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Alteration of S-nitrosothiols mediates seed shattering in rice

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Abstract

Background

Seed shattering in rice (*Oryza sativa*) is considered as one of the major factors responsible for domestication of rice. The shattering trait of rice results in severe reduction in yield related parameters such as number of tillers and number of spikelets. A few genes have been characterized for their involvement in the regulation of seed shattering in rice, however, the importance of seed shattering from yield perspectives necessitates the exploration of novel genes responsible for seed shattering in field crops like rice.

Results

We identified the *RICE HIGH SHATTERING 1 (RHS1)* from a greenhouse screen involving 130 rice lines. High seed shattering phenomena was observed in *rhs1* plants. Moreover, absence of *RHS1* resulted in sensitivity to nitrosative stress. In addition, *rhs1* plants demonstrated differential expression levels of genes responsible for nitric oxide (NO) production (*OsNIA1*, *OsNIA2*, *OsNOA1* and *OsGSNOR*) and seed shattering (*SHAT1*, *qSH1*, *qSH4* and *CPL1*). Of those, the *CPL1* expression was correlated with *S*-nitrosothiol (SNO) alteration in *rhs1*. Interestingly *noe1*, rice mutant with high SNO levels, exhibited low seed shattering, whereas *rhs1* resulted in low SNO level with high seed shattering.

Conclusion

This study reports the involvement of a novel gene *RHS1* in seed shattering. Most of the seed shattering related genes or QTLs reported so far in the literature positively regulate seed shattering as their knockout or loss of function mutants exhibit non-shattering phenotypes. Whereas, *RHS1* negatively regulates shattering as the loss of function *rhs1* plants exhibit high seed shattering and perturbed SNO contents. The molecular and mechanistic control of *RHS1* mediated regulation of seed shattering and its interaction with nitric oxide needs to be investigated further.

Keywords: Seed shattering, rice, nitric oxide, *S*-nitrosothiol (SNO)

Background

Rice (*Oryza sativa*), one of the major crops used as staple food around the world, feeds more than 50% of the world's population and is the most rapidly growing cereal source to overcome the scarcity of food after wheat [1]. Traits like water requirement, early maturity, growth speed and habit, plant height, strength of the stem, seed size and nutrient composition, starch and protein content in the grain, and various other properties related to plant architecture played very important roles in the domestication of rice in Asia and Africa, leading to the adaptation of two major types of rice species i.e. *Oryza sativa* and *Oryza glaberrima*, respectively [2]. Beside these, seed shattering is considered as one of the major factors responsible for domestication of rice [3] through conscious and/or unconscious selection process throughout the history. The shattering trait of wild rice results in severe reduction in yield related parameters. On the other hand, if upon maturity, the seeds stay attached to the panicle for longer duration or until harvesting and threshing, will increase the yield significantly [4, 5]. Seed shattering thresholds are also considered important in designing harvesting and threshing machinery in order to minimize yield loss. Various studies on rice seed shattering have been conducted based on molecular markers describing seed shattering as a quantitative trait controlled by unknown genes [6]. However, Zhou et al [7] showed that the loss of function mutant of the quantitative trait locus on chromosome 1 (*qSH1*) which encodes a BEL1-type homeobox protein, exhibits loss of seed shattering by inhibiting the formation of an abscission layer at the base of the grain. Similarly *SH4*, which encodes a trihelix family transcription factor has been shown to promote hydrolysis of abscission zone (AZ) cells during the abscission process [3]. Besides, *OsCPL1*, a repressor of abscission layer differentiation and a recessive gene for seed shattering has been reported [8] Afterwards, the *SEED SHATTERING ABORTION 1* (*SHAT1*) which encodes an APETALA2 (AP2) transcription factor, was shown to be required for seed shattering in rice as it controls the development of AZ in spikelets and

its expression is positively regulated by *SH4* [7]. The AP2 transcription factors are conservatively spread across the plant kingdom and are involved in diverse physiological processes, metabolic pathways, hormone biosynthesis, and biotic and abiotic stresses [9]. In addition, most of the seed shattering genes have been reported such as *SHATTERPROOF1* (*SHP1*), *SHP2* and *INDEHISCENT* (*IND*) from Arabidopsis, *SHATTERING 1-5* and *PDH1* from soybean, *qSH1* and *SH4* from rice are categorized as transcription factors [10]. Thus, these transcription factors have been considered valuable targets for genetic manipulation in order to incorporate desired traits in crop plants through genetic engineering [11].

