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

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ABSTRACT

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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.

Keywords: *Phalaris coerulescens* thioredoxin gene (*PTrx*), Aluminum, Transgenic barley, Oxidative stress

1. INTRODUCTION

Go to:

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 (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

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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′-TTCTGTGCCAGCCATGCTTAT-3′) to target the endosperm-specific α-gliadin promoter region and P2 (5′-GTCCCAGTCCTCTTTGGTAGTTATG-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 15 000×*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

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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.

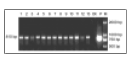

Fig. 1

PCR analysis of transgenic barley lines (LSY-11-1-1)

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.

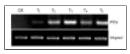

Fig. 2

RT-PCR analysis of *PTrx* expression in seeds of transgenic barley 15 d after anthesis

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.

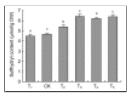

Fig. 3

Sulfhydryl content in seeds of transgenic and non-transgenic barley seeds

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.

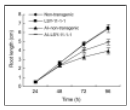

Fig. 4

Root length as a function of time of transgenic barley (LSY-11-1-1) and non-transgenic barley under Al^{3+} stress during germination

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.

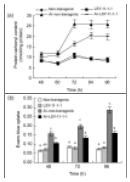

Fig. 5

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

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 ($P<0.01$).

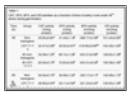

Table 1

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

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 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

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

$$O_2^- \xrightarrow{\text{SOD}} H_2O_2 \tag{1}$$

;

$$H_2O_2 \xrightarrow{\text{CAT}} H_2O + 1/2O_2 \tag{2}$$

;

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

;

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

.

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.

FOOTNOTES

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REFERENCES

Go to:

1. Asada K. Chloroplasts: formation of active oxygen and its scavenging. Methods Enzymol. 1984;105:422–429. doi: 10.1016/S0076-6879(84)05059-X. [Cross Ref]

2. Balmer Y, Vensel WH, Tanaka CK, Hurkman WJ, Gelhaye E, Rouhier N, Jacquot JP, Manieri W, Schürmann P, Droux M, et al. Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. PNAS. 2004;101(8):2642–2647. doi: 10.1073/pnas.0308583101. [PMC free article][PubMed] [Cross Ref]

3. Boscolo PRS, Menossi M, Jorge RA. Aluminum-induced oxidative stress in maize. Phytochemistry. 2003;62(2):181–189. doi: 10.1016/S0031-9422(02)00491-0.[PubMed] [Cross Ref]

4. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72(1-2):248–254. doi: 10.1016/0003-2697(76)90527-3.[PubMed] [Cross Ref]

5. Broin M, Rey P. Potato plants lacking the CDSP32 plastidic thioredoxin exhibit over-oxidation of the BAS1 2-Cys peroxiredoxin and increased lipid peroxidation in thylakoids under photooxidative stress. Plant Physiol. 2003;132(3):1335–1343. doi: 10.1104/pp.103.021626. [PMC free article][PubMed] [Cross Ref]

6. Cakmak I, Horst WJ. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxides activities in root tips of soybean (*Glycine max*) Physiol Plantarum. 1991;83(3):463–468. doi: 10.1034/j.1399-3054.1991.830320.x. [Cross Ref]

7. Delisle G, Champoux M, Houde M. Characterization of oxalate oxidase and cell death in Al-sensitive and tolerant wheat roots. Plant Cell Physiol. 2001;42(3):324–333. doi: 10.1093/pcp/pce041.[PubMed] [Cross Ref]

8. Ellman GD. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70–77. doi: 10.1016/0003-9861(59)90090-6.[PubMed] [Cross Ref]

9. Goodwin SB, Sutter TR. Microarray analysis of *Arabidopsis* genome response to aluminum stress. Biol Plantarum. 2009;53(1):85–99. doi: 10.1007/s10535-009-0012-4. [Cross Ref]

10. Jung BG, Lee KO, Lee SS, Chi YH, Jang HH, Kang SS, Lee K, Lim D, Yoon SH, Yun DJ, et al. A Chinese cabbage cDNA with high sequence identity to phospholipid hydroperoxide glutathione peroxidases encodes a novel isoform of thioredoxin-dependent peroxidase. J Biol Chem. 2002;277(15):12572–12578. doi: 10.1074/jbc.M110791200.[PubMed] [Cross Ref]

11. Kochian LV. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu Rev Plant Physiol Plant Mol Biol. 1995;46(1):237–260. doi: 10.1146/annurev.pp.46.060195.001321. [Cross Ref]

12. Laloi C, Mestres-Ortega D, Marco Y, Meyer Y, Reichheld JP. The *Arabidopsis* cytosolic thioredoxin h5 gene induction by oxidative stress and its W-box-mediated response to pathogen elicitor. Plant Physiol. 2004;134(3):1006–1016. doi: 10.1104/pp.103.035782. [PMC free article][PubMed] [Cross Ref]

13. Lemaire SD, Collin V, Keryer E, Issakidis-Bourguet E, Lavergne D. *Chlamydomonas reinhardtii*: a model organism for the study of the thioredoxin family. Plant Physiol Biochem. 2003;41(6-7):513–521. doi: 10.1016/S0981-9428(03)00079-2. [Cross Ref]

14. Levine RL, Garland D, Olive CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. 1990;186:464–478. doi: 10.1016/0076-6879(90)86141-H.[PubMed] [Cross Ref]

15. Li XM, Nield J, Hayman D, Langridge P. Thioredoxin activity in the C terminus of Phalaris S protein. Plant J. 1995;8(1):133–138. doi: 10.1046/j.1365-313X.1995.08010133.x.[PubMed] [Cross Ref]

