Phylogenetic analysis and photoregulation of siroheme biosynthesis genes: uroporphyrinogen III methyltransferase and sirohydrochlorin ferrochelatase of *Arabidopsis thaliana*

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Abstract Uroporphyrinogen III methyl transferase (*UPM1*) and Sirohydrochlorin ferrochelatase (*SIRB*) are the important genes involved in the biosynthesis of siroheme, the prosthetic group of nitrite reductases (NiR) and sulfite reductases (SiR) involved in nitrogen and sulfur assimilation. Both *UPM1* and *SIRB* could be potential candidate genes targeted for sustainable agriculture especially in N-deficient soil. The phylogenetic analysis revealed that these genes are highly conserved among algae, bryophytes and vascular plants including dicots and monocots. The *Arabidopsis* proteins UPM1 and SIRB have close similarity with *Camelina sativa* followed by *Brassica napus*, *Brassica rapa*, and *Brassica oleracea* of the family brassicaceae. The tissue specific expression studies revealed that both the gene are expressed in stem, flower and silique and have highest expression in leaves where the protein content is quite high. The in silico promoter analysis revealed the presence of several light-responsive elements like GATA box, G box, I box, SORLIP2, SORLIP5, SORLREP3 and SORLREP4. Therefore, expression of both the genes was minimal in etiolated seedlings and was upregulated in light. Photo-regulation of transcript abundance of *UPM1* and *SIRB* involved in the biosynthesis of siroheme the cofactor involved in 6 electron reduction of NO_2^- and SO_3^{2-} by NiR and SiR is crucial as the gene expression of latter two enzymes along with other N and S assimilatory enzymes are also modulated by light.

Keywords *Arabidopsis thaliana* · Light responsive elements · Phylogenetic tree · Uroporphyrinogen III methyltransferase · Sirohydrochlorin ferrochelatase

Introduction

Plants are sessile organisms and need to adapt themselves to a constantly changing environment. There are many stresses plants undergo in their life cycle that affects their survival and in turn their productivity. N is a macro nutrient that is a constituent of essential biomolecules carrying out vital functions in the cell. It is often the limiting factor for plant growth and crop productivity (Robertson and Vitousek 2009). Artificial supplementation with chemical fertilizers comes at a great cost and a great damage to the ecosystem. Therefore development of N deficiency tolerant crops is one of the key ways towards sustainable agriculture in future to meet the ever growing demands of the human population. A way is to identify the key genes involved in N assimilation and exploit them for increasing nitrogen utilization and better tolerance to nitrogen starvation conditions. Nitrogen assimilation in non leguminous plants is primarily mediated by a series of enzymes i.e., Nitrate reductase, Nitrite reductase, Glutamine synthetase, Glutamate synthase etc. Siroheme is an iron containing modified tetrapyrrole essentially required as a cofactor for nitrite reductase and sulfite reductase responsible for N and S assimilation (Murphy et al. 1974). Siroheme shares its biosynthetic pathway with chlorophyll and heme. Uroporphyrinogen III is the substrate for both Chl and siroheme biosynthesis. Its decarboxylation reaction leads to chlorophyll synthesis or its methylation reaction diverts it to siroheme biosynthesis.

The methylation reaction is catalysed by s-adenosyl-methionine dependant Uroporphyrinogen III methyl

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transferase (UPM1). This enzyme converts uroporphyrinogen III to precorrin1 and subsequently to precorrin 2. Precorrin 2 is converted to sirohydrochlorin by a dehydrogenase. The metal ion Fe^{2+} is inserted into sirohydrochlorin by sirohydrochlorin ferrochelatase (SIRB) to form siroheme. The *UPM1* and *SIRB* are absolutely essential for plant survival as the knockout mutant for either of them is embryo lethal (Tripathy et al. 2010).

The enzyme UPM1 is one of the few genes that is highly induced by nitrate especially following chronic nitrogen starvation (Bi et al. 2007). UPM1 is the translational product of a single copy gene on chromosome five. Sirohydrochlorin ferrochelatase is also encoded by a single gene on chromosome one.

