The Degradation of Starch in Transgenic Wheat Seeds with Antisense Trx s during Germination

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Abstract: Thioredoxin h (trx h) in plant seeds has an important role in seeds germination. The degradation of storage starch was retarded in transgenic wheat seeds with antisense trx. The aim of this study was to clarify the mechanism on the slow degradation of starch in transgenic wheat seeds during germination. The results show that the activities of α-amylase and β-amylase were markedly slowed in transgenic wheat seeds, and that the activity of amylopectase has no distinct change. XIP-I can interact with trx h, so it can be reduced into XIP-I precursor by trx h. It had been proved that the content of XIP-I in transgenic wheat seeds is more than that in control wheat seeds. Therefore, the interaction between XIP-I and trx h is an important reason for the lower activity of α-amylase in transgenic wheat seeds.

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1. Introduction

Preharvest sprouting (PHS) is one of the most important factors affecting the cereal production in the regions where have rainfall and high humidity during harvest season. Molecular breeding may be an effective method to reduce the serious worldwide problem. Thioredoxin h (trx h) in plant seeds can regulate of redox environment of the cell through catalyzing thiol-disulfide interchange. The thioredoxin s (trx s) gene from Phalaris coerulescens and the trx h gene from wheat are highly homologous, and they have similar biological functions. The expression of trx h in the transgenic wheat seeds with antisense trx s is lower than that in wild type (Ren et al, 2007), and that the transgenic wheat has lower PHS susceptibility (Zhou et al, 2006).

Some study had proved that the degradation of storage proteins and starch was retarded in transgenic wheat seeds, which is responsible for transgenic wheat seeds having high resistance to PHS (Guo et al, 2011). 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 protease activities either directly by reduction or indirectly by inactivating inhibitor proteins (Marx et al, 2003; Wong et al, 2003; Kobrehel et al, 1992). In transgenic wheat seeds, protein disulfide isomerase induced easily forming glutenin macropolymers and the resistance of storage proteins to degradation. Serine protease inhibitor might be responsible for the decreased activity of thiocalsin during the seeds germination. But some work is still necessary to clarify the slow degradation of starch in transgenic wheat seeds during germination.

2. Material and Methods

2.1 Plant material and treatment

After being sterilized, trx s antisense transgenic wheat and control wheat seeds (cv. Yumai 18) were allowed to germinate at room temperature on sterile filter papers soaked with water [12]. At 0 and 2 d, seeds were sampled, de-embryonated and used for further analysis.

2.2 The determination of starch content

The starch content was determined according to the method (1985).

2.3 The determination of activities of α-amylase, β-amylase and amylopectase

After seeds were ground in liquid nitrogen, α-amylase and β-amylase were extracted for 20 min in distilled water at a ratio of 1:100 (wt/vol). The extract was centrifuged for 10 min at 3,000g, and the supernatant fraction was used to determine the activities of α-amylase and β-amylase (Chen, 2002).

After seeds were ground in liquid nitrogen, amylopectase were extracted overnight in phosphate buffer (0.1 mol·L^{-1} pH 7.2) at a ratio of 1:4 (wt/vol). The extract was centrifuged for 15 min at 10,000g, and the supernatant fraction was used to determine the activity of amylopectase (Iwaki and Fuwa, 1981).

2.4 The interaction of Trx h and XIP-I

Cysteines Disulfide Bonding State and Connectivity Predictor was used for predicting the disulfide bonding state of XIP-I. BiFC method was used for analyzing the interaction of Trx h and XIP-I (Walter et al, 2004). Trxh (GI:24637237) and XIP-I (GI:284178233) were cloned from wheat seeds for

constructing the vector. A-nLuc/B-cLuc was used for positive control, and Trxh-nLuc/cLuc was used for negative control.

3. Results and discussion

3.1 The degradation of starch in transgenic wheat seeds during germination

Starch degradation is the main material metabolism during wheat seed germination and its metabolic products provide the necessary nutrients and energy for seed germination. As shown in Figure 1, starch degradation in transgenic wheat seeds is markedly slower than that in control wheat seeds. The necessary nutrients and energy were not timely provided for seed germination, therefore, the germination of transgenic wheat seeds was slower than control wheat seeds.

