Trends in Plant Science

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Review

Sparking a sulfur war between plants and pathogens

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The biochemical versatility of sulfur (S) lends itself to myriad roles in plant–pathogen interactions. This review evaluates the current understanding of mechanisms by which pathogens acquire S from their plant hosts and highlights new evidence that plants can limit S availability during the immune responses. We discuss the discovery of host disease-susceptibility genes related to S that can be genetically manipulated to create new crop resistance. Finally, we summarize future research challenges and propose a research agenda that leverages systems biology approaches for a holistic understanding of this important element's diverse roles in plant disease resistance and susceptibility.

Highlights

Genome analysis has revealed surprising diversity in mechanisms through which pathogens obtain sulfur (S) from their hosts during infection, and transcriptomics has uncovered variation in the modes of S uptake and metabolism during pathogen infection cycles.

Functional genomics of pathogen virulence proteins points toward mechanisms through which the pathogen is directly manipulating a plant S transport gene. In turn, this gene has been engineered to resist pathogen manipulation and provide disease resistance.

Mounting evidence indicates that plants can interpret S deficiency as a signal of pathogen invasion and can withhold S from pathogens as a component of the immune response. This aspect of 'competition' is emerging for other nutrients and represents a major area of growth in our understanding of the interrelated nature of pathogen virulence and plant immunity.

Toward a broader view of sulfur's myriad roles in plant–pathogen interactions

The importance of S in plant–biotic interactions has been studied since 1802, when William Forsyth recommended elemental S as an effective fungicide [3]. The importance of S in plant immunity and resistance against diverse pathogens was further underscored in the 1980s, when legislation was mandated in Europe to reduce S emissions from industry. The resultant reduction in atmospheric S, although beneficial overall, had unintended consequences on agricultural productivity because some high-S-demanding crops became more susceptible to disease [4]. This susceptibility was mitigated by the application of S fertilizer, leading to the concept of 'S-induced resistance' that has been investigated further in laboratory studies. The relationship between S nutrition and plant immunity has subsequently been well studied and is documented in several review articles [4–7].

Importantly plant S metabolism could also support pathogen virulence in various ways and thereby promote plant disease susceptibility. These aspects of S metabolism are much less understood. Here, we summarize the current understanding of how plant-associated microbes obtain S from plant hosts during colonization and how plants and microbes compete for S (Figure 1, Key figure). We emphasize several exciting new studies, highlight important emerging questions, and introduce a systems approach to provide a holistic perspective on plant–S–pathogen interactions. We also highlight strategies for translating this fundamental understanding into the development of new sources of genetic resistance to crop diseases.

How do pathogens obtain sulfur and what is it used for?

Sulfur metabolism in bacteria

While bacteria and plants share S assimilation pathways, they also differ significantly (Box 1 and Figure 2). For example, some bacteria can assimilate both inorganic and organic S, whereas plants primarily assimilate inorganic S (e.g., sulfate). Sulfate metabolism pathways are best studied in model organisms such as *Escherichia coli* and *Salmonella typhimurium* [8,9]. For bacteria in general, sulfate is typically the primary S source, while xenobiotics, sulfonates ($R–SO_3$),

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and sulfate esters (R–O–SO_3) are minor sources. However, sulfonates and sulfate esters are abundant in soil organic matter and can serve as nutrients for bacterial assimilation (Figure 2) [10].

Among plant-associated bacteria, S uptake and metabolism are best understood for nitrogen-fixing **rhizobial** (see Glossary) bacterial mutualists. Such bacteria are enclosed in **symbiosomes** within root **nodules** in legume plant hosts. Therein, the bacteria differentiate into **bacteroids** that fix nitrogen and exchange this for carbon from the host. S uptake into symbiosomes is important for the iron–S clusters in rhizobial nitrogenase enzymes that fix atmospheric nitrogen in a low oxygen environment. Accordingly, sulfate is transported at a high rate across the symbiosome membrane [11]. In *Lotus japonicus*, this transport is supported by a plant-expressed sulfate transporter called SST1, which localizes to the symbiosome membrane and was genetically validated to play an important role in the interaction [12]. The bacteroids incorporate plant-derived sulfate into nitrogenase. Moreover, bacteroids reduce a considerable proportion of the sulfate to thiols, which are exported back across the symbiosome membrane and transported throughout the plant [13]. In this way, S metabolized in the bacteroid can benefit other parts of the plant, thereby extending the benefits of the legume–rhizobia mutualism beyond nitrogen and carbon to include S.

Linkages between S metabolism and the beneficial effects of plant growth-promoting bacteria (PGPB) have also been established recently. In one report, Arabidopsis placed under salt stress displayed signatures of S starvation and salt stress was mitigated by application of sulfate, implicating S metabolism as a key component of salt stress [14]. The PGPB *Enterobacter* sp. SA187 reprogrammed the plant's S starvation response and mitigated the detrimental effects of salt stress in salt-sensitive mutants. This effect was accompanied by activation of S transport and metabolism in the microbe, revealing a key role for coordinated S metabolism in the plant and PGPB in abiotic stress tolerance. This PGPB also promotes growth in alfalfa and is particularly effective at promoting salt tolerance [15]. S metabolism has also been associated with growth promotion by *Pseudomonas fluorescens*, and it will be of great interest to further generalize the coordination in S metabolism between PGPB and plants [16].

