Chapter 8
Exogenous Glutathione-Mediated Abiotic Stress Tolerance in Plants

Fangbin Cao, Manman Fu, Runfeng Wang, Pedro Diaz-Vivancos, and Mohammad Anwar Hossain

Abstract Glutathione (GSH), a major non-protein low-molecular-weight thiol tripeptide in plant cells, is involved in a variety of life processes, including cell differentiation, removal of free radicals and hydroperoxides, thiol-disulfide exchange, and the synthesis of phytochelatin. Along with its oxidized form (GSSG), GSH plays key roles in maintaining cellular redox homeostasiss and signaling, as well as in defense reactions. As a component of ascorbate-glutathione (AsA-GSH) and glyoxalase pathways, GSH is involved in the regulation of hydrogen peroxide and methylglyoxal levels, ensuring their signaling functions, which are necessary for normal growth, development, and stress tolerance. In plants, GSH metabolism also plays important functions in determining the degree of expression of defense-related genes during abiotic and biotic stresses. Plants easily uptake exogenously applied GSH, which is transported into cellular compartments inducing a series of physiological and biochemical processes, including the modulation of abiotic stress tolerance. Recent studies have shown the multiple roles of exogenous GSH in improving abiotic stress tolerance through the regulation of multiple stress responsive pathways; however, the precise molecular mechanisms of exogenous GSH-induced abiotic stress tolerance are largely unknown. This chapter provides an overview to highlight the involvement of exogenous GSH in modulating abiotic stress tolerance. We also highlight the possible mechanisms of uptake and transport of the exogenously applied GSH under stressful conditions.

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Contents

1 Introduction

Abiotic stresses seriously restrict plant growth and development through the unrestrained accumulation of reactive oxygen species (ROS) and reactive carbonyl species (RCS), which can cause oxidation of lipids, proteins, inactivation of enzymes, and DNA damage, and finally cell death (Miller et al. 2010; Avery 2011; Hoque et al. 2012a; Biswas and Mano 2015; Hossain et al. 2015). Plants have developed efficient enzymatic and non-enzymatic defense systems to counter the deleterious effects of ROS and RCS as well as to maintain its optimum level in order to trigger specific protective responses needed to ensure normal growth and development (Hossain et al. 2011, 2015; Baxter et al. 2014; del Río 2015; Li et al. 2017). Recently, the role of glutathione (GSH; γ-L-glutamyl-L-cysteinylglycine) has attracted considerable interest from the scientific community due to its broad range of functions in plant growth, development, and stress tolerance (Chen et al. 2012; Cheng et al. 2015; Noctor et al. 2012; Munné-Bosch et al. 2013). GSH refers only to the reduced glutathione, whereas the term glutathione refers to the total pool (GSH plus glutathione disulphide; GSSG). Glutathione is present in various plant tissues in concentrations up to 2–3 mM; it plays an important role in many life processes, such as cell differentiation, enzymatic regulation, cell signaling, and cell death, and acts as an antioxidant (Srivalli and Khanna-Chopra 2008; Diaz-Vivancos et al. 2010, 2015; Cai et al. 2011a; Chen et al. 2012; Schnaubelt et al. 2013). Furthermore, glutathione is used as a marker of oxidative stress, acts as a major reservoir of reduced sulfur, and plays crucial roles in biotic and abiotic stress responses and tolerance in plants (Tausz et al. 2004; Zechman et al. 2014; Cheng et al. 2015).

GSH is synthesized by the sequential addition of cysteine and glutamate followed by the addition of glycine via two ATP-dependent steps catalyzed by γ-glutamylcysteine synthetase (γ-ECS) and GSH synthetase (GSHS), respectively (Noctor et al. 2002). γ-ECS is located in plastids whereas GSHS is located in cytosol and plastids, and both are encoded by single-copy genes in *Arabidopsis* (Cairns et al. 2006). In *Arabidopsis thaliana*, knockout mutations of γ-ECS and GSHS induce embryo and seedling lethal phenotypes, respectively (Cairns et al. 2006; Pasternak et al. 2008), whereas overexpression of *γ-ECS* and *GSHS* significantly elevate GSH content and abiotic stress tolerance (Zhu et al. 1999; Liedschulte et al. 2010; Choe et al. 2013). Müller et al. (2004)

used electron microscopic immunogold cytochemistry to investigate the distribution of GSH in plant cells and reported that the highest level of GSH was found in mitochondria in different plant tissues. As a component of the ascorbate-glutathione (AsA-GSH) cycle and glyoxalase cycle, GSH is involved in removing excess hydrogen peroxide (H_2O_2) and methylglyoxal (MG) levels as well as in the regulation of their signaling functions (Szalai et al. 2009; Hossain and Fujita 2009; Hossain et al. 2010, 2011; Baxter et al. 2014; Mostofa et al. 2015a, b; Hoque et al. 2016; Li et al. 2017). Along with its oxidized form (GSSG), the GSH system plays a key role in maintaining cellular redox homoeostasis and is also considered as a redox sensor of environmental stimuli (Cairns et al. 2006; Szalai et al. 2009). In addition, GSH can also modulate gene expression, cell division, reproductive growth and development, and protein activity (Foyer et al. 2001; Zechmann et al. 2011; Noctor et al. 2012; Marquez-Garcia et al. 2014). Cai et al. (2011b) found that application of exogenous GSH affects the accumulation pattern of many proteins under cadmium (Cd) stress in rice (*Oryza sativa* L.), and showed a genotypic- dependent effect. Besides its antioxidant functions, GSH is also the direct precursor of phytochelatins (PCs), which play key roles in heavy metal sequestration, chelation, and tolerance (Zhu et al. 1999; Hossain et al. 2012; Clemens and Ma 2016). Although significant progress has been made in learning about the multiple roles of GSH in abiotic stress tolerance, many aspects of GSH-mediated abiotic stress responses remain elusive. This chapter concentrates on the functions of exogenous GSH in defense against different abiotic stresses, and also briefly describes how exogenous GSH is absorbed and transported in regulating abiotic stress tolerance.

2 Glutathione Metabolism-Related Enzymes Conferring Abiotic Stress Tolerance

As an important non-protein sink of reduced sulfur, glutathione content is significantly affected by abiotic stresses in plants. Glutathione utilizing and regenerating enzymes such as glutathione reductase (GR), glutathione peroxidases (GPXs), glutathione *S*-transferases (GSTs), dehydroascorbate reductase (DHAR), glyoxalase I (Gly I), glyoxalase II (Gly II), and phytochelatin synthase (PCS) play central roles in scavenging abiotic stress-induced accumulation of ROS and MG as well as in the sequestration of toxic heavy metals into the vacuoles. An overview of the multiple functions of the glutathione and its related enzymes during abiotic stress conditions are shown in Fig. 8.1.

