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Transgenic barley with overexpressed *PTrx* increases aluminum resistance in roots during germination*

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Abstract: A transgenic barley line (LSY-11-1-1) with overexpressed *Phalaris coerulescens* thioredoxin gene (*PTrx*) was employed to measure the growth, protein oxidation, cell viability, and antioxidase activity in barley roots during germination on the presence of 2 mmol/L $AlCl_3$ on filter paper. The results show that (1) compared with the non-transgenic barley, LSY-11-1-1 had enhanced root growth, although both were seriously inhibited after $AlCl_3$ treatment; (2) the degree of protein oxidation and loss of cell viability in roots of LSY-11-1-1 were much less than those in roots of non-transgenic barley, as reflected by lower contents of protein carbonyl and Evans blue uptakes in LSY-11-1-1; (3) activities of catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR) in LSY-11-1-1 root tips were generally higher than those in non-transgenic barley root tips, although these antioxidase activities gave a rise to different degrees in both LSY-11-1-1 and non-transgenic barley under aluminum stress. These results indicate that overexpressing *PTrx* could efficiently protect barley roots from oxidative injury by increasing antioxidase activity, thereby quenching ROS caused by $AlCl_3$ during germination. These properties raise the possibility that transgenic barley with overexpressed *PTrx* may be used to reduce the aluminum toxicity in acid soils.

Key words: *Phalaris coerulescens* thioredoxin gene (*PTrx*), Aluminum, Transgenic barley, Oxidative stress
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1 Introduction

Numerous environmental stresses induce the formation of reactive oxygen species (ROS) in plant cells (Boscolo *et al*., 2003). Excessive metallic ions are a well-known example of environmental stress, and aluminum is a particularly important ion because it is involved in a number of physiological processes, such as increasing antioxidase activity (Cakmak and Horst, 1991; Yamamoto *et al*., 2001), and inducing the expression of various genes related to oxidative stress (Richards *et al*., 1998; Maron *et al*., 2008; Goodwin and Sutter, 2009). Corresponding with this, there is a complex scavenging system to cope with excessive production of ROS in plant cells, including low molecular weight compounds such as ascorbic acid and glutathione, and various antioxidases (Vieira Dos Santos and Rey, 2006) such as guaiacol peroxidase (POD), catalase (CAT), superoxide dismutase

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(SOD), glutathione peroxidase (GPX), and glutathione reductase (GR) (Mittler, 2002). The ROS scavenging system efficiently reduces ROS under normal conditions, but complete reduction might not occur in stress environments, and the result may be a state of oxidative stress causing oxidative damage of biomolecules, such as lipids, proteins, and DNA, or even causing cell death (Delisle *et al*., 2001; Pan *et al*., 2001; Boscolo *et al*., 2003; Shao *et al*., 2007a; 2007b; 2008; 2009).

Thioredoxin (Trx) is a family of small, 12 kDa proteins, and can be found in all plants. The active site (Trp-Cys-Gly(Pro)-Pro-Cys) of these proteins contains a redox-active disulfide group (Lemaire *et al*., 2003; Wong *et al*., 2004). The exact function of Trx is still largely unknown. There are more and more reports, however, on the participation of plant Trx in the response to oxidative stress (Rey *et al*., 2005; Vieira Dos Santos and Rey, 2006; Shao *et al*., 2007a; 2007b; 2008; 2009).

The development of molecular biology has provided new insights into plant Trx functions in the oxidative stress response. ROS induces the expression of plant *Trx* genes. The amount of *Arabidopsis Trxh5* expression, for example, increases remarkably under oxidative stress conditions (Laloi *et al*., 2004), and methyl viologen induces an increase in *Trxh* transcript abundance in rice seedlings (Tsukamoto *et al*., 2005; Vieira Dos Santos and Rey, 2006). As well, target proteins of plant Trx are involved in antioxidative mechanisms. One of the 2-Cys peroxiredoxins (Prxs) and B-type methionine sulfoxide reductase (MsrB), for example, have been identified as targets of CDSP32 (the chloroplastic drought-induced stress protein, composed of two Trx modules) (Rey *et al*., 2005). Several plant GPXs display peroxidase activity preferentially in the presence of Trx (Vieira Dos Santos and Rey, 2006). In addition, there are some Trx-regulated enzymes, such as CAT, SOD, and germin-like proteins (Balmer *et al*., 2004). Finally, Trx may protect plant from oxidative damage as antioxidants in vivo. The sensitivity and lipid peroxidation levels of transformed plants lacking CDSP32 are higher, for example, than those of wild-type plants upon photo-oxidative stress exposure (Broin and Rey, 2003).

