Review

A Revised View of the *LSU* Gene Family: New Functions in Plant Stress Responses and Phytohormone Signaling

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Abstract: *LSUs (RESPONSE TO LOW SULFUR)* are plant-specific proteins of unknown function that were initially identified during transcriptomic studies of the sulfur deficiency response in Arabidopsis. Recent functional studies have shown that LSUs are important hubs of protein interaction networks with potential roles in plant stress responses. In particular, LSU proteins have been reported to interact with members of the brassinosteroid, jasmonate signaling, and ethylene biosynthetic pathways, suggesting that LSUs may be involved in response to plant stress through modulation of phytohormones. Furthermore, *in silico* analysis of the promoter regions of *LSU* genes in Arabidopsis has revealed the presence of cis-regulatory elements that are potentially responsive to phytohormones such as ABA, auxin, and jasmonic acid, suggesting crosstalk between LSU proteins and phytohormones. In this review, we summarize current knowledge about the *LSU* gene family in plants and its potential role in phytohormone responses.

Keywords: sulfate deficiency; LSU; response to low sulfur; abiotic stress; sulfur nutrition; ethylene; auxin; phytohormones; transcription factors

1. Introduction

Sulfur (S) is an essential macronutrient required for plant growth since it is a constituent of relevant biomolecules such as the amino acids methionine and cysteine, the antioxidant glutathione, coenzymes and prosthetic groups [1]. Therefore, plants cannot adequately complete their life cycle when subjected to an S-deficiency condition [2]. The symptoms mainly appear in the young parts of the plant and are characterized by reduced height, chlorosis of the leaves, and accumulation of anthocyanins [1]; [3]. Unlike other nutritional deficiencies, S deficiency typically results in reduced shoot growth compared to root growth [3].

At the molecular level, the response to S-deficiency can be divided into two main stages based on the duration and severity of the deficiency [3]. In the initial stage, plants alter the expression of primary genes involved in S-assimilation and uptake from the soil, and mobilize stored inorganic S from the vacuole [3]; [4]. However, if S remains a limiting factor, plants intensify organic S fluxes and activate stress defense responses, followed by the down-regulation of genes responsible for nitrogen uptake and assimilation [5].

The advent of transcriptomics studies has allowed considerable progress in the identification of S-responsive genes, mainly in the model plant *Arabidopsis thaliana* [6]. An integrative meta-analysis of transcriptomic data from five different S experiments in public

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databases uncovered a robust set of genes whose expression depends only on the availability of S in Arabidopsis [7]. Interestingly, the biological function of approximately 45% of these robust S-responsive genes is currently unknown. A small gene family, "*RESPONSE TO LOW SULFUR*" (*LSU*), belongs to this group of consistently S-responsive genes, suggesting that these genes could be an essential component of this nutritional response [7].

Several studies have shown that LSUs are important hubs of protein interaction networks with potential function in the plant stress response [8]; [9]; [10]. Phytohormones play a critical role in helping plants adapt to adverse environmental conditions, including abiotic and biotic stresses [11]. Interestingly, it has been reported that LSU proteins interact with members of brassinosteroid signaling [12], jasmonate signaling [8], and the ethylene biosynthetic pathway [13], suggesting that LSUs could be involved in the response to plant stress by modulating phytohormones. Furthermore, *in silico* analysis of the promoter regions of *LSU* genes in Arabidopsis showed that they have cis regulatory elements which are potentially responsive to phytohormones such as ABA, auxin, and jasmonic acid [14]. This evidence suggests a possible crosstalk between LSU proteins and phytohormones.

The molecular functions of LSU proteins are currently unknown, but in recent years, several studies have shed light on their putative functions and evolution. In this review, we summarize the current knowledge about the *LSU* gene family in plants and their potential role in phytohormone responses.

2. General features and evolutionary history of the *LSU* gene family

2.1. The discovery of the LSU gene family

LSU genes were first described in the context of the S-deficiency response in *Arabidopsis thaliana* by Maruyama-Nakashita [15]. This study identified *LSU1* and *LSU2* as two of 15 S-responsive genes that were significantly upregulated at multiple time points after plants were transferred to S-free medium [15]. Specifically, *LSU1* was significantly induced 4, 8, 12, and 24 h after S-deficiency, while *LSU2* was upregulated 8, 12, and 24 h, indicating that the response of *LSU* genes to this nutritional deficiency is maintained during the first 24 hours in Arabidopsis roots [15]. In the same year, members of this gene family were also identified in tobacco plants in the context of S-deficiency using the suppression subtractive hybridization approach [16], suggesting that the response of *LSU* genes to this nutritional deficiency may be conserved in plants.

