Serine hydroxymethyltransferase localised in the endoplasmic reticulum plays a role in scavenging H$_2$O$_2$ to enhance rice chilling tolerance

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Abstract

Background: Rice is a chilling-sensitive crop that would suffer serious damage from low temperatures. Overexpression of the Lsi1 gene (Lsi1-OX) in rice enhances its chilling tolerance. This study revealed that a serine hydroxymethyltransferase (OsSHMT) mainly localised in the endoplasmic reticulum (ER) is involved in increasing tolerance to chilling.

Results: A higher transcription level of OsSHMT was detected in Lsi1-OX rice than in the wild type. Histone H1 and nucleic acid binding protein were found to bind to the promoter region of OsSHMT and regulate its expression, and the transcription levels of these proteins were also up-regulated in the Lsi1-OX rice. Moreover, OsSHMT interacts with ATP synthase subunit α, heat shock protein Hsp70, mitochondrial substrate carrier family protein, ascorbate peroxidase 1 and ATP synthase subunit β. Lsi1-encoded protein OsNIP2;1 also interacts with ATP synthase subunit β, and the coordination of these proteins appears to function in reducing reactive oxygen species, as the H$_2$O$_2$ content of transgenic OsSHMT Arabidopsis thaliana was lower than that of the non-transgenic line under chilling treatment.

Conclusions: Our results indicate that ER-localised OsSHMT plays a role in scavenging H$_2$O$_2$ to enhance the chilling tolerance of Lsi1-OX rice and that ATP synthase subunit β is an intermediate junction between OsNIP2;1 and OsSHMT.

Keywords: Rice, Chilling, Serine hydroxymethyltransferase, ROS, Protein-protein interactions

Background

Plants require a given temperature range for normal growth and development, and sudden changes in temperature can cause growth inhibition and even death [1]. Cold spells in late spring are examples of sudden drops in temperature over a short time and can adversely affect the growth of rice. Some varieties of rice, mainly those in the indica subspecies, are particularly sensitive to low temperatures [2, 3]. Continuous chilling of crop plants has been shown to result in increased reactive oxygen species (ROS) and cell membrane peroxidation and to affect the expression of chloroplast genes,
inhibiting photosynthesis [4]. Such ROS include \( \text{O}_2^\cdot \), \( \text{H}_2\text{O}_2 \), and \( \text{OH}^- \) [5].

Fang et al. [6] documented that chilling treatment inhibited the expression of chlorophyll synthesis genes and promoted the expression of protease genes from temperature-sensitive Dular rice (Oryza sativa ssp. indica), leading to chloroplast damage, chlorophyll degradation, loss of green colour and partial degradation of RNA in leaves. Cui et al. [7] found that the whitening of Dular leaves under chilling stress was associated with the deletion of the gene coding sequence of pentatricopeptide (LOC_Os09g29825.1), which is an RNA-binding protein, and the gene coding sequence missed 8 bases relative to that from Nipponbare rice, resulting in a frameshift mutation of the coding sequence and deactivation of the protein function. The mutant gene was named DUA1. Inactivation of the DUA1 protein in Dular rice leads to a loss of RNA editing capacity under chilling stress, resulting in rice seedlings that exhibit chloroplast development defects and leaf chlorosis. When the silicon-absorbing gene (Lsi1) was overexpressed in Dular rice, the chilling tolerance of the transgenic line was significantly improved, and the leaves maintained their fresh green colour under chilling treatment. The expression of genes from the photosynthesis pathway of transgenic rice was enhanced under low temperature stress, and the expression of genes involved in the protease was down regulated. In addition, the transcription level of the gene encoding serine hydroxymethyltransferase (SHMT, LOC_Os03g52840) was upregulated in the transgenic rice, but down-regulated in the wild-type, and the expression of its corresponding miRNA changed in an opposite way, indicating that OsSHMT may be involved in regulating the chilling-tolerance of Dular [6].