16. Li YC, Ren JP, Cho MJ, Zhou SM, Kim YB, Guo HX, Wong JH, Niu HB, Kim HK, Morigasaki S, et al. The level of expression of thioredoxin is linked to fundamental properties and applications of wheat seeds. Mol Plant. 2009;2(3):430–441. doi: 10.1093/mp/ssp025.[PubMed] [Cross Ref]

17. Livingstone DR, Lips F, Garcia Martinez P, Pipe PK. Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis* . Mar Biol. 1992;112(2):265–276. doi: 10.1007/BF00702471. [Cross Ref]

18. Maron LG, Kirst M, Mao C, Milner MJ, Menossi M, Kochian LV. Transcriptional profiling of aluminum toxicity and tolerance responses in maize roots. New Phytol. 2008;179(1):116–128. doi: 10.1111/j.1469-8137.2008.02440.x.[PubMed] [Cross Ref]

19. Meyer Y, Verdoucq L, Vignols F. Plant thioredoxins and glutaredoxins: identity and putative roles. Trends Plant Sci. 1999;4(10):388–394. doi: 10.1016/S1360-1385(99)01475-2.[PubMed] [Cross Ref]

20. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7(9):405–410. doi: 10.1016/S1360-1385(02)02312-9. [PubMed] [Cross Ref]

21. Pan JW, Zhu MY, Chen H. Aluminum-induced cell death in root-tip cells of barley. Environ Exp Bot. 2001;46(1):71–79. doi: 10.1016/S0098-8472(01)00083-1.[PubMed] [Cross Ref]

22. Rey P, Cuiné S, Eymery F, Garin J, Court M, Jacquot JP, Rouhier N, Broin M. Analysis of the proteins targeted by CDSP32, a plastidic thioredoxin participating in oxidative stress responses. Plant J. 2005;41(1):31–42. doi: 10.1111/j.1365-313X.2004.02271.x.[PubMed] [Cross Ref]

23. Richards KD, Schott EJ, Sharma YK, Davis KR, Gardner RC. Aluminum induces oxidative stress genes in *Arabidopsis thaliana* . Plant Physiol. 1998;116(1):409–418. doi: 10.1104/pp.116.1.409. [PMC free article][PubMed] [Cross Ref]

24. Sato K, Shin-I T, Seki M, Shinozaki K, Yoshida H, Takeda K, Yamazaki Y, Conte M, Kohara Y. Development of 5006 full-length CDNAs in barley: a tool for accessing cereal genomics resources. DNA Res. 2009;16(2):81–89. doi: 10.1093/dnares/dsn034. [PMC free article][PubMed] [Cross Ref]

25. Schaedle M, Bassham JA. Chloroplast glutathione reductase. Plant Physiol. 1977;59(5):1011–1012. doi: 10.1104/pp.59.5.1011. [PMC free article][PubMed] [Cross Ref]

26. Schenk H, Klein M, Erdbrugger W, Droge W, Schulze-Osthoff K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-κB and AP-1. PNAS. 1994;9l(5):1672–1676. [PMC free article][PubMed]

27. Shao HB, Jiang SY, Li FM, Chu LY, Shao MA, Li F. Some advances in plant stress physiology and their implications in the systems biology era. Colloids Surf B Biointerfaces. 2007;54(1):33–36. doi: 10.1016/j.colsurfb.2006.05.011.[PubMed] [Cross Ref]

28. Shao HB, Chu LY, Lu ZH, Kang CM. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. Int J Biol Sci. 2007;4(1):8–14. [PMC free article][PubMed]

29. Shao HB, Chu LY, Shao MA. Calcium as a versatile plant signal transducer under soil water stress. Bioessays. 2008;30(7):634–641. doi: 10.1002/bies.20770.[PubMed] [Cross Ref]

30. Shao HB, Jaleel CA, Manivannan P, Panneerselvam R, Shao MA. Understanding water deficit stress-induced changes in the basic metabolism of higher plants–biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. Crit Rev Biotechnol. 2009;29(2):131–151. doi: 10.1080/07388550902869792.[PubMed] [Cross Ref]

31. Tamás L, Budíková S, Šimonovičová M, Huttova J, Siroka B, Mistrik I. Rapid and simple method for Al-toxicity analysis in emerging barley roots during germination. Biol Plantarum. 2006;50(1):87–93. doi: 10.1007/s10535-005-0079-5. [Cross Ref]

32. Tsukamoto S, Morita S, Hirano E, Yokoi H, Masumura T, Tanaka K. A novel *cis*-element that is responsive to oxidative stress regulates three antioxidant defense genes in rice. Plant Physiol. 2005;137(1):317–327. doi: 10.1104/pp.104.045658. [PMC free article][PubMed] [Cross Ref]

33. Vieira Dos Santos C, Rey P. Plant thioredoxins are key actors in the oxidative stress response. Trends Plant Sci. 2006;11(7):329–334. doi: 10.1016/j.tplants.2006.05.005.[PubMed] [Cross Ref]

34. Wang GL, Fang HJ. Principle and Technique of Plant Genetic Engineering. Beijing: Science Press; 1998. (in Chinese)

35. Wong JH, Cai N, Balmer Y, Tanaka CK, Vensel WH, Hurkman WJ, Buchanan BB. Thioredoxin targets of developing wheat seeds identified by complementary proteomic approaches. Phytochemistry. 2004;65(11):1629–1640. doi: 10.1016/j.phytochem.2004.05.010.[PubMed] [Cross Ref]

36. Yamamoto Y, Kobayashi Y, Matsumoto H. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. Plant Physiol. 2001;125(1):199–208. doi: 10.1104/pp.125.1.199. [PMC free article][PubMed] [Cross Ref]

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