Journal of Plant Physiology 211 (2017) 13–26

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Ascorbic acid deficiency leads to increased grain chalkiness in transgenic rice for suppressed of L-GalLDH

Le Yu[a,1], Yonghai Liu[a,1], Lina Lu[b], Qilei Zhang[b], Yezheng Chen[a], Liping Zhou[a], Hua Chen[a], Changlian Peng[b,*]

[a] College of Life Sciences, Zhaoqing University, Zhaoqing 526061, People's Republic of China
[b] College of Life Sciences, South China Normal University, Guangzhou 510631, People's Republic of China

ARTICLE INFO

Article history:
Received 26 August 2016
Received in revised form
25 November 2016
Accepted 30 November 2016
Available online 17 January 2017

Keywords:
Ascorbic acid
Grain chalkiness
Antioxidant
L-Galactono-14-lactone dehydrogenase
Starch
Rice

ABSTRACT

The grain chalkiness of rice (*Oryza sativa* L.), which determines the rice quality and price, is a major concern in rice breeding. Reactive oxygen species (ROS) plays a critical role in regulating rice endosperm chalkiness. Ascorbic acid (Asc) is a major plant antioxidant, which strictly regulates the levels of ROS. L-galactono-1, 4-lactone dehydrogenase (L-GalLDH, EC 1.3.2.3) is an enzyme that catalyzes the last step of Asc biosynthesis in higher plants. Here we show that the L-GalLDH-suppressed transgenic rice, GI-1 and GI-2, which have constitutively low (between 30% and 50%) leaf and grain Asc content compared with the wild-type (WT), exhibit significantly increased grain chalkiness. Further examination showed that the deficiency of Asc resulted in a higher lipid peroxidation and H_2O_2 content, accompanied by a lower hydroxyl radical scavenging rate, total antioxidant capacity and photosynthetic ability. In addition, changes of the enzyme activities and gene transcript abundances related to starch synthesis were also observed in GI-1 and GI-2 grains. The results we presented here suggest a close correlation between Asc deficiency and grain chalkiness in the L-GalLDH-suppressed transgenics. Asc deficiency leads to the accumulation of H_2O_2, affecting antioxidant capacity and photosynthetic function, changing enzyme activities and gene transcript abundances related to starch synthesis, finally leading to the increased grain chalkiness.

1. Introduction

Grain chalkiness is a major concern in rice breeding because it affects the appearance quality of milled rice and is one of the key factors determining grain price (Yoshioka et al., 2007). The formation of grain chalkiness is controlled by multiple factors, including starch synthesis, starch granule structure and arrangement (Myers et al., 2000; Kang et al., 2005; Yamakawa et al., 2007) and various external stresses during the grain-filling stage. Recent works showed that reactive oxygen species (ROS) may play a critical role in regulating rice endosperm chalkiness (Xu et al., 2010; Liu et al., 2010). For example, an endogenous H_2O_2 burst was observed at 15 days after flowering (DAF) in the rice endosperm during the grain-filling stage (Xu et al., 2010). In another study, a higher concentration of H_2O_2 in the rice endosperm has been observed in a near-isogenic line which has higher grain chalkiness characteristics compared with its normal parental line (Liu et al., 2010). These observations suggest a close correlation between ROS homeostasis and grain chalkiness.

L-Ascorbic acid (Asc) is an important antioxidant of ROS scavenging mechanism to reduced peroxides (Potters et al., 2010). In higher plants, Asc acts as one of the most abundant non-enzymatic antioxidants and an excellent hydrophilic antioxidant in plants, which strictly regulates the levels of ROS (Gest et al., 2013). Studies have shown that 30–40% of the Asc within a plant cell is localized in the chloroplast and is thus broadly associated with photosynthetic function and detoxification of ROS (Noctor and Foyer, 1998). Asc scavenges ROS, H_2O_2 in particular, and is a substrate for ascorbate peroxidase, which leads to the formation of dehydroascorbate (DHA), the oxidized form of Asc (Foyer and Noctort, 2005; Asada, 2006). During seed development and the aging period, Asc limits oxidative stress, which occurs naturally during desiccation and

