Tomato Genotype in Relation to Nitrogen Utilization and Yield

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Twelve different tomato (*Lycopersicum esculentum* Mill.) genotypes were grown in a controlled greenhouse to determine the influence of genotype variation on the efficiency of nitrogen utilization and fruit yield. The results showed that the variation of genotype in tomatoes influences the utilization and assimilation of foliar NO_3^-. The genotypes that proved highly efficient in this utilization were G7, G9, G11, and G12. These genotypes had the highest foliar NO_3^- concentrations, greatest nitrate reductase activities, and highest protein and organic nitrogen contents. Genotype also influenced marketable and nonmarketable yields. Genotypes G2, G3, G6, and G8, which ranked intermediate in the efficient utilization of N, had the highest marketable and lowest nonmarketable yields.

Keywords: *Lycopersicum esculentum; genotypes; nitrogen efficiency and metabolism; yield*

INTRODUCTION

Nitrogen fertilizer use is a major issue in agriculture today. Nitrogen fertilizers are relatively expensive and can contribute to ground and surface water pollution through leaching and soil erosion (Sisson et al., 1991).

The processes of nitrate uptake by plant roots, translocation to the shoot, storage in the vacuoles, and assimilation are interdependent and closely regulated in higher plants (Huber et al., 1996; Sivasankar and Oaks, 1996). Nitrate reductase (NR) catalyzes the reduction of nitrate to nitrite and is considered a limiting factor in the growth and development of plants (Campbell, 1996; Sivasankar and Oaks, 1996; Ruiz et al., 1998).

Genetic variability in nitrogen use efficiency has been recognized for many years (Smith, 1934). The genetic variation for nitrogen use efficiency has been partitioned into differences in the uptake and use of nitrogen (Pollmer et al., 1979; Reinink et al., 1987). Teyker et al. (1989), by quantifiying genetic variance for nitrogen use efficiency in corn, demonstrated that selection for increased efficiency was possible. The presence of genotypic variation for traits related to N accumulation and use has also been demonstrated in wheat (Dhugga and Waines, 1989). Thus, the potential for developing superior nitrogen-efficient cultivars does exist in some crops.

The aim of the present work was to evaluate the response of different genotypes of tomato plants, all cultivated under identical conditions, in relation to the efficiency in N utilization and to marketable and nonmarketable yields.

MATERIALS AND METHODS

Crop Design. Twelve tomato (*Lycopersicum esculentum* Mill.) cultivars (cv) and lines (G1, Búfalo; G2, Corindon; G3, Dembelo; G4, GC773; G5, GC775; G6, Nancy; G7, Noa; G8, Sarky; G9, Yunque; G10, Volcani; G11, 617/83; G12, 2084/81) were seeded in cell flats (cell size 3 × 3 × 10 cm) filled with peat-lite mixture and kept on benches under the greenhouse conditions described below, for a period of 8 weeks; seedlings were then transplanted and grown under controlled conditions in an experimental greenhouse at Centro de Investigación y Desarrollo Hortícola, El Ejido, Almería, Spain. The plants were transplanted on August 15, 1997, and the cultivation ended at the close of January 1998. The climate is semiarid, and the lands are intensively used for agriculture. The soil used was loamy-sand with the following characteristics: sand, 37.3%; silt, 48.6%; and clay, 10.1%; $CaCO_3$ equivalent, 26.82%; $CaCO_3$ active, 14.35%; total N, 3.5 g kg^{-1}; total organic C, 36.1 g kg^{-1}; PO_4^{3-}, 890 mg kg^{-1}; K^+, 5.34 g kg^{-1}; pH, (H_2O) 8.45, (KCl) 8.01; electrical conductivity (EC), 4.63 dS m^{-1}. The relative humidity was 60−80% and the temperature range 24 ± 4 °C, with extremes of 15 and 30 °C in the greenhouse. The experimental design was a factorial arrangement in a randomized complete block with 12 treatments, corresponding to the 12 tomato cultivars studied. Each treatment was replicated three times in three individual plots of 4 m × 2 m wide (36 plots). Each plot contained 16 plants. The irrigation water had the following properties: pH, 8.05; EC, 2.03 dS m^{-1}; Cl^-, 483.90 mg L^{-1}; Na^+, 305.76 mg L^{-1}; K^+, 10.16 mg L^{-1}; HCO_3^-, 278.15 mg L^{-1}. Plants received, at each irrigation, macro- and micronutrients in the following forms and dosages: KNO_3, 2 mM; $Ca_2NO_3 \cdot 4H_2O$, 4 mM; K_2SO_4, 3 mM; $CaCl_2 \cdot 2H_2O$, 2 mM; $PO_4H_2Na \cdot 2H_2O$, 2 mM; $MgSO_4$, 1.5 mM; Fe-EDDHA, 5 μM; $MnSO_4 \cdot H_2O$, 2 μM; $ZnSO_4 \cdot 7H_2O$, 1 μM; $CuSO_4 \cdot 5H_2O$, 0.25 μM; $(NH_4)_6Mo_7O_2 \cdot 4H_2O$, 0.1 μM; H_3BO_3, 5 μM. The pH of the nutrient solution ranged from 5.0 to 6.0.