These results indicate that plant Trx fulfils an important function in protecting the plant from oxidative damage, and raises the possibility that transgenic barley with overexpressed *PTrx* will improve tolerance of aluminum stress. The *PTrx* gene belongs to the thioredoxin family because it shares nearly 94% sequence identity with *Trxh*, and its expression product has the same bio-functions and active site as Trxh (Li *et al*., 1995). In the present study, we measured the change of the growth, protein oxidation, cell viability, and antioxidase activity in roots of germinating barley seeds treated with 2 mmol/L $AlCl_3$ on filter paper. The main aim was to determine whether the transgenic barley with overexpressed *PTrx* activates protective responses to aluminum stress through increasing antioxidase activity during germination.

2 Materials and methods

2.1 Materials

Non-transgenic barley variety (*Hordeum vulgare* L. cv. LSY) and its third generation transgenic lines (LSY-11-1-1) transferred with exogenous *PTrx* (isolated from *Phalaris coerulescens*, mRNA No. GB: AF159388) by particle bombardment, were used in this study.

2.2 Polymerase chain reaction (PCR) analysis

Genomic DNA was extracted from leaf tissue according to cetyltrimethylammonium bromide (CTAB) method described by Wang and Fang (1998). Specific primers were designed according to the coding sequence of *PTrx* gene. The sequences of the primers were P1 (5′-TTCTGTGCCAGCCATGCT TAT-3′) to target the endosperm-specific α-gliadin promoter region and P2 (5′-GTCCCAGTCCTCT TTGGTAGTTATG-3′) to target the *PTrx* sequence. PCR products with loading dye were separated on a 1.5% (w/v) agarose gel by electrophoresis and scanned using a gel scanner JEDA801E (Jieda Science and Technology, Jiangsu, China).

2.3 Reverse transcriptase PCR (RT-PCR) analysis and sulfhydryl content in seeds

Seed samples of transgenic and non-transgenic lines were prepared and total RNA was isolated from the samples using the RNeasy kit (Sangon, Shanghai, China) according to the manufacturer's instructions.

RT-PCR analysis was used to determine transcript levels both in transgenic and non-transgenic lines according to the method of Li *et al*. (2009). Specific primers P3 (5′-CTCCCGATCCCAGGGCCTTCA-3′) and P4 (5′-GGCGGAAAAGACACGGAAACTG-3′) for *PTrx* were used in the RT-PCR analysis. The reaction included an initial 3 min of denaturation at 94 °C, followed by 27 cycles of 30 s at 94 °C, 30 s at 59 °C, and 40 s at 72 °C, a final extension of 10 min at 72 °C. As a control, a 415-bp PCR fragment of the barley constitutively-expressed actin gene *Hvpro1* (AK248710) (Sato *et al*., 2009) was amplified from the above samples. The primers for *Hvpro1* were HvPr-1 (5′-CAGCATTGTAGGAAGGCCACG-3′) and HvPr-2 (5′-CCACCACTGAGAACAACATTACCG-3′). The PCR parameters for amplifying *Hvpro1* fragments were the same as those for *PTrx*. PCR products were separated and photographed as described in PCR analysis. Two bands, 415 and 285 bp, were detected for the *Hvpro1* and *PTrx* genes, respectively.

The sulfhydryl content was measured by the method of Ellman (1959). Flours (0.15 g) were homogenized in 10 ml of Tris-glycine buffer (0.08 mol/L, pH 8.0) containing 3 mmol/L ethylene diamine tetraacetic acid (EDTA), and then 4 ml denaturation reagent (8 mol/L urea) and 0.1 ml Ellman's reagent [Tris-glycine buffer containing 0.4% (w/v) 2,2′-dinitro-5,5′-dithiodibenzoate (DTNB)] were added in 1 ml samples. Thionitrobenzoate, released by sulfhydryl groups of proteins reacted with DTNB, was measured at 412 nm, and the results were expressed as μmol sulfhydryl/g dry weight (DW).

