Sulfur Assimilation and Abiotic Stress in Plants

Nafees A. Khan • Sarvajeet Singh • Shahid Umar
Editors

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Springer

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ISBN 978-3-540-76325-3 e-ISBN 978-3-540-76326-0

Library of Congress Control Number: 2007938886

Cover Design: WMX Design GmbH, Heidelberg, Germany

Printed on acid-free paper

5 4 3 2 1 0

springer.com

Dedicated to Professor Samiullah, who nurtured the study of plant physiology and instilled the values of perseverance

Preface

Sulfur is one of the four major essential elements in the plant life cycle. Its assimilation in higher plants and its reduction in metabolically important sulfur compounds are crucial factors determining plant growth and vigor and resistance to stresses. The range of biological compounds that contain sulfur is wide. Sulfur serves important structural, regulatory, and catalytic functions in the context of proteins and as a cellular redox buffer in the form of tripeptide glutathione and certain proteins such as thioredoxin, glutaredoxin, and protein disulfide. In a cascade of enzymatic steps inorganic sulfur is converted to the nutritionally important sulfur-containing amino acid cysteine. Cysteine is the essential precursor of all organic molecules containing reduced sulfur; these range from the amino acid methionine to peptides such as glutathione, or phytochelatins, protein, vitamins, cofactors such as s-adenosyl methionine (SAM), and hormones. Cysteine and derived metabolites have the ability to regulate and repair abiotic stress-induced reactive oxygen species. They regulate the expression of many gene-encoding antioxidants, defense proteins, and signaling proteins. The information on sulfur assimilation can be exploited in tailoring transgenics for efficient sulfur utilization and in applied approaches for the sustenance of agricultural productivity through nutritional improvement and increased stress tolerance. The chapters in this book deal with the importance of sulfur in sustainable crop production, the role of sulfur-derived compounds in abiotic stress tolerance, and the enzymology of sulfur assimilation and its importance in stress tolerance. The physiology of sulfur assimilation in lower plants has also been discussed. Chapters 1 to 4 include the physiological aspects of sustainable crop production with sulfur. In addition, Chapter 4 deals with sulfur deficiency in agricultural soils and its impact on crop yield loss. Chapters 5, 6, and 7 describe the physiology of sulfur-metabolizing enzymes in abiotic stress management. Chapter 8 deals with stress-induced redox signals generated in chloroplast and modulation with sulfur metabolism. Chapters 9 and 10 are concerned with the role of cysteine and glutathione, respectively, in abiotic stress tolerance. The aspects of metal tolerance and its relationship with sulfur assimilation are described in Chapters 11, 12, and 13. Chapters 14 and 15 describe the physiology of sulfur assimilation in lower plants. Chapter 16 addresses the key problem of xenobiotic detoxification, as well as the potential role of the apoplast and possible links with sulfur metabolism. Chapter 17 is concerned with the interaction of sulfur and nitrogen.

Sincere thanks are expressed to the team at Springer-Verlag for their efficient and friendly cooperation in bringing out this volume. Thanks are also due to well-wishers, research students, and family for contributing productively in the completion of this task.

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Chapter 1
Responses to Sulfur Limitation in Maize

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Abstract Maize (*Zea mays* L.) is a widely cultivated major cereal crop, and a model for a monocotyledonous C_4 plant with a substantial physiological and anatomical information base. Studies on sulfur uptake and metabolism indicate that uptake is comparable with other species, while metabolism is characterized by a segregation of components of both carbon and sulfur assimilatory pathways between different cell types. These patterns for distribution and subsequent assimilation are unique and require further elucidation. Ten distinct members of the maize sulfate transporter gene family are reported here; however specific expression and characterization data only exist for two of these. Varietal variation in uptake characteristics has been reported and may represent a potential for breeding improved sulfur use efficiency. Responses to sulfur-limitation which occur at several levels in overlapping succession are described. These include changes in gene expression focussed on cellular processes such as uptake through to wholesale changes in root: shoot biomass allocation and influences on cell death programming and the formation of aerenchyma. These provide mechanisms to maximise uptake, enhance utilization efficiency and moderate, although ultimately cannot prevent, an enhanced susceptibility to abiotic and biotic stresses.

1 Introduction

Sulfur (S) fertilization has become an issue due to reduced industrial emissions of S to the atmosphere and the consequent decreased deposition of S onto agricultural land in many areas of the world (McGrath et al. 1996). Sulfur nutrition plays an important role in the growth and development of higher plants, and sulfur limitation results in decreased yields and quality parameters of crops (Hawkesford 2000). Adequate sulfur nutrition is also required for plant health and resistance to pathogens (Rausch and Wachter 2005).

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

In all plant species studied to date, a series of specific responses aimed at optimizing acquisition and utilization are induced by sulfur limitation (Hawkesford 2000, Hawkesford and De Kok 2006). *Arabidopsis* has proved a useful model for basic molecular studies including the elucidation of the genes involved in these responses. The molecular knowledge from this model has been applied to several crop species, notably cereals (wheat, barley, rice) and Brassicas in relation to sulfur use efficiency in a physiological context. This review focuses specifically on sulfur nutrition in maize (*Zea mays* L.), a monocotyledonous species of the Poaceae family and a typical C_4 plant, and on responses of maize to limiting sulfur availability.