Nitric oxide (NO), a free radical, has drawn attention as a key signaling molecule in animals as well as in plants. Recently, comprehensive transcriptomic studies have been released to help understand the incorporating mechanisms of NO. These studies demonstrated that exogenous NO application affects regulation of genes which are involved in many important physiological pathways such as redox signaling, hormone signaling, various protein kinases, and transcription factors [12, 13]. Various nitrogen-containing compounds influence seed dormancy and germination [14]. As seed dormancy is regulated by different phytohormones, the role of NO in modulating the levels of different phytohormones under normal and stress conditions is well known. Significant amount of research exploring the role of nitric oxide (NO) in mediating different physiological processes in plants has been conducted. However, scientific information about the relationship of NO with seed shattering is not available. NO-mediated signaling is carried out by various NO derivatives such as NO radical ($\cdot\text{NO}$), nitroxyl (NO^-), peroxynitrite (ONOO^-), and *S*-nitrosothiols [15]. These molecules react with oxygen, superoxide, and proteins especially to a cysteine residue which is called as *S*-nitrosylation [16]. *S*-nitrosylation, a main source of NO transferring in cellular levels, has been found in many proteins as post-translational regulation in plants as well as in mammals [17-19]. The crucial roles of SNO in mammals have been reviewed by MW Foster, DT Hess and JS Stamler [20].

Although concept of the research being originated from mammals, various roles of *S*-nitrosylated or de-nitrosylated proteins in regulating plant biological and physiological processes, including plant development and defense responses, have been substantiated. One of the well-known bioavailable NO sources in plant cells is *S*-nitrosoglutathione (GSNO), the endogenous *S*-nitrosothiol; which releases NO when and where it is required in cells and is responsible for its homeostasis via GSNO reductase, which controls SNO levels in plants through de-nitrosylation. Plant cells exposed to biotic or abiotic stresses undergo oxidative modification by ROS and these modifications might be avoided by NO through *S*-nitrosylation. However, excessive oxidative stress might result in dysregulation of NO mediated processes, leading to perturbed physiology [21]. Underlying mechanisms between SNO and physiological responses are still largely unknown. Recently, rice NO accumulation mutant, *nitric oxide excess 1 (noe1)* exhibiting higher contents of NO, increased the H₂O₂ levels and these factors lead to leaf cell death in rice suggesting a role of NO and SNO in regulation of H₂O₂-induced cell death [22].

Significant amount of research exploring the role of nitric oxide (NO) in mediating different physiological processes in plants has been conducted. However, scientific information about the relationship between NO and SNO levels with seed shattering and defense responses has not been revealed yet. In this study, we characterized 130 different rice Ac/Ds transposon mutant lines in Dongjin background and for the first time we report a linkage between nitric oxide, endogenous SNO levels and seed shattering in rice.

Results

***RHS1* is required for normal growth and development in rice**

While screening the Ac/Ds transposon mutant rice lines we found that a rice line with loss of function mutation in the locus *LOC_Os04g41250 (rhs1)* exhibits high seed shattering along

with other interesting phenotypic characteristics. *RHS1* is a zinc finger gene encoding an armadillo/beta catenin repeat family protein. The gene consists of 5' and 3' UTR regions and 4 introns between the translational start and stop codons (Additional File 1). Quantitative real time PCR analysis showed that *RHS1* is mainly expressed in seeds with at least 1000-folds high expression compared to leaves and stems. Highest expression was recorded in mature seeds followed by seeds in the milking stage and then roots. (Figure 1A & 1A inset). Rice *rhs1* plants were relatively shorter in height (Additional file 1). Loss of *RHS1* function also resulted in significant reduction in the number of tillers as compared to WT plants (Figure 1B) and spikelets per spike (Figure 1C) while these plants also produced shorter culms and panicles (Figure 1D).

