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Glutathione homeostasis as an important and novel factor controlling blossom-end rot development in calcium-deficient tomato fruits

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ABSTRACT

Based on previous results in which oxidative metabolism was suggested as a possible inducer of blossom-end rot (BER), the main questions addressed here were whether calcium deficiency is the main factor that induces BER or whether this physiological disorder a general stress-related phenomenon? Tomato plants were grown under optimal or deficient calcium concentrations. Only the application of 0.1 mM calcium resulted in BER induction, although only half of the fruits grown under this treatment had this disorder. Having fruits showing or not showing BER in the same plant and treatment provided us with a powerful tool that we used to investigate whether calcium deficiency operates alongside another mechanism in the induction of BER. Whether or not this other mechanism was the one controlling BER incidence was also investigated. We performed a complete study of the oxidative metabolism in the pericarp of healthy fruits and in the healthy portion of BER-affected fruits. Calcium deficiency led to an induction of NADPH oxidase, superoxide dismutase, dehydro- and monodehydroascorbate reductase, and to an inhibition of catalase, ascorbate peroxidase and glutathione reductase, with a concomitant accumulation of hydrogen peroxide and an increase in lipid peroxidation. While the ascorbate redox state was not affected by calcium deficiency, the glutathione redox state was markedly reduced. We conclude that calcium deficiency fundamentally affected the activity of the ascorbate–glutathione enzymes, with special importance to the inhibition of GR, which lead to a reduction of the glutathione redox state. This could cause the breakdown of cellular homeostasis, the inhibition of other enzymes responsible for H_2O_2 detoxification, and ultimately an increase of lipid peroxidation. Therefore, BER is defined here as the visual symptom of a massive lipid peroxidation event caused by the breakdown of cellular glutathione homeostasis.

Introduction

Blossom-end rot (BER) of tomato was first identified as a physiological disorder more than 100 years ago (Selby, 1986). BER has also been identified in other Solanaceae fruits such as pepper, eggplant and watermelon (Casado-Vela et al., 2005). BER may occur in all the tomato-producing areas of the world and has been shown to create losses of up to 50% of production (Casado-Vela et al., 2005). BER has been the subject of numerous studies, but the main mechanism or mechanisms that trigger the damage are not yet clear. BER incidence has been linked to reduced translocation of calcium to the fruit tip, so it is believed to be a calcium-related disorder (Ho et al., 1993; Olle and Bender, 2009).

Calcium plays very important roles as one of the essential macroelements necessary for plant growth. It is used for various processes, such as the maintenance of the plant cell structure, resistance to environmental stresses (salinity, drought, chilling, heat, etc.), and most importantly, as a secondary messenger in signal transduction in plants (Buchanan and Engman, 2002; Chaney et al., 2008; Chao et al., 2009). Calcium is transported into plants via the transpiration stream. Since calcium is not freely mobile in the plant, short periods of calcium deficit rapidly affect actively growing tissues (Kleemann, 1999; Olle and Bender, 2009). Calcium deficiency fruit disorders have become more important in recent years, possibly due to the intensification of production practices. However, physiological calcium deficiency is not generally prevented by calcium Fertilization. Therefore, it is very difficult to protect plants against this disorder (Olle and Bender, 2009). In recent years, several authors have shared the common opinion that calcium nutrition is neither the primary, nor an independent factor, in the development of BER (Saure, 2001; Ho and White, 2005). This is because: (a) of discrepancies in the published values for calcium

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbate; BER, blossom-end rot; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; MDHAR, monodehydroascorbate peroxidase; PDX, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

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in fruit with and without BER (Nonami et al., 1995); (b) BER can be also induced by maintaining the calcium concentration at optimum levels, and changing the concentration of other nutrients (Nukaya et al., 1995); and (c) there is no conclusive evidence revealing the role of calcium when BER is induced by one or more environmental stresses (Saure, 2001; Aktas et al., 2005; Chao et al., 2009).

Any circumstance in which cellular redox homeostasis is disrupted can lead to oxidative stress or the generation of reactive oxygen species (ROS) (Asada, 1994). Overproduction of ROS during environmental stresses is one of the main factors that leads to decreases in productivity, injury and death in plants. Uncontrolled ROS production can result in cell damage caused by enzyme inactivation, DNA/RNA nicking, protein oxidation, and lipid peroxidation, among other affected processes. Various abiotic stresses lead to the overproduction of ROS, ultimately resulting in oxidative stress in the plant. Among the ROS produced in plants, superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2) are the most important due to their permeability across the membranes and their levels of cellular accumulation. H_2O_2 is moderately reactive, and its excess in plant cells leads to the occurrence of oxidative stress (Gill and Tuteja, 2010). It is well established that the cellular accumulation of H_2O_2 leads to the peroxidation of lipids, which is considered to be the most damaging process known to occur in every living organism. The resulting lipid hydroperoxide can be easily decomposed into several reactive species, including lipid alkoxyl radicals, malonyldialdehyde, alkanes, lipid epoxides and alcohols (Davis and Swanson, 2001; Fam and Morrow, 2003). The overall effects of lipid peroxidation are a decrease in membrane fluidity, which makes it easier for phospholipids to exchange between the two halves of the bilayer; an increase in the leakiness of the membrane to substances that would not normally cross it, and damage to membrane proteins, which leads to the inactivation of receptors, enzymes, and ion channels (Gill and Tuteja, 2010).

