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Redox Metabolism in Response to Environmental Stimuli for Flowering

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ABSTRACT

It is generally known that flowering is affected by various stresses such as chilling, strong light, drought, wounding, and pathogen infection. Such stresses increase the cellular levels of reactive oxygen species (ROS) altering the cellular redox state of the plant. Recent studies have shown that, in plants, ROS are crucial molecules for signaling responses to biotic and abiotic stresses and for regulation of growth and development, probably including flowering. In this review, we provide information on the regulation of flowering and its relation of the cellular redox state. Based on this information, we discuss how flowering processes are influenced by environmental stimuli.

Keywords: Flowering, glutathione (GSH), reactive oxygen species (ROS), redox, stress
Abbreviations: ABA, abscisic acid; **APX**, ascorbate peroxidase; **AsA**, ascorbate; **BSO**, buthionine sulfoximine; **γ-ECS**, γ-glutamylcysteine synthetase; **GA**, gibberellin; **GSH**, glutathione; **JA**, jasmonic acid; **LOX**, lipoxygenase; **PSI and PSII**, photosystem I and II; **ROS**, reactive oxygen species; **SA**, salicylic acid

CONTENTS

INTRODUCTION

Plants are always exposed to various environmental stimuli and sense environmental changes to adapt themselves to the given situation. They adapt not only their size and shape, but also time of germination and flowering to the changing environment. When environmental stimuli, such as temperature, light, humidity, water availability and soil nutrients, exceed or do not reach their demand (we call such a situation "plants are exposed to environmental stress"), they become dormant or flower earlier to leave their descendants. Furthermore, plant growth and development are altered in response to insect herbivory, fungal and bacterial infection. Moreover, some plants have to undergo stress stimuli, such as winter and drought period, for flowering. However, how such stress stimuli influence plant growth and development is still not fully understood.

The flowering process is one of the keys for plants to leave their descendants. Thanks to recent progress in studies on genetic pathways controlling flowering, numerous genes involved in several flowering pathways have been identified mainly in *Arabidopsis* (Bäurle and Dean 2006). Nevertheless, the molecular mechanisms of stress-induced flowering are poorly understood.

Although reactive oxygen species (ROS) are toxic to cells (Noctor and Foyer 1998; Asada 1999), they are continuously produced as byproducts of photosynthesis and respiration in chloroplasts, mitochondria and peroxisomes in plant cells (Apel and Hirt 2004; Mittler *et al*. 2004) and cellular levels of ROS are increased by environmental stress due to restriction of the electron acceptor of photosynthesis and respiration. Recent studies have shown that ROS play crucial roles in signaling responses to biotic and abiotic stress and in developmental regulation (see a review by

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Invited Review

Henmi *et al*. 2007). Cellular levels of ROS are kept low by various enzymatic and non-enzymatic mechanisms involving glutathione (GSH). GSH has recently been suggested to be associated with flowering (Ogawa *et al*. 2001). This is a cue to understanding how flowering is influenced by environmental stress since GSH metabolism is regulated by ROS. In this review, we introduce redox metabolism and flowering regulation to give further insight into how stress stimuli influence flowering.

CELLULAR REDOX STATE AND FLOWERING

Redox homeostasis

ROS is a generic term for singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH). Since ROS, except for H_2O_2, is unstable and short-lived. Since $^1O_2$ and ·OH are short-lived and highly reactive and high concentrations of cellular components such as proteins increase the viscosity of the cellular fluid, these ROS thus react with any cellular components in the vicinity of their generation site, so that they cannot diffuse so far from their generation site. O_2^- and H_2O_2 have life enough to diffuse as signal molecule, but the charged O_2^- cannot translocate through the lipid membrane and its half life is remarkably reduced at low pH conditions such as apoplasts (pH 5.5). Therefore H_2O_2 is most likely a long-distance signal. *In vitro*, O_2^- is spontaneously disproportionated to H_2O_2 and O_2 but in plant cells, even in the low-pH compartment apoplast, O_2^- cannot be converted to H_2O_2 without superoxide dismutase (SOD) that catalyzes the disproportionation of O_2^- at a diffusion-controlled rate (Ogawa *et al*. 1997). In this regard, SOD exists for H_2O_2 generation and H_2O_2 is the most potent signal for developmental regulation. Plants have developed antioxidant systems to control cellular levels of ROS (Asada and Takahashi 1987; Mittler 2002; Apel and Hirt 2004). The major scavenging system is the ascorbate (AsA)-glutathione (GSH) cycle (Asada and Takahashi 1987; Noctor and Foyer 1998). The AsA-GSH cycle has so far been shown to exist in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes. In the cycle, as shown in **Fig. 1**, O_2^- is disproportionated into H_2O_2 and O_2 by SOD, and then H_2O_2 is reduced to H_2O by ascorbate peroxidase (APX) using AsA as an electron donor. Monodehydroascorbate and dehydroascorbate following scavenging of H_2O_2 are enzymatically reduced back to AsA using GSH and NADPH as electron donors. Oxidized glutathione (GSSG) is reduced by GSSG reductase. Owing to this redox buffering system, the cellular redox state is constantly kept reduced.