2.4 Germination conditions

Germination and growth conditions were conducted according to the method of Tamás *et al*. (2006). Seeds were surface sterilized with 12% H_2O_2 for 10 min and then rinsed three times with sterilized distilled water. After 4 h of incubation in 8.2 mmol/L $CaCl_2$ solution (pH 4.0, untreated) or in 6.2 mmol/L $CaCl_2$ containing 2 mmol/L $AlCl_3$ solution (pH 4.0, Al-treated) at 25 °C in darkness, the seeds were germinated between two layers of filter paper fully moistened with the same solutions, and the germinating seeds were transferred on the freshly moistened filter papers with appropriate solutions every 20 h. Root growth was measured by the length of the main roots.

2.5 Protein oxidation and cell viability

The degree of protein oxidation was measured by the reaction of carbonyls with 2,4-dinitrophenylhydrazine (DNPH), according to the method of Levine *et al*. (1990). Fresh root tips (0.2 g) were homogenized in 2 ml phosphate buffer solution (25 mmol/L, pH 7.0) containing 0.1 mmol/L EDTA and 1 mmol/L phenylmethanesulfonyl fluoride (PMSF), and then centrifuged at 15000×*g* for 15 min. After the supernatant reaction with DNPH, the carbonyl content was calculated by absorbance of aliphatic hydrazones [extinction coefficient: 22.1 L/(mmol·cm)] at 374 nm and expressed as nmol carbonyl/mg protein.

The cell viability loss was investigated though Evans blue staining method as described by Yamamoto *et al*. (2001) and Boscolo *et al*. (2003), with minor modifications. Fresh roots were washed three times with deionized water and stained with 0.25% (w/v) Evans blue aqueous solution for 15 min. After rinsing with deionized water for 30 min, 20 root tips (5 mm) were excised and the accumulated Evans blue was released by dipping the root sections in 3 ml of *N*,*N*-dimethylformamide reagent for 1 h at 37 °C, and then centrifuged at 3000×*g* for 5 min. The optical density (OD) of the supernatant was determined spectrophotometrically at 600 nm.

Soluble protein concentrations were measured using the method of Bradford (1976).

2.6 Enzyme activity

Enzyme activity was measured spectrophotometrically, and temperature was controlled to (25±0.5) °C.

CAT activity was determined as outlined by Cakmak and Horst (1991), and the activity was analyzed by measuring the degradation of H_2O_2, which was monitored at 240 nm. One unit of CAT decomposed 1 μmol/L of H_2O_2 [extinction coefficient: 39.4 L/(mmol·cm)] per minute. GR activity was assayed following the procedure of Schaedle and Bassham (1977), measuring the decrease of optical density at 340 nm, with the extinction coefficient of 6.22 L/(mmol·cm). One unit of activity is defined as the amount of enzyme that oxidizes 1 μmol of nicotinamide adenine dinucleotide phosphate (NADPH) per minute. GPX activity was determined following the method of Livingstone *et al*. (1992). The assay was

monitored at 340 nm and one unit of activity is defined as the amount of enzyme that oxidizes 1 μmol of NADPH [extinction coefficient: 6.22 L/(mmol·cm)] per minute. APX activity was measured according to Asada (1984) and the activity was recorded by the decrease of optical density at 290 nm, taking 2.8 L/(mmol·cm) as the extinction coefficient, and one unit of activity is defined as the amount of enzyme that oxidizes 1 μmol of ascorbate per minute.

2.7 Statistical analysis

Each experiment was repeated at least five times. Data were expressed as mean±standard error (SE). Variance analysis between different treatments was carried out with the SPSS 11.5 program using Duncan's multiple range test at $P<0.01$.

3 Results

3.1 PCR analysis

The presence of exogenous *PTrx* gene in transgenic barley was examined with PCR analysis. DNA from leaves of the transgenic barley lines (LSY-11-1-1) was amplified by PCR reaction using primers specific for exogenous *PTrx*. The transgenic plants exhibited the polymorphic band 810 bp in length, the same size as the positive control (plasmid), but non-transgenic plant DNA was negative (Fig. 1). The results showed that exogenous *PTrx* gene had been transferred in barley genome.