SHMT is widely distributed in plants [8]. The enzyme catalyses the reversible exchange between serine and glycine (glycine \( \text{CH}_2\text{THF} \rightarrow \text{H}_2\text{O} \rightarrow \text{serine THF} \)) [9, 10], and these two amino acids are precursors of chlorophyll, tryptophan and ethanolamine [11]. Therefore, SHMT plays an important role in the photoreparation processes of plants [12]. Studies have found that a mitochondrial serine hydroxymethyltransferase gene mutation in rice (ossshm1) causes blockage of the photoreparation pathway, thereby affecting the Calvin cycle and the efficiency of light energy; excess light in the chloroplast then leads to the accumulation of ROS, resulting in disruption of chloroplast development and fewer and smaller chloroplasts with less grana [13].

In addition, SHMT plays a positive role in regulating plants’ resistance to stress. In Arabidopsis, expression of NADH dehydrogenase was shown to be inhibited by chilling, leading to the continuous accumulation of ROS, suppressing the transcription of cold-response genes and hypersensitivity to chilling [14]. SHMT activity contributes to a reduction of ROS accumulation in the chloroplast and therefore reduces oxidative damage [15]; as a second messenger, a reduction in ROS would also prevent the transmission of the chilling signal and reduce the damage caused by ROS. The recessive mutation shmt1–1 in Arabidopsis results in abnormal regulation of cell death, leading to chlorotic and necrotic diseases under various environmental conditions, and mutants that carry the shmt1–1 allele exhibit more \( \text{H}_2\text{O}_2 \) accumulation than wild-type plants under salt stress, resulting in greater chlorophyll loss [16]. To the best of our knowledge, the specific role of OsSHMT in the regulation of rice cold resistance has not been reported.

In this study, an OsSHMT gene (LOC_Os03g52840) from rice was amplified, the subcellular localisation of OsSHMT was investigated, and proteins that bind to the promoter region of OsSHMT were obtained. The proteins that interact with OsSHMT were also investigated to indicate the regulation network of OsSHMT in rice under chilling stress, revealing the possible role of OsSHMT in scavenging \( \text{H}_2\text{O}_2 \) to improve cold resistance in rice.

Results

**Chilling tolerance of Dular and Lsi1–OX rice**

The Dular and Lsi1–OX transgenic lines presented different tolerances to the chilling treatment of 4 °C for 36 h. Most of the leaves from the Dular rice became whiter, whilst the Lsi1–OX line maintained tolerance to chilling (Fig. 1a). Further studies showed that the Lsi1 overexpression vector was driven by the ubiquitin promoter, and the translate OsNIP2;1 was localised in the cytoplasm (Fig. 1b), which differs significantly from the original localisation in the cell membrane [17]. This result suggests that OsNIP2;1 may have more roles than its initial function, which belongs to the aquaporin family and controls silicon accumulation in rice [17]. Scanning electron microscope (SEM) images of the leaves revealed that silica bodies in the Lsi1–OX rice leaves were bigger than those of the Dular rice (Fig. 1c).

**Subcellular localisation of OsSHMT**

Overexpression of Lsi1 in Dular rice results in changes in the expression of thousands of genes [6], including the OsSHMT gene. Expression of OsSHMT was up-regulated in the Lsi1–OX rice in comparison with the Dular rice (Fig. 2a). Subcellular localisation of OsSHMT (LOC_Os03g52840) in the rice protoplast then showed that yellow fluorescence was concentrated around the nucleus, and no obvious endonuclear fluorescence was observed, whereas significant yellow fluorescence was seen in the whole nucleus in the rice protoplast transformed with the eYFP vector (Fig. S1). This suggested
that OsSHMT was localised on the endoplasmic reticulum around the nucleus. Further co-localisation of the endoplasmic reticulum marker protein with mcherry indicated that OsSHMT was widely distributed in the endoplasmic reticulum, as the mcherry fluorescence from the marker protein and yellow fluorescence from OsSHMT infused with eYFP was completely overlapping (Fig. 2b). OsSHMT is involved in the photorespiratory processes of plants, and existing studies have suggested that the protein is localised to mitochondria, chloroplasts and cytoplasm. The results of this study complement those findings.

