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Quantitative trait locus analyses of ozone-induced grain yield reduction in rice

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ABSTRACT

Reduction of grain yield (total seed weight) by ozone in rice (*Oryza sativa* L.) is believed to be caused by ozone-induced reduction of photosynthetic activity followed by growth inhibition. Here, *japonica* rice cultivar Sasanishiki and *indica* rice cultivar Habataki showed different responses to ozone. When exposed to ozone, the leaves of Habataki exhibited no critical damage, whereas those of Sasanishiki developed lesions. Conversely, ozone exposure reduced total seed weight by 19% in Habataki, but not significantly in Sasanishiki. Chronic ozone exposure also significantly decreased culm length, number of primary rachis branch, and number of spikelets per panicle in Habataki. QTL analysis in Sasanishiki/Habataki chromosome segment substitution lines identified a single locus associated with the yield loss caused by ozone on chromosome 6 of Habataki close to marker RM3430 (107.6 cM). A QTL for reduction of primary rachis branch number and total spikelet number was found in the same position. These results indicate that a QTL on chromosome 6 has an important role in ozone-induced yield loss, and is also involved in primary rachis branch formation and total spikelet number in ozone-exposed rice.

1. Introduction

Ozone is the main photochemical oxidant that causes leaf damage, and exposure to it can decrease the productivity of crops and forests. Ashmore et al. (2006) predicted that the tropospheric ozone concentration will continue to increase in Eastern Asia to 2020, and that crop yield may decrease by up to 60% in China as a result. In Japan, the tropospheric ozone concentration has continued to increase, although the concentrations of nitrogen oxide and non-methane hydrocarbons, which are the major precursors of ozone, have decreased over the past 20 years (Ohara, 2007).

Generally, acute exposure to ozone can result in chlorosis and necrosis. Thus, many studies have focused on the mechanisms of ozone-induced leaf injury as a means of understanding the effects of ozone on plants. Consequently, the physiological and molecular aspects of visible leaf injury by ozone are well understood. When ozone enters the plant leaf through the stomata, it is rapidly reacts with components of the cell wall, plasma membrane and apoplastic fluids (Kangasjärvi et al., 1994). In the apoplast, ozone is decomposed to reactive oxygen species (ROS) such as the hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$), and singlet oxygen (Rao and Davis, 2001; Baier et al., 2005). Furthermore, these ROS induce the production of further ROS by plant itself, designated as the oxidative burst (Wohlgemuth et al., 2002). As a consequence, visible ozone damage of leaves can occur due to (i) direct necrotic tissue damage caused by ROS or (ii) ROS-induced programmed cell death that resemble the hypersensitive response observed in pathogen infection (Kangasjärvi et al., 2005). Long-term exposure to relatively low concentration of ozone (chronic ozone exposure) accelerates senescence of plant cell, which leads increase oxidative stress in chloroplasts, and degradation of ribrose-1,5-bisphosphate carboxylase/oxigenase (Pell et al., 1997). Chronic ozone exposure, followed by disturbances in sugar metabolism, inhibition of photosynthesis, and imbalances in the redox states results in reduction in plant growth and/or crop yield (Schraudner et al., 1997). The ozone sensitivity, evaluated as visible leaf injury, of many rice cultivars has been assessed (Sohn and Lee, 1997; Frei et al., 2008), and the mechanism of leaf damage by ozone has been studied (Lin et al., 2001; Frei et al., 2008). The degree of visible leaf

Abbreviations: QTL, quantitative trait loci; ROS, reactive oxygen species; NA, natural air; LBS, leaf bronzing score; CSSL, chromosome segment substitution line; EM, expectation-maximization; LOD, likelihood-of-odds; *APO1*, *ABERRANT PANICLE ORGANIZATION 1*.

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injury did not correlate with the grain yield reduction by ozone in 20 rice cultivars (Sawada and Kohno, 2009). This result suggests that the leaf injury and grain yield loss may be regulated by different mechanisms. However, the mechanisms underlying ozone-induced grain yield loss have not been investigated in rice.

In recent studies, genes involved in rice yield have been identified by quantitative trait locus (QTL) analysis (Ashikari et al., 2005; Fan et al., 2006; Song et al., 2007; Xue et al., 2008). For example, the expression level of cytokinin oxidase/dehydrogenase gene (*OsCKXs*), encoding an enzyme that degrades the phytohormone cytokinin, in inflorescence meristems regulates the number of reproductive organs and grain yield (Ashikari et al., 2005). Furthermore, at least eleven putative *CKX* genes have been found in rice, but only the *OsCKX2* gene has important role for grain yield (Ashikari et al., 2005). Despite the identification of several genes involved in rice grain yields, no research has clarified the mechanism of ozone-induced yield loss in rice. The objective of this study was to identify the gene loci determining rice yield loss under high-ozone conditions by using Sasanishiki/Habataki chromosome segment substitution lines (CSSLs). Toward this end, we used 39 chromosome segment substitution lines (CSSLs) from a cross between Sasanishiki, as the recurrent parent and Habataki, as the donor (Ando et al., 2008). Previous study showed that ozone-induced grain yield loss in Sasanishiki, a parent of CSSLs, was less than that in an indica variety, Takanari (Sawada and Kohno, 2009). In addition, Takanari is sibling variety of Habataki (Imbe et al., 2004), indicating that ozone responses, including grain yield loss, are expected to be same between Takanari and Habataki. Therefore, we use Sasanishiki/Habataki CSSLs, in this study, to determine the gene loci that affect ozone-induced grain yield loss.

