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Roles of Reactive Oxygen Species and Glutathione in Plant Development

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ABSTRACT

Endogenous levels of reactive oxygen species (ROS), which are toxic to cells, increase in response to various environmental and developmental stimuli, inducing changes in cellular redox state that is maintained by the redox buffer glutathione. Such redox changes are used in signals for the regulation of plant growth and stress responses. In this review, in order to give further insight into the mechanism underlying ROS- and glutathione-associated physiological events, we present recent findings on and discuss the roles of ROS and glutathione in plant growth and development.

Keywords: *S*-glutathionylation, life cycle, redox
Abbreviations: ABA, abscisic acid; **AsA**; ascorbic acid; **BSO**, L-buthionine sulfoximine; **DHAR**, dehydroascorbate reductase; **γ-ECS**, γ-glutamylcysteine synthetase; **FBA**, fructose-1,6-bisphosphate aldolase; **GR**, glutathione reductase; **Grx**, glutaredoxin; **GSH**, reduced form of glutathione; **GSSG**, oxidized form of glutathione; **GST**, glutathione *S*-transferase; **PCD**, programmed cell death; **ROS**, reactive oxygen species; **TPI**, triose-phosphate isomerase; **Trx**, thioredoxin

CONTENTS

INTRODUCTION

Plant development is largely dependent on the energy that is acquired from photosynthesis and influenced by abiotic and biotic stresses. How stresses influence plant development is concerned with metabolic pathways of redox low-molecular-weight molecules such as reactive oxygen species (ROS), glutathione and ascorbic acid (AsA), which maintain the

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Invited Review

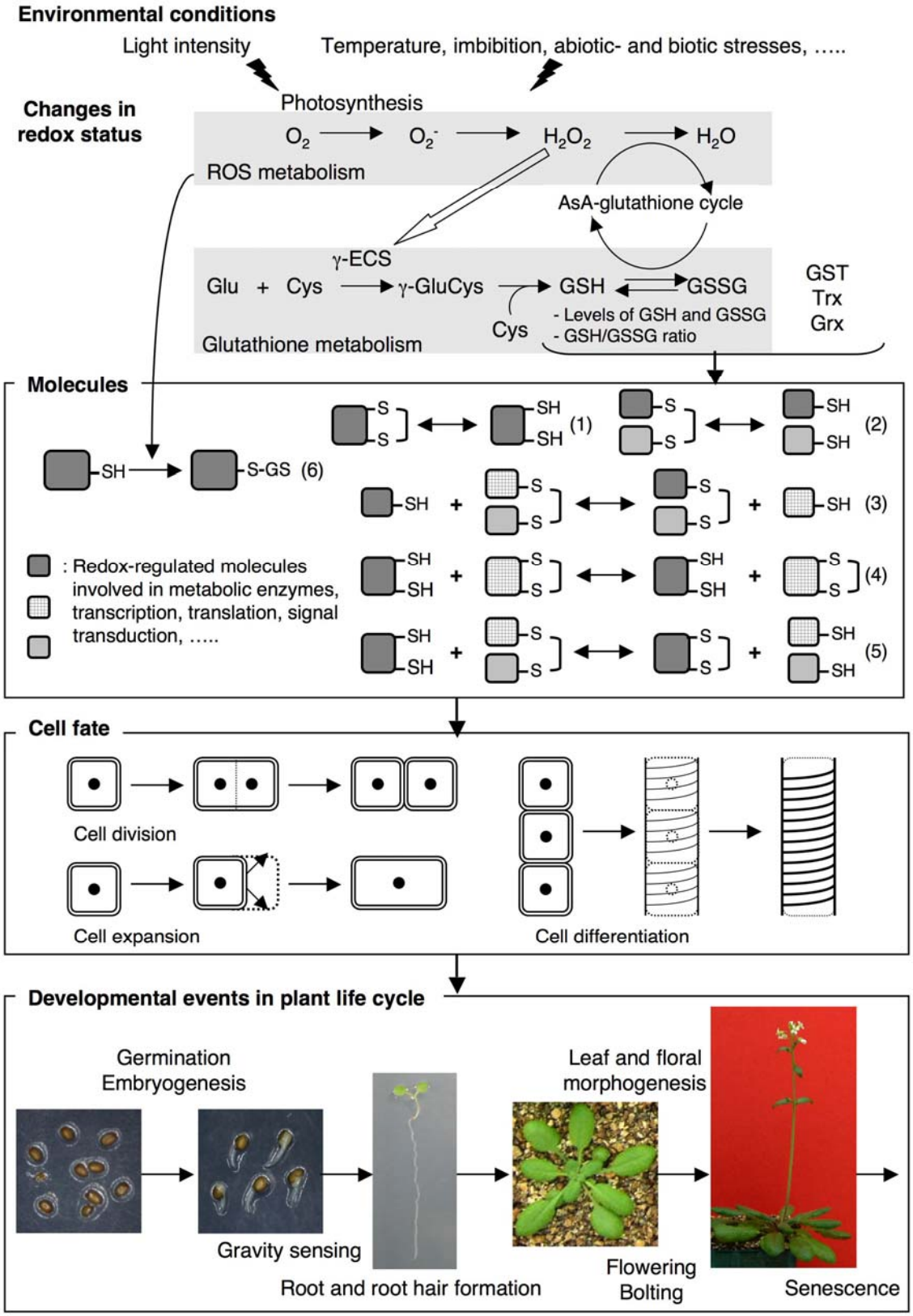

Fig. 1 Scheme for plant development by redox regulation. Plants sense changes in cellular redox state in response to environmental changes. Such redox changes alter the physiological functions of redox-regulated molecules, determining the cell fate in developmental event. See the text for details.

cell in a reduced redox state. Since the stresses change cellular redox state, plants have come to utilize the redox molecules as signals for sensing environmental changes to adapt themselves to the changing environment. A transient oxidative state following the stresses is one of the signals for sensing environmental changes. Such a redox change in cells induces posttranslational redox modifications of various proteins, leading to pleiotropic developmental responses (summarized in **Fig. 1**). In some mammalian systems, it has been indicated that redox signal transduction is necessary for cell proliferation and programmed cell death (PCD) processes (Sen 2000). Taking into account the ubiquity of ROS and glutathione, it is assumed that physiological events are redox-regulated in all aerobic organisms.

Recent studies accumulate new findings on the redox regulation of plant development. In this review we introduce pleiotropic roles of ROS and glutathione in the plant life cycle and discuss their significance in changes in the cellular redox status.

CELLULAR REDOX STATUS AND THE ENVIRONMENT

Determinants of cellular redox state in plants

ROS is a generic term for singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). Since ROS, except for H_2O_2, are short-lived, H_2O_2 is the most potent signal for developmental regulation. In plant cells, $O_2^{\cdot-}$ is disproportionated into H_2O_2 and O_2 by superoxide dismutase (SOD), and then H_2O_2 is reduced to H_2O by ascorbate peroxidase using AsA as an electron donor. Monodehydroascorbate and dehydroascorbate, following the scavenging of H_2O_2, are enzymatically reduced back to AsA using GSH and NAPDH as electron donors (Noctor and Foyer 1998). Oxidized glutathione (GSSG) is reduced by GSSG reductase. These metabolic couplings are called the ascorbate-glutathione cycle. Thanks to this cycle, the cellular redox state of the plant is kept reduced. In non-stressful conditions, plant GSSG does not exceed 10% of the total amount of glutathione (GSH+GSSG).