More recently, S-deficiency has been reported to also induces the expression of the *LSU3 and LSU4* genes in Arabidopsis roots and leaves [17]. However, the degree of induction was not the same among members of this family: the induction of *LSU4* by S-deficiency is lower than *LSU1/2/3 LSU1/2/3* in both organs [17]. Furthermore, this study also showed that the mRNA levels of all tomato *LSU* genes and three wheat *LSU* genes increased by S-deficiency [17], supporting the idea that this gene family is associated with S-deficiency and the response to this nutritional deficiency could be conserved in angiosperm plants.

Analysis of the Arabidopsis genome revealed four members of the *LSU* family (*LSU1–4*), distributed in two chromosomes [14]: *LSU1* and *LSU3* are located on chromosome 5, and *LSU2* and *LSU4* are on chromosome 3. These two pairs of *LSU* genes are separated by a small distance of about 2 Kb [14]. In addition, *LSU* genes are characterized by their small size (approximately 300 bp of coding sequence) and the absence of introns [14].

2.2. Evolution of LSU gene family

The evolutionary history of the LSU gene family has recently been analyzed using genomic information from 134 plant species that include representatives of the major phylogenetic groups of the *Viridiplantae* clade [17]. The first notable finding of this study was that the *LSU* family probably originated from the common ancestor of seed plants [17]. As shown in Figure 1, no homologous *LSU* sequences were found in the genomes of ancient vascular plants such as *Selaginella moellendorffii*, non-vascular plants, or microalgae. This

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result contrasts with the evolutionary history of other genes involved in the S-deficiency response, such as sulfate transporters, or genes encoding enzymes of S assimilation, such as ATP sulfurylase (APS) or APS reductase (APR), which are present in all Viridiplantae from microalgae to angiosperms (Figure 1) [17]. Furthermore, the family of the central transcriptional regulator of plant S-response, *ETHYLENE-INSENSITIVE3-LIKE3* (*EIL3*), is present in all analyzed land plant genomes (Figure 1), indicating that the evolutionary appearance of *LSU* family is recent compared to other genes involved in the S-deficiency response [17]. In addition, several experimental-verified interactors of *LSU* genes in Arabidopsis, such as *APS1*, *GAPC1*, *RAF2*, *FSD2*, and RAP1, are also present in all analyzed *Viridiplantae* genomes [17].