S uptake and metabolism have been studied much less intensively in plant pathogenic bacteria, compared with rhizobia. Genome analysis of the citrus pathogen *Xanthomonas citri* revealed similarities and differences with model bacteria: a canonical sulfate transporter and systems responsible for transport and oxidoreduction of alkanesulfonates or organosulfur compounds are evident, but a taurine transporter is absent, suggesting that taurine is not an important S source [17]. Deletion analyses of genes for two alkane sulfonate-binding proteins indicated that alkanesulfonate uptake is necessary for xanthan gum synthesis, adhesion, biofilm production, and full virulence on the plant [18,19]. Thus, organic S compounds are likely to be important for *X. citri* during host infection. Sulfate uptake could be important too, because the *X. citri* sulfate transporter is induced during infection [20].

Sulfur metabolism in fungi

The molecular genetics of S assimilation in model filamentous fungi has been well studied [21]. Fungi can take up inorganic S, including SO_4 as well as more reduced forms, and can assimilate organic S in the forms of amino acids, sulfones, sulfonates, sulfones, sulfonamides, and sulfamates [22,23]. Research on fungal pathogens of humans has leveraged the knowledge from model systems to validate the importance of S-containing compounds for virulence and to propose strategies for therapeutics that target S metabolism [24]. Corresponding studies for plant pathogens have been initiated, albeit at a relatively limited scale so far. For example, knockouts of genes for methionine biosynthesis reduce pathogenicity in tomato leaf mold

Glossary

Bacteroids: differentiated rhizobia that are capable of fixing atmospheric nitrogen.
Biotrophy: a pathogenic lifestyle in which the microbe extracts nutrients from living host plant cells.
Effectors: proteins that are secreted by pathogens to target plant proteins on the outside or inside of plant cells. Dozens of these proteins have been characterized experimentally, and most appear to be dedicated to interference with plant immune responses [1].
Effector-triggered immunity: results when plants recognize pathogen effectors, inside or outside of plant cells. Surveillance for effectors inside plant cells is carried out by NLR proteins, while effectors in the apoplast can be recognized by PRR proteins.
Effector-triggered susceptibility (EST): results when pathogens secrete effectors that collectively render plant cells more conducive to pathogen exploitation.
Hemibiotrophy: a pathogenic lifestyle in which the microbe begins the infection cycle as a biotroph but later shifts to necrotrophy.
Microbe-associated molecular patterns (MAMPs): comprise pathogen-derived macromolecules that are recognized as signaling of microbial colonization. Such recognition often occurs in the apoplast via PRR proteins.
Necrotrophy: a pathogenic lifestyle in which the microbe destroys and extracts nutrients from host plant cells.
NLR (Nod-like receptor) proteins: contain nucleotide-binding sites and leucine-rich repeats. These proteins carry out surveillance for microbial effectors inside plant cells.
Nodules: differentiated organs in the roots of legume species that provide appropriate conditions for rhizobial nitrogen fixation.
Obligate pathogens: derive nutrients only from living cells of a compatible host and cannot be cultured in synthetic media. Many important pathogens have evolved to an obligate lifestyle.
Oomycetes: microbes that resemble fungi but reside within the Stramenopile kingdom. This group includes many important plant pathogens, including *P. infestans*, the cause of the Irish potato famine.
PRR proteins: proteins that contain an extracellular domain that binds to apoplastic microbe-associated

Cladosporium fulvum, the rice blast fungus *Magnaporthe oryzae*, the wheat/barley head scab pathogen *Fusarium graminearum*, and the corn smut pathogen *Ustilago maydis* [25–30]. Deletion of the *met6* gene from *M. oryzae* causes defects in host cell penetration and invasive infectious growth [25]. These defects could be rescued by the addition of methionine, suggesting that the pathogen cannot access sufficient Met or Met derivatives from its interface with the plant [25]. More generally, this study illustrates how the generation of auxotrophic mutants is a valuable approach toward defining the nutrient conditions that plant pathogens encounter during infection [28].

Sulfur metabolism in oomycetes

S metabolism in **oomycetes** is not well understood compared with bacteria and fungi, due to the lack of well-established model species. Studies with defined nutrient media indicate that sulfate is a preferred S source for species in the *Phytophthora* genus [31], while species in the *Pythium* genus can utilize a broader range of inorganic and organic sources [32]. These differences align with the contrasting lifestyles of these pathogens: *Phytophthora* species are **hemibiotrophic** with a narrow host range, while *Pythium* species are **necrotrophic** with a broad host range and grow **saprophytically** in soil. The capacity of *Pythium* to utilize diverse S sources might be a factor in its versatility, while the *Phytophthora* species have evolved a more restricted palate. Interestingly, modeling to identify potential metabolic vulnerabilities in the late blight oomycete *Phytophthora infestans* highlighted sulfate uptake as a process that could be targeted for disease control via sulfate restriction, mirroring similar perspectives on human pathogens such as *Mycobacterium tuberculosis* [33,34].

At the genomic level, sulfate permease domains (SulP, IPR011547) are over-represented in the genomes of oomycete pathogens, with an average abundance of 13.5 domains compared with 8.58 for all other eukaryote species [35]. A total of 16 SulPs were classified in *P. infestans* and ten in *Pythium ultimum*, compared with only six in the fungus *M. oryzae* [36]. The biological significance of the larger sulfate permease gene family in oomycetes, compared with fungi, is unclear but suggests that sulfate uptake is precisely regulated. Accordingly, transcription profiling of *Py. ultimum* and *P. infestans* revealed differential regulation of sulfate uptake during different infection stages: genes that are responsible for the reduction of inorganic sulfate into cysteine are induced early during necrotrophic infection of potato tubers by *Py. ultimum*. These sulfate assimilation genes were not induced during the early **biotrophic** growth of *P. infestans* but were activated later during the necrotrophic stage [37]. These observations suggest that sulfate assimilation is accelerated from lysed plant cells during necrotrophic growth and that *P. infestans* might take up S in alternative forms during the biotrophic phase of its growth.