Plant Sampling. Fully expanded leaves from plants of the same size were taken from the top third of the plant at the onset of flowering, and then 2 weeks later, from each plot. Leaves were rinsed three times in distilled water after disinfection with nonionic detergent at 1% (Decon 90, Merck) (Wolf 1982) and then blotted by filter paper. Approximately half of the plant material was dried in a forced-air oven at 70 °C for 24 h and then ground in a Wiley mill. The remaining plant material was used to analyze the enzymatic activity NR, amino acid, and protein levels.

Plant Analysis. *NO_3^- Determination.* NO_3^--N was analyzed from an aqueous extraction of 0.2 g of dried and ground leaf material in 10 mL of Millipore-filtered water. A 100 μL aliquot was taken for NO_3^--N determination and added to 10% (w/v) salicylic acid in sulfuric acid at 96%, and the NO_3^--N concentration was measured by spectrophotometry as per-

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Table 1. Nitrate Reductase Activity and Accumulation of Various N Compounds in 12 Tomato Genotypes[a]

genotype	NR activity [μmol of NO_2^- (g of fw)$^{-1}$ h^{-1}]	amino acids [mg (g of fw)$^{-1}$]	proteins [mg (g of fw)$^{-1}$]	nitrate [mg (g of dw)$^{-1}$]	organic N [mg (g of dw)$^{-1}$]
G1 (Búfalo)	1.46 c	3.15 b	20.1 de	5.47 e	31.5 d
G2 (Corindon)	1.95 b	3.69 a	22.8 c	6.15 d	34.0 c
G3 (Dembelo)	1.91 b	3.55 a	22.5 cd	7.23 c	34.1 c
G4 (GC773)	1.53 c	3.49 a	18.8 e	6.01 de	30.0 de
G5 (GC775)	1.61 c	3.67 a	20.2 d	5.35 e	30.1 d
G6 (Nancy)	1.85 b	3.21 ab	21.7 d	6.77 cd	34.3 c
G7 (Noa)	2.45 a	3.42 a	24.7 b	10.39 a	38.3 a
G8 (Sarky)	1.79 bc	3.24 ab	23.1 c	6.89 c	33.9 cd
G9 (Yunque)	2.31 ab	3.38 a	25.9 ab	9.94 ab	36.6 ab
G10 (Volcani)	1.11 d	3.18 b	17.8 e	5.03 e	28.1 e
G11 (617/83)	2.31 ab	3.24 ab	27.4 a	9.71 b	36.5 ab
G12 (2084/81)	2.31 ab	3.24 a	24.1 bc	10.03 a	36.0 bc
analysis of variance significance	***	ns	***	***	***

[a] Values followed by the same letter within a column are not different by DMRT at the 5% level. Levels of significance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and $P > 0.05$ not significant (ns).

formed by Cataldo et al. (1975). The results were expressed as milligrams per gram of dry weight (dw).

Detection of in Vivo NR Activity. The basic method was an adaptation of the in vivo NR assay by Jaworski (1971) and Mauriño et al. (1986). Leaves were cut into 5 mm sections, and the sample (0.5 g) was placed in 10 mL of incubation buffer (100 mM potassium phosphate buffer, pH 7.5) and 1% (v/v) propanol. The sample was infiltrated, and the intracellular spaces of the tissues were flushed with buffer, using a vacuum (0.8 bar). After 10 min, the vacuum was released and the samples were reevacuated. The samples were incubated at 30 °C in darkness for 1 h and placed in a boiling water bath to stop the NR activity. The resulting NO_2^- was measured according to the method of Snell and Snell (1949), and the NR activity was expressed as micromoles of NO_2^- per gram of fresh weight (fw) per hour.

Amino Acid and Soluble Protein Determination. Fresh leaf samples (0.5 g) were crushed with cold phosphate buffer (50 mM KH_2PO_4, pH 7) and centrifuged at 12000*g* for 15 min. The resulting supernatant was used for the determination of total amino acids according to the ninhydrin method as described by Yemm and Cocking (1955); total free amino acids were expressed as milligrams of glycine per gram of fw. Soluble proteins were measured by using Bradford G-250 reagent (Bradford, 1976) and expressed as milligrams of bovine serum albumin per gram of fw.

Organic N Determination. Subsample (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 salicylate/sodium nitroprusside, and 5.35% (v/v) sodium hypochlorite. Samples were incubated at 37 °C for 15 min, and organic N was measured by spectrophotometry according to the method of Baethgen and Alley (1989). The results were expressed as milligrams per gram of dw.