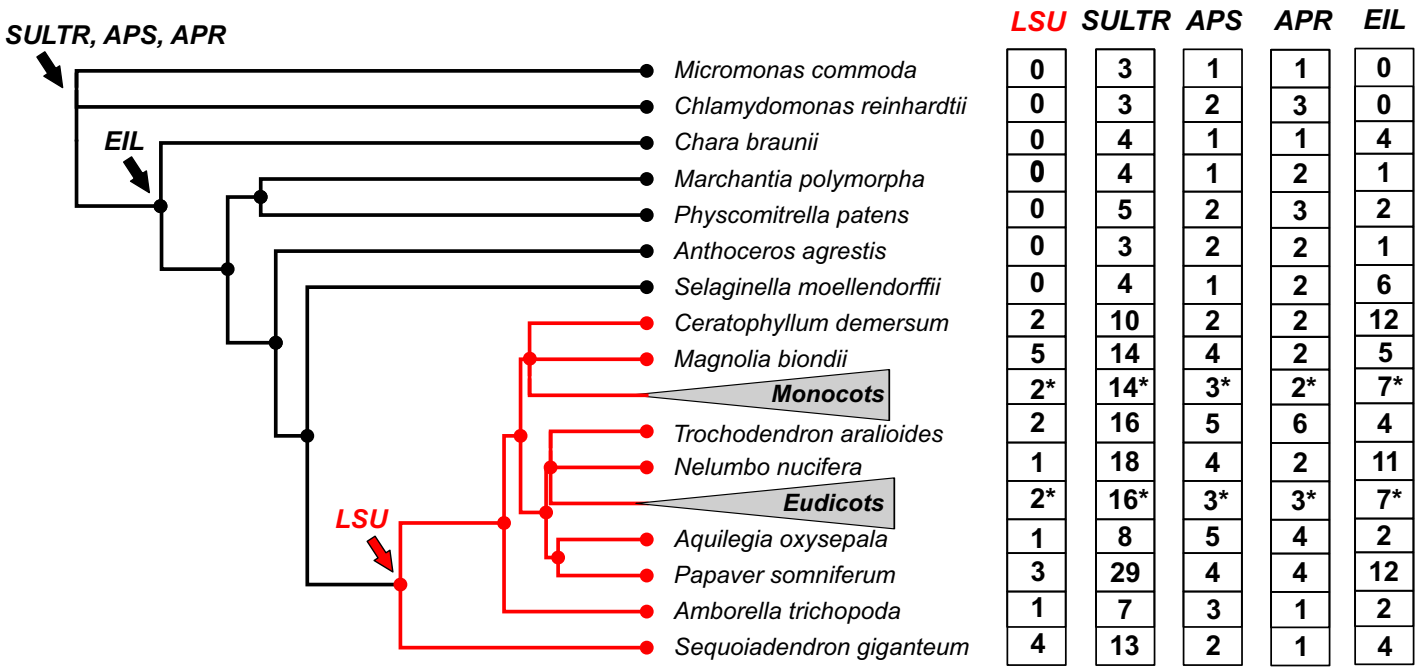

Figure 1. The *LSU* gene family appeared recently in plant evolution compared to other S-responsive genes. The phylogenetic tree was constructed according to [17]. To improve visualization, 73 eudicotyledon and 31 monocotyledon species collapsed in the phylogenetic tree (triangle), and the average number of *LSU* genes are indicated with an asterisk. The copy number of *LSUs*, sulfate transporters (*SULTR*), ATP sulfurylases (*APS*), *adenosine 5′-phosphosulfate reductase* (APR), and *ethylene-insensitive3-like* transcription factors (*EIL*) was obtained from the PLAZA 5.0 database [18].

The number of *LSU* genes varies between angiosperm plants, ranging from 1 to 9 members [17]. This variation is mainly due to genome size, as a significant positive correlation has been found between the number of *LSU* genes and the size of genome [17]. Furthermore, the analysis of the distribution of normalized *LSU* gene numbers in monocotyledon and eudicotyledons revealed no significant differences between these clades [17], suggesting that the *LSU* family does not expand during the evolution of angiosperm plants. Unlike the LSU copy number, the evolutionary distance between *LSU* genes of the same species in monocotyledons is more significant than in eudicotyledons, indicating a potential functional divergence of *LSU* genes within monocotyledon species such as wheat [17].

Phylogenetic analysis revealed that *LSU* genes could be divided into three main phylogenetic groups: Group A, which includes most of the monocotyledon species; Group B, including most of the malvid species; Group C, including most of the rosid species [17]. Protein sequence analysis based on 270 LSU sequences showed that the central region of LSU proteins has two highly conserved domains and also revealed the presence of three additional motifs that further support the classification by phylogenetic analyses [17]. The significance of conserved and group-specific motifs in LSU proteins is currently un-

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known, and further research should be undertaken to reveal the molecular function of these domains [17].

3. Functional analyses of the *LSU* family

3.1. Subcellular localization of LSU proteins

Biochemical fractionation has shown that LSU1 and LSU2 proteins localize in multiple cell compartments, including nuclear, cytosolic, and microsomal fractions [19], while LSU dimers are most probably located in the cytosol [12]. Data from the SUBA4 database [20] support a mainly nuclear and cytoplasmic localization for LSU1, and nuclear, cytoplasmic, chloroplastic, and mitochondrial localization for LSU2 and LSU3 (data for LSU4 is not available) (from Cell eFP viewer, ePlant, [21]. In tobacco, UP9C has a reported nuclear and cytosolic localization, and, generally, a putative nuclear localization signal has been found in this protein [14]. Although no nuclear localization signal has been found in Arabidopsis LSUs, their small size probably allows them to readily cross the nuclear pore [14].

3.2. Different members of the LSU gene family showed tissue-specific expression

Analysis of *LSU* tissue expression has been limited to *LSU1* and *LSU2*, showing that these proteins present specific tissue expressions consistent with a specialized role. For example, *LSU1* is diffusely expressed in roots and strongly expressed in guard cells, indicating a role in stomata function, while *LSU2* is ubiquitously expressed in leaves and roots [19]. Additionally, we performed a correlation analysis of *LSU* expression data across 69 samples of the Arabidopsis developmental atlas included in the eFP browser [21]; [22]. *LSU1*, *LSU2*, and *LSU3* showed a high and significant positive correlation (p-value<0.01, Figure 2A), indicating that they have similar expression patterns in the developmental atlas. In contrast, no significant correlation was found between *LSU4* and *LSU1/LSU2/LSU3*, indicating that Arabidopsis *LSU* genes are grouped into two clusters according to developmental and tissue-specific expression (Figure 2A).