2 Characteristics of Maize

2.1 Architecture

The root system of maize comprises embryonic and postembryonic components (Abbe and Stein 1954). The embryonic root system consists of a single primary root and a variable number of seminal roots, while the postembryonic root system is made up of shoot-borne roots: the crown roots formed at consecutive underground nodes and the brace roots formed at consecutive aboveground nodes of the shoot. Lateral roots which emerge from all major root types also belong to the postembryonic root system. Later in development the postembryonic shoot-borne root system becomes dominant and, together with its lateral roots, is responsible for water and nutrient uptake. Although the anatomical structures of the different root types are very similar, they are initiated from different tissues during embryonic and postembryonic development (Hochholdinger et al. 2004).

The maize shoot consists of a superposition of elementary units, the phytomers. Each of these consists of a leaf, the internode below it, and the node with the axillary branch at the base of the internode. Phyllotaxy is opposite: each leaf includes the blade and the sheath. The blade unrolls progressively, while the sheath remains rolled, forming the sheath tube. Each phytomer develops within the cylinder formed by the rolled leaf of the preceding phytomer (Morrison et al. 1994).

2.2 Functional Anatomy

The maize leaf is characterized by Kranz anatomy, with a prominent bundle sheath cell (BSC) layer comprising concentric layers of cells having an intensely green color and, immediately surrounding, more loosely packed mesophyll cells (MC). CO_2 is initially fixed into malate in the MC and then transported into the BSC, where the formation of glycerate 3-phosphate is localized (Black 1973).

The spatial separation of phosphoenolpyruvate carboxylase and ribulose-1, 5-bisphosphate carboxylase/oxygenase (rubisco) is achieved by the anatomical differentiation of MC and BSC and cell-specific localization of the enzymes. The enzymes involved in the primary CO_2 fixation and malate and/or aspartate synthesis, such as cytosolic carbonic anhydrase, phosphoenolpyruvate carboxylase, pyruvate phosphate dikinase, and NADP-malate dehydrogenase, are localized predominantly in the MC, whereas NAD(P)-dependent malic enzyme, rubisco, rubisco activase, and some enzymes of the Calvin cycle are found exclusively in BSC (Sheen 1999, Edwards et al. 2001). BSC chloroplasts lack photosystem II and therefore exhibit very little oxygen evolution (Hatch and Osmond 1976). Consequently, noncyclic electron flow and the capacity for NADPH formation are restricted in BSC chloroplasts. The reduction of nitrate occurs exclusively in the MC (Moore and Black 1979). This combination of anatomy and physiology and the consequent division of labor is a primary factor contributing to high rates of carbon assimilation (Black 1973) and nitrogen use efficiency (Brown 1978) in C_4 plants. Evolved in the tropics in conditions of high temperatures, high light intensity, and low availability of water, maize utilizes CO_2 more efficiently than C_3 plants, and it can maintain a photosynthetic rate comparable to that of C_3 plants with reduction in water loss (Press 1999).

Maize root anatomy is typical of a monocotyledonous plant. Mature primary and seminal roots as well as shoot-borne roots exhibit a central cylinder (protostele) with many ribs of xylem. The pericycle forms the outermost layer of the central cylinder. The ground tissue consists of one layer of endodermal tissue with the suberized and often lignified casparian band and several layers of parenchymatous cortex tissue. The outermost cell layer is formed by the epidermis (rhizodermis), which consists of root-hair-forming trichoblasts and non-root-hair-forming atrichoblasts. In older roots the short-lived epidermis is replaced by a lignified and/or suberized exodermis, which develops from the outermost cells of the cortex and forms an additional casparian band. In above-ground-formed brace roots the epidermis persists and forms a protective cuticula. Maize roots do not show secondary growth of the root (Hochholdinger et al. 2004). The root apical meristem has a closed organization with three distinct tiers or layers of initials. The longitudinal structure of the maize root includes various partially overlapping specialized zones of development including the root cap, the root apical meristem, the distal elongation zone, the elongation zone and the maturation zone (Ishikawa and Evans 1995).

3 Sulfur Metabolism in Maize

3.1 Sulfate Uptake and Transport

Higher plants use inorganic sulfate as their major source of sulfur. Sulfate is actively taken up from the external environment into the symplast of the root by high-affinity sulfate transporters, and is reduced and assimilated into cysteine by

the reductive sulfate assimilation pathway (Hell 1997, Leustek and Saito 1999, Hawkesford and Wray 2000).

The plant sulfate transporter gene family is divided into five distinct groups, and although not all of the respective gene products have confirmed sulfate transport activity based upon localization, functional and expression data, many may have distinct roles in S-assimilation and transport within the plant (Hawkesford 2003). Much of the data on individual isoforms has come from studies on *Arabidopsis* and *Brassica* (Buchner et al. 2004b, Buchner et al. 2004c, Hawkesford 2003, Takahashi et al. 1997), with studies on cereal sulfate transporters focused on uptake into the plant by Group 1 transporters (Buchner et al. 2004a, Smith et al. 1997, Vidmar et al. 1999). Group 1 includes the high-affinity sulfate transporters, which are responsible primarily, but not exclusively, for the transport of sulfate from the external environment into the root cells. One Group 1 isoform appears to be phloem-specific (Yoshimoto et al. 2003). Group 2 sulfate transporters have a lower affinity for sulfate, and are apparently involved in the movement of sulfate around the plant toward and between sink tissues (Hawkesford and Wray 2000). Group 3 is more enigmatic, and one isoform has been reported to be involved in a heterodimer structure, facilitating increased activity (Kataoka et al. 2004a). The Group 4 transporters are tonoplast-located and appear to be involved in efflux of sulfate from the vacuole (Kataoka et al. 2004b). Little information exists on the Group 5 transporters, which based on sequence alone are the most divergent isoforms, except that they are tonoplast-located (Buchner, Takahashi and Hawkesford, unpublished). Long-distance, inter-, and intracellular transport of sulfate around the plant depend on the coordinated expression of many of these sulfate transporters (Buchner et al. 2004b, Clarkson et al. 1993, Hawkesford and De Kok 2006).