Promoter region of OsSHMT and the binding proteins

The promoter region 2597 bp upstream of the CDS of the OsSHMT gene from Dular rice was amplified and labelled with biotin at the 5′ flanking region of this DNA fragment (Table S1). According to the DNA pull-down results, compared with the control group at room temperature, chilling treatment of Lsi1-OX induced more proteins to bind to the promoter region of OsSHMT. In contrast, chilling treatment of the Dular rice had no significant effect on the proteins binding to the promoter (Fig. 3a). Identification of the proteins showed that retrotransposon protein (Ty3-gypsy subclass), glyceraldehyde-3-phosphate dehydrogenase, and AT hook motif protein were identified from both the Lsi1-OX and Dular rice under chilling treatment or room temperature conditions; however, some proteins, such as histone H1, nucleic acid binding protein (NABP) and tubulin/FtsZ domain-containing protein (LOC_
Os03g51600.1) were only identified from the chilling-treated Lsi1-OX group; another tubulin/FtsZ domain-containing protein (LOC_Os05g34170.2) was identified from the chilling-treated Lsi1-OX and Dular groups and from the Dular group at room temperature. AAA-type ATPase family protein was identified from the Dular group at room temperature but not from the chilling-treated Dular group, and this protein was induced in the chilling-treated Lsi1-OX group in comparison with its control group at room temperature (Table 1). The tubulin/FtsZ domain-containing proteins (LOC_Os03g51600.1, LOC_Os05g34170.2, LOC_Os07g38730.1), histone H1 and NABP were selected to analysis their gene expression level on the two rice. A comparison of the gene transcription levels in Dular and Lsi1-OX rice after chilling treatment for 12, 24 and 36 h in comparison with 0 h, revealed opposite trends in the two rice lines, and the gene expression level was up-regulated in the Lsi1-OX rice in comparison with Dular rice under the same treatment conditions (Fig. 3b).

The results indicate that these genes act in combination to exert a positive role in the regulation of OsSHMT expression.

**Proteins interacting with OsSHMT**

Based on the GFP-TRAP method to obtain the proteins interacting with OsSHMT, it was found that several proteins co-precipitated with OsSHMT, in comparison with the GFP-vector control (Fig. 4; Table S2). Some of these proteins, including ATP synthase α subunit, ATP synthase β subunit, heat shock protein 70, mitochondrial substrate carrying family protein E, ascorbate peroxidase 1, are defense proteins that interact with OsSHMT protein (Table 2). Further determination of the interaction of OsSHMT with ATP synthase subunit α, ATP synthase subunit β, Hsp70, MSCP and APX from the rice showed that yellow fluorescence was detected in the leaves of tobacco infected with OsSHMT and each of these proteins.
proteins respectively. No fluorescence was detected in the control group, demonstrating that OsSHMT positively interacts with the above proteins (Fig. 5). The results indicate that OsSHMT interacted with defence-related proteins in rice, including APX, MSCP, HSP70 and ATP synthase, to jointly regulate chilling resistance.

H$_2$O$_2$ content in the OsSHMT transgenic A. thaliana and wild type

To further indicate the function of OsSHMT in scavenging H$_2$O$_2$, wildtype A. thaliana and positive transgenic T$_3$ A. thaliana seedlings were exposed to a temperature of 4°C for 12, 24 and 36 h. The leaves of the wild type showed more reddish-brown spots after Diaminobenzidine (DAB) staining. The transgenic line also showed reddish-brown spots, but the spots were small in size and number (Fig. 6a). Determination of the leaf H$_2$O$_2$ content of the transgenic line and wild type of A. thaliana showed that the increase in H$_2$O$_2$ in the transgenic line of A. thaliana after chilling treatment was significantly lower than that of the wild type that underwent the same treatment (Fig. 6b).

