



# Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance

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**Abstract** Glutathione (GSH;  $\gamma$ -glutamyl-cysteinyl-glycine) is a small intracellular thiol molecule which is considered as a strong non-enzymatic antioxidant. Glutathione regulates multiple metabolic functions; for example, it protects membranes by maintaining the reduced state of both  $\alpha$ -tocopherol and zeaxanthin, it prevents the oxidative denaturation of proteins under stress conditions by protecting their thiol groups, and it serves as a substrate for both glutathione peroxidase and glutathione *S*-transferase. By acting as a precursor of phytochelatin, GSH helps in the chelating of toxic metals/metalloids which are then transported and sequestered in the vacuole. The glyoxalase pathway (consisting of glyoxalase I and glyoxalase II enzymes) for detoxification of methylglyoxal, a cytotoxic molecule, also requires GSH in the first reaction step. For these reasons, much attention has recently been directed to elucidation of the role of this molecule in conferring tolerance to abiotic stress. Recently, this molecule has drawn

much attention because of its interaction with other signaling molecules and phytohormones. In this review, we have discussed the recent progress in GSH biosynthesis, metabolism and its role in abiotic stress tolerance.

**Keywords** Antioxidant · Glyoxalase system · Oxidative stress · Phytochelatin · Thiol · Reactive oxygen species

## Abbreviations

$\gamma$ -ECS	$\gamma$ -Glutamylcysteine synthetase
ABA	Abscisic acid
APX	Ascorbate peroxidase
AsA	Ascorbate
CAT	Catalase
Cys	Cysteine
DHAR	Dehydroxyascorbate reductase
Glu	Glutamine
Gly I	Glyoxalase I (Lactoylglutathione lyase)
Gly II	Glyoxalase II (Hydroxyacylglutathione hydrolase)
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSHS	Glutathione synthase
GSNO	<i>S</i> -Nitrosoglutathione
GSSG	Glutathione disulfide
GST	Glutathione <i>S</i> -transferase
JA	Jasmonic acid
MAP	Mitogen activated protein
MDA	Malondialdehyde
MG	Methylglyoxal
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric oxide
PC	Phytochelatin
PCS	Phytochelatin synthase

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POD	Peroxidase
ROS	Reactive oxygen species
SA	Salicylic acid
SLG	S-D-Lactoylglutathione
SOD	Superoxide dismutase

## Introduction

Due to the climate change environmental stresses are frequently associated with plant production significantly. Worldwide, abiotic stresses have been estimated to reduce average crop yields by 50% (Hasanuzzaman et al. 2012a). Understanding plants responses to stress and developing tools for improving stress tolerance are keys to prevent crop loss under abiotic stress conditions.

Abiotic stresses generally result in overproduction of reactive oxygen species (ROS) such as singlet oxygen ( $^1\text{O}_2$ ), superoxide ( $\text{O}_2^-$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\text{OH}\cdot$ ). These ROS cause oxidative damage that leads to peroxidation of lipids, oxidation of proteins, inhibition of enzymes, and damage to DNA/RNA. The ROS are also recognized for their signaling action in regulating developmental processes and in the development of stress tolerance (Mittler 2002; Sharma and Dubey 2005). Regulation of ROS is therefore vital for improving the stress resistance of plants and is implemented by an antioxidant defense system composed of a number of antioxidant enzymes and non-enzymatic antioxidants.

Antioxidants are among the key elements those protect plants from the oxidative damages caused by abiotic stresses. Among the non-enzymatic antioxidants, glutathione (GSH;  $\gamma$ -glutamyl-cysteinyl-glycine) is a low molecular weight, water soluble thiol compound that is distributed widely in most plant tissues. Apart from the role in storage and transport of reduced sulfur GSH takes part in the detoxification of ROS, directly or indirectly (Foyer and Noctor 2005a, b). Apart from the ROS detoxification, GSH participates in methylglyoxal (MG) detoxification (Hasanuzzaman et al. 2017). It acts as a co-factor in different biochemical reactions, it interacts with hormones, signaling molecules, and its redox state triggers signal transduction (Foyer and Noctor 2005a, b). Another major function of GSH is the formation of phytochelatin (PCs) that bind heavy metals for safe transport and sequestration in the vacuole (Sharma and Dietz 2006). Thus it plays vital role in detoxifying toxic metals/metalloids and xenobiotics (Srivalli and Khanna-Chopra 2008). The various biochemical properties of GSH give it the potential for involvement in plant growth and development, both under normal growing conditions and under different stress conditions. Glutathione modulates cell proliferation, apoptosis, fibrogenesis, growth, development, the cell

cycle, gene expression, protein activity and immune function (Noctor and Foyer 1998; Ogawa 2005; Shao et al. 2008; Nahar et al. 2015a).

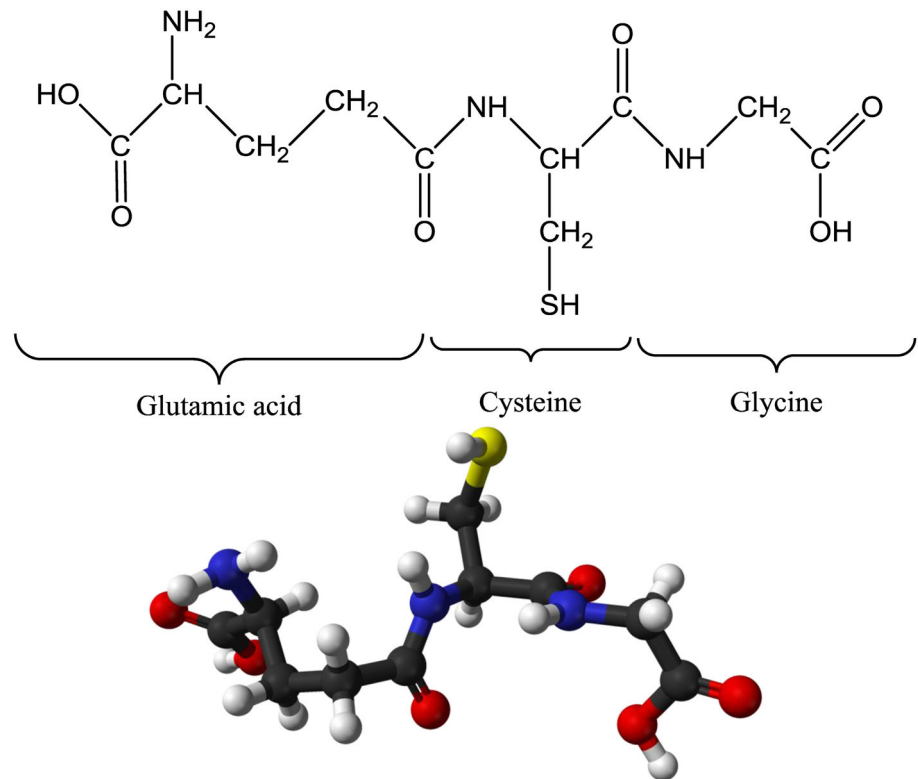
Glutathione enhances plant tolerance to different abiotic stresses, including salinity, drought, high temperature (HT), low temperature, and toxic metal stress (Hasanuzzaman and Fujita 2011; Hasanuzzaman et al. 2011, 2012a, b; Luo et al. 2011; Hasanuzzaman and Fujita 2013). Exogenously applied GSH can improve abiotic stress tolerances in plants (Kattab 2007; Salama and Al-Mutawa 2009; Wang et al. 2011; Wu et al. 2011; Chen et al. 2012; Nahar et al. 2015a, b, c). In this review, we concentrate on the biosynthesis and metabolism of GSH and its roles in plants under environmental stress conditions, focusing on recent research findings on GSH and abiotic stress tolerance.

## Biochemical nature of glutathione

Glutathione was first identified in yeasts in 1888 but its structure was not described until 1935. Intensive study began in the 1960s due to the discovery of its functions in human body fluids. The clarification of GSH metabolism was credited to Dr. Alton Meister due to his indisputable contribution (Meister and Anderson 1983). Glutathione is often termed as a nonprotein reduced sulfur and is a strong water-soluble antioxidant.

Glutathione is a ubiquitous tripeptide ( $\gamma$ -glutamyl-cysteinyl-glycine;  $\gamma$ -Glu-Cys-Gly) that contains long hydrophilic groups. The structure of GSH includes a bond between the carboxyl group of the glutamate (Glu) side-chain and the amine group of cysteine (Cys) which is attached to a glycine by a peptide bond (Fig. 1). Nevertheless, the thiol group is the main chemically reactive group with respect to its biological and biochemical functions (Zagorchev et al. 2013). The Glu linkage of GSH leads to an increased reactivity with respect to its participation in the  $\gamma$ -glutamyl cycle and protects GSH against attack by aminopeptidases (Wonisch and Schaur 2001). Glutathione is a low molecular weight tripeptide found at high (millimolar) concentration in all aerobic organisms (Hasanuzzaman et al. 2012a; Gill et al. 2013). It is found in the cytosol, endoplasmic reticulum, vacuoles, mitochondria, chloroplasts, peroxisomes, and the apoplast (Noctor and Foyer 1998). For instance, in tobacco mesophyll cells, GSH is distributed as 76, 17, and 7% in chloroplast, vacuole, and cytoplasm, respectively (Berthe-Corti et al. 1992). Some plant species (e.g. soybean, pea, peanut) do not primarily contain GSH; instead, they contain another thiol called homoglutathione ( $\gamma$ -glutamylcysteinyl- $\beta$ -alanine), which functions in a similar way to GSH and shows the same redox reaction.