Loss of inorganic sulfate assimilation is associated with obligate biotrophy

Many important oomycete and fungal pathogens have evolved to an **obligate** lifestyle. Genome analyses have revealed that obligate oomycetes and fungi have lost genes for sulfite reductase (SiR; Figure 2), which produces sulfide that is then incorporated into cysteine. For example, the SiR gene has been deleted from the genomes of several obligate oomycete downy mildew and white blister pathogens, as well as fungal rust and powdery mildew pathogens [38–40]. Loss of S assimilation has also been noted for bacterial, oomycete, and protozoan pathogens of animals and humans [41,42]. Importantly, deficiencies in sulfate assimilation have evolved independently in several different evolutionarily lineages; this convergent evolution indicates that the loss of sulfate assimilation confers a fitness benefit and that these pathogens can utilize organic S nutrients from their hosts. The obligate rust pathogen *Puccinia striiformis* f. sp. *tritici* expresses a transporter for S-methylmethionine strongly and specifically in haustoria, suggesting that

molecular patterns or less commonly to secreted effector proteins.
Rhizobia: bacteria that establish mutualistic symbioses with plant hosts, in which the bacteria fix atmospheric nitrogen, provide this to the plant, and receive carbon.
Saprophytic microbes: can grow on dying or dead plant material.
Symbiosomes: membrane-bound, organelle-like structures within nodules that house rhizobia and serve as the site for nitrogen fixation.
Transcription activator-like (TAL) effector proteins: secreted effector proteins from bacteria that act as molecular mimics of plant transcriptional activators. These modular proteins contain leader sequences that enable their secretion into plant cells, followed by modular DNA-binding domains and transcriptional activator domains that are sufficient to activate plant genes proximal to the binding site for the TAL effector [2].

