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Physiological and genotype-specific factors associated with grain quality changes in rice exposed to high ozone☆

Liquan Jing [a], Vitalij Dombinov [b,1], Shibo Shen [a], Yanzhen Wu [a], Lianxin Yang [a], Yunxia Wang [c], Michael Frei [b,*]

[a] Key Lab of Crop Genetics & Physiology of Jiangsu Province, Yangzhou University, PR China
[b] Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Germany
[c] College of Environmental Science and Engineering, Yangzhou University, PR China

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ABSTRACT

Rising tropospheric ozone concentrations in Asia affect the yield and quality of rice. This study investigated ozone-induced changes in rice grain quality in contrasting rice genotypes, and explored the associated physiological processes during the reproductive growth phase. The ozone sensitive variety Nipponbare and a breeding line (L81) containing two tolerance QTLs in Nipponbare background were exposed to 100 ppb ozone (8 h per day) or control conditions throughout their growth. Ozone affected grain chalkiness and protein concentration and composition. The percentage of chalky grains was significantly increased in Nipponbare but not in L81. Physiological measurements suggested that grain chalkiness was associated with a drop in foliar carbohydrate and nitrogen levels during grain filling, which was less pronounced in the tolerant L81. Grain total protein concentration was significantly increased in the ozone treatment, although the albumin fraction (water soluble protein) decreased. The increase in protein was more pronounced in L81, due to increases in the glutelin fraction in this genotype. Amino acids responded differently to the ozone treatment. Three essential amino acids (leucine, methionine and threonine) showed significant increases, while seven showed significant treatment by genotype interactions, mostly due to more positive responses in L81. The trend of increased grain protein was in contrast to foliar nitrogen levels, which were negatively affected by ozone. A negative correlation between grain protein and foliar nitrogen in ozone stress indicated that higher grain protein cannot be explained by a concentration effect in all tissues due to lower biomass production. Rather, ozone exposure affected the nitrogen distribution, as indicated by altered foliar activity of the enzymes involved in nitrogen metabolism, such as glutamine synthetase and glutamine-2-oxoglutarate aminotransferase. Our results demonstrate differential responses of grain quality to ozone due to the presence of tolerance QTL, and partly explain the underlying physiological processes.

1. Introduction

Ozone plays an ambivalent role in terrestrial ecosystems. While stratospheric ozone protects life on the earth's surface from excessive UV radiation and helps to maintain the climatic balance (Robinson and Erickson, 2015), tropospheric ozone has long been recognized as an air pollutant affecting human health and plant productivity (Ashmore et al., 2006; Ashmore, 2005; The Royal Society, 2008), and likewise acts as a greenhouse gas (IPCC, 2014). Tropospheric ozone is formed in photo-oxidative reactions of precursor gases originating largely from anthropogenic gas emissions, including NO_x, CO, and volatile organic compounds (The Royal Society, 2008). In many traditionally industrialized parts of the world, such as North America or Europe, environmental policies have been implemented in recent decades to restrict the emissions of these precursor gases (Oltmans et al., 2013; The Royal Society, 2008). However, in many transitional economies, high rates of economic growth are strongly correlated with increases in fossil fuel combustion, leading to rising tropospheric ozone levels (IPCC, 2014). As Asia has many such economies in transition, including

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* Corresponding author. Karlrobert Kreiten Strasse 13, 53115 Bonn, Germany.
E-mail address: mfrei@uni-bonn.de (M. Frei).
[1] Present affiliation: Institut für Bio-und Geowissenschaften, Forschungszentrum Jülich, Germany.

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China and India, it will likely experience rising tropospheric ozone levels, at least until the middle of the 21st century (IPCC, 2014; The Royal Society, 2008).

Tropospheric ozone affects the growth, yield and quality of agricultural crops (Fuhrer, 2009). With the rising ozone levels in Asia, the effects of ozone on rice as Asia's major staple crop are becoming a matter of concern. Similar to other crop species such as wheat or soybeans (Feng et al., 2008; Morgan et al., 2003), ozone causes oxidative stress in rice tissue and decreases the photosynthetic carbon assimilation, ultimately leading to reductions in grain and biomass yield and changes in grain and straw quality (Ainsworth, 2008; Frei, 2015; Frei et al., 2011). Grain yield losses exceeding 50 percent have been reported in artificial environments such as fumigation chambers (Frei, 2015), while yield losses of up to 20 percent are expected with the seasonal ozone doses encountered in natural rice growing areas (Ainsworth, 2008; Frei, 2015; Shi et al., 2009). It has been estimated that yearly rice grain yield losses may be equivalent to 3–4 percent of the global rice production (Van Dingenen et al., 2009), which would currently correspond to 22–29 million tons of paddy rice (FAOSTAT, 2015). Therefore, breeding of ozone resistant rice is essential for the food security of large parts of Asia. Rice shows tremendous genotypic variability in the response to ozone (Sawada and Kohno, 2009; Ueda et al., 2015a), thus providing a resource for adaptive breeding. Quantitative trait loci (QTLs) associated with ozone tolerance in rice have previously been reported and characterized (Frei et al., 2010, 2008; Tsukahara et al., 2015; Tsukahara et al., 2013), and the marker assisted introgression of two QTLs *OzT8* and *OzT9* into ozone-sensitive genetic background was shown to mitigate ozone-induced biomass losses (Wang et al., 2014a).