ROS generation

The major compartment for ROS generation is the chloroplast (Asada 1999). When the absorbed photon energy exceeds the capacity of photosynthesis that is dependent on the electron flux to carbon fixation, excess electrons reduce dioxygen to $O_2^{\cdot-}$ in photosystem I (PSI), followed by the disproportionation of $O_2^{\cdot-}$ into H_2O_2 and O_2 that is catalyzed by CuZn-SOD microcompartmented at the vicinity of PSI (Ogawa *et al*. 1995). In photosystem II (PSII), the ground state oxygen is exited to $^1O_2$ (Hidég *et al*. 2001; Asada 2006) even though it would be rapidly scavenged by β-carotene. It is therefore easy to postulate that the rate of ROS generation would be enhanced with increasing light intensity, but there has been no report considering whether photoperiod influences ROS generation or not. However, considering that the night length of prevailing photoperiod that promotes the vegetative growth increases Rubisco contents resulting in increase of the electron flux from PSI to the Calvin cycle, ROS would be more liable to be generated in the night length of photoperiod that promotes the reproductive growth. Since light harvesting antenna chlorophylls absorb light most strongly in the blue and red portions of electromagnetic spectrum and absorption maxima of PSII and PSI are 680 and 700 nm, respectively, the rate of ROS generation would be relatively enhanced by red and blue light. C4 and CAM plants equip systems to concentrate and reserve CO_2 and thus CO_2 availability is higher in CAM and C4 plants than in C3 plants. Furthermore, photorespiration which accompanies ROS generation is not measurable in C4 and CAM plants. Considering these, ROS seems to be more easily generated in C3 plants than in C4 and CAM plants and that CAM and C4 plants appear more advantageous in that they can drive the Calvin cycle in more stressful conditions. However, we cannot easily conclude it because many of desert plant species are C3 plants.

Other compartments of ROS generation are the plasma membrane, mitochondria and peroxisome (Mittler *et al*. 2004). In rice, plasma membrane NADPH oxidase generates ROS as signal molecules for the resistance against pathogen attack (Ono *et al*. 2001). The generation of $O_2^{\cdot-}$ and its disproportionation into H_2O_2 and O_2 take place in complex I and III (Rhoads *at al*. 2006). In peroxisomes, enzymatic reactions such as glycolate oxidase and flavin oxidase generate H_2O_2 as a byproduct (del Río *et al*. 2006). In peroxisomes, $O_2^{\cdot-}$ is generated by xanthine oxidase and an electron transport chain composed of a flavoprotein NADH:ferricyanide reductase and a cytochrome *b* (del Río *et al*. 2006).

Because of the physical traits of ROS, it is technically impossible to measure ROS *in vivo*. Nevertheless, it is estimated that the chloroplast is the major compartment of ROS production and that $O_2^{\cdot-}$ concentration in the stroma below 10 nM (Ogawa *et al*. 1995; Asada 1999; Asada 2000; Asada 2006). Dioxygen concentration in chloroplasts is estimated to reach to 300 μM when photosynthesis takes place, while that of mitochondria is estimated around 1 to 10 nM (Asada and Takahashi 1987). Therefore, it is reasonable to consider that other compartments generate much less ROS than chloroplasts.

Glutathione synthesis

GSH is synthesized into two ATP-dependent reactions that are catalyzed by γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase. The *ch1-1* (*chlorina1-1*) mutant, defective in the light-harvesting complex in photosystem II, accumulates the GSH precursor cysteine with a decreased level of GSH (Ogawa *et al*. 2004). Considering this together with the fact that cysteine is synthesized in three compartments (chloroplasts, mitochondria and cytosol) and negatively feed-back regulated in mitochondria and cytosol but not in chloroplasts (Noji *et al*. 1998), it can be concluded that GSH is synthesized in chloroplasts and that the γ-ECS reaction is a limiting step for GSH synthesis (Ogawa *et al*. 2004).

Cellular redox changes in response to environmental changes

Biotic and abiotic stress such as drought, chilling, heat shock, pathogenic infection and wounding restrict the availability of electron acceptor $NADP^+$ in PSI, leading to increase in ROS generation in PSI. In response to increased ROS generation, GSSG production is also increased and the GSH/GSSG ratio decreases. As mentioned above, chloroplasts are the major compartment of ROS generation and GSH synthesis. Therefore, chloroplasts are the primary compartment that determines cellular redox state.

REDOX-REGULATED MOLECULES

Mechanisms sensing cellular redox status

In order to sense cellular redox state, some proteins may be directly oxidized by ROS like yeast Gpx3, an H_2O_2 sensor (Delaunay *et al*. 2002), although there has been no evidence showing any functional modification of plant proteins by ROS. Another possibility of sensing the cellular redox state is functional modifications mediated by thiol compounds (Reactions 1 to 6, **Fig. 1**).

$$2\ GSH + RS_2 \leftrightarrow GSH + (SH)RS\text{-}SG \leftrightarrow GSSG + R(SH)_2 \quad (1)$$

$$2\ GSH + RS\text{-}SR' \leftrightarrow GSH + RS\text{-}SG + R'SH \leftrightarrow GSSG + RSH + R'SH \quad (2)$$

$$RSH + R'S\text{-}SR'' \leftrightarrow RS\text{-}SR' + R''SH \quad (3)$$

$$R(SH)_2 + R'S_2 \leftrightarrow (SH)\ RS\text{-}S\ R'SH \leftrightarrow RS_2 + R'(SH)_2 \quad (4)$$

$$R(SH)_2 + R'S\text{-}SR'' \leftrightarrow (SH)\ RS\text{-}SR' + R''SH \leftrightarrow RS_2 + R'SH + R''SH \quad (5)$$

$$RSH + GSH + H_2O_2 \rightarrow RS\text{-}SG + 2\ H_2O \quad (6)$$

Reactions 1 to 5 are all thiol-disulfide exchange reactions; Reactions 1 to 3 are a simple monothiol mechanism, while Reactions 4 to 5 are a dithiol mechanism. Reaction 6 can be mediated by peroxidases. When the GS moiety is covalently incorporated into Cys residue(s) in proteins, the reaction is called *S*-glutathionylation, *S*-thiolation or mixed (protein-nonprotein) disulfide formation. If there is/are any accessible free Cys residue(s) in proteins, the redox state of the cysteine residue(s) is changeable in response to the redox state of glutathione and reducing proteins, thioredoxins (Trxs) and glutaredoxins (Grxs).

