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Tiller number is altered in the ascorbic acid-deficient rice suppressed for L-galactono-1,4-lactone dehydrogenase

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ABSTRACT

The tiller of rice (*Oryza sativa* L.), which determines the panicle number per plant, is an important agronomic trait for grain production. Ascorbic acid (Asc) is a major plant antioxidant that serves many functions in plants. L-Galactono-1,4-lactone dehydrogenase (GLDH, EC 1.3.2.3) is an enzyme that catalyzes the last step of Asc biosynthesis in plants. Here we show that the GLDH-suppressed transgenic rices, GI-1 and GI-2, which have constitutively low (between 30% and 50%) leaf Asc content compared with the wild-type plants, exhibit a significantly reduced tiller number. Moreover, lower growth rate and plant height were observed in the Asc-deficient plants relative to the trait values of the wild-type plants at different tillering stages. Further examination showed that the deficiency of Asc resulted in a higher lipid peroxidation, a loss of chlorophyll, a loss of carotenoids, and a lower rate of CO_2 assimilation. In addition, the level of abscisic acid was higher in GI-1 plants, while the level of jasmonic acid was higher in GI-1 and GI-2 plants at different tillering stages. The results we presented here indicated that Asc deficiency was likely responsible for the promotion of premature senescence, which was accompanied by a marked decrease in photosynthesis. These observations support the conclusion that the deficiency of Asc alters the tiller number in the GLDH-suppressed transgenics through promoting premature senescence and changing phytohormones related to senescence.

Introduction

L-Ascorbic acid (Asc) is the most abundant water-soluble antioxidant in plant tissue and exerts a strong influence on plant growth and development for serving as a co-factor for a number of enzymes and contributing to the detoxification of reactive oxygen species (ROS) (Kerchev et al., 2011). In recent years, the endogenous level of Asc has been suggested to be important in the regulation of developmental senescence (Barth et al., 2004, 2006), plant defense against pathogens (Pastori et al., 2003; Pavet et al., 2005) and control of flowering time (Attolico and De Tullio, 2006; Kotchoni et al., 2009). Asc also appears to be involved in a complex phytohormone-mediated signaling network that ties together ozone and pathogen responses and influences the onset of senescence (Mukherjee et al., 2010).

The major Asc biosynthesis pathway in plants was first proposed by Wheeler et al. (1998) and is the most commonly described in higher plants. In this pathway, L-galactono-1,4-lactone dehydrogenase (GLDH, EC 1.3.2.3) is an enzyme that catalyzes the last step of Asc biosynthesis (Bartoli et al., 2000; Millar et al., 2003). GLDH is attached to complex I of the mitochondrial electron transport chain, which represents a major fragment of the "membrane arm" of complex I (Schertl et al., 2012). GLDH 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). Although no clear relationships among the Asc content, GLDH protein content, and GLDH activity have been observed in wheat (*Triticum aestivum* L.) (Bartoli et al., 2005) and tobacco (*Nicotiana tabacum* L.) (Imai et al., 2009) plants, the deficiency of Asc and GLDH has been observed to affect not only the division and growth of tobacco BY-2 (*N. 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), *Arabidopsis* (Pineau et al., 2008), and rice (Yu et al., 2010; Liu et al., 2011). Transgenic tobacco cells expressing antisense RNA for GLDH were abnormal

Abbreviations: ABA, abscisic acid; Asc, L-ascorbic acid; Car, carotenoids; Chl, chlorophyll; Ci, intercellular CO_2 concentration; DHA, dehydroascorbate; L-GalL, L-galactono-1,4-lactone; GI, GLDH-suppressed transgenic rice; GLDH, L-galactono-1,4-lactone dehydrogenase; JA, jasmonic acid; MDA, malondialdehyde; PC, principal component; PCD, programmed cell death; ROS, reactive oxygen species; TCA, trichloroacetic acid; Tr, transpiration rate.

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in both phenotype and cell structure (Tabata et al., 2001), GLDH-suppressed transgenic tomato plants resulted in a dwarf phenotype (Alhagdow et al., 2007), and an *Arabidopsis* mutant interrupted in the GLDH gene did not develop beyond the cotyledon stage in the absence of Asc supplementation (Pineau et al., 2008). Recently, we reported that homozygous GLDH-suppressed transgenic rice plants (GI-1 and GI-2) with approximately 30–50% of the Asc content of wild-type plants were developed by RNA interference (RNAi) (Yu et al., 2010). Further study showed that GLDH-suppressed rice plants (GI-1 and GI-2) displayed a reduced plant growth rate and seed set (Liu et al., 2011). Low tissue Asc content is also 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, smaller rosettes were observed in Asc-deficient *Arabidopsis thaliana* mutants (*vtc1-1* and *vtc2-1*) compared with those of the wild type at later stages of development, which showed intracellular structural changes that were consistent with PCD (Olmos et al., 2006). It was reported that the Asc-deficient *A. thaliana* mutant *vtc2* exhibit enhanced photo inhibition and oxidative damage (Müller-Moulé et al., 2003, 2004), while recent studies showed that the Asc deficient *A. thaliana* mutant *vtc1-1* is conditionally hypersensitive to ammonium (NH_4^+) and the short-root phenotype in *vtc1-1* is independent of Asc deficiency and oxidative stress (Barth et al., 2010; Li et al., 2010). The prevention of stress-induced premature senescence is an important target in crop improvement programs, so it is important to have a better understanding of how Asc interacts with the processes that control plant growth and development (Foyer et al., 2006).

