Journal of Plant Physiology 216 (2017) 164–173

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Original article

Study of phytohormone profile and oxidative metabolism as key process to identification of salinity response in tomato commercial genotypes

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ARTICLE INFO

Keywords:
Salt stress
Oxidative metabolism
Phytohormones
Solanum lycopersicum L.

ABSTRACT

Climatic change, intensive agriculture, and worsening water quality induce abiotic stress conditions for plants. Among these factors, salinity stress is a limit factor for plant growth. Therefore, the purpose of this study was to analyze the phytohormones role and oxidative metabolism in response to salt stress of two genotypes of tomato cv. Grand Brix and cv. Marmande RAF, the crops were carried out in a growth chamber. Salinity stress reduces biomass and relative growth rate (RGR) in both genotypes, this effect being greater in cv. Marmande RAF. These results, together with main stress indicator response, the $O_2^{\cdot -}$, indicate that cv. Marmande RAF is more sensitive to Saline stress. Grand Brix showed less oxidative stress, because it presented greater detoxification of the $O_2^{\cdot -}$, due to SOD enzyme activity induction and greater antioxidant capacity. Furthermore, Grand Brix has a better hormonal profile adapted to salt stress resistance, the accumulation of IAA, GA4 and CKs and their beneficial role against oxidative stress could make the difference between resistance and sensitivity to salt stress. On the other hand, a lower ACC concentration, ethylene precursor, combined with a greater $O_2^{\cdot -}$ detoxification in the cv. Grand Brix could play a fundamental role in tolerance to saline stress. Besides, an increase in ABA levels promotes better stomatal closure, better photosynthesis control and a lower rate of water loss. This data could be essential to select plants with greater resistance to saline stress.

1. Introduction

Tomato is a crop with the greatest economic importance in the world. According to the FAO, in 2014 roughly 4,888,880 t of tomato were produced only in Spain, 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 of 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. Coming years, several factors could exacerbate this situation, such as the intensive agriculture and the use of poorer quality water. Therefore, it is great importance 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 crops of high quality and commercial value, such as the tomato (Lynch and Clair 2004). Growth conditions under salinity stress, trigger osmotic and ionic imbalances, prompt oxidative stress, and upset the plant's metabolism.

The capacity of the plant to tolerate salinity is determined by multiple biochemical and physiological mechanisms, in particular by controlling the generation of reactive oxygen species (ROS) and readjusting the cell redox state (Gong et al., 2013). ROS negatively affect biological structures, provoking DNA damage, protein and amino acid oxidation, and lipid peroxidation (Asada 1999). The ROS generated under stress conditions in plants should be eliminated and, for this purpose, plants have mechanisms to detoxify ROS. These can be classified as enzymatic or non-enzymatic. In addition, phytohormones also are related to ROS generation/detoxification processes.

Plant hormones are structurally diverse compounds involved in multiple processes. Phytohormones thus have a vital role in mediating plant response to abiotic stress, the enzymes play a role in the regulation of oxidative stress. (Fahad et al., 2015). It has been observed that in tomato plants under salt stress a decrease in IAA concentration. A low IAA content could stimulate senescence, this compound has been generally seen as a senescence-retarding factor (Ghanem et al., 2008). The

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http://dx.doi.org/10.1016/j.jplph.2017.05.016
Received 31 March 2017; Received in revised form 18 May 2017; Accepted 22 May 2017
Available online 26 May 2017

beneficial effect of auxins on the prevention of damage caused by oxidative stress has been known for some years (Noctor et al., 2015). The auxins can help detoxify ROS, this is observed in plants with decreased catalase (CAT) activity (Noctor et al., 2015). Other authors point out how ROS alter gradients and auxin signaling (Raja et al., 2017).

Cytokinins (CKs) such as trans-zeatine (tZ) and isopentenyl adenine (iP), are also known to alleviate the adverse effects of salinity on plant growth (Fahad et al., 2015). It has been observed that an increase of the CKs can decrease the damages caused by the ROS, this can help you to be more tolerant of stress (Pogány et al., 2004). Ghanem et al. (2011) underwent two varieties of tomato to salt stress and they showed that the concentration of CKs in the plants increase in the leaves in saline treatment. These authors concluded that a greater accumulation of CKs, could improve the resistance to salt stress by delay leaf senescence, would improve maintaining stomatal conductance.