In the present study the authors analysed the key genes involved in the biosynthesis of siroheme, a cofactor of the plant sulfite and nitrite reductases from an evolutionary perspective and studied the expression pattern of these genes in different tissues of *Arabidopsis thaliana*. The in silico analysis revealed the presence of light regulatory elements (LRE) in the promoter regions of *UPM1* and *SIRB* suggesting their possible regulation by light. We confirmed their regulation by studying the photo-modulation of the gene expression.

Materials and methods

Amino acid alignment

The complete amino acid sequence of AtUPM1 and AtSIRB was used for pBLAST search to identify the related sequences in other species. The sequences were retrieved and exported to the Molecular Evolutionary Genetics Analysis version 6 application (MEGA6) integrated tool for phylogenetic analysis (Tamura et al. 2013) and peptide sequences were aligned using Multiple Sequence Comparison by Log-Expectation MuSCL; (Edgar 2004) with the following constraints: Gap Penalties: Open = 22.9, Extend = 0, Hydrophobicity Multiplier = 1.2. Memory/iterations: Max Memory in Mb = 4095, Max iterations = 50.

Phylogeny construction

The evolutionary history was inferred using the Neighbor-joining method, the bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Branches corresponding to partitions reproduced in less than 75 % bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown

next to the branches (Felsenstein 1985). The evolutionary distances were computed using the Poisson model (Zuckerkandl 1965) and are in the units of the number of amino acid substitutions per site. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013). Numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees; only values >60 % are shown.

Promoter analysis

Putative light responsive elements and conserved motifs in the promoters of UPM and SIRB were identified using the AGRIS database that contains information about the putative/experimentally validated cis regulatory elements of the *Arabidopsis thaliana* curated genes. The information can be accessed at arabidopsis.med.ohio-state.edu/AtcisDB/

Plant growth conditions

For light dependant expression, the stratified WT (Col-0) *Arabidopsis* seeds were germinated on 1/2X MS media in dark for 6 days at 21 °C. The etiolated seedlings were then exposed to low light (60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for different durations and then samples were harvested for further analysis.

For the tissue dependant expression the WT plants were grown on pots containing agropeat and vermiculite (3:1) at 21 °C and 14 h light/10 h dark photoperiod and grown under 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The tissues were then harvested and processed for further experiments.

Semi quantitative RT PCR

Total RNA was extracted from tissues using TRI reagent (Sigma, St Louis) and treated with RNase- free DNase I (Promega, Madison, WI, USA). Semiquantitative RT-PCR was performed as described by Dutta et al. (2009). Actin was used as internal control. The cDNA was diluted 1:5, and 1 μL of cDNA was used as a template for PCR amplification in a 20- μL reaction mixture. Reactions were carried out with selected couples of the gene-specific primers. PCR was performed for 25–29 cycles within a linear range of amplification of *UPM1*, *SIRB* and *ACTIN2* genes. For each primer pair, the optimization was done for the annealing temperature and the number of cycles of PCR reaction. Ten microliters of the PCR products were loaded and separated on 0.8 % agarose Tris–acetate EDTA gel.

Primer sequence for SIRB

F: GCGGATCCATGACGACTCAGTCGCAGT and *SIRB*
R: GCCTAAGAACTATTGTAGAGCTTGCAC.

ACTIN 2F: ATGGCTGATGGTGAAGACATT and
ACTIN 2R: TCAGAAGCACTTCCTGTGAACA.

Primer sequence for UPM1

UPM1 F: ATGGCTCTTGTTTCAGCGGATTC
UPM1 R: CTACCGGGTCTCTACAAGGCA

The densitometric analysis of the bands was performed using Alpha Ease FC software. Each data point is an average of three biological replicates. Asterisks indicate significant differences determined by Students' *t* test compared with the control (**P* < 0.05, ***P* < 0.01)

qRT-PCR

Plants were grown in etiolated conditions for 6 days and then exposed to low levels of light (60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for different time points and then samples harvested. Total RNA was isolated from the samples using Tri reagent (Sigma) from 3 different plants (three biological replicates). The first strand cDNA was synthesized using cDNA synthesis kit (Verso, USA). Relative expression of different genes was studied by performing qRT-PCR on ABI Prism 7500 Sequence Detection System (Applied Biosystems, USA). Three technical replicates were taken per sample. Three biological replicates were used for the experiment. 10 μl reaction mixture contained 0.5 μl cDNA, 5 μmol primers and 5 μl 2xSYBR Green PCR Master Mix (Applied Biosystems, USA). The reference gene used was *Actin 2*. The relative gene expression data were analyzed using $2^{-\Delta\Delta C_t}$ quantitation methods (Livak and Schmittgen 2001). Primer sequence for *UPM1 F*: CCCAAACCCATATAAACGACAAG and *UPM1 R*: GTAAGGTTTCTCACTGGTCATTCA.