2.1 Glutathione Reductase

Glutathione reductase (GR; EC 1.8.1.7) belongs to the NADPH-dependent oxidoreductase family and plays key roles in plant cell defense against ROS by reducing GSSG to GSH (Gill et al. 2013). Edwards et al. (1990) purified and isolated different subcellular isoforms of GR and detected GR activity in mitochondrial, cytosolic, and chloroplastic

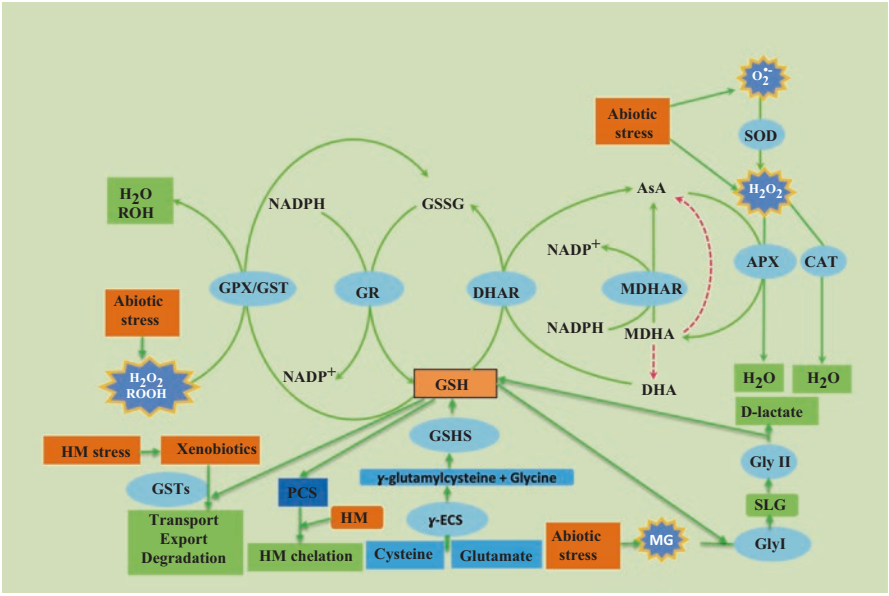

Fig. 8.1 Comprehensive scheme of GSH synthesis, interaction of GSH with its utilizing and regenerating enzymes in improving abiotic stress tolerance through stringent regulation of ROS and MG levels and heavy metal detoxification and chelation (modified from Hossain et al. 2012). GSH is synthesized from its constituent amino acids through two ATP-dependent reactions catalyzed by γ-ECS and GSHS. Superoxide ($O_2^{\bullet-}$) produced in cells is converted to H_2O_2 by SOD. H_2O_2 is then directly converted to H_2O by CAT or converted to H_2O by APX at the expense of AsA, depending on the cell compartment. The oxidized forms of ascorbic acid (MDHA and DHA) produced during the process are then converted to AsA by MDHAR and DHAR. GSSG is converted to GSH by GR at the expense of NADPH. GPX and GST catalyze the reduction of ROOH and H_2O_2, including lipid peroxides, to H_2O or alcohols. GSTs also catalyze the conjugation of metal-induced xenobioics and its transport into vacuoles. PCs sequester the metal to form a complex that is then transported into the vacuole. MG is degraded to form D-lactate through the glyoxalase pathway by the action of the enzymes Gly I and Gly II, which are GSH-dependent. R may be an aliphatic, aromatic, or heterocyclic group. *AsA* ascorbate, *DHA* dehydroascorbate, *γ-ECS* γ-glutamylcysteine synthetase, *HM* heavy metal, *NADPH* nicotinamide adenine dinucleotide phosphate, *APX* ascorbate peroxidase, *SOD* superoxide dismutase, *CAT* catalase, *GPX* glutathione peroxidase, *GR* glutathione reductase, *GSHS* GSH synthetase, *GSTs* glutathione *S*-transferases, *MG* methylglyoxal, *MDHA* monodehydroascorbate, *GSSG* oxidized glutathione, *GSH* reduced glutathione, *Gly II* glyoxalase II, *Gly I* glyoxalase I, *NADPH* nicotinamide adenine dinucleotide phosphate, *PCS* Phytochelatin synthase, *PCs* phytochelatins, *SLG S*- D-lactoylglutatione. For further discussion see the text

fractions of pea (*Pisum sativum* L.). GR is encoded by two genes, *GR1* and *GR2*. *GR1* encodes the protein that is detected in peroxisome and cytosol, while *GR2* encodes mitochondrial and chloroplastic GR (Kataya and Reumann 2010; Noctor et al. 2012). The positive function of GR in plant cells against abiotic stress has been widely reported. The major function of GR in conferring abiotic stress tolerance is the recycling of GSH and the maintaining of GSH/GSSG homeostasis (Noctor et al. 2012; Gietler et al. 2016). For instance, GR activity was increased in many plant species under abiotic stresses, such as heavy metal stress (Dazy et al. 2009), chilling (Turan and Eknekci 2011), salinity (Yazici et al. 2007), drought (Rapala-Kozik et al. 2008), and dehydration tolerance

(Gietler et al. 2016). However, some studies also reported that GR activity was decreased or not changed under abiotic stresses (Almeselmani et al. 2006; Hossain et al. 2010). It has been reported that the *GR1* deletion mutant showed normal growth and development whereas the *GR2* deletion mutant produced a lethal phenotype and showed growth arrest (Diaz-Vivancos et al. 2015). By using chloroplastic GR RNAi plants, Ding et al. (2009) showed that the activity of GR is very important for maintaining glutathione and the ascorbate pool under oxidative stress conditions. Additionally, it has been reported that the knockdown of *GR2* leads to early leaf senescence in *Arabidopsis* due to elevated levels of H_2O_2 and altered glutathione status (Ding et al. 2016a). Recently, Yin et al. (2017) reported that transgenic plants over-expressing the GR gene showed higher aluminum toxicity tolerance by increasing the ROS and RCS detoxification.

