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Activities of Sulfhydryl-Related and Phenylpropanoid-Synthesizing Enzymes during the Germination of *Arabidopsis thaliana* Seeds

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Seeds of *Arabidopsis thaliana* were sowed in a Murashige and Skoog's medium and incubated in a tissue culture chamber at 26.2°C. They appeared to germinate at 60-72 hours after sowing. We measured the time-course activities of sulfhydryl-related enzymes such as thioredoxin, thioltransferase (glutaredoxin), thioredoxin reductase, and glutathione reductase. The activities of two enzymes, phenylalanine ammonia-lyase and tyrosine ammonia-lyase, which are involved in phenylpropanoid synthesis, were also measured and compared. Phenylalanine ammonia-lyase activity increased from 48 hours after sowing, whereas tyrosine ammonia-lyase activity increased transiently at the early stage and decreased slightly afterwards. Thioredoxin activity gradually decreased during the germination process. However, thioltransferase activity increased drastically from 60 hours after sowing. Thioredoxin reductase and glutathione reducatase increased rapidly from 36 hours after sowing. Thioredoxin activity was found to be relatively high in the dormant seeds.

Keywords: *Arabidopsis thaliana*, germination, glutathione reductase, phenyalanine ammonia-lyase, thioltransferase, thioredoxin

INTRODUCTION

Seed germination is an important development switch in which quiescent seed cells initiate oxidative phosphorylation for further development and differentiation. Enzyme activities in plants undergo rapid changes during germination. Several nutritive factors such as vitamin concentrations and bioavailability of trace elements and minerals increase (Lintschinger *et al.*, 1997). The time course changes of amino acids and protein during seed germination in *Terminalia arjuna* showed initial decrease in protein followed by increase at subsequent stages (Srivastava *et al.*, 1997). The seeds contained a high level of serine and glutamic acid, which later decreased as the germination progressed. Variations in the activities of various enzymes such as phosphoenolpyruvate carboxylase (Gonzalez *et al.*, 1998), lipoxygenase (Beaudoin and Rothstein, 1997), catalase (Guan and Scandalios, 1995), glutamine synthetase (Watanabe *et al.*, 1994), phytase (Laboure *et al.*, 1993), carboxypeptidase (Washio and Ishikawa, 1992), alpha-amylase (Karrer *et al.*, 1991), and malate synthase (Graham *et al.*, 1990) were demonstrated during seed germination.

Sulfhydryl biochemistry plays a remarkably broad and important role in cells, because the redox status of cysteine sulfhydryl groups dictates the native structure and/or activity of many enzymes, receptors, transcription factors, and transport proteins (Starke *et al.*, 1997). Two well-characterized systems involved in thiol/disulfide interchange exist in the cytosolic fraction of various cells. Thioltransferase (glutaredoxin) recieves reducing equivalents from glutathione (Nikkola *et al.*, 1991), which, in turn, is reduced by NADPH and glutathione reductase. Thioredoxin is directly reduced by NADPH via thioredoxin reductase (Holmgren, 1979). Thioltransferase and thioredoxin were reported to serve as regenerative systems for oxidatively damaged proteins *in vivo* as well as *in vitro*, but they contained different substrate specificities (Yoshitake *et al.*, 1994). More recently, thioredoxin was found to perform a range of regulatory functions from the germination of seeds to the division and development of animal cells (Buchanan *et al.*, 1994). Thioltransferase has been

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isolated and characterized from several plant cells in spinach leaves (Morell *et al.*, 1995), rice (Sha *et al.*, 1997), kale (Sa *et al.*, 1998), *Arabidopsis thaliana* seeds (Cho *et al.*, 1998), and the Chinese cabbage (Cho *et al.*, 1998). Thioltransferase purified from *A. thaliana* seeds appear to have an atypical molecular size (22 kDa). Five different clones encoding thioredoxin h were isolated from *A. thaliana* cDNA libraries (Rivera-Madrid *et al.*, 1995).

Phenylalanine ammonia-lyase and tyrosine ammonia-lyase are involved in phenylpropanoid metabolism. Phenylalanine ammonia-lyase, usually identified as a tetrameric enzyme, catalyzes the conversion of L-phenylalanine to *trans*-cinnamic acid in the first step of the phenylpropanoid pathway, which supplies the precursors for flavonoid pigments, lignins, stilbenes, some alkaloids, and coumarins (Hahlbrock and Scheel, 1989). It also plays an important role in plant development and pathogen defense (Dixon and Lamb, 1990). It is induced by various stress-related stimuli including wounding, heavy-metal, light, phytotoxin, and phytochrome (Brödenfeldt and Mohr, 1988; Dubery and Smit, 1994; Smith *et al.*, 1994).