ACTIN2 F: CTTGAAGTATCCTATTGAGCATGGTGT
 TT and *ACTIN2 R*: CGGGAGAGTTAAAGGTCTCAAA
 CATGA.

Statistical analysis

Microsoft Excel was used to perform unpaired Students' *t* test. Asterisks indicate significant differences as compared with the control (**P* < 0.05, ***P* < 0.01).

Results

Phylogenetic tree of UPM1

The phylogenetic tree reflects the specific position of particular motif in evolutionary pattern in comparison with those of other plants. The phylogenetic analysis of UPM1 indicates that the protein is conserved across all eukaryotes. The protein is highly conserved across plant

kingdom i.e., algae, mosses, dicots and monocots. The protein sequence of UPM1 of green filamentous alga *Klebsormidium flaccidum* is close in homology to bryophytic moss *Physomitrella patens*. The protein sequence of UPM1 is highly conserved among solanaceous plants i.e., *Solanum pennellii*, *Solanum lycopersicum*, *Solanum tuberosum*, *Nicotiana tomentosiformis*, *Nicotiana sylvestris*, *Nicotiana tabacum*. Closely related dicotyledonous families i.e., pedaliaceae (*Sesamum indicum*), phrymaceae (*Erythranthe guttata*) and amaranthaceae (*Beta vulgaris*) have conserved UPM1 aminoacid sequences. The *Arabidopsis* proteins delineated early from a basal angiosperm *Amborella*. The protein was found to have greatest similarity with the other eudicot *Camelina sativa* followed by *Brassica napus*, *Brassica rapa*, and *Brassica oleracea*, of the same family Brassicaceae. The protein sequence leguminous plants belonging to the family fabaceae i.e., *Medicago truncatula*, *Cicer arietinum*, *Vigna radiata*, *Glycine soja* and *Glycine max* are clustered together. The protein is also conserved among rosaceae and its closely related families. Protein sequence of cucurbits having epigynous ovary are clustered together and closely related to plants belonging to rosaceae having perigynous ovary. Most of the evolved monocots of family poaceae are clustered together among different species, i.e., *Brachypodium distachyon*, *Setaria italic*, *Zea mays*, *Oryza sativa*, *Oryza brachyantha*, *Hordeum vulgare* and *Triticum aestivum*. Numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees; only values >60 % are shown (Fig. 1).

Phylogenetic tree of SIRB

The phylogenetic analysis indicates that similar to UPM1, the *Arabidopsis thaliana* SIRB protein shares greatest similarity with *Camelina sativa*, a member of Brassicaceae family. Other closely related members are *Brassica oleracea*, *Brassica rapa* and *Brassica napus* in the Brassicaceae family. Amongst monocots which diverged early in the evolution, members of poaceae i.e., *Zea mays*, *Oryza sativa*, *Hordeum vulgare* and *Brachypodium distachyon* are clustered together. Another monocot *Zostera marina*, a type of sea grass and *Musa acuminata*, a member of musaceae evolved in parallel to the *Arabidopsis* protein. However unlike UPM1, the members of solanaceae i.e. *Solanum pennellii*, *Solanum lycopersicum*, *Solanum tuberosum*, *Nicotiana tomentosiformis*, *Nicotiana sylvestris*, *Nicotiana tabacum* share less homology with the *Arabidopsis* counterpart. The families of myrtaceae (*Eucalyptus grandis*), euphorbiaceae (*Ricinus communis*), moraceae (*Morus alba*) and vitaceae (*Vitis vinifera*) evolved independently alongwith SIRB. This protein is also conserved in the legumes of the family fabaceae

