Tropical Plant Biol. (2015) 8:60–73
DOI 10.1007/s12042-015-9152-1

Sulfur Metabolism and Stress Defense Responses in Plants

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Received: 18 October 2014 / Accepted: 23 June 2015 / Published online: 12 July 2015

Abstract Sulfur management is an important issue in crop plant nutrition. Sulfur has a role in fundamental processes such as electron transport, structure and regulation. It is also associated with photosynthetic oxygen production, abiotic and biotic stress resistance and secondary metabolism. Sulfate uptake, reductive assimilation and integration into cysteine and methionine are the central processes that direct oxidized and reduced forms of organically bound S into their various functions. Sulfur-containing defense compounds that are crucial for plant survival during biotic and abiotic stress include elemental sulfur, hydrogen sulfide, glutathione, phytochelatins, S-rich proteins and various secondary metabolites. Formation of these compounds in plants is closely related to the supply, demand, uptake and assimilation of S. This review will highlight the role of S during the stress response in plants and the relationship between S metabolism and primary S nutrition.

Keywords Abiotic stress · Antioxidants · Oxidative stress · Plant nutrition · Sulfur uptake and metabolism

Communicated by: Paulo Arruda

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Abbreviations

ABA	Abscisic acid
ACCO	1-aminocyclopropane-1-carboxylic acid oxidase
ACCS	1-aminocyclopropane-1-carboxylic acid synthase
Al	Aluminum
$AlCl_3$	Aluminum chloride
APR	Adenosine 5′ phosphosulfate reductase
APX	Ascorbate peroxidase
ATP	Adenosine triphosphate
ATP-S	Adenosine triphosphate-sulfurylase
AU	Auxins
C	Carbon
CAS	β-cyanoalanine synthase
CAT	Catalase
CbL	Cystathionine β-lyase
Cd	Cadmium
CgS	Cystathionine γ-synthase
cDNA	Complementary deoxyribonucleic acid
CK	Cytokinins
Cys	Cysteine
CysK	Cysteine synthase
ET	Ethylene
GA	Gibberellins
DMSP	Dimethylsulfonio-propionate
GLU	Glutamate or glutamic acid
GLY	Glycine
GPX	Guaiacol peroxidase
GR	Glutathione reductase
GS	Glucosinolates
GSH	Glutathione
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
HMT	Homocysteine S-methyltransferase
HPLC	High-performance liquid chromatography

JA	Jasmonates
K	Potassium
LCD	L-cysteine desulfhydrase
MDA	Malondialdehyde
MeJA	Methyl jasmonate
Met	Methionine
mRNA	Messenger ribonucleic acid
MS	Methionine synthase
MTs	Metallothioneins
N	Nitrogen
NaHS	Sodium hydrosulfide
NR	Nitrate reductase
OAS	O-acetylserine
OASS	O-acetylserine sulfhydrylase
OASTL	O-acetylserine(thiol) lyase
OPHS	O-phosphohomoserine
P	Phosphorus
PA	Polyamines
PAPS	3′-phosphoadenosine 5′-phosphosulfate
PCs	Phytochelatins
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/ oxygenase
S	Sulfur
S^{2-}	Sulfide
SA	Salicylic acid
SAM	S-adenosyl methionine
SAMDC	S-adenosylmethionine decarboxylase
SAMS	S-adenosyl methionine synthetase, SAM synthetase
SAT	Serine acetyltransferase
SiR	Sulfite reductase
SLC13	Sodium/ SO_4^{2-} co-transporter
SLC26	SO_4^{2-}/anion exchanger in animals
SMM	S-methylmethionine
SO_2	Sulfur dioxide
SO_3^{2-}	Sulfite
SO_4^{2-}	Sulfate
SOD	Superoxide dismutase
SOT	Sulfotransferase protein family
STs	Sulfotransferases
SUL	Proton/ SO_4^{2-}co-transporter in yeast
SULTR	Proton/ SO_4^{2-}co-transporter in plants
TS	Threonine synthase

Introduction

Environmental variation triggers plant acclimation, adaptation or death. Natural and anthropogenic activities induce biotic and abiotic stresses during agricultural and forestry operations. Plant metabolism is often damaged by toxic compounds and hazardous chemicals present in soils, water and air (Paiva et al. 2009; Su et al. 2014; Iannone et al. 2015). Thus, acclimation and adaptation processes are crucial to plant survival, and the identification and understanding of plant tolerance mechanisms are of major importance. Stress can be defined as any alteration in normal plant growth conditions (Boaretto et al. 2014). Some of these alterations are related to temperature, salinity, water supply, ozone, soil acidification and heavy metal toxicity, among others (Azevedo et al. 1998; Bulbovas et al. 2014; Medici et al. 2014; Nogueirol et al. 2015). Studies of plants have been conducted to evaluate the effects of these changes on growth and development. Plants possess very efficient defense pathways that allow the scavenging of reactive oxygen species (ROS), protecting the cells from oxidative damage (Gratão et al. 2005).

The primary function of regulatory mechanisms is to manage fluxes of sulfur (S) in response to developmental and environmental changing conditions. The goal for the plant is to optimize the use of available S to match the demands for growth and development, and resistance to stress (Hawkesford 2012). Sulfur assimilation starts from the uptake of external sulfate by the activity of sulfate transporter (SULTR) in roots. On the other hand, plants are able to use foliar absorbed hydrogen sulfide as S source for growth, especially under conditions where the S to the roots is limited (Koralewska et al. 2008). Sulfate is activated by adenosine-5′-triphosphate sulfurylase (ATPS, EC 2.7.7.4) and then catalyzed by adenylyl-sulfate reductase (APR, EC 1.8.99.2) and sulfite reductase (SiR, EC 1.8.7.1) to produce sulfide. Regarding the primary metabolism in plants, nitrate and sulfate need to be reduced prior to their incorporation into various essential organic nitrogen (N) and S compounds. The uptake and assimilation of S and N are strongly related since the major proportion of the reduced N and S in plants is incorporated into amino acids and subsequently into proteins (Stulen and De Kok 2012). An important coordination with C/N metabolism occurs at the level of cysteine and methionine biosynthesis, with the cysteine synthase complex (serine acetyltransferase (SAT, EC 2.3.1.3) and O-acetylserine(thiol)lyase (OAS-TL; EC 2.5.1.47; also named cysteine synthase)) acting as both a sensor and a regulator, mediated by a reversible association/ dissociation of the complex. SAT is active when associated with OAS-TL, but inactive when dissociated. As the dissociation is promoted by excess O-acetylserine (OAS), the complex effectively senses both OAS and S availability and self regulates further OAS production accordingly (Hawkesford 2012). Thus, OAS is a signal mediating substrate availability and flux, and OAS-TL, which is in excess, will catalyze synthesis of cysteine given the availability of OAS and sulfide. The formation of these compounds is closely related to the supply, demand, uptake and assimilation of S in plants. In this review, we provide an overview on the role of these S-containing compounds, particularly with regard to abiotic stress acclimation and plant tolerance adaptations.

