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trxS 基因对大麦发芽籽粒中蛋白质降解的影响

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摘　要: 对转 *trxS* 基因大麦籽粒发芽过程中蛋白酶活性、不同蛋白组分含量和贮藏蛋白 SDS-PAGE 图谱的变化进行了研究。结果表明：与对照相比，转基因籽粒中的蛋白酶活性提高；清蛋白、球蛋白、醇溶蛋白和谷蛋白含量低于对照。SDS-PAGE 图谱也表明，转基因籽粒中贮藏蛋白降解快于对照。

关键词: *trxS* 基因, 蛋白质, 蛋白酶, SDS–PAGE

Effects of *trxS* gene on protein degradation in germinating barley seeds

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Abstract: We assessed the effects of *trxS* gene on changes of proteinase activity, contents of different protein fractions and SDS-PAGE profiles in germinating seeds of contrasting transgenic and nontransgenic barley variety. Proteinase activity was enhanced by 70.28% in transgenic than nontransgenic barley seeds, whereas contents of albumin, globulin, hordein and glutelin in transgenic seeds were 3.68%, 23.52%, 31.37%, and 21.04%, lower than those in nontransgenic seeds. Degradation rates of hordein and glutelin in transgenic seeds were faster than those in nontransgenic seedlings as indicated by the SDS-PAGE profiles. Our data imply that the transformation of *trxS* gene could promote the degradation of protein, providing theoretic basis for the use of *trxS* gene and barley quality breeding.

Keywords: *trxS* gene, proteniase, protein content, SDS-PAGE

Introduction

Protein is the major component which stored in the endosperm and constitutes about 8%–15% (*W*/*W*) of gain seed[1]. Originally, Osborne classified proteins into four groups according to soluble characteristics: albumin, globulin, hordein and glutelin. The degradation of protein is dependent on proteinase activity, accelerating the process of protein degradation will generate more amino acids, which will facilitate seed germination and early seedling development. Meanwhile, more soluble amino acids and α-ammonia nitrogen generated during seed germination will provide more available nitrogen sources for yeast growth and finally increase Kolbach Index in beer brewing.

Extra proteinase is usually used in domestic beer brewing industry in order to accelerate the degradation of proteins, but it may increase the cost and excessive proteinase may promote the formation of turbidity during beer storage process[2]. Biotechnology provides a new way to accelerate the degradation of protein by

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modifying seed germination characteristics so as to generate more proteinases[3]. It was reported that thioredoxin h could reduce the intramolecular or intermolecular disulfide bonds and increase the sulfhydryl (–SH) content of proteins, prompting protein solubility and hydrolyzation[4,5]. A putative *S* gene has been isolated from the pollen of the grass *Phalaris coerulescens* [6], a database search showed that *S* gene has high homology with thioredoxin h proteins at its conserved C terminus. This was functionally confirmed by demonstration of thioredoxin-like activity in the *Escherichia coli* expressed C terminal protein[7]. The *trxS* gene was transformed into barley by particle bombardment and its expression was confirmed by RT-PCR analysis [8], the thioredoxin h activity was also increased in germinating barley grains[9]. From the previous research, it is possible that increasing thioredoxin h activity may change the solubility and hydrolyzation of storage protein.

The present study was undertaken to illuminate the effects of *trxS* gene on proteinase activity, protein solubility and protein degradation in order to provide scientific basis for modifying barley quality with *trxS* gene.

1 Materials and Methods

1.1 Plant material

Dry mature seeds from transgenic and nontransgenic (as CK) lines of barley (*Hordeum vulgare cv.* Jinyin 6) were surface-sterilized with 70% aqueous ethanol for 5 min and then washed 3 times with sanitized water. The grains were incubated at 25°C for 6 days for germination, the germinated seeds were freezen in liquid nitrogen and then stored at −80°C.

1.2 Proteinase activity

Proteinase extraction and determination were carried out according to Ouyang *et al* [10]. 0.2g endosperms of transgenic and nontransgenic grains were ground to fine powder in liquid nitrogen and the powder was suspended in 3 mL extraction buffer (0.1 moL/L Tris·HCl, 0.01 moL/L $CaCl_2$, 1%的 PVP, pH 8.0). The suspension was ashaked at 4°C for 1 h and then centrifuged for 10 min at 14 000 r/min, 4°C. The supernatant was saved at −20°C as proteinase extracts.

Proteinase activity was assayed using BAPNA (N-benzoyl-DL-arginine-4-nitroanilide) as substrate. 0.2 mL of proteinase extracts was incubated with 3 mL reaction buffer containing substrate (0.1 moL/L Tris·HCl，0.01 moL/L $CaCl_2$，0.001 moL/L BAPNA, pH 8.0) with frequently shaking at 30°C for 1 h. Then, 0.5 mL of 30% acetic acid was added to stop the reaction. The reaction mixture was centrifuged for 15 min at 14 000 r/min and the supernatant was used to read the absorbance at 410 nm. The absorbance was used to weigh the proteinase activity. 0.1 mL of proteinase extract was used to read the absorbance at 595 nm to determinate protein content [11]. OD_{410}/OD_{595} was used to indicate proteinase activity in per unit protein. The result was identified by repeating for three-times (the following experiments were the same).