**Fig. 1** Chemical structure (above) and ball-and-stick model of the glutathione molecule, C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>6</sub>S



## Glutathione biosynthesis and metabolism

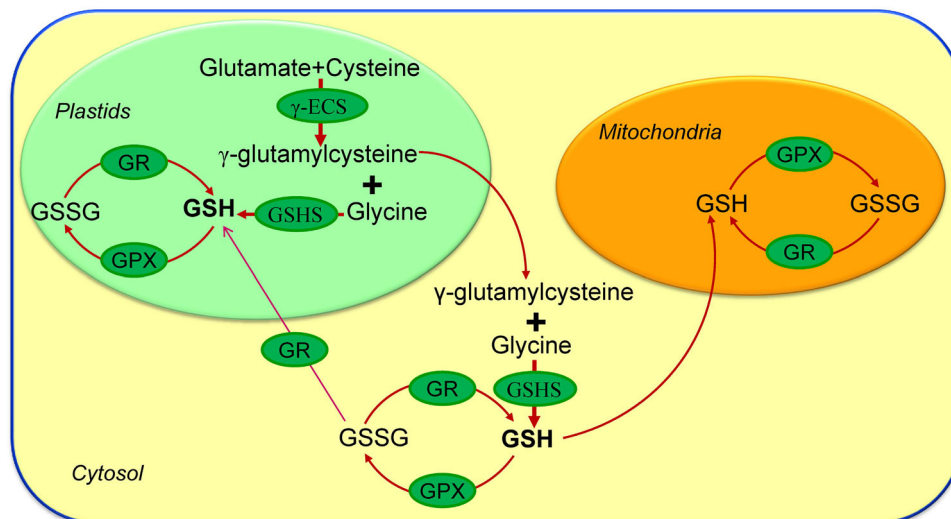
In general, GSH synthesis takes place in the chloroplast, cytosol, and mitochondria (Mahmood et al. 2010; Zechmann and Müller 2010). However, the major processes occur in the chloroplast and consist of a two-step reaction. The reaction starts with three kinds of amino acids Glu, Cys, and glycine. The synthesis of GSH is catalyzed by two enzymes [ $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS) and glutathione synthase (GSHS)]. This synthesis process occurs through a two-step reaction at the expense of two molecules of ATP (Fig. 2). The  $\gamma$ -ECS is found in plastids and GSHS is found both in the cytosol and plastids; for this reason, plastids are considered the site of GSH production in higher plants (Pasternak et al. 2008). The earliest step of GSH biosynthesis consists of a reaction between the  $\gamma$ -carboxyl group of Glu and  $\alpha$ -amino group of Cys to create an amide bond to yield  $\gamma$ -ECS; and the  $\gamma$ -ECS enzyme catalyzes this reaction. The next step is GSH formation; which occurs in the presence of the GSHS enzyme by amide bond formation between the  $\alpha$ -carboxyl group of the cysteine moiety in  $\gamma$ -glutamylcysteine and the  $\alpha$ -amino group of glycine, to form GSH (Hell and Bergmann 1990; Xiang and Oliver 1999). It is also possible to export the  $\gamma$ -ECS from the plastids to the cytosol, where it serves as the precursor to GSH biosynthesis. After the biosynthesis of GSH in the cytosol, it can be efficiently reimported into the plastids or transported into mitochondria (Mahmood et al. 2010).

Once synthesized in the cytosol, GSH can be imported directly or in other forms, into other organelles to meet metabolic requirements. However, rapid conversion of this reduced GSH into oxidized glutathione or glutathione disulfide (GSSG) can occur through different biochemical reactions within different compartments, and especially during adverse or stress conditions. The activities of glutathione reductase (GR) and glutathione peroxidase (GPX) maintain a balanced state of GSH/GSSG (Mahmood et al. 2010). Different enzyme activities are responsible for the GSH homeostasis and the GSH/GSSG ratio, which are also significantly affected by abiotic stress conditions. The AsA-GSH cycle plays a substantial role in this process. The synthesis of GSH is controlled by several factors; the most vital of these is feedback inhibition of  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS).

## Glutathione-induced abiotic stress tolerance

### Salinity

Glutathione can aid in improving plant performance under salt stress. Seedling growth in media containing salt is regulated by GSH or the activities of glutathione-dependent and regulating enzymes (Roxas et al. 1997; Hussain et al. 2008). Transgenic tobacco plants overexpressing both GST and GPX biosynthesis genes increased seedling



**Fig. 2** Biosynthesis and metabolism of glutathione.  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -ECS). First step (in plastid): Glutamate and cysteine together form  $\gamma$ -glutamylcysteine through a reaction catalyzed by the enzyme  $\gamma$ -ECS ( $\gamma$ -glutamylcysteine synthetase). Second step (in cytosol or in plastid):  $\gamma$ -glutamylcysteine and glycine bond together to form GSH via a reaction catalyzed by GSHT: glutathione

synthase. The synthesized GSH can be utilized by cellular organelles like the plastids or mitochondria or in the cytosol. GSH participates in ROS scavenging and is converted into GSSG by the enzyme GPX. GSSG can be reconverted/recycled again into GSH by the activity of GR. Nahar et al. (2016b), with Permission from Wiley

establishment, and seedlings grew faster under salt stress. This effect could be caused by regulation of the glutathione pool (Roxas et al. 1997). The antioxidative function of glutathione reduces oxidative stress, prevents lipid peroxidation, and protects the plasma membrane. Stabilization of the plasma membrane by glutathione helps to reduce passive  $\text{Na}^+$  influx, which enhances plant salt tolerance. Glutathione also helps to maintain cellular redox balance and performs signaling functions in plants under salt stress (Foyer and Noctor 2005a). Enhanced GSH level was also correlated with enhanced salt tolerance in several studies. For example, wild type canola seedlings showed a three-fold increase in GSH and Cys synthesis during exposure to salt stress when compared to transgenic plants that lacked the genes for GSH and Cys synthesis; the wild type also showed greater salinity tolerance, suggesting a protective mechanism for GSH against salt stress (Ruiz and Blumwald 2002).

Glutathione is also associated with the detoxification of methylglyoxal. It acts as a cofactor in the glyoxalase system and helps to detoxify methylglyoxal in step-by-step reactions involving two enzymes viz. glyoxalase I (Gly I) and glyoxalase II (Gly II). Research findings have demonstrated that GSH acts as a methylglyoxal detoxifier that confers salt stress tolerance (Singla-Pareek et al. 2003). An experiment by El-Shabrawi et al. (2010) conducted with Pokkali (salt tolerant) and IR64 (salt sensitive) rice cultivars, indicated that the GSH level and both Gly I and II activities were significantly higher in Pokkali than in IR64. When both cultivars were exposed to salt

stress, Pokkali showed higher GSH/GSSG ratio and ascorbate (AsA)/dehydroascorbate (DHA) ratio, together with higher activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and GPX, when compared to IR64. The higher GSH level and homeostasis (i.e., GSH/GSSG ratio) and the enhanced activity of the antioxidant and glyoxalase systems were associated with the alleviation of salt induced damage in the salt tolerant Pokkali cultivars, as indicated by the reduced Na/K ratio, the status of ROS, and the extent of oxidative DNA damage when compared to the sensitive IR64 cultivar (El-Shabrawi et al. 2010). The protective function against NaCl-induced oxidative stress in tobacco arose by a reduction in protein carbonylation and an improved antioxidant defense and MG detoxification systems triggered by exogenous proline and glycinebetaine application. Increased GSH level, GSH redox state, and activity of GPX, glutathione-S-transferase (GST) and Gly I were associated with salt stress tolerance in tobacco (Hoque et al. 2008). Exogenous GSH (0.5 mM) ameliorated onion bulb epidermis damage, improved membrane permeability, prevented protoplasmic swelling, and maintained cell viability under salt stress (150 mM NaCl).

Salama and Al-Mutawa (2009) described the roles of GSH as ROS scavenger in the improvement of salt stress tolerance. They mentioned a GSH-triggered mitigation in salt-induced alterations in the plasma membrane of onion epidermal cells. Salt stress imposed on two cultivars of canola (*Brassica napus* L. cv. Serw and cv. Pactol) significantly reduced several plant growth parameters, as well

as contents of pigments, total soluble carbohydrates, free amino acids, and total soluble proteins. The amounts of different antioxidant components like GSH, AsA, carotenoids, phenols, proline, and glycine betaine also changed in response to salt stress. Seed priming with exogenous GSH and polyadenylic acid improved the tolerance of *Brassica* seedlings to salt stress and was associated with increased levels of antioxidants and enhanced activities of SOD and phenol peroxidase antioxidant enzymes (Kattab 2007).

A role of GSH as an antioxidant has been confirmed in several studies on plant growth under salt stress. GSH applied exogenously during salt stress regulated other antioxidant components (both enzymatic and non-enzymatic) and compatible organic solutes or osmoprotectants (Foyer and Noctor 2005a; Kattab 2007; Salama and Al-Mutawa 2009). Glutathione could prevent the loss of photosynthetic pigments under salt stress (Kattab 2007). These responses point to the underlying mechanisms responsible for improved plant growth under salt stress induced by GSH. The GSH redox signaling process enhances salt tolerance, as seen in an *Arabidopsis* mutant that showed adaptive responses to salt stress, where GSH functioned as a downstream component of signal transduction pathways. This mutant showed hyposensitivity to ABA (abscisic acid) during seed germination which was associated with higher accumulation of GSH. It also reduced stomatal aperture and transpiration rate and performed better root and vegetative development which were attributed due to higher GSH and ABA levels (Shao et al. 2008).