2.2 Glutathione peroxidases

Glutathione peroxidases (GPXs; EC 1.11.1.9) are a family of enzymes that catalyze the reduction of H_2O_2 and organic hydroperoxides using GSH as a reducing reagent (Diao et al. 2014). Unlike animals, most GPXs in plants are non-selenium dependent (Diao et al. 2014). Plant GPXs have been recognized as the fifth class of peroxiredoxins and are expressed in various subcellular compartments, such as cytosol, mitochondria, endoplasmic reticulum, and chloroplasts (Milla et al. 2003; Navrot et al. 2006). In *Arabidopsis*, eight related protein GPX isoenzymes, termed AtGPX1–AtGPX8, have been identified (Gaber et al. 2012). Gaber et al. (2012) found that the transcript and protein levels of AtGPX8, localized at the nucleus and cytosol, were up-regulated under oxidative damage induced by high-light stress and paraquat. Expression of *CsGPX2* was significantly up-regulated in *Camellia sinensis* plants under many abiotic stresses, including heavy metal, drought, and salinity (Fu 2014). Chen et al. (2004) reported that a tomato phospholipid hydroperoxide GPX (LePHGPX) not only inhibited cell death induced by oxidative stress in yeast, but also inhibited heat, salt, and Bax (a pro-apoptotic member of the Bcl-2 family of proteins) induced programmed cell death in *Nicotiana tabacum*. Transgenic tomato plants over-expressing GPX gene showed improved abiotic stress tolerance (Herbette et al. 2011) and transgenic *Arabidopsis* over-expressing *AtGPX8* showed higher oxidative stress tolerance and maintained cellular redox homeostasis (Gaber et al. 2012).

2.3 Glutathione S-transferases

Glutathione *S*-transferases (GSTs; EC 2.5.1.18) are a ubiquitous superfamily of enzymes that play important roles in many detoxification reactions (Dixon and Edwards 2010; Kumar et al. 2013). GSTs are also GSH-dependent detoxifying enzymes and constitute more than 1% of soluble protein in the leaves of maize (Marrs 1996). GSTs have multifunctional roles in plant cells: they catalyze GSH-dependent biotransformation processes, serve as binding and carrier protein for intracellular transport, and catalyze

conjugation reactions (Edwards et al. 2000). The GSH-based transferase activity is involved in the conjugation of GSH with cytotoxic, electrophilic, and hydrophobic substrates (Soranzo et al. 2004). Functioning as GPX, plant GSTs can catalyze the reduction of hydroperoxides to less harmful alcohols and safeguard the protein function from oxidative damage, whereas its DHAR activity is involved in the maintenance of the redox homeostasis by regenerating AsA from DHA (Dixon and Edwards 2010). In plants, GSTs can be induced by abiotic stresses and elevated GST levels, contributing to maintaining the cell redox homeostasis (Kumar et al. 2013; Cao et al. 2014). Cao et al. (2014) found that GST activity was increased >50% in Cd-tolerant barley genotype, while no change in a sensitive genotype was observed under Cd toxicity. Kumar et al. (2013) reported that the expression of *OsGSTL2* in *Arabidpsis* provides tolerance for salt, osmotic, cold, and heavy metal stress. Similarly, transgenic tobacco over-expressing the sweet orange (*Citrus sinensis*) tau type glutathione transferases (*CsGSTUs*) showed higher salt, drought, and herbicide tolerance (Lo Cicero et al. 2015).

2.4 Dehydroascorbate Reductase

Dehydroascorbate reductase (DHAR; EC 1.8.5.1), the GSH-dependent enzyme in the AsA-GSH pathway, maintains the redox pool of ascorbate by recycling dehydroascorbate (DHA) to AsA and limits ROS-induced damage in plant cells (Gallie 2013; Noshi et al. 2016). Apart from recycling of DHA, this enzyme also plays diverse roles in plant growth and different plant physiological processes such as photosynthesis (Chen and Gallie 2008). In guard cells, the signaling function of H_2O_2 is regulated by both its AsA content and DHAR (Gallie 2013). Under abiotic stress conditions, susceptible plants showed lower DHAR activity and AsA/DHA ratio than tolerant plants (Mittova et al. 2003a, b; Ren et al. 2016). Transgenic plants over-expressing *DHAR* also showed higher abiotic stress tolerance that is accompanied by higher DHAR activity, AsA levels, as well as ascorbate redox state (Kim et al. 2014). Importantly, *DHAR* knock-down mutant showed higher sensitivity to high light stress due to a lower AsA level as well as DHAR activity; however, the redox state of GSH was markedly affected. These results suggest that both AsA and GSH redox states are altered by DHAR (Noshi et al. 2016). Additionally, under stressful conditions, when MDHAR activity is inhibited, the DHAR activity increases and acts as a functional back-up to maintain the cellular redox balance (Locato et al. 2009; Noshi et al. 2016). Hence, higher AsA content by AsA recycling through DHAR optimizes the AsA utilization and modulates abiotic oxidative stress tolerance.

2.5 Glyoxalase System Enzymes

Thy glyoxalase system is a ubiquitous GSH-dependent detoxification system in plants. In this system the glyoxalase I (Gly I; EC 4.4.1.5) and glyoxalase II (Gly II; EC 3.1.2.6) enzymes transform MG, a cytotoxic compound produced in ample

amounts under stressful conditions, to D-lactate in different cellular organelles through two steps of irreversible reactions (Hossain et al. 2011; Hoque et al. 2016). In the first step, MG reacts with GSH forming hemithioacetal that is then converted to *S*-D-lactoylglutathione (SLG) in a reaction catalyzed by Gly I. In the second step, SLG is converted to D-lactate by the enzyme Gly II, being then the GSH regenerated in the system (Fig. 8.1). Recently, it has been reported that glyoxalase III can detoxify MG to D-lactate without of the participation of GSH (Ghosh et al. 2016). Although the glyoxalase system is involved in various plant physiological processes, its involvement in plant abiotic stress response and tolerance is considered crucial (Hossain et al. 2009, 2014a, b; Hossain and Fujita 2009; Kaur et al. 2014; Hoque et al. 2016). The glyoxalase system not only regulates MG levels in plants under stressful conditions but also regulates glutathione redox state through the recycling of GSH. A higher level of cellular GSH and GSH/GSSG ratio are required for stress defense against oxidative stress (Yadav et al. 2005a, b; Noctor et al. 2012). A large number of studies have shown a close link between the antioxidant and glyoxalase systems in plants (Hossain et al. 2010, 2011; Mostofa et al. 2015a, b; Nahar et al. 2015a, b, c). Recent studies in plants further demonstrated the diverse roles of this pathway in plant abiotic stress tolerance through the regulation of MG and ROS levels, allowing their signaling functions and improving stress tolerance through the expression of stress responsive genes (Hoque et al. 2012b, 2016; Li et al. 2017).