1.3 Protein content and SDS-PAGE analysis

The extraction of four protein fractions (albumin, globulin, hordein, glutelin) was according to Chen [12]. The protein content was determinated by the methods described by reference [11]. OD_{595} was used to indicate protein content. Hordein and glutelin were separated by SDS-PAGE according to Wang *et al* [13].

2 Results

2.1 Changes of proteinase activity

The changing trend of proteinase activity in transgenic and nontransgenic seeds was similar (Fig. 1). From 1 d to 5 d after seed germination, the proteinase activity increased with seed germination and reached the highest level on 5 day after seed germination, then reduced on day 6 after seed germination, the proteinase activity in transgenic seeds was higher than in nontransgenic seeds. On each time point, the proteinase activity in transgenic seeds was 2.29%, 21.28%, 29.30%, 59.91%, 130.40%, 178.49% higher than in nontransgenic seeds and reached significant level (P<0.05).

2.2 Changes of albumin, globulin, hordein and glutelin content

The degration of protein is mainly relayed on proteinase activity. The contents of four group protein were analyzed during seed germination (Table 1). Table 1 showed that the content of albumin was high from 1 d to 3 d after seed germination, then reduced from four day after seed germination, the content of albumin in transgenic seeds was lower than in nontransgenic seeds except on two day after seed germination. By t pair detection, the difference of albumin content between transgenic and non transgenic seed was not significant. The contents of

globulin in transgenic barley seeds were lower than in nontransgenic seeds from 1 d to 6 d after seed germination, the reducing rate was from −5.9% to −40%, and reached significant level (P<0.01); the hordein content in transgenic seed was higher than in nontransgenic seed on 1d after seed germination, but it was lower than in nontransgenic seed from 2 d to 6 d after seed germination, which indicated that the degradation rate of hordein in transgenic seed was faster than in nontrasgenic seed, the difference of hordein content between transgenic and nontransgenic seed was significant by t detection (P<0.05). The glutelin content in transgenic seeds was also lower than in nontransgenic seeds during seed germination process, the reducing rates was from −2.53% to −66.7%, and reached significant level (P<0.01), demonstrating that higher proteinase activity promoted the degradation of four protein fractions.

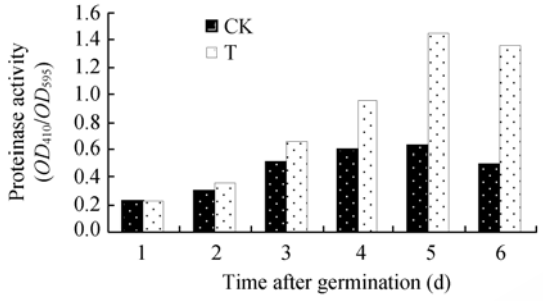

图 1 大麦发芽种子蛋白酶活性变化

Fig. 1 Changes of proteinase activity in germinating barley grains.

From the SDS-PAGE profiles of hordein and glutelin (Fig. 2), we knew that the differences of the protein bands of hordein between transgenic and nonreansgenic seed were not obviously significant from one day to two day after seed germination (Fig. 2, left). From three day after seed germination, the bands in transgenic seed were fewer and weaker than in nontransgenic seed, especially those bands between 31.0 kD and 66.2 kD. Similar changes were found in glutelin SDS-PAGE profile (Fig. 2, right).

3 Discussion

With the development of domestic beer industry, more and more malting barley have been used to produce beer. The beer quality is closely related to the quality of malting barley, so imropving barley quality is essential to beer industry. The hydrolysis of protein plays a key role in beer quality, because the characteristics such as the speed of wort filtration, Diastaic power and Sol-Nitrogen have close relationship with the hydrolysis of protein.

Genetic modfication provides a new way for barley breeding. During germinating of barley seed, the degradation of protein is related to the protease activity. In our experiments we found that the proteinase activity in transgenic seed was higher comparing to nontransgenic seed after seed germination, indicting that the transformation of *trxS* gene could modulate the proteinase activity in transgenic barley seeds. The reasons might be that there were proteinase inhibitors in mature grains which had several intramolecular disulfide bonds (S–S)[14] and their degradation after germination was mainly dependent on cysteine proteases[15] and the deoxidized sulfhydryl (–SH) was

表 1 大麦发芽种子中清蛋白、球蛋白、醇溶蛋白和谷蛋白含量变化

Table 1 Changes of albumin, globulin, hordein and glutelin content in germinating barley seeds