Redox sensing molecules

Phytohormone signaling

Both ABI1 and ABI2 of *Arabidopsis thaliana*, which are involved in abscisic acid (ABA) signaling, are type 2C of Ser/

Thr phosphatases and their enzymatic activity is reversibly regulated by GSH and H_2O_2 *in vitro*, although it remains to be investigated *in vivo* (Meinhard and Grill 2001; Meinhard *et al*. 2002). The function of *Arabidopsis* ETR1, which is an ethylene receptor, is regulated via redox-sensing Cys65 in H_2O_2-induced stomatal closure, but Cys65 is not located in the catalytic domain (Desikan *et al*. 2005).

Phytohormone-regulated developmental events might be associated with the ascorbate-glutathione cycle. AsA is a cofactor of GA_{20} 3β-hydroxylase involved in gibberellic acid (GA) biosynthesis (Smith *et al*. 1990). The subunits of 26S proteasome undergo *S*-glutathionylation (Dixon *et al*. 2005). In this regard, redox regulation might involve some of 26S proteasome substrates involved in the regulation of morphology, such as NAC1 (a member of NAM, ATAF and CUC transcription factors) for auxin-responsive lateral root formation (Xie *et al*. 2002), SLR1 (SLENDER RICE 1) for GA-responsive stem elongation (Ueguchi-Tanaka *et al*. 2005), PIN1 (Pin-formed 1) for auxin efflux carrier and some members of Aux/IAA (Auxin/indole-3-acetic-acid) transcriptional repressors for auxin signaling (Gray *et al*. 2001; Petrášek *et al*. 2006).

Metabolic enzymes undergoing S-glutathionylation

Sugar metabolism including reductive and oxidative pentose phosphate pathways is a crucial determinant of plant growth and development. A cytosolic triosephosphate isomerase (TPI) and two plastidic fructose-1,6-bisphosphate aldolases (FBAs) from *A. thaliana*, which are enzymes involved in sugar metabolism, are identified as proteins undergoing *S*-glutathionylation *in vivo* (Ito *et al*. 2003; Ogawa *et al*. 2005; Matsumoto and Ogawa 2008). TPI activity is suppressed by GSSG and recovered by excess GSH. In contrast, one FBA isozyme is activated by GSH or GSSG at pH 8, while, at pH 7, the activity is inhibited by GSH and not affected by GSSG (Ogawa *et al*. 2005; Matsumoto and Ogawa 2008). In addition to TPI and FBAs, many enzymes involved in sugar metabolism are identified as proteins undergoing *S*-glutathionylation (**Table 1**). These may suggest that sugar metabolism is subjected to cellular redox changes following environmental changes.

Recent proteomic analysis has revealed that numerous proteins including enzymes, structural proteins and stress-induced proteins are *S*-glutathionylated in plants (Dixon *et al*. 2005). Although the physiological significance of *S*-glutathionylation is not fully understood, proteins undergoing *S*-glutathionylation may be subjected to cellular redox changes following environmental stresses. Since glutamine synthetase is a key enzyme in ammonium assimilation of the GS-GOGAT (glutamine synthetase-glutamate synthase) cycle and it is well known that nitrogen availability positively correlates to growth and photosynthesis, *S*-glutathionylation of glutamine synthetase, identified by Dixon *et al*. (2005), may suggest that nitrogen fixation is redox regulated for plant development and photosynthesis. Considering that glutamate is the precursor of GSH, it may be redox regulated for GSH synthesis.

Transcription

Two Cys residues in maize P1 regulator (ZmP1), a member of R2R3 MYB proteins, participates in redox-dependent DNA binding (Heine *et al*. 2004). In contrast, although sunflower HAHR1 and Hahb-10, homeodomain transcription factors, form a homodimer through the intermolecular disulfide bridge, reduction of these factors increases the DNA-binding activity like ZmP1 (Tron *et al*. 2002). NPR1 (nonexpressor of *PR-1* gene) protein, which forms an inactive oligomer through an intermolecular disulfide bond in normal conditions, is reduced to an active monomer in systemic acquired resistance (SAR) induction, resulting in the translocation to the nuclei and the induction of *PR-1* gene expression (Despres *et al*. 2003; Mou *et al*. 2003).

Unlike ZmP1, the transcription of two chloroplastic genes is indirectly regulated by the GSH/GSSG redox state (Baena-González *et al*. 2001). In that case, the transcriptional activity depends on the phosphorylation of RNA polymerase by a GSH/GSSG-regulated kinase (Baena-González *et al*. 2001).

Translation

In the chloroplast of *Chlamydomonas*, the translation of the Rubisco large subunit is arrested in response to a decrease in the GSH/GSSG ratio following increase in ROS levels (Irihimovitch and Shapira 2000). It is not inconsistent with the findings that some elongation factors are potential Trx and Grx targets (Buchanan and Balmer 2005; Rouhier *et al*. 2005), although whether the proteins are redox regulated remains to be elucidated.

Possible second messengers of redox signaling

Glutathione *S*-transferase (GST), glutathione peroxidase and formaldehyde dehydrogenase use GSH as an electron donor and possible producer of second messengers. Possible signaling mechanisms involving these proteins have been discussed in Ogawa (2005).

Thioredoxin and glutaredoxin

Trxs and Grxs reduce the redox-sensitive disulfide bridge in numerous proteins, participating in the regulation of protein functions (Meyer and Hell 2005). Like Trxs and Grxs, the

Table 1 *S*-glutathionylated enzymes involved in sugar metabolism in *Arabidopsis thaliana*.

Enzyme	Reference
Cytosolic triosephosphate isomerase	Ito *et al*. 2003
Plastidic fructose-bisphosphate aldolase	Ito *et al*. 2003
Fructose-bisphosphate aldolase (outer mitochondrial membrane)	Dixon *et al*. 2005
Plastidic A_4-glyceraldehyde 3-phosphate dehydrogenase	Zaffagnini *et al*. 2007
Glyceraldehyde 3-phosphate dehydrogenase (mitochondrial membrane)	Dixon *et al*. 2005
Sucrose synthase	Dixon *et al*. 2005
Decarboxylase	Dixon *et al*. 2005
Enolase	Dixon *et al*. 2005
Malic enzyme	Dixon *et al*. 2005
UDP-Glucose pyrophosphorylase	Dixon *et al*. 2005
UDP-Glucose dehydrogenase	Dixon *et al*. 2005
Fumarase	Dixon *et al*. 2005
Alcohol dehydrogenase	Dixon *et al*. 2005
Arginosuccinate synthase	Dixon *et al*. 2005
3-Isopropylmalate dehydratase	Dixon *et al*. 2005
Aconitate hydratase-like	Dixon *et al*. 2005
Sorbitol dehydrogenase	Dixon *et al*. 2005
dTDP-Glucose 4,6-dehydratase	Dixon *et al*. 2005

strong reductant dithiothreitol (DTT) reduces the protein Cys residue(s) to modulate their function *in vitro*, but is unable to regulate some physiological events that are under the control of GSH (Yanagida *et al*. 2004). These suggest that those events are not regulated by a simple thiol-disulfide exchange reaction. Intensive studies provide candidate lists of Trx and/or Grx target proteins in various plants (Rouhier *et al*. 2004; Buchanan and Balmer 2005; Gelhaye *et al*. 2005; Rouhier *et al*. 2005).