A rapid accumulation of Gibberellins (GAs) is characteristic of plants exposed to abiotic stresses, and this phytohormone can impart stress tolerance, including salinity. Under abiotic stress GAs regulates metabolic process such as of sugar signaling and antioxidative enzymes. Likewise, they act in stress response since these hormones are antagonistic with respect to abscisic acid (ABA) (Iqbal and Ashraf 2013).

ABA is known as a hormone that increases their concentration in stressed plants and is key to coordinate stress responses. In maize plants subjected to salt stress, the most resistant hybrid to salt stress had higher ABA concentration. These authors describe how increasing ABA could benefit to plants under salt stress conditions, inducing a lower rate of transpiration, so the tissues accumulate less Na^+ (Zörba et al., 2013). ABA is also closely related to H_2O_2 and Ca^{2+} ion, both of which interact in the stomatal closure/opening process. In addition, H_2O_2 stimulates the accumulation of ABA. Finally the accumulation of H_2O_2 stimulates the accumulation of ABA, this ABA promotes the stomatal closure thus avoiding the loss of water that can cause saline stress (Mittler and Blumwald, 2015).

Ethylene interacts with other hormones (GA and ABA) in homeostasis processes (Rzewuski and Sauter 2008). It accumulates alongside ROS. The ethylene and $O_2\cdot^-$ in plants under abiotic stress are the main cause of programmed cell death. The ethylene and $O_2\cdot^-$ in plants under abiotic stress behaves as an indicators of stress. An increase in the hormone jasmonic (JA) can lead to an increase in ethylene levels (Overmyer et al., 2003).

Other two phytohormones relevant in stress response are JA and salicylic acids (SA). JA is commonly associated with stress by pathogens. However, pretreatment with JA diminished the inhibitory effect of high salt concentration on growth and photosynthesis in barley. However, there is no information about how salinity affects endogenous JA levels in natural plant. SA has antagonistic effects on JA by preventing its accumulation in injury response. On the other hand, SA is usually associated with the chemical defense of plants against microbes and herbivores (Singh and Gautam 2013). The role of hormones in defense against abiotic stress and ROS is becoming clearer. In spite of this, more studies are necessary to understand this process well.

In this context, considering the importance of phytohormones and role oxidative metabolism in plant resistance to saline stress, we investigate here the response of oxidative metabolic process and phytohormones concentrations in two tomato genotypes submitted to salinity stress. The aim is to determine whether the oxidative metabolism and hormonal profile are determinant to define the cultivar with the strongest tolerance to saline stress. Also, understand how different hormones and oxidative metabolism are related with salt stress tolerance.

2. Experimental

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 3 L volume, 8 plants/tray. Throughout the experiment the plants were treated 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, pH 5.5–6.0.

2.2. Experimental design

Treatment of saline stress started 38 days after germination, this treatment was maintained for 15 days. The control treatment received the growth solution, described in Section 2.1, this solution was renewed every three days. Saline treatment received the growth solution plus 100 mM NaCl, this solution was renewed every three days. 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 saline-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. Determination of the concentration of promoters and indicators of oxidative stress (MDA, H_2O_2 and $O_2\cdot^-$), lipoxygenase (LOX) activity

For the extraction of MDA, 0.1 g of frozen leaf material was grounded, in 1 mL of buffer 50 mM (0.07% of $NaH_2PO_4{\cdot}2H_2O$ and 1.6% of $Na_2HPO_4{\cdot}12H_2O$). The extract was centrifuged to 20000*g* for 25 min. Subsequently, an aliquot of supernatant was mixed in test tubes with 4 mL of trichloroacetic acid 20% containing 0.5% of thiobarbituric acid. The resulting mixture was heated to 95 °C for 30 min. Then it was rapidly cooled in an ice bath. The absorbance of the supernatant was measured at 532 nm. The value for the non-specific absorption at