Plant Physiology and Biochemistry 109 (2016) 262–272

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Research article

The efficient physiological strategy of a tomato landrace in response to short-term salinity stress

Tommaso Michele Moles [a], Antonio Pompeiano [b], Thais Huarancca Reyes [a], Andrea Scartazza [c], Lorenzo Guglielminetti [a, d, *]

[a] Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy
[b] Laboratory of Ecological Plant Physiology, Global Change Research Institute CAS, Brno, Czech Republic
[c] Institute of Agro-environmental and Forest Biology, National Research Council, Monterotondo Scalo, RM, Italy
[d] Interdepartmental Research Center Nutrafood-Nutraceuticals and Food for Health, University of Pisa, Pisa, Italy

ARTICLE INFO

Article history:
Received 8 August 2016
Received in revised form
10 October 2016
Accepted 10 October 2016
Available online 12 October 2016

Keywords:
Salt tolerance
Tomato landrace
Chlorophyll *a* fluorescence
Gas exchange
Soluble sugars
Antioxidants

ABSTRACT

Landraces represent an important part of the biodiversity well-adapted under limiting environmental conditions. We investigated the response of two Southern Italy tomato landraces, the well-known San Marzano (our commercial standard) and a local accession called “Ciettaicale”, to different levels of sodium chloride in water irrigation (from 0 up to 600 mM) for a short-time exposure (one week). The combination of the chlorophyll *a* fluorescence and gas exchange analyses suggested that Ciettaicale maintained a higher efficiency of photosystem II (PSII) photochemistry and CO_2 utilization at high salinity concentrations than San Marzano. Stomatal and non-stomatal limitations occurred in San Marzano according to the reduction of maximum efficiency of PSII photochemistry and the increase of intercellular CO_2 concentration. Higher Na^+/K^+ ratio and higher concentration of total soluble sugars contributed to non-stomatal limitations in San Marzano leaves. These effects were significantly less evident in Ciettaicale. We also observed changes in total antioxidant capacity and leaf pigment content that emphasized the occurrence of modifications in the photosynthetic apparatus according to salt gradient. The more efficient assimilates supply and an integrated root protection system provided by sugars and antioxidants can explain the significantly higher root/shoot ratio in Ciettaicale. Overall, our results suggest that a comprehensive assessment of salinity tolerance in a genotypes comparison could be also obtained evaluating plant response to high salinity levels at early vegetative stage. In addition, further studies will be carried out in order to evaluate the possibility of using Ciettaicale in tomato improvement programs.

1. Introduction

Soil salinization is a growing problem in Mediterranean basin resulting from seawater intrusion into freshwater aquifers and irrigation with brackish water (Carillo et al., 2011). Salinity affects not only crop production but also several aspects of the plant's physiology and biochemistry. Thus, high levels of ions in the soil solution induce quickly osmotic effects reducing the water uptake by roots (physiological drought), and the increasing excess uptake of ions starts to produce cytotoxicity and pH/nutrient imbalance interfering with physiological and cellular processes (Hunsche et al., 2010; Munns and Tester, 2008).

Plants regulate cell biophysics promoting cellular turgor decrease and leaf stomatal closure to avoid water losses due to evapotranspiration, as well as a large root system to reach and absorb accessible water conserving the inner water balance (Rigano et al., 2014). Consequently, a reduction of stomatal conductance limits CO_2 uptake and CO_2 availability as substrate for photosynthesis (Chaves et al., 2009). Under salinity the chloroplast is exposed to excessive light excitation energy that triggers the formation of reactive oxygen species (ROS) (Apel and Hirt, 2004). The resulting oxidative burst can affect plant photosynthesis that is associated with the change of source-sink demand and the inhibition of growth. Photosystem II (PSII) is a crucial point in the chloroplast energy management and the estimation of its activity in

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* Corresponding author. Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy.
E-mail address: lorenzo.guglielminetti@unipi.it (L. Guglielminetti).

http://dx.doi.org/10.1016/j.plaphy.2016.10.008
0981-9428/

combination with CO_2 fixation can reflect plant health state during stress events (Krall and Edwards, 1992). In fact, excessive radiation destabilizes PSII electron transport activity promoting photoinhibition and photodamage events (Degl'Innocenti et al., 2009). However, despite ROS generation participates to detrimental effects of salt stress, new evidences are emerging about the involvement of ROS production in salt stress signalling (Apel and Hirt, 2004; Miller et al., 2010; Xiong et al., 2002). Crops show a gradient of salt tolerance that is the result of a combination of anatomical, physiological and molecular strategies more or less efficient to minimize the detrimental effects of salinity. The main salt tolerance target is keeping the ionic excess away from the metabolic active tissues to preserve leaf photosynthesis and meristematic activity. This aim is obtained generally thanks to the regulation of membrane ionic channels and pumps at different structural levels (Deinlein et al., 2014; Munns and Tester, 2008). Evidences supported the important role of ion transporters in salt tolerance (Apse et al., 1999; Shi et al., 2000; Zhang and Blumwald, 2001). In addition, the biosynthesis of chaperones and osmolytes such as proline, betaine, polyalcohol compounds and sugars can improve osmotic adjustment and, at the same time, act as signals part of an intense cross-talking (Munns, 2002; Cardi et al., 2015; Mittal et al., 2016). However, salt tolerance is a complex trait that is strictly related to salt concentration, stress exposure, genotype and plant growth stage (Flowers, 2004).