Monothiol Grxs having the CXXS motif (and other proteins having reactive Cys residue(s), e.g., GST and dehydroascorbate reductase (DHAR)) are able to catalyze *S*-glutathionylation. Although an extensive *S*-glutathionylation is observed *in vivo* (Dixon *et al*. 2005; Ogawa *et al*. 2005), there have so far been no information on *in vivo* mediators of *S*-glutathionylation and no evidence showing that monothiol Grx is a mediator of *S*-glutathionylation.

Interestingly, it has been reported that some of Trx and Grx are identified as proteins undergoing *S*-glutathionylation (Gelhae *et al*. 2004; Michelet *et al*. 2005). The homodimeric alternative oxidase is activated through the reduction of its intermolecular disulfide bond by PtTrx*h*2, a poplar mitochondrial Trx that is shown to undergo *S*-glutathionylation *in vitro* (Gelhae *et al*. 2004). Some AtDHARs and AtGSTs that have been shown to undergo *S*-glutathionylation are induced by redox-perturbing reagents such as BSO and AsA or oxidative stresses in *Arabidopsis* (Dixon *et al*. 2002, 2005). These might suggest that these proteins are mediators of *S*-glutathionylation.

HOW DOES CELLULAR REDOX STATE INFLUENCE CELL FATE?

Cell division

An adequate level of GSH is required for the G_1-to-S phase transition in synchronized tobacco BY-2 cells (Vernoux *et al*. 2000). Exogenous application of GSH raised the number of root meristematic cells undergoing mitosis, while depletion of GSH by buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, had the opposite effect in *Arabidopsis* (Sánchez-Fernández *et al*. 1997). High levels of GSH were associated with the epidermal and cortical initials and markedly lower levels in the quiescent center (Sánchez-Fernández *et al*. 1997; Fricker *et al*. 2000). These experimental observations may imply an interrelation between endogenous GSH and proliferation activity, and some cell cycle machinery is dependent on GSH. Although it has not been reported yet in plants, ribonucleoside reductase is regulated by Grx1 in *Escherichia coli* (Holmgren 1976).

Cell expansion

Cell size is influenced by ROS and/or glutathione. Exogenous application of GSH makes short trichoblasts with long hairs, while depletion of GSH by BSO has the opposite effects in *Arabidopsis* (Sánchez-Fernández *et al*. 1997). In the growing zone of the maize root, cell wall loosening is markedly caused by the generation of $^{\cdot}OH$, which may be catalyzed by the wall-locating peroxidase (Liszkay *et al*. 2004). These may suggest that redox regulation of the plasma membrane and cell wall is required for cell expansion.

Recent proteomic analysis has identified cytoskeletal proteins such as tubulin and actin as targets of *S*-glutathionylation in *Arabidopsis* (Dixon *et al*. 2005). Since G-actin polymerization is modulated by growth factor-induced deglutathionylation in a human cell line (Wang *et al*. 2001), some proteins undergoing *S*-glutathionylation may contribute to the regulation of cytoskeleton formation in cell expansion.

Cell differentiation

ZeRAC2, a Rac homologue as regulatory components of NADPH oxidase complex, is preferentially expressed in the cells facing developed tracheary elements (TEs) in *Zinnia elegans* (Nakanomyo *et al*. 2002). Taking into account the requisition of $O_2^{\cdot-}$ and H_2O_2 in the spinach vascular tissue, ROS are necessary for the lignification of secondary walls during vascular formation (Ogawa *et al*. 1997). In cotton fiber formation, H_2O_2 is involved in the differentiation of secondary walls (Potikha *at al*. 1999). ROS are important not only for the cell wall synthesis as substrates but also for intracellular signal molecules. The transdifferentiation of isolated mesophyll cells into TEs is induced by auxin and cytokinin in the *Zinnia* experimental system (Fukuda and Komamine 1980). ROS scavengers or an inhibitor of NADPH oxidase suppresses TE differentiation of cultured mesophyll cells (Henmi *et al*. 2001; Ogawa *et al*. 2001). And application of GSSG at an early culture period (before expression of Zen1, an S1 endonuclease involved in autolysis, Pesquet *et al*. 2004) promotes the differentiation, but that of GSH or GSSG at an advanced period (but still before Zen1 expression) suppresses it (Henmi *et al*. 2001). The sensitivity of the cultured cells to glutathione during the differentiation period is consistent with the changes in endogenous GSH and GSSG and mRNA levels of DHAR and GR, which determine the cellular redox state of glutathione (Henmi *et al*. 2005). Assuming that ROS affect TE differentiation through the ascorbate-glutathione cycle and Zen1 expression is an event marker of autolysis that is an essential step for the cell to be TE, an early step of TE differentiation is regulated by changes in the redox state of glutathione.

REDOX-REGULATED DEVELOPMENTAL EVENTS

A developmental event is the integration of elementary processes such as cell division, cell size control, cell differentiation (**Fig. 1**). However, we cannot so far provide extensive information on how elementary processes occurring in individual cells are integrated to regulate any physiological event. Thus, we only provide (probable) information on which developmental events are regulated by cellular redox state and discuss how each physiological event is regulated by cellular redox state. Information regarding redox metabolism in flowering and bolting processes are coming in a review by Hatano-Iwasaki and Ogawa (2007).

Germination

In dormant pea and pine seeds, the ratio of GSSG to total glutathione is high, although GSSG is enzymatically reduced to GSH during imbibition (Kranner and Grill 1993; Tommasi *et al*. 2001). Considering that the oxidized form of transcriptional factors such as ZmP1 and Hahb-10 are inactive, an oxidative state of glutathione in dormant seeds may contribute to the maintenance of the dormant phase. Seed germination is promoted by GSH in barley seeds (Fontaine *et al*. 1995), but inhibited in *Arabidopsis* (Ogawa *et al*. 2001). Considering that exogenous H_2O_2 promotes germination in *Zinnia* and barley seeds (Fontaine *et al*. 1995; Ogawa and Iwabuchi 2001) and that endogenous ROS are essentially required for seed germination (Ogawa and Iwabuchi 2001), each cellular compartment and/or each cell are differentially regulated by redox state. In addition, some proteins, such as embryo globulin 1, are identified as Trx *h*-specific targets in germinating embryo and the oxidative pentose phosphate pathway are activated following imbibition to supply NADPH, from which we can conclude that successive reduction of proteins by Trx system occurs after imbibition (Marx *et al*. 2003).

Since GA and ABA regulate seed germination and dormancy, GA and ABA signaling may be associated with the redox regulation of seed germination and dormancy. During seed germination, the degradation of PIL5 (PHYTOCHROME INTERACTING FACTOR 3-like 5) protein by proteasome is promoted by light signal through GA accumulation (Oh *et al*. 2006). ABA prevents the degradation of ABI5 (ABA insensitive 5) protein, a repressor of germination, from the photolytic activity of proteasome (Lopez-

Molina *et al*. 2001). Considering the fact that α- and β-subunits consisting of 20S proteasome undergo *S*-glutathionylation (Dixon *et al*. 2005), it might be concluded that GA and ABA signaling for seed germination and dormancy are redox regulated.

