Chapter 8
Response of Photosynthetic Organelles to Abiotic Stress: Modulation by Sulfur Metabolism

Basanti Biswal(✉), Mukesh K. Raval, Udaya C. Biswal and Padmanabha Joshi

Abstract Sulfur metabolism mediated modulation of plant response to various abiotic stress factors is the focus of this review. Since chloroplast is extremely sensitive to abiotic stress factors and at the same time a major location of sulfur assimilation, the organelle plays a major role in the modulation of stress response. The photosynthetic organelle coordinates carbon, nitrogen, and sulfur metabolic pathways and provides the essential precursors for synthesis of sulfur compounds. The abiotic stress factors like high light, low light, temperature extremes, drought, and UV radiations which the organelle experiences lead to creation of an oxidative environment and production of reactive oxygen species (ROS). Sulfur metabolites containing thiol residues with reversible oxidation-reduction potential effectively scavenge ROS in a series of biochemical reactions. Abiotic stress factors cause up- and downregulations of several stress-related genes. The stress signals, their transmission, and downstream signaling network regulating gene expression are complex. The stress-induced redox signals generated in chloroplast play a major role in different signal transduction systems and expression of stress-responsive genes in green plants.

1 Introduction

In recent years, the molecular physiology of sulfur assimilation has become one of the major studies in plant science. Sulfur assimilates not only play key roles in the primary metabolism of plants and provide structural components of essential cellular molecules, some of these assimilates act as signaling molecules for cellular communication with the environment. The synthesis of an initial metabolic sulfur compound like cysteine (Cys) involves sulfur uptake through specific transporters and its subsequent assimilation in different locations of the cell. The important secondary metabolic sulfur compounds like glutathione (GSH), phytochelatins

Basanti Biswal
School of Life Sciences, Sambalpur University, Jyotivihar-768019, Orissa, India
basanti_b@hotmail.com

N.A. Khan et al. (eds.), *Sulfur Assimilation and Abiotic Stress in Plants.*

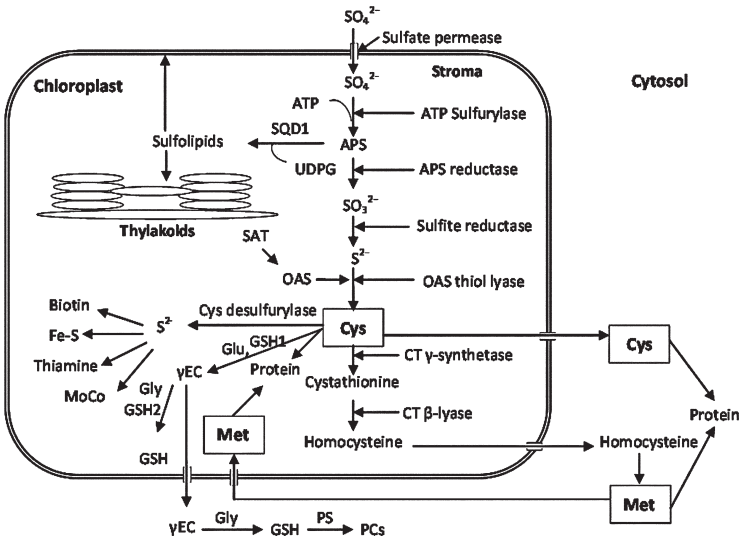

Fig. 8.1 A scheme for sulfur assimilation in chloroplast. ATP: adenosine triphosphate, APS: adenosine phosphosulfate, Cys: cysteine, CT: cystathionine, γEC: γ-glutamylcysteine, GSH1: γEC synthetase, GSH2: glutathione synthetase, Met: methionine, MoCo: molybdenum cofactor, OAS: O-acetyl serine, PC: phytochelatin, PS: phytochelatin synthase, SAT: serine acetyltransferase, SQD1: enzyme for catalysis of formation of sulfolipid- 6-sulfo-α-D-quinovosyl diacylglycerol, UDPG: uridine diphophoglucose. (Modified after Mullineaux and Rausch 2005, Pilon-Smits and Pilon 2006)

(PCs), sulfolipids, vitamins, and many other regulatory compounds are subsequently synthesized in different metabolic routes with Cys as the precursor. The assimilatory pathways of sulfur in chloroplast and sulfur metabolism as a part of cellular metabolic network for synthesis of other secondary sulfur metabolites are shown in Fig.8.1. The sulfur assimilatory pathway involves the assimilatory pathways of an carbon and nitrogen. It also needs involvement of an electron transport pathway that provides adenosine triphosphate (ATP) and reducing power. In this respect, cells of green plants are unique for developing a distinct metabolic combination of the pathways that effectively provide the requirements for the assimilation of this essential nutrient.

The level of crucial metabolites that regulates the sulfur assimilation process is dynamic and is sensitive to environmental changes. Therefore, the pool size of the assimilated sulfur in green plants depends not only on the availability of sulfur but also on the environmental conditions that affect the regulatory mechanism associated with the process of its assimilation. The plants, however, possess different sensory mechanisms that respond to environmental signals and tend to effectively modulate the pool. The pool size of some of the sulfur compounds, especially the compounds with thiol groups which are sensitive to oxidized environment, is important in sulfur metabolism. The thiol-containing sulfur metabolites are well known as potential modulators of the stress response. Therefore, green plants experiencing abiotic stress need these metabolites to develop effective adaptive mechanisms to counter the stress effect. The stress-induced increase in expression of

sulfur transporters and enzymes involved in its assimilatory pathway have been recently investigated in many laboratories.

There are different channels of sulfur-mediated plant-adaptive mechanisms to counter the abiotic stress effects. The adaptation may involve repair of sulfur complexes, including incorporation of Fe-S cluster to apoproteins and stabilization of biomembranes with sulfolipids. The other channel of adaptation is chelation of heavy metals by sulfur compounds. The heavy metals are toxic to plants, and their exposure may induce O-acetyl serine(OAS) thiolyase gene (*atcys3A*) (Dominguez-Solis et al. 2001), ATP sulfurylase, adenosine phosphosulfate(APS) reductase, GSH, and PC synthetic pathways (Cobbett 2000, Pickering et al. 2000, Harada et al. 2002, Mendoza-Cozatl et al. 2005). PCs act as metal chelators. S^{2-}, being a soft base, can selectively ligate to soft acids like, Cu^{+}, Cd^{2+}, Hg^{2+}, As^{3+}. PC-metal chelates are excreted to vacuoles. The most important aspect of sulfur-mediated stress adaptation is modulation of stress effect by redox active thiol residues of its compounds. The redox active thiol residues can exist either in reduced or in oxidized forms, and the reversibility of the forms is an important property that provides a switch for initiating different downstream signaling cascades in the metabolic network for appropriate stress adaptation.