Abbreviations: Asc, L-ascorbic acid; Chl, chlorophyll; DAF, days after flowering; DHA, dehydroascorbate; GBSS, granule-bound starch synthase; GI, L-GalLDH-suppressed transgenic rice; GL, grain length; GLWR, grain length-to-width ratio; GW, grain width; GMP, GDP-mannose pyrophosphorylase; L-GalL, L-galactono-1,4-lactone; L-GalLDH, L-galactono-1,4-lactone dehydrogenase; MDA, malondialdehyde; PCD, programmed cell death; ROS, reactive oxygen species; SBE, starch branching enzyme; SS, soluble starch synthase; SEM, scanning electron microscope; T-AOC, total antioxidant capacity; TCA, trichloroacetic acid.

* Corresponding author.
E-mail address: pengchl@scib.ac.cn (C. Peng).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jplph.2016.11.017
0176-1617/

consequently counteracts enzyme denaturation, lipid peroxidation, and the loss of plasma membrane integrity, delaying increased leakage of ions, decreased turgor, and cell death (Gest et al., 2013). Low tissue Asc content is linked to premature senescence (Barth et al., 2004) and programmed cell death (PCD) in plants (Pavet et al., 2005; De Pinto et al., 2012). For example, Asc-deficient *Arabidopsis thaliana* mutants (*vtc1-1* and *vtc2-1*) show intracellular structural changes that are consistent with PCD (Olmos et al., 2006). Asc-deficient mutant *vtc2* also exhibits enhanced photoinhibition and oxidative damage (Müller-Moulé et al., 2003, 2004). Recently, it was reported that knock-down of GDP-mannose pyrophosphorylase (GMP) gene involved in Smirnoff-Wheeler's pathway significantly decreased Asc contents and altered the phenotype of tomato plants with lesions and further senescence (Zhang et al., 2013). Smirnoff-Wheeler's pathway has been proved to be the major Asc biosynthetic pathways by biochemical and genetic approach (Gallie, 2013). In this pathway, L-galactono-1, 4-lactone dehydrogenase (L-GalLDH, EC 1.3.2.3) is an enzyme that catalyzes the ultimate step of Asc biosynthesis in higher plants (Bartoli et al., 2000). L-GalLDH has been proven to be attached to complex I of the mitochondrial electron transport chain (Schertl et al., 2012), which uses L-galactono-1, 4-lactone (L-GalL) as an electron donor to reduce cytochrome *c* between complexes III and IV, while L-GalL is converted into Asc (Bartoli et al., 2000). The role of L-GalLDH in the control of cell, organ, and plant growth has been studied using mainly antisense or RNA interference (RNAi) approaches, and the deficiency of Asc and L-GalLDH has been observed to affect not only the division and growth of tobacco BY-2 (*Nicotiana tabacum* cv. Bright Yellow 2) cell culture (Tabata et al., 2001) but also the growth and development of tomato (*Solanum lycopersicum*) (Alhagdow et al., 2007) and *Arabidopsis* (Pineau et al., 2008). Recently, we have reported that homozygous L-GalLDH-suppressed transgenic rice (GI) plants with approximately 30%–50% of the foliar Asc content of wild type (WT) plants were developed by RNAi (Yu et al., 2010). Further study has shown that GI plants display a reduced plant growth rate, tiller number and seed set (Liu et al., 2011; Liu et al., 2013). Our latest study shows that the enhanced level of Asc reduces grain chalkiness in the L-GalLDH-overexpressing transgenic by maintaining photosynthetic function and affecting phytohormones associated with grain filling (Yu et al., 2015). However, it is not clear whether there is a link between Asc deficiency and grain chalkiness. The present work was designed to assess the role of Asc during the interactions between ROS and chalkiness formation. The consequences of a decrease in Asc (by using homozygous Asc-deficient transgenic rices, GI-1 and GI-2, which have constitutively very low or moderately leaf and grain Asc contents) compared with the WT plants on grain chalkiness, L-GalLDH activity and gene expression, lipid peroxidation, H_2O_2 accumulation, antioxidant capacity, photosynthetic function, grain-filling rate, enzyme activities and gene transcript abundances related to starch synthetic pathway were studied. The results suggest a close correlation between Asc deficiency and grain chalkiness in the L-GalLDH-suppressed transgenics. Asc deficiency leads to the accumulation of H_2O_2, affecting antioxidant capacity and photosynthetic function, changing enzyme activities and gene transcript abundances related to starch synthesis, finally leading to the increased grain chalkiness.