Sulfur Uptake and Assimilation

Most soils currently used for agricultural and forest crops are naturally low in fertility, and chemical fertilization should be implemented to provide the crop requirements for essential nutrients such as N, phosphorus (P), potassium (K) and S. S uptake is directly driven by demand. Inadequate S nutrition can cause the inefficient use of other nutrients, such as carbon (C) and N, leading to deficiencies and decreases in protein biosynthesis, chlorophyll content and eventually crop yield (Lunde et al. 2008; Mazid et al. 2011; Iqbal et al. 2013). On the other hand, environmental pollution from sulfur dioxide (SO_2), H_2S, sulfite (SO_3^{2-}) and sulfate (SO_4^{2-}) is a serious global problem and can be toxic to plants (Krischan et al. 2012).

S is a component of proteins, the amino acids cysteine (Cys) and methionine (Met), vitamins (biotin and thiamin), cofactors (Co-A and S-adenosyl methionine, SAM) and a range of secondary metabolites (Mazid et al. 2011). S is an essential macronutrient for living organisms and has multiple roles in plant development, including catalytic, regulatory and structural functions (such as in protein disulfide bonds, cellular membrane SO_4^{2-} esters and electron transport through Fe-S groups). S-containing compounds such as phytochelatins (PCs) and glutathione (GSH) also have a role in trace element homeostasis (Na and Salt 2011). S is an important substrate/reductant in reactions during abiotic stress processes; GSH, a major antioxidant in plant stress defense and the major non-protein S source in plants (Kopriva and Rennenberg 2004; Ghelfi et al. 2011; Rennenberg and Herschbach 2012; Seth et al. 2012) is present in all root and leaf cell compartments, with the exception of the apoplast in the absence of stress (Josefczak et al. 2012).

S is taken up from the soil solution predominantly as SO_4^{2-} in an energy-dependent process mediated by specific membrane-bound SO_4^{-} transporters (Buchner et al. 2004; Davidian and Kopriva 2010). Plants can also obtain organic forms of S, such as S-containing amino acids, organic SO_4^{2-} and elemental S, from the soil solution. Although of less significance, S in the form of atmospheric SO_2 can be absorbed by plant leaves and fruits (Mazid et al. 2011), and atmospheric H_2S can be absorbed through leaf stomata (Riemenschneider et al. 2005).

The translocation of SO_4^{-} into plastids for assimilation, storage in vacuoles, and long-distance transport among organs requires specific transporters; the mechanism of plasma membrane transport is proton-coupled co-transport (Buchner et al. 2004). There are approximately 12 to 16 reported genes encoding SO_4^{2-} transporters (SULTR) in plant species. SULTR proteins can be classified according to their protein sequence similarities into SULTR 1 to 5 (for a review: Buchner et al. 2004; Davidian and Kopriva 2010). These transporters can move SO_4^{2-} into the plant when soils are deficient in S; SULTR1;1 (skilled in trace SO_4^{2-} uptake) and SULTR1;2 (major component) have been identified on root hairs and root epidermal and cortical cells of knockout mutants of *Arabidopsis thaliana* (Takahashi et al. 2011). From the structural perspective, SULTR are members of a family of membrane-bound solute transporters provided to 12 domains that cross the plasma membrane (Takahashi et al. 2012). SULTR2;1 is a low-affinity SULTR that appears to be involved in SO_4^{2-} translocation from roots to shoots. The initial uptake by the root epidermis and cortical cells depends on high-affinity transport, as well as on the displacement of SO_4^{2-} into tissues and/or organs, which can be very specialized. Low-affinity transporters (in *A. thaliana*: AtSULTR2;1 and AtSULTR2;2) were shown to be more related to vascular transport of SO_4^{2-} and to the regulation of the cytoplasmic SO_4^{2-} concentration during accumulation in the vacuole (Buchner et al. 2004; Davidian and Kopriva 2010).

The coordination and the dynamics between the pathways of short- and long-distance transporters require specific signaling mechanisms to control and regulate a range of genes encoding specific proteins involved in S uptake, transport and assimilation (Davidian and Kopriva 2010). Expression of these transporters is regulated by internal and external sulfate signals and by the N, C and S reductive assimilation pathways, including phytohormones and variable metabolites (Gojon et al. 2009; Davidian and Kopriva 2010).

After uptake, $SO_4^{2-}$is assimilated into Cys, an amino acid at the intersection of primary metabolism, protein synthesis and the formation of low molecular weight S-containing defense compounds (Rausch and Watcher 2005; Gotor et al. 2014). Excess SO_4^{2-} transported to leaves is stored in vacuoles and constitutes a large S reserve for plant metabolism (Iqbal et al. 2013).

Cys synthesis is required for GSH biosynthesis and occurs in plastids, mitochondria and the cytosol. Activated SO_4^{2-} also forms 3′-phosphoadenosine 5′-phosphosulfate (PAPS, EC 1.8.99.2), the S-donor for sulfonation, sulfation or sulfuryl-transfer reactions; the reaction is catalyzed by sulfotransferases (STs) that play important roles in cell communication, plant development and defense (Negishi et al. 2001). In parallel with S assimilation, some 'dissimilatory' reactions, such as the release of H_2S from Cys 1 and 2, might contribute to defense processes (Rausch and Watcher 2005; Lisjak et al. 2011; for more information on H_2S see Lisjak et al. 2010 and Lisjak et al. 2013).

SO_4^{2-} assimilation is critical for providing reduced S for various cellular redox processes (for review: Jacob and Anwar 2008; Takahashi et al. 2011) and for the synthesis of GSH (Kopriva and Rennenberg 2004; Ghelfi et al. 2011; Seth et al. 2012). GSH has many distinct functions in plant cell metabolism, including controlling gene expression linked to the redox state of cells or subcellular compartments; being an important reducing cofactor of many enzymes related to ROS detoxification (for review: Noctor 2006; Foyer and Shigeoka

2011); and directly controlling the S assimilation pathway. Reduced forms of S decrease significantly during S uptake and assimilation (Kopriva 2006; Chan et al. 2013).