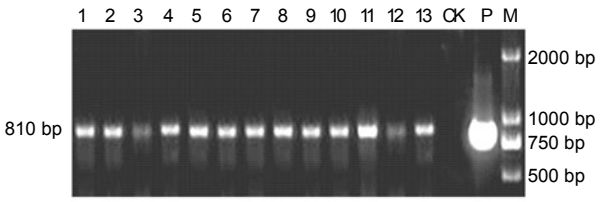

Fig. 1 PCR analysis of transgenic barley lines (LSY-11-1-1)
1–13: transgenic plants of LSY-11-1-1 (the next generation from T_5 in Figs. 2 and 3); CK: non-transgenic plants; P: positive control (plasmid); M: marker

3.2 RT-PCR analysis and sulfhydryl content

To investigate the expression of exogenous *PTrx* gene in LSY-11-1-1, the template cDNAs from transgenic and non-transgenic barley seeds 15 d after anthesis were assayed by RT-PCR and the sulfhydryl content in the mature seeds was measured. The specific band of 215 bp was exhibited in transgenic seeds, while non-transgenic seeds were negative (Fig. 2). These results indicate that exogenous *PTrx* gene can be transcribed and expressed normally in transgenic barley seeds.

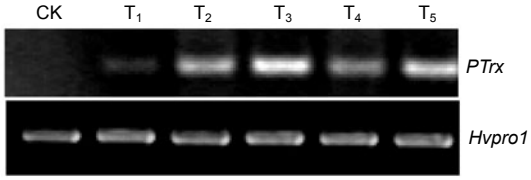

Fig. 2 RT-PCR analysis of *PTrx* expression in seeds of transgenic barley 15 d after anthesis
CK: non-transgenic plants; T_1–T_5: seeds of different transgenic lines of the third generation (T_5 is the plants of LSY-11-1-1)

The sulfhydryl content in seeds of transgenic barley was generally higher than that in non-transgenic (Fig. 3, $P<0.01$), and, in LSY-11-1-1, was 1.3 times that of non-transgenic barley.

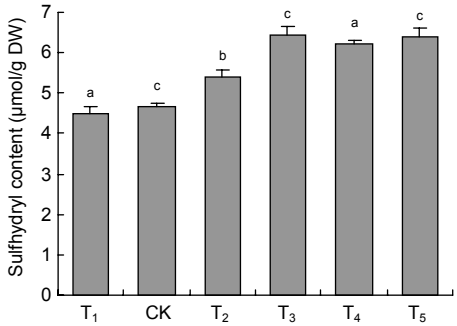

Fig. 3 Sulfhydryl content in seeds of transgenic and non-transgenic barley seeds
Data are expressed as mean±SE (n=5); Bars (i.e. means) with different letters are significantly different ($P<0.01$). CK: non-transgenic; T_1–T_5: seeds of different transgenic lines of the third generation (T_5 is the seeds of LSY-11-1-1)

3.3 Effect of Al-treatment on root growth

As seen in Fig. 4, the root growth of germinating barley seeds was obviously inhibited between 72 and 96 h after the onset of seed imbibition treated by 2 mmol/L $AlCl_3$. Although a continuous decrease of the root growth of both transgenic and non-transgenic germinating barley seeds was observed, the root length of LSY-11-1-1 was obviously longer than that of non-transgenic barley.

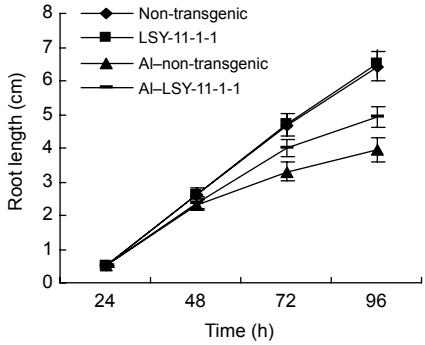

Fig. 4 Root length as a function of time of transgenic barley (LSY-11-1-1) and non-transgenic barley under Al^{3+} stress during germination
Data are expressed as mean±SE (n=5)

3.4 Protein oxidation and cell viability

Carbonyl group content in the transgenic and non-transgenic barley root tips were presented in Fig. 5a. The result showed that protein oxidation of LSY-11-1-1 was clearly lower than that of non-transgenic barley. The carbonyl content of LSY-11-1-1 was decreased, for example, by 37.0%, 20.8%, and 23.3% after 2 mmol/L $AlCl_3$ stress for 72, 84, and 96 h, respectively, compared with the results of non-transgenic barley.

The Evans blue uptakes in root tips were significantly increased after $AlCl_3$ treatment (Fig. 5b). The non-transgenic barley showed the highest value after 96 h treatment, and it was 2.9-fold higher than that of the untreated sample. Compared to the non-transgenic barley, the Evans blue uptakes in LSY-11-1-1 were significantly lower (P<0.01), only 66.0%, 68.5%, and 56.1% that of non-transgenic barley after 48, 72, and 96 h treatments, respectively.