During germination process of plant seeds, many kinds of proteins should be synthesized in a consecutive manner. Therefore, sulfhydryl-related enzyme activities might be required during the germination process. Phenylalanine ammonia-lyase is known to be involved in the development of plants. However, the activities of sulfhydryl-related and phenylpropanoid-synthesizing enzymes have not been determined during the germination process of plants. In this article, experiments were carried out to explore the possible involvement of sulfhydryl-related enzymes and phenylpropanoid-synthesizing enzymes in the seed germination of *A. thaliana.*

MATERIALS AND METHODS

Chemicals

Bovine serum albumin (BSA), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase (yeast), NADPH, Tris, 5,5'-dithio-2-nitrobenzoic acid (DTNB), acrylamide, N,N'-methylenebisacrylamide, N,N,N',N',-tetramethylethylenediamine (TEMED), ammonium persulfate, and EDTA were obtained from Sigma Chemical Co. (St. Louis, USA). L-Phenylalanine, L-tyrosine, oxaloacetate, and Coomassie Brilliant Blue R-250 were also from Sigma Chemical Co. (St. Louis, USA). 2-Hydroxyethyl disulfide was purchased from Aldrich Chemical Co. (Milwaukee, USA). *Escherichia coli* thioredoxin reductase was kindly provided by Prof. James A. Fuchs, University of Minnesota, St. Paul, Minnesota, USA. All other chemicals and reagents used were of the highest grade commercially available.

Seed Germination

Seeds of *A. thaliana* ecotype Columbia were kindly provided by Prof. Hong-Gil Nam, Pohang University of Science and Technology, Pohang, Korea. The seeds were cultivated in soil, a 1:1:1 mixture of vermiculite, perlite, and peat moss, and a large quantity of seeds was obtained at a later stage of growth. The seeds of *A. thaliana* were sowed in a Murashige and Skoog's medium, and incubated in a tissue culture chamber at 26.2°C. During the germination, time-course withdrawal of the germinating seeds was performed.

Preparation of Seed Extract

The germinating seeds (0.05 g) of *A. thaliana* were ground up with buffer A [20 mM Tris-HCl, 2 mM EDTA, pH 8.7] containing sea sand (about 1 g) in a mortar. The mixture was then clarified by centrifugation (10,000 g, 20 min) at 4°C, and the supernatant was saved for enzyme assays.

Enzyme Assays

Thioltransferase activity. Thioltransferase catalyzes the reduction of certain disulfides in the presence of glutathione and thus has GSH-disulfide-transhydrogenase (Nagai and Black, 1968). In the present study, 2-hydroxyethyl disulfide was used as a substrate. Two quartz semimicro cuvettes with 1 cm light path contained 500 μl of mixture at room temperature. To both cuvettes were added 50 μl of 15 mM 2-hydroxyethyl disulfide, 100 μg/ml bovine serum albumin, 1 mM GSH, 6 μg/ml yeast glutathione reductase, 0.4 mM NADPH, and 0.1 M Tris-HCl (pH 8.0)-2 mM EDTA. The absorbance at 340 nm was recorded for 2 min to ensure that both cuvettes were balanced with respect to the nonenzymatic spontaneous reaction between glutathione and 2-hydroxyethyl disulfide. A leaf extract was added to the sample cuvette, and an equal volume of buffer A was added to the reference cuvette. The decrease in absorbance was then recorded for a few minutes. The result was calculated as ΔA_{340}/min.

Phenylalanine ammonia-lyase activity. Phenylalanine ammonia-lyase activity was measured by a

modification of the spectrophotometric assay described previously (Lim *et al.*, 1998). The reaction mixture contained 50 mM Tris-HCl (pH 9.0), 2 mM L-phenylalanine and leaf extract in a total volume of 1.5 mL. The assay was carried out at 30°C, and the reaction was stopped by the addition of 1 mL 2 N HCl. The *t*-cinnamic acid formed was extracted into 2 mL of toluene by vortexing for 10 seconds and centrifuging at 1,500 g for 10 min. The absorbance at 290 nm of *t*-cinnamic acid recovered in the toluene phase was measured using toluene as a blank.

Glutathione reductase activity. The oxidation of NADPH was followed spectrophotometrically at 340 nm (Carlberg and Mannervik, 1985). The reaction mixture contained 0.1 M phosphate buffer (pH 7.0) - 1 mM EDTA, 0.1 mM NADPH, and 1 mM GSSG in a total volume of 1 mL. The reaction was initiated by the addition of the leaf extract to the cuvette, and the decrease in absorbance at 340 nm followed. The glutathione reductase activity was expressed as ΔA_{340}/min.