Many studies have emphasized the importance of N in S assimilation and plant stress defenses (Kopriva and Rennenberg 2004; Siddiqui et al. 2008, 2012; Salvagiotti et al. 2009; Carfagna et al. 2011). SO_4^{2-} assimilation declines under nitrate (NO_3^-) deficiency, and the capacity to reduce NO_3^- and the activity of nitrate reductase (NR, EC 1.6.6.1-3) are diminished in plants that are starved for SO_4^{2-} (Kopriva and Rennenberg 2004; De Bona et al. 2011). In tobacco plants, for example, SO_4^{2-} uptake by the roots was drastically reduced when NR was inactivated (Kruse et al. 2007; Siddiqui et al. 2012). Moreover, in N-starved plants, the activities of enzymes responsible for S assimilation and the mRNA levels associated with related genes decreased, but the addition of two distinct N sources (NO_3^- and ammonium, NH_4^+) quickly restored the enzymatic function (Koprivova et al. 2000).

In cereal plant species, increases in S fertilization can enhance the efficiency of N uptake and use because S is a constituent of some enzymes involved in N metabolism (Salvagiotti et al. 2009; De Bona et al. 2011). Wheat plants exposed to distinct N and S levels revealed an important relationship between N and S. When N was less limiting, N uptake was high at the highest S concentration. This increase in uptake was more directly correlated with recovery efficiency than with internal use efficiency (Salvagiotti et al. 2009). Nonetheless, it has been demonstrated that APR, the key enzyme for SO_4^{2-} assimilation, is regulated by carbohydrates (Lewandowska and Sirko 2008; Chan et al. 2013). The availability of Cys is another crucial factor in GSH synthesis, but an adequate supply of glutamate and glycine is also important (Kopriva and Rennenberg 2004).

The activity and expression of SO_4^{2-} transporters and APR in plants are modulated by their S status and the demand for growth (Koralewska et al. 2008). The key enzyme of plant S metabolism, OAS-TL, catalyzes the formation of Cys from the sulfide ion (S^{2-}) and O-acetylserine, as illustrated in Fig. 2 (Youssefian et al. 2001). Cys biosynthesis can be regarded as the exclusive function of S reduction in plants and is a key limiting step in the production of GSH and in tolerance to biotic and abiotic stresses (Youssefian et al. 2001; Mera et al. 2014). OAS-TL plays a key role in the synthesis of Cys and GSH, which are required for regulation of plant responses in response to oxidative stress (Youssefian et al. 2001; Gotor et al. 2014).

Studies of barley plants demonstrated that N or S deficiency altered GSH levels in leaves. In N- and S-starved plants, GSH levels doubled, and the Cys concentration was shown to increase by 50 % (Carfagna et al. 2011). In *Brassica juncea*, N and S enhanced the activity of adenosine triphosphate-sulfurylase (ATP-S, EC 2.7.7.4), a key enzyme in the S assimilation pathway, which activates SO_4^{2-} via an ATP-dependent reaction. In response to an environmental N deficit, the addition of NO_3^- or NH_4^+ rapidly improved ATP-S and OAS-TL function (Siddiqui et al. 2012). N addition positively affected OAS-TL activity in plant roots, and a precise sequence of N metabolism and S assimilation is necessary to provide the N precursors for Cys biosynthesis (Carfagna et al. 2011). Thus, S assimilation is significantly related to assimilation of NO_3^- and C (Yoshimoto et al. 2007). Some transcriptional factors responsible for SO_4^{2-} uptake and assimilation have been identified, demonstrating a relationship between mRNA levels (for APR and ATP-S), protein biosynthesis and enzyme activity (Koprivova et al. 2000; Hesse et al. 2003; Davidian and Kopriva 2010).

Phytohormones in S Assimilation

Some studies have reviewed the importance of the relationship between S assimilation and phytohormones (Maruyama-Nakashita et al. 2004; Maruyama-Nakashita et al. 2005; Kopriva 2006). Phytohormones are essential for plant acclimation and adaptation to environmental changes (Peleg and Blumwald 2011). The signaling pathway of phytohormones is linked to efficient nutrient use, plant defense pathways and plant developmental processes and metabolism (Fatma et al. 2013). Phytohormones such as cytokinins (CK), gibberellins (GA), auxins (AU), ethylene (ET), jasmonates (JA) and salicylic acid (SA) can interact with mineral nutrients under both normal and stress conditions, playing an essential role in salt stress control and affecting plant growth recovery, cell division, germination and seed production, even when applied exogenously (Fatma et al. 2013).

CK is known to be related to the N cycle and metabolism, being involved in N and P assimilation. Therefore, it seems that CK has a general role in the assimilation of nutrients, including S (Kopriva 2006).

The expression of indole-3-acetic-acid-amido synthetase (IAA-amido synthetase, EC 6.3.2.-) in rice seedlings was correlated with an increase in expression of LEA (late embryogenesis abundant) genes, which have been shown to promote drought stress tolerance (Zhang et al. 2009). The expression of many other genes related to auxin synthesis and to enzyme biosynthesis, transporters and activity can also be regulated by ET, whilst auxin seems to affect ET biosynthesis (Peleg and Blumwald 2011). ET has an important role in improving N use efficiency, photosynthetic rates and plant growth in N-optimal and N-deficient *Brassica juncea* plants (Khan et al. 2008). ET signaling increases with SA and/or JA, resulting in expression of a wide range of genes related to plant defense.

GA enhances the effects of salt stress in soybean, perhaps by regulating the availability of other phytohormones. Abscisic acid (ABA) reduced Na^+ and Cl^- content and,

consequently, the Na^+/K^+ ratio and increased the Ca^{2+}, K^+, soluble sugar and proline contents in rice crops (Khorshidi et al. 2009; Iqbal et al. 2013). Phytohormones can alleviate the effects of salt stress and improve plant tolerance by influencing proline metabolism. N and/or Ca^{2+} accumulation are altered, and a link between Ca^{2+} signaling and SA content improves proline content (Du et al. 2009; Al-Whaibi et al. 2012). Proline accumulation seems to be regulated by ABA-dependent and ABA-independent pathways (Iqbal et al. 2013).

ABA appears to be the phytohormone that responds most rapidly to plant stress. ABA synthesis and expression of ABA-inducible genes cause stomatal closure in plants under drought stress (Peleg and Blumwald 2011). Many genes associated with ABA biosynthesis and ABA receptors were identified in *A. thaliana* (Brocard-Gifford et al. 2004) and maize (Peleg and Blumwald 2011).