Loss of *RHS1* function leads to seed shattering and increases sensitivity to nitrosative stress

Seed shattering data revealed that *rhs1* seeds needed 35 and 50 percent less vertical and horizontal force respectively to pluck them from inside the spikelet as compared to WT (Dongjin) and the other two cultivars, Wonkwang and Drimi-2ho (Figure 2A) being the most cultivated rice varieties in South Korea. Wonkwang and Drimi-2ho were used as control check of WT and further experiments were focused in the comparison of *rhs1* and either WT. In a previous study in our lab [23], the transcriptomic analysis of rice induced by 1 mM CysNO, revealed *RHS1* to be down-regulated (Additional file 2; Table S2). Accordingly, NO was applied exogenously to investigate any linkage among NO, *RHS1* and shattering related known genes. As a result, the expression of shattering related genes—*qSH1* and *SHAT1* was significantly lower in *rhs1* plants compared to WT plants under normal conditions. Apart from that, nitrosative stress negatively regulated the expression of *qSH1* and *SHAT1* in both WT and *rhs1* plants but positively regulated *qSH4* expression in *rhs1* plants only (Figure 2B).

For this purpose, WT and *rhs1* plants were exposed to 10 and 50 mM CySNO. The *rhs1* seedlings showed an overall poor growth and exhibited acute sensitivity to nitrosative stress with leaves turning yellow within 12h of treatment and leaf tips became dried, necrotic and turned brown eventually leading to death of the plants within 24 h (Figure 3A). On the other hand, WT plants showed less symptoms of nitrosative stress. This indicates that the absence of *RHS1* function makes plants sensitive to nitrosative stress either due to higher accumulation of NO at cellular level or its associated forms or due to loss of NO scavenging ability. Quantitative real time PCR analysis of nitric oxide related genes showed that in *rhs1* plants, the expression levels of genes responsible for NO production (*OsNIA1*, *OsNIA2*, and *OsNOA1*) were lower as compared to *OsGSNOR* under normal conditions (Figure 3B-E). However, after treatment with the nitric oxide donor, we found a gradual increase in the expression of *OsNIA2* which is responsible for the maximum amount of nitrate reductase activity or NO production through the reductive pathway (Figure 3E). Though the *OsNIA1* (Figure 3B) and *OsNOA1* (Figure 3C) showed slight variations in their expression following nitrosative stress, the 10 mM CysNO proved to be the terminal dose for a significant increase in their expression at 48 h post treatment. These results were further supported by the lower expression of *OsGSNOR* in the *rhs1* mutants (Figure 3D).

High seed shattering in *rhs1* is associated with SNO contents

The higher relative expression of *OsGSNOR* and the lower expression of NO production genes in *rhs1* plants under normal conditions indicated a possible involvement of nitric oxide or its derivatives in seed shattering. Our results revealed significantly higher shattering value for the *noe1* (*nitric oxide excess 1*) mutant as compared to the WT and *rhs1*, indicating that the *noe1* need higher force both vertically and horizontally to pluck the seeds from the spikelet (Figure 4A). Therefore, in order to investigate a possible role of endogenous S-nitrosothiol (SNO) content and seed shattering, we measured the total SNO content of *rhs1*, WT (Dongjin and

Nipponbare) and the NO overproducing mutant *noe1* plants. The results showed significantly lower SNO levels in the *rhs1* mutant lines while *noe1* exhibited significantly higher SNO contents as compared to the wild type and *rhs1* (Figure 4B) indicating that alteration of SNO content affect the seed shattering. This result was further supported by the significantly lower expression of *CPL1* (a negative regulator of seed shattering which represses the development of the abscission layer required for seed shattering) in *rhs1* plants and significantly higher expression in *noe1* plants (Figure 4C). Together all, our findings reveal that the loss of *RHS1* function improves seed shattering in rice where endogenous SNO contents of *rhs1* plants may play an important role directly or indirectly.

Discussion

Seed shattering is the natural detachment and dispersal of seeds upon reaching physiological maturity. Scientific studies in the literature suggest that loss of shattering in crops resulted due to genetic variations or mutations occurring over a long period of time due to which plants tend to retain the seeds after physiological maturity, hence making harvesting possible [24]. Genetic control of seed shattering has been investigated in all major crops such as barley [25, 26], rice [3, 27-29], millet [30], buckwheat [31], wheat [32-35], maize [36], rye [37], sorghum [38] and many others. Among them, most of the genes are affiliated with transcription factors.