OsNIP2;1 interacts with ATP synthase subunit β from rice

Lsi1 encodes the aquaporins protein OsNIP2;1, which is a nodulin 26-like intrinsic protein (NIP) and is localised in the membrane of the cell. Overexpression of Lsi1 in Dular rice using a ubiquitin promoter resulted in the cytoplasm localisation of this protein, enabling OsNIP2;1 to play multiple roles. The interaction between OsNIP2;1 and ATP synthase subunit β was confirmed by immunoblotting.
1 and OsSHMT was also investigated. The results showed no direct interaction between OsNIR2;1 and OsSHMT. However, OsNIP2;1 interacted with ATP-synβ, a protein that also interacts with OsSHMT, and the results indicate that ATP-synβ acts as an intermediate junction between OsNIP2;1 and OsSHMT (Fig. 7).

**Discussion**

Plant suffers from low-temperature frequently results in the accumulation of ROS, which would lead to lipid peroxidation of the cell membrane. Our studies here indicated that OsSHMT functions in scavenging H₂O₂ in the plant. OsSHMT has been declared to be localised in the mitochondria, chloroplast, cytoplasm, nucleus, plasma membrane and cytosol [13, 18, 19], and participates in the photosynthesis pathway. Besides, our present study indicated that OsSHMT is also localised in the endoplasmic reticulum, which is the main organelle for protein and lipid synthesis, membrane biogenesis, xenobiotic detoxification and cellular calcium storage.