Embryogenesis

A homozygous mutation of *Arabidopsis GSH1* encoding γ-ECS results in embryo-lethal, showing that *de novo* synthesis of GSH is essential for embryo development and seed maturation (Cairns *et al*. 2006). In white spruce, the number and quality of embryos generated from primary embryonic tissues are improved by supplementation with 0.1 mM GSH and 1 mM GSSG, respectively (Belmonte *et al*. 2005). A transfer of tissues treated with GSH for seven days to GSSG containing medium results in synergistic improvement in embryo number and quality. Increased mitotic activity and starch accumulation are observed in the treated embryo. This is consistent with the positive effect of glutathione on cell division. Starch accumulation might be associated with redox regulation of sugar metabolism.

Organ morphogenesis

Root and root hair growth

Pharmaceutical alteration of glutathione levels affect root growth, trichoblast (hair-forming cells) length and root hair density and length (Sánchez-Fernández *et al*. 1997). Exogenous application of GSH causes decreased trichoblast length and increased hair density and length. This observation is consistent with that of genetic alteration of glutathione levels (Xiang *et al*. 2001). Severe mutation of *Arabidopsis GSH1* causes loss of the root meristem as shown in *rml1* (*root meristemless 1*) mutants (Cheng *et al*. 1995; Vernoux *et al*. 2000). Overexpressing the GR gene in *Arabidopsis* plants suppresses redox changes in glutathione and makes the root meristematic region narrow (Henmi *et al*. 2005). These suggest that cell division and differentiation of the root is regulated by redox changes in glutathione. This conclusion is supported by the phenotype of *atgr1* mutant: decreased density of lateral roots (Henmi *et al*. 2005).

ROS generated from NADPH oxidase *AtrbohD* (*Arabidopsis thaliana respiratory burst oxidase homolog D*) and *AtrbohF* genes contribute to root growth (Kwak *et al*. 2003). The requisition of ROS in root elongation is consistent with the fact that ROS are detected in the proliferating and elongating parts during the germination of *Zinnia* seeds (Ogawa and Iwabichi 2001). It is also consistent with the ROS-dependent regulation of plasma membrane channels and cell wall loosening during cell elongation (Demidchik *et al*. 2003; Liszkay *et al*. 2004).

In contrast, ROS generated from the NADPH oxidase *RHD2* (*ROOT HAIR DEFFECTIVE2*)/*AtrbohC* gene are detected in root-hair tips and contribute to root-hair growth (Foreman *et al*. 2003; Carol *et al*. 2005; Jones *et al*. 2007). Localization of ROS generation is associated with SCN1 (SUPERCENTIPEDE1)/RhoGDI (RhoGTPase GDP DISSOCIATION INHIBITOR) function that allows the activation of RHD2/AtrbohC in root hair tips. In the endodermis of the maize primary root, a transient and local accumulation of ROS participates in root gravitropism (Joo *et al*. 2001). SCN1/RhoGDI may function in root gravitropism as well.

Although there has been no protein that is directly regulated by ROS or glutathione during root growth and root hair development, the *OXI1* (*OXIDATIVE SIGNAL-INDUCIBLE1*) gene that encodes a Ser/Thr kinase is induced in H_2O_2-treated roots and root hair cells (Rentel *et al*. 2004). OXI1 is a candidate for factors functioning downstream of RHD2/AtrbohC, judging from the short-root- hair phenotype of *oxi1* mutant.

Floral and leaf organs

ROXY1, a dithiol Grx, determines the expression area of the class C gene *AGAMOUS*, which is involved in the petal development in *Arabidopsis* (Xing *et al*. 2005). This is suggestive that petal morphogenesis is regulated by glutathione. Assuming that cellular ROS levels also regulate ROXY1, it is reasonable that *Rboh* antisense tomato plants exhibit increased numbers of petal and fasciated styles and ovaries (Sagi *et al*. 2004). The *Rboh* antisense tomato plants also show the leaflets with smoother edges and less lobed, which may suggest that leaf morphogenesis is also associated with ROS-dependent regulation (Sagi *et al*. 2004). Like Rboh in tomato, BOP (BLADE-ON-PETIOL) proteins are involved in leaf and floral morphogenesis (Ha *et al*. 2004; Hepworth *et al*. 2005; Norberg *et al*. 2005). BOP1 and BOP2 are NPR1-like proteins with two conserved Cys residues, resembling the SH group-mediating transcriptional regulation in SAR response. Although whether BOP proteins are controlled like NPR1 remains to be elucidated, redox-regulation of BOP proteins seems likely, considering that both leaf and floral morphogenesis involve the same enzymes regarding ROS generation.

In *Arabidopsis* leaves, oxidative stress induces ectopic trichome formation, and this is suppressed by the *ttg* (*transparent testa glabrous*) mutation (Nagata *et al*. 1999). Considering that the *TTG* gene is required for the initiation of trichome formation, ROS is required for the initial stage, at least upstream of TTG function, of trichome development. The genes involved in GSH synthesis are highly expressed and cytosolic GSH content in trichome is much higher than that in the trichome basement and epidermal cells (Gutierréz-Alcalá *et al*. 2000). ROS may be associated with the redox state of glutathione in trichome development. Interestingly, *TTG* is also required for root hair patterning (Galway *et al*. 1994; reviewed in Teixeira da Silva and Nhut 2003). Trichome and root hair development is partially regulated by a common mechanism via glutathione and ROS metabolism.

Death process

Senescence

BSO completely prevents chilling-induced senescence and the inhibitory effect of BSO is abolished by GSH, but not by DTT, in *Eustoma grandiflorum*, suggestive that senescence is regulated specifically by GSH (M. Yanagida and K. Ogawa, unpublished data). In leaf senescence of pea, total glutathione increases until chlorophyll content starts to decrease, and decrease after that (Vanacker *et al*. 2006).

Anthocyanins are accumulated during senescence. Transgenic *Arabidopsis* plants with reduced glutathione content accumulate reduced levels of anthocyanins (Xiang *et al*. 2001). This is consistent with the fact that phenylpropanoid biosynthesis is regulated by PAP1/AtMYB75 and PAP2/AtMYB90, members of R2R3 MYB family, with conserved two Cys residues like ZmP1 (Borevitz *et al*. 2000). In addition, TT19 (TRANSPARENT TESTA 19), a member of *Arabidopsis* GST family, is essential for vacuolar accumulation of anthocyanins (Kitamura *et al*. 2004). Thus, anthocyanin accumulation during senescence is strongly associated with glutathione.

Ethylene, one plant hormone promoting senescence, is synthesized from methionine as a precursor. Interestingly, methionine synthase and 1-aminocyclopropane-1-carboxylic oxidase involved in ethylene biosynthesis are found to undergo *S*-glutathionylation (Dixon *et al*. 2005), implying that senescence is redox-regulated at the level of plant hormone. Ethylene induces respiratory oxidative burst (de Jong *et al*. 2002) and such an oxidative condition may accelerate *S*-glutathionylation of the enzymes. This may be indicative that there is a positive feed-back regulation in ethylene signaling.