The performances of salt-tolerant (Pusa Vishal) and salt-sensitive (T44) mung bean cultivars were examined following salicylic acid (SA) treatment. Salt tolerant cultivars showed better performance in response to SA application under salt stress when compared to the salt sensitive cultivar; this improved performance was due to a reduction in oxidative stress and improved physiological performance. The tolerant cultivars showed higher GSH content and higher activities of AsA-GSH cycle components, restricted  $\text{Na}^+$  and  $\text{Cl}^-$  content in the leaves, higher efficiency of PSII, better photosynthetic N-use efficiency, and improved water relations (Nazar et al. 2011). Recently, Nahar et al. (2015c) showed that exogenous GSH could result in coordinated induction of the antioxidant and glyoxalase systems which effectively reduced oxidative damage and MG toxicity, and improved the physiological adaptation of *V. radiata* seedlings to short-term salt stress (200 mM NaCl, 24 and 48 h).

Hormone induced regulation of GSH and their contribution in abiotic stress tolerance have been proved in several research findings. Increased glutathione level results in salt stress tolerance and global translational

changes in *Arabidopsis*. Both exogenous and endogenous GSH delayed senescence and flowering time. Translatome analysis also exposed that ABA, auxin and jasmonic acid (JA) biosynthesis, and signaling genes were activated during GSH treatment (Cheng et al. 2015). A direct link between cellular redox status and auxin signaling has been reported during characterization of a triple mutant of *Arabidopsis* (Bashandy et al. 2010). The GST and GPX activities were higher in the auxin autotrophic tobacco callus lines which were suggested to enhance salt stress (100 mmol/L NaCl) tolerance. In the autotrophic tissues, the upregulated antioxidant enzyme activities led to reduced levels of  $\text{H}_2\text{O}_2$  and MDA levels under NaCl stress. The auxin autotrophic line also showed higher tolerance to exogenous  $\text{H}_2\text{O}_2$  (Csiszár et al. 2004). Cytokinin (in the form of 6-benzyladenine, 6-BA) application showed protective effect against salt stress by improving in photosynthesis and antioxidant capacity. Exogenous 6-BA increased AsA, GSH and proline contents, activities of SOD and POD and decreased  $\text{O}_2^-$  production rate and MDA content in eggplant under salt stress (Wu et al. 2014). Polyamines (spermidine, spermine and putrescine) application conferred salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. In antioxidant defense system, exogenous polyamines improved GSH level and GSH/(GSSG, glutathione disulfide) ratio, activities of GR and GPX which defended overproduction of ROS. In glyoxalase system, increased activities of glyoxalase II (which is a GSH dependent enzyme) and enhanced GSH level contributed to reduce toxic methylglyoxal level (Nahar et al. 2016a, b).

## Drought

Drought-induced impairment of stomatal conductance, membrane electron transport rate,  $\text{CO}_2$  diffusion, carboxylation efficiency, and photosynthesis lead to the generation of ROS which causes oxidative damage. Limited growth and developmental processes, reduced growth, and lower final crop yield are some common effects of drought (Pinheiro and Chaves 2011). Glutathione and its metabolic enzymes interact with plant growth regulators, which are regarded as vital in the scheme of plant establishment both under normal and stressful conditions. GST expression in plants is induced by phytohormones such as SA, ethylene, cytokinin, auxin, ABA (Marrs 1996), methyl jasmonate (Moons 2003), and brassinosteroids (Deng et al. 2007). Chen et al. (2012) demonstrated that GST overexpressed in *Arabidopsis thaliana* plants had a signaling role and it regulated plant development by maintaining GSH pools. The mutant plant atgstu17 synthesized higher levels of GSH and ABA when compared to the wild type. Moreover,



supplementation of wild type plants with exogenous GSH resulted in higher ABA content and a similar phenotype to that seen in the mutant, as well as greater drought tolerance.

Glutathione application induced higher levels of ABA, the plant hormone that controls seed germination, regulates stomatal aperture, and reduces transpiration rate (Chen et al. 2012). Drought stress resulted in leaf rolling in *Ctenanthe setosa* (Rosc.) Eichler (Marantaceae); this leaf rolling was considered as an adaptive nature and was accompanied by an enhanced GSH content and a decreased GSSG content. The level of GSH and the enzymes of the AsA-GSH cycle were associated with ROS detoxification. High GSH content was also correlated with regulation of leaf water content, which may indicate a role in controlling leaf rolling (Saruhan et al. 2009). Glutathione supposedly has growth regulating functions, as elevation of the endogenous GSH level enhances cell division in the root meristematic region (Vernoux et al. 2000); this elongation is an important morphological adaptation to drought.

Numerous studies have confirmed that elevated endogenous GSH levels can confer drought stress tolerance and that GSH ameliorates ROS-induced damages. Drought stress was responsible for severe oxidative stress in *B. napus*, as indicated by a pronounced rise in  $H_2O_2$  and lipid peroxidation levels. Drought stress significantly increased GSSG content in the seedlings. Exogenously applied selenium had a beneficial effect because it reduced oxidative stress and improved drought stress tolerance by increasing endogenous GSH levels. The GSH/GSSG ratio and the activities of AsA-GSH cycle enzymes were also increased (Hasanuzzaman and Fujita 2011). Alam et al. (2013) also found similar results for endogenous GSH under drought stress.

Osmotic stress is a primary effect of drought stress. Glutathione and its related antioxidants were observed to reduce osmotic stress in maize and in wheat plants. In wheat, imposition of osmotic stress at the seedling stage increased total GSH and hydroxymethylglutathione. By contrast, in maize, the same level of osmotic stress enhanced GR activity and increased the total amount of hydroxymethylglutathione. Osmotic stress resulted in a great increase in the total GSH content in wheat seedlings and the GR activity in maize seedlings (Kocsy et al. 2002). GSH and glutaredoxins are concerned with detoxification of ROS, but they also transmit redox signals under drought stress (Meyer 2008). The GSH pools in the *Arabidopsis* atgst17 mutant were 35% higher compared with wild-type plants (Jiang et al. 2010). Application of exogenous betaine and proline application improved GSH level and maintained high activities of GST and Gly I, thereby protecting lentil plants from oxidative damage and toxic effects of MG (Molla et al. 2014).

Koffler et al. (2014) demonstrated the signaling roles of GSH in *Arabidopsis thaliana* under drought stress. During drought stress, compartment specific changes in GSH and AsA contents were recorded. *Arabidopsis thaliana* Col-0, and the GSH and AsA deficient mutants, pad2-1 and vtc2-1, respectively, were exposed to drought stress for 10 days. It was observed that the GSH content strongly decreased in both mutants at the early stages of drought stress, when the stress in leaves was not yet measurable. The authors concluded that GSH was used to indicate drought stress perceived by the roots to the leaves at early time points. The wild type plants also showed better performance under drought stress, compared to mutants. The mutants had reduced GSH and AsA contents, so they had lower activities of antioxidative enzymes, i.e., GR and DHAR (dehydroascorbate reductase) in chloroplasts and peroxisomes. This lower activity of these enzymes occur due to the Calvin cycle malfunctioning and the occurrence of photorespiration under drought stress, which caused greater accumulation of reactive oxygen species compared with the wild type (Koffler et al. 2014).

Glutathione (GSH) was involved in stomatal closure induced by ABA, as its presence in guard cells regulated ABA signaling in the *Arabidopsis* cad2-1 mutant (Okuma et al. 2011). Akter et al. (2012) showed an involvement of GSH in stomatal closure induced by ABA and methyl jasmonate (MeJA) in *Arabidopsis thaliana*. They found that exogenously applied GSH decreasing chemicals viz. p-nitrobenzyl chloride (PNBC), iodomethane (IDM), resulted in the depletion of GSH in guard cells of stomata. However, this decrease in GSH could increase the ROS significantly.

Involvement of hormone and GSH in improving drought stress tolerance was mentioned in several previous studies. Higher glutathione content improved drought stress tolerance and global translational changes in *Arabidopsis*. Abscisic acid, auxin and JA biosynthesis, and signaling genes were also activated during GSH treatment as revealed from transcriptomic study (Cheng et al. 2015). Drought-induced glutathione transferase, GST8 was identified in *Arabidopsis*. GST8 RNA levels were strongly induced by 2,4-D and NAA (two synthetic auxins) and by IAA (Bianchi et al. 2002). The *iaaM-OX* transgenic lines with higher endogenous indole-3-acetic acid (IAA) level and IAA pre-treated wild type *Arabidopsis* plants exhibited enhanced drought stress resistance. Moreover, endogenous and exogenous auxin positively modulated the expression levels of multiple abiotic stress-related genes and ROS metabolism, contents of GSH and GSSG and activity of GR. Some carbon metabolites including amino acids, organic acids, sugars, sugar alcohols and aromatic amines were also significantly affected by auxin under drought stress. Endogenous and exogenous auxin positively

modulated root architecture especially the lateral root number (Shi et al. 2014).

### Extreme temperature

Extreme temperatures at either high or low ranges are major environmental adversities that limit plant production (Hasanuzzaman et al. 2013). A beneficial role of GSH under HT stress has been investigated in few studies. The GSH pools, with their temporal and spatial changes, together with the GSH redox state and its regulation and roles in redox signaling and defense processes, are important components for the thermotolerance (Szalai et al. 2009). The glutathione pool is associated with the HT stress response of maize at the seedling stage. Total GSH levels and the GSH/GSSG ratio were increased by HT stress (40 °C, 1 day) treatment in chilling-sensitive maize genotypes, whereas GR activity and total hydroxyl methylglutathione content increased in both chilling sensitive and tolerant maize genotypes (Kocsy et al. 2002). *Coleus blumei* and *Fagus sylvatica* L. plants were exposed to heat stress (35 °C), which resulted in oxidative stress. However, after a short-term acclimation to low temperature, the seedlings of both species showed modulations in the GSH pool and activity of GR, although in different ways, which was correlated with the HT stress tolerance of these species. The GSH level and GR activity were higher in *F. sylvatica* than in *Coleus blumei*, so *F. sylvatica* also showed better tolerance to HT. The HT susceptibility of *C. blumei* was associated with a differential loss of GR and GSH at low temperature (Peltzer et al. 2002).