In addition to its participation as a modulator of oxidative stress induced by various abiotic stress factors, its deficiency in plants manifests in oxidative stress and enhances activity of enzymes like sulfate permease, ATP sulfurylase, and APS reductase participating in the sulfur assimilation and formation of various sulfur compounds (Gutierrez-Marcos et al. 1996, Leustek et al. 2000, Takahashi et al. 2000). ATP sulfurylase is limiting for sulfur uptake, as its over expression enhances sulfur uptake by plants (Pilon-Smits et al. 1999). It is encouraging to find that there is scope for genetic manipulation by upregulating the enzymes associated with sulfur assimilation and synthesis of redox state regulating sulfur compounds to combat stress (Link 2003).

For the last several years we have been working on photosynthesis of higher plants and the response of chloroplast to several abiotic stress factors. Since chloroplast is the major location of sulfur metabolism in green plants, the review focuses primarily on the possible participation of sulfur compounds in modulating the response of the photosynthetic organelle to the abiotic stress. The review very briefly describes the rationale for nature's selection of stress-sensitive chloroplasts for sulfur assimilation, structure and function of chloroplast, role of chloroplast in sulfur assimilation, oxidative stress induced by various abiotic stress factors and its modulation by chloroplast-mediated sulfur metabolism.

2 Stress-Sensitive Chloroplast as the Site for Major Sulfur Metabolism

Chloroplast contains the machine for synthesis of many sulfur compounds, including Cys and GSH. The synthesis of these compounds is directly linked to the availability of primary photochemical reaction products like ATP and

reducing equivalents, and organic carbon skeletons from so-called dark reactions. At the same time, the organelle is known to be extremely sensitive to abiotic stress factors because of its unique structural and functional features. Evolution of O_2, presence of pigments, and photoelectron transport in different environmental conditions could create an oxidative environment and result in the production of various oxygen-free radicals. These conditions possibly have forced plants to develop internal redox control mechanisms which not only counter the stress effect but also exploit redox status as one of the important sensors in the cellular redox signaling network. In the chloroplast, the sulfur-rich compounds appear to play a major role in the background of potential of some of the redox-sensitive sulfur compounds to scavenge ROS and also reduce the stress-induced oxidative environment. At the same time, stress signals generated in the chloroplast are communicated to the cytoplasm through the redox sensing mechanism for expression of stress-responsive nuclear genes. It appears that retention of major sulfur metabolism in the oxygenic photosynthetic system is an evolutionary selection of nature.

3 Structure and Function of Chloroplasts

3.1 Structure and Primary Photochemistry

Chloroplasts are cellular organelles usually located in the green leaves of plants. A chloroplast consists of a continuous double membrane structure known as an envelope and the granular fluid matrix called stroma enclosed by the envelope. The stroma contains proteins, enzymes, and various metabolites. Chloroplast DNA strand is located in stroma. A membrane system known as lamellae is interspersed in the stroma. Lamellae stack at places to form piled sac like structures known as grana. Protein complexes, namely photosystem I (PS I), photosystem II (PS II), cytochrome b_6f (Cyt b_6f), and ATP synthase, are embedded in the membrane system. Photosystems absorb light and carry out electron transport. PS II oxidizes $2H_2O$ to O_2 and liberates $4H^+$, consequently reducing quinones Q_A^-/Q_B^-. Electron transport from the reduced quinone pool to PS I is mediated by Cyt b_6f complex. PS I generates reduced species NADPH at the end of the electron transport chain. The proton gradient created during photoelectron transport is utilized by ATPase to produce ATP from ADP and conserve energy in chemical form. Chloroplast, thus, is the solar powerhouse of green plants. It absorbs photon and converts it to electrical energy through a series of photoredox reactions. The energy conserved in ATP and redox potential gained by photoelectron transport is finally utilized for various metabolic processes in chloroplast, namely carbon assimilation, fatty acid synthesis, nitrogen assimilation, and sulfur assimilation.

3.2 Carbon Assimilation

Carbon is assimilated to produce sugar by a number of enzymes in the Calvin-Benson cycle. Carboxylation of ribulose-1,5-bisphosphate molecule by an enzyme ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) present in the stroma is the first step towards carbon assimilation (see Spreitzer and Salvucci 2002). Rubisco consists of 8 large subunits (LSU) and 8 small subunits (SSU) forming an oligomer L_8S_8 (Knight et al. 1990, Taiz and Zeiger 1998). Though the LSU may assemble as an octamer L_8 and contains the catalytic active site, it needs to incorporate SSUs into the complex for the normal carboxylase activity (Andrews 1988). Further, Mg^{2+}, CO_2, and an ATP-dependent activase play important roles in activation of Rubisco (Andrews and Lorimer 1987, Salvucci and Ogren 1996).

3.2.1 Regulation of Carbon Dioxide Fixation

3.2.1.1 Light

The pH of stroma increases from 7 to 8 with simultaneous increase in the level of Mg^{2+} upon exposure to light. Higher pH facilitates formation of HCO_3^- by dissolution of CO_2 in the alkaline aqueous medium. HCO_3^- carbamylates deprotonated ε-NH_2 group of Lys201 of Rubisco. It leads to Mg^{2+} binding to the active site along with HCO_3^- as a ligand to Mg^{2+}. The photosynthetic CO_2 assimilation is regulated by light through the change of pH of stroma (see Biswal et al. 2003).

3.2.1.2 Thioredoxin

It is a 12 kD protein, which contains a disulfide bridge. Reduced ferredoxin (Fd) in the presence of ferredoxin-thioredoxin reductase (FTR) reduces the disulfide group of thioredoxin to sulfyhydryl groups upon illumination. Reduced thioredoxin activates a number of enzymes in the Calvin-Benson cycle by reducing disulfide bridges in the enzymes (Raines et al. 1999). Activation of sedoheptulose-1,7-bisphosphatase (SBPase) is one of the examples (Raines et al. 1999).

3.2.1.3 Starch Synthesis

Starch is synthesized in chloroplast during exposure to light and broken down in dark. CO_2 fixation in the Calvin-Benson enzyme cycle yields fructose-1,6-bisphosphate. It is dephosphorylated to fructose-6-phosphate and then isomerizes to glucose-1-phosphate. ADP-glucose pyrophosphorylase adenylates glucose-1-phosphate. Starch synthase and branching enzymes act upon ADP-glucose to form starch (Emes and Tobin 1993).