3.5 Effect of Al-treatment on antioxidase activity

The changes in antioxidase activities are shown in Table 1. Our experimental results demonstrated that the activities of CAT, GPX, GR, and APX were significantly increased after aluminum stress, except that of GR at 48 h (P<0.01).

Compared to non-transgenic barley, the activities of these antioxidases were enhanced significantly in LSY-11-1-1 root tips (P<0.01). In the presence of 2 mmol/L Al^{3+} treatment, the CAT activity of LSY-11-1-1 was 11.8%, 18.9%, 32.6%, and 17.6% higher than that of non-transgenic barley at 60, 72, 84, and 96 h after treatment, respectively. *PTrx* overexpressing

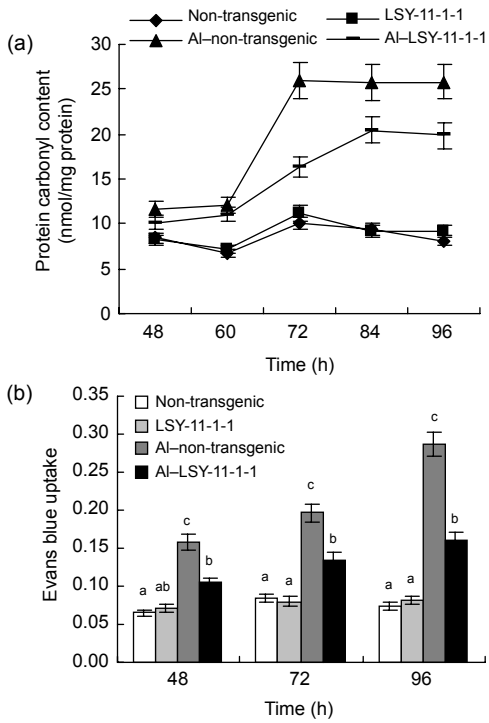

Fig. 5 Contents of protein carbonyls (a) and Evans blue uptakes (OD_{600}) (b) as a function of time in transgenic (LSY-11-1-1) and non-transgenic barley roots under Al^{3+} stress during germination
Data are expressed as mean±SE (n=5); Bars (i.e., means) with different letters are significantly different (P<0.01)

induced similar activity changes of GPX, the maximal value of GPX in LSY-11-1-1 was 96.7 U/mg protein at the activity peak, while it was 76.8 U/mg protein in non-transgenic barley. Compared with non-transgenic barley, the GPX activity of LSY-11-1-1 was significantly increased by 14.6%, 40.1%, and 30.9% after 72, 84, and 96 h treatments, respectively. APX activity of LSY-11-1-1 was 7.9%, 13.9%, 10.2%, and 11.3% greater than that of non-transgenic barley at 60, 72, 84, and 96 h after treatment, respectively. Although the GR activity was not significantly different between LSY-11-1-1 and non-transgenic barley from 48 to 72 h after Al^{3+} stress, it was significantly higher in LSY-11-1-1 at the other time sampled. The GR activity of LSY-11-1-1 was 1.2 and 1.1 times of that of non-transgenic barley at 84 and 96 h, respectively.

4 Discussion

We transferred exogenous *PTrx* gene into malting barley variety and obtained transgenic plants

Table 1 CAT, GPX, APX, and GR activities as a function of time in barley roots under Al^{3+} stress during germination