Thioredoxin activity. Thioredoxin catalyzes NADPH-dependent reduction of the disulfide bond in DTNB (Luthman and Holmgren, 1982). The assay mixture contained 100 mM Tris-HCl (pH 8.0), 2 mM DTNB, and 0.24 mM NADPH in a volume of 1.0 mL. Leaf extract was added into the sample cuvette, whereas buffer A was added into the reference cuvette. The reaction was initiated by adding thioredoxin reductase. An increase in absorbance at 412 nm was directly monitored using spectrophotometer. Thioredoxin activity was expressed as ΔA_{412} / min.

Tyrosine ammonia-lyase activity. The deamination of tyrosine by tyrosine ammonia-lyase was monitored as described previously (Abell and Shen, 1987). For tyrosine ammonia-lyase activity, 2.5 mL of a saturated solution of 12 mM L-tyrosine in 0.1 M Tris-HCl (pH 8.5) buffer was added as a substrate to leaf extract. The blank did not contain L-tyrosine. Increase in absorbance at 333 nm was recorded spectrophotometrically after incubation. The activity was expressed as ΔA_{333}/min.

Malate dehydrogenase activity. $NADP^+$-malate dehydrogenase activity was measured as described previously (Jacquot *et al.*, 1995). The reaction cuvette contained 0.1 M Tris-HCl buffer (pH 8.0), 0.75 mM oxaloacetate, and 0.15 mM NADPH in a volume of 1 mL. The reaction was initiated by the addition of a leaf extract. It was followed spectrophotometrically by the decrease of absorbance at 340 nm. The activity was expressed as ΔA_{340} / min.

Thioredoxin reductase activity. The activity of thioredoxin reductase was assayed in the presence of thioredoxin as a substrate (Lim and Lim, 1995). The reaction mixture contained 0.1 M Tris-HCl (pH 8.0), 0.5 mM DTNB, 0.24 mM NADPH, 0.1 mg/mL BSA, *E. coli* thioredoxin, and thioredoxin reductase. The reaction was started by adding thioredoxin reductase, and the absorbance increase at 412 nm was recorded.

Protein Determination

The protein content in the seed extract was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

The germination of plants is directly related with several metabolic pathways, which are governed by various enzymes. In some cases of enzymes, their contents are regulated at a gene level or modulated. Whether enzyme activities are modulated and their syntheses are regulated, it is important to understand the variations in enzyme activities during germination. This article describes the time-course variation in sulfhydryl-related enzymes and phenylpropanoid-synthesizing enzymes during the seed germination of *A. thaliana*, a well-known model plant, which would be very important for further approach. In our experimental conditions, it appeared that they germinated at 60-72 hrs after sowing.

Enzymes Involved in the Phenylpropanoid Pathway

During normal plant development, internal and external factors influencing cellular differentiation cause cell groups to become distinct tissue types and organs. During differentiation, specific branches of the phenylpropanoid pathway are initiated and activated according to environmental and hormonal stimuli (Liang *et al.*, 1989). The two enzymes which head phenylpropanoid metabolism, phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), are highly regulated by events taking place during various plant development stages (Wiermann, 1981). PAL and TAL convert L-phenylalanine and L-tyrosine to *t*-cinnamic acid and *p*-coumaric acid, respectively. There which are further modified in

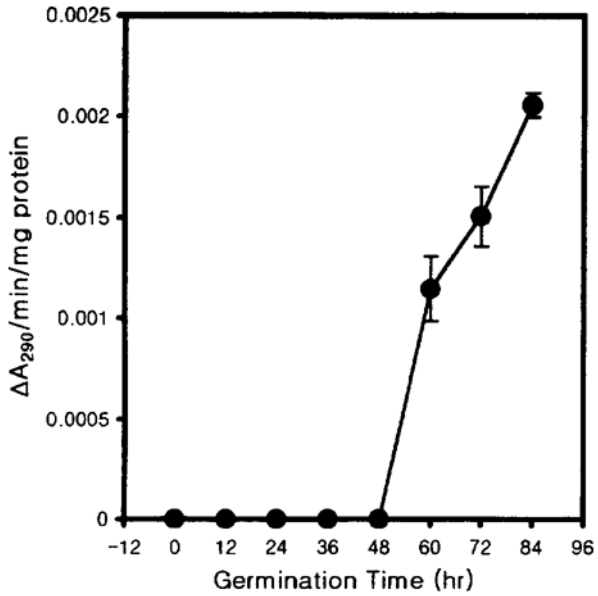

Fig. 1. Time-course variation in specific activities of phenylalanine ammonia-lyase during the seed germination of *A. thaliana*. Activity of phenylalanine ammonia-lyase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{290}/min/mg protein.