### Table 1  Proteins binding on the OsSHMT gene promoter from Dular and Lsi1-OX

| Protein ID | Unique peptide number | Unique spectra number | Coverage | Description |
|------------|----------------------|-----------------------|----------|-------------|
| LOC_Os03g58470.1 | 11 | 121 | 0.3481 | retrotransposon protein, putative, Ty3-gypsy subclass, expressed |
| LOC_Os04g58730.1 | 4 | 6 | 0.1527 | AT hook motif domain containing protein, expressed |
| LOC_Os03g03720.1 | 4 | 4 | 0.1261 | glyceraldehyde-3-phosphate dehydrogenase, putative, expressed |
| LOC_Os12g37260.1 | 3 | 3 | 0.0434 | lipoxygenase 2.1, chloroplast precursor, putative, expressed |
| LOC_Os11g47970.1 | 3 | 3 | 0.0687 | AAA-type ATPase family protein, putative, expressed |
| LOC_Os04g42320.1 | 3 | 3 | 0.0414 | AT hook motif family protein, expressed |
| LOC_Os07g08710.1 | 3 | 3 | 0.1871 | AT hook-containing DNA-binding protein, putative, expressed |
| LOC_Os03g58470.1 | 10 | 130 | 0.3311 | retrotransposon protein, putative, Ty3-gypsy subclass, expressed |
| LOC_Os04g58730.1 | 4 | 8 | 0.1527 | AT hook motif domain containing protein, expressed |
| LOC_Os07g08710.1 | 4 | 9 | 0.223 | AT hook-containing DNA-binding protein, putative, expressed |
| LOC_Os05g34170.2 | 4 | 7 | 0.1036 | tubulin/FtsZ domain containing protein, putative, expressed |
| LOC_Os03g03720.2 | 3 | 4 | 0.1226 | glyceraldehyde-3-phosphate dehydrogenase, putative, expressed |
| LOC_Os01g72049.1 | 5 | 5 | 0.1975 | retrotransposon, putative, centromere-specific, expressed |
| LOC_Os05g34170.2 | 3 | 4 | 0.0811 | tubulin/FtsZ domain containing protein, putative, expressed |
| LOC_Os07g38730.1 | 2 | 2 | 0.0444 | tubulin/FtsZ domain containing protein, putative, expressed |
| LOC_Os04g42320.1 | 6 | 9 | 0.0674 | AT hook motif family protein, expressed |
| LOC_Os05g34170.2 | 4 | 7 | 0.1975 | retrotransposon, putative, centromere-specific, expressed |
| LOC_Os04g49990.1 | 4 | 5 | 0.2507 | AT hook motif domain containing protein, expressed |
| LOC_Os05g34170.2 | 4 | 5 | 0.1059 | tubulin/FtsZ domain containing protein, putative, expressed |
| LOC_Os04g49990.1 | 4 | 5 | 0.1059 | tubulin/FtsZ domain containing protein, putative, expressed |
| LOC_Os08g40150.1 | 3 | 3 | 0.1102 | AT hook motif domain containing protein, expressed |
| LOC_Os04g38600.2 | 3 | 3 | 0.1111 | glyceraldehyde-3-phosphate dehydrogenase, putative, expressed |
| LOC_Os06g04020.1 | 3 | 3 | 0.1458 | histone H1, putative, expressed |
| LOC_Os03g52490.1 | 2 | 2 | 0.098 | nucleic acid binding protein, putative, expressed |
The activity of OsSHMT in scavenging H$_2$O$_2$ is considered to be prominent when the rice undergoes chilling stress. With scavenging of H$_2$O$_2$, OsSHMT was found to interact with a couple of proteins. Among these interacted proteins, APX is the one with ROS-scavenging activity. In Arabidopsis thaliana, the cytosolic ascorbate peroxidase 1 (APX1) is a central component of the H$_2$O$_2$-scavenging system, absence of APX1 in the knock-out lines lead to the collapse of the chloroplast H$_2$O$_2$ clearance system, thereby increasing levels of H$_2$O$_2$ and oxidative proteins [21]. The heat shock protein Hsp70 also interacts with OsSHMT. Hsp70 is a molecular chaperone protein and plays a critical role in stress tolerance [22]; in rice, chloroplast-localised Hsp70 is essential for chloroplast development under high-temperature conditions, whilst overexpression of mitochondrial HSP70 in rice suppresses programmed cell death [23, 24]. It is therefore suggested that the multiple functions of Hsp70 contribute to enhancing rice chilling tolerance. As ATP is required in the catalytic reaction to scavenging ROS, OsSHMT also interacts with subunit $\alpha$ and subunit $\beta$ of ATP synthase. These subunits are widely distributed in mitochondria and chloroplasts, and the synergistic effect of subunits function in produced ATP from ADP in the presence of a proton gradient across the membrane, which is generated by electron transport complexes of the respiratory chain [25], and the mitochondrial substrate carrier family proteins catalyse the passage of hydrophilic compounds such as ADP/ATP across the inner mitochondrial membrane [26]. The coordination of these proteins appears to provide ATP for OsSHMT activating in scavenging H$_2$O$_2$.

As the dominant role of OsSHMT in scavenging H$_2$O$_2$, increasing expression level of OsSHMT contributes to enhance its capacity. Gene expression of OsSHMT was higher in the Lsi1-OX rice than that in its wild-type Dular [6]. The different expression level of OsSHMT from these two rice lines mainly attributes to the expression of transcription regulators to OsSHMT. NABP and histone H1 were two transcription regulators binding on the promoter of OsSHMT. NABP has a dual role in regulation of gene expression at the transcriptional and posttranscriptional levels [27, 28]. Histones in eukaryotes are structural proteins that bind to DNA to construct chromatin nucleosomes. Histone H1 is commonly considered a transcriptional repressor because it prevents transcription factors and chromatin remodelling complexes from entering DNA [29]. Some other proteins, including AAA-ATPase family proteins and tubulin/FtsZ domain containing protein, were co-occurrence in these DNA-binding proteins. Tubulin is the main component of plant microtubules, which plays an important part in the regulation of stress tolerance and thus responds to a variety of intracellular and external stimuli [30–32]. Higher transcriptional levels of these genes in the Lsi1-OX rice than those in Dular, suggesting that these factors have a positive impact in

![Table 2](image)

**Table 2** The target protein from rdr6 interacted with OsSHMT identified by LC-MS
regulating the expression of OsSHMT gene. Whether these proteins are precisely or indirectly linked to the OsSHMT gene promoter remains to be revealed.