Fig. 1 Phylogenetic tree of protein sequence of uroporphyrinogen III methyl transferase (AtUPM1) of *Arabidopsis thaliana*, Accession no: NP_198901.1. The tree was constructed using MEGA version 6 using the Neighbor-joining method, the bootstrap consensus tree inferred

from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees; only values >60 % are shown

Fig. 2 Phylogenetic tree of Sirohydrochlorin ferrochelatase of *Arabidopsis thaliana* (AtSIRB) Accession no NP_564562.1. The tree was constructed using MEGA version 6 (Tamura et al. 2013) using the Neighbor-joining method, the bootstrap consensus tree inferred from

1000 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). Numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees; only values >60 % are shown

(*Medicago truncatula*, *Cicer arietinum*, *Vigna radiata*, *Glycine soja* and *Glycine max* (Fig. 2).

Tissue specific expression of *UPM1* and *SIRB*

The expression dynamics of the genes were studied in different tissues. The stem, rosette leaf, cauline leaf, flower and unripe siliques were studied. The expression pattern was almost similar for both *UPM1* and *SIRB*. They are expressed in all tissues but the predominant expression was found in leaves (Fig. 3a–d).

Promoter analysis of *UPM1* and *SIRB* for the presence of light responsive elements (LRE)

The in silico analyses revealed the presence of LRE in the promoter of both the siroheme biosynthesis genes, *SIRB* and *UPM1*. *SIRB* was found to have GATA Box and I box elements (Table 1). *UPM1* has several light responsive elements GATA box, G-box, SORLREP3, 4 and SORLIP2 and 5.

Light dependant expression of *UPM1* and *SIRB*

The presence of LRE in the promoters of *SIRB* and *UPM1* led us to study their light-dependant gene expression. The *UPM1* expression increased by 80 % after two hours of light exposure of etiolated seedlings. The transcript abundance of *UPM1* did not increase further, rather remained constant at longer hours of light exposure (up to 24 h).

The gene expression pattern for *SIRB* was a little different from that of *UPM1*. The etiolated seedlings when exposed to light ($60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) showed upregulation of *SirB* expression by 50 % at the initial three hours of light exposure. The light dependant upregulation for *SIRB* was observed up to 24 h of light exposure and it was increased by 150 % at 24 h of light exposure (Fig. 4a–c).

Discussion

The phylogenetic trees of *UPM1* and *SIRB* clearly demonstrate that they are well conserved in algae, mosses, dicots and monocots and among related genera and families. *UPM1* and *SIRB* catalyse the reactions involved in the biosynthesis of siroheme. Siroheme participates in the multielectron reduction reaction of nitrite to ammonia and sulfite to sulphide by serving as the cofactor of plant nitrite reductase and sulfite reductase respectively (Murphy et al. 1974). NiR and SiR are essentially chloroplast localized and therefore *UPM1* and *SIRB* enzymes are also predicted to be chloroplast/plastid localized. In the higher plants when

Fig. 3 Tissue specific expression of *UPM1* and *SIRB*. **a** The differential expression of *UPM1* in different tissues and **b** its densitometric analysis. **c** The differential expression of *SIRB* in different tissues and **d** its densitometric analysis. *A. thaliana* plants were grown in cool-white fluorescent light ($100 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) at 22°C in 14 h L and 10 h D photo-period and total RNA was isolated from different tissues (stem, rosette leaf, cauline leaf, flower and silique). Semi quantitative RT-PCR was done using *AtUPM1* gene specific primers. Actin was used as internal control. The densitometric analysis of the bands was performed using Alpha Ease FC software. Each data point is an average of three biological replicates