2.6 Phytochelatin Synthase

Phytochelatins [PCs; (γ-Glu-Cys)n-Gly (n = 2–11)] are widely accepted as the best-characterized heavy metal chelators and the major product for heavy metal detoxification and tolerance in plants, fungi, and other living organisms (Chia et al. 2013). PCs are cysteine-rich polypeptides that have high affinity for heavy metals (Lee and Hwang 2015). PCs are synthesized by the action of phytochelatin synthase (PCS) in cytosol with GSH as the precursor. Both GSH and PCs chelate heavy metals and metalloids such as Cd, copper (Cu), and arsenic (As), facilitating their sequestration into vacuoles (Cobbett and Goldsbrough 2002; Pilon-Smits 2005). In *Arabidopsis*, there are two genes encoding PCs, *AtPCS1* and *AtPCS2*. *AtPCS1* has been reported as the major player in PC synthesis, while the expression level of *AtPCS2* is much lower than *AtPCS1* in most tissues (Cobbett and Goldsbrough 2002; Blum et al. 2007). Meanwhile, AtPCS1 was ubiquitously present in *Arabidopsis* seedlings, while AtPCS2 was only found in the root tip (Blum et al. 2010). Blum et al. (2007) found that AtPCS1 had two cellular functions, mediating toxic heavy metal tolerance and GSH-conjugate degradation. Transgenic plants over-expressing *Arabidopsis* PCS gene (*AtPCS1*) in a non-accumulator plant *N. tabacum* improved Cd stress tolerance, and this response was further enhanced through the application of exogenous GSH (Pomponi et al. 2006). Besides heavy metals, PCs also play important roles in salinity, drought, heat, and UV-B tolerance (Chaurasia et al. 2016).

3 Uptake and Transport of Exogenously Applied GSH in Plant System

Uptake and transport of glutathione play central roles in many life processes, including sulfur assimilation, developmental processes, and tolerance against abiotic and biotic stresses. Glutathione-specific uptake systems have been found in plasma membranes of plant cells (Foyer et al. 2001). GSH uptake was observed in both protoplasts and cells (Noctor et al. 2012). Jamai et al. (1996) found that GSH was taken up by one saturable transporter with Km of 0.4 mM, while GSSG showed two systems with Km of 0.7 μM and 3.7 mM. In addition, it was also suggested that GSH and GSSG were taken up through proton symport. GSH uptake can be suppressed by GSSG and GS conjugates, while GSSG uptake can also be inhibited by GSH and GS conjugates (Zhang et al. 2004). Zhang et al. (2004) complemented a GSH-deficient yeast mutant with a GSH transporter cDNA from *O. sativa* and observed a strong increase in GSH uptake. Furthermore, the uptake activity showed a linear increase in the first 2–3 h. Noctor et al. (2000) incubated intact wheat chloroplasts with 100 and 1 μM ^{35}S-labelled GSH and found a time-dependent uptake within the initial 15 min. GSH concentration increased in all tissues of bean seedlings roots exposed to 1 mM GSH (Kumar et al. 2010). Moreover, GSH content in roots, leaves, and apex was increased 22-, 5-, and 3.5-fold after 4-h treatment, respectively. The results demonstrated that GSH is translocated to shoot and root systems through xylem.

GSH has been identified as a major form of long distance transport of reduced sulfur in xylem and phloem in plants, and can be readily exchanged between xylem and phloem in both directions (Schneider et al. 1994; Zhang et al. 2004). Different studies have suggested that GSH transport systems are present in membranes with fast exchange rates (Noctor et al. 2002; Tausz et al. 2004). The first high affinity GSH transporter (Hgt1p) was identified in *Scacharomyces cerevisiae* (Bourbouloux et al. 2000). In *Arabidopsis*, there are nine *Hgt1* homologues located in different chromosomes, and the homologues were also found in cotton (*Gossypium* sp.) and rice (*O. sativa)* (Foyer et al. 2001).

Intracellular transport between cytosol and organelles plays key roles in maintaining GSH homeostasis. Chloroplasts can synthesize GSH, and also uptake GSH from cytosol (Foyer et al. 2001). Noctor et al. (2012) suggested that γ-EC is produced exclusively in chloroplast, and then converted to GSH in chloroplast or transported to cytosol where the GSH can be transported to different organelles, including chloroplasts. Maughan et al. (2010) also reported that GSH biosynthesis was regulated by plastids and identified a plastid thiol transporter homologous to the *Plasmodium falciparum* chloroquine-resistance transporter (*Pf*CRT) in *Arabidopsis*. Arabidopsis mutants of the transporters were GSH-deficient and heavy metal-sensitive. In addition, knockout of the transporter family led to GSSG accumulation in cytosol, but not in plastids. In accordance with the literature, we suggest that exogenous GSH can be taken up through the root or leaf, then transported to different tissues via xylem and phloem, and finally transported to cytosol and different organelles via GSH transporters, which then play a positive role against abiotic stresses in plants.

4 Roles of Exogenous GSH in Modulating Abiotic Stress Tolerance

Abiotic stresses, in general, induce an overproduction of ROS and MG in plant cells and seriously limit different plant physiological process such as plant growth and development, leading to a reduced yield (El-Shabrawi et al. 2010; Saito et al. 2011; Hussain et al. 2016). Although GSH biosynthesis can be induced by abiotic stresses, this process can also be inhibited under serious stress conditions (Zhou et al. 2017). However, application of exogenous GSH can effectively compensate the decrease of endogenous GSH and improve abiotic stress tolerance in plants (Cai et al. 2010, 2011a; Zhou et al. 2017). A few recent studies focused on the effects of exogenous GSH in heavy metal, salinity, drought, heat, chilling, and low nutrient stresses through the assessment of different biochemical parameters related to stress tolerance (Chen et al. 2010; Cai et al. 2011a; Mostofa et al. 2014a; Nahar et al. 2015a, b, c; Hussain et al. 2016; Akram et al. 2017; Zhou et al. 2017). In the following section we will discuss the possible roles and mechanisms of exogenous GSH-mediated abiotic stress tolerance in plants.