Plants exposed to SA and ABA exhibited higher GSH concentrations and glutathione reductase activity (GR, EC 1.8.1.7, also named glutathione-disulfide reductase, GSR) (Kopriva 2006; Nazar et al. 2011a, b; Pál et al. 2014). This result confirms the relationship among GSH content, S assimilation and stress defense. ABA is related to environmental stress adaptation and SA plays a key role in plant stress tolerance, expression of genes that encode chaperones, heat-shock proteins and antioxidants, and genes related to secondary metabolites (Kopriva 2006; Pál et al. 2014; Peleg and Blumwald 2011; for a review on the role of phytohormones in stress tolerance, see Carvalho et al. 2011). ABA increases the levels of mRNA encoding cytosolic OAS-TL, a key enzyme in S assimilation and metabolism (Kopriva 2006).

The increased demand for GSH can be met by activation of pathways involved in S assimilation and Cys biosynthesis. Microarray analyses have indicated that the messenger ribonucleic acid (mRNA) transcript level of OAS-TL was upregulated in response to zinc stress in *A. thaliana* (Becher et al. 2004) and was constitutively elevated in its metal-tolerant relative, *A. halleri* (Weber et al. 2004).

Many studies address the production and influence of GSH and Cys in relieving biotic and abiotic stress in plants, such as in the protective response to oxidative stress resulting from various factors (Ruiz and Blumwald 2002; Rahoui et al. 2014; Zhang et al. 2014). Under abiotic stress, GSH demand increases (to promote stress tolerance), activating enzymes in the S assimilation pathway. Enzymatic activity, genetic manipulation of enzymes involved in S assimilation and external S supply can lead to abiotic stress tolerance in plants (Nazar et al. 2011a, b).

Cys content and GSH content and biosynthesis at the transcriptional level are regulated by JA (known to participate in the transduction of stress response) and methyl jasmonate (MeJA) (Shan and Liang 2010; Gfeller et al. 2011). Treatment of *A. thaliana* with MeJa increased the mRNA levels corresponding to many genes involved in S assimilation and GSH synthesis, without affecting the content of S metabolites or of mRNA levels associated with SO_4^{2-} transporters (Kopriva 2006). These signaling compounds also increased the accumulation of mRNA associated with genes that are involved in S metabolism (Takahashi et al. 2011) and associated with S deficiency. This suggests that JA has a signaling role for inducing S assimilation under S deficiency (Sasaki-Sekimoto et al. 2005; Srivastava et al. 2013).

Several limitations of the suggested stress response have to be emphasized because of the importance of the GSH system with respect to other components of the photoprotective and antioxidative defense systems. The roles of JA, SA, GA and ABA in regulating S assimilation enzymes are crucial for acquired abiotic stress tolerance in plants.

Sulfur Oxidation States in the Cell

Under stressful conditions, the induction or increase in S compounds related to plant defense is also crucial for detoxification of excessive ROS (Noctor et al. 2012), resulting in diverse modes of action for S-containing secondary metabolites (Figs. 1 and 2). In the cell, S can be found in several oxidation states mediated by different enzyme families. For example, the sulfotransferase protein family (SOT, EC 2.8.2) catalyzes the transfer of sulfonate molecules in the highest oxidation state to an appropriate hydroxyl group of many substrates using 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the sulfuryl donor. The SOT also catalyzes the sulfonation of a wide range of compounds and produces SO_4^{2-} esters and conjugates (Klein and Papenbrock 2004).

Cys is synthesized in the last stage of photosynthetic assimilation of sulfate in plant cells and is the first organic compound containing reduced S. Cys has essential roles in the function, structure and regulation of proteins, being the precursor of many important S-containing compounds involved in plant defense signaling and plant development (Gotor et al. 2014).

Macroarray analysis revealed an integrated signaling pathway in plant defense gene expression in *A. thaliana*, with upregulation by MeJA of several genes related to S metabolism, GSH, Cys and Met biosynthesis and S-rich defense proteins involved in glucosinolates (GS) metabolism (Jost et al. 2005; Guo et al. 2013).

Methionine Biosynthesis

Met synthesis links Cys biosynthesis to the aspartate-derived amino acid biosynthetic pathway (for review, see Hawkesford and De Kok 2006; see also other papers published by the group of M. Hawkesford; for reviews on the aspartate