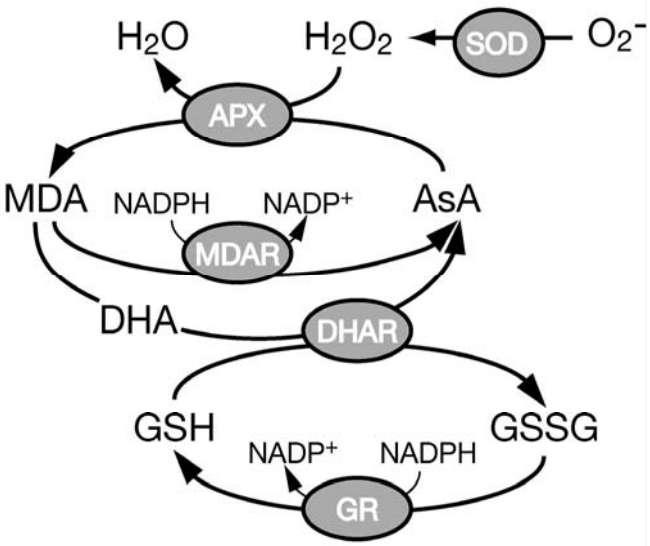

Fig. 1 The ascorbate-glutathione cycle. O_2^- is disproportionated into H_2O_2 and O_2 by superoxide dismutase (SOD), and then H_2O_2 is reduced to H_2O by ascorbate peroxidase (APX) using AsA as an electron donor, followed by oxidation of AsA to monodehydroascorbate (MDA). The MDA is re-reduced to AsA by MDA reductase (MDAR) using NADPH. Part of MDA is spontaneously disproportionated to dehydroascorbate (DHA) and AsA. DHA is regenerated to AsA by DHA reductase (DHAR) using GSH as an electron donor, and the resulting oxidized glutathione (GSSG) is reduced to GSH by glutathione reductase (GR) using NADPH. AsA, ascorbate**;** GSH, reduced glutathione.

Glutathione biosynthesis

GSH is a ubiquitous tripeptide that is synthesized from cysteine, glutamate and glycine in two reactions 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 (PSII), accumulates the GSH precursor cysteine with decreased levels of GSH (40 to 80% of wild-type level) (Ogawa *et al*. 2004). Considering this together with the fact that cysteine synthesis takes place in three compartments (chloroplasts, mitochondria and cytosol) and is negatively feed-back regulated in mitochondria and cytosol but not in chloroplasts (Noji *et al*. 1998), it is suggested that GSH is synthesized in chloroplasts and that γ-ECS reaction is a limiting step for GSH synthesis (Ogawa *et al*. 2004).

Compartments generating ROS

The major compartment for ROS generation is the chloroplast (Asada 1999). Electrons abstracted from H_2O in PSII are transmitted to $NADP^+$ in photosystem I (PSI) to produce NADPH. When the electron flux to the Calvin cycle is suppressed and/or excess photons are available for photosynthesis, $NADP^+$ availability is limited and excess electrons univalently reduce O_2 to O_2^- in PSI. When plants are exposed to strong light, the available photon energy exceeds the demand for photosynthesis resulting in excess electrons. The consumption of NADPH for CO_2 fixation is suppressed by environmental stresses, such as high and low temperatures, drought, submergence, high salinity, excess heavy metals, nutrient deficiency, etc. Chilling stress limits enzymatic activities for CO_2 fixation in the Calvin cycle. Drought stress induces stomatal closure, so that it restricts CO_2 availability for the Calvin cycle. Since the Calvin cycle is liable to be inactivated by oxidation, oxidative stress brought by high salinity, excess heavy metals, nutrient deficiency, etc. would restrict it. Biotic stress has been suggested to downregulate gene expression of proteins involved in photosynthesis, which may also increase ROS generation in chloroplasts. Therefore, under such stress conditions, the availability of the electron acceptor $NADP^+$ in PSI is restricted and excess electrons reduce O_2 to generate O_2^- in PSI.

ROS are also generated from the plasma membrane NADPH oxidase, mitochondrial alternative oxidase and peroxisomal oxidase (Mittler *et al*. 2004). Biotic stresses, including pathogen infection and insect herbivory induce ROS generation. In addition to preformed physical barriers such as the cuticle and cell walls and biochemical defenses such as antimicrobial toxins, plants have evolved a rapid inducible defense mechanism that is activated by pathogen attack. This activation process includes rapid activation of plasma-membrane NADPH oxidase to generate ROS, mainly O_2^- and H_2O_2, for defense signaling (Doke 1985; Lamb and Dixon 1997). Besides NADPH oxidases, peroxidase, and amine oxidase may be involved in ROS generation (reviewed in Apel and Hirt 2004; Mittler *et al*. 2004). Wounding such as insect herbivory induces ROS generation (Bi and Felton 1995; Orozco-Cárdenas and Ryan 1999), which is suggested to be attributed to NADPH oxidase (Orozco-Cárdenas *et al*. 2001). In these ways, abiotic and biotic stresses provoke oxidation, affecting the cellular redox state of plants.