Programmed cell death (PCD)

Prior to induction of aleuronic PCD during cereal seed germination, GA decreases antioxidants activity such as ascorbate peroxidase, catalase and SOD to increase the sensitivity to the death signal H_2O_2 (Fath *et al*. 2002), indicating that the aleuronic PCD is concomitantly regulated by ROS metabolism and plant hormonal control like TE differentiation process, another developmental PCD (see section on cell differentiation). Since PCD including hypersensitive-response-like response has been suggested to involve glutathione (Senda and Ogawa 2004; Henmi *et al*. 2005), aleuronic PCD might also be regulated by glutathione.

CONCLUDING REMARKS

The findings described above may be a cue to improving agriculturally and horticulturally important traits that are labile to environmental changes. Since environmental changes determine whether redox-sensing molecules regulating the traits are reduced or oxidized, modifications making the molecules free from redox regulation might make the traits stable regardless of stressful conditions. In order to realize this, it should be necessary to identify which redox-regulated protein regulates the subject traits.

Proteins undergoing *S*-glutathionylation are ubiquitously identified among eukaryotic and prokaryotic cells (Klatt and Lamas 2000; Lillig *et al*. 2003). Considering that certain proteins are reversibly regulated via *S*-glutathionylation/deglutathionylation (Ito *et al*. 2003; Matsumoto and Ogawa 2008), the *S*-glutathionylation/deglutathionylation regulation may be a molecular switch for modulating protein functions. It is similar to the phosphorylation/dephosphorylation system in that both are reversible reactions, but seems more advantageous in that a broad range of proteins are simultaneously regulated by cellular redox changes in response to environmental stimuli. In this regard, glutathione is not only a simple antioxidant but also a key molecule to adapt the plants to the changing environments. Since a large amount of dioxygen is evolved following photosynthesis, ROS levels in chloroplasts are much higher than those in mitochondria. In this respect, plants may have developed the sensing system of cellular redox changes for a survival strategy.

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REFERENCES

Asada K (1999) The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 601-639

Asada K (2000) The water-water cycle as alternative photon and electron sinks. *Philosophical Transactions of the Royal Society of London Biology Series* **355**, 1419-1432

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* **141**, 391-396

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmond CB, Arntzen CJ (Eds) *Photoinhibition*, Elsevier Science Publishers, Amsterdam, The Netherlands, pp 227-287

Baena-González E, Baginsky S, Mulo P, Summer H, Aro EM, Link G (2001) Chloroplast transcription at different light intensities. Glutathione-mediated phosphorylation of the major RNA polymerase involved in redox-regulated organellar gene expression. *Plant Physiology* **127**, 1044-1052

Belmonte MF, Donald G, Reid DM, Yeung EC, Stasolla C (2005) Alterations of the glutathione redox state improve apical meristem structure and somatic embryo quality in white spruce (*Picea glauca*). *Journal of Experimental Botany* **56**, 2355-2364

Borevitz JO, Xia Y, Blount J, Dixon RA, Lamb C (2000) Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. *Plant Cell* **12**, 2383-2394

Buchanan BB, Balmer Y (2005) Redox regulation: a broadening horizon. *Annual Review of Plant Physiology* **56**, 187-220

Cairns NG, Pasternak M, Wachter A, Cobbett CS, Meyer AJ (2006) Maturation of arabidopsis seeds is dependent on glutathione biosynthesis within the embryo. *Plant Physiology* **141**, 446-455

Carol RJ, Takeda S, Linstead P, Durrant MC, Kakesova H, Derbyshire P, Drea S, Zarsky V, Dolan L (2005) A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature* **438**, 1013-1016

Cheng JC, Seeley KA, Sung ZR (1995) *RML1* and *RML2*, *Arabidopsis* genes required for cell proliferation at the root tip. *Plant Physiology* **107**, 365-376

de Jong AJ, Yakimova ET, Kapchina VM, Woltering EJ (2002) A critical role for ethylene in hydrogen peroxide release during programmed cell death in tomato suspension cells. *Planta* **214**, 537-545

del Río LA, Sandalio LM, Corpas FJ, Palma JM, Barroso JB (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant Physiology* **141**, 330-335

Delaunay A, Pflieger D, Barrault MB, Vinh J, Toledano MB (2002) A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. *Cell* **111**, 471-481

Demidchik V, Shabala SN, Coutts KB, Tester MA, Davies JM (2003) Free oxygen radicals regulate plasma membrane Ca^{2+}- and K^+-permeable channels in plant root cells. *Journal of Cell Science* **116**, 81-88

Desikan R, Hancock JT, Bright J, Harrison J, Weir I, Hooley R, Neill SJ (2005) A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. *Plant Physiology* **137**, 831-834

Despres C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR (2003) The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. *Plant Cell* **15**, 2181-2191

Dixon DP, Davis BG, Edwards R (2002) Functional divergence in the glutathione transferase superfamily in plants. *The Journal of Biological Chemistry* **277**, 30859-30869

Dixon DP, Skipsey M, Grundy NM, Edwards R (2005) Stress-induced protein *S*-glutathionylation in Arabidopsis. *Plant Physiology* **138**, 2233-2244

Fath A, Bethke P, Beligni V, Jones R (2002) Active oxygen and cell death in cereal aleurone cells. *Journal of Experimental Botany* **53**, 1273-1282

Fontaine O, Billard JP, Fuault C (1995) Effect of glutathione on dormancy breakage in barley seeds. *Plant Growth Regulation* **16**, 55-58

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Doan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* **422**, 442-446

Fricker MD, May M, Meyer AJ, Sheard N, White NS (2000) Measurement of glutathione levels in intact roots of *Arabidopsis*. *Journal of Microscopy* **198**, 162-173

Fukuda H, Komamine A (1980) Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans*. *Plant Physiology* **65**, 57-60

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW (1994) The *TTG* gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. *Developmental Biology* **166**, 740-754

Gelhaye E, Rouhier N, Navrot N, Jacquot JP (2005) The plant thioredoxin systems. *Cellular and Molecular Life Sciences* **62**, 24-35

Gelhaye E, Rouhier N, Gerard J, Jolivet Y, Gualberto J, Navrot N, Ohlsson PI, Wingsle G, Hirasawa M, Knaff DB, Wang H, Dizengremel P, Meyer Y, Jacquot JP (2004) A specific form of thioredoxin *h* occurs in plant mitochondria and regulates the alternative oxidase. *Proceedings of the National Academy of Sciences USA* **101**, 14545-14550

Gutierréz-Alcalá G, Gotor C, Meyer AJ, Fricker M, Vega JM, Romero LC (2000) Glutathione biosynthesis in *Arabidopsis* trichome cells. *Proceedings of the National Academy of Sciences USA* **97**, 11108-11113