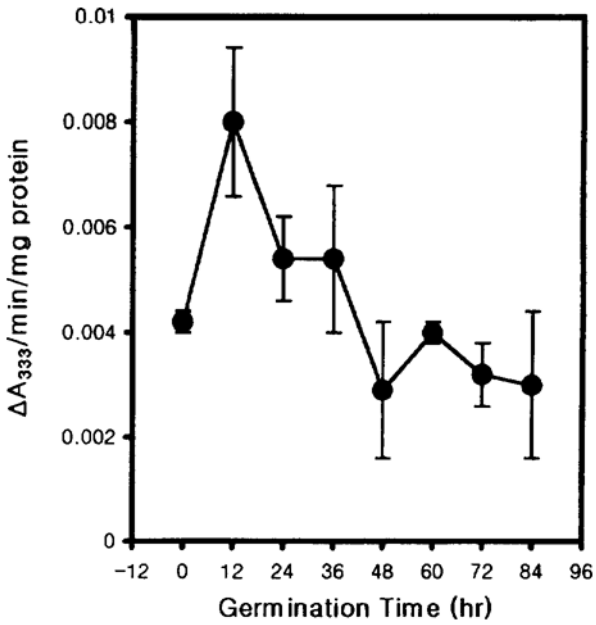

Fig. 2. Time-course variation in specific activities of tyrosine ammonia-lyase during the seed germination of *A. thaliana*. Activity of tyrosine ammonia-lyase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{333}/min/mg protein.

phenylpropanoid metabolism to precursors used in secondary pathways producing lignin, flavonoids, anthocyanins, phytoalexins, and tannins.

Expression of an *Arabidopsis* cDNA clone encoding 4-coumarate:CoA ligase, a key enzyme of phenylpropanoid metabolism, was activated early during seedling development (Lee *et al.*, 1995). The onset of 4-coumarate:CoA ligase expression was correlated with the onset of lignin deposition in cotyledons and roots 2-3 days after germination. Time-course PAL activity during the seed germination of *A. thaliana* is shown in Fig. 1. No PAL activity was observed early during germination. Its activity appeared 48 hrs after sowing, and then gradually increased afterward. Previously, in developing *Arabidopsis* seedlings, flavonoid genes such as PAL were demonstrated to be transiently expressed during germination in a light-dependent manner, with maximal mRNA levels occurring in 3-day-old seedlings (Kubasek *et al.*, 1998). This previous result corresponded with our time-course PAL activity changes during germination. Variation in TAL activity during the germination is shown in Fig. 2. Its activity was contained in the dormant seeds on a relatively high level, and it increased early during germination. After the transient increase it decreased slightly later during the germination. There results indicate that PAL and TAL are regulated in a different manner during the germination process. Genomic clones for three *A. thaliana* PAL genes containing the entire protein-coding region and upstream and downstream sequences have been obtained and completely sequenced (Wanner *et al.*, 1995).

Sulfhydryl-Related Enzyme Activities

In the chloroplasts of plant cells, the activities of several enzymes of the Calvin cycle are regulated by light (Pradel *et al.*, 1981). This control is effected through light-induced modifications of the levels of substrates and cofactors (Leegood and Walker, 1980). In addition to this type of control, activation of chloroplast enzymes by light also involves the reduction of enzyme disulfide bridges, in a process that is thought to be mediated by thioredoxins (Buchanan, 1980). In this system, the disulfide bridge of thioredoxin is reduced by a specific ferredoxin-thioredoxin reductase in the presence of photoreduced ferredoxin (Droux *et al.*, 1987). Time-course variation in thioredoxin activity during the seed germination of *A. thaliana* is shown in Fig. 3. It appeared that the thioredoxin activity decreased during the germination. Previously, it was also found that thioredoxin activity gradually decreased as the *Arabidopsis* leaves were getting older (data not

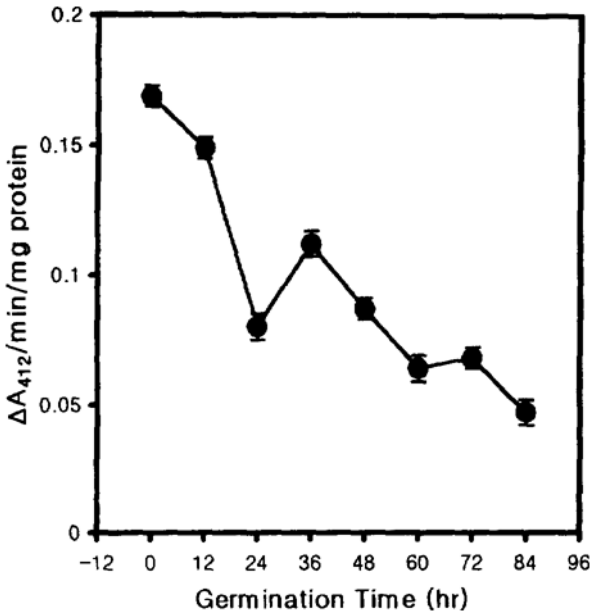

Fig. 3. Time-course variation in specific activities of thioredoxin during the seed germination of *A. thaliana*. Activity of thioredoxin was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{412}/min/mg protein.