ROS metabolism in flowering

Despite their toxicity, multiple roles of ROS have been suggested. ROS regulate plant growth and developmental processes such as seed germination and are required for biochemical processes such as lignification (Ogawa *et al*. 1997; Ogawa and Iwabuchi 2001; Foreman *et al*. 2003). Furthermore, ROS are essential molecules for phytohormone signaling and stress response, for example, abscisic acid (ABA)-induced stomatal closure, salicylic acid (SA)-related plant defense reaction (Chen *et al*. 1993; Zhang *et al*. 2001).

At the time of bolting and flowering in *Arabidopsis*, APX activity has been reported to decrease, being accompanied with lipid peroxidation by lipoxygenase (LOX) (Ye *et al*. 2000). Down-regulation of APX activity may increase ROS levels. Lipid peroxides are generated by oxidative stress and enhance the oxidative stress. When *Pharbitis nil* is grown at various temperatures from 15 to 35°C, 30°C is the most suitable temperature for flower bud formation and opening. LOX activity is also maximally induced when the plant is grown for 4 weeks at 30°C (Nam *et al*. 2005). The flowering inducer theobroxide, which was early identified as a natural product to promote potato tuber growth by means of inducing tuberonic acid, increases LOX activity in *P. nil* (Yoshihara *et al*. 2000; Gao *et al*. 2003). Thus, LOX activity is likely associated with flowering in plants.

Association of GSH with flowering

GSH has been found to play multiple roles in plant growth and development, and flowering (Ogawa 2005).

GSH is associated with the regulation of flowering in some plants. In *Arabidopsis*, the *cad2-1* mutation and gene silencing of γ-ECS reduce the levels of GSH (Cobbett *et al*. 1998; Ogawa *et al*. 2004) and delay flowering time with an increase in the number of rosette leaves at flowering that is an index of flowering (Ogawa *et al*. 2001, 2004). Under long-day conditions, the late-flowering phenotype in the *cad2-1* mutant is hastened by the application of buthionine sulfoximine (BSO), a specific inhibitor of γ-ECS, and restored partially by supplementation with GSH (Ogawa *et al*. 2001). These results suggest that a certain amount of GSH synthesized *de novo* is required for regulation of flowering in *Arabidopsis*.

In contrast, down-regulation of GSH biosynthesis and/or GSH levels during the late growth period is required for promoting flowering as well as up-regulation during the early growth period. An *Arabidopsis* mutant *of FCA* (*FLOWERING TIME CONTROL LOCUS A*) involved in the autonomous pathway exhibits the late-flowering phenotype that is hastened by vernalization (Koornneef *et al*. 1998; Levy and Dean 1998). The *fca-1* mutant has high levels of GSH (Ogawa *et al*. 2001). Under long-day conditions, flowering of the *fca-1* mutant is promoted by 7-day treatment with BSO when the treatment is started at 17 days after imbibition but retarded when it is started at 12 days after imbibition (Ogawa *et al*. 2001). This suggests that down-regulation of GSH biosynthesis at late stages and a crucial amount of GSH synthesized during early growth period are important for promotion of flowering. Overexpression of γ-ECS increases plant GSH with increasing light intensity from 25 to 500 μE/m^2/s (Hatano-Iwasaki and Ogawa, unpublished data) and delays flowering of plants grown at lower intensity of light (50 μE/m^2/s, **Fig. 3**), supporting the above conclusion. Under short-day conditions, flowering of wild-type Landsberg *erecta* (L*er*) plants is promoted by application of BSO (**Fig. 2**), which may sug-