A higher content of total GSH was suggested to confer HT tolerance in *T. aestivum*, *Z. mays* and *V. radiata* (Nieto-Sotelo and Ho 1986; Dash and Mohanty 2002; Nahar et al. 2015a). Efficient elimination of H<sub>2</sub>O<sub>2</sub> was possible due to the elevated GSH level in heat stressed mustard seedlings, which was facilitated by improved GR activity (Dat et al. 1998). Higher GSH levels at the reproductive stage in apple plants enhanced their capacity for HT acclimation. Apple peels exposed to HT and excessive solar radiation showed increased endogenous GSH levels, which improved the acclimation capacity under HT stress. Exogenous application of AsA + benzoic acid and SA increased the endogenous GSH level by 33.97 and 31.81%, respectively, compared to the control, and acclimation was again enhanced (Zhang et al. 2008). The AsA–GSH cycle was up-regulated in response to elevated temperatures in two-year-old *Malus domestica* Borkh. plants exposed to 40 °C for 0, 2, 4, 6, and 8 h. Highest amount of total GSH, and total and reduced AsA contents were recorded under HT exposure for 2 h. Activity and expression of DHAR, ascorbate peroxidase (APX), and GR increased following up to 4 h of HT. Thus, upregulation of the AsA–GSH cycle

showed protective effects HT-induced oxidative damages at the vegetative stage of *M. domestica* (Ma et al. 2008).

Higher total GSH contents increased antioxidative protection and electron consumption in *Soldanella alpina* and *Ranunculus glacialis*, which in turn enhanced thermotolerance (Laureau et al. 2011). Hasanuzzaman et al. (2012b) investigated the regulatory role of exogenous nitric oxide (NO) in alleviating HT-induced damage of *T. aestivum* seedlings. High temperature (38 °C) induced oxidative damage to the wheat seedlings, whereas application of exogenous NO protected the seedlings from oxidative damage due to increases in GSH levels, GSH/GSSG ratio, and other components of the AsA–GSH cycle (e.g., APX and GR activities), AsA content, and activity of GSH metabolizing enzymes, including GPX and GST. Increased chlorophyll content and better phenotypic appearance were also documented in NO supplemented plants exposed to HT, when compared to plants exposed to HT stress alone (Hasanuzzaman et al. 2012b). Nahar et al. (2015b) showed the pretreatment of *V. radiata* seedlings with GSH (1.0 mM) resulted in better physiological performance along with improved antioxidant and glyoxalase systems. They also observed the reduced MG and oxidative stress under short term heat stress (42 °C, 24 and 48 h). Heat stressed seedlings those were supplemented with GSH improved chl content and leaf RWC and also increased APX (only after 24 h), MDHAR, DHAR, GR, glutathione peroxidase (GPX), GST (increased only after 24 h), catalase (CAT). Increased Gly I and Gly II activities and improved endogenous GSH content and the GSH/GSSG ratio, while a reduction of GSSG content were also observed in those seedlings. The supplementation of GSH with drought stress resulted in significant decrease in MDA, H<sub>2</sub>O<sub>2</sub> and MG content, O<sub>2</sub><sup>-</sup> generation rate and LOX activity (Nahar et al. 2015b).

The relationship between protection from chilling stress (5 °C, 7 days) and total GSH or GR activity was studied by Kocsy et al. (2001) in *Zea mays*. When compared to an untreated control, a combination of two herbicide safeners improved the GSH pool and its precursors, Cys and  $\gamma$ -ECS. An increase in the total GSH level was correlated with enhanced GR activity. These alterations in GSH precursors or pools resulted in a 50–75% increase in protection of maize plants against chilling. Cold treatment of maize at different low temperatures (2, 5, 10, or 15 °C) also induced a greater increase in total GSH content and GR activity in tolerant genotypes than in a sensitive genotype. The GSH/GSSG ratio and the ratio of reduced to oxidized hydroxymethylglutathione (hmGSH/hmGSSG) were increased by the same low temperature treatments in a frost-tolerant wheat variety (Kocsy et al. 2002).

When compared to the normal (25/18 °C) growth condition, a chilling (15/8 °C) temperature resulted in

withering symptoms, with significantly increased  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^-$ , and malondialdehyde (MDA) levels in *Cucumis sativus* cv. Jinchun 4 seedlings. Supplementation of low-temperature-treated seedlings with exogenous silicon (Si) resulted in an improved phenotype and reduced oxidative damage, which were correlated with an increase in GSH and AsA levels, and enhanced activities of enzymes involved in AsA-GSH cycle (i.e., GPX and SOD) (Liu et al. 2009). Higher GSH levels and higher activities of enzymes of the AsA-GSH cycle also enhanced the tolerance to low temperature stress (0 °C for 2, 4, 6, 8, 12, 24, 48, and 72 h) in strawberry cultivars (Luo et al. 2011). Low temperature stress (13 °C) in *Dunaliella salina* increased the total GSH pool by 20–100% and the total ascorbate pool by 10–50%, as well as increasing SOD; these were considered vital responses to suit that environment (Haghjoua et al. 2009). Over-expression of GST in transgenic rice enhanced the germination and growth rate at low temperature (Takesawa et al. 2002). Studies on GSH under extreme temperature stress confirmed that GSH enhanced tolerance in different growth stages of plants including the seedling, vegetative and reproductive stages. However, most of the research findings regarding responses to extreme temperatures showed that the antioxidant activity of GSH enhanced temperature stress tolerance. Studies also showed GSH to regulate other antioxidant components that also induced temperature stress tolerance. As with other stresses, GSH is thought to act as a signaling molecule or as a growth regulator to improve extreme temperature stress tolerance in plants (Hasanuzzaman et al. 2012b). This hypothesis deserves further study.

### Toxic metals/metalloids

Several transition metals/metalloids are essential for plant growth (this is also the case for most other organisms); however, many of these compounds are toxic when present in excess amounts (Zitka et al. 2013). Unfortunately, due to rapid industrialization, emissions of metals to the environment have increased and now exceed those from natural sources. Metal/metalloid toxicity often disrupts the normal growth and physiology of plants, thereby substantially hampering productivity. One major consequence of metal/metalloid toxicity is the excess generation of ROS and subsequent oxidative stress (Hasanuzzaman and Fujita 2013). However, as already described, plants can reduce this damage by activating defense strategies to enhance antioxidative defenses and restore physiological processes (Hall 2002).

Glutathione and related thiols are potential components that reduce metal/metalloid toxicity in plants (Fig. 3). In line with its important functions in the maintenance of cellular redox homeostasis, GSH also participates in the

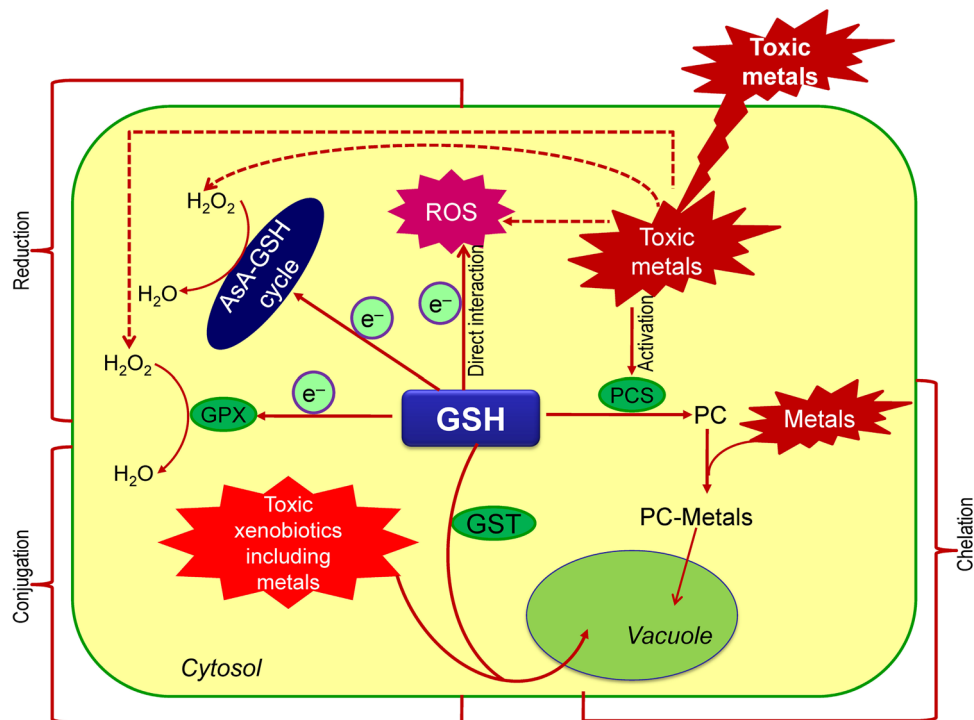
detoxification of toxic metals/metalloids. Glutathione can protect the plant cells from metal/metalloid toxicity in three possible ways: (1) direct quenching of ROS; (2) conjugation of toxic metals and other xenobiotics to GST; and (3) acting as a precursor for the synthesis of phytochelatins (PCs).