Table 1 Light Responsive elements found in the promoters of *UPM1* and *SIRB*

BS name	BS genome start	BS genome end	Binding site sequence
Summary of LRE for uroporphyrinogen III methyl transferase: promoter ID (At5g40850)			
GATA promoter motif [LRE]	16381472	16381477	tgatag
GATA promoter motif [LRE]	16381480	16381485	tgatag
GATA promoter motif [LRE]	16381490	16381495	agataa
GATA promoter motif [LRE]	16381606	16381611	agataa
GATA promoter motif [LRE]	16381723	16381728	agataa
GATA promoter motif [LRE]	16382109	16382114	tgataa
GATA promoter motif [LRE]	16382159	16382164	tgatag
GATA promoter motif [LRE]	16382236	16382241	tgataa
GATA promoter motif [LRE]	16382244	16382249	tgataa
GATA promoter motif [LRE]	16382382	16382387	tgataa
GATA promoter motif [LRE]	16382438	16382443	tgataa
GATA promoter motif [LRE]	16383551	16383556	tgatag
GATA promoter motif [LRE]	16382075	16382080	tgataa
G-box promoter motif [LRE]	16381823	16381828	cacgtg
G-box promoter motif [LRE]	16381822	16381827	cacgtg
SORLREP3	16381540	16381548	tgtatatat
SORLREP4	16383406	16383414	ctcctaatt
SORLIP2	16381910	16381914	gggcc
SORLIP2	16381997	16382001	gggcc
SORLIP2	16382984	16382988	gggcc
SORLIP2	16382976	16382980	gggcc
SORLIP5	16381756	16381762	gagttag
Summary of LRE for sirohydrochlorin ferrochelatase: promoter ID (At1g50170)			
GATA promoter motif [LRE]	18647366	18647371	agataa
I box promoter motif	18647367	18647372	gataag

nitrate is the source of nitrogen, most of nitrate assimilation occurs in the leaves (Guohua et al. 2012). Therefore leaves have the highest gene expression for these enzymes as compared to the flowers or siliques. In *Arabidopsis*, per gm fresh weight, leaves have higher amount of protein content than roots, flowers and siliques mostly due to the presence of rubisco which accounts for 50 % of total protein. (Parry et al. 2003). The higher demand of huge amount of nitrogen is met by the nitrogen and sulfur assimilation pathway operating chiefly in leaves. Hence our observation of leaves expressing the highest amount of *UPM1* and *SIRB* reflects the N assimilation status in the leaf cell.

Light is indispensable for plants as it is used not only as the primary energy source but also as one of the key signals that determines the development of young seedlings. The light responsiveness of a promoter is in effect due to a specific combination of different LRE (Puente et al. 1996; William and Anthony 1995). Photo-regulation of *UPM1* and *SIRB* is crucial as they encode proteins involved in the biosynthesis of siroheme, a cofactor for NiR and SiR whose expression is also modulated by light. NR and NiR contain several light responsive elements in their promoter

region (Bi et al. 2007). The in silico promoter analysis of *Arabidopsis* NiR (At2g15620) contains cis acting LREs i.e., GATA elements, G-Box, SORLREP3, SORLIP1 and evening element responsible for circadian rhythm. Similarly, *Arabidopsis* SiR (At5g04590) contains GATA elements, G-Box, I box and SORLIP2 LREs. These demonstrate that the biosynthesis of both the apoproteins and cofactors are light modulated. The primary step of nitrogen assimilation from soil is the reduction of nitrate to nitrite catalysed by nitrate reductase, a well known enzyme that is light regulated and under the circadian control (de Cires et al. 1993; Deng et al. 1990; Huber et al. 1992; Raghuram et al. 1999). The light response and regulation of nitrate reductase is mediated by phytochromes (Rajasekhar et al. 1988). Light also stimulates the activity of nitrite reductase of mustard, tomato and maize leaves via the phytochrome mediated signaling pathway (Becker et al. 1992; Rajasekhar and Mohr 1986).

The major genes of sulfate assimilation were found to be upregulated in green leaves as compared to etiolated tissues (Hell and Bork 1997). The key enzyme of sulfur assimilation Adenosine 5' phosphosulphate reductase (Brunold

Fig. 4 Light dependant expression of *UPM1* and *SIRB* **a** Effect of light on the expression of *UPM1* in *Arabidopsis thaliana*. The changes in gene expression of *UPM1* was monitored by qRT PCR. Actin2 was taken as reference gene. **b** Effect of light on the expression of *SIRB* in *Arabidopsis thaliana*. The changes in gene expression was monitored by semi quantitative RT-PCR. **c** The densitometric analysis was done using Alpha Ease FC software. Each data point is an average of three biological replicates. The stratified WT (Col-0) *Arabidopsis* seeds were germinated on 1/2X MS media in dark for 6 days at 21 °C. The etiolated seedlings were then exposed to light (60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for different durations and samples were harvested for gene expression studies. Asterisks indicate significant differences determined by Students' *t* test compared with the control (**P* < 0.05, ***P* < 0.01)