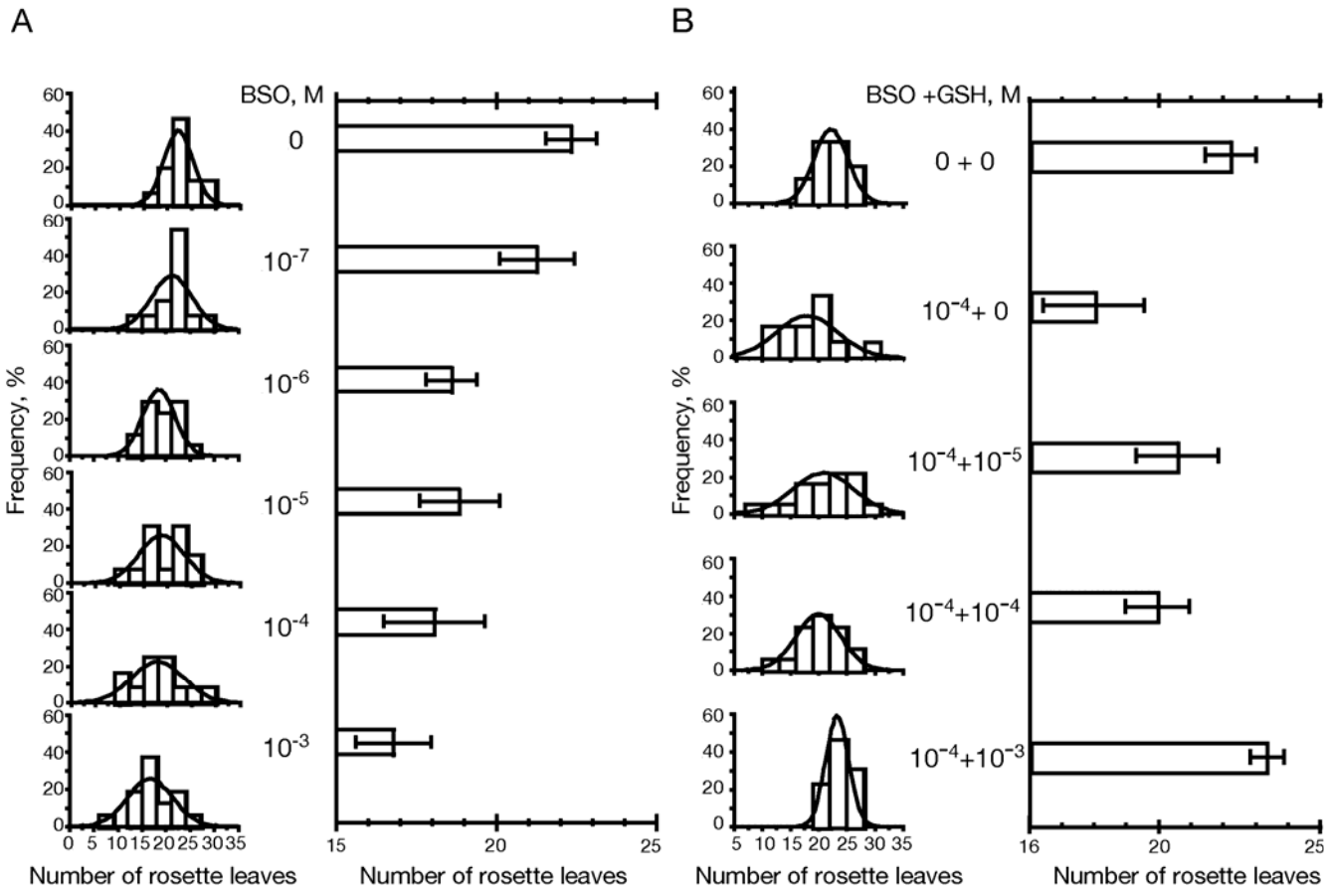

Fig. 2 Effects of BSO, an inhibitor of glutathione biosynthesis, on flowering of wild-type (L*er*) plants. Plants grown on soil under short-day conditions were treated with BSO alone (A) or together with GSH (B) at the indicated concentration (M) from 21 to 26 day after sowing. The number of rosette leaves was counted at flowering. Left panels indicate distributions of number of rosette leaves in wild-type (L*er*) populations and right panels indicate mean ± SE. Flowering of L*er* was promoted by application of BSO and the promotional effects of BSO are eliminated by addition of GSH. BSO, buthionine sulfoximine. Ogawa K *et al.* (unpublished).

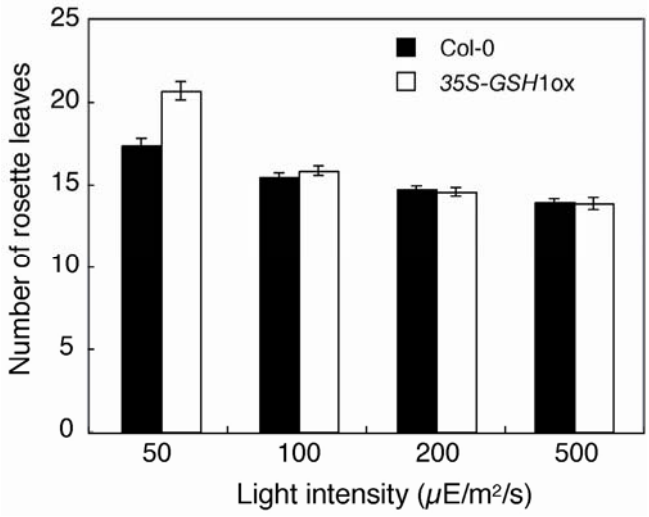

Fig. 3 Dependency of flowering of *GSH1*-overexpressing plants on light intensity. Plants were grown on soil at the indicated intensities of light until the number of rosette leaves at flowering was counted. Each value is the mean ± SE (n = 25-50). Flowering of *GSH1*-overexpressing plants (*35S-GSH1*ox) was delayed compared to that of wild-type (Col-0) plants. Hatano-Iwasaki A and Ogawa K (unpublished).