Key figure

A holistic model of sulfur in plant–pathogen interactions

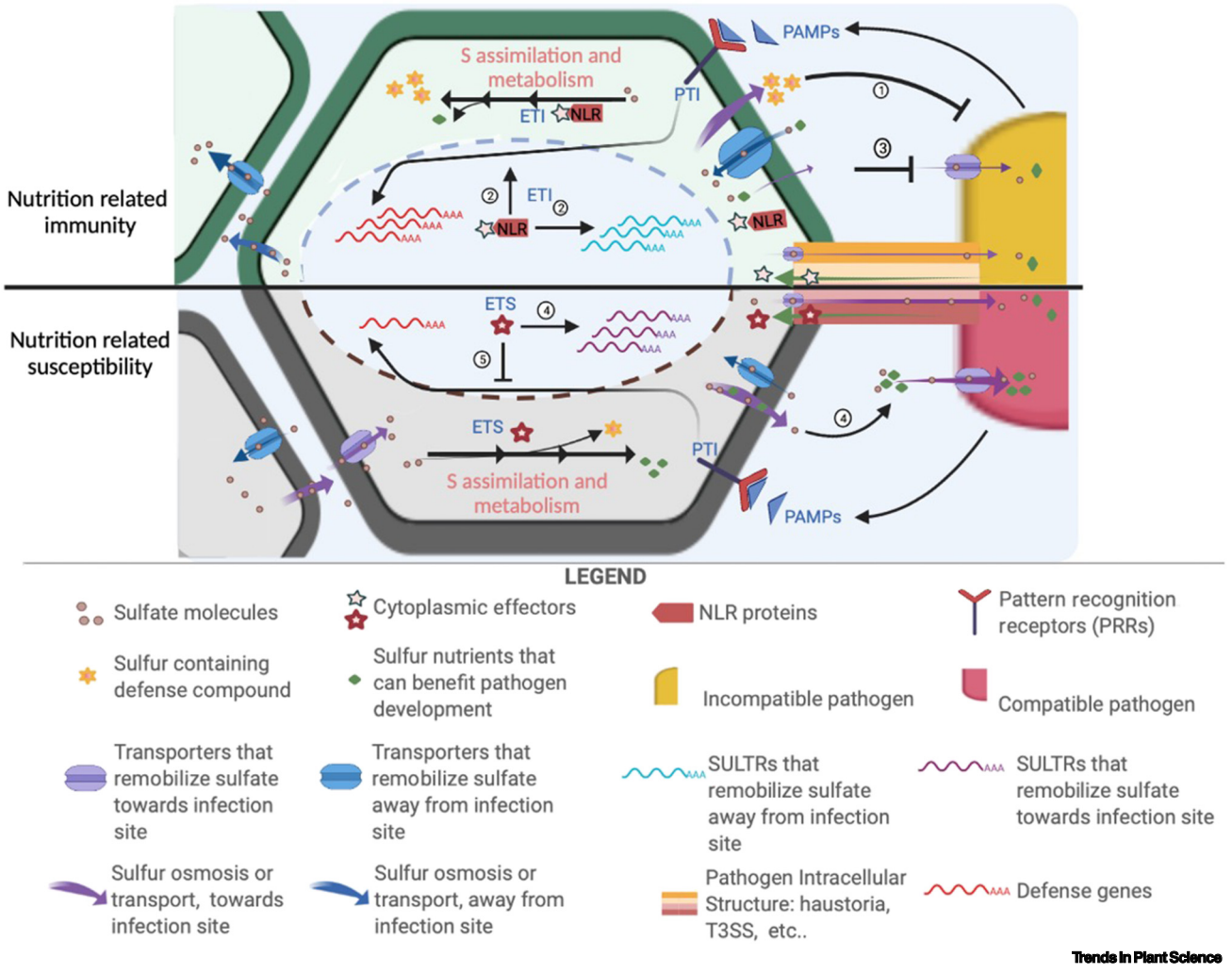

Figure 1. Plant immune responses are activated when plant cells detect microbe-associated molecular patterns or secrete pathogen virulence effector proteins and activate multicomponent immune responses (Box 2). Some sulfur compounds are integral components of the plant immune system, (1) acting as antioxidants, direct inhibitors of pathogen growth, or (2) inducing defense gene expression, which can include (3) genes that mediate nutritional restrictions to pathogens. Adapted pathogens can secrete effectors to (4) manipulate host sulfur transport to promote nutrient acquisition and metabolism and (5) inhibit immunity by disrupting immune signaling and defense gene expression. Abbreviations: ETI, effector-triggered immunity; ETS, effector-triggered susceptibility; NLR, Nod-like receptor; PAMP, pathogen-associated molecular pattern; S, sulfur; T3SS, type III secretion systems.

S-methylmethionine could be a S source [43,44]. However, no other clues exist to understand how obligate pathogens take S from the host during infection.

How do plants and pathogens compete for sulfur?

Recent evidence suggests that plant immunity triggers nutrient sequestration in plant tissues, aimed at interfering with pathogen nutrient acquisition. For example, the Arabidopsis *Sugar*

Box 1. There is no life without sulfur

S is an essential element for all organisms due to its biochemical versatility. S resides directly below oxygen in the periodic table, but S can maintain a much wider range of oxidation states owing to its electronic configuration with 3p electrons and empty 3d orbitals [114]. Thus, S stably shares electrons and presents reducing power [115]. The reducing power of S was a key component during the evolution of life in anaerobic environments, and the evolution of S-containing antioxidant compounds has been proposed as a key innovation enabling the expansion of life during the great oxidation event around 2 billion years ago. For example, S atoms comprise a key component of antioxidant compounds that are essential for detoxification of reactive oxygen species that are generated from electron transport chains (e.g., through oxidative phosphorylation). S is present in the proteogenic amino acids cysteine (Cys) and methionine (Met), and is found in vitamins, prosthetic groups, and secondary metabolites that collectively impact every aspect of growth, development, and responses to the environment [116–118]. Figure 2 in main text depicts aspects of S uptake and metabolism that are common or distinct between plants and their pathogens, while Figure 3A in main text displays the current state of understanding about the different roles of sulfate transporters in plants, based primarily on studies in the reference species *Arabidopsis thaliana*.

Transporter 13 (*STP13*) gene is activated during bacterial infection to translocate monosaccharides from the apoplast to intercellular stores, thereby reducing the food supply for bacteria [45]. This activity is regulated through phosphorylation of the transporter by a **pattern recognition protein (PRR)** complex that recognizes bacterial pathogen-associated molecular patterns (PAMPs). This regulatory relationship indicates that nutrient restriction is one of the responses triggered by the plant immune system to restrict pathogen growth. Conversely, pathogens can manipulate plant sugar transport to shift the balance in their favor. This is exemplified by bacterial virulence proteins that ectopically induce plant sugar transporters, discussed in more detail later.

This 'tug-of-war' for nutrients might also extend to S (Figure 1). Lovelace and colleagues [46] used transcriptomics for insight into the physiological stresses that pattern-triggered immunity (PTI) imposes on bacterial pathogens. Their approach was to examine the transcriptome of the bacterial pathogen *Pseudomonas syringae* (*Pst*) inoculated into Arabidopsis plants in which PTI had been activated by prior treatment with the PAMP flg22, a 22 amino acid subunit of flagellin. They compared the transcriptome of PTI-stressed bacteria with 'naive' bacteria grown in plants that were not pretreated with flg22. The most striking difference in this comparison was for genes associated with S transport and metabolism: 16 bacterial S importers including importers of alkanesulfonate, taurine, and sulfate were induced in PTI-stressed bacteria. Moreover, bacterial catabolism genes for sulfonates and taurine were induced in response to PTI. Because these are S starvation-responsive genes, the authors hypothesized that PTI limits S resources and thereby triggers S starvation [10]. The absence of this response in naive bacteria suggests that the pathogen can somehow counteract or interfere with the mechanism through which S starvation is imposed. An alternative hypothesis is that S starvation phenotypes observed could result

Box 2. The plant immune system

Plants have evolved a sophisticated immune system that can detect a wide variety of pathogen-associated signals of invasion with a relatively limited set of immune surveillance proteins. The foundation of this system rests on detection of **microbe-associated molecular patterns (MAMPs)** that are often broadly conserved among divergent taxa. The best characterized include motifs such as bacterial flagellin or fungal chitin that are perceived in the apoplast by plant pattern recognition receptors. These PRRs have extracellular domains that bind directly to corresponding MAMPs, causing conformational changes that trigger signaling events to induce production of reactive oxygen intermediates, plant structural reinforcements, and antimicrobial compounds, among which S defense compounds can play key roles. Pathogens interfere with MAMP-triggered immunity (MTI) by secreting effector proteins that interact with plant immune system proteins to disable their functionality [**effector-triggered susceptibility (ETS)**]. The second major component of the plant immune system is based on immune surveillance proteins that recognize these effectors inside plant cells. Such proteins are named '**NLR**' because of canonical nucleotide-binding sites and leucine-rich repeats. NLR proteins can bind effectors directly or, more commonly, can guard or mimic the plant proteins that effectors target. The resultant immune responses are similar to those induced by MAMP perception and can also include programmed cell death at the infection site (the hypersensitive response). Although often described as distinct immune responses, recently evidence suggests that MTI and **effector-triggered immunity** are closely linked and mutually reinforce each other.