Rice grain yields are determined by many factors, among which tiller number is generally regarded as a key factor (Wang and Li, 2011). The tiller number of rice, which determines the panicle number per plant, is an important agronomic trait for grain production (Li et al., 2003). In addition, phytohormones play key roles in regulating rice tiller occurrence (Kariali and Mohapatra, 2007), while Asc plays an important role in conjunction with various phytohormones in regulating gene expression during senescence (Barth et al., 2006). It has been reported exogenous Asc can benefit to wheat tiller number and increase yield (Amin et al., 2008; Jafar et al., 2012). Recently, we have reported that seed set and thousand-grain weight were affected in the Asc-deficient rice plants, which showed significantly reduced number of grains per plant (Liu et al., 2011). However, it is not clear whether endogenous Asc can provide a link between senescence and tiller number. The present work was designed to assess the role of Asc during the interactions between senescence and tiller number in rice. The consequences of decrease in Asc (by using homozygous AsA-deficient transgenic rices, GI-1 and GI-2, which have constitutively very low or moderately low leaf Asc contents) compared with the wild-type plants on tiller number, lipid peroxidation, photosynthetic function, phytohormones and their interactions were studied. The results suggest that Asc plays a key role in affecting tiller number through mediating the interactions of senescence and phytohormones.

Materials and methods

Plant materials

Oryza sativa L. cv. Zhonghua 11 and the previously described homozygous L-galactono-1,4-lactone dehydrogenase (GLDH)-suppressed transgenic rice plants GI-1 and GI-2 (Liu et al., 2011) were used for the physiological study. GI-1 has constitutively very low (about 30%) while GI-2 has constitutively moderately low (about 50%) leaf L-Ascorbic acid (Asc) content compared with the wild-type plants.

Growth conditions and treatments

Germinated seeds of Zhonghua 11 and the transgenics were pregrown with complete Kimura B nutrient solution (Yoshida et al., 1976) in a green house until 15 days. Seedlings were 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 (the contents of soil organic matter, alkaline hydrolytic nitrogen, effective phosphorus, available potassium was 14.2%, 66.2 mg kg^{-1}, 8.5 mg kg^{-1}, and 8.0 mg kg^{-1}, respectively, and soil pH 5.4) amended with 1.0 g $(NH_4)_2SO_4$, 0.8 g P_2O_5, and 0.6 g K_2O/kg soil to grow. The plants were grown under natural conditions with average temperature of 32 °C/24 °C (day/night), relative humidity 65–85%, photo synthetically active radiation 600–1200 μmol m^{-2} s^{-1} and photoperiod of 14/10 h (day/night). Young fully expanded leaves of Zhonghua 11 and transgenics from the top of plants were sampled at 40, 60, and 80 days after germination. The sampled leaves were stored at −70 °C for future analysis.

Measurement of Asc and dehydroascorbate (DHA)

For the determination of Asc (reduced form), 0.1 g of fresh leaves was homogenized in 1 mL 6% (w/v) trichloroacetic acid (TCA) solution in an ice bath, and the homogenate was centrifuged at 12,000 × *g* and 4 °C for 10 min. The supernatant was used for Asc and total ascorbic acid analysis as described by Kampfenkel et al. (1995). DHA was determined as the difference between total and reduced ascorbic acid.

Quantitative determination of photosynthetic pigments

Chlorophylls (Chl) a, b, and carotenoids (Car) were determined in the same leaf pigment extract using the re-determined extinction coefficients and equations established by Lichtenthaler (1987). In brief, fresh leaf material (0.1 g) was extracted with 80% (v/v) acetone, and the absorption of the extracts was measured at 663, 645, and 470 nm (UV–vis spectrophotometer, Shimadzu). The A_{663}, A_{645}, and A_{470} was determined and used to calculate Chla, Chlb, and Car content by the equations: Chla (mg L^{-1}) = 12.21 A_{645} − 2.81 A_{645}, Chlb (mg L^{-1}) = 20.13 A_{645} − 5.03 A_{645} and Car (mg L^{-1}) = (1000 A_{470} − 3.27 Chla − 104 Chlb)/229, respectively.

Lipid peroxidation assay

Fresh leaves (0.1 g) were homogenized in 2 mL of 0.1% (w/v) TCA solution on ice, and the suspension was rinsed into a centrifuge tube with an additional 1 mL of 0.1% (w/v) TCA. Then the homogenate was centrifuged at 10,000 × *g* for 5 min, and the supernatant was collected for analysis. The thiobarbituric acid test, which determines malondialdehyde (MDA) as an end-product, was used to analyze lipid peroxidation (Sunkar et al., 2003).

Gas exchange measurements

The net photosynthesis rate of rice leaves under natural conditions were determined by using a portable photosynthesis system (Li-6400, Li-Cor Inc., Lincoln, NE, USA) at PFD (photo flux density) of 1000 μmol m^{-2} s^{-1}, 32 ± 2 °C, 340 μmol mol^{-1} CO_2, and relative humidity 79–83%. The stomata conductance, transpiration rate (Tr) and intercellular CO_2 concentrations (Ci) value were also read from the portable photosynthesis system.

Detection of phytohormones

Young fully expanded leaves from Asc-deficient and wild type rice plants (0.5 g) were harvested for abscisic acid (ABA) and