In Figure 2B, we compared the expression patterns of *LSU2* (as a representative gene with a higher average expression of the *LSU1/LSU2/LSU3* cluster) and *LSU4* to illustrate the two different groups of Arabidopsis *LSU*s. In the case of *LSU2*, this gene is mainly expressed in leaf petiole, leaf vein and pod of the senescent silique 1 (Figure 2B). In contrast, the maximum expression of *LSU4* is detected in floral tissues (Figure 2C), supporting the contrasting correlation values between *LSU4* and other LSUs obtained in Figure 2A. We then asked whether the existence of two contrasting groups of *LSU* expression also occurs in other plant species. To this end, we performed the same analysis with wheat *LSU* genes [17] as an example of a monocotyledon plant. We also found two contrasting groups of *LSU* genes in wheat according to their expression patterns throughout development (71 samples; Figure 2A and Figure 2C), suggesting a possible functional divergence between members of this family in plants.

3.3. Functional analyses of LSUs in Arabidopsis

Insights into the role of individual LSU proteins in Arabidopsis have been obtained by characterization of available T-DNA insertional lines, mainly for *LSU2* and *LSU4*. Currently, no T-DNA lines for *LSU1* are available, and although insertional lines for *LSU3* exist, no reports have been published to date. The involvement of *LSUs* with biotic stress responses was first suggested in analyses of the Arabidopsis protein-protein interactome, showing that *LSUs* represented hubs in immune response-related networks [9]. Analysis of *lsu2* mutants showed that this protein was necessary for normal immune plant response to the bacterium *Pseudomonas syringae* DC3000 (avrRpt2) and the fungi *Hyaloperonospora arabidopsidis*. *LSU2* was identified as a target of pathogen effector proteins and was proposed to act as part of a growth-suppression mechanism mediated by the *P.syringae* 2 (RPS2) NB-LRR protein [9]. Later work using *lsu2* mutants showed that stomatal closure in response to *P. syringae* DC3000 and the human pathogen *Salmonella enterica subsp. enterica serovar Typhimurium* strain 14028s was significantly reduced.

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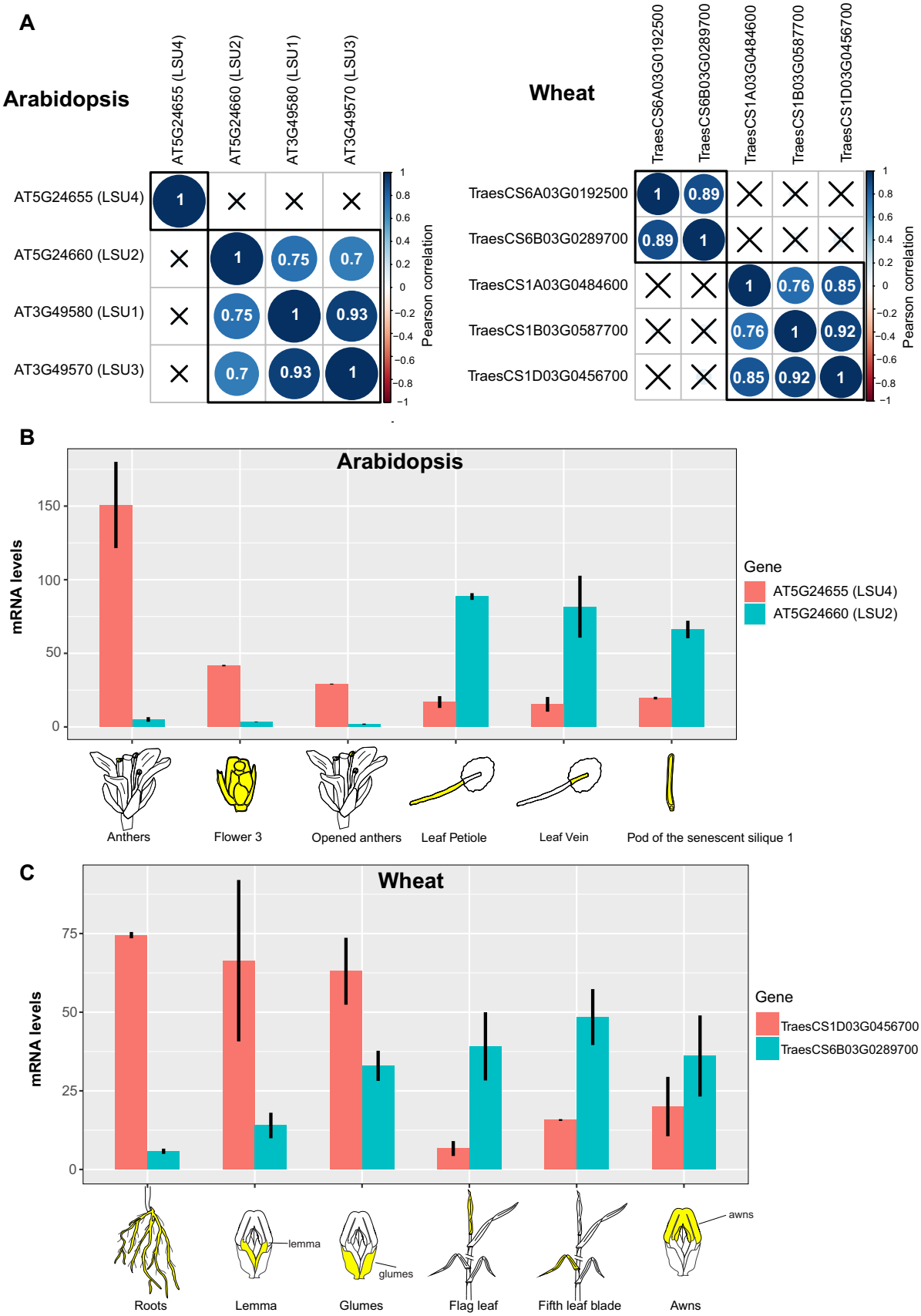