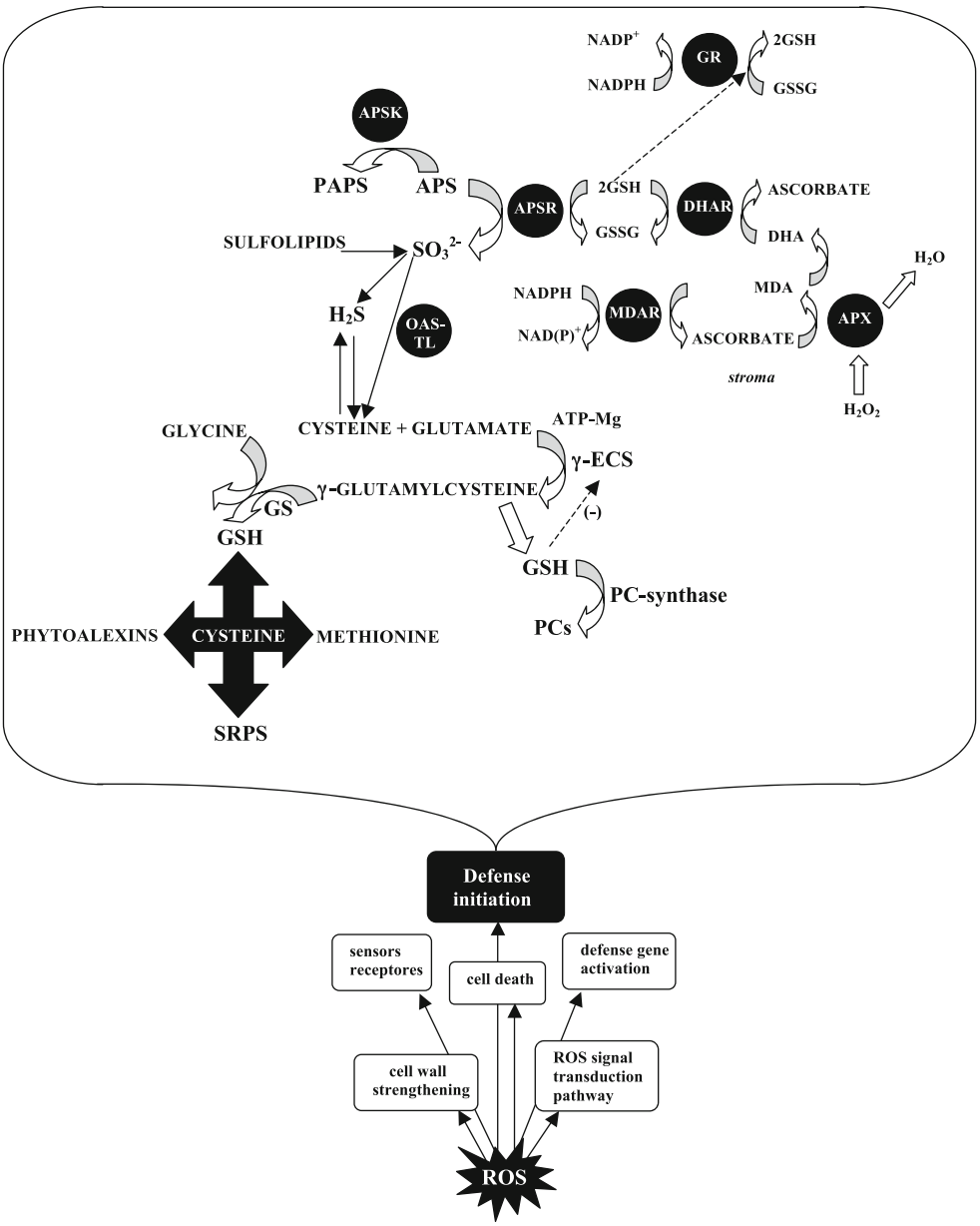

Fig. 1 Sulfur-containing defense compounds. *GR* Glutathione Reductase, *APX* Ascorbate Peroxidase, *GPX* Glutathione Peroxidase, *MDHAR* Monodehydroascorbate Reductase, *DHAR* Dehydroascorbate Reductase, *APS* 5′-adenylylsulfate, *PAP* 3′-phosphoadenylylsulfate, *APSK* APS kinase, *APSR* APS reductase, *OAS-TL* O-acetylserine thiol lyase (modified from Rausch and Watcher 2005; Mendoza-Cozatl et al. 2005)

pathway, see Azevedo et al. 1997, 2006). Biosynthesis of Met from Cys involves three enzymatic steps. O-phosphohomoserine (OPHS, EC 2.7.1.39) derived from the aspartate pathway is a common substrate for both threonine and Met synthesis, catalyzed by threonine synthase (TS, EC 4.2.3.1) and methionine synthase (MS, EC 2.1.1.13), respectively (Azevedo et al. 1997). Cystathionine γ-synthase (CgS, EC 2.5.1.48) catalyzes the synthesis of cystathionine from Cys and OPHS by trans-sulfuration (Hawkesford 2005). Cystathionine is then converted to homocysteine (a β-cleavage reaction) by cystathionine β-lyase (CbL, EC 4.4.1.8). Homocysteine is exported from chloroplasts and converted (by methylation) into Met through MS activity. The activity of CgS and TS will influence biosynthesis of Met and threonine, respectively. CgS activity almost certainly has a large effect on flux and is most likely feedback-regulated by Met or a derivative (Azevedo et al. 1997, 2006). Similarly, TS activity is regulated by S-adenosylmethionine (SAM, also known as S-AdoMet), which is a derivative of Met (Azevedo et al. 1997; Wang and Frey 2007). These controls effectively maintain the Met pool within close constraints. Rather small gene families encode the proteins of this pathway (CgS: 2 genes, CbL: 1 gene, MS: 3 genes). Furthermore, Met is a gateway to many other important S-containing metabolites, including S-methylmethionine (SMM), SAM and dimethylsulfonio-propionate (DMSP). SMM is a transportable derivative of Met. It can revert to Met by donating a methyl group to homocysteine in a reaction catalyzed by homocysteine S-methyltransferase (HMT, EC 2.1.1.10). Under some circumstances, SMM may be the major S constituent of

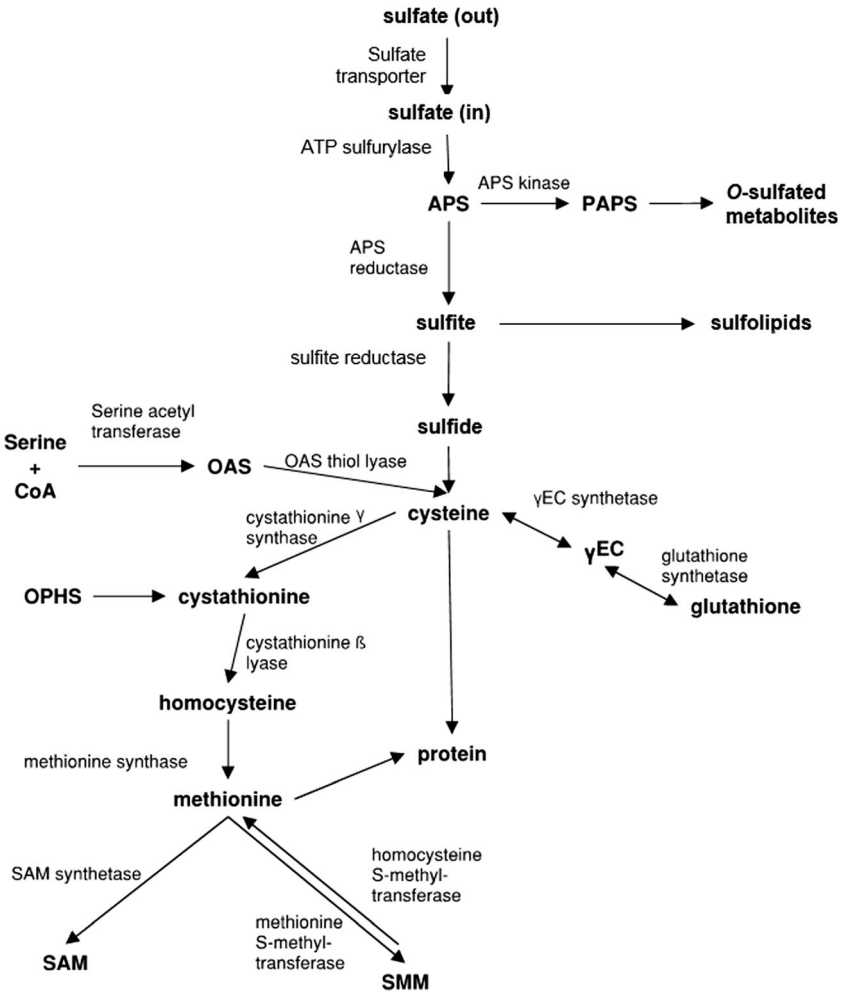

Fig. 2 Biosynthetic pathways for S-containing amino acids and their derivatives. A key enzyme of plant S metabolism, OAS-TL, also named cysteine synthase, catalyzes the formation of Cys from the sulfide ion (S^{2-}) and O-acetylserine. *APS* adenosine-5′-phosphosulfate; *PAPS* 3′-phosphoadenosine-5′-phosphosulfate; *γ-EC* γ-glutamyl-cysteine; *OAS* O-acetylserine; *CoA* acetyl coenzyme A; *SAM* S-adenosylmethionine (S-AdoMet); *SMM* S-methylmethionine (modified from Hawkesford 2005; Koprivova and Kopriva 2014)

the phloem sap, and it has a role in delivering S to sink tissues such as seeds (Hawkesford 2005).