2. Materials and methods

2.1. Plant materials and growth conditions

Rice (*Oryza sativa* L.) WT Zhonghua 11 and the previously described homozygous GI plants (GI-1 and GI-2) (Liu et al., 2011) were used as experimental plants. GI-1 has constitutively very low (about 30%) while GI-2 has constitutively moderately low (about 50%) leaf Asc content compared with the WT plants. Germinated seeds were pre-grown with complete Kimura B nutrient solution (Yoshida et al., 1976) in a greenhouse under natural conditions. Until the seedlings had four leaves they were taken out gently and then transferred to earthen pots of 30 cm in diameter and 32 cm in depth filled with 6.0 kg of sieved, sterilized dry paddy soil amended with 1.0 g $(NH_4)_2SO_4$, 0.8 g P_2O_5, and 0.6 g K_2O per kg soil to grow until the seeds were harvested. The plants were grown under natural conditions with average temperature of 32 °C/24 °C (day/night), relative humidity 65–85%, photosynthetically active radiation 600–1200 $\mu mol\ m^{-2}\ s^{-1}$ and a photoperiod of 14/10 h (day/night). Flag leaves of WT and GI were sampled at 10, 20, and 30 DAF, while grains were sampled at 10, 15, 20, 25 and 30 DAF. The sampled leaves and grains were stored at − 80 °C until further analysis.

2.2. Determination of chalkiness, starch and amylose content

Mature panicles were harvested and dried at 37 °C for at least 3 d to achieve 15% moisture content. The seeds were then manually threshed and machine dehulled. The degree of chalkiness, grain length (GL) and grain width (GW) were evaluated following the method of Xiao et al. (2001). Starch content and amylose content of GI and WT grains were determined as Fujita et al. (2007).

2.3. Scanning electron microscope (SEM)

Morphological properties of rice samples were examined. Rice samples were broken along natural fracture planes and the pieces mounted on stubs. The specimens were coated with gold using SC7610 Sputter Coater (Fisons Ins, England). These samples were observed by JSM-6380LV scanning electron microscope (Jeol, Akishima-Shi, Japan) at a magnification of 2000×.

2.4. Determination of Asc and DHA

For the determination of Asc (reduced form) and total Asc (Asc plus DHA), 0.1 g of fresh flag leaves or endosperms was homogenized in 1 mL 6% trichloroacetic acid (TCA) solution in an ice bath, and the homogenate was centrifuged at 12 000 rpm and 4 °C for 10 min. The supernatant was used for Asc analysis according to Kampfenkel et al. (1995). DHA was determined as the difference between total and reduced Asc.

2.5. Lipid peroxidation assay

Lipid peroxidation was evaluated by measuring the malondialdehyde (MDA) content from 0.1 g of fresh flag leaves or endosperms, according to the method of Heath and Packer (1968) with slight modification.

2.6. Measurement of H_2O_2 levels

H_2O_2 concentrations in GI and WT leaves and endosperms were measured with a non-enzymatic assay according to Snell and Snell (1949) with modifications. For the measurement of H_2O_2 production, rice flag leaves or endosperms (0.1 g) were ground with a mortar and pestle in liquid nitrogen to a fine powder and added to a 1 mL cuvette containing 0.8 mL of double distilled H_2O and 0.2 mL of 25 mM titanium sulfate and then incubated for 15 min at room temperature. Oxidation of titanium sulfate was recorded by reading A_{410} nm. Readings were converted to corresponding concentrations using a standard calibration plot.