A full-length cDNA encoding a Group 1 sulfate transporter (ZmST1;1) was isolated from maize roots (accession number AF355602, Hopkins et al. 2004), which shared 99.7% homology with the 701-bp partial sequence (accession number AF016306) reported by Bolchi et al (1999). ZmST1;1 is a 658 amino acid polypeptide (Mr 72209). This is the only maize sulfate transporter to be extensively characterized.

An extensive analysis of the databases reveals 10 maize sulfate transporter sequences, and these are shown in relation to the rice homologous gene family in Figure 1.1 and Table 1.1. Additional maize genes will most likely be identified to give a comparable number to that found for rice.

In addition to tissue specificity, there are local cellular patterns of specific expression (Hawkesford 2003). Maize represents an excellent model for the study of localization given the clear patterns of cellular organization. ZmST1;1 was expressed in epidermal cells and in the cell layer surrounding the central vascular bundle, in common with other homologous transporters of this group such as LeST1 (Howarth et al. 2003). Strongest expression away from the root tip was apparent in the epidermal and endodermal layers in common with the sites of highest expression of ZmST1;1 (Hopkins and Hawkesford 2003, Hopkins et al. 2004).

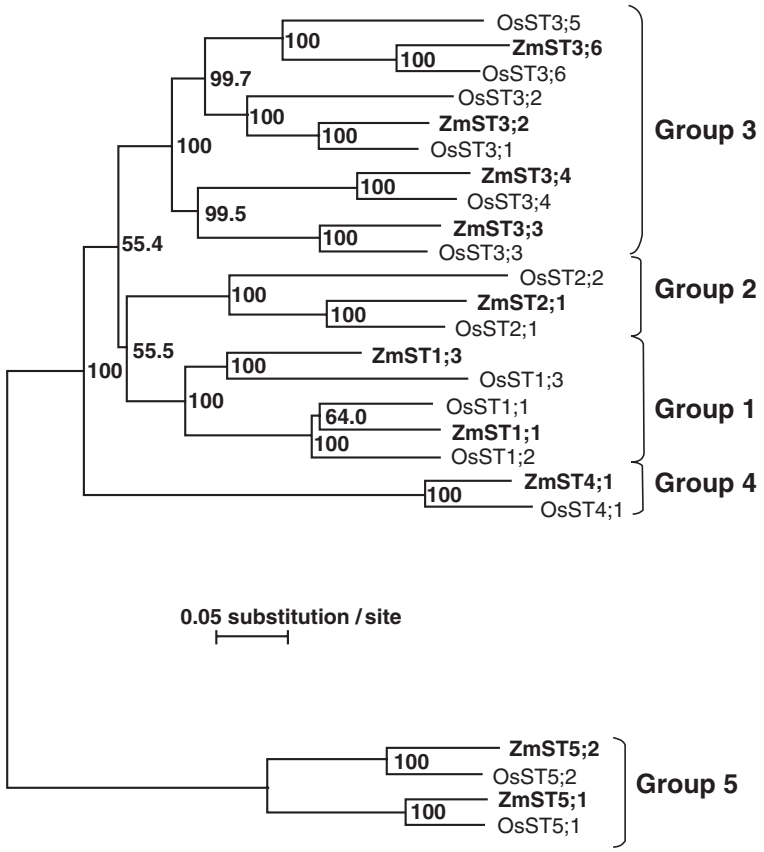

Fig. 1.1 Phylogenetic analysis of available sulfate transporter gene/mRNA sequences from maize compared to the rice sulfate transporter gene family. Neighbor joining tree (NJPLOT; Perrière and Gouy 1996) from the multiple alignment (Clustal V. 1.81; Thompson et al. 1997) of coding cDNA sequences of rice and maize sulfate transporters (see Table 1.1). The bootstrap values, expressed as a percentage, were obtained from 1,000 replicate trees

3.2 Sulfur Assimilation

Reductive sulfate assimilation is a multistep pathway. Sulfate is activated, reduced to sulfide, and incorporated into the amino acid cysteine, which is then used for the synthesis of other sulfur-containing compounds (Hawkesford and Wray 2000, Leustek and Saito 1999, Leustek et al. 2000). The sulfate assimilatory pathway includes ATP-sulfurylase (ATPS) and APS-reductase (APR). The fixation of the formed hydrogen sulfide is catalyzed by the serine acetyltransferase (SAT, EC 2.3.1.30)/O-acetylserine (thiol) lyase (OASTL, EC 4.2.99.8) bi-enzyme complex

Table 1.1 Accession numbers for rice and maize sulfate transporter mRNA and gene sequences. (DFCI = Dana Farber Cancer Institute; http://compbio.dfci.harvard.edu/tgi/)