Harsh environmental conditions, such as an endurable soil salt contamination, have certainly contributed to the selection of genetic or physiological traits in individuals among the sessile plant biodiversity. During evolution these traits have ensured a high fitness to tolerate and survive under stress (Rigano et al., 2014). However, natural selection has not been in agreement with crop improvement programs, mostly driven by market force to meet the consumers. The history of tomato (*Solanum lycopersicum* L.) breeding programs is an example of this controversy. The commercial tomato germplasm is the result of a series of genetic bottlenecks during domestication to which limited accessions were subjected (Koenig et al., 2013; Sacco et al., 2015). Yield, shelf-life, fruit size and organoleptic quality were the main targets of tomato breeders at the expense of some traits such as stress tolerance/resistance (Bai and Lindhout, 2007). Given its importance as crop characterized by its economic and nutritional relevance, tomato is a well characterized model system and the availability of its genome is useful for biological investigation (Tomato Genome Consortium, 2012). Most commercial tomato cultivars are classified as moderately sensitive to salinity (Foolad, 2007). Fortunately, potential drought/salt tolerance traits have been identified within ancestral tomato related germplasm (Liu et al., 2015; Mittova et al., 2002; Orellana et al., 2010) and landraces (Galmes et al., 2013; Rigano et al., 2014; Assimakopoulou et al., 2015). Landraces are also an important part of the cultural heritage (Hagenblad et al., 2012). Local accessions are often predisposed to low-input or organic farming lowering the farmer capital investments thanks to their high adaptability to the territory (Negri, 2003). Thus, the conservation of landrace germplasm appears as a sustainable strategy for scientists and breeders to obtain plants able to counteract the future climate change (Halford and Foyer, 2015). Ciettaicale is a landrace cultivated in Basilicata region (Southern Italy) where is affected by an intense drought fluctuation during summer and by salt-contaminated water from aquifers. However, Ciettaicale shows an interesting adaptation capacity in these harsh environmental conditions.

The objective of this work was to study salt tolerance observed in Ciettaicale ecotype. We selected San Marzano as standard industrial cultivar which is well adapted to the Mediterranean climate. In a growth chamber experiment we subjected plants at vegetative stage to strong sodium chloride concentrations (the highest NaCl concentration was 600 mM) in irrigation water for a short-term exposure to exacerbate differences on stress response between the two entries mimicking supplemental irrigation in field conditions in marginal Mediterranean coastal areas during drought period. We correlated the results provided by chlorophyll *a* fluorescence and gas exchange analyses with the content of photosynthetic pigments and the distribution of Na^+, K^+, sugars and total antioxidant activity between source and sink organs.

2. Materials and methods

2.1. Plant material and growth conditions

Ciettaicale seeds (De Angelis S.r.l., Tolve, Italy) and San Marzano seeds (Blumen, Milan, Italy) were germinated in rockwool plugs (Grodan, Roermond, the Netherlands) in growth chamber (temperature 22 ± 1 °C, 16 h photoperiod, irradiation intensity 100 μmol photons m^{-2} s^{-1}). After two weeks, germinated seedlings were transplanted in plastic pots containing a mixture of soil and perlite (3:1, v/v) and placed for other eight weeks in the same growth conditions. Plants received distilled water three times a week and nutrient solution (NPK 5-5-5) once a week. For each species, uniform plants were divided into four groups, each with a different NaCl concentration in irrigation water (0, 300, 450 and 600 mM). Distilled water for the control group and salt solutions were applied once a day to the top of the pots.

2.2. Biometric analysis and cations content

After one week of treatment, plants were separated into leaves, stems and roots and then weighed (fresh weight, FW). Root tissues were previously washed with water to remove soil and perlite. The dry weights (DW) of plant organs were recorded after drying at 60 °C for 72 h. Additional set of plants not used for testing biometric traits, were collected and immediately processed or ground in liquid nitrogen, then stored at −80 °C for further biochemical analyses. For determination of cations content, powdered dry leaf (youngest fully expanded one) or root samples were digested in a solution of HNO_3:H_2O_2 (2:1, v/v). Na^+ and K^+ were determined using atomic absorption spectrometry (AAnalyst-300, HGA-800, Perkin Elmer).

2.3. Leaf gas exchange and chlorophyll a fluorescence measurements

Leaf gas exchange and chlorophyll fluorescence parameters were measured simultaneously using an open-type portable measurement system (Li-6400, Li-Cor Inc., NE, USA) equipped with an integrated fluorescence chamber head (Li-6400-40 leaf chamber fluorometer, Li-Cor Inc.). Analysis setting was maintained at 25 °C and at growth chamber light condition. Cuvette CO_2 concentration was set at 400 ppm CO_2 and the relative humidity was maintained between 45 and 55%. The measurements were carried out after one week of treatment for all plants of the four compared experimental treatments on youngest fully expanded leaves with similar exposition to radiation. Instantaneous values of net CO_2 assimilation rate (A), transpiration rate (E), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) were determined. Intrinsic water-use efficiency (A/g_s) and instantaneous water-use efficiency (WUE) were calculated as the A to g_s and A to E ratios, respectively.

The potential efficiency of PSII photochemistry (F_v/F_m) was calculated on dark-adapted leaves as $F_v/Fm = (Fm - F_o)/F_m$, where F_o and F_m are the minimum and maximum fluorescence yield emitted by the leaves in the dark-adapted state respectively. The actual