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Research Paper

Comparative study of the toxic effect of salinity in different genotypes of tomato plants: Carboxylates metabolism

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ABSTRACT

Tomato is a crop with the greatest economic importance in the world and salinity stress causes a reduction in the quantity and quality of crop production. Today the main challenge in world agriculture is to sustain the continuously growing global population, and this becomes more difficult due to climatic change, as this imposes further abiotic stress. The aim of this study was to determine the involvement of the TCA cycle in resistance to salinity in two genotypes of tomato. We found that the cv. Grand Brix enhances the tricarboxylic acid (TCA) cycle activity as a mechanism of resistance to salt stress, while the cv. Marmande RAF does not have this mechanism. This causes a greater accumulation of organic acids in Grand Brix which favours even more resistance to salt stress. We propose an idea of how the oxaloacetate could help to control Na^+ excess in the cv. Grand Brix.

1. Introduction

Tomato is the crop with the greatest economic importance in the world. According to FAO (2017), in 2014 roughly 4,888,880 t of tomato were produced in Spain alone, cultivated on 54,750 Ha. A great part of this cultivation area is affected by salinity stress. In particular, salinity stress causes a reduction in the quantity and quality of crop production (Saito et al., 2008). Currently the main challenge in world agriculture is to sustain the continuously growing global population, and this becomes more difficult due to climatic change, as this imposes further abiotic stress. In the coming years, several factors could exacerbate this situation, such as the continual spread of intensive agriculture and the use of poorer quality water. Therefore, it is very important to ascertain the impact of saline stress in tomato cultivation. Greatly limiting crop yield in semiarid and arid regions, salinity affect roughly 397 million Ha of soils in the world (Gong et al., 2013). This is particularly true in the Mediterranean area, where cultivation tends to occupy small fields often with high quality and commercial value crops, such as tomato (Lynch and Clair, 2004). Organic acid metabolism is of fundamental importance at the cellular level for several biochemical pathways, including energy production, formation of precursors for amino acid biosynthesis and at the whole plant level modulating adaptation to the environment (López-Bucio et al., 2000). Nowadays we have a lot of information about the functioning of carboxylate metabolism, however there are few studies about the relationship between salt stress and the activity of TCA cycle enzymes.

Growth conditions under salinity stress, trigger osmotic and ionic imbalances, prompt oxidative stress, and upset the plant's metabolism. Some molecules are involved to resolve these imbalances, such as organic acids (Hossain and Dietz, 2016). Studies have been made in this regard in tomato plants, but especially these papers have focused on the responses of organic acids in fruits. These studies have generally shown that salinity produces fruits with a higher content of sugars and organic acids, which contribute to improve fruit market quality (Cuartero and Fernández-Muñoz, 1999). For example, Saito et al. (2008) observed an increase of organic acids in *Solanum lycopersicum* fruits under salt stress, after the overexpression of the aconitate synthase enzyme. These authors showed that organic acids accumulation, increases fruits quality, and also favours the accumulation of other compounds such as sugars. However, the study of the TCA cycle importance in salinity resistance in tomato plants has not been addressed.

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In recent years, in other species, some researchers have tried to overexpress enzymes of this metabolism in order to improve certain types of stress by the accumulation of organic acids (Ryan et al., 2001). In fact, it has been observed, in tobacco transgenic plants and *Arabidopsis thaliana*, a relationship between overexpression of the citrate synthase (CS) enzyme and citrate increase, the accumulation of organic acids and resistance to Al^{3+} stress (Ryan et al., 2001). Richter et al., 2015 in a study with two corn hybrids concluded that the TCA cycle is severely affected by salt stress, since the cycleís metabolites and carbon catabolism are reduced, it affects plant metabolism. In other species as pea plants or Arabidopsis, Jacoby et al. (2011) showed how an increase of malate dehydrogenase enzyme (MDH) increases salt stress tolerance.

In this context, considering the importance of the organic acids and carboxylate metabolism in plant, and its relationship with resistance to saline stress, we investigate here the response of these metabolic processes in two tomato genotypes submitted to salinity stress. The final aim is to determine whether the organic acids and the TCA cycle enzymes are key to select and/or generate the cultivar with the best tolerance to this type of stress.

2. Material and methods

2.1. Plant material and treatments

Seeds of *Solanum lycopersicum* cv. Gran brix and *Solanum lycopersicum* cv. Marmande Raf (Saliplant S.L., Spain) were germinated and grown for 30 days in cell flats of 3 cm × 3 cm × 10 cm filled with a perlite mixture substratum. The flats were placed on benches in an experimental greenhouse located in Southern Spain (Saliplant S.L., Motril, Granada). After 30 days, the seedlings were transferred to a growth chamber (Department of plant physiology, University of Granada) under the following controlled environmental conditions: Relative humidity 60–80%; Day/night temperatures 28/19 °C; 16/8 h photoperiod at a photosynthetic photon flux density (PPFD) of 350 μmol m^{-2} s^{-1} (measured at the top of the seedlings with a 190 SB quantum sensor, LI-CORInc., Lincoln, Nebraska, USA). Under these conditions the plants were grown in hydroponic cultivation in lightweight polypropylene trays (60 cm diameter top, bottom diameter 60 cm and 7 cm in height) of 31 volume, 8 plants/tray. Throughout the experiment the plants were cultured with a growth solution made up of 4 mM KNO_3, 3 mM $Ca(NO_3)_2{\cdot}4H_2O$, 2 mM $MgSO_4{\cdot}7H_2O$, 6 mM KH_2PO_4, 1 mM $NaH_2PO_4{\cdot}2H_2O$, 2 μM $MnCl_2{\cdot}4H_2O$, 1 μM $ZnSO_4{\cdot}7H_2O$, 0.25 μM $CuSO_4{\cdot}5H_2O$, 0.1 μM $Na_2MoO_4{\cdot}2H_2O$, 5 μM Fe-chelate (Sequestrene; 138 FeG100) and 50 mM H_3BO_3. This solution, with a pH of 5.5–6.0, was renewed every three days.