	Rice		Maize	
Group	Name	Accession Number	Name	Accession Number
1	OsST1;1	AF493792	ZmST1;1	AF355602
	OsST1;2	NM_001055796		
	OsST1;3	AP004691	ZmST1;3	TC341973 DFCI *Zea mays* Gene Index
2	OsST2;1	NM_001055793	ZmST2;1	AY106086
	OsST2;2	AK067353		
3	OsST3;1	NM_001055577	ZmST3;1	TC318119 DFCI *Zea mays* Gene Index
	OsST3;2	NM_001071158 / AK107671		
	OsST3;3	NM_001063313	ZmST3;3	BT018869
	OsST3;4	AK104831	ZmST3;4	AY105934
	OsST3;5	AP003253 gene 30249-33228		
	OsST3;6	AK121195	ZmST3;6	CG222112 / CG117703
4	OsST4;1	AF493793	ZmST4;1	AM711891
5	OsST5;1	AK100928	ZmST5;1	BM336167
	OsST5;2	AK106547	ZmST5;2	CG145534/ CG367893

(Bogdanova and Hell 1997). SAT acetylates L-serine using acetyl-CoA to form O-acetylserine (OAS), which is then combined with sulfide in a reaction catalyzed by OASTL to form L-cysteine (Saito 1999).

In maize, ATPS and APR are essentially restricted to the BSC (Schmutz and Brunold 1984), hence restricting sulfate assimilation to these cell types (Kopriva and Koprivova 2005). Sulfite reductase (EC 1.8.7.1) and OASTL activities are found in both cell types at comparable levels (Passera and Ghisi 1982, Burnell 1984, Schmutz and Brunold 1984, 1985). The localization of ATPS and APR in BSC of C_4 plants implies a transport system for reduced sulfur compounds from BSC to MC. Cysteine synthesis seems to be located in the BSC and spatially separated from glutathione synthesis in the mesophyll cells (Burgener et al. 1998).

3.3 Glutathione Synthesis

Glutathione (GSH) is an important store of reduced sulfur, is a major form of transported reduced sulfur, and is involved in resistance to many biotic and abiotic stresses. GSH has a specific role in maintaining a cellular redox status (Kopriva and Koprivova 2005). Glutathione synthetase activity is greater in MC than in BSC, thus leading to GSH synthesis predominantly in the MC (Burgener et al. 1998) and higher GSH levels in this cell type (Doulis et al. 1997, Burgener et al. 1998, Kopriva et al. 2001). Cysteine is the suggested transport metabolite between the BSC and the MC, although the mechanism for this is unknown; this would represent

a unique extracellular transport of this molecule in plants (Burgener et al. 1998). GSH content is affected by sulfur nutrition (Blake-Kalff et al. 2000).

The significance of compartmentation of sulfate assimilation in maize remains an open question. When maize plants were subjected to chilling stress, resistance to which is linked to GSH, APR activity and mRNA level were greatly increased in BSC, however, only mRNA but not enzyme activity was also detectable in MC, showing that posttranscriptional mechanisms also participate in the compartmentalization of sulfate assimilation in maize (Kopriva et al. 2001, Kopriva and Koprivova 2005).

4 Responses to Sulfur Limitation

4.1 Growth

In common with all other species examined, sulfur deprivation results in a shift of the biomass allocation program toward the root. When ten-day-old maize plants were deprived of a sulfur source, a small increase in shoot biomass was observed for the first four days, accompanied by a progressive increase in root biomass for the first six days, followed by a decline in both cases afterward (Louka and Bouranis, unpublished data; Fig. 1.2a). With regard to dry biomass, sulfur deprivation significantly reduced shoot growth and enhanced root proliferation (Bouranis et al. 2006). By day 6, the accumulation rate of dry mass in the shoot was reduced, resulting in a 44% decrease of the sulfur-deprived shoot by day 18. In contrast, dry mass accumulation in the root system was enhanced by day 18, resulting in a 63% increase of the sulfur-deprived root compared with the control. As a consequence, the root:shoot ratio of sulfur-deprived plants increased progressively from 0.53 at day 6 to 0.84 at day 18.

Growth of the crown roots of the sulfur-sufficient plants remained stable at 2.2 cm d^{-1} for 18 d. By contrast, sulfur deprivation resulted in an initial decrease of growth rate to 2 cm d^{-1} in the first 6 d followed by an increase to 3.5 cm d^{-1} up to day 12 and to 4.1 cm d^{-1} up to day 18. At day 12, sulfur-deprived root length was increased by 22% compared with the control. From day 12 to day 18, sulfur-sufficient and sulfur-deprived crown roots enlarged in length by 51.3%, and 74.3%, respectively. At day 18, sulfur-deprived root length was 40.2% longer than the control (Bouranis et al. 2006).

4.2 Leaf Anatomy

Sulfur deprivation affected leaf lignification. The lamina of the fully expanded second leaf of sulfur-deprived plants presented a more developed lower sclerenchyma