Figure 2. Analysis of the *LSU* developmental expression atlas in Arabidopsis and wheat suggests the existence of two LSU subgroups with contrasting expression profiles. A) Co-expression analyses of Arabidopsis (left panel) and wheat LSU family (right panel) performed with the r package *"corrplot"* [23] using all samples included in the developmental atlas of ePlant [21]. Only Pearson correlation values with a p-value<0.01 are shown in the correlation heatmaps. B) Expression profiles of Arabidopsis *LSU4* and *LSU2* in the three samples with the higher expression of each selected gene. C) Expression profiles of wheat *TraesCS1D03G0456700* and *TraesCS6B03G0289700* in the three samples with the higher expression of each selected gene.

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These results implicate *LSU2* as part of an important plant defense mechanism that occur in guard cells to prevent the entry of bacterial pathogens [24].

In addition to its role in the immune response to pathogens, *LSU2* works as an integrator of light and chloroplast signaling. *LSU2* is induced by light and lincomycin, a chloroplast biogenesis inhibitor [25]. *lsu2* mutants have more than 2-fold chlorophyll contents compared to wild-type plants when deetiolation is performed in a wide range of light fluences [25]. As such, *LSU2* (together with six other genes) was classified as an enhanced deetiolation (*end*) gene [25]. Consistent with the putative role of *LSU2* in integrating light and plastid signaling, *lsu2* mutants have a decreased expression of the photosynthesis-related genes *Lhcb1.4*, *RbcS1A*, *PsbS*, and *CHS* [25]. *LSU2* has also been shown to act as part of a common response module of genes involved in plastid performance and retrograde signaling [26]. These functions of *LSU2* are consistent with its subcellular localization in the chloroplast.

Regarding the *LSU4* function, *lsu4* mutants show a late flowering phenotype under short-day conditions, while flowers formed in the first flowering phase present aberrant developmental phenotypes and do not produce siliques [27]. This is accompanied by a decrease in the expression of critical flowering genes such as *LFY*, *AP1*, *AP3*, *PI*, and *SEP3* transcripts and an increase in the expression of *AP2*, *AG*, and *SEP2* [27]. Consistent with these phenotypes in the *lsu4* mutant, *LSU4* shows an induced expression during flowering and fruit formation [27]. The induction of *LSU4* is also evident during deficiencies in different nutrients (phosphorous, nitrogen, potassium, iron), indicating a possible role of *LSU4* as a coordinator of nutrient demand and flowering [27].

Given that no individual T-DNA lines exist for all *LSU* genes and to uncover phenotypes that can be masked by potential functional redundancy, Arabidopsis knock-down lines have been generated using artificial microRNAs (amiRNAs) targeting all *LSU* members (>80% reduction in *LSU1*, *LSU2* and *LSU3* and 50% for *LSU4*) [19]. These lines present no obvious phenotypes when grown in standard soil or in vitro conditions [19]. However, closing of abaxial stomata in response to S-deficiency was impaired in the knock-down lines, leading to increased water loss and indicating a role for *LSUs* in this response [19]. This phenotype is consistent with the expression of *LSU1* in guard cells [19] and the reported role of *LSU2* in stomata closure [24].

