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Ozone pollution and rice production in Asia: significance, physiological response of rice and development of tolerant genotypes

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Introduction

Surface ozone concentrations have been rising in many Asian countries in recent years due to environmental pollution accompanying economic and industrial development. Ozone is an air pollutant that is formed in the earth's troposphere as a consequence of photochemical reactions of precursor gases such as nitrous oxides, carbon monoxide, or volatile organic compounds. Apart from being detrimental to human health, ozone negatively affects vegetation. Ozone causes visible leaf damage ("bronzing") due to oxidative stress, and negatively affects plant growth by hampering photosynthesis. Average rice yield reductions around fourteen percent due to ozone pollution have been estimated at ozone concentrations above 60 nL L^{-1} (Ainsworth, 2008), a threshold that is frequently exceeded in India and China. A powerful strategy to prevent such yield losses is the development of tolerant rice varieties. We therefore aim to identify genetic factors associated with ozone tolerance in rice, and to understand the underlying physiological mechanisms.

Methods

We carried out a series of greenhouse experiments using three week old rice seedlings grown in hydroponic tanks. Plants were exposed to 120 nL L^{-1} ozone from 9.00 to 16.00 for 10 to 18 days in each experiment using an open top chamber. Control plants were grown in a separate greenhouse compartment where ozone concentration did not exceed 20 nL L^{-1}. Dry weight development and visible leaf damage (leaf bronzing) were used as tolerance indicators. We initially assessed genotypic differences for tolerance to elevated ozone using 23 different varieties. Based on these result a suitable mapping population for quantitative trait loci (QTL) was selected. We then mapped tolerance QTLs in a permanent mapping population of 98 backcross inbred lines derived from contrasting parents Kasalath (a tolerant *indica* landrace) and Nipponbare (an intolerant modern *japonica* variety). The effects of QTLs were confirmed in further experiments with chromosome segment substitution lines (SLs). These SLs carried tolerance alleles at the QTL positions in the genetic background of the intolerant parent Nipponbare. Subsequently, physiological experiments were carried out with SLs in order to characterize the physiological basis of QTLs. During these experiments, leaves were sampled for antioxidant analyses, and photosynthesis measurements were made using a LI6400-40 gas exchange system (Li-Cor Inc.Lincoln, NE, USA).

Results and Discussion

In an initial tolerance screening with 23 rice varieties we found that substantial genotypic differences existed in ozone tolerance (Frei *et al*., 2008). We used these results to select a QTL mapping population in which the parents showed contrasting ozone tolerance. The Nipponbare/Kasalath permanent mapping population was suitable, because Kasalath was highly tolerant in terms of leaf bronzing and dry weight development, and Nipponbare was moderately sensitive in terms of leaf bronzing and highly intolerant in dry weight.

Mapping of QTLs using the Nipponbare/Kasalath mapping population initially yielded four QTLs for leaf bronzing and one QTL for dry weight (Frei *et al*., 2008). The effect of these QTLs was tested in an independent experiment with chromosome segment substitution lines (SL). In this experiment the effect of two of the leaf bronzing related QTLs and the dry weight related QTL could be confirmed.

We then carried out experiments with substitution lines aimed at identifying the physiological basis underlying these QTLs. Our first hypothesis was that QTLs influencing leaf bronzing (a consequence of oxidative stress) would be linked to the plants oxidative stress response system. Indeed we found that the two QTLs strongly influenced ascorbic acid level in ozone treated plants (Frei *et al*., 2008). Ascorbic acid is a key antioxidant in plants stress response as it can detoxify radical oxygen species in various ways (Conklin, 2001). In further studies we now intend to elucidate by which mechanism our QTLs influence ascorbic acid metabolism.

Our second hypothesis was that a dry weight related QTL would influence photosynthetic carbon assimilation under ozone stress. Gas exchange measurements were carried out to investigate how biomass development would relate to photosynthetic performance. Plant dry weights under ozone exposure and control conditions were significantly correlated with the photosynthetic rate and stomatal conductance, although the latter correlation was much weaker (Frei *et al*., 2008). A tolerant substitution line (SL37) maintained significantly higher photosynthetic rate under ozone exposure than its intolerant parent Nipponbare (Frei *et al*., 2008). Further measurements of CO_2 response were made to break down factors limiting photosynthesis under ozone stress. A/Ci curves (Fig.1) plot the leaf internal CO_2 concentration (Ci) versus the photosynthetic rate (A) at varying Ci concentration. In other words they display the response of a leaf's carbon assimilation irrespective of stomatal limitations. They thus allow to partition biochemical and stomatal limitations on photosynthesis (Long and Bernacchi, 2003). A/Ci curves of ozone treated and control plants differed significantly, but more so in the intolerant parent Nipponbare (Fig.1). We therefore hypothesize that the major limitations on photosynthesis are biochemical factors such as Rubisco activity and the regeneration of RuBP, rather than stomatal conductance. Furthermore, ozone tolerance mechanisms in SL37 seem to be linked to these biochemical limitations on photosynthesis.