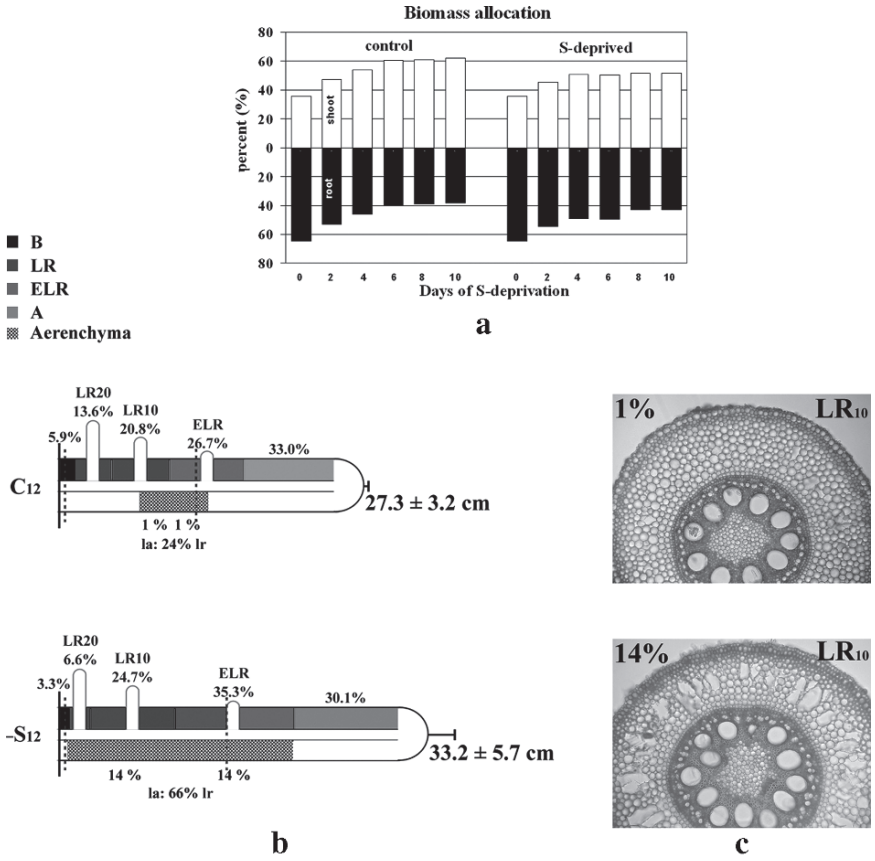

Fig. 1.2 Impacts of sulfur deprivation on root/shoot growth and morphology. (**a**) Progress of fresh biomass allocation (day 0: 10-day-old maize plants) between root and aerial part, and the corresponding effect of sulfur deprivation resulting in a shift of allocation toward the root after day 6 (Louka and Bouranis, unpublished data). (**b**) The shift in biomass allocation is accompanied by multiple progressively developed internal alterations, some of which are depicted in the schematic of the development of a crown root belonging to the second whorl in a complete (C) or sulfur-depleted (–S) nutrient solution 6 days later on. In the upper part of the cartoon (in gray scale), the various root sectors and the percentage of the total root length they occupy are given. LRx, root sector carrying lateral roots with mean length of x mm; ELR, root sector with emerging lateral roots; B and A, the basal and apical root sectors, respectively. In the lower part, the beginning and the end of aerenchyma formation are given, and within this range, the percentage of aerenchyma measured in the cortex of each root section included are provided. (**c**) Cross-sections of control (upper panel) and 12 d sulfur-deprived (lower panel) of LR10 (scale bar = 250 mm) with the percentage of aerenchyma measured in the cortex of this sector indicated (modified and used with permission from Bouranis et al. 2006)

and an intense lignification compared to sulfur-sufficient plants at day 6, mainly in the epidermal cells above the lower sclerenchyma as well as in the vascular bundles. In the lamina of the expanding fourth leaf of sulfur-deprived plants, vascular bundles were more developed, with more and larger xylem vessels compared with

S-sufficient plants (Bouranis et al. 2004). The functional significance of S-deprivation of leaf lignification is not known. Sulfur contributes in at least two key transformations of the lignification process: CoASH is required for the transformation of p-coumaric acid to p-coumaryl-SCoA, while S-adenosyl-methionine is used for the methylation process during biosynthesis of ferulic and sinapic acids.

4.3 Root Anatomy

Under well-oxygenated conditions, sulfate deprivation induced the formation of aerenchyma in maize roots (Fig. 1.2c), similar to nitrogen or phosphorus deprivation. When the beginning of sulfur deprivation coincided with the emergence of a crown root, aerenchyma started to form by day 6 of sulfur deprivation produced by lysigeny in the cortex of the root, and the first aerenchymatous spaces were created in the middle of the cortex of sulfur-deprived roots (Bouranis et al. 2003b). Hypodermis and endodermis were not affected at all by the lytic process. In a fully developed aerenchyma, chains of cells bridge hypodermis to endodermis and stele of roots. After 12 days of sulfur deprivation, aerenchyma covered the entire sector that carries the emerging lateral roots and a part of the nearby sector, with the expanded lateral roots being the 66% of root length and carrying aerenchyma in 14% of the cortex area. Developmentally, aerenchyma was disseminated toward the basal part of the S-sufficient root and toward the apical part in sulfur-deprived root. The basal and apical sectors had no aerenchyma at all (Fig. 1.2b, Bouranis et al. 2006). The functional significance of aerenchyma formation under S-deprivation is unknown and it may include redirection of scarce resources to the maintenance of essential sinks. Furthermore, it may be part of an adaptive program, which includes activation of adaptive pathways and disinvestment in nonessential sinks and pathways.