2.2. Experimental design

Treatment of saline stress started 38 days after germination and was maintained for 15 days. The control treatment received the nutrient solution, while the treatment saline stress received the nutrient solution plus 100 mM NaCl. The experimental design was a randomized complete block with two treatments, 8 plants per treatment and with 3 replications per treatment (n = 9).

2.3. Plant sampling and determination of the relative growth rate (RGR)

Plants of each treatment (53 days after germination) were divided into roots and leaves, washed with distilled water, dried on filter paper and weighed, thereby obtaining fresh weight (FW). Half of leaves from each treatment were frozen at −30 °C for further work and biochemical assays and the other half of the plant material was lyophilised for 48 h to obtain the dry weight (DW) and the subsequent analysis of the concentrations of nutrients. To determine the relative leaf growth rate (RGR), leaves from three plants per cultivar were sampled on day 38 after germination, immediately before starting the stress treatment (Ti). The leaves were dried in a forced-air oven at 70 °C for 24 h, and the dry weight (DW) was recorded as grams per plant. The remaining plants were sampled 53 days after germination (15 days of treatments, Tf). The relative growth rate was calculated from the increase in leaf DW at the beginning and at the end of the water-stress treatment, using the equation RGR = (ln DWf−ln DWi)/(Tf−Ti) where T is the time and the subscripts denote the final and initial sampling.

2.4. Carboxylate metabolism

Extracts for measuring enzyme activities were made following the method of Li et al. (2000), modified by grinding 0.1 g of plant material in liquid N with 1 ml of extraction buffer containing 1 mM EDTA-Na, 10% glycerol, 1% Triton X-100, 5 mM DTT and 1% polyvinylpyrrolidone (PVP) in 100 mM Tris–HCl pH 8.0. The slurry was centrifuged for 5 min at 14,700 rpm and 4 °C, and the supernatant was collected and analyzed immediately.

The activities of all enzymes were analyzed in 0.2 ml (final volume) of the media indicated below. The activity of MDH (EC 1.1.1.37) was determined with oxalate as substrate by measuring the decrease in absorbance at 340 nm due to the enzymatic oxidation of NADH (Dannel et al., 1995). The reaction was carried out with 0.1 mM NADH, 0.4 mM oxalate and 46.5 mM Tris–HCl, pH 9.5. The activity of CS (EC 4.1.3.7) was assayed spectrophotometrically according to Srere (1969) by monitoring the reduction of acetyl coenzyme A (CoA) to CoA with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) at 412 nm. The reaction was carried out in 0.1 mM DTNB, 0.36 mM acetyl CoA, 0.5 mM oxalate and 100 mM Tris–HCl, pH 8.1. ICDH (EC 1.1.1.42) activity was determined by monitoring the reduction of NADP at 340 nm in a reaction mixture containing 3.5 mM MgCl2, 0.41 mM NADP, 0.55 mM isocitrate and 88 mM imidazole buffer pH 8.0. Fumarase (EC 4.2.1.2) was assayed following the increase in optical density at 240 nm due to the formation of fumarate in 50 mM malate and 100 mM phosphate buffer, pH 7.4 (Bergmeyer et al., 1974). Finally PEPC (EC 4.1.1.31) activity was measured in a coupled enzyme assay with the MDH in 2 mM phosphoenolpyruvate (PEP), 10 mM NaHCO3, 5 mM MgCl2, 0.16 mM NADH and 100 mM of N,N-bis[2-hydroxyethyl]glycine (Bicine)-HCl, pH 8.5 (López-Millán et al., 2001).

2.5. Determination of the concentration of protein in the plant extracts

The concentration of proteins in the enzyme extracts was determined by the method of Bradford (1976), using serum-albumin as standard.

2.6. Concentrations of organic anions by U-HPLC–MS

Malic, citric and oxalic acids were analysed according to Gómez-Romero et al. (2010) with some modifications. Briefly, 75 mg of freeze-dried and ground plant material were dropped in 1 ml of cold (−20 °C) extraction mixture of methanol/water/acetic acid (80/19.5/0.5, v/v/v). Solids were separated by centrifugation (20.000g, 15 min) and re- extracted for 30 min at 4 °C in additional 1 ml of the same extraction solution. Pooled supernatants were passed through Sep-Pak Plus †C_{18} cartridges (SepPak Plus, Waters, USA) to remove interfering lipids and part of plant pigments and evaporated at 40 °C under vacuum to near dryness. The residue was dissolved in 1 ml water/methanol/acetic acid (94.5/5/0.5, v/v/v) solution using an ultrasonic bath. The dissolved samples were fil-