4.1 Salinity Stress

Soil salinity is worldwide an increasing constraint in agricultural production, and nearly 20% of irrigated land has been affected by salinity in the world (Yamaguchi and Blumwald 2005). Oxidative stress and MG stress is also an important phenomenon of salinity (Mittova et al. 2003a, b; El-Shabrawi et al. 2010; Mostofa et al. 2015a; Akram et al. 2017). It has been suggested that the salt-tolerant genotypes displayed higher endogenous GSH concentrations than the susceptible genotypes in rice, tomato, and groundnut (Mittova et al. 2003a; El-Shabrawi et al. 2010; Kumar et al. 2010). The analysis of salt-tolerant and salt-sensitive cultivars also showed that the endogenous GSH levels and GSH-utilizing and regenerating enzymes are key factors in improving salt stress tolerance. Mittova et al. (2003a, b) showed that salt-tolerant *Lycopersicon pennellii* showed higher GSH biosynthesis, GSH content, GSH/GSSG ratio, and higher GST and GPX activities when compared to the salt-sensitive *L. esculentum* genotype. The tolerant genotype also showed lower H_2O_2 and malondialdehyde (MDA, lipid peroxidation marker) levels as compared to the sensitive one. GSH is of intrinsic importance in the prevention of salt-induced oxidative stress in *L. pennellii*, a mechanism that may also be employed by other salt-tolerant species. Subsequently, El-Shabrawi et al. (2010) showed that the salt-tolerant rice genotype (Pokkali) maintained a higher GSH and GSH/GSSG ratio, as well as Gly I, Gly II, SOD, CAT, peroxidase (POX), and GPX activities as compared to the salt-sensitive genotype (IR64). The tolerant genotype also showed lower ROS accumulation and ROS-induced DNA damage. These findings suggested the intrinsic function of GSH and proved that the

coordinate induction of GSH biosynthesis and GSH-metabolizing enzymes is correlated with salt stress tolerance.

A large number of recent studies also elucidated the role of exogenous GSH in conferring salinity tolerance in tomato (*Solanum lycopersicum* L.), mung bean (*Vigna radiata* L.), rice (*Oryza sativa* L.), and cotton through enhancing antioxidant and glyoxalase pathway enzyme activities, GSH content, and photosynthetic capacity (Wang et al. 2014a; Nahar et al. 2015a; Hussain et al. 2016; Akram et al. 2017; Ibrahim et al. 2017). By using contrasting rice cultivars (Pokkal, salt tolerance, and Peta, salt sensitive), Wang et al. (2014a) showed that the application of either GSH or AsA modulates the salt-induced oxidative stress tolerance. Under salt stress, rice seedlings supplemented with GSH or AsA displayed lower ROS and MDA content, as well as higher endogenous levels of GSH and AsA and higher SOD, APX, and GR activities than non-treated salt-stressed seedlings. Nahar et al. (2015a) showed the importance of GSH in modulating salt stress tolerance in mung beans by analyzing ROS and MG metabolism. An abrupt increase in ROS, MG, and MDA levels was found in response to salt stress. The relative water content (RWC), chlorophyll (Chl), and AsA content, as well as the GSH/GSSG ratio was decreased by salt stress. The activities of CAT, DHAR, MDHAR, and Gly I decreased whereas the activities of APX, SOD, GST, GR and GPX increased. Seedlings treated with GSH + salt treatment resulted in better salt-induced (short-term) oxidative stress tolerance as indicated by lower ROS and MG levels; higher RWC, Chl, AsA, GSH, and GSH/GSSG ratio, and induced ROS and MG detoxification systems (Nahar et al. 2015a). Recently Zhou et al. (2017) confirmed the positive roles of exogenous GSH in improving salt-induced oxidative stress tolerance in tomato (*S. lycopersicum* L. cv. Zhongshu No. 4). Exogenous application of GSH increased the transcript level of GSH synthesis and metabolizing enzymes such as γ -ECS, GSHS, GST, GPX, and GR, the content of intracellular GSH and AsA, and the GSH/GSSG and AsA/DHA ratios in salt-stressed plants and in salt-stressed plants treated with buthionine sulfoximine (BSO, inhibitor of GSH synthesis key enzyme γ -ECS). Application of GSH also enhanced the activities of SOD, CAT, POD, and enzymes related to the AsA-GSH cycle including APX, DHAR, MDHAR, and GR, and decreased the content of H_2O_2 and $O_2^{\bullet -}$, and lipid peroxidation levels. Consequently, Ibrahim et al. (2017) showed the positive impact of exogenous GSH in modulating salt stress tolerance in cotton by using the contrasting salt-sensitive 'Zhongmian 41' and salt-tolerant 'Zhong 9806' cultivars. The application of salt stress (150 mM NaCl) produced a significant decrease in morphological (root and shoot characteristics), physiological (photosynthetic rate), and biochemical (MDA and chlorophyll levels) traits, and an altered leaf/root ultrastructure. Applications of exogenous GSH mitigated those deleterious effects, with a greater influence noticed in the salt-sensitive genotype.

Apart from improving salinity stress tolerance at the seedling stage, our recent study also showed that the application of exogenous GSH improves salinity stress tolerance in rice at the reproductive stage. Imposition of salt stress (200 mM NaCl) at the flowering stage resulted in a significant decrease in yield and yield-attributing traits, and a greater decrease was found in the salt-sensitive genotypes. Application

of exogenous GSH improves salt stress tolerance as indicated by higher effective tillers per plant, number of filled grains per panicle, spikelet fertility, 100-seed weight, and seed yield per plants as compared to non-treated salt-stressed seedlings. The beneficial effects of exogenous GSH were higher in salt-susceptible genotypes as compared to the salt-tolerant genotypes (Hussain et al. 2016). Subsequently, we further proved that exogenous GSH improved salinity stress tolerance at seedling as well as at reproductive stage in soybean [*Glycine max* (L.) Merrill]. The imposition of salt stress at reproductive stage decreased the yield and yield-contributing traits. Application of exogenous GSH improved plant height, number of branches per plant, number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight, and yield per plant. Importantly, application of exogenous GSH at seedling stage also improved the oxidative stress tolerance as indicated by lower H_2O_2 and MDA levels (Akram et al. 2017). The above studies clearly demonstrated the diverse function of exogenous GSH in modulating salt stress tolerance through the regulation of multiple stress responsive pathways.