In general, programmed cell death involves fragmentation of nuclear DNA, involvement of Ca^{2+}, changes in protein phosphorylation, increases in nuclear heterochromatin, and involvement of reactive oxygen species (ROS) (Jones and Dangle 1996). The early stages of the lytic process that lead to aerenchymatous spaces were detected by assessing the loss of nuclear staining with acridine orange during S-deprivation. This revealed that in the apical sector at day 6 and in sections 2 mm from the root tip, the nuclei of the cortex of sulfur-deficient roots were shrunken and near to the cell wall, while by day 12 there was no fluorescence due to the nuclei in the cortex of sulfur-deficient roots (Bouranis et al. 2003b). Formation of ROS was detected in whole cells of the root cortex of sulfur-deprived plants by day 6. ROS appeared in groups of intact midcortex cells, and formation of superoxide anion and hydrogen peroxide was found in degenerating cells of the mid-cortex. Inhibition of superoxide dismutase activity (treatment with N, N-diethyldithiocarbamate; DDC) increased the presence of superoxide anions in the same locations and less hydrogen peroxide was apparent. Treatment of roots with ascorbate or ascorbate + DDC resulted in an almost complete inhibition of hydrogen

peroxide production. By day 12, ROS were detected in the cell walls of endodermal, hypodermal, and epidermal cells of sulfur-deprived plants and were not detected in the cortical cells. The presence of hydrogen peroxide was located where superoxide anions appeared (Bouranis et al. 2003b). In the non-aerenchymatous basal sector under S-deprivation, when stained for ROS, plasma membranes of intact cortex cells fluoresced with increased intensity from day 6 onward (Bouranis et al. 2006). The alterations of calcium levels and pH in aerenchymatous sectors under sulfur deprivation were compared with the basal non-aerenchymatous region. There was a higher calcium concentration in the cell walls of the endodermis and epidermis than in the rest of the sulfur-deprived root tissues, and a higher pH was observed, mainly in the cell walls of the hypodermis and to a lesser extent in the cell walls of the endodermis of the sulfur-deprived roots (Bouranis et al. 2003b). The higher apparent Ca^{2+} concentrations may be linked to the elevated hydrogen peroxide levels: the plasma membrane NADPH oxidase, required for the controlled generation of hydrogen peroxide, is directly modulated by calcium fluxes. In addition to NADPH oxidase, pH-dependent cell wall peroxidases have been proposed as sources of hydrogen peroxide in the apoplast, activated by alkaline pH (Neill et al 2002).

The hydrogen peroxide may be directly utilized by wall-bound peroxidases in lignification and cell wall strengthening. Sulfur deprivation induced thickening of the cell walls of the endodermis, and after 12 days, thickness of the cell walls of endodermis of sulfur-deprived roots increased by 68%, estimated to be 2.7 μm. Furthermore, sulfate deprivation induced the lignification process in maize roots (Bouranis et al. 2003b). Lignified epidermal layers were located at the basal sectors, with a limited extension of the lignified layers toward the nearby lateral root carrying sector (Bouranis et al. 2006). Cell wall thickening may enhance mechanical strengthening of roots suffering sulfur deprivation.

4.4 Root Morphology

As found for other nutrients, root system architecture is affected by sulfur nutrition (see Fig. 1.2b, and Bouranis et al. 2006, Hopkins et al. 2004, Kutz et al. 2002). A proliferation of lateral roots has being reported for *Arabidopsis* in response to sulfur limitation (Kutz et al. 2002). In aeroponically-grown maize, both increased lateral root length and increased abundance of laterals near the tip of the main root occurred upon sulfur deprivation (Hopkins et al. 2004). In addition, in a hydroponic study, sulfur deprivation demonstrated shorter lateral roots in the sectors proximal to the root base. The lateral root proliferation is also linked to aerenchyma formation: aerenchyma found in the cortex along the root length was located particularly in the region of emerging or developing lateral roots (as discussed above, Section 4.3). The basal and apical sectors had no aerenchyma, and no aerenchyma connection was found with the shoot (Bouranis et al. 2006).

4.5 Sulfate Uptake and Transport

Maize responds to a limited sulfur supply by increasing influx capacity for sulfate in roots along with increased expression of genes encoding components of the uptake and assimilation pathway, a phenomenon observed in many other plant species. Removal of the sulfur source from the medium of maize seedlings led to a 3.8-fold increased capacity for sulfate uptake over a 10-min period (Hopkins et al. 2004, Quaggiotti et al. 2003). Influx was approximately equal in all sections of sulfur-sufficient and sulfur-deprived roots. In sections distal from the tip, the uptake may have been attributable to de novo influx from the external solution or from upward translocation; however, less than 0.5% of sulfate was recovered in the shoot tissues, indicating that translocation was minimal during the 10-min influx period and suggesting that all regions of the root in these young seedlings had a similar capacity for uptake (Hopkins et al. 2004). Expression analysis of abundance of sulfate transporter transcripts in the different root sections and in response to the removal of sulfur supply indicated an increased abundance of the ZmST1;1 mRNA in all root sections. There was a slightly higher apparent abundance in the sections 0-10 cm from the root base compared to the sections nearer the tip, which was most apparent 1 d after sulfur removal (Hopkins et al. 2004). Data on expression patterns of other members of the sulfate transporter gene family in maize are limited to ZmST2;1. (Hopkins et al. 2004) and a similar pattern of expression in roots (as observed by northern blotting) occurred for this transporter (Hopkins et al. 2004).

Sulfate uptake and transporter gene expression have been examined in two maize hybrids, chosen on the basis of their productivity at low nutritional inputs (Quaggiotti et al. 2003). Kinetic measurements of sulfate influx on S-deprived seedlings indicated contrasting adaptive strategies with either high affinity or high V_{max} in intact roots. Both varieties showed substantial increased capacity for uptake when S-deprived, although the response was more rapid and greater in magnitude in the variety with the higher V_{max}. Using a probe for ZmST1, a similar induction of mRNA abundance was observed for both varieties. As no other sulfate transporters were examined, the basis of the variation is not clear and deserves further attention. It may be that other members of the transporter family are involved or that variation exists in characteristics of the respective transporters. These alleles have not been isolated and sequenced; however, such variation is indicative of a potential for selection of genotypes with improved sulfur use efficiency.