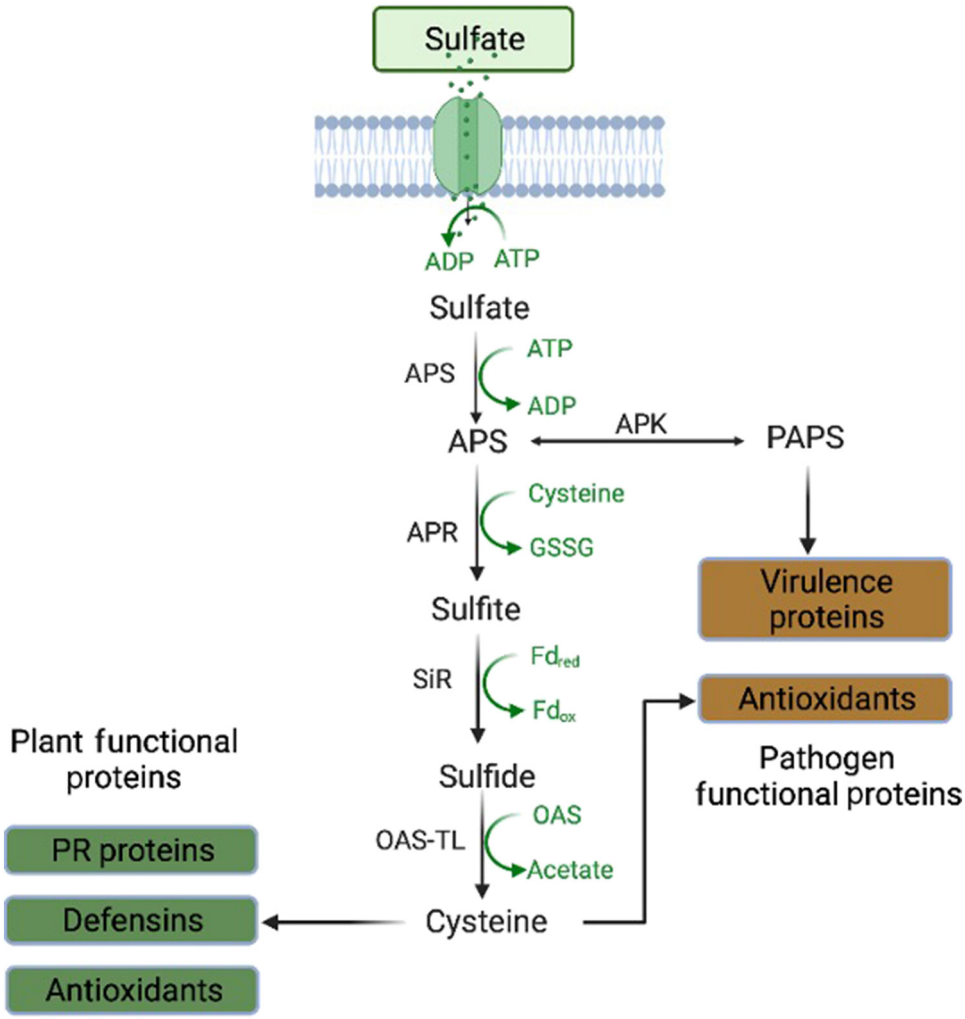

Figure 2. Core components of sulfur metabolism pathways in plant and pathogens. For plants, the primary source of sulfur is sulfate ions that are taken from the soil by sulfate transport proteins. Sulfate can also be transported through the vascular system and distributed to subcellular destinations by additional transporters (Figure 3A). Sulfate is transported by sulfur transporters in plants or permeases in pathogens with ATP as energy sources. Intracellular sulfate will be activated by the formation of APS driven by ATP hydrolysis. APS will undergo a series of reduction and produce cysteine which is the first sulfur-containing amino acid in the sulfur metabolism pathway. For plants, cysteine plays a central role as a precursor to various sulfur defense compounds and itself serves as a key regulator of immune signaling mediated by the defense hormone salicylic acid. Glutathione protects plants from oxidative damage and also regulates cellular redox status that impacts immune response signaling. Secreted, sulfur-rich proteins can directly or indirectly defend against pathogens through varied mechanisms, while sulfur-containing secondary metabolites (e.g., phytoalexins, glucosinolates in Brassicas) display potent antimicrobial activities. For pathogens, cysteine is important for antioxidants and PAPS provides sulfur groups for the sulfurylation of secreted proteins. Abbreviations: APK, APS kinase; APS, adenosine 5′-phosphosulfate; Fd_{ox}, ferridoxin, oxidized; Fd_{red}, ferridoxin, reduced; GSSG, glutathione disulfide; OAS, O-acetyl-L-serine; OAS-TL, O-acetylserine(thiol)lyase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PR, pathogenesis-related.

from damage to iron–S cofactors as a result of the PTI-associated oxidative burst. These two hypotheses are not mutually exclusive and additional research is necessary to precisely define the physiological cause of the S stress response in this interaction. Interestingly, regulation of *P. syringae* genes for iron–S clusters is differentially regulated when bacteria are grown in Arabidopsis mutants that affect immune signaling [47].