4.2 Drought Stress

Drought- or water stress-induced excessive accumulation of ROS due to impairment of photosynthesis has been well documented in plants (reviewed in Cruz de Carvalho 2008). Plenty of studies have shown that increased synthesis or recycling of GSH and high GSH/GSSG ratio might be essential for drought resistance in plants (Selote and Khanna-Chopra 2004; Gorantla et al. 2007; Garg et al. 2012; Cheng et al. 2015; Nahar et al. 2015b). Drought tolerant wheat cultivar showed a higher GSH redox pool due to higher GSH biosynthesis and AsA-GSH cycle enzyme activities as compared to sensitive cultivar (Garg et al. 2012). Expressed sequence tags (ESTs) analysis of drought-tolerant indica rice (Nagina 22) genotype also showed a high expression of GSH- and AsA-related stress defence genes such as *GSTs*, *GPX*, *Gly I*, and *APX* (Gorantla et al. 2007). Imposition of drought stress at the panicle development stage showed that the drought-tolerant genotype (N22) showed higher GSH and AsA levels and higher antioxidant enzyme (GR, SOD, APX) activities as compared to the sensitive genotype (Selote and Khanna-Chopra 2004). The function of GSH in modulating drought stress tolerance through the regulation of ROS and MG detoxification systems by using exogenous GSH has also been reported (Nahar et al. 2015b). Imposition of drought stress (−0.7 Mpa) in mung bean (*V. radiata* L.) seedlings resulted in a decrease in plant biomass, AsA content, GSH/GSSG ratio, DHAR, MDHAR, and CAT activities, but increased MDA, $O_2^{\bullet -}$, H_2O_2, proline, and MG content. The activities of Gly I and Gly II were also increased under drought stress. Application of exogenous GSH significantly alleviated drought-induced oxidative damage through enhancing the capacity of glyoxalase and antioxidant systems (Nahar et al. 2015b). Recently, Chen et al. (2012) reported that Arabidopsis *GST U17*-knockout mutant had higher drought and salinity stress tolerance due to higher accumulation of GSH and abscisic acid

(ABA). To explore how the mutant accumulated ABA, wild type plants were treated with exogenous GSH, and it was found that these plants accumulated higher ABA than those grown in the absence of GSH. Moreover, GSH-treated plants were more tolerant to salinity and drought, suggesting an interaction between GSH and ABA in increasing plant fitness under stressful conditions (Chen et al. 2012). More recently it has been reported that GSH modulates salt and drought stress tolerance by direct effects on global transcriptional changes as well as on ABA and JA biosynthesis and signaling (Cheng et al. 2015).

4.3 Heavy Metal Stress

Heavy metal or metalloid stress negatively affects plant growth and development and alters the physiological, biochemical, and molecular plant processes (reviewed in Hossain et al. 2012). The roles of GSH in modulating heavy metal or metalloid stress tolerance have been well documented in plants (Hossain et al. 2012; Anjum et al. 2014; Zhou et al. 2016). Studies with heavy metal tolerant or hyperaccumulator plants showed that the biosynthesis of GSH and the activities of GSH-regenerating and utilizing enzymes have significant effects on heavy metal tolerance. Iannelli et al. (2002) showed that high GSH and AsA content as well as APX, CAT, GR, GST, and GPX activities are key players in Cd tolerance in *Phragmites australis*. Recent transcriptomic analysis using low or high Cd-accumulating genotypes also showed the important roles of GSH in Cd stress tolerance (Zhou et al. 2016). Additionally, it has been reported that GSH-mediated ROS and MG metabolism are also involved in heavy metal tolerance in plants (Singla-Pareek et al. 2006; Hossain et al. 2010; Chen et al. 2010; Cai et al. 2011a; Mostofa et al. 2015b).

Numerous recent studies using exogenous application of GSH in barley, rice, cotton, and tobacco under different heavy metal toxicity conditions have shown the key role of GSH in heavy metal tolerance (Table 8.1). Our previous studies suggested that genotypic differences in Cd tolerance could be positively linked to the endogenous GSH content. Similarly, alleviation of Cd stress by exogenous GSH was significantly associated with increased endogenous GSH (Chen et al. 2010; Cai et al. 2011a). For instance, Cai et al. (2011a) investigated the effect of 50 μM GSH treatment on PCs, GSH, and cysteine content, and photosynthetic performance in different rice genotypes submitted to 5 and 50 μM Cd stresses. Exogenous GSH significantly increased GSH and PCs in the roots after 5 d exposure to 5 μM Cd, whereas GSH, cysteine, and PCs content decreased in plants submitted to 50 μM Cd. Nevertheless, external GSH markedly increased chlorophyll content, net photosynthetic rate, Fv/Fm, and effective PSII quantum yield, but decreased quantum yield of regulated energy dissipation and coefficient of non-photochemical quenching in both genotypes, compared with Cd treatments. Hasan et al. (2016) reported that foliar application of GSH significantly increased PCs content under Cd stress in tomato. GSH can also sequestrate heavy metal into cell walls (Hasan et al. 2016). Exogenous GSH also significantly alleviated Cr^{6+}-induced growth inhibition via

Table 8.1 Alleviating effects of exogenous GSH in modulating heavy metals stress response and tolerance

Plant species	Stress imposed	Factors responsible for exogenous GSH-mediated stress tolerance	Reference
Barley (*Hordeum vulgare)*	Cadmium	Exogenous GSH improved the capacity of antioxidant defense system and photosynthesis, ameliorated Cd-induced damage on ultrastructure, and reduced Cd-induced ROS accumulation and Cd concentration	Chen et al. (2010), Wang et al. (2011)
Tomato (*S. lycopersicum*)	Cadmium	Upregulated transcript level of several transcription factor and increased nitric oxide and S-nitrosothiol content, GSH:GSSG and AsA:DHA ratio, and sequestration of Cd into vacuoles and cell wall	Hasan et al. (2016)
Cotton (Gossypium spp.)	Cadmium	Alleviated Cd-induced growth inhibition, photosynthesis reduction, ROS accumulation, and microstructure damage of chloroplast	Daud et al. (2016)
Tobacco (Nicotiana tabacum)	Cadmium, Copper and Zinc	Elevated chlorophyll and rubisco content, but decreased rubisco activity except Cu	Son et al. (2014)
Rice (Oryza sativa)	Cadmium	Increased endogenous GSH, mineral element and chlorophyll content, induced up-regulation of PCs, regulated antioxidant defense enzyme activity and Cd-tolerant-related protein expression level and reduced Cd accumulation	Cai et al. (2010, 2011a, b), Cao et al. (2013a, 2015)
Rice (Oryza sativa)	Chromium	Increased secretion of organic acids and cell viability, changed the forms of Cr ions and distribution, alleviated Cr-induced damage on ultrastructure of root cell and chloroplast	Qiu et al. (2013)
Rice (Oryza sativa)	Copper	Decreased ROS and proline content, regulated antioxidant and MG detoxification system and reduced Cu uptake	Mostofa et al. (2014a)
Rice (Oryza sativa)	Cadmium+ Chromium	Reduced Cr uptake and translocation, improved H^+-ATPase activity and Fe, Zn and Mn uptake and translocation, regulated antioxidant enzyme activity and repressed MDA accumulation	Cao et al. (2013b)