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF^{TIR1}-dependent degradation of AUX/IAA proteins. *Nature* **414**, 271-276

Ha CM, Jun JH, Nam HG, Fletcher JC (2004) *BLADE-ON-PETIOL1* encodes a BTB/POZ domain protein required for leaf morphogenesis in *Arabidopsis thaliana*. *Plant and Cell Physiology* **45**, 1361-1370

Hatano-Iwasaki A, Ogawa K (2007) Redox metabolism in response to environmental stimuli for flowering. *Functional Plant Science and Biotechnology* **1**, 246-253

Heine GF, Hernandez M, Grotewold E (2004) Two cysteines in plant R2R3 MYB domains participate in REDOX-dependent DNA binding. *The Journal of Biological Chemistry* **279**, 37878-37885

Henmi K, Demura T, Tsuboi S, Fukuda H, Iwabuchi M, Ogawa K (2005) Change in the redox state of glutathione regulates differentiation of tracheary elements in *Zinnia* cells and *Arabidopsis* roots. *Plant and Cell Physiology* **46**, 1757-1765

Henmi K, Tsuboi S, Demura T, Fukuda H, Iwabuchi M, Ogawa K (2001) A possible role of glutathione and glutathione disulfide in tracheary element differentiation in the cultured mesophyll cells of *Zinnia elegans*. *Plant and Cell Physiology* **42**, 673-676

Hepworth SR, Zhang Y, McKim S, Li X, Haughn GW (2005) BLADE-ON-PETIOL-dependent signaling controls leaf and floral patterning in Arabidopsis. *Plant Cell* **17**, 1434-1448

Hidég E, Ogawa K, Kálai T, Hidég E (2001) Singlet oxygen imaging in *Ara-*

bidopsis thaliana leaves under photoinhibition by excess photosynthetically active radiation. *Physiologia Plantarum* **112**, 10-14

Holmgren A (1976) Hydrogen donor system for *Escherichia coli* ribonucleoside-diphosphate reductase dependent upon glutathione. *Proceedings of the National Academy of Sciences USA* **73**, 2275-2279

Irihimovitch V, Shapira M (2000) Glutathione redox potential modulated by reactive oxygen species regulates translation of Rubisco large subunit in the chloroplast. *The Journal of Biological Chemistry* **275**, 16289-16295

Ito H, Iwabuchi M, Ogawa K (2003) The sugar-metabolic enzymes aldolase and triose-phosphate isomerase are targets of glutathionylation in *Arabidopsis thaliana*: detection using biotinylated glutathione. *Plant and Cell Physiology* **44**, 655-660

Jones MA, Raymond MJ, Yang Z, Smirnoff N (2007) NADPH oxidase-dependent reactive oxygen species formation required for root hair growth depends on ROP GTPase. *Journal of Experimental Botany* **58**, 1261-1270

Joo JH, Bae YS, Lee JS (2001) Role of auxin-induced reactive oxygen species in root gravitropism. *Plant Physiology* **126**, 1055-1060

Kitamura S, Shikazono N, Tanaka A (2004) *TRANSPARENT TESTA 19* is involved in the accumulation of both anthocyanins and proanthocyanidins in *Arabidopsis*. *The Plant Journal* **37**, 104-114

Klatt P, Lamas S (2000) Regulation of protein function by S-glutathionylation in response to oxidative stress and nitrosative stress. *European Journal of Biochemistry* **267**, 4928-4944

Kranner I, Grill D (1993) Content of low-molecular-weight thiols during the imbibition of pea seeds. *Physiologia Plantarum* **88**, 557-562

Kwak JM, Mori IC, Pei ZM, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. *The EMBO Journal* **22**, 2623-2633

Lillig CH, Potamitou A, Schwenn J-D, Vlamis-Gardikas A, Holmgren A (2003) Redox regulation of 3'-phosphoadenylylsulfate reductase from *Escherichia coli* by glutathione and glutaredoxins. *The Journal of Biological Chemistry* **278**, 22325-22330

Liszkay A, van der Zalm E, Schopfer P (2004) Production of reactive oxygen intermediates ($O_2^{\cdot-}$, H_2O_2, and $^{\cdot}OH$) by maize roots and their role in wall loosening and elongation growth. *Plant Physiology* **136**, 3114-3123

Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* **98**, 4782-4787

Marx C, Wong JH, Buchanan BB (2003) Thioredoxin and germinating barley: targets and protein redox changes. *Planta* **216**, 454-460

Matsumoto M, Ogawa K (2008) New insight into the Calvin cycle regulation - Glutathionylation of fructose bisphosphate aldolase in response to illumination. In: Allen JF, Osmond B, Bolbeck JH, Gantt E (Eds) *Photosynthesis 2007: Energy from the Sun*, Springer, Heidelberg, Germany, in press

Meyer AJ, Hell R (2005) Glutathione homeostasis and redox-regulation by sulfhydryl groups. *Photosynthesis Research* **86**, 435-457

Meinhard M, Grill E (2001) Hydrogen peroxide is a regulator of ABI1, a protein phosphatase 2C from *Arabidopsis*. *FEBS Letters* **508**, 443-446

Meinhard M, Rodriguez PL, Grill E (2002) The sensitivity of ABI2 to hydrogen peroxide links the abscisic acid-response regulator to redox signalling. *Planta* **214**, 775-782

Michelet L, Zaffagnini M, Marchand C, Collin V, Decottignies P, Tsan P, Lancelin JM, Trost P, Miginiac-Maslow M, Noctor G, Lemaire SD (2005) Glutathionylation of chloroplast thioredoxin *f* is a redox signaling mechanism in plants. *Proceedings of the National Academy of Sciences USA* **102**, 16478-16483

Mittler R, Vanderauwera S, Gollery M, van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490-498

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. *Cell* **113**, 935-944

Nagata T, **Todoriki S**, **Hayashi T**, **Shibata Y**, **Mori M**, **Kanegae H, Kikuchi S** (1999) γ-Radiation induces leaf trichome formation in Arabidopsis. *Plant Physiology* **120**, 113-120

Nakanomyo I, Kost B, Chua NH, Fukuda H (2002) Preferential and asymmetrical accumulation of a Rac small GTPase mRNA in differentiating xylem cells of *Zinnia elegans*. *Plant and Cell Physiology* **43**, 1484-1492

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 249-279

Noji M, Inoue K, Kimura N, Gouda A, Saito K (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. *The Journal of Biological Chemistry* **273**, 32739-32745

Norberg M, Holmlund M, Nilsson O (2005) The *BLADE ON PETIOL* genes act redundantly to control the growth and development of lateral organs. *Development* **132**, 2203-2213

Ogawa K (2005) Glutathione-associated regulation of plant growth and stress response. *Antioxidants and Redox Signaling* **7**, 973-981

Ogawa K, Iwabuchi M (2001) A mechanism for promoting the germination of *Zinnia elegans* seeds by hydrogen peroxide. *Plant and Cell Physiology* **42**, 286-291

Ogawa K, Henmi K, Tasaka Y (2001) Cell- or organ-differentiation controllers and method of controlling morphogenesis by using the same. International patent No. WO 01/80638

