans Royal Soc B 355:1517–1529

Wolf B (1982) A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. Commun Soil Sci Plant Anal 13:1035–1059

Xu ZZ, Zhou GS (2006) Nitrogen metabolism and photosynthesis in *Leymus chinensis* in response to long-term soil drought. J Plant Growth Regul 25:252–266

Yang X, Wang X, Wei M, Hikosaka S, Goto E (2010) Response of ammonia assimilation in cucumber seedlings to nitrate stress. J Plant Biol 53:173–179

Yemm EW, Cocking EC (1955) The determination of amino acids with ninhydrin. Analyst 80:209–213