Several reports have showed GSH-induced protective effects in mitigating metal/metalloid toxicity. A few reports showed that GSH content is increased in response to metal exposure due to the enhanced activities of proteins responsible for GSH biosynthesis (Xiang and Oliver 1999). In an in vitro study, Ruegsegger et al. (1990) found 3- to 4-fold increase in GSH activity in *P. sativum* when exposed to Cd. Bergmann and Rennenberg (1993) also demonstrated a time-dependent increase in GSH activity in same species upon exposure to Cd. Wang et al. (2011) reported some injury symptoms, such as leaf chlorosis, necrosis and stunting in barley seedlings when subjected to Cd (5  $\mu\text{M}$ ). However, the addition of GSH (20  $\text{mg L}^{-1}$ ) in the Cd culture medium can alleviate the Cd-induced growth inhibition and leaf injury symptoms significantly. Exogenous GSH resulted in the increase of plant dry weight in the following two barley varieties: Weisubuzhi (by 42.3%) and Dong 17 (by 70.6%). The reduced shoot/root Cd concentrations of 33.8/42.2% in Weisubuzhi and 59.4/66.3% in Dong 17 relative to the Cd alone treatment reflects that GSH can suppress plant Cd uptake (Wang et al. 2011).

Exogenous GSH not only improved growth and nutrient uptake but also increased the endogenous GSH content, while decreasing the Cys content of leaf tissue of rice seedlings under chromium (Cr, 100  $\mu\text{M}$ ) stress. It also markedly increased the contents of acetic, lactic, citric, tartaric, and malic acid in the root, while decreasing the Cr concentration of root and shoot. The reduction in Cr concentration by exogenous GSH supports the metal chelation and sequestration activity of GSH. The growth-regulating functions of GSH were confirmed by the regulation of biomolecule contents and nutrient uptake, as well as plant growth (Qiu et al. 2013). Adding GSH in different concentrations (1, 5, 10 or 25  $\text{mg L}^{-1}$ ) to media containing different concentrations (1, 5, 50, 100 or 200  $\text{mg L}^{-1}$ ) of  $\text{Pb}(\text{NO}_3)_2$  can ameliorate the adverse effects of heavy metals on morphogenesis of *Spilanthes calva* L. by enhancing survival percentage, shoot number and shoot length (Shankar et al. 2012).

Glutathione improves both the growth parameters and physiological activities in barley. A combined supplementation of Dong 17 barley seedlings with Cd (5  $\mu\text{M}$ ) together with GSH (20  $\text{mg L}^{-1}$ ) increased photosynthesis by 130.2%, transpiration by 68.9% and stomatal conductance by 52.4%, when compared to seedlings exposed to Cd alone. Glutathione treatment also promoted the





**Fig. 3** GSH mediated toxic metal and xenobiotic detoxification in a plant cell; PC: phytochelatin, PCS: phytochelatin synthase. Dotted arrows indicate the induction of ROS production. Protection of plant cells from metal/metalloid toxicity by GSH occurs in three possible ways: (1) reduction: direct quenching of ROS which is occurred by AsA-GSH cycle together with other components of antioxidant system, (2) conjugation: toxic metals/xenobiotics conjugation by the

activity of glutathione *S*-transferase (GST) and transportation of conjugates into vacuole, (3) chelation: GSH acts as a precursor for the synthesis of phytochelatins (PC). Metals are bound to the thiol (–SH) group of GSH and forms PC by the activity of enzyme phytochelatin synthase (PCS,  $\gamma$ -Glu-Cys transpeptidase); the PC-Metal complex is also transported into the vacuole. Nahar et al. (2016b), with Permission from Wiley

maintenance of a better number and ultrastructure of plastids and mitochondria. In plastids, well-developed thylakoid membranes properties, parallel patterns of lamellae, and more unfolded starch grains have been demonstrated. Glutathione also resulted in well-structured mitochondrial cristae and uniform chromatin distribution in nuclei was observed. The improved membrane and anatomical structures were due to the antioxidant functions of GSH. The mechanisms underlying the improvement in physiological processes under stress indicates various others roles for GSH which deserve further research (Wang et al. 2011).

Chen et al. (2010) studied the antioxidant functions of GSH that enhanced Cd stress tolerance in rice. Oxidative stress is reduced due to the regulation of antioxidant enzymes by GSH which renders better survival of the rice plants. It increased POX activity and decreased CAT activity, while maintaining a better structure of the leaf and root, which aided in conferring tolerance (Cai et al. 2011). Dramatic depressions in  $O_2^-$ ,  $H_2O_2$ , and MDA accumulation were seen after the supplementation of GSH in rice seedlings subjected to Cd and the antioxidant system modulation is considered to be the main reason. When GSH application is done exogenously, it can increase the

endogenous GSH levels and can also decrease the AsA and nonprotein thiol levels, while maintaining activities of monodehydroascorbate reductase (MDHAR), DHAR and GPX similar to those of the non-treated seedlings. The activities of CAT and APX were significantly increased compared to Cd-stressed plants (Chen et al. 2010). The oxidative stress induced by Cd in *Ceratophyllum demersum* was alleviated by exogenous Zn application, which effectively restored thiols. The application of Zn also inhibited oxidation of AsA and GSH, and maintained the redox state balance. Zn addition also restored and enhanced the activities of AsA-GSH enzymes (i.e., APX, MDHAR, DHAR, and GR), GST, and GPX, which all together conferred tolerance to Cd stress (Aravind and Prasad 2005). Upregulation of GSH and GSH dependent enzymes, modulation of AsA-GSH cycle components by exogenous polyamines application improved toxic metal stress tolerance. An increased GSH content and activities of antioxidant enzymes such as SOD, CAT, GST, MDHAR, DHAR and GR which reduced oxidative stress and enhanced cadmium tolerance of exogenous spermine treated mung bean plant (Nahar et al. 2016a). In other study, exogenous spermidine increased AsA and GSH contents, activities of APX, DHAR, GR and CAT which reduced ROS

production and oxidative stress under aluminium stress (Nahar et al. 2017).

Beside playing a key role in the antioxidant defense system and improving the physiological processes under heavy metal toxicity conditions, GSH also binds the toxic metals. Transportation of toxic metals to safe sequestration sites within the plant cell not only ensures a sound cellular environment but also presents an ideal perspective to abolish the toxic metals from environment by the process of bioremediation and phytoremediation (Peuke and Rennenberg 2005). The reduction of the free metal content within the plant cell can be achieved by two ways: the synthesis of specific chelators and the sequestration of metal complexes (Smeets et al. 2009; Cuypers et al. 2010). The high affinity for metals imparted by the GSH thiol (–SH) group, coupled with the fact that GSH is a precursor of PCs. It is the reason why GSH is considered to be a very important biomolecule in the process of scavenging metals (Jozefczak et al. 2012). Enzymatic oligomerization of GSH results in phytochelatins formation to give a generalized structure of  $(\gamma\text{-Glu-Cys})_n\text{-Gly}$  ( $n = 2\text{--}11$ ). Phytochelatins can bind metal and then can be transported into the vacuole. The formation of PCs largely depends on whether heavy metals are present or not. It also varies with the activity of PC synthase (PCS,  $\gamma\text{-Glu-Cys}$  transpeptidase), a PC synthesizing enzyme (Fig. 3). The activation of the PCS enzyme by the metal itself mainly regulates the PC formation. Phytochelatins are reported to chelate Cd most effectively, followed by As (Jozefczak et al. 2012); the other preference order for chelation of metals are  $\text{Ag} > \text{Bi} > \text{Pb} > \text{Zn} > \text{Cu} > \text{Hg} > \text{Au}$  (Pasternak et al. 2008). The vacuole contains the sequestered PC-ion complexes, thereby reducing toxic metal effects.

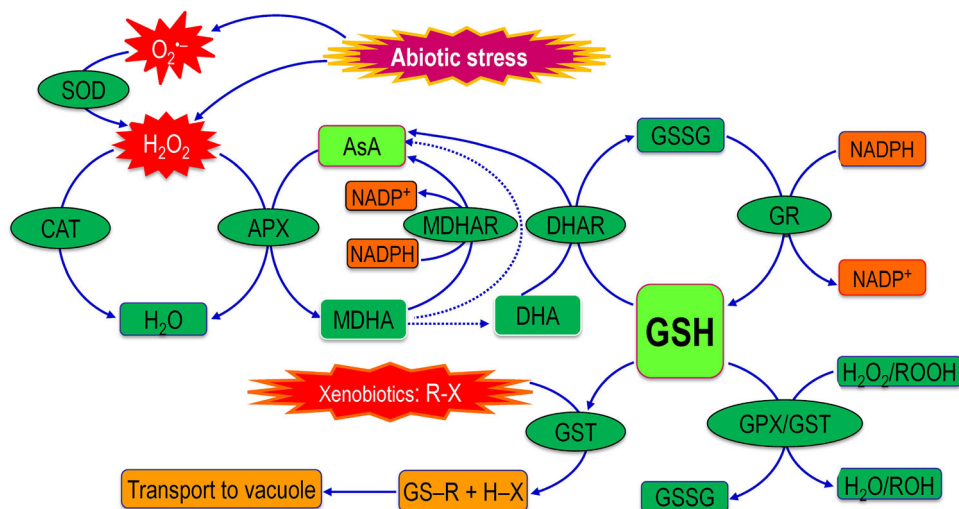
In plants, PCs can confer tolerance to heavy metal, as indicated by numerous recent studies, including Cd tolerance in *B. juncea* (Mohamed et al. 2012), *Populus nigra* (Iori et al. 2012), *Oenothera odorata* (Son et al. 2012), *T. aestivum* (Hentz et al. 2012), and *Linum usitatissimum* (Najmanova et al. 2012); As tolerance in *Nicotiana tabacum* (Shukla et al. 2012) and *Arabidopsis thaliana* (Huang et al. 2012); Zn and Cu tolerance in *Arabidopsis thaliana* (Huang et al. 2012); and Mn tolerance in *Vitis vinifera* (Yao et al. 2012). Glutathione conjugates metals by forming PCs, which can be translocated. In between roots and shoots of *Arabidopsis* plant, PCs go through a long distance transport and are also identified in the xylem and phloem saps of *B. napus*. The concentrations of PCs and Cd in the phloem sap is higher than xylem sap; which indicates that translocation of PCs occurs largely via the phloem. Again, in the phloem sap the high ratios of [PC]/[Cd] and [GSH]/[Cd] designate the PCs and GSH as the long-distance carriers for Cd (Mendoza-Cozatl et al. 2008). *Arabidopsis thaliana* overexpressing *AtPCS1* and *GSH1*

