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Research article

The role of thioredoxin h in protein metabolism during wheat (*Triticum aestivum L.*) seed germination

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ABSTRACT

Thioredoxin h can regulate the redox environment in the cell and play an important role in the germination of cereals. In the present study, the *thioredoxin s* antisense transgenic wheat with down-regulation of *thioredoxin h* was used to study the role of thioredoxin h in protein metabolism during germination of wheat seeds, and to explore the mechanism of the *thioredoxin s* antisense transgenic wheat seeds having high resistance to pre-harvest sprouting. The qRT-PCR results showed that the expression of protein disulfide isomerase in the *thioredoxin s* antisense transgenic wheat was up-regulated, which induced easily forming glutenin macropolymers and the resistance of storage proteins to degradation. The expression of serine protease inhibitor was also up-regulated in transgenic wheat, which might be responsible for the decreased activity of thiocalsin during the germination. The expression of WRKY6 in transgenic wheat was down-regulated, which was consistent with the decreased activity of glutamine oxoglutarate aminotransferase. In transgenic wheat, the activities of glutamate dehydrogenase, glutamic pyruvic transaminase and glutamic oxaloacetic transaminase were down-regulated, indicating that the metabolism of amino acid was lower than that in wild-type wheat during seed germination. A putative model for the role of thioredoxin h in protein metabolism during wheat seed germination was proposed and discussed.

1. Introduction

Thioredoxin h (trx h) is involved in the regulation of redox environment of the cell through reducing protein disulphide bonds. Lower trx h content indicates that there is lower reducing force in plant cells, which can decrease the reducing of protein disulphide bonds. Trx h was widely found in various plant species, including wheat seeds [1]. The biological functions of trx h had been well documented. Some researchers suggested that trx h plays an important role in the germination of cereals (e.g. wheat and barley) by reducing the intramolecular disulfide bonds of storage proteins and other proteins in the starchy endosperm. During seed germination, trx h can increase the susceptibility of storage proteins to proteolysis by breaking the intramolecular disulfide bonds, and can also change the activities of enzymes either directly by reduction or indirectly by counteracting the effect of the inhibitor proteins [2–9]. However, further work is needed to clarify the biological functions of trx h in wheat seed germination.

The *trx s* gene from *Phalaris coerulescens* and the *trx h* gene from *Triticum aestivum* belong to the thioredoxin gene family. The trx s from *Phalaris coerulescens* was highly conserved at the C terminus with significant homology to trx h from *T. aestivum* (Figure S1), and their expression products have similar biological functions [10–12]. In our previous work, transgenic wheat was formed after the antisense *trx* s was transferred into wheat with particle bombardment (Figure S2). In the *trx s* antisense transgenic wheat seeds, the expression of trx h was distinctly reduced compared with the wild-type seeds (Figure S3 and Table S1) [13,14], resulting in the low pre-harvest sprouting susceptibility of transgenic wheat seeds (Figure S4) [15]. Due to its lower germination ability, the *trx s* antisense transgenic wheat is a good material to study the biological functions of trx h in wheat seed germination.

Recently, we analyzed the differences in seed proteome between the *trx* s antisense transgenic wheat seeds and their wild type, and found 36 differentially expressed proteins, of which 11 differential proteins were involved in protein metabolism [16]. Especially, the expressions of serine protease inhibitor (Serpin) and

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protein disulfide isomerase (PDI) were up-regulated in the *trx s* antisense transgenic wheat seeds. Serpin is an inhibitor of serine protease [17], thus it may have an important effect on storage protein degradation during seed germination. PDI can catalyze the forming of disulfide bond and serve as molecular chaperones helping proteins fold, so it has a role in storage protein synthesis [18,19]. The other nine differential proteins were down-regulated in the *trx s* antisense transgenic wheat seeds. However, it is unclear how these differential expressed proteins participate in the regulation of transgenic wheat seed germination.

In the present study, to provide further evidence for the role of trx h in protein metabolism during wheat seed germination, several proteins or enzymes (glutenin polymer, thiocalsin, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), glutamate dehydrogenase (GDH) and glutamine oxoglutarate aminotransferase (GOGAT)) involved in seeds protein metabolism were analyzed. These results will be helpful to understand the mechanism of the *trx s* antisense transgenic wheat seeds having high resistance to pre-harvest sprouting.