Furthermore, H_2O_2 production in guard cell chloroplasts of knock-down lines was reduced compared to wild-type plants in response to S deficiency and other stresses such as high salt and Cu [19]. Consistent with this observation, the iron-dependent superoxide dismutase 2 (FSD2) was shown to physically interact with LSU1 and LSU2 in vitro and in vivo, and this interaction was shown to increase the enzymatic activity of FSD2; thus, the production of H_2O_2 from O_2^- [19]. Interestingly, the LSU1-FSD2 interaction is targeted and interfered by different virulence effectors, revealing a mechanism used by bacteria to abrogate Pathogen-Associated Molecular Pattern-triggered immunity [19]. As expected, the amiRNA lines are more susceptible to pathogen attack, and conversely, *LSU1* overexpressor lines present an enhanced disease resistance phenotype under standard conditions, as well as under conditions of abiotic stress [19]. Interestingly, *LSUs* have also been linked to the function of beneficial bacteria such as *Enterobacter* sp. SA187, an endophytic bacterium that protects plants from abiotic stresses. Plant colonization with SA187 can completely suppress the increased ROS levels and alleviate growth suppression in *LSU* knock-down plants subjected to high salt stress [28].

3.4. Functional analyses of LSUs in tobacco plants

Similarly to Arabidopsis, UP9 proteins, the *LSU* homologs in tobacco, play an important role in S-deficiency responses [14]. Knock-down of UP9 proteins using a *UP9C* antisense line alters glutathione levels in roots and mature tobacco leaves, especially under S-deficiency conditions [29]. The effect of *UP9* downregulation is organ-dependent, with mature leaves of *UP9* transgenic plants having higher levels of total S and glutathione (GSH) than wild-type plants in S-deficiency, similar to plants grown in S sufficiency [29]. In the

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case of roots, total S and glutathione are more affected, presenting significantly decreased levels in the *UP9* transgenics in both S conditions [29]. This resulted in knock-down plants presenting shorter roots, while shoot growth was unaffected. At the transcript expression level, *UP9* knock-down resulted in altered levels of S-related enzymes and transporters, as well as genes related to ethylene, jasmonic acid, and polyamines [29]. *UP9* knock-down plants also present altered metabolite profiles under S-deficiency, suggesting UP9s are key to adaptation to S-deficiency conditions [29]. Using these knock-down lines, *UP9* was also shown to be required for the increased ethylene production that occurs during S-deficiency [13] . This is partly due to its interaction with the ACC oxidase protein [13]. *UP9* downregulation affected the S-deficiency response of several genes, mainly involved in S metabolic processes, but also transcription regulation, defense response, and hormonal pathways such as ethylene, ABA, and CK [13].

4. LSU protein interactions and phytohormone signaling

4.1. LSU protein interactions suggest some degree of specialization within this family in Arabidopsis

Despite their high similarity, protein interaction analyses have shown that the LSU interactomes partially overlap [9]; [10], suggesting some degree of specialization. For example, from 100 protein interactors identified for LSU1 or LSU2, only 17 are shared by both LSU proteins [14]. All LSU proteins have been shown to homo- and hetero dimerize in planta; however, these results have been replicated in the Y2H heterologous system only for LSU1-LSU1, LSU2-LSU2, and for LSU1 and all other LSUs in the case of heterodimers, indicating that interaction efficiencies may vary between different LSU pairs, or that LSUs associate into multimeric complexes [12]. Multimer formation has also been suggested for tobacco UP9Cs, due to their stranded coiled–coil regions [14]; [29].

Computer modeling of different LSU proteins identified coiled-coil motifs in their structure [14]. Additional circular dichroism studies using a recombinant UP9C protein (tobacco LSU-like protein) suggest that this protein is mostly alpha-helical, which further supports a coiled-coil structure [29] and also by the predicted 3D structure using AlphaFold (https://alphafold.ebi.ac.uk/; Q9SCK1, Q9FIR9, Q9SCK2, and Q8L8S2 for the Arabidopsis LSUs). Although the 3D structure of LSUs has not been experimentally determined, the coiled-coil motif, which facilitates oligomerization, suggests that these small proteins can form multimers and interact with multiple kinds of proteins. Consequently, using the BIFC and yeast 2H approaches, it has been shown that LSU proteins can form heterodimers and homodimers, but the interaction efficiency differs in different pairs [12]. Additional structural modeling and spatial distribution of the electrostatic potential of LSU-LSU dimers revealed significant differences in homo- and heterodimer formation, suggesting that the dimer formation by LSU might have a regulatory function [12]. These analyses also suggested that dimers might bind to different molecular partners than monomeric forms [12]. Additionally, combined mutagenesis and Y2H analyses have identified that the conserved cysteine residues (C54) are not involved in the dimer stabilization and do not form S–S bridge between the monomers of LSUs. Since these cysteine residues were located on the surface of the protein and exposed to solvent, it has been proposed that they play a role in the recognition of other target proteins and the interaction with the coiled-coil structure [12].