SAM is one of the most important S-compounds in plant metabolism (Azevedo et al. 2006); it is involved in many processes and is the main methyl donor involved in transmethylation of proteins, nucleic acids, polysaccharides and fatty acids (Ma et al. 2003).

SAM is also a precursor of the polyamine (PA) synthetic pathway (spermidine/spermine biosynthesis pathway) and of nicotinamide biosynthesis (important for Fe nutrition in plants). SAM is known as the 'activated Met form' (Bürstenbinder and Sauter 2012), and up to 80 % of the Met pool may be converted to SAM at the expense of adenosine triphosphate (ATP) utilization (Ravanel et al. 1998; Iqbal et al. 2013) by SAM synthetase (SAMS, EC 2.6.1.6, five genes in the family). Spermidine and spermine have multiple proposed roles, including stress response, pH regulation, DNA replication and senescence processes. Consumption of SAM may increase S demands to meet these needs, although ultimately Met is recycled. SAM is also the precursor for ET, a potent modulator of plant growth and development that is involved in stress signaling (Wang et al. 2002). The synthesis of ET from SAM is catalyzed by 1-aminocyclopropane-1-carboxylic acid synthase (ACCS, EC 4.4.1.14) and ACC oxidase (ACCO, EC 1.14.17.4). Met is not consumed in this reaction but is recycled, resulting in no net S demand. A side product of the final biosynthetic step for ET is cyanide, which is detoxified to β-cyanoalanine by β-cyanoalanine synthase (CAS, EC 4.4.1.9), an isoform of OAS-TL (Hatzfeld et al. 2000). DMSP is produced in high concentrations in many marine algae and in some higher plants, such as marsh grasses in the genus *Spartina*, sugar cane and *Wollastonia biflora*. It is synthesized in higher plants via SMM, but it is generally present in low concentrations in other plant species. Several roles have been proposed, including salt tolerance and herbivore deterrence.

SAM, in decarboxylated form and catalyzed by S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50), provides 5′-desoxy-(5′-),3-aminopropyl-(1), a methylsulfonic salt required for PA biosynthesis (Roy and Wu 2002; for reviews on PA see Alcázar et al. 2010; Hussain et al. 2011; Bitrián et al. 2012). PA metabolites are essential to plant survival and have been correlated with biotic and abiotic stress resistance in many plant species; studies have employed

exogenous PA application and genetic manipulations of different plant species (Bitrián et al. 2012). Increased biosynthesis of putrescine and spermidine in transgenic tobacco plants that had human SAMDC inserted into their genomes resulted in greater resistance to salt, drought and biotic stress (Waie and Rajam 2003). Microarray, transcriptomic and proteomic studies have demonstrated the role of PA in signaling cascades that increase plant tolerance or resistance to biotic and abiotic stress (Hussain et al. 2011).

Sulfur Compounds Related to Plant Defense

Environmental stress usually affects plant cell homeostasis and development, increasing ROS production and leading to oxidative stress (Arruda and Azevedo 2009; Azevedo et al. 2011; Cia et al. 2012; Boaretto et al. 2014). Abiotic stress is a consequence of the effects of a wide variety of distinct external agents on plants, such as temperature (heat or chilling), water (drought or flooding), salinity, proton toxicity, heavy metals, overexposure to ultraviolet rays, ozone and others (Azevedo et al. 1998; Gratão et al. 2005; Monteiro et al. 2011; Bulbovas et al. 2014; Nogueirol et al. 2015). All living organisms have a series of pathways to combat environmental stress. In plant species, changes in photorespiration, enzymatic and non-enzymatic antioxidant pathways, regulation and responsive gene expression, and morphological and anatomical adaptations have been identified and investigated (Foyer and Shigeoka 2011). Excessive ROS generation has been considered a negative process for many years, but it is an essential component of signaling processes that prompt adjustments in gene expression and cellular structure in response to environmental changes (Shao et al. 2008; Foyer and Shigeoka 2011; Monteiro et al. 2011).

Plants can respond to abiotic stresses in a number of ways. These include, as a primary step, the induction of a network of signaling pathways and, at later stages, the response by specific proteins, metabolites and other compounds triggered by the signal transduction of the first step (Shulaev et al. 2008). Molecular analysis revealed that both short-term and long-term responses are important for understanding the progression of signaling events when the external and then the internal nutrient supply become depleted (Schachtman and Shin 2007). Similarly, it is critical to understand how experiments are designed because chronic and acute treatments with a stressor can produce completely different responses (Gratão et al. 2008). Furthermore, these distinct responses may improve the understanding or identification of the mechanisms involved in stress tolerance or of plants that are tolerant to the induced stressful condition. Signal transduction and detection networks that control plant responses to nutrient deprivation are not characterized for N and S to the extend it should considering how important these elements are. As already emphasized in this review, the S assimilation pathway is related to plant responses to abiotic stress and to defense mechanisms. It is a source of reduced S for many cellular processes and for synthesis of Cys, which is used in Met synthesis and/or incorporated into proteins or GSH (Siddiqui et al. 2012).

It is important to bear in mind that ROS is naturally produced by the cell metabolism. Oxidative stress occurs when the redox balance is disturbed and excess ROS induces a range of stress defense mechanisms (Gratão et al. 2005). ROS is an upstream mediator of nutrient signaling and increases rapidly after mineral nutrient deprivation, as indicated by Schachtman and Shin (2007). This is a major problem because the literature concerning the effect of an element/nutrient on a plant is extensive, but studies on the secondary responses and effects on the uptake and translocation of other essential elements are more limited. The resulting knowledge gap causes uncertainties regarding the full effects of the stressful conditions to which the plants were subjected. More integrated studies must be conducted.