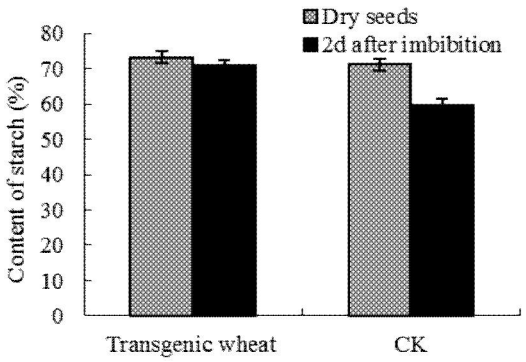
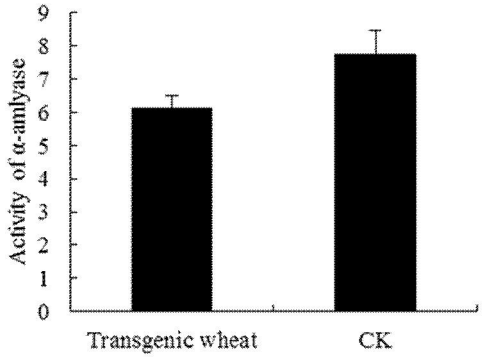


Figure 1. Change of starch content during wheat seeds germination.

3.2 The activity of amylase in transgenic wheat seeds



The three enzymes, α-amylase, β-amylase and amylopectase, are the key enzymes in starch degradation during seeds germination. As shown in Figure 2, the activities of α-amylase and β-amylase in transgenic wheat seeds were lower than that in control wheat seeds, and the activity of amylopectase has no distinct different between transgenic wheat and control wheat seeds during germination. So it is easy to understand the slow degradation of starch in transgenic wheat seeds.

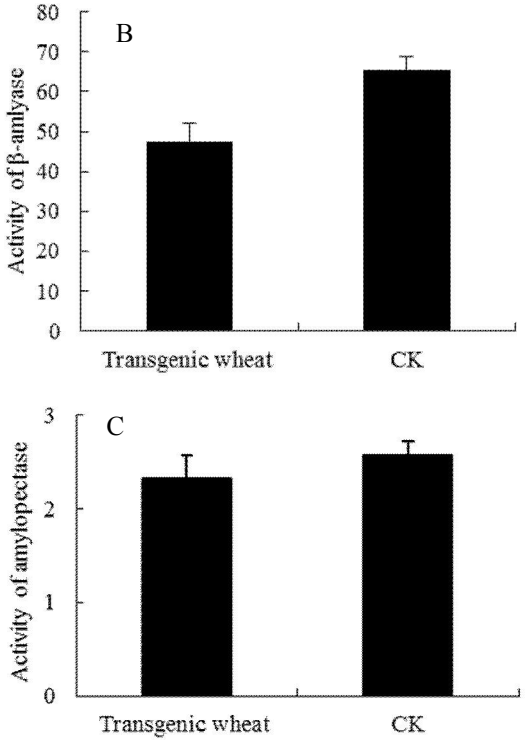


Figure 2 The activities of α-amylase, β-amylase and amylopectase. A, α-amylase, B, β-amylase, C, amylopectase

3.3 The interaction of Trx h and XIP-I

Figure 3. The prediction for disulfide bonding state of XIP-I.

Xylanase inhibitor protein I (XIP-I) is a bifunctional inhibiting protein which can inhibit both xylanase and amylase from barley (Payan et al, 2004). XIP-I contains two intermolecular disulfides, and

XIP-I precursor contains four cysteines (Figure 3). XIP-I precursor can be oxidized into XIP-I, and XIP-I can also be reduced into XIP-I precursor by some reducing substance, such as trx h. As shown in Figure 4, it was demonstrated that XIP-I can interact with trx h. There was a lower quantity of trx h in transgenic wheat, and the XIP-I precursor content in transgenic wheat is lower than control wheat (Guo et al, 2011). Therefore, XIP-I may play a role in decreasing the degradation of starch during transgenic seeds germination.

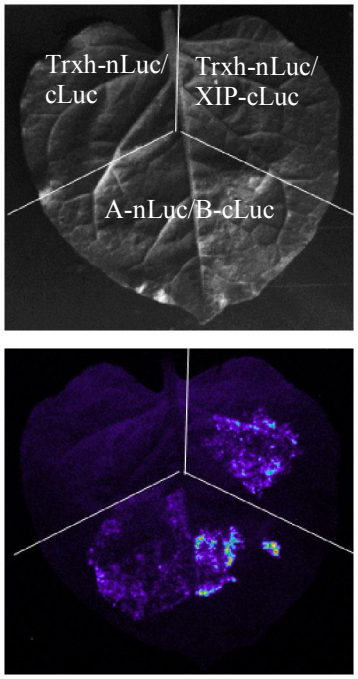


Figure 4. The interaction of Trx h and XIP-I.

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