The increased expression of the transcripts of the transporters involved in uptake may be due to the root proliferation but primarily represents an increase in density of the transporters in the root tissues. These responses together maximize the capacity for uptake from the soil under sulfur-limiting conditions (Hopkins et al. 2004).

Studies in maize, particularly using cell cultures, have contributed to the development of a model linking nutritional status with changes in gene expression which includes a role for OAS (Clarkson et al. 1999). The model proposes that increased expression of sulfate transporters' response to sulfur deprivation is not simply a de-repression model of regulation of sulfate uptake and assimilation

involving a decrease in a downstream product of assimilation which relieves repression, but acts together with an increase in abundance of an inducer, the cysteine precursor, OAS (Smith et al. 1997, Hawkesford and Wray 2000), although not all data in other plant species are consistent with OAS as the key nutritional signal (Hopkins et al. 2005).

The sulfate transporters, ZmST1;1 and 2;1 were induced in leaf tissues (Hopkins et al. 2004). One day after the removal of the sulfur supply, a strong increase in abundance of the ZmST2;1 was found specifically in the first leaf, while this increased abundance was a transient phenomenon for the ZmST1;1. A similar increase in transcript abundance of the two sulfate transporters in the other leaves in response to the removal of sulfur supply was also observed (Hopkins et al. 2004). The occurrence of the Group 1 sulfate transporters in shoot tissues is not normally observed, but this clearly indicates that this group is not root specific. ZmST2;1 falls into Group 2 of the sulfate transporters.

A systematic analysis of 12 isoforms identified in *Brassica oleracea* (Groups 1-4) indicated a complex tissue distribution and tissue-specific responses to sulfur availability (Buchner et al. 2004b). Generally Group 1 transporters were root specific except under sulfur deprivation. The 2;1 isoforms occurred in roots, stems, and leaves, and mRNA abundance increased under S-deprivation. These data are not inconsistent with that seen for maize (Hopkins et al. 2004); however, without a more complete picture of isoforms in maize, direct comparisons are difficult.

4.6 Sulfur Assimilation

Patterns of expression of ATPS, APR, and OASTL, components of the sulfur assimilation pathway, have been studied in both roots and shoots of young aeroponically-grown maize seedlings (Hopkins et al. 2004). Increased abundances of both ATPS and APR mRNA pools were seen in both roots and leaves in response to S-limitation. In young seedlings these responses could be seen within 24 h of the removal of the external S-supply, although responses may be expected to be slower if substantial S-pools occur within more mature tissues. Levels of expression were substantial in the roots, indicating a potential for substantial S-assimilation in these tissues under these conditions. Some spatial regulation of expression was apparent, with higher expression levels occurring away from the root tip region and in the youngest leaves. Transcript abundance of OASTL did not vary in response to sulfur deprivation in the leaves or the roots in any significant pattern. In situ analysis of OASTL transcripts showed a unique spatial pattern of expression, with strongest expression throughout the cortex and noticeably absent in the root cap/quiescent zone and vascular tissues (Hopkins et al. 2004), although comparable data for other genes of the assimilatory pathway are not available. The most significant induction was of ATPS in the leaves, in contrast to *Arabidopsis*, where APR showed the greatest induction (Takahashi et al. 1997, Vauclare et al. 2002), indicating that regulation may vary between species. This contrast in regulation may be related to

the spatial distribution of pathway components between different cell types, and distribution studies will be required to compare S-replete and S-deficient plant materials. Maize also differs from *Arabidopsis* in that regulation of gene expression of components of the assimilatory pathway appears to be mediated via cysteine (Bolchi et al. 1999) rather than glutathione as demonstrated for *Arabidopsis* and *Brassica* (Lappartient et al. 1999). The increased expression of enzymes of the assimilatory pathway, particularly ATPS and APR, represents an adaptation for the optimization of the assimilatory pathway under sulfur-limiting conditions and will maximize the flux of available sulfur from sulfate toward cysteine.

4.7 Glutathione Synthesis

Glutathione content of plant tissues is an indicator of sulfur nutritional status of the plant (Blake-Kalff et al. 2000), and indeed glutathione content of young maize seedlings is drastically reduced when plants are sulfur-deprived (Bolchi et al. 1999, Petrucco et al. 1996, Quaggiotti et al. 2003). As GSH is a transient store and a major transported form of reduced sulfur, sulfur-deficient conditions which limit its synthesis might be expected to cause depletion, either by dilution through growth (a minor influence if growth rate is retarded) or by consumption, as it is utilized for protein synthesis and other biosynthetic requirements for reduced sulfur. It has been suggested that glutathione plays an essential role as a signal for sulfur nutritional status; however, its importance relative to OAS has been questioned (Smith et al. 1997). As discussed above, in maize specifically, drastically reducing glutathione levels using a glutathione inhibitor (buthionine sulfoximine) did not induce a sulfate transporter or ATPS (Bolchi et al. 1999). A consequence of reduced glutathione would be impaired protection against stresses normally dependent upon its presence, including redox stress, metal exposure, chilling sensitivity, or pathogen infection. Under these conditions, other protective mechanisms may assume important roles. For example, using glutathione content as a marker, Petrucco et al. (1996) identified an isoflavone reductase by differential display, which was suggested to provide a redox protection role, compensating for decreased glutathione levels.