showed greater PC formation and enhanced tolerance for Cd and As accumulation than single-gene transgenic lines (Guo et al. 2008). The two glyoxalase pathway genes (Gly I and Gly II), individually and together, in *Nicotiana tabacum* plants enhanced the levels of both GSH and PCs and imparted tolerance against Zn stress (Singla-Pareek et al. 2006). Overexpression of the *AtPCS1* gene encoding PCS in *Brassica juncea* resulted in tolerance to Cd, As, and Zn stresses (Gasic and Korban 2007a, b). High intracellular GSH content and enhanced activities of PCS were observed in *Lotus japonicus*, where the biosynthesis of PCs and homophytochelatins are involved in regulation of heavy metal sequestration (Asada 1994). In the absence of PC formation, conjugation of GSH with both the exogenous and endogenous organic toxicants (hydrophobic) can be formed, which are then transported and sequestered in the vacuole (Fig. 3; Peuke and Rennenberg 2005; Hasanuzzaman et al. 2012a). Paulose et al. (2013) found that the *Arabidopsis* plants overexpressing  $\gamma\text{-glutamyl cyclotransferase}$  (*GGCT*) genes showed better tolerance to Cd and As which is due to higher turnover of GSH because GGCT is responsible for Glu recycling.

### Glutathione-induced oxidative stress tolerance

Glutathione is a strong antioxidant; hence, it has a vital role in antioxidant defense. Under adverse environmental conditions, excessive amounts of ROS are formed, which leads to oxidative stress. However, plants have developed well-equipped antioxidant defense systems, and GSH is one of the major molecules that forms an integral part of the AsA-GSH cycle that scavenges  $\text{H}_2\text{O}_2$ . Glutathione reacts with  $\text{O}_2^-$ ,  $\text{OH}^-$  and  $\text{H}_2\text{O}_2$  form adducts with reactive electrophiles (glutathiolation). Glutathione is also an efficient electron donor during the ROS detoxification process and oxidation to GSSG (Asada 1994). Further, GSH can be regenerated by GR using nicotinamide adenine dinucleotide phosphate (NADPH) as the substrate (Fig. 4; Noctor and Foyer 1998; Hasanuzzaman et al. 2012a). Because of its abundance in almost all of the cellular organelles GSH participates actively in various metabolic processes under stress conditions (Fig. 4). Glutathione not only detoxifies ROS, but also modulates antioxidant enzymes.

Glutathione helps to maintain the reduced state of constituents of the AsA-GSH pathway and thus detoxify ROS. The DHAR enzyme uses GSH to convert oxidized ascorbic acid (DHA) to AsA (Foyer and Noctor 2011); GSH also reduces DHA by a nonenzymatic process in the chloroplast at alkaline pH (Foyer and Halliwell 1976). Glutathione is a substrate for GPX when converting the lipid hydroperoxide or  $\text{H}_2\text{O}_2$  into a non-toxic form or water (Noctor et al. 2002). Besides, GST uses GSH to convert toxic xenobiotics to



**Fig. 4** Mechanisms of ROS detoxification by different antioxidant enzymes. AsA-GSH cycle consist of AsA (ascorbate) and GSH (glutathione), and antioxidant enzymes APX (ascorbate peroxidase), MDHAR (monodehydroascorbate reductase), DHAR (dehydroascorbate reductase), GR (glutathione reductase). MDHA-monodehydroascorbate, DHA-dehydroascorbate. Dotted lines denote non-enzymatic conversions. R may be an aliphatic, aromatic, or heterocyclic group; X may be a sulfate, nitrite, or halide group. Abiotic stresses generate reactive oxygen species/ROS. The SOD (superoxide dismutase) is considered the first line of defense in the ROS detoxification process and converts  $O_2^-$  to  $H_2O_2$ . This  $H_2O_2$  can be

converted to  $H_2O$  by CAT (catalase) activity or  $H_2O_2$  enters the AsA-GSH cycle, where APX, using AsA, converts  $H_2O_2$  to  $H_2O$ . While participating in the ROS detoxification process, AsA is oxidized to DHA (dehydroascorbate reductase). AsA is regenerated through the AsA-GSH cycle enzymes MDHAR and DHAR in a step-by-step reaction. In the AsA-GSH cycle, GSH takes part in detoxification of ROS (by the activity of enzyme GPX/glutathione peroxidase or GST/glutathione-S-transferase) or xenobiotics (by the activity of GST). The GSH is converted into GSSG (glutathione disulfide) during ROS detoxification and this GSSG is recycled again to GSH by the activity of GR. Adapted from Hasanuzzaman et al. (2012a)

non/less toxic complexes that are transported to the vacuole (Srivalli and Khanna-Chopra 2008). Apart from its direct role in ROS scavenging, GSH also maintains the reduced state of other antioxidants like tocopherol and zeaxanthin and indirectly protects cell membranes. Glutathione also protects proteins from denaturation under stressful conditions. It also transmits signals for maintaining cellular homeostasis in plants, thereby protecting the plant from stress-induced damage (Noctor 2006). A histochemical study of *Sedum alfredii* revealed that inhibition of GSH biosynthesis during Cd stress overproduced  $H_2O_2$  and  $O_2^-$  (Jin et al. 2008a). It is also reported that Zn toxicity may involve inhibition of NADPH oxidase, and GSH synthesis, which may lead to increased  $H_2O_2$  and  $O_2^-$  accumulation (Jin et al. 2008b). In the last couple of decades several researchers reported that exogenous application of GSH provided better protection against oxidative stress and improved physiology under environmental stress (Table 1).

### Methylglyoxal detoxification: a vital role of glutathione

One of the most important functions of GSH is its involvement in the MG detoxification system. MG is an organic compound that is developed under different abiotic stress conditions and cause toxicity to plants. It is a cytotoxic  $\alpha$ -

oxoaldehyde compound that is highly reactive and produced through different enzymatic and non-enzymatic reactions. Methylglyoxal can create oxidative stress and thus cause considerable damage to plant cells. The enzymatic functions of biochemical reactions including those of antioxidant enzymes can also be disrupted by MG (Wang et al. 2009; Desai et al. 2010). So, in case of conferring tolerance against the abiotic stress, MG detoxification should be considered as an important strategy (Hoque et al. 2008). Gly I (lactoylglutathione lyase; EC 4.4.1.5) and Gly II (hydroxyacylglutathione hydrolase; EC 3.1.2.6) these two enzymes, using GSH as a cofactor, make up the glyoxalase system that detoxifies MG in plants (Fig. 5). These two enzymes use GSH to convert toxic MG and other 2-oxoaldehydes to their 2-hydroxyacids. The detoxification process is a two-step reaction. Gly I first converts MG to *S*-D-lactoylglutathione (SLG) using GSH, and Gly II then converts SLG to D-lactic acid, simultaneously regenerating GSH (Yadav et al. 2008). This MG detoxifying action of GSH has been confirmed and supported by a number of studies.

### Signaling cross talk of glutathione

Glutathione is considered to be a signaling molecule in several studies. Several schemes have been suggested for GSH-induced signal transmission pathways by different

**Table 1** Protective effect of glutathione under abiotic stress conditions

Plant species	Stress level	Glutathione supplementation	Protective effects	References
<i>Allium cepa</i> cv. Giza 6	Salinity (150 mM NaCl, soaked epidermal cells for 3 h)	0.5 mM GSH, soaked epidermal cells for 3 h	Reduced cell membrane permeability coefficient (Ks)	Salama and Al-Mutawa (2009)
<i>B. napus</i> vs. Serw and Pactol	Salinity (100 and 200 mM NaCl)	100 mg L <sup>-1</sup> GSH, seed soaking for 24 h	Decreased Number of dead and swollen cells Improved shoot and root growth Increased SOD, CAT, POX, GPX and APX activities Increased the contents of total soluble carbohydrates, soluble proteins, phenols and free amino acid	Kattab (2007)
<i>V. radiata</i> cv. Binamoog-1	Salinity (200 mM NaCl, 24 and 48 h)	1 mM GSH, 24 and 48 h	Increased total photosynthetic pigment contents Reduced MDA and H <sub>2</sub> O <sub>2</sub> content, O <sub>2</sub> <sup>-</sup> generation rate, and LOX activity	Nahar et al. (2015c)
<i>O. sativa</i>	Salinity (200 mM NaCl)	2 mM GSH, foliar spray	Reduced Pro content Improved Chl b and total Chl contents Lessened the reduction rate of RWC Increased SOD, APX, GR, GST and GPX activities Increased the number of filled grains per panicle Increased spikelet fertility	Hussain et al. (2016)
<i>V. radiata</i> cv. Binamoog-1	Drought (25% PEG, -0.7 MPa)	1 mM GSH, 24 and 48 h	Increased yield per panicle Reduced MDA content, O <sub>2</sub> <sup>-</sup> generation rate and LOX activity Restored the decreased Chla and total Chl contents Increased SOD, CAT, APX, MDHAR, DHAR, GR, GST and GPX activities	Nahar et al. (2015a)
<i>Cicer arietinum</i> and <i>C. reticulatum</i> Ladiz.	Drought (withheld irrigation, 10 days)	10 and 100 mM, foliar spray	Reduced MG content and increased Gly I and Gly II activities Reduced CAT, APX and GR activities Increased AsA content	Çevik and Ünyayar (2015)
<i>V. radiata</i> cv. Binamoog-1	High temperature (42 °C, 24 h and 48 h)	0.5 mM GSH, 24 h seedling pretreatment	Restored leaf RWC Increased Chl b and total Chl contents	Nahar et al. (2015b)
<i>Hordeum vulgare</i> cvs. Dong 17 and Weisuobuzhi	Metal toxicity (5.0 µM CdCl <sub>2</sub> )	20 mg L <sup>-1</sup> GSH	Reduced MDA and H <sub>2</sub> O <sub>2</sub> contents along with LOX activity Increased CAT, GPX, DHAR, MDHAR and GR activities Reduced MG content and increased Gly I and Gly II activities Alleviated growth inhibition Reduced Cd concentrations in leaves and roots Increased net photosynthetic rate P(n), stomatal conductance G(s) and transpiration rate T(r)	Wang et al. (2011)
<i>O. sativa</i> lines 117 and 41	Metal toxicity (100 µM Cr <sup>6+</sup> )	300 µM GSH	Increased K, Mg, Fe and Mn contents in both shoot and root Reduced Cr contents in both shoot and root Increased GSH contents in leaf, stem and root	Qiu et al. (2013)