Overexpression of Lsi1 in the Dular results in the endoplasmic reticulum localisation of Lsi1-encoded Nod26-like intrinsic protein (OsNIP2;1). Since OsSHMT is also localised on the endoplasmic reticulum, the interactions of ATP synthase β subunit respectively with OsNIP2;1 and OsSHMT, indicating that OsNIP2;1 can indirectly cooperate with OsSHMT through the ATP synthase β subunit, and that OsSHMT interacts with defence and anti-oxidation related proteins to regulate the chilling resistance of rice and ensure its normal growth and development. The increase in OsSHMT expression

Fig. 5 Bimolecular fluorescence complementation validation of the bio-interaction of OsSHMT and ATP synthase subunit α, ATP synthase subunit β, Hsp70, MSCP and APX from rice. To validate the positive interaction of OsSHMT with ATP synthase α subunit (ATP-synα), ATP synthase β subunit (ATP-synβ), heat shock protein 70, mitochondrial substrate carrying family (MSCP) protein E and ascorbate peroxidase (APX) in rice, their genes were amplified from Dular rice and respectively infused with the N-terminal or C-terminal of YFP to construct YFP<sup>+</sup> and YFP<sup>+</sup>-containing recombinant vectors for bimolecular fluorescence complementation (BiFC), according to transient transformation in the leaves of *Nicotiana benthamiana*. The YFP fluorescence was detected using laser confocal microscopy under 488-nm excitation light.
Fig. 6 DAB staining of leaves of OsSHMT transgenic and wild type A. thaliana (a) and the H$_2$O$_2$ content in the leaves (b). OsSHMT transgenic Arabidopsis thaliana and its wild-type were exposed to 0 °C for 12, 24 and 36 h, and leaves were sampled and stained with DAB, after which the chlorophyll in the leaves was dissolved using ethanol, leaving dark brown precipitate on the leaf. Arrowheads indicate reddish-brown spots. Bar = 1 mm. The H$_2$O$_2$ content in the leaves was determined using an H$_2$O$_2$ determination kit (Solarbio Life Sciences).

Fig. 7 Bimolecular fluorescence complementation validates the bio-interaction of OsNIP2;1 and ATP-synthase Lsi1. Lsi1 was infused with the N-terminal domain of YFP and ATP synthase subunit β and OsSHMT was infused with the C-terminal domain of YFP. Lsi1 with N-terminal domain was then co-expressed with ATP synthase subunit β or OsSHMT with the C-terminal domain of YFP, respectively, in the leaves of Nicotiana benthamiana for 48 h, and YFP fluorescence was detected using laser confocal microscopy under 488-nm excitation light. The subcellular localisation of Lsi1 was also detected in the leaves of Nicotiana benthamiana.
and its constructive role in scavenging $\text{H}_2\text{O}_2$ provides partial assistance to rescue the loss of chilling resistance in Dular rice due to the inactivation of $\text{DUA1}$.

**Conclusions**

Overexpression of $\text{Lsi1}$ in chilling-sensitive Dular rice resulted in the plasma membrane-localized OsNIP2;1 protein expressed in the cytoplasm, which would enable OsNIP2;1 to perform multiple roles. The expression of OsSHMT was up-regulated in the $\text{Lsi1}-\text{OX}$ line in comparison with the Dular line. The differential gene expression level of OsSHMT may be transcriptionally regulated by NABP, and histone H1. In addition, OsSHMT interacts with APX, Hsp70, ATP- synthα, ATP- synthβ and MSCP to scavenge $\text{H}_2\text{O}_2$ and ATP- synthβ interacts with OsNIP2;1. Even OsSHMT does not directly interact with OsNIP2;1, ATP synthase subunit β is an intermediate junction between OsNIP2;1 and OsSHMT (Fig. 8).