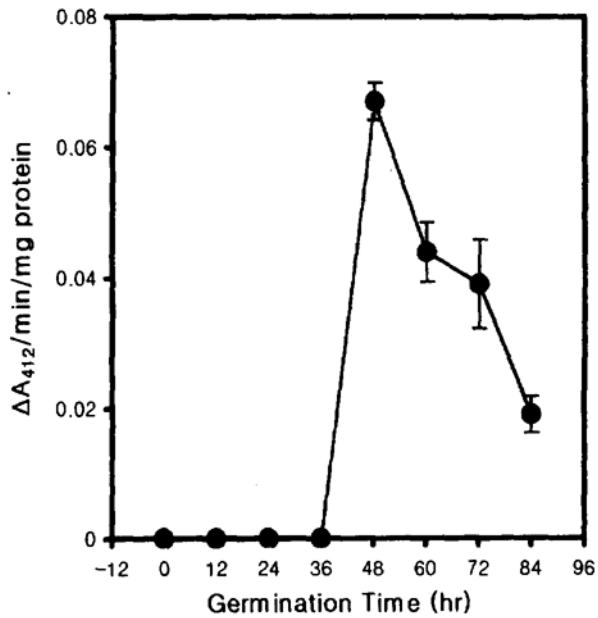

Fig. 4. Time-course variation in specific activities of thioltransferase during the seed germination of *A. thaliana*. Activity of thioltransferase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{340} / min / mg protein.

shown). These results indicate that thioredoxin may play an important role in the storage of the seeds. However, only the thioredoxin activity, which was able to interact with *E. coli* thioredoxin reductase, was measured.

Thioltransferase catalyzes the reversible thiol-disulfide interchange reactions. The enzyme has a major role in maintaining intracellular thiols in the reduced state and functions in this capacity by coupling to glutathione and glutathione reductase. Thioltransferase also has a role in cellular regulation by catalyzing the reversible modification of proteins by thiol-disulfide interchange. In result, thioltransferase is able to control the activity, stability, and correct folding of enzymes through disulfide/dithiol isomerization reactions. During the seed germination of *A. thaliana*., thioltranferase activity increased at the early stage, and then remained relatively constant until 60 hours (Fig. 4). After 60 hours, it increased drastically. There results suggest that thioltransferase may play a greater role in younger leaves than in seed germination. Thioltransferase shows an opposite manner of regulation compared with thioredoxin. Recently, one type of thioltransferase was purified and characterized from the seeds of *A. thaliana* (Cho *et al.*, 1998).

Thioredoxin reductase reduces the oxidized thioredoxin in various types of cells. Thioredoxin is also reduced by ferredoxin-thioredoxin reductase in

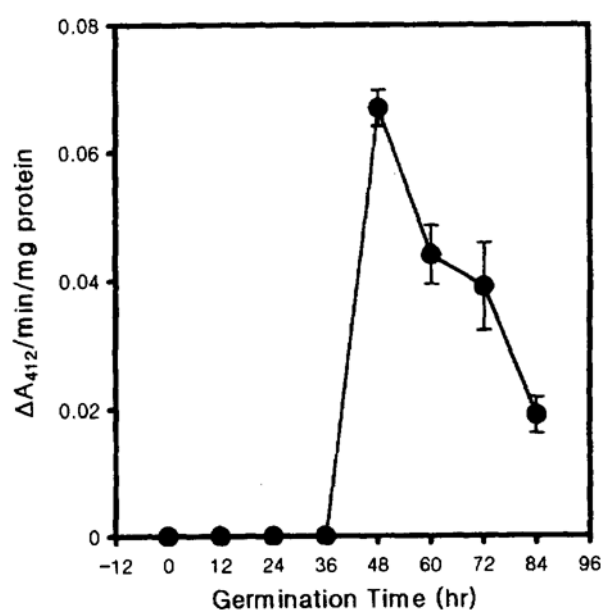

Fig. 5. Time-course variation in specific activities of thioredoxin reductase during the seed germination of *A. thaliana*. Activity of thioredoxin reductase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{412} / min / mg protein.