Different reports indicated that LSUs appear as multifunctional hubs in protein-protein interaction networks in Arabidopsis [10]; [12]. Using a variety of high-throughput and specific focused studies, it has been described multiple partners for LSU proteins under different growth conditions [9]; [8]; [30], which have been involved in different aspects of abiotic and biotic stress responses, including MYB51 involved in glucosinolate biosynthesis [31], iron (Fe)-dependent superoxide dismutase (SOD) FSD2 involved in plant immune responses [19], the selective autophagy cargo receptor Joka2/NtNBR1 [32], ATP sulfurylase APS1 involved in the sulfate assimilation pathway [12] and the C subunit of cytosolic

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GADPH GAPC1 enzyme, involved in the glycolytic pathway and signaling cascades induced by reactive oxygen species [12].

4.2. Crosstalk between LSUs and phytohormones

Different data indicated that LSU proteins also play an important role in different aspects of phytohormone signaling pathways. LSU1-4 has been shown to interacts with the 14-3-3 protein *GRF8* (general regulatory factor 8) [12], which is involved in brassinosteroid signaling [33], by modulating the nuclear localization of two key transcription factors, *BZR1* and *BZR2/BES1*, and with RAF2/SDIRIP a protein involved in Rubisco assembly, that also mediates abscisic acid-dependent stress responses [34]; [35]. In addition, mapping of the Arabidopsis interactome based on the Y2H system [8] has revealed that the interacting partners of LSU1 or LSU2 include members of the JAZ (jasmonate ZIM-domain) family of repressors, suggesting that LSU proteins also display functions in jasmonate signaling [14]. Furthermore, LSUs have been reported to play a key role in ethylene signaling and plant responses to S-deficiency through the interaction between UP9C and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO2A) [13]. Accordingly, they reported that tobacco antisense UP9C plants showed no increased ethylene levels induced by S limitation treatments [29]; [13]. Moreover, using transcriptomic data, the same work also demonstrated that many S-regulated genes in tobacco are misregulated in UP9C-antisense lines, suggesting that specific levels of LSU proteins are necessary for the full transcriptomic response to S limitation in tobacco.

In addition, it has been reported that the central regulator of the ethylene signaling pathway, EIN3, can bind the *LSU1* promoter in vivo and regulate its expression [36]. Specifically, a ChIP-qPCR assay showed that EIN3 protein bound strongly to the fragments of *LSU1* promoter, and this result was confirmed by EMSA and yeast one-hybrid analyses [36]. Furthermore, a transient dual-luciferase assay in Arabidopsis protoplast indicated that EIN3 transcriptionally represses *LSU1*, which agrees with higher *LSU1* mRNA levels in *ein3-1* mutants [36]. These results demonstrate that phytohormone signaling pathways can regulate *LSUs* in Arabidopsis.

4.3. Differential expression of Arabidopsis LSU genes in response to phytohormone treatment

To get new insights into the response of *LSU* genes to phytohormones, we reviewed the transcriptomic data of Arabidopsis *LSUs* in Plant Regulomics database [37], which integrates 11.090 Arabidopsis transcriptomic datasets, including phytohormone treatments. As shown in Figure 3A, *LSU* genes significantly respond to at least one of the following phytohormones: ABA, ethylene, auxin, and jasmonate. *LSU1* is the member of this gene family with the highest number of experiments as a differentially expressed gene (adjusted p-value <0.05) (8 experiments, Figure 3A). Specifically, *LSU1* is down-regulated by ethylene in 3 experiments, which is consistent with the previously reported repression of this gene by EIN3 [36]. In addition, *LSU1* is also down-regulated by auxin (except in the experiment GSE1491) and jasmonate (Figure 3A). In contrast, *LSU2* and *LSU4* showed a positive response to auxin and the *LSU3* gene to jasmonate, suggesting that the response to phytohormones differs among members of the LSU family in Arabidopsis (Figure 3A). These differences between members of the *LSU* family in response to phytohormones are also reflected in the regulatory network predicted for these genes (Figure 3B). The predicted TF-target interaction obtained from PlantRegMap [38] suggests that *LSU1* is mainly regulated by TFs from the ERF family, which are important regulatory components of ethylene signaling and are involved in plant development and stress responses by regulating the expression of ethylene-responsive genes [39]. On the contrary, *LSU2* is predicted to be regulated by ABA-associated TFs , such as ABF3, and *LSU3* by jasmonate-related TFs such as JAM2 (Figure 3B).