3.3 Nitrogen Assimilation

Chloroplast is the site for assimilation of most of the NH_3 (Emes and Tobin 1993). Nitrate reductase reduces NO_3^- to NO_2^- in the cytoplasm. The NO_2^- is transported into the chloroplast by nitrite translocator (Brunswick and Cresswell 1988), where it is further reduced to NH_3 by ferredoxin-nitrite reductase (Crawford 1995). Glutamate dehydrogenase may catalyze the reaction between α-ketoglutarate and NH_3 to produce glutamic acid (Glu). Plastidic glutamine synthetase 2 (GS2) may act upon Glu and NH_3 to form glutamine (Gln) (Lam et al. 1995). Nitrogen could be incorporated into other amino acids by the action of transaminase once it is assimilated into Gln or Glu.

3.4 Sulfur Assimilation

Sulfur is an essential element for growth and physiological functioning of plants. Its content varies from 0.1% to 1% of the plant's dry weight (Pilon-Smits and Pilon 2006). Sulfur is taken up by plants in the form of sulfate through the roots and is then transported to shoots. Sulfate is transported across the envelope of chloroplast through sulfate permease. Its subsequent reduction and incorporation into organic compounds to produce Cys takes place in the chloroplasts as shown in Fig.8.1. Cys serves as the precursor of most other organic sulfur compounds in plants. About 70% of total organic sulfur in plant is present in the protein fraction as Cys and methionine (Met) residues. Cys and Met play a significant role in the structure and function of proteins. Thiol (glutathione), sulfolipids and secondary sulfur compounds, namely alliins, glucosinolates, and phytochelatins, are other forms of sulfur in plants. These compounds play an important role in physiology, food quality of crops, phytopharmaceutics, and protection against environmental stress and pests (see Schung, 1998, Grill et al. 2001, Abrol and Ahmad 2003).

3.5 Cross Talk between Carbon, Nitrogen, and Sulfur Metabolic Pathways

Sulfur and nitrogen assimilation are coordinated to maintain a ratio of reduced sulfur to reduced nitrogen of about 1:20. Reduced sulfur compounds stimulate nitrate reductase, and reduced nitrogen compounds stimulate ATP sulfurase and APS reductase, establishing cross talk between the two pathways (Reuveny et al. 1980, Koprivova et al. 2000). The reduced sulfur and nitrogen compounds also inhibit key enzymes of their own biosynthetic pathways by feedback mechanism. Sulfur uptake is not stimulated under nitrogen-limiting condition (Yamaguchi et al. 1999). Although cross talk between carbon and nitrogen pathways is the basis of most of the

biochemical routes, the cross talk between carbon pathway and sulfur assimilation is also extensive and covers a complex network as depicted in Fig. 8.1.

The coordination of sulfur metabolism with the carbon and nitrogen assimilatory pathway is required for optimization of sulfur assimilation in chloroplasts, specifically when plants experience fluctuation in environmental conditions (Kopriva and Rennenberg 2004).

4 Sulfur Compounds in Chloroplasts

4.1 Sulfur Reduction in Chloroplast

Sulfate reduction predominantly takes place in the leaf chloroplasts (Pilon-Smits and Pilon 2006). Sulfate enters cytosol across sulfate permeases embedded in plasma membrane. The chloroplast envelope also contains sulfate permeases to import sulfate into stroma. Sulfate in stroma is activated to adenosine 5′-phosphosulfate (APS) prior to its reduction to sulfite. The activation is catalyzed by ATP sulfurylase, and APS is subsequently reduced to yield sulfite by APS reductase. GSH is the probable electron donor in this catalysis (Pilon-Smits and Pilon 2006). Sulfite is reduced to sulfide by sulfite reductase with Fd as a reductant.

4.2 Synthesis of Sulfur Compounds in Chloroplasts

Sulfolipids are synthesized from sulfite. A part of sulfite enters into the formation of sulfolipids. Sulfite is coupled to UDP-glucose catalyzed by sulfolipid-6-sulfo-α-D quinovosyl diacylglycerol (SQD1) enzyme to form UDP-sulfoquinovose, which is coupled to diacylglycerol to produce sulfolipid 6-sulfo-α-D-quinovosyl diacylglycerol (SQDG) (Pilon-Smits and Pilon 2006). The sulfolipids are unique to chloroplasts and are required for plastid functions including photosynthesis (Yu and Benning 2003).

Cysteine is the major form of organic sulfur compound in chloroplast. It is synthesized from OAS with incorporation of sulfide catalyzed by OAS lyase. Cys acts as source of formation of other sulfur compounds. Cys is transported from chloroplast to cytosol through an unknown transporter. Reaction of Cys with O-phosphohomoserine by cystathionine-γ-synthase yields cystathionine. Homocysteine is formed from cystathionine by the action of cystathionine-β-lyase. Homocysteine is transported across the envelope to cytosol, where it is converted to Met by the action of Met synthase. Met is transported into chloroplast. Cys and Met get incorporated into newly synthesized proteins in chloroplast as well as cytosol, as indicated in Fig. 8.1.

Cys-desulfurase acts on Cys to yield sulfide and Ala. Sulfide thus formed is utilized in synthesis of thiamine, biotin, molybdenum cofactor (MoCo), and Fe-S centers.

Fe-S clusters [2Fe-2S] or [4Fe-4S] are contained in proteins and enzymes associated with chloroplast function, namely PS I, Rieske protein of Cyt b_6f, Fd, import protein Tic55, and many enzymes in nitrogen and sulfur assimilation pathways. These centers are formed and incorporated into the apoproteins in chloroplast. Elements of *suf*-machinery, and some of *nif*- and *isc*- components, along with some special proteins like cystine lyase (C-DES) and P-loop ATPase (HCF101), are believed to exist in chloroplasts, which may be responsible for Fe-S cluster metabolism (Kessler and Papenbrock 2005). NifU proteins AtNFU1-3 are identified in stroma of *Arabidopsis* chloroplasts (Leon et al. 2003, Yabe et al. 2004). Suf proteins SufA-D and SufS are associated with Fe-S cluster biosynthesis. Complete Suf type proteins are believed to be present in chloroplast (Xu and Moller 2004). SufB, -C,-D and –S are identified in *Arabidopsis* chloroplasts (Xu and Moller 2004). AtNFU2 is supposed to carry the Fe-S cluster and transfer it to apoproteins. Mutant lacking in AtNFU2 shows drastic impairment of PS I activity and decreased level of Fd (Touraine et al. 2004, Yabe et al. 2004). HCF101 is associated with the formation of [4Fe-4S] cluster in PS I (Lezhneva et al. 2004, Stöckel and Oelmüller 2004).

Glutathione is a major sulfur compound maintaining the redox status of biosystems. GSH plays a role in chloroplastic gene expression via redox-mediated control of transcription (Link 2003). It is also synthesized in chloroplasts. Cys and Glu are acted upon by γ-glutamylcysteine synthetase (GSH1) to form γ-EC, which is bonded to glycine (Gly) by the action of glutathione synthetase (GSH2) to synthesize GSH (Mullineaux and Rausch 2005). However, the detailed mechanism and location of synthesis of GSH are under active investigation for further confirmation (Mullineaux and Rausch 2005).