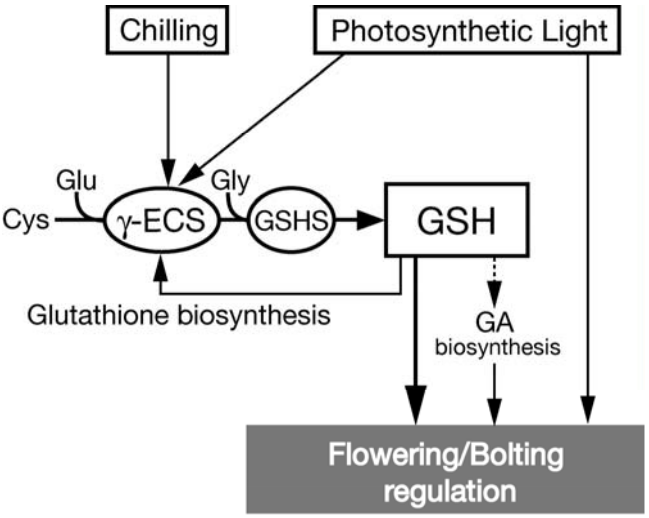

Fig. 4 A hypothetic scheme of flowering regulation via glutathione biosynthesis and regulation by photosynthetic light and chilling stress. The arrows with the thin lines indicate GSH biosynthesis and the existence of a link but do not necessarily positive regulation. The arrow with the dashed line indicates possible interaction of GSH with GA biosynthesis. Photosynthetic light and chilling stress are associated with flowering regulation via GSH biosynthesis. Both the level and redox state of glutathione are associated with flowering and/or bolting regulation. Cys, cysteine; γ-ECS, γ-glutamylcysteine synthetase; GA, gibberellin; Glu, glutamate; Gly, glycine: GSH, glutathione; GSHS, glutathione synthetase.

gest that GSH levels are kept high in the night length of prevailing photoperiod that promotes reproductive growth.

The difference in flowering between wild-type and ECS-overexpressing transgenic plants is reduced with increasing light intensity (100-500 μE/m^2/s) (**Fig. 3**). The γ-ECS activity is regulated by negative feedback by high levels of GSH, i.e. an over-reduced state (May and Leaver 1994; Noctor *et al*. 2002). Similarly, sulfur assimilation leads to the synthesis of cysteine, the precursor of GSH biosynthesis, and is stimulated by oxidants, such as GSSG and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and inhibited by reductants, such as GSH, dithiothreitol (DTT), and 2-mercaptoethanol (2-ME) (Bick *et al*. 2001). Thus, over-reduced states suppress GSH biosynthesis and suppress flowering under weak-light conditions, while under strong-light conditions, the cellular redox state becomes more oxidative, thus promoting flowering. Both levels and redox state of GSH seem important for flowering regulation (**Fig. 4**).

Ascorbate and flowering

Plants have high levels of AsA, which is vitamin C acting as a redox buffer (Foyer *et al*. 1983). AsA has also been suggested to influence flowering. Alteration of AsA levels affects *Arabidopsis* flowering. The *vtc-1* mutant deficient in AsA due to a mutation in GDP-mannose pyrophosphorylase, which is involved in the AsA biosynthesis pathway (Smirnoff *et al*. 2001), shows late-flowering phenotype under short-day conditions (Veljovic-Jovanovic *et al*. 2001; Pavet *et al*. 2005).

A phytohormone, gibberellin (GA), acts as a promoter of flowering through the induction of the floral integrator *LFAFY* (*LFY*) under short-day condition in *Arabidopsis* (Wilson *et al*. 1992; Blázquez *et al*. 1998). GA biosynthetic enzymes require AsA for its activities as a co-factor. Antisense transgenic *Arabidopsis* of the GA 20-oxidase gene, which encodes an enzyme of GA-biosynthesis, show late-flowering phenotype only under short-day conditions, as does *vtc-1* (Coles *et al*. 1999). Therefore, it is likely that cellular levels of AsA influence through the GA-mediated flowering pathway that is stronger in short-day conditions. Under short-day conditions, levels of ABA and GSH in *vtc-1* mutant plants are 1.6-fold and 1.3-fold higher, respectively, than those in wild-type plants (Veljovic-Jovanovic *et al*. 2001; Pastori *et al*. 2003; Pavet *et al*. 2005), although the ABA biosynthetic pathway requires AsA. The late-flowering phenotype of the *vtc-1* mutant might also be attributed to the high level of ABA because ABA acts negatively in flowering (see a review by Levy and Dean 1998). It is also possible that the level of GSH contributes to late-flowering phenotype in the *vtc1* mutant, based on the above observations that high levels of GSH retard flowering. It is likely that FCA negatively regulates GSH levels because of high levels of GSH in the *fca* mutant (Ogawa *et al*. 2001). Recently, FCA has been identified as a receptor for ABA (Razem *et al*. 2006). This might suggest an association of GSH with ABA via FCA regarding flowering regulation.