Days after germination (d)		Protein fractions							
		Albumin	± CK (%)	Globulin	± CK (%)	Hordein	± CK (%)	Glutelin	± CK (%)
1	T	0.138	−1.43	0.097	−5.825	0.160	3.896	0.270	−2.53
	CK	0.140		0.103		0.154		0.277	
2	T	0.159	3.247	0.061	−20.78	0.079	−8.140	0.234	−2.90
	CK	0.154		0.077		0.086		0.241	
3	T	0.155	−3.73	0.05	−26.47	0.051	−17.700	0.099	−10.00
	CK	0.161		0.068		0.062		0.110	
4	T	0.120	−4.76	0.039	−17.02	0.009	−43.800	0.040	−14.90
	CK	0.126		0.047		0.016		0.047	
5	T	0.080	−3.61	0.020	−31.03	0.006	−62.500	0.017	−29.20
	CK	0.083		0.029		0.016		0.024	
6	T	0.045	−11.80	0.009	−40.00	0.006	−60.000	0.008	−66.70
	CK	0.051		0.015		0.015		0.024	

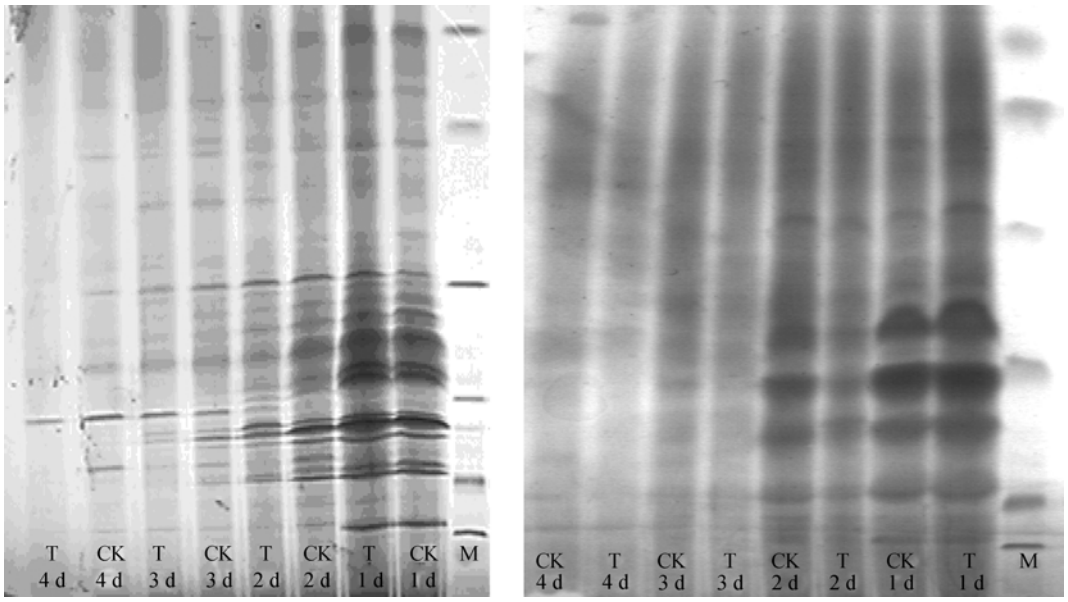

图 2 大麦发芽种子中醇溶蛋白和谷蛋白 SDS-PAGE 图谱

Fig. 2 SDS-PAGE separation of hordein (left) and glutelin (right) in transgenic and nontransgenic germinating barley seeds. Marker: 97.4 kD, 66.2 kD, 43.0 kD, 31.0 kD, 20.1 kD, 14.4 kD.

essential to cysteine protease activity. The expression of *trxS* gene increased the thioredoxin h activity and higher thioredoxin h activity facilitated remaining the deoxidized sulfhydryl (–SH) of cysteine proteases and deoxidizing disulfide bonds (S–S) of proteinase inhibitors to sulfhydryl (–SH), further released the inhibition to proteinase and then increased the proteinase activity. The enhancement of proteinase activity could facilitate the degradation of insoluble proteins, storage proteins and other proteins in grains.

Hordein and glutelin are main storage proteins in barley grains. According to amino acid compositions and electrophoretic mobility in SDS-PAGE, hordein protein can be separated into four groups and are named B hordein group (34–40 kD), C hordein group (44–74 kD), D hordein group (>100 kD) and three subsets of the γ hordein group (32–33 kD). The B hordein group and C hordein group are main hordein fractions and constitutes 65%–90% and 10%–20% of total hordein fraction (%), respectively[16]. Glutelin protein can be also separated into four groups and are named A group (80–120 kD), B group (42–51 kD), C group (30–40 kD) and D group (with a molecular weight between A group and B group) [17]. In our study, we found that the contents of hordein and glutelin in transgenic seeds were lower after germination and the degradation rates were also faster comparing to nontransgenic grains. The reasons might be that B hordein (constituting 65%–90% of total hordein fraction), D hordein and, γ hordein group were abundant in Cysteine except for C hordein group, which could become high molecular weight protein polymers by forming intramolecular or intermolecular disulfide bonds[16]. The glutelin were also be composed of polypeptide monomers by disulfide bonds[17]. The expression of *trxS* gene increased thioredoxin h activity, higher thioredoxin h activity could improve the reduction of disulfide bonds (S–S) to sulfhydryl (–SH) and depolymerized the polymers[18], then increased the protein solubility and accelerated the degradation of endosperm protein. The results would provide a new way for barley high quality breeding and be beneficial for beer malting industry.

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