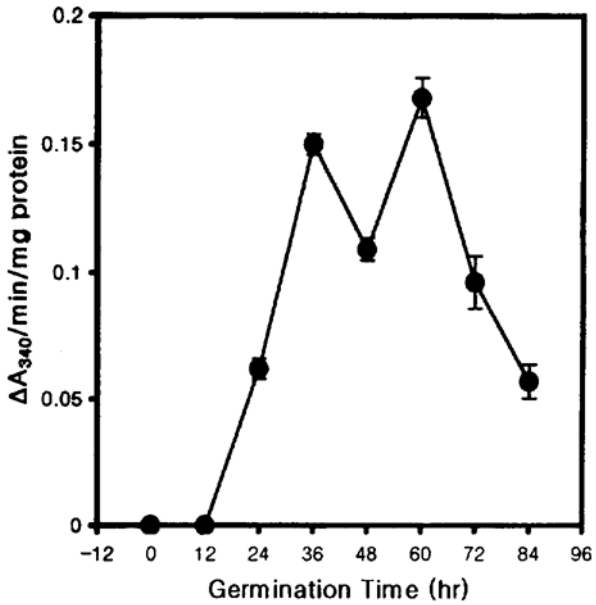

Fig. 6. Time-course variation in specific activities of glutathione reductase during the seed germination of *A. thaliana*. Activity of glutathione reductase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{340}/min/mg protein.

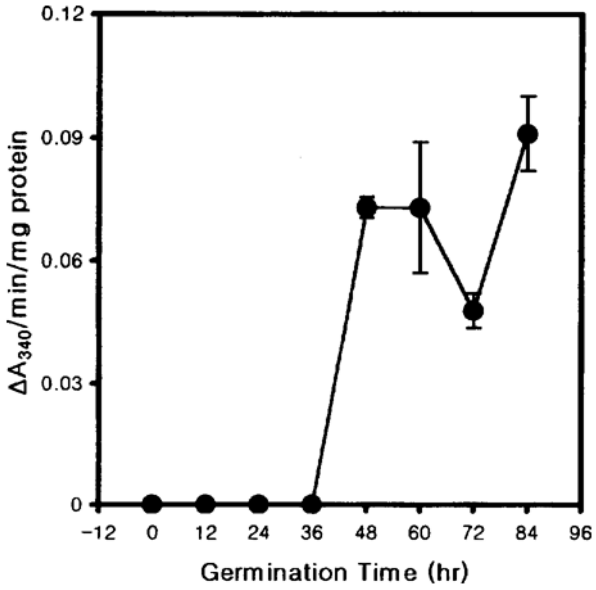

Fig. 7. Time-course variation in specific activities of $NADP^+$-specific malate dehydrogenase during the seed germination of *A. thaliana*. Activity of $NADP^+$-specific malate dehydrogenase was assayed as described in 'MATERIALS AND METHODS'. Its specific activity was expressed as ΔA_{340}/min/mg protein.

plant cells (Droux *et al.*, 1987). Thioredoxin reductase activity increased rapidly 36 hours during the seed germination of *Arabidopsis*, and it decreased at the later stages (Fig. 5). This variation pattern is not consistent with that of thioredoxin activity detected in this study. Considering that there are multiple kinds of thioredoxin in plant cells, the two different trends in the variation may be understood.

Glutathione reductase is a flavoprotein catalyzing the NADPH-dependent reduction of glutathione disulfide to glutathione. The reaction is essential for the maintenance of glutathione levels. Glutathione reductase activity increased 36 hours during the seed germination and remained relatively constant at the later stage (Fig. 6). This trend of variation is similar with that of thioltransferase activity.

$NADP^+$-Malate Dehydrogenase

$NADP^+$-specific malate dehydrogenase catalyzes the reduction of oxaloacetate using NADPH as a reductant. It occurs in high activity in C_4 variants in which malate is the principal short-term product of CO_2 assimilation and is located in the chloroplasts of mesophyll cells. In the germination process of *Arabidopsis* seeds, $NADP^+$-malate dehydrogenase activity increased rapidly at the earlier stage and decreased after the germination (Fig. 7). The activity did not give a large variation during the leaf growth (data not shown).

In this article, time-course variations of sulfhydryl-related enzymes and PAL and TAL were measured during the seed germination of *A. thaliana*. These enzyme activities were shown to be related closely with the seed germination process. Sulfhydryl-related enzymes may be necessary for the activation of several enzymes directly involved in seed germination. They might be also involved in the correct folding of proteins newly synthesized in the germination process. However, according to our results, thioredoxin activity decreases during the germination process, whereas thioltransferase activity increases. This indicates that thioredoxin activity plays more important role in seed themselves, and that thioltransferase activity acts as an important disulfide reductase during the germination process and in the leaves of *A. thaliana*. The results obtained in this study may be useful for regulation studies on these enzymes and germination process.

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LITERATURE CITED

Abell, C.W. and R.-S. Shen. 1987. Phenylalanine ammonia-lyase from the yeast *Rhodotorula glutinins. Methods Enzymol.* **142**: 242-253.

Beaudoin, N. and S.J. Rothstein. 1997. Developmental regulation of two tomato lipoxygenase promoters in transgenic tobacco and tomato. *Plant Mol. Biol.* **33**: 835-846.