Phytochelatins are polypeptides with a general structure $(\gamma\text{-GluCys})_n$Gly, where n varies from 2 to 5 (Cobbett 2000). Phytochelatins are synthesized from GSH by elongation of the γ-GluCys unit by phytochelatin synthase (PS). PCs chelate metal ions and detoxify excess metal ions, specifically heavy metal ions. PS is expressed constitutively, but it is activated by metal ions, especially Cd^{2+} or As^{3+} (Cobbett 2000, Pickering et al. 2000). PC-metal ion complex is transported to vacuoles by ABC-type transporters.

4.3 Significance of Sulfur Compounds in Structure and Function of Chloroplasts

Sulfur compounds like GSH or sulfur moieties in protein in its reduced state (oxidation state-2) are mostly responsible for maintaining the general redox status in chloroplasts. Electron transfer is essential for maintenance of redox status. This is performed by reversible –S-S- /SH couple or Fe-S clusters. A [2Fe-2S] is present in Rieske protein of $Cytb_6f$ complex in the photoelectron transport chain of thylakoid. [4Fe-4S] cluster F_X occurs in dimeric subunits PsaA/B of PS I. Besides, [4Fe-4S] centers,

F_A and $F_{B,}$ occur in the PsaC subunit of PS I, and a [2Fe-2S] cluster occurs in Fd associated with PS I. These clusters act as photoelectron transfer units. One Cys and one Met along with two His residues participate in Cu binding in plastocyanin associated with PS I. Thioredoxin plays a regulatory role for change in conformations of FBPase and malate dehydrogenase (MDH) enzymes in chloroplasts through disulfide-thiol switch (Raines et al. 1999, Pilon-Smits and Pilon 2006).

5 Chloroplast under Abiotic Stress

A mature chloroplast exists in a nonequilibrium stationary state. It tends to resist perturbation by the external forces, including variations in the temperature, intensity of light, concentration of CO_2, salinity, and osmotic pressure. The appropriate internal changes in the system tend to restore the state as far as possible. The external changes are designated as stress, and the internal changes in the organelle in response to the stress collectively constitute the adaptation process. Processes like absorption of photon, electron transport, proton translocation, CO_2 fixation, and assimilation of other nutrients operate in a coordinated manner in a mature chloroplast. Therefore, response to stress also operates in an integrated pattern (see Biswal et al. 2003).

5.1 Chloroplast as the Sensor of Stress

Light may act as a major external environmental factor for green plants. The light is sensed mainly by PS I and PS II, and subsequently the photosignals are transduced through various components of the organelle in chloroplasts. Further, the changes in other environmental factors in the presence of light may also be sensed by the photosystems, and the consequence is observed primarily through photoinhibition of PS II of thylakoid (Biswal 1997). The sensitivity of photosystems, especially PS II, makes chloroplast the sensor of stress in plants (see Biswal et al. 2003). Operation of a feedback mechanism via the redox state of the components associated with the electron transport system of PS I and PS II is reported during stress acclimation of the photosynthetic apparatus (Anderson et al. 1995, Biswal and Biswal 1999).

A shift in the redox steady state of the chloroplast system due to physiological variation induced by the stress may lead to the reduction of O_2 to toxic oxygen free radicals. A strong oxidant generated on the donor side of PS II in the stress-impaired system may oxidize pigments, proteins, or lipids of thylakoid. These special characteristics associated with the thylakoid make the photosynthetic organelle a major stress sensor in green plants (see Biswal et al. 2003).

5.2 All Abiotic Stress Factors Lead to Oxidative Stress

The key feature of chloroplast under abiotic stress is nonutilization of energy by the disturbed sink, which may create redox or excitation pressure on the photosystems. The building up of the excitation pressure initiates the events associated with the stress adaptation or stress-induced damage of the system. Chloroplasts under abiotic stress exhibit the high light stress syndrome (see Biswal et al. 2003). Ultimately, almost all abiotic stress may lead to photoinhibitory damage in chloroplasts resulting in oxidative stress as per the scheme provided in Fig. 8.2.

Models for signal transduction pathways for adaptation to stress involving various components associated with the electron transport chain, redox-responsive protein kinase, and thiol-regulated enzymes are proposed, and chlorophyll (Chl) precursors may mediate in transducing the signals in these pathways (Mullineaux and Karpinski 2002). In response to stress, the chloroplast system may employ multiple antioxidants, enzymes, or chemical species as an adaptation strategy (Lee et al. 2007, Lee YP et al. 2007).

The details of responses of chloroplasts to abiotic stress factors like high light, water stress, temperature extremes, and UV radiations that ultimately generate oxidative stress, and their modulation by sulfur metabolism are provided below.

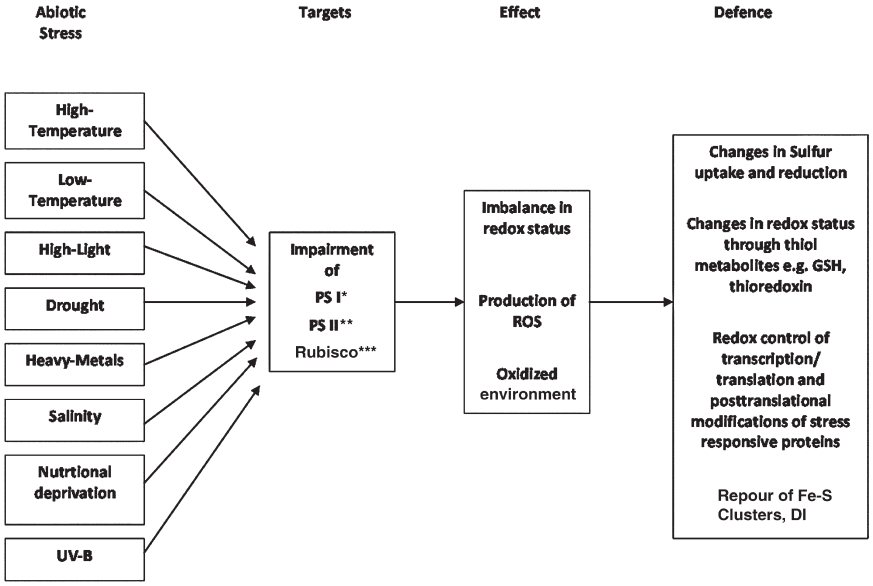

Fig. 8.2 A scheme showing that the abiotic stress leads to imbalance in the redox status of chloroplast. Consequently an oxidative stress results. Sulfur metabolism plays an important role in stress management in plant by providing a long-term and second line of defense to abiotic stress. * mark in the second box indicates the susceptibility of PSI, PS II, and Rubisco to abiotic stress factors. The higher the number, the greater the susceptibility of the system to stress