Ogawa K, Kanematsu S, Asada K (1997) Generation of superoxide anion and localization of CuZn-superoxide dismutase in the vascular tissue of spinach hypocotyls: their association with lignification. *Plant and Cell Physiology* **38**, 1118-1126

Ogawa K, Matsumoto M, Ito H (2005) Fructose-1,6-bisphosphate aldolase is a target protein of glutathionylation in Arabidopsis chloroplasts. In: van der Est A, Bruce D (Eds) *Photosynthesis: Fundamental Aspect to Global Perspectives* (Vol 1), Allen Press, Inc., Lawrence, USA, pp 468-470

Ogawa K, Hatano-Iwasaki A, Yanagida M, Iwabuchi M (2004) Level of glutathione is regulated by ATP-dependent ligation of glutamate and cysteine through photosynthesis in *Arabidopsis thaliana*: mechanism of strong interaction of light intensity with flowering. *Plant and Cell Physiology* **45**, 1-8

Ogawa K, Kanematsu S, Takabe K, Asada K (1995) Attachment of CuZn-superoxide dismutase to thylakoid membranes at the site of superoxide generation (PSI) in spinach chloroplasts: detection by immuno-gold labeling after rapid freezing and substitution method. *Plant and Cell Physiology* **36**, 565-573

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *The Plant Journal* **47**, 124-139

Ono E, Wong HL, Kawasaki T, Hasegawa M, Kodama O, Shimamoto K (2001) Essential role of the small GTPase Rac in disease resistance of rice. *Proceedings of the National Academy of Sciences USA* **98**, 759-764

Pesquet E, Barbier O, Ranocha P, Jauneau A, Goffner D (2004) Multiple gene detection by *in situ* RT-PCR in isolated plant cells and tissues. *The Plant Journal* **39**, 947-959

Petrášek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, Wiśniewska J, Tadele Z, Kubeš M, Čovanová M, Dhonukshe P, Skůpa P, Benková E, Perry L, Křeček P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zažímalová E, Fříml J (2006) PIN proteins perform a rate-limiting function in cellular auxin efflux. *Science* **312**, 914-918

Potikha TS, Collins CC, Johnson DI, Delmer DP, Levine A (1999) The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. *Plant Physiology* **119**, 849-858

Rentel MC, Lecourieux D, Ouaked F, Usher SL, Petersen L, Okamoto H, Knight H, Peck SC, Grierson CS, Hirt H, Knight MR (2004) OXI1 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature* **427**, 858-861

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. *Plant Physiology* **141**, 357-366

Rouhier N, Gelhaye E, Jacquot JP (2004) Plant glutaredoxins: still mysterious reducing systems. *Cellular and Molecular Life Sciences* **61**, 1266-1277

Rouhier N, Villarejo A, Srivastava M, Gelhaye E, Keech O, Droux M, Finkemeier I, Samuelsson G, Dietz KJ, Jacquot JP, Wingsle G (2005) Identification of plant glutaredoxin targets. *Antioxidants and Redox Signaling* **7**, 919-929

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. *Plant Cell* **16**, 616-628

Sánchez-Fernández R, Fricker M, Corben LB, White NS, Sheard N, Leaver CJ, van Montagu M, Inzé D, May MJ (1997) Cell proliferation and hair tip growth in the Arabidopsis root are under mechanistically different forms of redox control. *Proceedings of the National Academy of Sciences USA* **94**, 2745-2750

Sen CK (2000) Cellular thiols and redox-regulated signal transduction. *Current Topics in Cellular Regulation* **36**, 1-30

Senda K, Ogawa K (2004) Induction of PR-1 accumulation accompanied by runaway cell death in the *lsd1* mutant of *Arabidopsis* is dependent on glutathione levels but independent of the redox state of glutathione. *Plant and Cell Physiology* **45**, 1578-1585

Smith VA, Gaskin P, MacMillan J (1990) Partial purification and characterization of the gibberellin A_{20} 3β-hydroxylase from seeds of *Phaseolus vulgaris*. *Plant Physiology* **94**, 1390-1401

Teixeira da Silva JA, Nhut DT (2003) Control of plant organogenesis: Genetic and biochemical signals in plant organ form and development. In: Nhut DT, Van Le B, Tran Thanh Van K, Thorpe T (Eds) *Thin Cell Layer Culture System*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 135-190

Tommasi F, Paciolla C, de Pinto MC, de Gara L (2001) A comparative study of glutathione and ascorbate metabolism during germination of *Pinus pinea* L. seeds. *Journal of Experimental Botany* **52**, 1647-1654

Tron AE, Bertoncini CW, Chan RL, Gonzalez DH (2002) Redox regulation of plant homeodomain transcriptional factors. *The Journal of Biological Chemistry* **277**, 34800-34807

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow T, Hsing YC, Kitano H, Yamaguchi I, Matsuoka M (2005) *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. *Nature* **437**, 693-698

Vanacker H, Sandalio LM, Jiménez A, Palma JM, Corpas FJ, Meseguer V,

Gómez M, Sevilla F, Leterrrier M, Foyer CH, del Río LA (2006) Roles for redox regulation in leaf senescence of pea plants grown on different sources of nitrogen nutrition. *Journal of Experimental Botany* **57**, 1735-1745

Vernoux T, Wilson RC, Seeley KA, Reichheld JP, Muroy S, Brown S, Maughan SC, Cobbett CS, van Montagu M, Inzé D, May MJ, Sung ZR (2000) The *ROOT MERISTEMLESS1/CADMIUM SENSITIVE2* genes defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. *Plant Cell* **12**, 97-109

Wang J, Boja ES, Tan W, Tekle E, Fales HM, English S, Mieyal JJ, Chock PB (2001) Reversible glutathionylation regulates actin polymerization in A431 cells. *The Journal of Biological Chemistry* **276**, 47763-47766

Xiang C, Werner BL, Christensen EM, Oliver DJ (2001) The biological functions of glutathione revisited in arabidopsis transgenic plants with altered glutathione levels. *Plant Physiology* **126**, 564-574

Xie Q, Guo HS, Dallman G, Fang SY, Weissman AM, Chua NH (2002) SINAT5 promotes ubiquitin-related degradation of NAC1 to attenuate auxin signals. *Nature* **419**, 167-170

Xing S, Rosso MG, Zachgo S (2005) *ROXY1*, a member of the plant glutaredoxin family, is required for petal development in *Arabidopsis thaliana*. *Development* **132**, 1555-1565

Yanagida M, Mino M, Iwabuchi M, Ogawa K (2004) Reduced glutathione is a novel regulator of vernalization-induced bolting in the rosette plant *Eustoma grandiflorum*. *Plant and Cell Physiology* **45**, 129-137

Zaffagnini M, Michelet L, Marchand C, Sparla F, Decottignies P, le Marechal P, Miginiac-Maslow M, Noctor G, Trost P, Lemaire SD (2007) The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-phosphate dehydrogenase is selectively regulated by glutathionylation. *FEBS Journal* **274**, 212-226