increasing GSH concentration and secretion of organic acids (Qiu et al. 2013). GSH can alter forms of Cr ions in rhizosphere and their distribution among different subcellular components (Qiu et al. 2013).

Heavy metal injury is mainly attributed to the over-accumulation of ROS, including H_2O_2, superoxide radical ($O_2^{\bullet -}$), and hydroxyl radical (•OH). Several studies have showed that exogenous GSH reduced ROS accumulation through counteracting heavy

metal-induced alterations of certain antioxidant enzymes and maintaining increased AsA/DHA and GSH/GSSG ratios (Chen et al. 2010; Cao et al. 2013a, b; Mostofa et al. 2014; Hasan et al. 2016). For instance, exogenous GSH significantly decreased $O_2\bullet^-$, H_2O_2 and MDA content in Cd-treated barley via: increasing extracellular GSH reduction, bringing root GPX, DHAR, and MDHAR activities down towards control levels, and increasing APX and CAT activities (Chen et al. 2010). External GSH also markedly increased MnSOD, sAPX, and tAPX activities, and up-regulated the expression level of certain APX and CAT isoenzymes, compared with Cd-treated plants. Similar results were also found in rice under copper (Cu) and combined Cd and chromium (Cr) stresses (Cao et al. 2013b; Mostofa et al. 2014a).

Exogenous GSH has the ability to decrease heavy metal uptake and transport, and ameliorate heavy metal-induced damage on root/leaf ultrastructure (Cai et al. 2010, 2011a; Wang et al. 2011; Cao et al. 2013a, b, 2015; Mostofa et al. 2014a). As mentioned above, GSH can be involved in regulating gene and protein expression. Cai et al. (2011b) investigated the effect of external GSH on 2-D based protein profiles under Cd stress in rice and found several proteins which levels were decreased by Cd treatment but increased in GSH + Cd-treated plants. These proteins included aminopeptidase N, clpA/clpB family protein, glycolipid transfer protein-like, and heat shock proteins. Hasan et al. (2016) found that foliar spray of exogenous GSH induced Cd tolerance; this response is related to the up-regulation of several transcription factors, including MYB transcription factors and ethylene-responsive transcription factors.

Based on the above discussion, it can be concluded that the mechanisms by which exogenous GSH alleviates heavy metal toxicity are mainly related to: the scavenging of the induced ROS production by regulating the antioxidant system; converting to PCs, which transport heavy metals into the vacuole; increasing photosynthesis performance; inducing organic acids secretion, which can chelate heavy metals; decreasing heavy metal uptake and transport and maintaining ion homeostasis; and up-regulating the expression of stress response genes.

4.4 Heat Stress

High temperature-induced oxidative and MG stress, and the involvement of GSH in improving heat stress tolerance have been well documented in plants (Mostofa et al. 2014b; Nahar et al. 2015c). Several studies using tolerant and susceptible genotypes have also shown the importance of GSH and its related enzymes in improving heat stress tolerance. Heat-tolerant wheat genotype (C 306) showed high SOD, CAT, APX, GR, and POD activities in response to heat stress at various stages (vegetative, anthesis, and 15 days after anthesis) of plant growth, whereas the activities of CAT, GR, and POX showed a significant decrease in the susceptible genotype (PBW 343). The level of H_2O_2 was also higher in the susceptible genotype due to the imposition of heat stress at various growth stages (Almeselmani et al. 2009). Heat acclimation-induced thermotolerance studies in tall fescue (*Festuca arundinacea*) and perennial

rye grass (*Lolium perenee*) also showed the importance of GSH and AsA in improving heat-induced oxidative stress tolerance (Xu et al. 2006). Wang et al. (2014b) showed that heat stress tolerance of wheat (*Triticum aestivum* L.) was associated with high GR, SOD, and POD activities. Recent studies also showed the positive roles of exogenous GSH in modulating heat stress tolerance. Nahar et al. (2015c) reported the intrinsic functions of GSH in conferring short-term heat stress tolerance in mung beans (*Vigna radiata* L.). Heat stress (42 °C, 24–48 h) resulted in a severe oxidative stress and MG stress as indicated by high H_2O_2, MG, $O_2^{\bullet -}$, Pro, MDA content and lipoxygenase (LOX) activity as well as lower chlorophyll and relative water content. The activities of MDHAR, DHAR, GPX, CAT, and Gly I decreased whereas the activities of APX, GR, and GST increased. Importantly, pre-treatment with exogenous GSH led to improved stress tolerance as indicated by lower ROS, MG, and MDA levels and LOX activity. The endogenous level of GSH and GSH/GSSG ratio was higher in GSH-pretreated heat-stressed seedlings. Most of the enzymes of antioxidative and glyoxalase systems showed higher activities as compared to heat-stressed seedlings. These findings demonstrated the positive roles of exogenous GSH in improving short-term heat stress tolerance. Recently, Ding et al. (2016b) also showed that the application of exogenous GSH improved heat stress tolerance in cucumber (*Cucumis sativus* L.) seedlings by regulating morphological, physiological, and biochemical parameters. Heat stress resulted in a significant decrease in plant height, shoot growth characteristics, chlorophyll content, and lower photosynthetic rates, whereas increased plant growth, chlorophyll content, and photosynthetic rates were observed in the GSH-treated heat-stressed seedlings. Proline content increased in response to heat stress but a greater increase in Pro content was observed in GSH-treated seedlings. Importantly, heat stress led to severe oxidative stress as indicated by lower GSH content, GSH/GSSG ratio, and higher $O_2^{\bullet -}$ and MDA levels, whereas GSH-treated plants showed lower oxidative damage and higher GSH levels and GSH/GSSG ratio. GSH-treated heat-stressed plants also showed a significant increase in the activities of APX, POD, and GR as compared to heat-stressed seedlings. Heat stress significantly reduces the expression of most of the calvin cycle enzymes whereas a significant increase in these enzymes was observed in GSH-treated heat-stressed plants (Ding et al. 2016b). The above findings clearly demonstrated the multiple functions of GSH in plant growth and heat stress tolerance.