5.3 High Light- Induced Stress

5.3.1 PS II is the Target of High Light Stress

High light stress results when light is absorbed by photosystems of thylakoid, and its utilization in CO_2 fixation are not properly coordinated. Normally, exposure to light intensity between 1000-2000 μM $m^{-2}s^{-1}$ for a plant is considered as high light in experimental conditions. PS II is the main target of high light stress. The light absorbed by the special pair pigment P680 of the PS II reaction center (RC II) leads to the formation of a charge pair $Pheo^-$ (pheophytin) and $P680^+$. The latter is reduced by Y_Z (Tyr161, D1), yielding Y_Z^+. Both $P680^+$ and Y_Z^+ are strong oxidants. Y_Z^+ oxidizes O_2 evolving complex (OEC). The OEC has a very delicate structure and is a soft target to stress. The instability of metal cluster Mn_4Ca in OEC under stress makes the complex prone to damage. Damaged OEC does not effectively donate electrons to Y_Z^+ and $P680^+$. The life-times of these strong oxidants consequently become longer, and they may oxidize pigments, lipids, and amino acids of proteins in the vicinity of PS II (Andersson and Barber 1996, Biswal et al. 1997). D1 protein is oxidized by the strong oxidant $P680^+$ and is subsequently degraded (Jegerschold and Styring 1996). The charge recombination process between Q_A^-/Q_B^- and long-lived $P680^+$ brings about the formation of $^3P680$ and the subsequent production of highly toxic $^1O_2$. Since the half-life of $^1O_2$ is short, its primary target is PS II, particularly its reaction center proteins, especially D1 (Keren et al. 1997). D1 protein is prone to oxidation at the stromal side of the second helix and lumenal side of the fourth helix, probably due to $^1O_2$ produced at RC II as revealed by the mass spectrometric studies (Barber and Sharma 2000). Oxidation of D1 protein may lead to proteolytic degradation of the protein (Barber and Sharma 2000).

5.3.2 Turnover of Reaction Center II Proteins

The turnover of D1 protein of RC II during high light stress has been considered as one of the major adaptive responses of PS II to stress. In addition to high light, the light-dependent turnover of the protein has been reported during water stress (Giardi et al. 1996, Deo and Biswal 2001) and nutritional stress (Godde and Hefer 1994). The association of sulfur metabolism with amelioration of stress is indicated by the fact that D1 biosynthesis and reassembly require sulfate uptake (Vasilikiotis and Melis 1994, Melis and Chen 2005).

5.3.3 Gene Expression in Response to High Light

The redox signaling system is believed to regulate *lhcb* gene expression at varying light intensities (Durnford and Falkowski 1997). The level of D1 protein is enhanced under high light (Shapira et al. 1997) and decreased under low-light intensity

(Sailaja and RamaDas 1995). A distant relative of *lhca* is also reported to express under low- or high light stress. A novel one-transmembrane helix containing protein Ohp2 is identified, which is a distant relative of light-harvesting proteins associated with PS I (Andersson et al. 2003). Light stress triggers *ohp2* gene expression. Other stress conditions did not upregulate the expression of o*hp2*. The accumulation of Ohp2 might be a novel photoprotective strategy induced within PS I in response to light stress.

Proteomic analysis of *Arabidopsis* exposed to normal (100 μM $m^{-2}s^{-1}$) high light (1000 μM $m^{-2}s^{-1}$) conditions implicates 64 proteins associated with high light stress (Phee et al. 2004). Out of the 52 proteins selected for analysis, 35 photosynthetic proteins are downregulated, 14 proteins including heat shock proteins (HSPs) and dehydroascorbate reductase (DHAR) are upregulated, and 3 novel proteins are synthesized with unknown functions.

5.4 Water Stress

The term water stress is used for a water deficit condition and is very often referred to as drought stress. Under drought stress leaf stomata are closed to restrict CO_2 assimilation and H_2O loss (Cornic 1994), resulting in the inhibition of photosynthesis (Bradford and Hsiao 1982). Although the stress causes alterations in different biochemical pathways (Graan and Boyer 1990, Lauer and Boyer 1992, Lu and Zhang 1998, Ramachandra Reddy et al. 2004), PS II is known to be the primary target of drought stress. OEC of PS II (Canaani et al. 1986, Toivonen and Vidaver 1988) and the PS II reaction center proteins and their turnover pathways (He et al. 1995, Giardi et al. 1996) are reported to be affected. The stress also induces reduction in the levels and activities of the enzymes of the photosynthetic carbon reduction cycle, including the key enzyme, Rubisco (Ramachandra Reddy et al. 2004). Under stress conditions the differential loss in the efficiency of photoelectron transport and carbon reduction cycle may result in imbalance in the redox state of photosynthetic organelles. This situation still worsens if the light- driven electron transport proceeds unabated.

The effect of water stress is known to increase the level of H_2O_2 and $O_2^{\bullet -}$ radicals and to enhance the activities of SOD, ascorbate peroxidase, and glutathione reductase (GR) (Jiang and Zhang 2002). However, Mahan and Wanjura (2005) have observed an increase in the amount of ascorbate and ascorbate peroxidase activity without any change in GSH concentration in field-grown cotton. They have argued that the GSH metabolism in field-grown cotton is enough to meet the challenge of water-stress-mediated oxidative damage. On the other hand, species-dependent contradictory reports on the level of GSH under water stress are available. While the level of GSH is found to increase by 3.2 times in pea (Iturbe-Ormataexe et al. 1998), it declines two-fold in rice (Srivalli et al. 2003). The content in ascorbate and reduced GSH is also known to increase in response to the stress. Water stress stimulates the *de novo* synthesis of GR (Pastori and Trippi 1992). In general there is a higher

level of GSH and ascorbate in water deficit plants. An elevated GSH content is usually correlated with the increased adaptive response of plants to abiotic stress (Kocsy et al. 1996, Okane et al. 1996)

Therefore, most of what we know about plant defense associated with sulfur metabolism is determined by the synthesis and reduction level of GSH. In association with Cys, GSH induces the accumulation of transcripts of Cu/Zn superoxide dismutase (SOD) enzyme and elevates the activities of SOD and ascorbate-GSH cycle (McKersie et al. 1996, Hawkesford and De Kok 2006), which are known to scavenge the ROS. However, under acute stress conditions there is a drop in GSH concentration, resulting in an oxidized redox state that initiates the degradation of the organelle (Tausz et al. 2004).