**Methods**

**Plant materials and treatments**

In this study, Dular rice ($\text{Oryza sativa L. subsp. indica}$) and its transgenic line with $\text{Lsi1}$ overexpression ($\text{Lsi1-OX}$) were used; Dular rice is saved at Fujian Agriculture and Forestry University (Fuzhou, China) and the $\text{Lsi1-OX}$ transgenic line of Dular was germinated in our previous studies [33]. *Arabidopsis thaliana* with an RNA-dependent RNA polymerase 6 gene mutation ($\text{rdr6}$) [34] and *Nicotiana benthamiana* [35] were also used in this study.

The Dular and $\text{Lsi1-OX}$ rice seeds were sterilised with 25% sodium hypochlorite solution (W/V) for 30 min and washed with sterile water to remove any residues. The sterilised seeds were soaked overnight at 30°C in an incubator. The seeds were germinated and sown in black pots filled with a hydroponic nutrient solution suspended in a polyethylene mesh. The pots were placed in an artificial climate chamber for 10 days, during which a temperature of 26°C was maintained for 14 h and darkness was maintained for 10 h at 22°C. The relative humidity in the chamber was maintained at about 85%. The nutrient solution was changed weekly, and the pH was maintained between 5.5 and 6.0 throughout the experiment.

When the rice had reached the three-leaves stage, the $\text{Lsi1-OX}$ and wild-type Dular that treated at 12°C/10°C (day/night) were set as treatment groups, and the control groups were these two rice lines that placed in another chamber with day/night temperatures of 26°C/22°C. Both the treatment and the control groups for each rice line had four replicates and they were both repeated three times in the same growth chamber.

**Protein sub-localisation**

The coding DNA sequence of OsSHMT (LOC_Os03g52840) was amplified and fused with $\text{eYFP}$ in the pCambia2300 to construct a recombinant 35S::OsSHMT-$\text{eYFP}$ vector for rice protoplast transformations. Subcellular localisation of OsSHMT was detected using confocal laser scanning microscopy. An organelle-
specific protein marker (mcherry) was co-localised with OsSHMT to validate the above results. At the same time, a blank vector that contained only the yellow fluorescent protein gene was separately transferred into the rice protoplast to serve as a reference. The rice protoplasts were cultured at 28°C for 48 h. The distribution of yellow fluorescence in the protoplast was observed by laser confocal microscopy to determine the subcellular localisation of OsSHMT protein.

DNA pull-down fishes transcription regulators binding to the promoter of OsSHMT
The second-topmost leaves of the four replicates were respectively sampled at 48 h after chilling treatment. These rice leaves were quickly frozen in liquid nitrogen. The natural leaf proteins of Dular and Lsi1-OX were extracted using Pi-IP buffer (50 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA pH 8.0, 1% Triton X-100, 1 mM PMSF, 1x EDTA-free Protease Inhibitor Cocktail, Roche, Merck) (Method S1). The promoter region (2549 bp upstream of the CDS of OsSHMT) was cloned from the genomic DNA of Dular, and the interacting proteins on the promoter were obtained following the protocols of our previous studies [36].