Plants have developed powerful antioxidant defense machinery that protects them against oxidative stress damages. This machinery consists of efficient enzymatic and non-enzymatic antioxidant defense systems, which work in concert to control the cascades of uncontrolled oxidation, thereby protecting plant cells from oxidative damages by scavenging of ROS. Enzymatic antioxidants include superoxide dismutases (SODs), catalase (CAT), peroxidases (PDXs) and the enzymes belonging to the ascorbate–glutathione cycle, such as ascorbate peroxidase (APX), dehydro- and monodehydroascorbate reductase (DHAR, MDHAR), and glutathione reductase (GR). The most important non-enzymatic antioxidants include ascorbate (AsA), glutathione (GSH), phenolic compounds, non-protein amino acids and α-tocopherol. Changes in turnover rates during the ascorbate–glutathione cycle may manifest as altered redox ratios of AsA/dehydroascorbate (DHA) or GSH/oxidized glutathione (GSSG) (Tausz et al., 2009; Rivero et al., 2007). Several studies conducted in a number of plant species under abiotic stress conditions have elucidated the fact that a high ratio of GSH/GSSG and/or AsA/DHA sustained by increased GSH and AsA or diminution of GSSG and DHA, may be the key factor for the efficient protection against abiotic stress-induced accumulation of ROS (Szalai et al., 2009).

In recent years, several studies have focused on the possible relationship between BER occurrence and oxidative stress, with the aim of finding a primary cause of BER appearance in tomato fruits other than calcium deficiency. Casado-Vela et al. (2009) showed a possible role of some antioxidant enzymes and the pentose phosphate pathway through a proteomic approach to BER in tomato fruits. Also, Di Matteo et al. (2010) showed that the ascorbic acid concentration in tomato fruits was associated with the expression of genes involved in pectin degradation and BER occurrence. Turhan et al. (2006) showed that salinity-induced BER increased ascorbic acid and PDX levels as part of a protective anti-oxidation mechanism that determines cultivar sensitivity to BER. However, to date, there is no scientific evidence that oxidative stress is the primary cause of BER incidence. Here, for the first time, we describe a complete study of the oxidative metabolism in tomato fruits, showing not only that oxidative stress could be the main cause of BER development, but also that glutathione homeostasis is probably the main factor inducing the oxidative stress observed.

Materials and methods

Plant material and growth conditions

Solanum lycopersicon L. cv. Microtom seeds were sown in vermiculite. Once the plants reached 4–5 cm, they were transferred to 18 L containers and grown in an aerated hydroponic system containing a modified Hoagland solution (Supplemental Table S1) in a growth chamber under controlled conditions of light (600 μmol m^{-2} s^{-1}), photoperiod (16/8 h day/night), humidity (65/80% day/night) and temperature (25/22 °C day/night). The pH of the nutrient solution was kept between 5.5 and 6.1 and the solution was renewed every week. From the start date, the plants grew with the different calcium under these controlled conditions until the first flowers set. Three different treatments were applied to the tomato plants: 1 mM calcium (optimal concentration), and two treatments below the optimal (0.5 mM and 0.1 mM) with the goal being the induction of BER in these plants. Each calcium treatment was applied to 5 containers, with each container containing 3 tomato plants, so each treatment had a total of 15 tomato plants randomly distributed in the growth chamber (Supplemental Fig. S1). The flowers that had set were marked and the tomato fruits were collected 10 days, 15 days and 20 days after the flower set was detected, with the aim of detecting possible changes in the oxidative metabolism of these fruits (Supplemental Fig. S2). BER incidence was observed 12 days after fruit set (Supplemental Fig. S3). Finally, no changes were detected in the different parameters analyzed at the different fruit set dates, so the data were the mean of the values obtained at the three sampling dates.

The fruits collected were checked for BER incidence and were weighed and stored at −80 °C for further analyses. Parts of these fruits as well as a sample of the leaves from the plants were dried in a forced air oven at 70 °C for calcium concentration determination. For fruits affected by BER, all of the analyses and determinations described below were performed using the healthy the tissue from these fruits (Supplemental Fig. S3C). From the 0.1 mM of calcium treatment, only half of the fruits showed BER, so we analyzed fruits with BER and fruits without BER separately.

Calcium concentration measurement

Dried plant material (leaves and fruits) was digested with HNO_3:$HClO_4$ (2:1, v:v). Calcium concentrations were determined by atomic absorption spectrometry (Perkin-Elmer 5500, Waltham, MA, USA). The data reported are the mean ± SE of 5–7 values per treatment.

H_2O_2 concentration

H_2O_2 was extracted as described by McNervin and Uron (1953) with some modifications (Brennan and Frenkel, 1977; Rivero et al., 2007). The concentration of peroxide in the extracts was determined by comparing the absorbance against a standard curve representing a titanium–H_2O_2 complex from 0.1 to 1 mM. The hydroperoxides represent the total peroxides.