Time (h)	Group	CAT activity (U/mg protein)	GPX activity (U/mg protein)	APX activity (mU/mg protein)	GR activity (mU/mg protein)
48	Non-transgenic	25.65±0.66[a]	41.46±1.19[a]	398.77±3.38[a]	151.44±0.83[a]
	LSY-11-1-1	24.47±0.68[a]	39.93±0.81[a]	418.66±9.84[ab]	145.48±1.83[a]
	Al–non-transgenic	50.69±1.23[b]	68.14±1.39[b]	455.27±6.89[b]	148.62±1.38[a]
	Al–LSY-11-1-1	50.04±0.74[b]	70.63±0.65[b]	453.01±9.55[b]	150.58±1.92[a]
60	Non-transgenic	35.04±0.75[a]	39.99±1.32[a]	340.17±4.11[a]	130.69±1.72[a]
	LSY-11-1-1	38.96±0.93[a]	39.11±0.90[a]	321.33±2.40[a]	139.93±1.09[b]
	Al–non-transgenic	47.56±0.92[b]	76.77±1.91[b]	377.33±5.24[b]	203.44±1.99[c]
	Al–LSY-11-1-1	53.19±1.69[c]	77.85±1.01[b]	407.33±4.67[c]	209.59±2.11[c]
72	Non-transgenic	30.48±1.46[a]	36.88±1.38[a]	381.67±5.24[b]	134.60±2.56[a]
	LSY-11-1-1	32.59±1.80[a]	41.85±1.06[a]	344.00±3.21[a]	123.79±1.63[a]
	Al–non-transgenic	46.30±1.10[b]	56.96±1.48[b]	493.65±5.04[c]	198.72±1.29[b]
	Al–LSY-11-1-1	55.07±1.03[c]	65.26±0.63[c]	562.66±3.38[d]	208.18±2.39[b]
84	Non-transgenic	32.18±1.21[a]	44.00±1.74[a]	452.78±5.59[b]	161.14±1.71[a]
	LSY-11-1-1	31.38±1.14[a]	44.30±2.09[a]	402.66±6.84[a]	153.59±2.00[a]
	Al–non-transgenic	55.00±1.61[b]	68.99±0.71[b]	693.66±8.84[c]	239.11±1.74[b]
	Al–LSY-11-1-1	72.92±1.72[c]	96.66±2.17[c]	764.43±10.49[d]	275.01±2.08[c]
96	Non-transgenic	38.11±1.49[a]	46.29±0.99[a]	410.00±6.43[a]	136.63±0.38[a]
	LSY-11-1-1	34.40±1.41[a]	39.79±0.76[a]	441.63±4.94[a]	140.01±1.07[a]
	Al–non-transgenic	52.24±1.26[b]	70.87±1.15[c]	677.25±3.76[b]	234.40±2.41[b]
	Al–LSY-11-1-1	61.44±2.03[c]	92.78±1.45[d]	753.66±18.22[c]	269.41±1.23[c]

Data are expressed as mean±SE (n=5). Means followed by different letters are significantly different (P<0.01)

by the detection of PCR analysis. In order to confirm the expression of *PTrx*, the specific band of 215 bp in the transgenic line was lifted by RT-PCR, while the non-transgenic barley was negative. This revealed a positive result, indicating that exogenous *PTrx* gene had been successfully transferred and expressed in transgenic barley. The active site Trp-Cys-Gly(Pro)-Pro-Cys of Trx contains a highly reactive dithiol, which may reduce disulfide bridges of many proteins by thiol-disulfide interchange (Lemaire *et al*., 2003), so overexpressed *PTrx* will enhance the reducing ability in the transgenic barley seeds. Our experimental data demonstrate that the sulfhydryl content in the seeds of transgenic barley is higher than that in the non-transgenic barley, which also explains the *PTrx* expression.

Root growth of both transgenic barley and non-transgenic barley was inhibited seriously in the presence of Al^{3+}, consistent with the results reported by Kochian (1995) and Boscolo *et al*. (2003), but the transgenic barley showed a stronger root growth than non-transgenic barley on filter paper with $AlCl_3$ treatment during germination, as reflected by the greater root length. These results demonstrate that the phytotoxic effects of Al^{3+} on root growth are less severe in transgenic barley root growth than in non-transgenic barley root growth.

Oxidative stress is possibly an important component of the plant's reaction to aluminum toxicity (Pan *et al*., 2001). Several studies have shown that aluminum treatment causes excessive ROS formation (Cakmak and Horst, 1991; Boscolo *et al*., 2003). In our experiment, the increased activities of SOD, POD, CAT, APX, GPX, and GR induced by aluminum treatment suggest that Al^{3+} induces ROS excessive production.

The result of excessive ROS formation may cause biomolecule oxidation or even cell death (Cakmak and Horst, 1991; Delisle *et al*., 2001; Pan *et al*., 2001; Yamamoto *et al*., 2001). We investigated protein oxidation through a determination of the amount of carbonyl, an indicator that describes protein oxidation degree caused by ROS (Levine *et al*., 1990; Boscolo *et al*., 2003), and cell death through changes of Evans blue uptakes, a parameter of loss of cell viability, another parameter of oxidative stress damage in plant (Yamamoto *et al*., 2001; Boscolo *et al*., 2003). The lower protein carbonyl contents and

Evans blue uptakes in roots of LSY-11-1-1 indicate that excessive ROS, caused by $AlCl_3$ stress, was eliminated more efficiently in the transgenic barley roots than in the non-transgenic barley roots during germination, so the degree of the damage caused by $AlCl_3$ stress in transgenic plants was less than that in non-transgenic plants, which may be due to its effective antioxidase system.