Research on oxidative stress induced by heavy metals is increasing dramatically. The number of studies being published is astonishing, although the majority confirms known information. Nonetheless, it appears that the stress induced by metals increases demand for reduced S, activating the expression of SO_4^{2-} transporters and enzymes of the assimilatory pathway (Hawkesford 2005; Hawkesford and De Kok 2006; see also other papers published by the group of M. Hawkesford). Recent studies indicate that SO_4^{2-} transport in the plant vascular system, its assimilation in leaves and the recycling of S-containing compounds are related to drought stress signaling and response (Takahashi et al. 2011; Hawkesford 2012).

Plants are known to synthesize and release H_2S in a process catalyzed by L-cysteine desulfhydrase (LCD, E.C. 4.4.1.1) and involving conversion of L-Cys to H_2S, pyruvate and ammonia (García-Mata and Lamattina 2010). H_2S was formerly considered toxic to plant development, inducing excessive production of ROS. However, as understanding of its role in metabolic stress responses has increased, several studies have shown that its main function in plants is as a signaling molecule that controls physiological and biochemical processes (Jin et al. 2011; Li et al. 2012). For example, a study of spinach plants fumigated with H_2S gas demonstrated that approximately 40 % of the H_2S was converted into GSH in plant leaves (Lisjak et al. 2011). H_2S had an essential role in alleviating the stress damage caused by aluminum chloride ($AlCl_3$) in germinating wheat seedlings, increasing esterase and amylase activity and maintaining low malondialdehyde (MDA) and H_2O_2 levels (Zhang et al. 2010). Pre-treatment with sodium hydrosulfide (NaHS, a H_2S donor) resulted in increased activity of guaiacol peroxidase (GPX, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) and

decreased aluminum (Al) uptake in Al pre-treated seeds of wheat; this confirmed H_2S as a signaling molecule in response to abiotic stress (Zhang et al. 2010). In *Vicia faba*, *A. thaliana* and *Impatiens walleriana,* H_2S also induced stomatal closure and ABA-dependent signaling, possibly through the regulation of ABC transporters in guard cells under drought, and enhanced tolerance of water stress (García-Mata and Lamattina 2010). Tobacco cells pretreated with NaHS also exhibited heat tolerance and regrowth after stress exposure (Li et al. 2012). *A. thaliana* exposed to the same pretreatment produced more H_2S, exhibited drought tolerance by limiting stomatal aperture and increased the production and expression of drought marker genes (Jin et al. 2011).

In plants, incorporation of O-acetylserine sulfhydrylase (OASS, EC 2.5.1.65) and serine acetyltransferase (SAT, EC 2.3.1.30) into the cysteine synthase (CysK, EC 2.5.1.47) complex plays a regulatory role in S assimilation and Cys biosynthesis (Francois et al. 2006). The molecular mechanisms for the coordination of S, nitrogen and carbon assimilation are not yet known in detail. O-acetylserine, a precursor of Cys, was proposed as the signal regulating SO_4^{2-} assimilation, but it is not likely to be the outgoing signal for N and C metabolism. In S-deprived plants, for example, the level of glucose, fructose and phosphoenolpyruvate decreased, while starch concentration increased (Lunde et al. 2008). The reduction in photosynthetic rate, increase in oxidation of the ferredoxin:thioredoxin system, changes in starch synthesis and degradation, and the lower use of carbohydrates as an energy source can explain the changes in carbohydrates content (Lunde et al. 2008).

Complementary deoxyribonucleic acid (cDNA) array analysis revealed that expression of genes involved in auxin synthesis is induced upon S-starvation, suggesting a possible role of phytohormones (Kopriva and Rennenberg 2004). Clearly, and despite significant progress in understanding the regulation of SO_4^{2-} assimilation and GSH synthesis, their coordination with N and C metabolism is not yet fully understood, and the several potential signal molecules identified are still far from being sufficiently explanatory (Kopriva and Rennenberg 2004).

Stressed plants usually exhibit decreased rates of cellular division and elongation and, consequently, reduced or inhibited growth. This response may not only be a means of preserving energy for the defense process but may also function as protection against hereditary damage (Li et al. 2014). The chloroplasts play a major role in modulating the plant response, being both sensitive to abiotic stress factors and a major site for S assimilation (Biswal et al. 2008). These organelles are also important for ROS production because of the interaction between electrons escape from the photosynthetic electron transport chain and molecular oxygen (Foyer and Noctor 2012). Chloroplasts can therefore coordinate the C, N and S metabolic pathways, providing essential precursors for the synthesis of S-containing compounds (Jamal et al. 2006; Biswal et al. 2008). Another interesting aspect is that Fe-deficiency can result in severe disruption to the thylakoid lamellae, with loss of grana, but such damage to the photosynthetic apparatus can be diminished by S nutrition. Photosynthetic activity and sucrose synthase and ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO, EC 4.1.1.39) activity are also closely related to S status (Muneer et al. 2014).

Glutathione, Metallothioneins and Phytochelatins

The stress response involves enzymatic antioxidant and other defense systems, including sulfur-containing compounds such as the essential macronutrient sulfur (S); glutathione (GSH: a S-containing thiol tripeptide, γ-L-glutamyl-L-cysteinyl-glycine); a class of phytochelatins [PCs: (g-Glu-Cys)n-Gly, n=2 to 5 usually]; S-rich proteins; S-amino acids; hydrogen sulfide (H_2S); and a range of secondary metabolites (Gratão et al. 2012).

The GSH pool determines the degree of expression of genes linked to defense. It is controlled by many signaling pathways before and during stress, establishing a direct link between stress defense gene expression and GSH biosynthesis. Microarray, reverse transcription polymerase chain reaction (RT-PCR) and high performance liquid chromatography (HPLC) analyses of *A. thaliana* plants exposed to cadmium (Cd) revealed that plants activate the S assimilation pathway by increasing transcription of specific genes that enhance the supply of GSH for PCs synthesis (Kawashima et al. 2011; Jobe et al. 2012). Moreover, roots and leaves have also been shown to exhibit distinct responses to Cd stress (Herbette et al. 2006; Gallego et al. 2012).