2. Results and discussion

2.1. Transcript analysis of trx h, Serpin, WRKY6, PDI, GOT and GDH

The qRT-PCR analysis for six transcripts including *trx h* (GenBank Database ID: EU706448.1), *WRKY6* (GenBank Database ID: EU665435.1), *Serpin* (GenBank Database ID: Z49890.1), *PDI* (GenBank Database ID: HQ911363.1), *GOT* (GenBank Database ID: EU346759.1) and *GDH* (GenBank Database ID: HQ821868.1) are shown in Fig. 1. The transcript level of *trx h* was significantly lower in transgenic wheat than that in wild-type wheat, which was consistent with the lower expression level of trx h in the *trx s* antisense transgenic wheat seeds [13,14]. The transcript levels of *Serpin* and *PDI* were significantly higher (11.5 and 2.25 folds, respectively) in transgenic wheat than that in wild type wheat, which was consistent with our previous results on the elevated expression of *Serpin* and *PDI* (8.9 and 10.5 folds, respectively) in the *trx s* antisense transgenic wheat seeds [16]. The transcript levels of *WRKY6*, *GOT* and *GDH* were reduced for 3, 7.5 and 11.7 folds in transgenic wheat, which was consistent with our previous results on the decreased expression of *WRKY6*, *GOT* and *GDH* (9.4, 5.6 and 11 folds, respectively) [16]. The results indicated that trx h can regulate the expression of *WRKY6*, *Serpin*, *PDI*, *GOT* and *GDH* by transcriptional regulation during seed germination.

2.2. The effect of trx h on the activity of thiocalsin during wheat seed germination

Thiocalsin is a thioredoxin-linked serine protease and catalyzes the degradation of disulfide-rich seed storage proteins, only after which have been reduced during seed germination [3]. As shown in Fig. 2, the activity of thiocalsin gradually increased during wheat

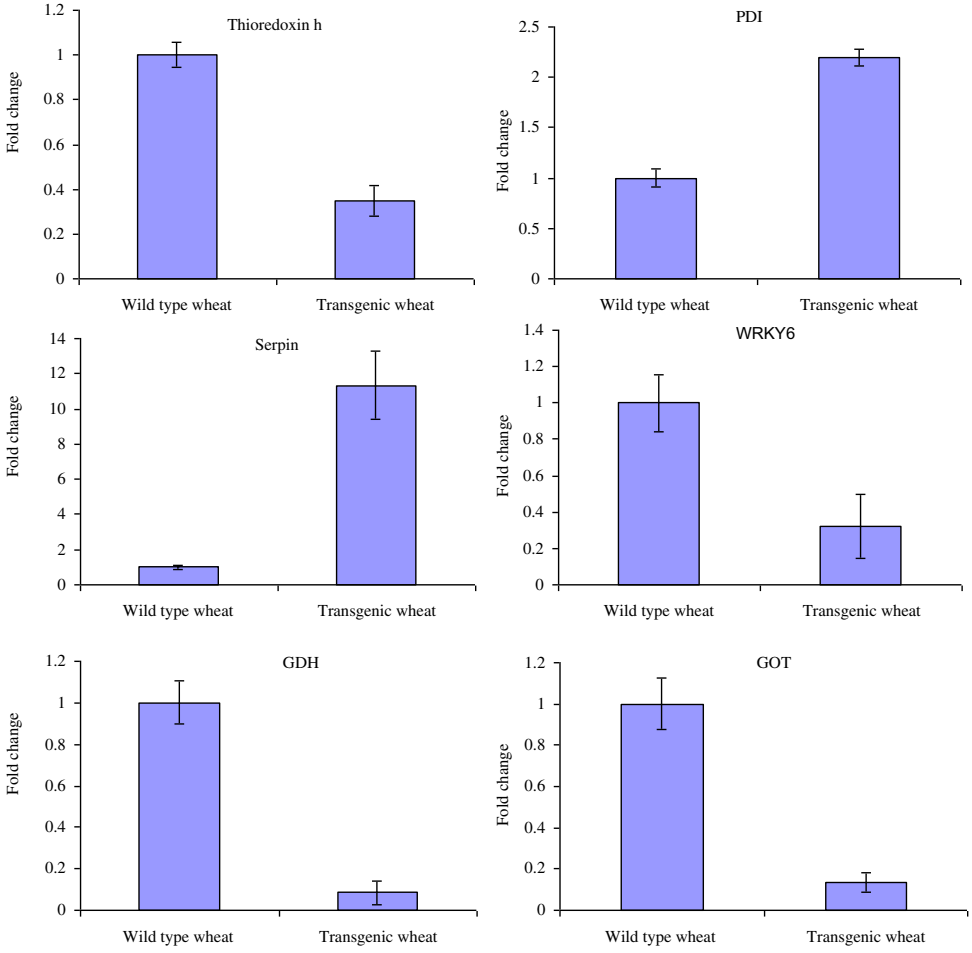

Fig. 1. qPCR analysis of *trx h*, *Serpin*, *WRKY6*, *GDH*, *GOT* and *PDI* transcripts at 2 d after seed germination. Fold change was determined using the $2^{-\triangle\triangle Ct}$ method and error bars represent the standard deviation of the mean (SD).