Brödenfeldt, R. and H. Mohr. 1988. Time course for phytochrome-induced enzyme levels in phenylpropanoid metabolism (phenylalanine ammonia-lyase, maringenin-chalcone synthase) compared with time courses for phytochrome-mediated end-product accumulation (anthocyanin, quercetin). *Planta.* **176**: 383-390.

Buchanan, B.B. 1980. Role of light in the regulation of chloroplast enzymes. *Annu. Rev. Plant Physiol.* **31**: 341-374.

Buchanan, B.B., P. Schürmann and J.-P. Jacquot. 1994. Thioredoxin and metabolic regulation. *Semin Cell Biol.* **5**: 285-293.

Carberg, I. and B. Mannervik. 1985. Glutathione reductase. *Methods Enzymol.* **113**: 484-490.

Cho, Y.-W., E.-H. Park and C.-J. Lim. 1998. Thioltransferase (glutaredoxin) from Chinese cabbage : purification and properties. *J. Biochem. Mol. Biol.* (in press).

Cho, Y.-W., J.C. Kim, C.D. Jin, T.J. Han and C.-J. Lim. 1998. A thioltransferase from *Arabidopsis thaliana* seed : purification to homogeneity and characterization. *Mol. Cells.* (in press).

Dixon, R.A. and C.J. Lamb. 1990. Molecular communication in interactions between plants and microbial pathogens. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41**: 339-367.

Droux, M., M. Miginiac-Maslow, J.-P. Jacquot, P. Gadal, N.A. Crawford, N.S. Kosower and B. Buchanan. 1987. Ferredoxin-thioredoxin reductase : a catalytically active dithiol group links photoreduced ferredoxin to thioredoxin functional in photosynthetic enzyme regulation. *Arch. Biochem. Biophys.* **256**: 372-380.

Dubery, I.A. and F. Smit. 1994. Phenylalanine ammonia-lyase from cotton (*Gossypium hirsutum*) hypocotyls : properties of the enzyme induced by a *Verticillium dahliae* phytotoxin. *Biochim. Biophys. Acta.* **1207**: 24-30.

Gonzalez, M.C., L. Osuna, C. Echevarria. J. Vidal and F.J. Cejudo. 1998. Expression and localization of phosphoenolpyruvate carboxylase in developing and germinating wheat grains. *Plant Physiol.* **116**: 1249-1258.

Graham, I.A., L.M. Smith, C.J. Leaver and S.M. Smith. 1990. Developmental regulation of expression of the malate synthase gene in transgenic plants. *Plant Mol. Biol.* **15**: 539-549.

Guan, L. and J.G. Scandalios. 1995. Developmentally related responses of maize catalase genes to salicylic acid. *Proc. Natl. Acad. Sci. USA* **92**: 5930-5934.

Hahlbrock, K. and D. Scheel. 1989. Physiology and molecular biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**: 347-369.

Holmgren, A. 1979. Reduction of disulfides by thioredoxin. Exceptional reactivity of insulin and suggested functions of thioredoxin in mechanism of hormone action. *J. Biol. Chem.* **254**: 9113-9119.

Jacquot, J.-P., E. Issakidis, P. Decottignies, M. Lemaire and M. Miginiac-Maslow. 1995. Analysis and manipulation of target enzymes for thioredoxin control. *Methods Enzymol.* **252**: 240-252.

Karrer, E.E., J.C. Lifts and R. L. Rodriquez. 1991. Differential expression of alpha-amylase genes in germinating rice and barley seeds. *Plant Mol. Biol.* **16**: 797-805.

Kubasek, W.L., F.M. Ausubel and B.W. Shirley. 1998. A light-independent developmental mechanism potentiates flavonoid gene expression in *Arabidopsis* seedlings. *Plant Mol. Biol.* **37**: 217-223.

Laboure, A.M., J. Gagnon and A.M. Lescure. 1993. Purification and characterization of a phytase (myo-inositol-hexakis-phosphate phosphohydrolase) accumulated in maize (*Zea mays*) seedling during germination. *Biochem. J.* **295**: 413-419.

Lee, D., M. Ellard, L.A. Wanner, K.R. Davis and C. J. Douglas. 1995. The *Arabidopsis thaliana*. 4-coumarate : CoA ligase (4CL) gene : stress and developmentally regulated expression and nucleotide sequence of its cDNA. *Plant Mol. Biol.* **28**: 871-884.

Leegood, R.C. and D.A. Walker. 1980. Autocatalysis and light activation of enzymes in relation to photosynthetic induction in wheat chloroplasts. *Arch. Biochem. Biophys.* **200**: 575-582.

Liang, X., M. Dron, C.L. Cramer, R.A. Dixon and C.J. Lamb. 1989. Differential regulation of phenylalanine ammonia-lyase genes during plant development and by environmental cues. *J. Biol. Chem.* **264**: 14486-14492.