5 Combined Effects

5.1 Sulfur Deprivation and Nitrogen

Proteins contain both nitrogen and sulfur, and a deficiency of either will severely restrict protein synthesis and plant growth. An imbalance of supply leads to perturbations of pools of nitrate and sulfate ions and of intermediary metabolites. Plants

respond at the level of gene expression and possibly enzyme activity to moderate these metabolite imbalances, and this implies interaction and coordination of the nitrogen and sulfur uptake and assimilatory pathways.

Induction (or de-repression) of the sulfate uptake and assimilatory pathway in response to sulfur deficiency will only occur in the presence of nitrogen (see, for example, Brunold 1993, Clarkson et al. 1989, Koprivova et al. 2000, Reuveny et al. 1980, Yamaguchi et al. 1999), Similarly, under sulfur-limiting conditions decreased expression and activity are seen for many enzymes of the nitrate assimilatory pathway (Friedrich and Schrader 1978, Prosser et al. 2001). In spite of the cross-coordination, accumulations of either nitrate or sulfate are seen in the vacuoles and perturbations in many amino acid pools, particularly the basic amino acids (Amancio et al. 1997, Migge et al. 2000, Prosser et al. 2001). An elegant study using ^{15}N to NMR monitor incorporation into amino acids indicated that the elevated amino acid pools were a consequence of de novo synthesis, not of protein breakdown (Amancio et al. 1997).

OAS is a key metabolite linking the nitrogen and sulfur pathways (Clarkson et al. 1999). It is the immediate precursor for cysteine combining with sulfide in a reaction catalyzed by OASTL. OAS pools will accumulate if nitrogen assimilation is greater than sulfate reduction, and the OAS has been suggested to be a positive regulator of sulfate transporter and other gene expression (for example, Smith et al. 1997). In addition, a sophisticated mechanism of control has been suggested in which SAT, which synthesizes OAS, is only active when in a complex with OASTL, and the complex is disrupted by excess OAS or cysteine and stabilized by sulfide. Hence cysteine synthesis is facilitated when sulfate reduction is active and when there are adequate sinks for the cysteine produced but also excess build-up of OAS is prevented. The complex, therefore, acts as a sensor of N/S balance (Hawkesford et al. 2006 and references therein).

5.2 Sulfur Deprivation and Iron

Maize has been a useful model for the examination of the interactions of iron and sulfur nutrition (Astolfi et al. 2003, 2004a, 2006a, Bouranis et al. 2003a). Sulfur metabolism is dependent upon adequate iron nutrition, for example, the upregulation of ATPS and OASTL in response to sulfur depletion (Astolfi et al. 2003, 2004a). In contrast, sulfate uptake capacity was increased by iron deficiency (Astolfi et al. 2004a, 2006b), while root cysteine content was elevated, apparently due to shoot to root translocation (Astolfi et al. 2006b). In studies on barley, sulfur deficiency has been shown to decrease phytosiderophore release and iron uptake (Astolfi et al. 2006a). Iron acquisition in graminaceous species is dependent upon phytosiderophore production, which in turn is dependent upon the sulfur assimilation pathway and methionine biosynthesis specifically. An early consequence of sulfur deficiency is therefore a decrease in iron content (Astolfi et al. 2003, Bouranis et al. 2003a).

5.3 Sulfur and Resistance to Stresses

Plant health as well as growth is dependent upon sulfur. Many sulfur-containing compounds are essential components of resistance mechanisms to abiotic and biotic stresses. Limitations in sulfur supply will influence partitioning of available sulfur. Allocation to sulfur-containing compounds of secondary metabolism may, for example, be secondary to partitioning to roots to aid proliferation. Pools of glutathione are known to be reduced under sulfur-limiting conditions (Blake-Kalff et al. 2000). Manipulation of GSH biosynthesis increases resistance to oxidative stress (Blaszczyk et al. 1999, May and Leaver 1993, May et al. 1998, Sirko et al. 2004, Youssefian et al. 2001). As discussed in section 4.3, ROS increase upon sulfur deprivation in maize. Resistance to metal stress (Astolfi et al. 2004b) or to high irradiance (Astolfi 2001) in maize has been shown to be dependent upon sulfur nutrition. Studies in other species have demonstrated clear requirements for adequate or "more than adequate" sulfur fertilization for resistance to pathogens (Cooper and Williams 2004, Rausch and Wachter 2005, Williams et al 2002).

6 Future Prospects

Maize is a unique model for studying the molecular physiology of sulfur nutrition in a monocotyledonous C_4 plant. It is an important crop, and such knowledge will be valuable for the development of low-input fertilizer strategies. The physiological information base, including detailed knowledge of anatomy and architecture, facilitates the understanding of interactions between cells and organs for the optimization of nutritional use efficiency, including sulfur. A next step will be to localize expression in relation to this physiological knowledge. As a model for the study of source sink interactions, the mature maize crops offer many opportunities, both for improving sulfur use efficiency and crop quality by optimizing sulfur nutritional content.

The ongoing genome sequencing project will give access to the required genes and will facilitate the analysis of their expression as has been determined for *Arabidopsis* and *Brassica*. No work has been undertaken examining genetic variability, which will be a vital resource for the development of low-input sustainable agriculture.

Acknowledgements Rothamsted Research receives grant-aided support from the Biotechnology and Biological Science Research Council (BBSRC) of the UK.

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