Under long-day conditions, *vtc1-1* shows a late-flowering phenotype only at a low light intensity of 25 μE/m^2/s and its phenotype is restored by high light intensity of 100-500 μE/m^2/s, as do the plants with altered GSH levels (**Fig. 5**). This might suggest that GSH has something to do with the late-flowering phenotype in the *vtc1-1* at low light in-

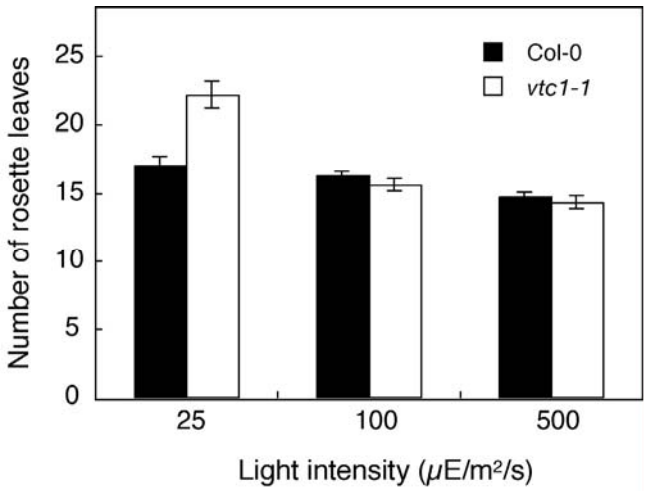

Fig. 5 Flowering behavior in the *vtc1-1* mutants depended on light intensity. Plants were grown on soil at the indicated intensities of light until the number of rosette leaves at flowering was counted. Each value is the mean ± SE (n = 12-37). The *vtc1-1* mutants show the late-flowering phenotypes compared to wild-type (Col-0) plants at low light intensity but the phenotypes restore at higher light intensities. Hatano-Iwasaki A and Ogawa K (unpublished).

tensities. However, another report showed the early flowering phenotype of the *vtc-1* mutants (Conklin and Barth 2004). One explanation for the opposite phenotype of flowering in the *vtc-1* mutants may be the different growth conditions used in those studies, in particular, light intensity, because, as shown in **Fig. 5**, light intensity remarkably affects the flowering behavior of the *vtc-1* mutants. In Conklin and Barth's conditions in which light intensity is probably sufficient for promoting flowering, application of the AsA precursor L-galactono-1,4-lactone increases AsA levels, and delays flowering and *LFY* expression in *Arabidopsis* (Attolico and de Tullio 2006; Barth *et al*. 2006). In addition, high levels of SA in *vtc-1* might contribute to the early-flowering phenotype (Martinez *et al*. 2004; described below).

FLOWERING BY PHOTOSYNTHETIC LIGHT

GSH is associated with flowering regulation through photosynthesis. In *Arabidopsis*, the late-flowering phenotypes of plants having reduced levels of GSH are reduced with an increase in light intensity. GSH levels increase with increasing the light intensity in the ranges of 25-100 $\mu E/m^2/s$, accompanied with the promotion of flowering (Ogawa *et al*. 2004). The late-flowering phenotype of the *ch1-1* mutant defective in photosynthetic light-harvesting is reduced by increasing light intensity or overexpression of γ-ECS (Ogawa *et al*. 2004). Interestingly, it was also suggested in the same paper that light has a suppressive effect on flowering through photosynthesis. The *ch1-1* mutant flowers earlier than wild-type plants at high light intensities.

INTERACTION BETWEEN ENVIRONMENTAL STIMULI AND FLOWERING

Chilling

Vernalization (experiencing a certain period of chilling) is required for flowering of many biennial and winter annual plants. Chilling stress causes changes in GSH levels (Walker and McKersie 1993; Kocsy *et al*. 1996; O'Kane *et al*. 1996) and enhances GSH biosynthesis in plants (Kocsy *et al*. 2000). *Eustoma grandiflorum* is a rosette plant and requires a certain period of chilling for bolting. In *E. grandiflorum*, application of 1 mM GSH to the growth medium is able to induce bolting without vernalization but other thiols, DTT and 2-ME are not (Yanagida *et al*. 2004). Furthermore, the inductive effect of vernalization on bolting is eliminated by the application of BSO to the growth medium and BSO-mediated inhibition is abolished by application of GSH in a dose-dependent manner (0.1 to 1 mM) (Yanagida *et al*. 2004). During the vernalization period, GSH levels increase with lipid peroxides and γ-ECS activity. Thus, vernalization stimulates GSH biosynthesis for bolting and flowering in this plant.

The late-flowering phenotype of the *Arabidopsis fca-1* mutant, which has high levels of GSH, is partially restored by vernalization with a transient decrease of GSH (Ogawa *et al*. 2001). These findings in *Eustoma* and *Arabidopsis* suggest that chilling stress regulates flowering and/or bolting through a GSH-associated flowering pathway (**Fig. 4**).

GA plays a key role in promotion of flowering by vernalization in rosette plants (Hillman 1969). Chilling stress modulates the metabolism and turnover of GA precursors in plants, such as *Thlaspi arvense* (Metzger 1990; Hazebroek *et al*. 1993) and *Raphanus sativus* (Nakayama *et al*. 1995) and also GA-responsible genes and sensitivity to GA in *Arabidopsis* and *Eustoma* (Oka *et al*. 2001). Genes involved in GA biosynthesis, such as genes encoding GA20-oxidase and GA3-β-hydroxidase, are up-regulated during and after chilling (Mino *et al*. 2003). Given the fact that these genes are active in presence of the reductant (Lange *et al*. 1994), GSH-regulated pathways of bolting and flowering are promoted by vernalization, probably via GA biosynthesis (**Fig. 4**).