Lintschinger, J., N. Fuchs, H. Moser, R. Jager, T. Hlebeina, G. Markolin and W. Gossler. 1997. Uptake of various trace elements during germination of wheat, buckwheat and quinoa. *Plant Foods Human Nutr.* **50**: 223-237.

Lim, H.W. and C.-J. Lim. 1995. Direct reduction of DTNB by *E. coli* thioredoxin reductase. *J. Biochem. Mol. Biol.* **28**: 17-20.

Lim, H.-W., J.-H. Sa, T.-S. Kim, E.-H. Park, S.-S. Park, and C.-J. Lim. 1998. Purification and properties of phenylalanine ammonia-lyase from Chinese cabbage. *J. Biochem. Mol. Biol.* **31**: 31-36.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenyl reagent. *J. Biol. Chem.* **193**: 265-275.

Luthman, M. and A. Holmgren. 1982. Rat liver thioredoxin and thioredoxin reductase : purification and characterization. *Biochemistry* **21**: 6628-6633.

Morell, S., H. Follmann and I. Höberlein. 1995. Iden-

tification and localization of the first glutaredoxin in leaves of a higher plant. *FEBS Lett.* **369**: 149-152.

Nagai, S. and S. Black. 1968. A thiol-disulfide transhydrogenase from yeast. *J. Biol. Chem.* **243**: 1942-1947.

Nikkola, M., F.K. Gleason, M. Saarinen, T. Joelson, O. Björnberg and H. Eklund. 1991. A putative glutathione-binding site in T4 glutaredoxin investigated by site-directed mutagenesis. *J. Biol. Chem.* **266**: 16105-16112.

Pradel, J., J.-M. Soulié, J. Buc, J.-C. Meunier and J. Ricard. 1981. On the activation of fructose-1, 6-bisphosphatase of spinach chloroplasts and the regulation of Calvin cycle. *Eur. J. Biochem.* **113**: 507-511.

Rivera-Madrid, R., D. Mestres, P. Marinho, J.-P. Jacquot, P. Decottignies, M. Miginiac-Maslow and Y. Meyer. 1995. Evidence for five divergent thioredoxin h sequences in *Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA* **92**: 5620-5624.

Sa, J.-H., M.-Y. Yong, B.-L. Song and C.-J. Lim. 1998. Characterization of thioltransferase from kale. *J. Biolchem. Mol. Biol.* **31**: 20-24.

Sha, S., K. Minakuchi, N. Higaki, K. Sato., O. Kozo, A. Kurata, H. Yoshikawa, M. Kotaru, T. Masumura, K. Ichihara and K. Tanaka. 1997. Purification and characterization of glutaredoxin (thioltransferase) from rice (*Oryza sativa* L.). *J. Biolchem.* **121**: 842-848.

Smith. C.G., M.W. Rodgers, A. Zimmerlin, D. Ferdinando and G. P. Bolwell. 1994. Tissue and subcellular immunolocalization of enzymes of lignin synthesis in differentiating and wounded hypocotyl tissue of French bean (*Phaseolus vulgaris* L.). *Planata* **192**: 155-164.

Srivastava, N., D. Prakash and H.M. Behl. 1997. Biochemical contents, their variation and changes in free amino acids during seed germination in *Terminalia arjuna. Int. J. Food Sci. Nutr.* **48**: 215-219

Starke, D.W., Y.C. Chen. C.P. Bapna. E.J. Lesnefsky and J.J. Mieyal. 1997. Sensitivity of protein sulfhydryl repair enzyme to oxidative stress. *Free Radic. Biol. Med.* **23**: 373-384.

Wanner, L.A., G. Li, D. Ware, I.E. Somssich and K.R. Davis. 1995. The phenylalanine ammonia-lyase gene family in *Arabidopsis thaliana. Plant Mol. Biol.* **27**: 327-338.

Washio, K. and K. Ishikawa. 1992. Structure and expression during the germination of rice seeds of the gene for a carboxypeptidase. *Plant Mol. Biol.* **19**: 631-340.

Watanabe, A., K. Hamada, H. Yokoi and A. Watanabe. 1994. Biphasic and differential expression of cytosolic glutamine synthetase genes of radish during seed germination and senescence of cotyledons. *Plant Mol. Biol.* **26**: 1807-1817.

Wiermann, R. 1981. *Secondary plant products. The biochemistry of plants : A comprehensive treatise,* Vol. 7 (Stumf, P. K. and E. E. Conn Ed.) pp. 85-116. *Academic Press, New York.*

Yoshitake, S., H. Nanri, M.R. Fernando and S. Minakami. 1994. Possible differences in the regenerative roles played by thioltransferase and thioredoxin for oxidatively damaged proteins. *J. Biochem.* **116**: 42-46.

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