Metallothioneins (MTs) are proteins with two structural domains (Cys-rich and metal-binding) involved in metal homeostasis and detoxification (Majic et al. 2008; Choppala et al. 2014; Gu et al. 2014). But in the case of metal stress, the action of PCs is essential. PCs are Cys-rich peptides related to GSH and most likely are synthesized in the same pathway (Qureshi et al. 2007; Zagorchev et al. 2013). Plants can inactivate the toxic effects of excessive ROS by intracellular chelation of the metallic ion by GSH and/or PCs in the cytosol. Depending on the plant species, these complexes can be transported into the vacuole by a specific metal-requiring enzyme (Pál et al. 2006; Yadav 2010; Hossain and Komatsu 2013). Increased GS can be considered a sulfate reserve for PCs synthesis, however the identity of the S-compounds that are reduced when GS increase and the impact of metals on their metabolism are unknown, despite the hypothesis that GS provides an additional S source under metal stress condition (Ernst et al. 2008; Bell and Wagstaff 2014). Additionally, a common response by metal-stressed plants may be the activation of the ascorbate–glutathione cycle, either for the removal of H_2O_2 or to ensure the availability of GSH for the synthesis

of these metal-binding proteins (Vitória et al. 2001; Josefczak et al. 2012).

Glucosinolates

Glucosinolates (GS) are amino acid-derived secondary metabolites consisting of a thioglucose moiety, a sulfonated aldoxime, and a side chain derived from either aliphatic or aromatic amino acids (Halkier and Geshenzon 2006). When plant tissues are disrupted the GS are hydrolyzed by the highly active plant enzyme myrosinase (MYR, EC 3.2.3.1), a thiolglucosidase. The cleavage of the glucose thioester linkage produces an unstable intermediate that rearranges into biologically active thiocyanates, isothiocyanates, nitriles and oxalidine-2-thiones, depending on reaction conditions and presence of additional proteins (Hell and Kruse 2006). These products are chemically very reactive and may interfere with proteins and free amino acids. They are generally caustic and potentially toxic, hence their antimicrobial activities in plant defence. Although the GS also occur in a number of other plant families, the economic importance of oilseed rape, mustard, and the cabbage subspecies raised further interest in the biochemistry and molecular biology of GS biosynthesis and degradation (Hell and Kruse 2006).

In the reductive S assimilation pathway, the activities of enzymes in the pathway are influenced by S supply, with some (in vitro) evidence that ATPS and APR can form a complex to by-pass a branch point in the S assimilation pathway catalyzed by adenosine-5′-phosphosulfate kinase (APSK). APSK forms 3′-phosphoadenosine 5′-phosphosulfate (PAPS), an important substrate for the formation of the secondary S-containing metabolites including GS, and is therefore a significant enzyme in members of *Brassicaceae* (Leung et al. 2006). Four APSK genes have been cloned from *A. thaliana*, for example, APSK1, APSK2, and APSK4, all localized in the plastid, while APSK3 is a cytoplasmic isoform (Mugford et al. 2009). The use of mutants has shown that APSK1 is able to produce sufficient PAPS to maintain normal plant growth (Mugford et al. 2010), while disruption of APSK1 and APSK2 expression reduces the biosynthesis of GS (Mugford et al. 2009) demonstrating that the expression of APSK genes are strongly linked to the biosynthesis of GS. However, in non-glucosinolate accumulating species, including for example onion, the secondary (APSK-mediated) pathway must also operate to generate important pools of sulfate esters.

In order to elucidate the synthesis and degradation of GS in plants, (Mugford et al. 2009) observed highly significant alterations in the levels of GS and their desulfo-precursors in *A. thaliana* apk1, apk2, apk3, apk4 and wild-type mutants. The levels of each individual GS were reduced in the leaves of the mutant so that total GS levels reached only 15 % of that in wild-type mutant. The reduction was accompanied by a massive increase in desulfo-precursors, which reached a ten-fold higher concentration than the mature GS in wild-type leaves. A similar reduction in GS levels was detected in the seeds of the mutant plants, however, the sulfo-precursors did not accumulate in the seeds. These results confirm that GS are not synthetized in seeds but are transported in the mature sulfate form (Magrath and Mithen 1993).

Final Considerations

S is an essential chemical nutrient for plant growth and survival. S-containing defense compounds play significant roles in plant metabolism, stress response, cellular acclimation and adaptation (Fig. 3). They have gained much attention worldwide as biochemical genetics, plant physiology and breeding have been integrated to produce stress-tolerant plants and to identify preferential tolerance mechanisms. The development of transgenic plants with multi-stress tolerance should be the focus of research in the near future because stress factors rarely occur singly. Yet, the use of natural occurring or induced mutants cannot be left out.

Our knowledge of the effects of transgenic S-containing compounds is still limited, partially because of the complex

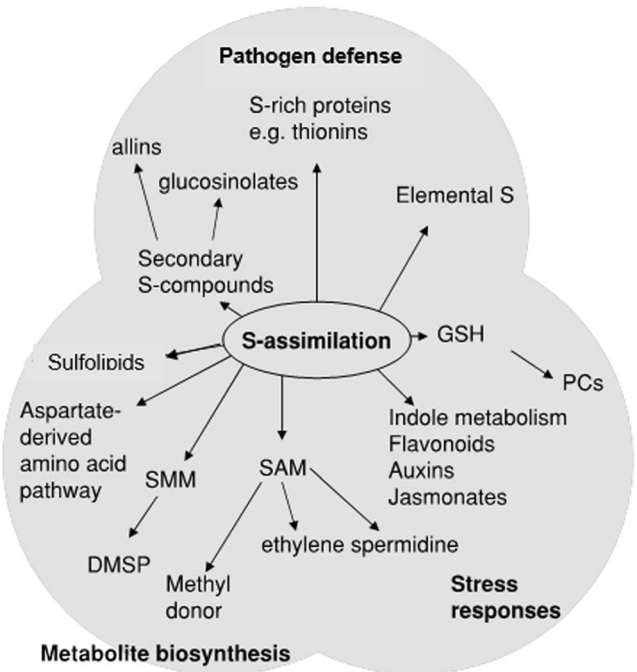

Fig. 3 Sulfur assimilation is linked to multiple metabolic pathways responsible for a diverse range of physiological functions. Three major areas include primary metabolite biosynthesis, stress responses and pathogen defense. *GSH* glutathione; *PCs* phytochelatins; *SAM* S-adenosylmethionine (S-AdoMet); *SMM* S-methylmethionine; *DMSP* dimethylsulfonioproprionate (Modified from Hawkesford 2005)

regulatory mechanisms described previously. Significant advances in metabolic analyses with the "omics" techniques can improve identification of non-target gene products, enabling simultaneous consideration of gene expression, enzyme activity and metabolites.

In conclusion, this literature review is expected to improve our understanding of the essential mechanisms involved in oxidative stress in plants and the induction of S-containing compounds. The increased understanding may aid researchers in overcoming problems that occur in contaminated environments.

Acknowledgments We would like to thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – Brazil) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES - Brazil) for continuous financial support over the years for our projects on this and related subjects.

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