1993), is well known to be regulated under circadian rhythm and under light control in *Arabidopsis* and maize (Kocsy et al. 1997; Kopriova et al. 1999). In addition mRNA levels of APS kinase, SiR, O Acetyl serine thiol lyase and serine acetyl transferase were many fold higher in the green tissues as compared to the etiolated leaves of *Arabidopsis thaliana* (Hell and Bork 1997).

The promoters of *UPM1* and *SIRB* are predicted to contain 13 and 1 GATA motifs respectively. GATA elements are the most extensively studied motifs and those are most

common in light regulated genes (Grob and Stuber 1987). G box like elements have been reported in the light regulated genes of small subunit of Ribulose 1,5 bisphosphate carboxylase/oxygenase, Chlorophyll a/b binding proteins and chalcone synthase (Cashmore AEMaAR 1994). G box promoter motifs are predicted in the promoter of *UPM1*. These cis elements are binding sites for G box binding factors (GBF), a family of bZIP transcription factors involved in gene regulation in the major developmental or physiological processes and respond to light or hormones (Menkens et al. 1995). Both the G box motif and the GATA motif alone and in combination confer higher reporter gene expression in light grown seedlings as compared to dark grown seedlings (Puente et al. 1996). Light directly modulates activity of promoters containing LREs via phytochrome signaling (Puente et al. 1996). The LREs in conjunction with the *trans*-factors are regarded as signal interaction points in the network that mediates both light and developmental regulation of gene expression (Puente et al. 1996). GATA elements also serve as the binding sites of factors involved in other biological processes like response to nitrate, greening process and carbon metabolism. The GNC/GNL (GATA NITRATE-INDUCIBLE, CARBON-METABOLISM INVOLVED and CGA1/GNL (CYTOKININ-INDUCED GATA1/GNC-LIKE; GNL) are the paralogous B-GATA factors implicated in greening process, nitrogen availability, response to phytohormones and light (Behringer and Schwechheimer 2015).

The I box has been predicted in the promoter of *SIRB*. The I box is sufficient and necessary for transcriptional activation of many genes in response to light (Giuliano et al. 1988). There were many sequences that were found to be overrepresented in the Phytochrome A-induced promoters (Hudson and Quail 2003). These elements are named as 'sequences over-represented in light induced promoters (SORLIPs)'. The *UPM1* promoter contains SORLIP2 and SORLIP5 which might contribute to the light responsiveness of the promoters. Besides *UPM1* contains SORLREP3 and SORLREP4 elements. These elements were characterized for the first time (Hudson and Quail 2003), when they found that certain consensus sequences were strongly conserved in statistically significant number in the promoters of Phy-A repressed genes. Termed as term sequences over-represented in light-repressed promoters (SORLREPs), these elements might contribute to promoter response to light. Interestingly SORLREP 4 is also found in promoters that are circadian-regulated (Hudson and Quail 2003). Thus the presence of various LRE's in *UPM1* caused the fast induction i.e. within 2 h of light exposure. The gene expression pattern for *SIRB* was different. The light induced gene expression was observed at 3 h that continued till 24 h. The presence of only two LRE i.e. GATA and I box could have

determined the observed response of the *SIRB* promoter to light in the etiolated seedlings.

In conclusion our studies demonstrate that siroheme biosynthesizing proteins UPM1 and SIRB, well conserved across plant kingdom, are mostly expressed in leaves where most N and S assimilation occurs. The photomodulation of these genes suggest their co-regulation with N and S assimilating enzymes i.e., NiR, NR and SiR etc. and other light-regulated genes involved in photosynthesis and circadian rhythms. *UPM1* and *SIRB* could be of potential interest for developing crop plants for enhanced nitrogen use efficiency and higher sulfur assimilation potential.

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Authors' contribution BCT designed the experiments. SG and NCJ performed the experiments and analyzed the data. SG and BCT prepared the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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