

Genotypic variation in the expression levels of antioxidative genes in *Triticum aestivum* and their association with carbon assimilation related traits in abiotic stress prone environments

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ABSTRACT

An elevated level of reactive oxygen species (ROS) is often produced in plants under abiotic stress conditions. These ROS molecules at high concentrations are harmful to cells and can inhibit carbon assimilation. Plants possess a number of antioxidative systems to prevent ROS accumulation. In this study, we have examined genotypic differences in the expression levels of some antioxidative genes in several recombinant inbred wheat progeny lines. Our study identified genotypic variation in the mRNA levels of a number of antioxidative genes among the progeny lines, and their positive association with carbon assimilation related traits in wheat grown under rain-fed conditions in northern Australia.

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide, H₂O₂ and hydroxyl radical, are generated as by-products during photosynthesis and respiration in plants (Mitter, 2002). ROS can accumulate under abiotic stress conditions, including drought stress, salt stress and high light intensity (Mitter, 2002; Blokhina et al., 2003; Foyer and Noctor, 2005). At high concentrations, these ROS molecules can inactivate various enzymes and damage important cellular components. Consequently, oxidative stress can potentially reduce carbon assimilation and plant growth. Plants possess a number of antioxidative systems to prevent ROS accumulation (Mitter, 2002; Blokhina et al., 2003; Foyer and Noctor, 2005). In this study, we examined genotypic differences in the expression levels of some antioxidative genes in several recombinant inbred lines from a cross between Seri and Babax (SB) using Affymetrix GeneChip data (Xue et al., 2008) and evaluated their potential association with carbon assimilation related traits.

MATERIALS AND METHODS

Recombinant inbred lines, derived from the Seri M82/Babax cross, were grown under rain-fed conditions with two field replicates of each line in 2005 at Gatton, Queensland, Australia, as described by Xue et al. (2008). The upper stem internodes and leaf sheaths (peduncle and penultimate internodes with leaf sheath attached) of 8 SB lines with similar anthesis dates were sampled at 50% anthesis between 12:00 and 1:00 pm about 2 days after 30 mm of rain. Each sample contained 7 to 8 stems from main tillers that were randomly sampled from each

plot. RNA isolation, Affymetrix wheat genome GeneChip analysis and WSC measurement were as described previously (Xue et al., 2008).

RESULTS AND DISCUSSION

To evaluate the potential role of antioxidative genes in carbon assimilation related traits, such as grain weight and stem WSC concentration, we have examined the genotypic differences in the expression levels of genes associated with antioxidative systems in the top-two-internode stems and leaf sheaths of 8 SB lines varying in grain weight, stem WSC concentration and maturity biomass using Affymetrix GeneChip expression analysis (Xue et al., 2008). This genome-wide expression analysis revealed that the expression levels of a large number of potential antioxidative genes appeared to be positively associated with grain weight, stem WSC concentration and maturity biomass in these SB lines. The correlation coefficients between the mRNA levels of many of these positively associated genes and grain weight, stem WSC concentration or maturity biomass were statistically significant and some were also significantly correlated with grain yield in these lines. These positively associated antioxidative genes include metallothionein, ascorbate peroxidase, monodehydroascorbate reductase, thioredoxin, glutaredoxin, thioredoxin reductase, catalase, phospholipid hydroperoxide glutathione peroxidase, NADPH:quinone reductase and γ -tocopherol methyltransferase (data not shown). In this communication, expression data on genes involved in three antioxidative systems, metallothionein, ascorbate peroxidase and thioredoxin/glutaredoxin, and their association with carbon assimilation related traits are presented.

EXPRESSION LEVELS OF METALLOTHIONEIN GENES POSITIVELY ASSOCIATED WITH GRAIN WEIGHT AND STEM WSC CONCENTRATION

Metallothioneins are a group of low molecular weight and cysteine-rich proteins (Kumari et al., 1998) and are divided into several types (Coyle et al., 2002). These cysteine-rich proteins have redox and metal-binding capabilities (Kumari et al., 1998; Coyle et al., 2002) and have been implicated in protection against oxidative damage and heavy metal toxicity. An examination of the

expression levels of metallothionein genes using Affymetrix GeneChip array revealed that the type I and II isoforms of wheat metallothionein genes were extremely highly expressed in the stems and leaf sheaths of wheat (data not shown) and that considerable differences in the mRNA levels of a number of these genes were observed among the 8 SB lines. Correlation analysis showed that the mRNA levels of many of these metallothionein genes were positively associated with the grain weight and stem WSC concentration in these SB lines grown under water-limited rain-fed conditions. Three metallothionein genes representing type I, II and III are shown in Figure 1.