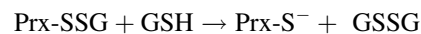
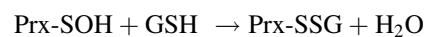
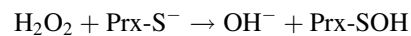
**Table 1** continued

Plant species	Stress level	Glutathione supplementation	Protective effects	References
<i>Spilanthes calva</i>	Metal toxicity (1, 5, 50, 100, or 200 mg L <sup>-1</sup> Pb, 28 days)	10 mg L <sup>-1</sup> GSH	Minimized the toxic effects of Pb Produced multiple shoots	Shankar et al. (2012)
<i>Gossypium hirsutum</i> cvs. Coker 312 and TM-1	Metal toxicity (50 µM Cd, 7 days)	50 µM GSH	Minimized the contents of various Stress markers (TTC reduction, TSP, MDA, H <sub>2</sub> O <sub>2</sub> ) Reduced leaf Cd content Increased SOD, POD, APX and GR activities	Daud et al. (2016)
<i>Gossypium hirsutum</i> cv. TM-1	Metal toxicity (500 µM Pb)	50 µM GSH	Increased net photosynthetic rate, stomatal conductance, intercellular CO <sub>2</sub> concentration and transpiration rate Reduced MDA, H <sub>2</sub> O <sub>2</sub> , total soluble proteins contents and Pb uptake in leaves Increased CAT, SOD, POD, GR and APX activities	Khan et al. (2016)

researchers, but no complete pathways or mechanisms for GSH-induced signal transmission have been discovered yet. The following is a discussion of some feasible proposed pathways:

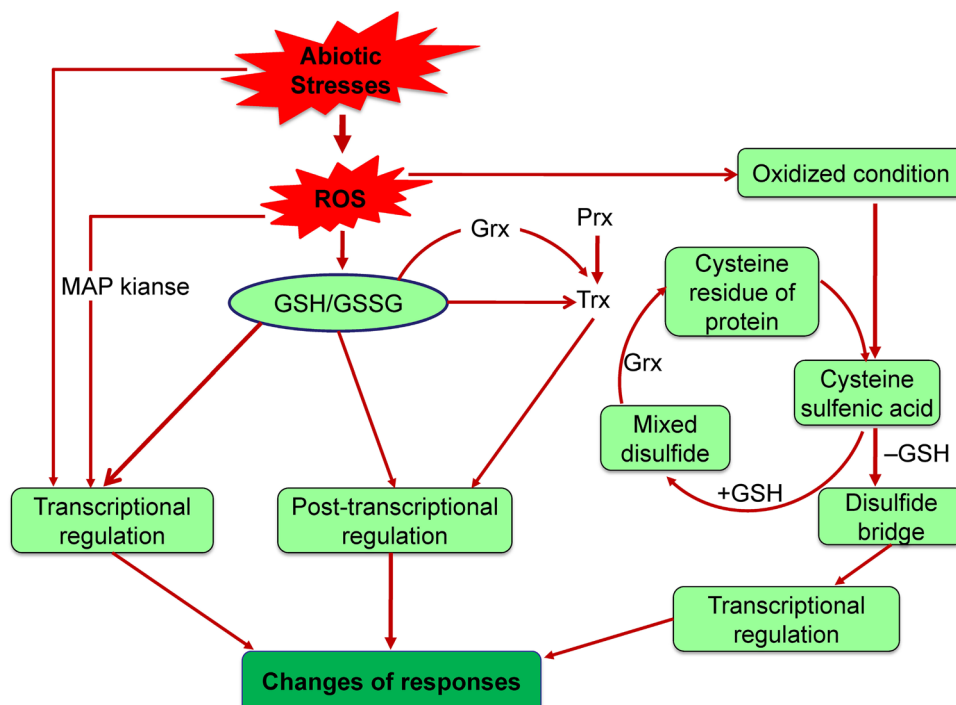
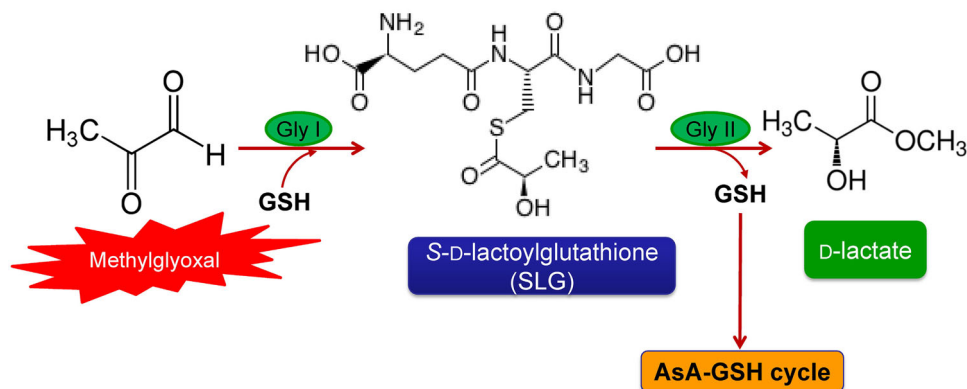
Under different abiotic stress conditions, GSH (either alone or with H<sub>2</sub>O<sub>2</sub> or some other ROS) triggers adaptive or cell death processes through an intercellular signaling system (Foyer and Noctor 2005a, b). Glutathionylation is the posttranslational modification of protein cysteine residues by the addition of glutathione. An involvement of glutathione is suggested in redox signaling processes, where glutathionylation of protein affects the processes of transcription. Glutathione can execute protection against abiotic stress conditions. Stress conditions cause disturbance to the cellular redox homeostasis, thereby influencing transcription factors. It also influences the translation, post-translational modification of proteins, regulation of metabolic processes, and the expression of genes. GSH is actively involved in this signaling process (Szalai et al. 2009; Kumar et al. 2010) but the details of signal transduction pathway is still unknown. A generalized model is presented in Fig. 6 (Szalai et al. 2009).

Glutathione modulates the transcriptional or post-translational levels by maintaining interaction with other redox systems directly or indirectly. Stress generates the ROS and creates an oxidative environment. The family of proteins, peroxiredoxins (Prxs) can catalyze the reduction of H<sub>2</sub>O<sub>2</sub> in the presence of GSH and/or other thiols. Prx, with Cys, in its thiolate (S<sup>-</sup>) form in their active sites (as below).



Prx-SOH characterizes the sulfenic acid intermediate that perpetuate to react with GSH to detoxify OH<sup>-</sup> and to form Prx-SSG. Later on Prx-S<sup>-</sup> is formed. The sulfenic acid intermediate or combined thiolate (Prx-S<sup>-</sup>) are linked with signaling process. Thiol chemistry, GPX reaction or nonenzymatic metal catalyzed oxidations are described for signal transmission. H<sub>2</sub>O<sub>2</sub> does not react with thiols. In case of H<sub>2</sub>O<sub>2</sub> or peroxyxynitrite reactions with a thiol, which are catalyzed by metal, produce either a sulfinic (-SO<sub>2</sub>H) or sulfonic (-SO<sub>3</sub>H) acid. These cannot be reduced easily. H<sub>2</sub>O<sub>2</sub> in low concentrations, react spontaneously with the thiolate anion (S<sup>-</sup>), which results in the formation of sulfenic acid that has been described to participate in signal transmission (Foyer and Noctor 2005a, b; Szalai et al. 2009; Kumar et al. 2010). Cysteine sulfenic acid and some other fates of this compound might be controlled by GSH (Kumar et al. 2010). The gradual oxidation of this Cys sulfenic acid to Cys sulfinic acid and Cys sulfonic acid can

**Fig. 5** Roles of glutathione in methylglyoxal (MG) detoxification. First step: The enzyme GlyI (glyoxalase-I) utilizes GSH to convert MG to *S*-*D*-lactoylglutathione (SLG). Second step: *S*-*D*-lactoylglutathione (SLG) is converted to *D*-lactate by the activity of GlyII (glyoxalase-II) and GSH is regenerated in this step