Plant yield was expressed as the mean of fruit weight. Harvested mature fruits from each plant wre separated into marketable and nonmarketable and then weighed. Marketable yield represents fruits with acceptable color and caliber, whereas those without represent nonmarketable yield (Monteiro, 1983; Abad and Guardiola, 1986); total yield is the sum of both types of yield.

Statistical Analysis. Analysis of variance was used to assess the significance of treatment means. Differences between treatment means were compared using the LSD and Duncan's multiple-range test (DMRT).

RESULTS AND DISCUSSION

One of the major and limiting stages of NO_3^- assimilation is NR activity (Huber et al., 1996; Sivasankar and Oaks, 1996). The highest in vivo assay values were found in G7 and G12, with a 123% increase over the lowest values recorded for genotype G10 (Table 1). One of the principal factors regulating de novo NR synthesis and activity is the presence of NO_3^- (Campbell, 1996; Sivasankar and Oaks, 1996; Ruiz et al., 1998). The NO_3^- concentrations (Table 1) were similar to those of NR activity, with highest foliar concentrations in G7 and G12 and the lowest in G10. The relationship between two factors was $r^2 = 0.81$ **.

The principal products of NO_3^- assimilation are amino acids and proteins (Barneix and Causin, 1996). Amino acid concentrations were not affected by cultivar (Table 1). However, protein concentrations reflected the greatest NO_3^- reduction (Table 1), with G7, G9, G11, and G12 having the highest values. Organic nitrogen (Table 1) showed the same trend as that of the proteins. Both the protein and organic nitrogen concentrations increased proportionally to the rise in NR activity (Table 1), the relationships between these factors being positive and significant (NR activity, proteins, $r^2 = 0.78$ *; NR activity, organic N, $r^2 = 0.83$ **).

Overall, genotypes G7, G9, G11, and G12 had the greatest uptake, translocation, and assimilation of NO_3^-, reflecting the highest foliar contents of NO_3^-, the highest NR activity, and the greatest protein and organic N contents (Table 1). These trends appear to correspond to improved efficiency in the utilization of nitrogenous fertilizer applied in the form of NO_3^-. The opposite trend characterized genotypes G1, G4, G5, and G10, whereas G2, G3, G6, and G8 remained intermediate.

Genotype influenced marketable, nonmarketable, and total yields (Table 2). The highest nonmarketable yields occurred in G9, G10, and G12, exceeding the levels of G2, G3, G6, and G8 by up to 205%. G6 registered the highest levels of marketable yield, surpassing that of G10 by 75%. The highest total yield was harvested from G6 and the lowest from G5 and G10.

Nitrogenous fertilizer and its utilization can be determining factors for yield (Mattson et al., 1991; MacDonald et al., 1996; López-Cantarero et al., 1997). The highest marketable yields were recorded from G2, G3, G6, and G8 (Table 2), the genotypes defined above as having intermediate efficiency in the utilization of N (NO_3^-). In contrast, genotypes that were highly efficient in N utilization (G7, G9, G11, and G12) had low marketable and high nonmarketable yields. High N

Table 2. Marketable, Nonmarketable, and Total Fruit Yields in 12 Tomato Genotypes[a]

genotype	yield (kg/plant)		
	nonmarketable	marketable	total
G1 (Búfalo)	0.93 b	3.43 c	4.36 b
G2 (Corindon)	0.56 c	4.21 ab	4.77 ab
G3 (Dembelo)	0.57 c	4.14 b	4.73 ab
G4 (GC773)	0.95 b	3.19 cd	4.14 bc
G5 (GC775)	0.67 bc	3.01 cd	3.68 c
G6 (Nancy)	0.48 c	4.62 a	5.10 a
G7 (Noa)	0.94 b	3.54 bc	4.48 b
G8 (Sarky)	0.53 c	4.31 ab	4.84 ab
G9 (Yunque)	1.11 ab	3.68 bc	4.79 ab
G10 (Volcani)	1.04 ab	2.66 d	3.70 c
G11 (617/83)	0.81 bc	3.69 bc	4.50 b
G12 (2084/81)	1.46 a	3.61 bc	5.07 a
analysis of variance signficance	**	***	*

[a] Values followed by the same letter within a column were not measurably different by DMRT at the 5% level. Levels of significance are represented by * at $P < 0.05$, ** at $P < 0.01$, *** at $P < 0.001$, and $P > 0.05$ not significant (ns).

utilization of these genotypes can encourage excessive vegetative growth and less fruiting (Davenport, 1996). In addition, López-Cantarero et al. (1997) found that heavier N fertilization and therefore increased N utilization by eggplant (*Solanum melongena*) boosted nonmarketable yield. Finally, the genotypes defined as having low efficiency in N utilization (G1, G4, G5, and G10) behaved similarly to those of high efficiency, falling substantially in marketable and total yields. These facts indicate the close relationship between yield and N metabolism.

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