Strong light and drought

As described above, environmental stresses such as strong light and drought induce ROS generation. GSH biosynthesis is activated by oxidative stress induced by strong light and drought stresses. Exposure of *Arabidopsis* plants that have been grown at a light intensity of 50 $\mu E/m^2/s$ to relatively strong light (450 $\mu E/m^2/s$) increases plant GSH and the increase is 2- or 3-fold higher than that in case of moderate light (150 $\mu E/m^2/s$) (Karpinski *et al*. 2003). In *Begonia* × *erythrophylla*, the acclimation of 10-day shaded leaves to sunlight 30% increases leaf GSH (Burritt and Mackenzie 2003).

It has been reported that GSH levels increase in response to drought stress. In *Myrothamnus flabellifolia*, a short woody shrub from Africa, GSH levels are increased by 10-day desiccation (Kranner *et al*. 2002). In a C_4 plant, *Sorghum bicolor*, it has been shown that GSH levels increase following seven-day drought treatment (Zhang and Kirkham 1996). In a CAM (Crassulacean acid metabolism) plant, *Sedum album* L., a 12-day drought stress increases APX activity (Castillo 1996).

In these ways, such stress stimuli may influence flowering regulation through GSH-regulated flowering pathway, although the mechanism is not fully understood.

INFLUENCE OF BIOTIC STRESS ON FLOWERING

Phytohormones such as jasmonic acid (JA) and SA are involved in stress response to biotic stresses including insect herbivory and pathogen infection, these being followed by ROS generation. These phytohormones are also associated with regulation of various developmental processes and GSH metabolism. It is suggested that there is a relation between these phytohormones and flowering.

Salicylic acid

SA is a well-known phytohormone as a marker of biotic stress responses, such as pathogen infection. SA induces ROS production following response to pathogen defense reaction, such as systemic acquired resistance (Chen *et al*. 1993). It has been proposed that SA is associated with regulation of flowering in plants (Cleland 1974; Cleland and Ajami 1974). In *Arabidopsis*, UV-C light irradiation promotes the flowering with increased SA levels and SA-deficient plants show late-flowering phenotype (Martínez *et al*. 2004). SA is closely related to the level and redox state of GSH. In plant-pathogen interactions, GSH redox state changes with SA levels (Vanacker *et al*. 2000; Mou *et al*. 2003). GSH levels are changeable by SA with SA-inducible genes for pathogen defense, such as pathogen-related protein 1 (PR1) gene, via nonexpressor of pathogenesis-related protein 1 (NPR-1) dependent pathway (Mou *et al*. 2003; Ball *et al*. 2004; Gomez *et al*. 2004; Senda and Ogawa 2004). Plants grown under strong-light conditions have high levels of SA and GSH than those grown under weak-light conditions (Karpinski *et al*. 2003; Ogawa *et al*. 2004). Recently, It has been reported that levels of SA and GSH are mutually correlated and interact with each other (Mateo *et al*. 2006). SA-deficient plants have low levels of GSH and the mutants harboring high levels of SA have high levels of GSH (Mateo *et al*. 2006). Therefore, the SA-regulated flowering pathway might be associated with the GSH-regulated flowering pathway. It is possible that SA acts downstream of the GSH-regulated pathway, because the late-flowering phenotype of SA-defective plants is not restored by UV-C irradiation and high light intensity. Based on the observations that SA deficiency does not affect promotion of flowering by vernalization and that exogenous SA does not promote flowering in the *fca* mutant, it might be suggested that SA regulates flowering via the GSH-regulated pathway.

Jasmonic acid

JA is an essential phytohormone that regulates plant responses to biotic stresses, such as wounding and pathogen infection (Creelman and Mullet 1997; Thomma *et al*. 1998). JA is associated with regulation of many physiological and developmental processes, including root growth, fruit ripening, senescence, and pollen development. JA is synthesized via the 13-LOX-catalyzed oxygenation of α-linolenic acid. LOXs are associated with flowering regulation (Ye *et al*. 2000; Nam *et al*. 2005). Theobroxide, which is a natural product and known as a flowering inducer, induces not only flowering but also JA formation (Yoshihara *et al*. 2000; Kong *et al*. 2005). Another flowering inducer, 9-hydroxy-10-oxo-12(*Z*),15(*Z*)-octadecadienoic acid (KODA), is synthesized from α-linolenic acid by 9-LOX, which is different from the enzymes in the JA biosynthesis pathway, and allene oxide synthase (AOS), which acts in the JA biosynthesis pathway (Yokoyama *et al*. 2000; Suzuki *et al*. 2003). It has been suggested that AOS plays a role in flowering in *Pharbitis nil* (Kong *et al*. 2005). Thus, it is suggested that JA biosynthesis is closely related to flowering.