Additional evidence of S starvation in pathogens during infection was documented in Arabidopsis and lettuce for a bacterial pathogen of humans [48]. An example on the fungal side is provided by *Colletotrichum gloeosporioides* (*Cgm*) on round-leaved mallow [49]. Colletotrichum gloeosporioides arylsulfatase (cgars) is an enzyme responsible for degrading S-containing compounds to supplement sulfate availability under a sulfate starvation environment. Arylsulfatase is repressed under S-replete conditions and induced by S deficiency so that cgars is widely utilized as a reporter for S stress. In *Cgm*, cgars was expressed at a high level in the biotrophic stage but decreased during the necrotrophic stage of infectious growth *in planta* [49]. This indicates that during the early stage of infection, the pathogen was under S starvation stress that was overcome gradually as the pathogen accessed S more efficiently during necrotrophy.

The mechanism through which plants restrict S at the infection site remains to be determined but, by analogy to the STP13 case described earlier, could involve sequestration of plant S compounds by plant transporters. One candidate gene for such a role is tomato sulfate transporter 2 (ST2), encoding a high-affinity transporter that takes sulfate from the soil and is induced by sulfate starvation. ST2 is also induced by flagella but repressed by **effectors** during infection by *Pst* [46,50]. The fungal pathogen *Verticillium dahliae* also induces ST2 and another high-affinity transporter LeST1-2 in tomatoes [51,52]. This expression pattern indicates that induction of the sulfate assimilation pathway is involved in PTI responses and *Pst* can counteract this mechanism. However, it is unknown whether ST2-mediated transport is important for immunity. Data from *st2* mutants are critically important to address this question. Additional follow-up questions include what pathways PTI utilizes to regulate ST2; does *Pst* induce equivalent transporters in other plants, including Arabidopsis AtSULTRs; what other components from the S metabolism pathways besides transporters are involved in PTI; and why and how is ST2 expression suppressed by *Pst*. Initial clues to such questions can be found in publicly available transcriptome data from Arabidopsis–pathogen interactions (Figure 3) and can be tested by the facile reverse genetics in Arabidopsis. Interestingly, S deprivation triggers activation of defenses in Arabidopsis, suggesting that the plant might interpret S deprivation as a signal of pathogen infection [53].

On the other side of the tug-of-war, host nutrient metabolism networks can be targeted by pathogen effector proteins, perhaps to counteract the plants' attempts at nutrient restriction (Figure 1). The best-characterized examples of the virulence strategies are from **transcription activator-like (TAL) effector proteins** from bacteria that activate plant genes encoding transmembrane transporters of plant-synthesized sugars. Such upregulation results in the translocation of sugars from plant cell cytoplasm to the apoplasm, where it is available for uptake by bacteria that inhabit the apoplast [54].

Recently, this virulence strategy was extended to a rice sulfate transporter, called *OsSULTR3;6* [55]. This gene is ectopically activated by the Tal2g effector from the bacterial rice streak pathogen *Xanthomonas oryzae*. Deletion of Tal2g led to a major loss in bacterial virulence that could be rescued by a synthetic TAL effector that specifically targets *OsSULTR3;6*. These results suggested that the pathogen's capacity to manipulate *OsSULTR3;6* is a major factor in its ability to cause disease. In a subsequent study, targeted mutation of *OsSULTR3;6* was shown to significantly reduce bacterial growth and disease symptoms, demonstrating the potential utility of editing sulfate transporter genes for disease resistance [56].

These findings should inspire other studies to investigate whether other pathogens can deploy effectors to manipulate S transport or metabolism. The ortholog of OsSULTR3;6 in *Populus trichocarpa* (PtSultr3;5) is induced during interactions with the fungal rust pathogen *Melampsora*