In general, the major ROS-scavenging enzyme pathways of plants include SOD [Eq. (1)], CAT [Eq. (2)], the ascorbate-glutathion cycle [Eq. (3)], and GPX cycle [Eq. (4)] (Mittler, 2002).

$$O_2^- \xrightarrow{\text{SOD}} H_2O_2; \quad (1)$$

$$H_2O_2 \xrightarrow{\text{CAT}} H_2O + 1/2\,O_2; \quad (2)$$

$$\begin{aligned} &H_2O_2 + \text{ascrobate} \xrightarrow{\text{APX}} H_2O + \text{MDA}, \\ &\text{MDA} + \text{NAD(P)H} \xrightarrow{\text{MDAR}} \text{ascrobate} + \text{NAD(P)}^+, \\ &\text{Dehydroascrobate} + \text{GSH} \xrightarrow{\text{DHAR}} \text{ascrobate} + \text{GSSG}, \\ &\text{GSSG} + \text{NAD(P)H} \xrightarrow{\text{GR}} \text{GSH} + \text{NAD(P)}^+; \end{aligned} \quad (3)$$

$$\begin{aligned} &\text{GSSG} + \text{NAD(P)H} \xrightarrow{\text{GR}} \text{GSH} + \text{NAD(P)}^+, \\ &H_2O_2 + \text{GSH} \xrightarrow{\text{GPX}} H_2O + \text{GSSG}. \end{aligned} \quad (4)$$

In our experiment, $AlCl_3$ treatment induced a significant increase in SOD, POD, CAT, GPX, APX, and GR activities, although the activities of SOD and POD were similar changes in both LSY-11-1-1 and non-transgenic barley (data not shown). It is apparent that all the ROS-scavenging enzyme pathways of plants were indispensable for ROS detoxification in roots during barley seed germination in the presence of 2 mmol/L $AlCl_3$, but the CAT pathway, ascorbate glutathione cycle, and GPX cycle played a more important role on the progress of quenching ROS caused by Al^{3+} stress in transgenic barley, and the increased antioxidase activity contributed to a more effective reduction in the oxidative damage in roots during germination.

It is suggested that the redox state of proteins, including enzymes and regulatory components, is controlled by cellular redox agents, the paramount one among which is Trx (Wong *et al*., 2004). Several antioxidases, protecting plant cells from oxidative stress, are potential targets of Trxh, such as CAT, SOD (Cu-Zn), and GR (Jung *et al*., 2002), almost all of the which contain conserved cysteine, e.g., the numbers of conserved cysteine of CAT, GPX, POD, SOD (Cu-Zn), APX, 2-Cys Prxs, and 1-Cys Prxs are 5, 1, 8, 2, 2, 1, and 1, respectively (Wong *et al*., 2004). Trx might be involved in modulating redox-dependent signaling cascades, as shown by Schenk *et al*. (1994), or reduced Trx transfers its electrons to a target disulfide (Meyer *et al*., 1999; Wong *et al*., 2004), as a result providing reducing equivalents for regulating activity and increasing the antioxidase activity (Wong *et al*., 2004). Moreover, the thioredoxin gene and several genes encoding Cys-rich proteins were previously shown to be induced by aluminum stress (Maron *et al*., 2008; Goodwin and Sutter, 2009), suggesting that thioredoxins could play similar role as antioxidants.

Aluminum is the most abundant metal in the earth's crust, and aluminum toxicity is a very important factor limiting crop growth in acidic soil (Kochian, 1995). Thus, it is necessary to enhance the aluminum endurance of plants. Increased activities of antioxidases endow the transgenic barley with more effective elimination of excessive ROS induced by aluminum stress. This decreases the damage of biomolecules by oxidation and contributes to the integrity of membrane structure. In turn, aluminum is prevented from exerting its full physiological toxicity in the roots, as reflected by the fact that the roots of transgenic barley geminating seeds showed better growth than those of non-transgenic barley. It is possible that, in the future, *PTrx*-overexpressed barley can grow in aluminum-polluted environments without decreasing production because of the enhanced aluminum resistance.

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