Moreover, it has been reported that the metabolic pathway-related genes of GSH and AsA are induced by exogenous JA (Xiang and Oliver 1998; Sasaki-Sekimoto *et al*. 2005). Regulation of development and stress response, which involves JA signaling, might be associated with GSH and AsA (**Fig. 6**). It is horticulturally known that wounding stress, such as cutting, is able to induce bolting and flowering in plants. It is possible that JA is associated with induction of the flowering response to such stress stimuli.

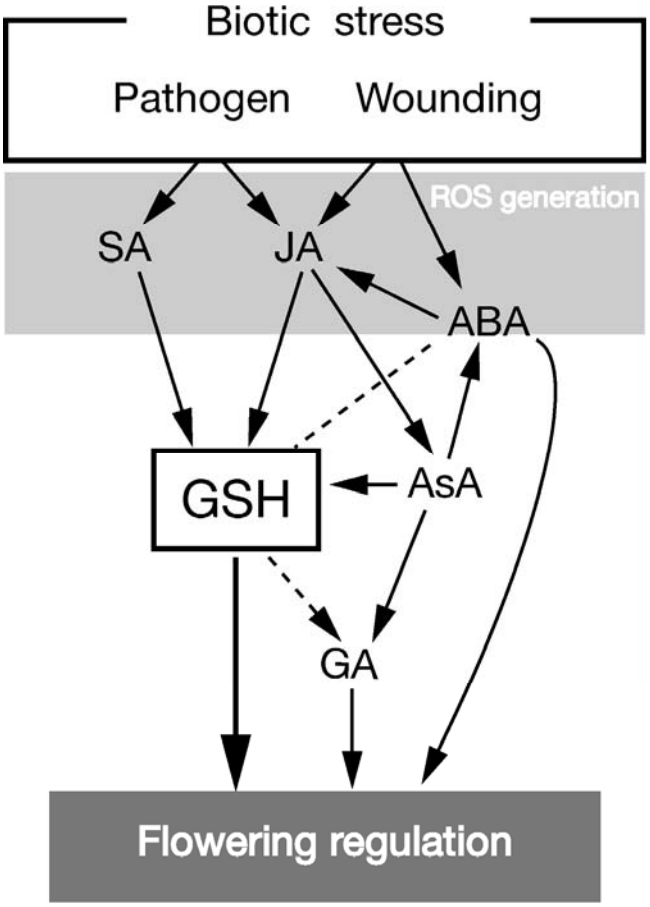

Fig. 6 A putative scheme of flowering regulation via glutathione and phytohormones, SA, JA, and ABA. The arrows with the thin lines indicate the existence of a link but do not necessarily positive regulation. Possibly interactions of GSH with ABA or GA indicate as the dashed line and the arrow with the dashed line, respectively. Stresses induce to generation of ROS and signaling pathways response to stresses are involved in phytohormones. Moreover, it has been proposed that phytohormones can affect GSH metabolism, resulting in alteration of the redox status and /or the leves of GSH. Therefore, flowering affected by such stresses seems to be associated with GSH-related regulation via phytohormones. ABA, abscisic acid; AsA, ascorbate; GA, gibberellin; GSH, glutathione; JA, jasmonic acid; ROS, reactive oxygen species; SA, salicylic acid.

CONCLUDING REMARKS

Agriculture and horticulture are not necessarily based on scientific principles but also on accumulation of empirical knowledge. The finding of the GSH-associated flowering pathway has provided a cue to understanding some empirical knowledge at the molecular level. The more empirical knowledge is understood at the molecular level, the easier agricultural and horticultural improvement of culture method and breeding are. Since many plants including ornaental flowers and vegetable crops require vernalization stiuli for flowering, an artificial temperature control for chiling treatment has been required to control flowering. Based on empirical knowledge, it has also been carried out to perorm shoot- or root-cutting, GA-treatment, and injuring bark in order to promote flowering and fruit and seed production. The more the mechanisms of such stresses are known, the more endogenous factors associated with stress-induced flowering will be able to be monitored for optimization of manipulation of flowering. Therefore, we expect further progress in studies on the mechanism for how cellular redox state regulates plant growth and development. Since it has been known that stress stimuli also influence insect dormancy and animal development, progress in understanding of how plant growth and development is influenced by stress stimuli might contribute to a more general understanding of stress physiology.

Despite cellular levels of AsA being high enough to act alone as an antioxidant in plant cells (Foyer *et al*. 1983) and that regeneration of AsA does not necessarily require glutathione, cellular levels of GSH are also high and therefore it seems to be the backup system for AsA regeneration in the AsA-GSH cycle. The finding of a physiological function of GSH in flowering regulation also opens new insight into GSH. Among several possibilities of the mechanism of GSH-associated flowering regulation have been discussed (Ogawa 2005), we expect that one of key regulations is glutathionylation of proteins, i.e. the formation of a mixed disulfide bond between GSH and specific cysteine residues of proteins. It is noteworthy that plants have a diversified protein family of glutaredoxins (Grxs) (Fernandes and Holmgren 2004; Buchanan *et al*. 2005), which mediate glutathionylation as well as reduction of proteins. This might be important for further understanding of the function of GSH in flowering regulation.

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