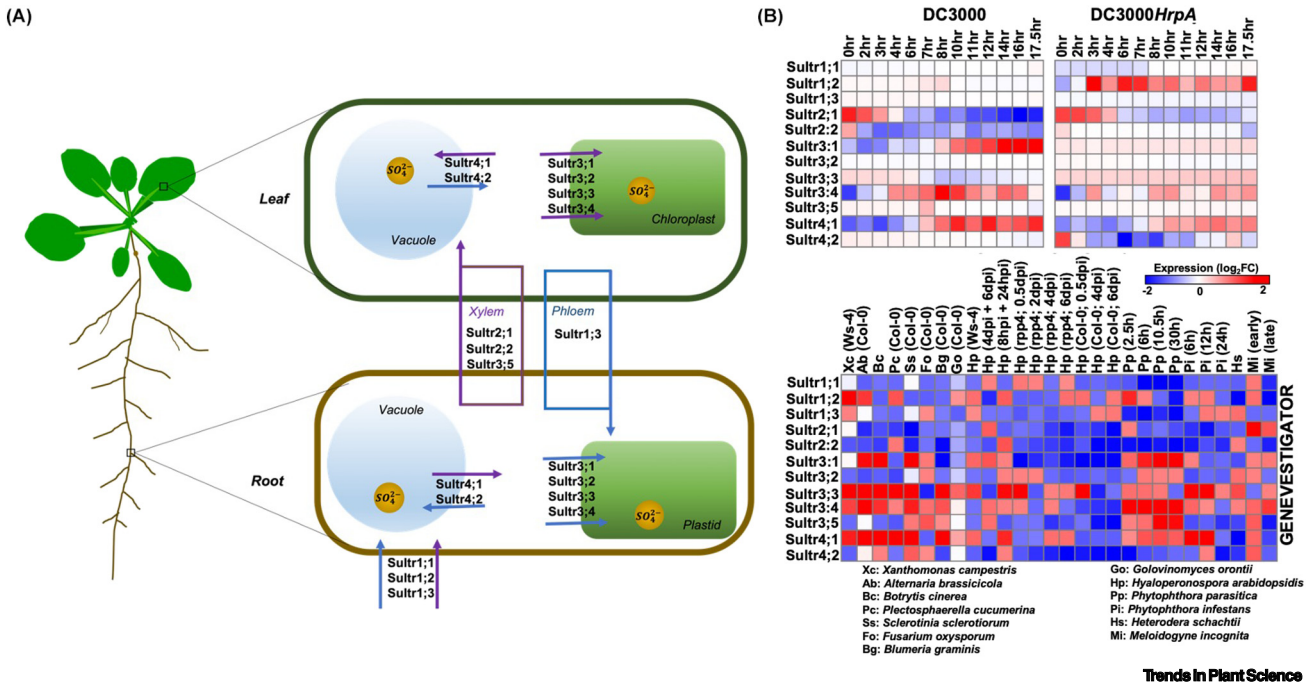

Figure 3. Functions and pathogen-induced expression of Arabidopsis sulfur transporter genes (SULTRs). (A) Arabidopsis SULTR subcellular localization and function. Sulfate is initially taken up from the environment by root SULTRs: SULTR1:1, SULTR1:2, and SULTR1:3. SULTR1:3 is also expressed in phloem cell to load sulfate into phloem that is transported from the leaves to the roots. SULTR2:1, SULTR2:2, and SULTR3:5 are responsible for loading sulfate into xylem that is subsequently transported into the leaves. SULTR3:1, SULTR3:2, SULTR3:3, and SULTR3:4 primarily transport sulfate across the chloroplast envelope into the leaves but are also expressed in root plastids to support sulfur metabolism in the root. SULTR4;1 and SULTR4;2 are localized on the tonoplast membrane to store extra sulfate into the vacuole or to unload sulfate from vacuole to support local or long-distance sulfate needs [110,111]. (B) Differential expression of Arabidopsis SULTR genes during infection by diverse pathogens. Expression patterns of Arabidopsis SULTR genes during infection by various bacteria, fungi, oomycetes, or nematodes. The heatmaps depict relative expression (log_2-fold change) compared with uninfected controls. The heatmap on the top was extracted from a published data set [112] of Arabidopsis treated with virulent *Pseudomonas syringae* DC3000 or the nonpathogenic mutant HrpA that is incapable of secreting effector proteins. The heatmap on the bottom was extracted from public data sets curated in GENEVESTIGATOR [113] and comprises Arabidopsis treated with diverse pathogens as indicated in legends.

larici-populina [55,57]. PtSultr3;5 is strongly induced during compatible and incompatible interactions, leading the authors to hypothesize that induction is due to manipulation by the fungus. Similarly, a PtSultr3;5 ortholog in grapevine is strongly induced by the oomycete pathogen *Plasmopara viticola* [58]. As noted earlier, plant S transport gene may also play an important role in mutualistic interactions: PtSultr3;5 is induced during the interaction between poplar and the mutualist fungus *Laccaria bicolor*, and the ortholog of PtSultr3;5 is the SST1 symbiosome S transporter that was described earlier [12]. It will be of great interest to investigate whether these diverse detrimental and beneficial microbes utilize effectors to induce *SULTR3.5* genes in their respective hosts, and to understand how this induction benefits the microbes.

Another potential target for effector-mediated manipulation of host S is the hub protein, low S up-regulated (LSU) [59]. LSU proteins serve as scaffolds in multiprotein regulatory hubs that mediate plant responses to various environmental challenges such as S deficiency and plant pathogens [59,60]. Arabidopsis ATP sulfurylase (Figure 1) is a direct interactor with LSU proteins *in planta* and other S metabolism proteins are in the LSU protein interaction network. Moreover, the LSU proteins are targeted by effectors from a diverse range of pathogens including bacteria, fungi, and oomycetes [61,62]. However, it remains to be clarified whether these effectors target the LSU hub to manipulate S metabolism, plant immunity, or both.

Multiomics approaches to decipher sulfur-related defense or susceptibility in plants

Recent technological advances in systems biology provide new insights into the intricate relationships between plants and their pathogens, which is mostly facilitated by the innovation in diverse '-omics' [63,64]. However, leveraging such an integrative multiomics framework to unravel the interplay of S metabolism-related pathways during different stages of host–pathogen interactions remains an uncharted territory. The first line of evidence linking S with the plant immune system comes from the pioneering transcriptome-wide studies in conjunction with S deprivation that identified numerous biotic stress-responsive genes including *pathogenesis-related gene* 1 (PR1) and SULTR [65–70]. In addition, O-acetyl-L-serine-clustered genes implicate the underlying association between sulfate metabolism and hydrogen peroxide, a primary reactive oxygen species source in plant–pathogen interactions [67]. The central question is how these suites of defense-responsive genes are regulated? Remarkably, a study identified 21 S-responsive transcription factors (TFs) that are implicated in plant–pathogen interactions [71–74]. Whether these TFs can regulate the diverse responses of SULTR genes to different pathogens (Figure 3B) is another important question for future study. Besides, single-cell transcriptional profiling of Arabidopsis roots revealed that a fine-tuned cell–cell communication for stress response contributed to root-patterning regulations under nutrient deficiency involving S, suggesting that more precise transcriptomic investigations based on cell identities are required [75]. Further, the co-transcriptomics landscape will allow us to reveal the novel regulatory genes required for plants and pathogens in S-mediated nutritional competition [46,76,77]. Taken together, comprehensive system-level investigations are necessitated to decipher the canonical regulatory networks of S in plant–pathogen tug-of-war.