2. Materials and methods

2.1. Plant material and growth conditions

To detect QTLs for ozone resistance, we used a mapping population consisting of 39 CSSLs developed from backcrosses of rice (*Oryza sativa* L.) cultivars Sasanishiki (recurrent parent) and Habataki (donor parent) (Ando et al., 2008). Seeds of the two parents and the 39 CSSLs, designated SL401–SL439, were obtained from the Rice Genome Resource Center (RGRC, Tsukuba, Japan). Seeds were sown in plastic boxes (80 plants per box; 28 cm × 21 cm × 9 cm) filled with seedbed soil on 10 April 2009. The seedlings were first grown for 6 weeks in a glasshouse in natural (ambient) air at the Akagi Testing Center of the Central Research Institute of the Electric Power Industry (Gunma Prefecture, Japan, 36°28′N, 139°11′E, 540 m above sea level). Six weeks after sowing (21 May 2009), the seedlings were transplanted into pots (four plants per pot; 0.05 m^2 surface area and 0.015 m^3 volume) and grown in glasshouses (five pots per glasshouse for each line) under conditions of natural air (NA) or elevated ozone (×2). For the ×2 treatment, artificially generated ozone was added to natural air via a mass flow controller. The mean ozone concentration during the daytime (from 06:00 to 18:00) was 32.0 nl l^{-1} (ppb) under NA and 76.5 nl l^{-1} under ×2 condition, with a daily peak of 43.1 nl l^{-1} and 91.0 nl l^{-1}, respectively (Fig. 1). Fertilizer was supplied at N:P:K = 15:15:15 g m^{-2} at 2 weeks after transplanting. Plants were grown in a glasshouse under natural light until harvest (24 or 25 September 2009). For total RNA isolation, plants were cut off at the base at 10 days before heading, and young panicles (about 5 cm long) enclosed by a leaf sheath were isolated and frozen at −80 °C until use.

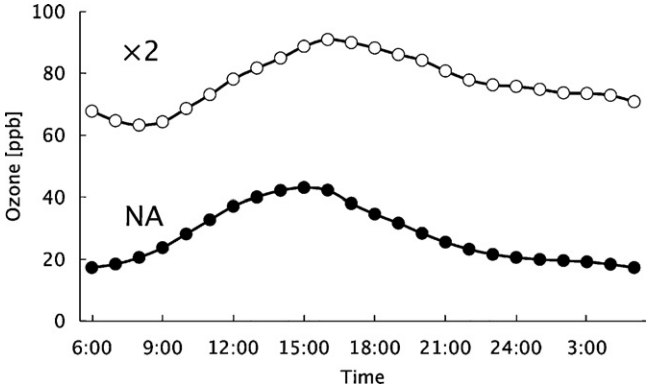

Fig. 1. Daily ozone exposure in glasshouse. Values represent mean ozone concentration (ppb) each hour of the day averaged from 21 May to 25 September in 2009. NA, natural air condition; ×2, high-ozone condition.

2.2. Measurement of visible leaf injury

At 19 days after transplanting, ozone-induced visible leaf injury was quantified by assigning a leaf bronzing score (LBS) to the fourth leaf from the base of five plants per treatment. LBS was defined using the following scale: 0, no visible damage; 1, very few small chlorotic spots; 2, very few small brown stipples; 3, 10%–20% of leaf area with chlorotic or brown stipples; 4, 20%–40% of leaf area with chlorotic or brown stipples; 5, >40% of leaf area with brown lesions; 6, >40% of leaf area with brown lesions and large necroses; 7, entire leaf dying (Sawada and Kohno, 2009; Fig. 2a).

2.3. Measurement of gas exchange

On 29 July and 4 August 2009, 3 pots of samples of the two parental cultivars, Sasanishiki and Habataki, were selected from each glasshouse, and gas exchange of two flag leaves per pot was measured with a Li-Cor LI-6400P Portable Photosynthesis System with a 3 cm × 2 cm (6 cm^2) leaf chamber. Illumination was provided by an attached LI-6400-02B LED light (Li-Cor, Inc., Lincoln, NE, USA); the photosynthetic photon flux density in the leaf chamber was 2000 ± 1 μmol m^{-2} s^{-1}. Temperature, CO_2, and water vapor concentration in the leaf chamber were set to 25.2 ± 0.2 °C, 380 ± 0.5 μmol mol^{-1}, and 22 ± 0.5 μmol mol^{-1}, respectively.

2.4. Measurements of yield and harvest indexes

During cultivation, the day of heading of each plant was recorded. Plants were cut off at the base of the culm between 24 and 25 September 2009, and the length of the longest leaf blade, culm length, culm number, and biomass were measured. Panicles were cut off at the neck of the spike, and the panicle length and number of primary rachis branch were measured. Then, grains were removed from the panicles and sorted into filled and unfilled grains. The total spikelet number, number of spikelets per panicle, number of filled grains, filling rate, total seed weight, and 1000-seed weight were measured. The total seed weight was calculated from filled grains only, and was used as the measure of total seed weight in this report.

2.5. Identification of putative gene loci by QTL analysis

We performed linkage analysis by interval mapping (Lander and Botstein, 1989) as implemented in the program R/qtl (Broman et al., 2003), using the expectation-maximization (EM) algorithm (Haley and Knott, 1992). The genotype of each CSSL had been determined previously by using 166 DNA markers