In addition to the modulation of drought-induced oxidative stress, sulfur metabolism may be involved in maintaining structural integrity of biomembranes. In response to water stress, there is an increase in accumulation of SQDG in drought-resistant plants (Okanenko and Taran 1998). These lipids, besides stabilizing light harvesting complex II under stress conditions, are localized as the prosthetic group at the surface of the D_1/D_2 reaction center and help in holding them together (Sigrist et al.1988). Therefore, an enhanced level of SQDG accumulation in water stress could help in maintaining membrane fluidity and stabilizing PS II organization.

5.5 Temperature Stress

5.5.1 Low Temperature Targets Photosystem I

PS I is the target of stress under low temperature (4 °C), at low light (100-200 µmol $m^{-2}s^{-1}$), and in the presence of oxygen (Terashima et al. 1994, Sonoike 1996). Chilling stress inhibits PS I activity but does not significantly inhibit PS II activity (Terashima et al. 1994). Chilling impairs iron- sulfur centers: F_A/F_B and F_X of PS I (Sonoike 1996). The impairment may be caused by $O_2^{\bullet -}$ generated on the reducing side of PS I (Miyake and Asada, 1992, Ogawa et al. 1995). Finally, a serine type protease may be involved in proteolysis of the subunit B of PS I and may cleave the protein to yield 45, 51 and 18 kD fragments (Sonoike et al. 1997). Though the event is similar to the D1 degradation of PS II, PsaB turnover rate is extremely slow compared to that of D1 and requires several days (Sonoike 1996).

5.5.2 High-Temperature Stress

The high temperature stress impairs PS II (Vani et al. 2001). Temperature in the range of 45-60 °C inactivates Rubisco (Li et al. 2002). The inactivation may be related to the thermal denaturation of activase (Salvucci et al. 2001). High temperature

may lead to sequestration of Rubisco activase to thylakoid membrane. SBPase activity prevents the sequestration of the activase and maintains the activity of Rubisco. Over expression of SBPase enhances photosynthesis against high-temperature stress (Feng et al. 2007). There is also report that heat may induce expression of a novel gene for activase, and this novel form of activase may play a role in the acclimation process (Law et al. 2001). Inactivation of PS II and Rubisco due to heat stress results in similar stress-induced manifestations, as in case of high light stress. The link is suggested by the fact that the plastid-specific heat shock proteins are linked to the protection of the photosynthetic organelle during light and heat stress (Biswal 1997, Schroda et al. 1999, Lee BH et al. 2000). Heat stress (42 °C) in rice induces expression of the *oshsp26* gene, which encodes chloroplast-localized small heat shock protein (smHSP). This gene is also expressed when rice plant is treated with oxidative agents like methyl viologen in light or H_2O_2 in light (or dark). The results indicate the protective role of smHSP against both oxidative and heat stresses (Lee BH et al. 2000).

The nature of the function of heat shock proteins to protect chloroplasts is not clear. However, there is a report that conserved Met in chloroplast Hsp21 may be involved in the formation of groove for sequence-independent recognition of hydrophobic domains in peptides (Sundby et al. 2005). Hsp21 may bind to hydrophobic domains of proteins, preventing its aggregation during stress. Debel et al. (1997) have observed the accumulation of a 23 kD nuclear encoded heat shock protein in the mitochondria under light stress. These authors have proposed a possible coupling of the chloroplast and mitochondrial functions in protecting the photosynthetic organelle against the stress.

5.6 UV Radiation

A lot of work has been conducted recently to understand the mechanisms of damage and repair of the photosynthetic apparatus of green leaf exposed to UV radiation (McKenzie et al. 2003). The radiation is known to affect the growth, photosynthetic function, and alteration in gene expression (Bornman 1989, Jordan 1996, Teramura and Ziska 1996). The radiation reduces the activities of chloroplast ATPase (Brosche and Strid 2003), Rubisco (Jordan et al. 1992) and violaxanthin de-epoxidase (Pfundel et al. 1992, Bischof et al. 2002). The PS II of photosynthetic apparatus is affected the most among all thylakoid complexes. The radiation damages the D_1/D_2 reaction center proteins (Friso et al. 1994 a, b), OEC and impairs oxidizing as well as reducing sides of PS II at multiple sites (Jordan 1996, Teramura and Ziska 1996). In the process, the radiation, through dismantling of thylakoid complex and loss of Rubisco, generates ROS, which are considered to be the sensor of UV-B responses (Brosche and Strid 2003)

The ascorbate-GSH cycle, which regenerates a large pool of ascorbate, contributes enormously to the management of oxidative stress (Salin 1987, Kunert and Foyer

1994). The enhancement in the level of ascorbate, GSH (Takeuchi et al. 1996, Jansen et al. 1998), and in the activities of SOD (Rao et al. 1996, Jansen et al. 1996), GR (Jansen et al. 1996, Rao et al. 1996), and ascorbate peroxidase (Landry et al. 1995, Takeuchi et al. 1996) in response to UV-B stress could, therefore, be considered as an adaptive process involving metabolic adjustment. In the process of adjustment it is not necessary that all these parameters increase. Even a significant rise in the ratio of reduced GSH to oxidized GSH without any alteration in ascorbate content in response to UV-B stress is sufficient to maintain the redox status of the cell to meet the challenge posed by oxyfree radicals (Costa et al. 2002). Rao and Ormrod (1995), on the other hand, have observed that *Arabidopsis thaliana*, which possesses an effective free radical scavenging system, is susceptible to UV-B stress when its flavonoid biosynthesis is blocked. But the observation made by A-H-Mackerness et al. (1998) that the antioxidant enzyme activities depend on the rate of free radical generation make us believe that the enzymatic activities in plants depend on efficient organization of different metabolic processes, including sulfur metabolism.

6 Sulfur Metabolism Mediated Modulation of Oxidative Stress

Chloroplasts under various kinds of stresses, as discussed above, ultimately experience oxidative stress. The plants, therefore, develop a second line of defense mechanism against ROS, like $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$, and $^1O_2$. Disulfide/thiol exchange reactions involving the GSH pool and the production of H_2O_2 are believed to be crucial for regulation of stress adaptation at the molecular level (Foyer et al. 1997). The oxidative stress induces a remarkable increase in the transcripts of two ascorbate peroxidase genes. These enzymes are cytoplasmic and are involved in regulating the redox level of the GSH pool. SODs, ascorbate peroxidase, DHAR, and GSH are associated with defense mechanisms against stress. It is important to note that sulfur deprivation leads to oxidative stress in plants (Pilon-Smits and Pilon 2006).