Apart from yields, the grain quality is particularly important in rice for several reasons. Firstly, around eighty percent of the rice produced globally is directly consumed by humans, unlike with other cereal crops, where a larger proportion is fed to domestic animals or processed into non-food products (GRiSP, 2013). Secondly, rice is the staple crop in many low or middle income countries, where it often accounts for the majority of people's daily energy intake, and plays an important role as a source of protein and micronutrients due to the low consumption of animal products (de Pee, 2014; GRiSP, 2013). And thirdly, unlike with other cereal crops, rice is mostly marketed and consumed as intact grains rather than in a processed form, making its appearance and physicochemical properties critical in terms of consumer acceptance (Lyman et al., 2013; Zhou et al., 2002). It is thus of the utmost importance to understand the effects of ozone on rice grain quality in detail. A few studies have previously explored this topic. Similar to observations in other cereal crops such as wheat (Broberg et al., 2015; Piikki et al., 2008), exposure led to a decrease in the starch concentration in grains and an increase in the protein concentrations in rice (Frei et al., 2012; Wang et al., 2014b, 2012; Zhou et al., 2015b). However, the protein yield, *i.e.* the amount of protein produced per area unit, decrease as grain yield losses outweighed the positive effects of higher protein concentrations (Frei et al., 2012). In addition, decreases in grain size (Frei et al., 2012), increases in lipid concentration of brown rice (Frei et al., 2012) and deterioration of the visual appearance due to grain chalkiness (Wang et al., 2014b) have been reported.

Various reasons have been put forward to explain the physiological processes underlying these changes in rice quality. Increases in cereal grain protein concentration due to ozone stress have often been ascribed to a ‘concentration effect’, since a lower amount of grain is produced with the same amount of available nitrogen (Wang and Frei, 2011). On the other hand, reductions in protein yield occurred because negative effects of ozone on photosynthesis lead to lower biomass and grain formation (Broberg et al., 2015). Moreover, ozone was assumed to favor processes involved in plant senescence and nitrogen remobilization towards the grain, while simultaneously limiting carbohydrate metabolism and thus starch synthesis during grain filling (Wang and Frei, 2011). Together, these processes could explain higher protein concentrations and lower starch concentrations due to ozone exposure, although little experimental evidence exists for these hypotheses. Grain chalkiness in rice typically results from low density of starch granules, and has often been associated with high temperatures during grain filling (Lin et al., 2014). Ozone-induced grain chalkiness has been ascribed to incomplete grain filling due to a shortened maturity period (Wang et al., 2014b), but detailed investigations of carbohydrate metabolism during grain filling are lacking to date. In summary, rice grain quality has shown consistent responses to ozone in several previous experiments, but the underlying physiological mechanisms and genotypic differences in these processes are poorly understood.

The aim of this study was to expand our understanding of ozone effects on rice grain quality by specifically addressing the following questions: (i) Can we explain changes in rice grain quality with physiological processes during the reproductive growth stage? This question was addressed through physiological measurements of carbohydrate and nitrogen metabolism during the heading, milky and dough stages, in addition to detailed measurements of grain quality. (ii) Are the effects of ozone on rice grain quality and the underlying physiological processes affected by previously identified ozone tolerance QTL *OzT8* and *OzT9*? This question was addressed by comparative analysis of the ozone sensitive rice variety Nipponbare (NB) and a breeding line (L81) containing introgressions of two QTL in NB genetic background.

2. Materials and methods

2.1. Plant material and cultivation

Two previously characterized rice (*Oryza sativa* L.) genotypes contrasting in ozone tolerance were used: Nipponbare (NB), an ozone-sensitive *japonica* variety, and L81, a breeding line with a NB genetic background and two introgressions at quantitative trait loci (QTL), which make it more resistant to ozone in terms of leaf symptom formation and biomass yield (Wang et al., 2014a). Seeds were germinated and nursed in a field for 28 days. Thereafter, seedlings were transplanted at 18 × 17 cm spacing to concrete tanks filled with paddy soil (Wang et al., 2014a), which were placed inside four independent glasshouse-type fumigation chambers (3 × 3 × 1.7 m). Within each chamber, U-shaped concrete tanks were divided into three blocks containing around 20 plants of each genotype. Throughout the experiment, plants were exposed to natural light, and the soil was kept water-saturated. Fertilizer was applied at a rate 15 g nitrogen m^{-2}, 7 g P_2O_5 m^{-2}, and 7 g K_2O m^{-2}, respectively. One day before transplanting, 9 g nitrogen m^{-2}, 7 g P_2O_5 m^{-2} and 7 g K_2O m^{-2} was applied as basal dressing, and additionally 6 g nitrogen m^{-2} was applied at 45 days after transplanting (DAT45).

2.2. Ozone fumigation

Two chambers were assigned to the elevated ozone treatment, while another two chambers were used as a control. Ozone fumigation was conducted using a fumigation system described previously (Wang et al., 2014a, 2014b). In brief, a temperature and humidity control unit in each glasshouse chamber enabled simulation of designated meteorological conditions, while a gas distribution system simulated the targeted ozone concentration. The main control system (S7-200, Siemens, Nürnberg, Germany)