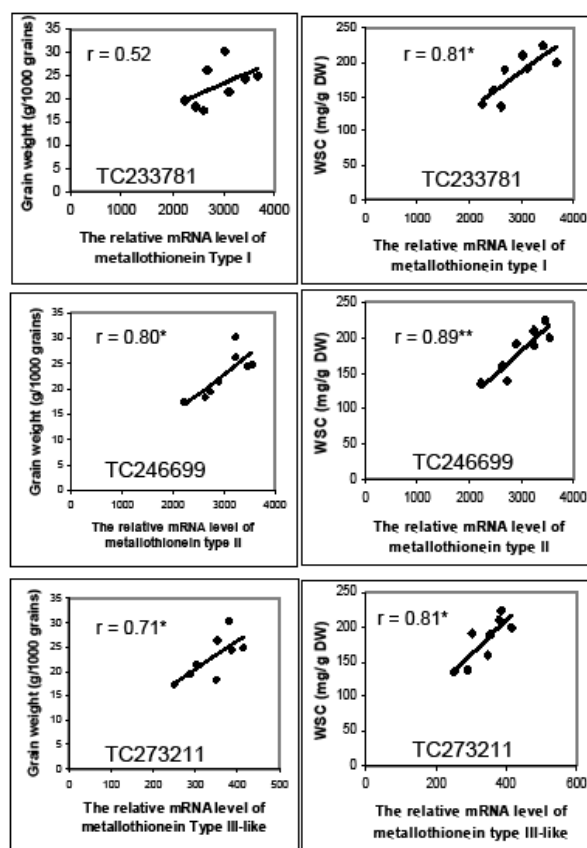


Figure 1. Positive association of the expression levels of metallothionein genes with grain weight and stem WSC concentration among 8 SB lines grown under rain-fed conditions. DW, dry weight. * $P < 0.05$; ** $P < 0.01$.

ASCORBATE PEROXIDASE GENES AND THEIR ASSOCIATION WITH GRAIN WEIGHT AND STEM WSC CONCENTRATIONS

Ascorbate is one of the major and most powerful redox buffers in plants. Ascorbate peroxidase catalyses the reduction of H_2O_2 to water by using ascorbate as an electron donor. This enzyme has several isoforms, present in cytoplasm, chloroplast, mitochondrion, etc. All isoforms of ascorbate peroxidase have been implicated to have a role in protecting plants from oxidative injury (Shigeoka et al., 2002; Davletova et al., 2005). Our Affymetrix data showed that the mRNA levels of a number of ascorbate peroxidase genes in the stems and leaf sheaths of the SB lines are positively associated with grain weight or stem WSC concentration. Three representative genes are shown in Figure 2.