The origins and factors responsible for production of ROS in chloroplast in light are complex. The studies on photosynthetic response to various abiotic stress factors however, indicate that Rubisco is the most susceptible component of the organelle to the stress. This consequently results a loss in the efficiency of Calvin-Benson cycle compared to the loss in primary photochemical reaction associated with the thylakoid membrane. A weak sink (Calvin-Benson cycle) for photoelectron transport leads to accumulation of excess unused quanta and excess electrons in the organelle. Under this condition, O_2 through the components of PS I receives the electrons and is reduced to $O_2^{\bullet -}$, which subsequently, through a series of redox reactions involving ascorbate-GSH cycle, are converted to H_2O as summarized in Fig.8.3.

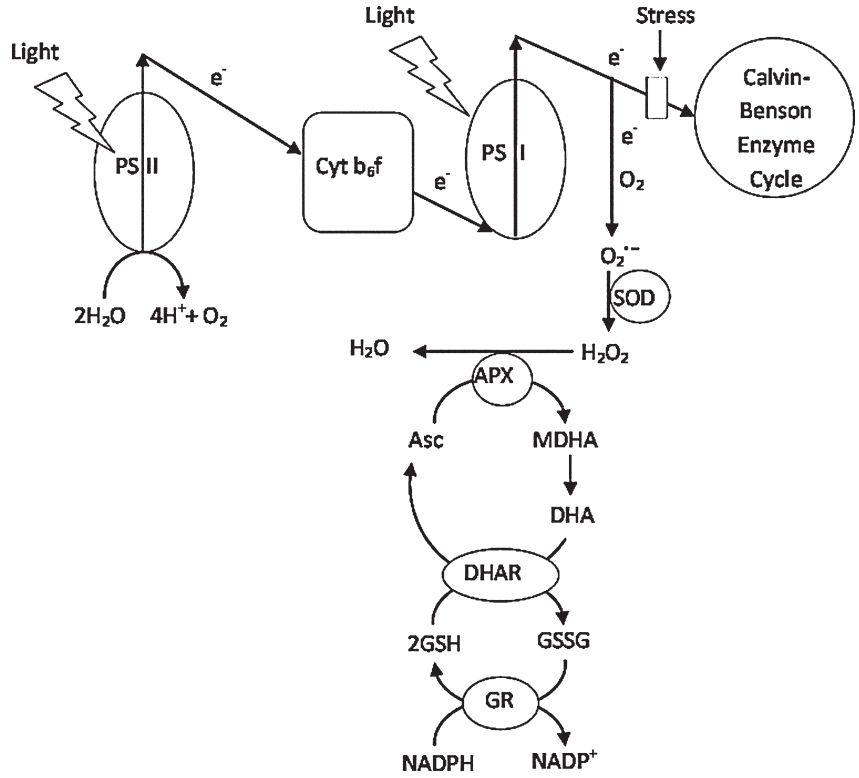

Fig. 8.3 A scheme depicting glutathione-mediated scavenging of reactive oxygen species produced under stress. APX: ascorbate peroxidase, DHA: dehydroascorbate, DHAR: dehydroascorbate reductase, GSH: Glutathione, GSSG: oxidized glutathione, GR: glutathione reductase; MDHA: monodehydroascorbate, $NADP^+$: nicotinamide adenine dinucleotide phosphate (oxidized), NADPH: reduced $NADP^+$, SOD: superoxide dismutase. (Modified after Asada 2000.)

7 Stress-Induced Redox Signals Generated in Chloroplasts, Their Transmission and Gene Expression

Light-induced redox signals generated in chloroplast are known to participate in the expression of photosynthetic genes (see Biswal et al. 2003). The signals not only modulate gene expression in chloroplast but are also transmitted to cytoplasm for expression of stress-related nuclear genes.

7.1 Signal Transduction and Modulation of Plastid Gene Expression

The best example of stress-induced plastid gene expression is the high light-induced turnover of D1 protein of PS II of thylakoid. Although the precise mechanism of

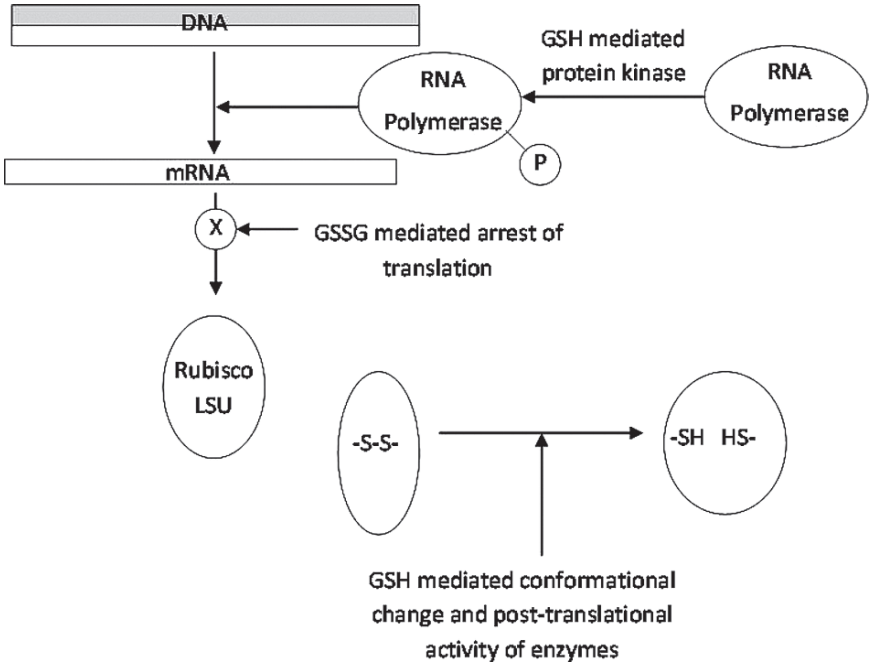

Fig. 8.4 A schematic representation of glutathione-mediated redox regulation of expression of chloroplastic gene. GSH: glutathione, GSSG: oxidized glutathione, P: phosphate, Rubisco LSU: large subunit of Rubisco. X represents blockage of pathway

redox signaling for synthesis of D1 protein is not known, high light is known to activate the *psbA* gene that codes for D1 protein through a signal induced by a high level of reduced quinone, which on oxidation leads to the inactivation of gene transcription. This suggests plastid gene expression to be under the control of a kind of redox signaling system. The involvement of redox-regulated thylakoid protein kinase and subsequent downstream signaling to the level of gene expression could be a possibility. Similarly, redox control of translation of *psbA* is known to remain under the control of Fd-thioredoxin system (see Mullineaux and Rausch 2005).

RNA polymerase phosphorylation through GSH-mediated signaling, as depicted in Fig.8.4, may be considered as one of the thiol redox controls of plastid gene expression. The ROS-induced enhancement in the oxidized pool of GSH is likely to arrest translation of the Rubisco large subunit (Irihimovitch and Shapira 2000).