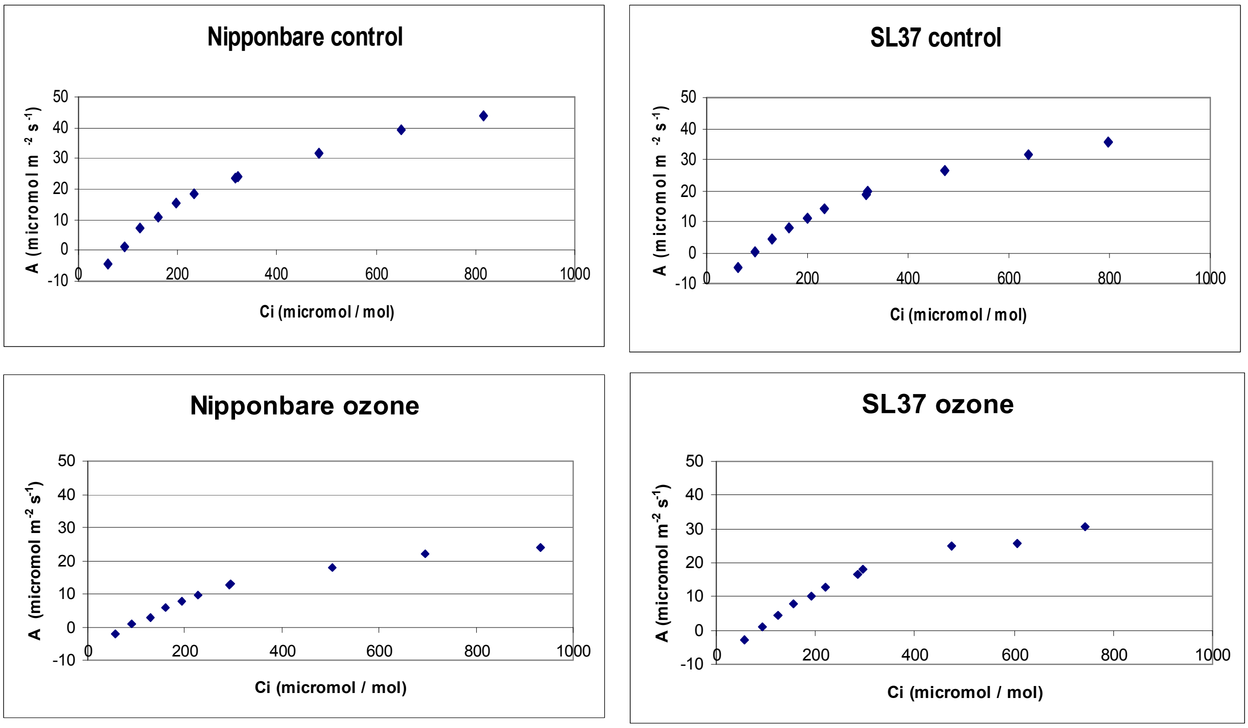

Figure 1: CO_2 response curves of rice plants exposed to 120 nL L^{-1} ozone and control plants grown without ozone fumigation; SL37 is a substitution line carrying an ozone tolerance QTL in the genetic background of Nipponbare. Measurements were taken with a LI-6400 gas exchange system. A = photosynthetic rate, Ci = leaf internal CO_2 concentration.

Conclusions and Outlook

Substantial natural genetic variation in tolerance to ozone was observed in rice. We were able to dissect this genetic variation into QTLs that show highly reproducible effects in greenhouse experiments. QTLs are linked to distinct tolerance mechanisms, and further research will now aim at further characterizing the physiological basis of these QTLs and evaluating potentially important genes in the QTL areas by gene expression studies. Based on converging evidence from these approaches we aim at identifying distinct ozone tolerance genes. Moreover, the effect of our QTLs on the harvestable rice grain yield needs to be assessed in field based studies. The ultimate goal would be to develop rice varieties that produce enhanced grain yield in ozone affected areas compared to conventional varieties.

References

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