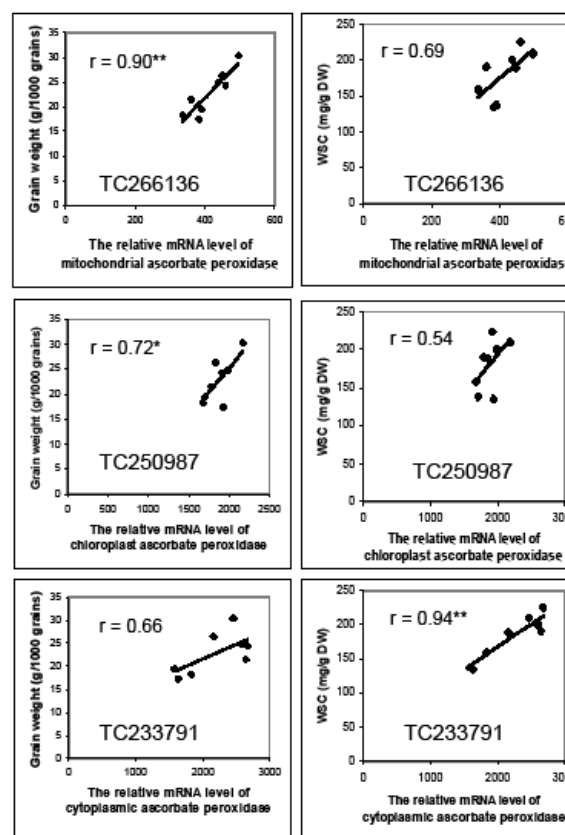


Figure 2. Positive association of the expression levels of ascorbate peroxidase genes with grain weight and stem WSC concentration among 8 SB lines grown under rain-fed conditions. * $P < 0.05$; ** $P < 0.01$.

THIOREDOXIN AND GLUTAREDOXIN GENES AND THEIR ASSOCIATION WITH GRAIN WEIGHT AND STEM WSC CONCENTRATION

Thioredoxins and glutaredoxins are $-(SH)_2$ redox site-containing proteins and are involved in antioxidative defense, including in regulating the activity of enzymes implicated in photosynthetic carbon assimilation (Meyer et al., 2007). As shown in Figure 3, the mRNA levels of two representative genes in wheat stems and leaf sheaths were positively associated with grain weight or stem WSC concentration among 8 SB lines.

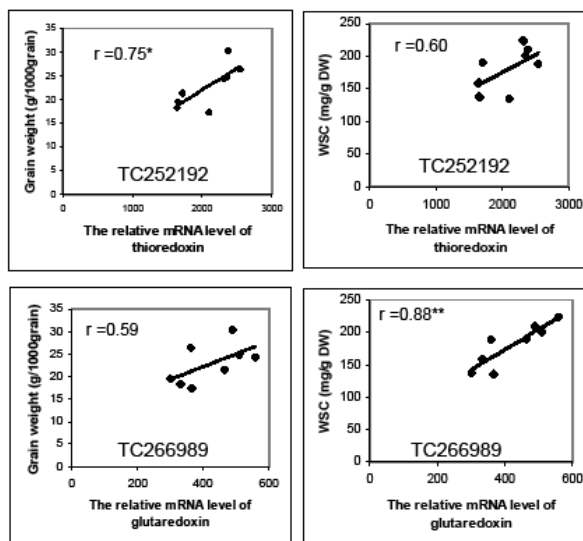


Figure 3. Positive association of the expression levels of thioredoxin and glutaredoxin genes with grain weight and stem WSC concentration among 8 SB lines grown under rain-fed conditions. * $P < 0.05$; ** $P < 0.01$.

These data imply that a higher expression level of antioxidative genes may potentially contribute to an improved carbon assimilation capacity in wheat plants grown in abiotic-stress-prone environments in northern Australia. However, further biochemical and physiological analyses and field trials with an extended group of SB lines are required to demonstrate relationship between the expression levels of antioxidative genes and the oxidative stress tolerance in wheat and their contribution to the improvement of carbon assimilation under abiotic stress conditions.

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REFERENCES

- Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Ann. Bot.* 91: 179-194.
- Coyle P, Philcox JC, Carey LC, Rofe AM (2002) Metallothionein: the multipurpose protein. *Cell. Mol. Life Sci.* 59: 627-647.
- Davletova S, Rizhsky L, Hongjian L, Zhong S, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. *Plant cell* 17: 268-281.
- Foyer C, Noctor G (2005). Oxidant, antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant cell Environ.* 28 :1056-1071.
- Kumari MV, Hiramatsu M, Ebadi M (1998) Free radical scavenging actions of metallothionein isoforms I and II. *Free Radical Res.* 29: 93-101.
- Meyer Y, Siala W, Bashandy T, Riondet C, Vignols F, Reichheld JP (2008) Glutaredoxins and thioredoxins in plants. *Biochim. Biophys. Acta* 1783: 589-600.
- Mitter R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7: 405-410.
- Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. *J. Exp. Bot.* 53: 1305-1319.
- Xue GP, McIntyre CL, Jenkins CLD, Glassop D, van Herwaarden AF, Shorter R (2008) Molecular dissection of variation in carbohydrate metabolism related to water soluble carbohydrate accumulation in stems of wheat (*Triticum aestivum* L.). *Plant Physiol.* 146:441-454.