**Fig. 6** Schematic representation of GSH induced signal transmission under abiotic stress condition. Several pathways are proposed for signal transmission by GSH. Stress induced ROS and the subsequent stress signal may be transmitted through a mitogen activated protein (MAP) kinase cascade to modulate transcriptional regulation. The GSH/GSSG redox system can control redox signaling and can directly participate in transcriptional regulation. Oxidative stress and GSH/GSSG-modulated glutathionylation has been confirmed for a number of proteins including Grx (glutaredoxin) and several GSTs. Redox changes in Trxs (thioredoxin) are important because they target the intercellular disulfide bonds of proteins. Trxs are inactivated by glutathionylation. The reduction of Trxs may be regulated by GPXs (glutathione peroxidases) and Prxs (peroxiredoxins). Trxs can also be activated by deglutathionylation, which is activated by Grxs

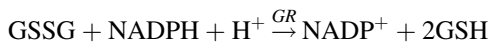
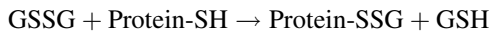
(glutaredoxins, members of the Trx superfamily). Trx and the GSH/Grx redox systems have important roles in signaling. Interactions between GSH, GSH/GSSG, Trx, Grx, and Prx in stress signaling pathways still require intensive study. Glutathione directly or indirectly regulates the transcriptional or post-translational processes by interacting with other redox systems. Abiotic stress generates ROS/oxidative conditions in the cell. The oxidative environment helps to convert cysteine residues of proteins into cysteine sulfenic acid, which is regulated by GSH and Grx (glutaredoxin). Oxidation of protein then occurs by conversion of cysteine sulfenic acid into cysteine sulfinic acid and cysteine sulfonic acid. Deglutathionylation of the cysteine sulfenic acid then occurs to form the disulfide bridge, which transmits the oxidative stress signal to regulate transcriptional factors. Nahar et al. (2016a), with Permission from Wiley

occur and results in oxidation of the protein. The formation of Cys sulfenic acid can be reversed by GSH. Deglutathionylation of Cys sulfenic acid forms a disulfide bridge that can transmit the signal of oxidative stress and influence

the transcriptional factors (Fig. 6). Szalai et al. (2009) suggested that the GSH/GSSG redox pair mainly controls redox signaling. Glutathione may be involved in the transcriptional regulation. The stress-induced ROS can have

effects on gene expression which may be imparted through a mitogen activated protein kinase (MAP) cascade for transcriptional regulation.

GSSG (oxidized form of GSH) can exchange with protein sulfhydryls and proceed to produce protein–glutathione mixed disulfides (protein–SSG) (protein–SSG can be reconverted into protein-SH reacting with GSH. GS indicates Glu-Cys ligase catalytic subunit) (Szalai et al. 2009).



Mixed disulfides, i.e., protein–SSG, possess a longer half-life than GSSG (because of protein folding). For the action of GSH in cell signaling this exchange reaction renders a vital mechanism. Significant numbers of protein containing critical thiols are involved in the signaling. The functions of the receptors/proteins involved in ubiquitinylation, several protein kinases and transcription factors can be altered upon formation of mixed disulfides. However, once GSSG is produced upon reaction between GSH and ROS, it can be reduced to GSH (by NADPH) by GR.

The function of GSH as an antioxidant that confers abiotic stress tolerance is well established, but the mechanism underlying this defense response is unknown. Cross-communication of GSH with other signaling molecules helps in adaptation to abiotic stress (Ghanta and Chattopadhyay 2011), as suggested by several research findings on GSH cross-talk with signaling molecules. Glutathione and its redox state have major functions in the regulation of plant growth, development, and stress responses (Noctor and Foyer 1998; May et al. 1998). The antioxidative property of GSH is involved in the reduction of  $\text{H}_2\text{O}_2$ , which in turn alters the redox state of the GSH/GSSG couple. The GSH/GSSG ratio and  $\text{H}_2\text{O}_2$  perform a signaling role by affecting proteins during transcription, translation, post-translational processes, and the metabolic processes conducted by the proteins (Dietz 2008; Quan et al. 2008).  $\text{H}_2\text{O}_2$  regulates the GSH pool in maize under various abiotic stresses and imposes posttranslational regulation of  $\gamma$ -ECS and GR transcript levels, thereby controlling GSH synthesis, the GSH/GSSG ratio, and GR activity (Kellós et al. 2008). This interaction between  $\text{H}_2\text{O}_2$  and GSH served as a stress signal that enhanced chilling tolerance in mungbean (Yu et al. 2003). Noctor et al. (2002) established the involvement of GSH in redox signaling because inter and intracellular GSH were linked by transport across the membranes. Oxidative stress-induced glutathionylation has been recognized as a mechanism for induction and regulation of several proteins, including Grx (glutaredoxin), Trxs (thioredoxin) and GSTs, which are involved in environmental stress responses (Dixon et al.

2005; Reichheld et al. 2007). For example, Grx and GST proteins were induced in *Arabidopsis* under oxidative stress (Dixon et al. 2005).

Different hormones (e.g. ethylene, JA, and NO) can affect expression of GST at the transcript level. However, this response depends on the growth stage and the tolerance level of the plant (Moons 2005). Abscisic acid may regulate redox signaling by affecting the GSH/GSSG ratio (Pastori and Foyer 2002). Intracellular GSH regulates stomatal movement by acting together with signal molecules. Jasmonic acid regulates *Arabidopsis* GSH concentration and genes for GSH metabolism (Sasaki-Sekimoto et al. 2005). Glutathione functions as a signal molecule during MeJA signaling in guard cells. In *Arabidopsis*, intracellular GSH regulates MeJA-induced stomatal movement (Jahan et al. 2008). Signaling roles for GSH were confirmed in carrot vacuoles, where GSH modulates a slow vacuolar channel activity that is also dependent on cytosolic pH (Scholz-Starke et al. 2004). Exogenous SA caused differential changes in the  $\gamma$ -ECS transcript and GSH levels, GR transcript levels, and GR activity in maize genotypes that varied in stress tolerance (Kellós et al. 2008). Salicylic acid altered GSH level and GR activity in soybean cell suspension (Knörzer et al. 1999). Overexpression of the SA metabolism gene resulted in changes in both SA and GSH concentrations in rice that were correlated with oxidative stress tolerance (Kusumi et al. 2006). Nitric oxide can regulate GSH synthesis by regulating genes related to GSH biosynthesis or metabolism (Innocenti et al. 2007). Interaction of GSH with NO forms S-nitrosoglutathione (GSNO) which is involved in nitrogen-based signaling pathways (Neill et al. 2002). Cadmium stress activated a GSNO signal that effectively conferred cadmium stress tolerance in pea plants (Barroso et al. 2006; Hasanuzzaman et al. 2010). Expression of the  $\gamma$ -ECS and GSH synthase genes have been upregulated by NO application in *Medicago trunculata* roots which led to a significant increase in root GSH level (Kusumi et al. 2006). An involvement of glutathione is suggested in the  $\text{Ca}^{2+}$ -dependent protein kinase activation which is associated with stress signal pathways. In tobacco GSH and GSSG treatment modulated  $\text{Ca}^{2+}$  level, calcium signaling and gene expression (Gómez et al. 2004). Treatment with  $\text{H}_2\text{O}_2$  increased the  $\text{Ca}^{2+}$  concentration in low temperature stress affected tobacco plant (Price et al. 1994).

## Concluding remarks and future perspectives

Glutathione occupies a central position in antioxidant defense; thus, it represents a potent molecule for plant protection against various abiotic stresses. The function of GSH as an antioxidant that confers abiotic stress tolerance

is being well established, with evidence from many plant studies. Increased levels of endogenous GSH allow efficient scavenging of ROS, thereby providing the plant cell with relief from oxidative stress experienced during abiotic stresses. Exogenous application of GSH also confers abiotic stress tolerance by maintaining normal physiological functions and improving growth and development. Increases in endogenous GSH levels and GSH/GSSG ratios or application of exogenous GSH are correlated with sound plant health due to maintenance of better plant physiology and biochemistry from many points of view. Higher GSH levels maintain osmotic balance by induction of osmoprotectants and they prevent damage to lipids, amino acids, and polysaccharides, thereby preventing damage to membranes, photosynthetic pigments, mitochondria, and other organelles which ultimately confer tolerance to abiotic stresses (Nahar et al. 2015a, b, c). GSH is indispensable in the detoxification of xenobiotics and heavy metals and for enhancing toxic metal tolerance, as demonstrated by many research findings.

Considerable attempt has been made in underlying GSH biosynthesis, metabolism, and the role played by GSH in plant stress tolerance. Nevertheless, the results with respect to overall stress responses are still extremely variable. The mechanism underlying the GSH defense response is still not established. Thus, future studies need to identify the genes responsible for GSH biosynthesis and the cellular mechanisms underlying the regulation of enzymes related to GSH metabolism or degradation. How the amount of GSH is regulated by biosynthesis/degradation and the required amount of GSH and its regulation in different components of cell are not known. Cross-talk between GSH and other signaling molecules would be anticipated during adaptation to abiotic stress, but this aspect is still poorly studied. The involvement of GSH in signaling pathways, as well as the molecular basis of interactions between GSH and other molecules (e.g., phytohormones, NO, H<sub>2</sub>O<sub>2</sub>, and Ca<sup>2+</sup>) during signaling, still need to be resolved. Some plant species show limitations in GSH synthesis under adverse condition, so the potential for using exogenous GSH to increase GSH levels in stressed plants is now being investigated by many researchers. However, the exact doses and the optimal methods of application are still matters for further research. Various posttranslational modifications such as nitrosylation, hydroxylation, and glutathionylation are involved in stress signaling. The involvement of GSH in these processes is not clearly elucidated. Transcriptomics, proteomics, metabolomics, ionomics and micromics together have been a bloom in revealing plant stress responses and the mechanisms that underlie these responses in different stress response studies. These fields can be incorporated with GSH, its interaction with other hormonal/signaling

molecules and cellular redox induced stress signal response and abiotic stress tolerance in plants.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no conflicts of interest.

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