4.5 Cold Stress

Cold stress that includes chilling and/or freezing temperatures adversely affects plant growth and development, with GSH and its related enzymes playing an important role in regulating cold temperature-induced oxidative stress tolerance (Walker and McKersie 1993; Kocsy et al. 2001; Yu et al. 2002, 2003; Kaur et al. 2008; Li et al. 2013; Ao et al. 2013a, b). Chill-tolerant tomato (*L. hirsutum)* showed higher GSH content, GSH/GSSG ratio, and GR activity than the sensitive *L. esculentum* (Walker and McKersie 1993). A strong relationship between tissue

GSH content and chill tolerance has been reported in maize (*Zea mays*) and wheat (*Triticum aestivum* L.) (reviewed in Kocsy et al. 2001). Yu et al. (2002, 2003) reported that cold-acclimation or H_2O_2-induced chill tolerance is associated with higher GSH biosynthesis and antioxidant enzyme activities. Later, Hung et al. (2007) reported in a chill-sensitive mung bean (*Vigna radiata* L.) cultivar that H_2O_2 treatment induced a chill tolerance comparable to cold acclimation, and this response was correlated with increased GSH content. Opposite results were observed if the seedlings were pre-treated with a GSH biosynthetic inhibitor, buthionine sulfoximine (BSO). Cold-tolerant chickpea (*Cicer arietinum* L.) breeding lines showed higher activities of CAT, APX, and GR as compared to sensitive lines (Kaur et al. 2008). Li et al. (2013) showed that cold-priming induced cold tolerance in *Jatropha curcas* L. that was associated with higher activities of APX, GPX, GR, and SOD as well as higher GSH, AsA, GSH, Pro, and glycinebetaine (GB) levels. Consequently, Ao et al. (2013a, b) found that the cold-acclimation induced cold stress tolerance was due to increased AsA and GSH content, higher POD, CAT, SOD, APX, and GR activities, and higher expression levels of Pro and GB biosynthetic genes. The exogenous application of GSH also improved chill stress tolerance. In this context, Lukatkin and Anjum (2014) observed that the application of 100 μM exogenous GSH decreased $O_2^{\bullet-}$ generation, electrolyte leakage, and lipid peroxidation intensity, and improved chill stress tolerance in cucumber (*Cucumis sativus* L.).

4.6 Low or Excessive Nutrient Stress

Like other abiotic stresses, low or excessive amounts of essential nutrients also induce oxidative stress in plants, affecting sustainable agricultural production (Ruiz et al. 2003; Cervilla et al. 2007; Han et al. 2009). GSH and its associated enzymes were also found to play key roles in protecting plants from nutrient deficiency and toxicity stress (Ruiz et al. 2003; Cervilla et al. 2007; Han et al. 2009; Ramírez et al. 2013). The activities of SOD and AsA-GSH cycle enzymes and the levels of GSH and AsA increased in response to boron (B) deficiency stress in citrus (Han et al. 2009). However, the synthesis of GSH was inhibited due to excess B in sunflower (*Helianthus annuus* L.), whereas the application of exogenous GSH or cysteine significantly reduces B toxicity as indicated by a similar foliar biomass to that of control plants (Ruiz et al. 2003). Cervilla et al. (2007) also showed that the higher synthesis of GSH and AsA and the activities of AsA-GSH cycle enzymes play an important role in B-toxicity tolerance in tomato (*Solanum lycopersicum* L.). Iron (Fe) is required for many biological processes in plants, such as photosynthesis, electron transport, and nucleic acid synthesis (Ramírez et al. 2013). Fe deficiency can also induce oxidative stress as indicated by a decreased GSH level and higher accumulation of ROS in leaf and root tissues as well as higher chlorophyll degradation (Ramírez et al. 2013). Exogenous

application of GSH alleviated Fe deficiency-induced chlorosis and restricted the over-accumulation of ROS. Additionally, exogenous GSH recovered the activity of APX to control level and preserved the level of ferredoxin2 (Ramírez et al. 2013). Later, Shanmugam et al. (2015) used a GSH-deficient mutant to investigate the mechanism of Fe-deficiency tolerance in *Arabidopsis*. The results showed that the mutant accumulated lower Fe than the wild type because of a lower expression level of Fe uptake-related genes under the Fe-deficiency condition. They also found that the nitric oxide-mediated induction of these genes was dependent of GSH addition in the mutant under the Fe-limited condition (Shanmugam et al. 2015). The results suggested that GSH supplementation can maintain cell redox homeostasis, activate the expression of Fe-uptake related genes, and increase internal Fe availability under Fe deficiency condition.

5 Conclusion and Perspectives

Oxidative and MG stress are a common characteristic of abiotic stresses, and pose a serious threat for normal plant growth and development, restricting full genetic potential to deal with the stress. Importantly, the pathways of GSH biosynthesis, transport, and metabolism have been well established in plants and plenty of research evidences suggest that the redox-state of glutathione is at the hub of the stress-signaling pathways modulating abiotic stress response and tolerance. Moreover, exogenous GSH plays an essential role during abiotic stress tolerance at various stages of plant growth. In summary, under abiotic stress conditions, GSH is mainly involved in: (1) antioxidant defense and ROS signaling, (2) MG detoxification and MG signaling, (3) direct or indirect metal chelation and sequestration, (4) increasing the expression of genes related to abiotic stress tolerance or nutrient transport, (5) xenobiotic detoxification, and (6) protecting cellular structures and reproductive development. However, key questions related to how exogenous GSH is absorbed and transported in different cell compartments, including cytoplasm, chloroplast, and mitochondria, need further investigation. In addition, GSH can induce ABA accumulation under stressful conditions. Nevertheless, the interaction of GSH with other plant hormones (such as jasmonic acid, salicylic acid, and ethylene) and signaling compounds like nitric oxide and Ca^{2+} needs to be elucidated. Additionally, more studies are needed to apply the current knowledge in practical agricultural production and breeding.

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