Quantitative PCR to determine gene expression level
Total RNA from Dular and Lsi1-OX rice exposed to a temperature of 15°C for 12, 24 and 36 h was extracted using Trizol and reverse-transcribed into cDNA using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix. The control groups were grown at 26°C for 12, 24 and 36 h. Specific primers for tubulin/FtsZ domain containing protein (LOC_Os03g51600, LOC_Os05g34170, LOC_Os07g38730), histone H1 and nucleic acid binding protein (NABP) are listed in Table S3; the β-actin gene was taken as the reference. The qPCR reaction system was prepared using TransStart Tip Green qPCR SuperMix and an Eppendorf reaplex4 instrument. The reaction process was as follows: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing at 55°C for 15 s, extension at 72°C for 10 s; 42 cycles. When the amplification was finished, analysing of the melting curve was conducted and specificity of the product was determined based on the melting curve. Each candidate mRNA was set with four independent replicates. The relative expression of the gene was calculated by the 2–△△Ct method with the threshold cycle values (Ct) of each candidate mRNA in both the control and test samples [37].

Arabidopsis thaliana transformation
The CDS of OsSHMT was amplified and inserted into modified pCambia3301 (with 35 s promoter) to construct the recombinant vector for Arabidopsis thaliana transformation using the floral dip protocols described by Clough and Bent [38]. Natural leaf proteins were extracted from a T3 generation homozygote of OsSHMT transgenic A. thaliana and incubated with GFP-Trap agarose (Chromotek) to collect putative interacting proteins.

Bimolecular fluorescence complementation (BiFC) validates rice protein interactions
BiFC was conducted to validate the possible interactions between OsSHMT and the proteins identified from the Co-IP results. The genes that encode these proteins were cloned from Dular rice, construction of recombinant vectors for each gene and the subsequent protocols for BiFC are available in our previous studies [36].

DAB staining and determination of H2O2 content
Detection of H2O2 in leaves was conducted using diaminobenzidine (DAB) staining; OsSHMT transgenic A. thaliana and its wild type were treated at 4°C for 12, 24 and 36 h. The treatment was repeated three times in the same growth chamber. Leaves from these two lines were sampled and immersed in 50 mg/L DAB solution by vacuum-pumping for 1 h and incubated overnight at room temperature. These leaves were then decolourised with 95% ethanol in a water bath at 80°C, and the reddish brown spots in the transgenic leaves and wild-type leaves were observed using an integrated microscope (Nikon, SMZ18). The H2O2 content in the 12 h-treated leaves of the transgenic line and the wild type were determined using an H2O2 determination kit (Solarbio Life Sciences).

Primer sequences
A list of the primers is provided in Table S3.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12870-020-02446-9.

Additional file 1 Fig. S1 Subcellular localisation of OsSHMT protein in rice protoplast
Additional file 2 Table S1 Sequence of OsSHMT gene promoter from Dular. Table S2 Proteins interacted with OsSHMT in the OsSHMT transgenic A. thaliana using GFP-Trap Co-IP. Table S3 Primers used in this study
Additional file 3 Methods S1 Extraction of rice leaf protein
Additional file 4 Electronic Supplementary Material 1. Full length gel presents proteins binding on the OsSHMT-promoter in Dular
Additional file 5 Electronic Supplementary Material 2. Full length gel presents proteins binding on the OsSHMT-promoter in Lsi1-OX transgenic line
Additional file 6 Electronic Supplementary Material 3. Full length gel presents protein interactions with OsSHMT in Arabidopsis thaliana

Abbreviations
APX: Ascorbate peroxidase; BiFC: Bimolecular fluorescence complementation; DAB: Diaminobenzidine; ER: Endoplasmic reticulum; Lsi1: Low silicon gene 1;
Ox: Oxidative stress; SHMT: Serine hydroxymethyltransferase; NIP: Nodulin 26-like intrinsic protein; ROS: Reactive oxygen species; NABP: Nucleic acid binding protein; HSP70: Heat shock protein 70

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Authors’ contributions

CF and WL designed the experiments. PZ, LL and CF performed most of experiments and analyzed the data. LY, DM, XY and ZL assisted in experiments and discussed the results. CF and WL wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated during this study are included in this published article and its supplementary information files, and the raw data used or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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