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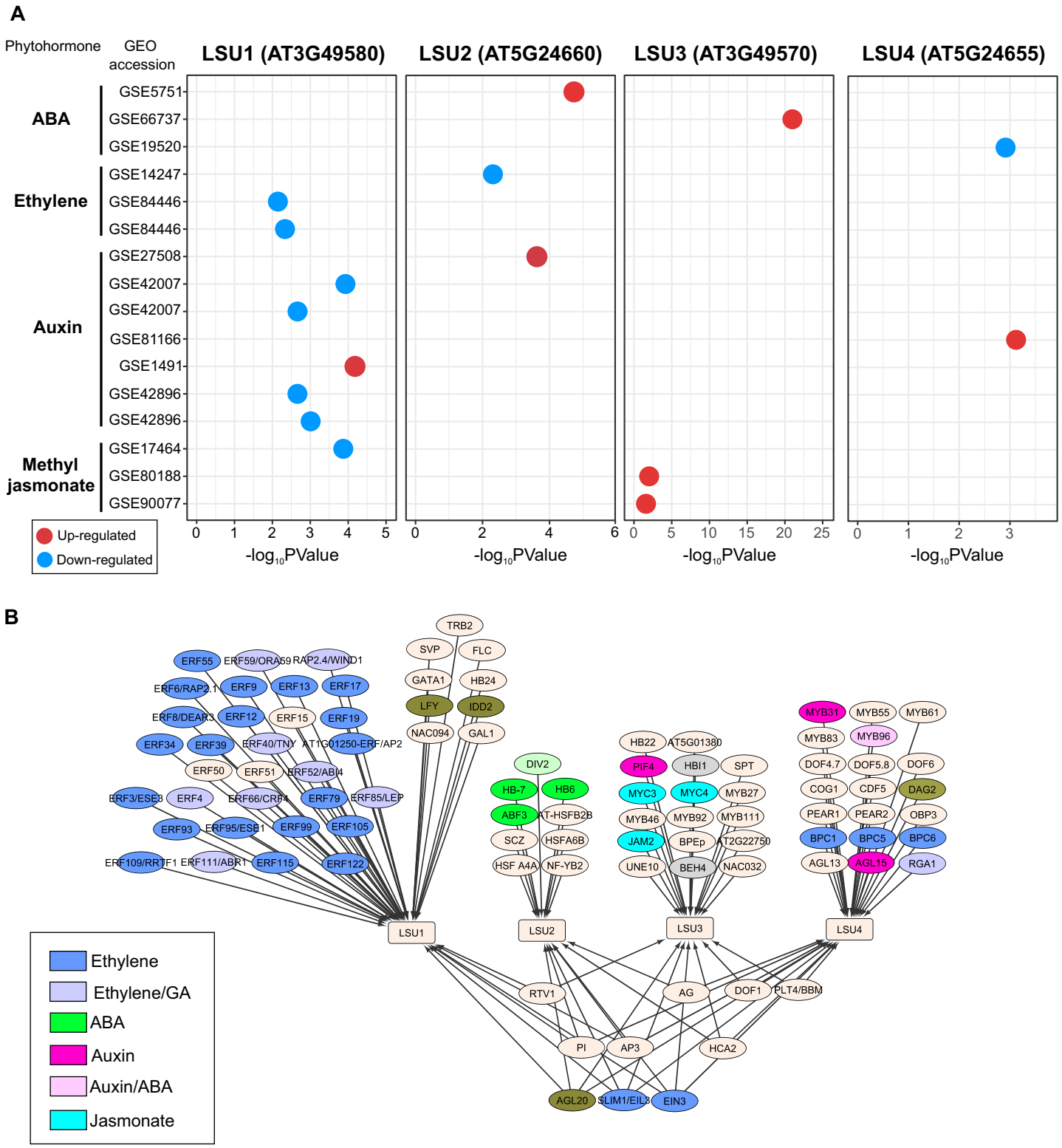

Figure 3. The Arabidopsis *LSU* genes significantly respond to different phytohormone treatments (A), and the analysis of gene regulatory network (B) predicted that contrasting groups of phytohormone-related transcription factors regulate each *LSU* gene. Identification of the significant response of *LSU* genes to phytohormone treatments was performed using transcriptome data sets available in the Plant Regulomics database [37]. The regulatory interactions between *LSU* genes and transcription factors were obtained PlantRegMap [38].

5. Conclusions

Although some information has been gathered about the *LSUs*, there are still many open questions about their functions. In this review, we have provided evidence that this group of proteins appears to display more multifaceted roles than previously expected.

Nevertheless, only a few plant LSU proteins have been functionally characterized. Members of the *LSU* family are likely to participate in fine-tuning responses to the different plant stresses, especially S limitation, and in various aspects of plant development, such as flowering and fruit formation. By modulating a variety of LSU in their protein–protein interactions, *LSU* might act in the crosstalk of various signaling pathways directly or indirectly linked to S metabolism.

It would be interesting to analyze the molecular mechanisms by which *LSUs* orchestrate metabolic homeostasis, plant stress responses, and plant growth and development. In this regard, we expect significant advances in connecting the structure and functions in this family of plant proteins in the following years.

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