7.2 Chloroplast to Nucleus Signal Transduction is Primarily Redox Controlled

Nuclear gene expression through a plastid signal generated by the photooxidative environment of the organelle induced by high light stress has long been known

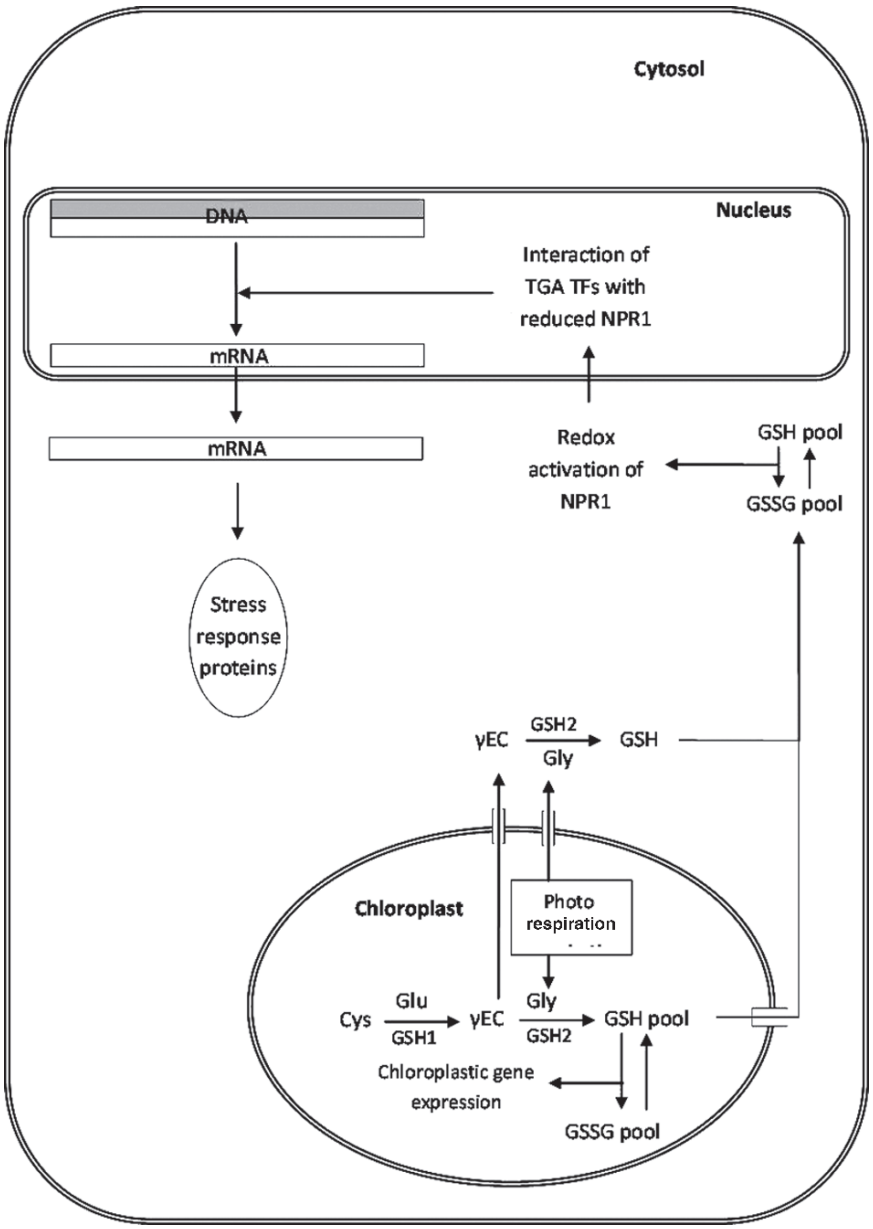

Fig. 8.5 A scheme representing the stress-responsive nuclear gene expression with a redox signal from chloroplast. Cys: cysteine, γEC: γ-glutamylcysteine, Gly: glycine, GSH1: γEC synthetase, GSH2: glutathione synthetase, GSH: glutathione, GSSG: oxidized glutathione, NPR1: nonexpresser of pathogenesis-related genes- a stress related protein, TGA TFs: transcription factors. Gly produced by photorespiration is provided for GSH synthesis, which results in photosynthetic regulation of GSH pool

(see Biswal et al. 2003). As discussed earlier, chloroplast is the most sensitive organelle to abiotic stress. The abiotic stress factors affecting the organelle produce a cellular oxidative environment, which may alter the redox status of cytoplasm. GSH possibly plays a major role in the chloroplast-to-nucleus communication system during oxidative stress. The redox state of cytoplasmic GSH system significantly affects gene expression through various redox-sensitive regulatory proteins, including NPR1, which are transported to the nucleus, interact with the transacting factors, and regulate the expression of stress-responsive genes. In this background, the pool of GSH in cytoplasm, which plays important role in nuclear gene expression, is modulated by the activity of chloroplast. Chloroplast provides the organic carbon skeleton for synthesis of GSH in cytoplasm, which also imports precursors of GSH namely, γEC from chloroplast. It is therefore likely that stress-induced perturbation in photosynthetic activity of chloroplast brings a change in the GSH pool and its redox status in cytoplasm and, consequently, alteration in nuclear gene expression. Fig.8.5 shows the possible transmission of plastid-specific redox signal and regulation of expression of nuclear genes.

8 Conclusion and the Future

1. Some of the fundamental features of sulfur uptake and its subsequent assimilation are known. The expression and activities of the enzymes that participate in its metabolism are now better understood in the light of information available with *Arabidopsis thaliana* as a model system. At the same time, extensive use of advanced techniques like DNA microarray, proteomics, metabolomics, and techniques relating to production of transgenics have helped to expand our understanding of the importance of sulfur assimilates in plant metabolism in general. However, the regulatory mechanisms of its metabolism specifically in photosynthetic tissue still remain complex and need attention.
2. It is almost clear that the redox changes induced by abiotic stress factors lead to changes in expression of stress-responsive genes, but the precise nature and identification of signal transduction pathways are poorly understood. Although some of the redox signaling systems mediated by redox-active thiols are known, their precise origin in chloroplast, subsequent transmission, and their cross talk with other cellular transduction systems for plastid and nuclear gene expression have to be worked out.
3. More detailed studies are required to understand the stress response modulated by sulfur metabolism at molecular and mechanistic levels in order to develop an effective strategy to raise transgenic species for stress resistance. It is necessary to identify the stress-relevant genes and the redox active proteins and define their functions. Once the mechanism at the level of genes is understood, its manipulation in the direction of biotechnological application will be possible. It is important to note that redox homeostasis is a very complex and interwoven metabolic network. Careful and judicious manipulation can only lead to biotechnological manipulation for producing stress resistance traits without a gross misbalance in the network.

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