The discovery of S-responsive genes, which are implicated in the phytohormone signaling network, highlights the existence of crosstalks between S and plant hormonal pathways. Indeed, phytohormone–S nexus was characterized by transcriptional signatures of salicylic acid, jasmonate, auxin, and flavonoids under S deprivation conditions [70,78]. Considering the indispensable functions of phytohormones in plant defense against a wide spectrum of pathogens [79–82], addressing how pathogens interfere with plant S metabolism via rewiring phytohormone pathways should be the next focal point of the research. Such an experimental design may also benefit if explored in conjunction with other micro- and macronutrients including iron, zinc, and sugar owing to the essential roles of these trace elements and glucose in response to S availability and biotic stress [78,83–86]. Apart from the crosstalks among biochemical pathways, the specific cross-regulation between S and essential elements, involving nitrogen, potassium, and phosphorus, is also reported [66,87,88], indicating a high degree of commonality in response to multiple nutrient deficiencies. Alongside determining the crosstalk of S-responsive genes in other nutrients and metabolic pathways, the emerging challenge is to investigate how this cross-regulation changes once plants are imposed by biotic stress, particularly during pathogen infection.

Despite the accumulation of S-related transcriptomics, the available data with respect to other '-omics' remain fragmented. By far, a limited number of *cis*-elements and *trans*-elements have been found located in promoter regions of S-responsive genes [89–91], necessitating genome-wide studies combining experimental and *in silico* analyses to identify S-responsive promoters. In addition to regulatory elements, the current status of global miRNA (miR) studies is only confined to one particular miR, miR395 [92–95]. In this wide-open area of research, revealing the roles of additional miRs in S-mediated nutritional immunity will fill another gap in knowledge in plant immune systems. Moving forward with the discovery of additional species of small RNAs, including siRNA, piwi-interacting RNA (piRNA), and tRNA-derived small RNAs (tsRNAs) as well as long noncoding RNAs (lncRNAs), the interplay of such small and long

noncoding and coding RNAs in conjunction with TFs will provide a comprehensive insight into the intricate regulatory mechanisms of S's involvement in plant defense [96–100].

Compared with transcriptomics, fewer metabolomics analyses have been accomplished under S deprivation [66,68,78]. This led to the discovery of S secondary metabolites such as Cys and Ser, which have been implicated in plant–pathogen interactions previously [101,102]. The recent advances, opportunities, and challenges pertinent to S-containing metabolites (S metabolites) are previously discussed in a recent review article [103]. For instance, the hyphenated techniques combining liquid chromatography and Fourier transform ion cyclotron resonance and mass spectrometry and the targeted metabolomic analysis of S metabolites (S-omics) contribute to systemically and universally profile S-containing compounds in a wide range of plant species based on the discrepancies among ^{32}S and ^{34}S isotopes in ultrahigh-resolution metabolome data [103]. Moreover, this approach was applied to a wide range of plants and preferably those tough to be labeled by stable isotopes. Nevertheless, the restricted extraction confined only to the S monoisotopic ions with certain abundance turns out to be a current issue [103]. Similarly, a series of techniques following S-containing compound profiling were aimed to conduct the structure and spatial distribution analysis of known and unknown compounds. While the automatic annotation based on metabolite database [104] functions as the ideal high-throughput way of chemical assignment of known metabolites, a sophisticated workflow integrating structural analysis techniques, such as nuclear magnetic resonance [105] and imaging mass spectrometry [106], with isolation of metabolites is necessitated to achieve the efficient and fast elucidation of S metabolites. Efficient usage of S metabolomics in association with genomics and transcriptomics will enable researchers to exploit the gene–metabolite relationships [107] as well as advance synthetic biology using newfound S-containing compounds [108].

Taken together, an integrative multiomics approach including co-transcriptomics, epitranscriptomics, epigenomics, proteomics, metabolomics, ionomics, and large-scale image-based phenomics will allow a comprehensive understanding of S-mediated plant–pathogen interactions, which will provide deep insights into the crosstalk of micronutrients and plant immune systems.

Concluding remarks

The roles of S in plant immunity are broadly appreciated and increasingly well understood. However, many important aspects of S metabolism and transport during successful pathogen colonization, as well as competition for S during infection, remain understudied and are crucial for a complete understanding of plant–pathogen interactions (see Outstanding questions). New avenues for disease control may also be forthcoming from this foundational understanding. We hope to have sparked interest in these topics and look forward to an explosion of new data in the near future.

Outstanding questions

Which host-derived S sources (e.g., S defense compounds [109]) can be utilized by pathogens and how are these sources taken up and metabolized during infection?

How do obligate pathogens compensate for their deficiencies in metabolizing inorganic S?

Which S metabolism pathways in host plants are important for supporting pathogen virulence, and how do pathogens manipulate these pathways? Are effectors involved?

How do plants restrict S to pathogens during infection, how do restriction mechanisms differ for apoplastic and haustorial pathogens, and how are S restriction mechanisms balanced with the imperative for S metabolism into S-containing defense compounds?

How are SULTR genes transcriptionally regulated during the S tug-of-war between plant and pathogens? Are the relevant transcription factors manipulated by pathogens?

To what extent do pathogens manipulate plant S metabolism to dampen defense responses, such as protection against reactive oxygen species, altering phytohormone production (e.g., abscisic acid synthesis), or to changing composition and distribution of other elemental nutrients (phosphorus, copper, zinc, manganese)?

How can we utilize answers to these questions to promote accommodation of beneficial microbes and exclude detrimental organisms?

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Declaration of interests

The authors have no interests to declare.

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