Nitric oxide is a free radical and acts as a small signaling molecule. A number of studies with exogenous NO application demonstrated that it affects regulation of genes involved in diverse physiological pathways such as redox signaling, hormone signaling, various protein kinases, and transcription factors [12, 13]. Also known to regulate seed dormancy, germination and pollen development by controlling hormone signaling along with other features of plant reproductive structures [39]. However, information about NO-mediated seed shattering is not available so far. To investigate linkage between nitric oxide and seed shattering, we screened

145 Ac/Ds transposon mutant rice lines in *Dongjin* background for their phenotypical characteristics where we found a rice mutant exhibiting abnormal growth and high seed shattering trait (fig 1. A). *rhs1* encodes a putative armadillo/beta-catenin repeat family protein and was found in our previous transcriptomic data (Mun et al. 2018), to be down-regulated by almost 2 folds (Table S2 in additional file 2). In addition, according to its annotation, is known to be involved in cadherin-associated cell adhesion to the cytoskeleton in *Drosophila* and that these proteins are involved in a broad range of biological functions. Recently, some genes of ARM repeat family have been reported to be involved in GA signaling and ABA-dependent gene expression in plants [40, 41]. Moreover, based on protein sequence similarity, *RHS1* orthologues revealed their presence in *Arabidopsis*, maize and other plant species. Depending on the gene expression result shown in Fig.1 B, *rhs1* is highly expressed in the seeds compared to stem, leaves and roots. Interestingly. Shattering related genes such as *qSH1* (LOC_Os01g62920), *SH4* (LOC_Os04g57530) and *SHAT1* (LOC_Os04g55560) also follow the same expression patterns with maximum RNA-Seq FPKM expression values in the inflorescence for *qSH1* and *SH4* and in the endosperm for *SHAT1* (<http://rice.plantbiology.msu.edu/index.shtml>). However, the unique function of *RHS1* is that it negatively regulates seed shattering as contrary to the other genes mentioned above that positively regulate seed shattering, as their loss-of-function mutant plants exhibited the non-shattering phenotypes.

The *RHS1* locus identified in this study not only regulates seed shattering but also affects other growth parameters (Fig. 1. C-D). In general, plants with impaired redox balance or lose-of-function of essential gene(s) show abnormal growth and phenotype. With ROS, Nitric oxide is an important redox-active molecule which plays important role in various physiological processes as has been revealed by numerous studies involving plants accumulating high or low levels of NO in *Arabidopsis*. Literature indicates that plants with a stunted phenotype

(Additional File 1), perturbed pollination, and various disturbed floral and seed phenotypes are caused due to changes of endogenous NO [42]. In rice, Lin et al. reported that *noe1* which generate more NO in plant exhibited PCD (programed cell death) -like phenotype. Taken all together, we expected that the abnormal phenotype and high seed shattering of *rhs1* could be caused by either change in the NO contents or NO related signaling pathway. Therefore, we investigated the response of *rhs1* plants to nitrosative stress. As revealed by Fig 2B, the expression levels of the two transcription factor genes, *qSH1* and *SHAT1* were down regulated in the presence of NO in both WT and *rhs1*. However, absence of *rhs1* resulted in increased expression of *qSH4*. In addition, high dose of nitrosative stress to *rhs1* plants resulted in higher sensitivity. These results suggest that NO, as one of the master signaling molecule, possibly mediates regulation of shattering related genes and also *rhs1* may associate with NO either directly or indirectly. Furthermore, *rhs1* seedlings exhibited a gradual increase in the expression of *OsNIA1* and *OsNIA2* in response to NO (Figure 3). Our results also reveal the higher expression of *OsGSNOR* and lower expression of NO production associated genes in *rhs1* as compared to the WT under normal conditions. We found that absence of *RHS1* leads to failure of adjustment in response to exogenous NO application. We further found that the loss of *RHS1* function leads to a reduction in endogenous SNO contents. Upon demonstration, it was revealed that the high SNO containing rice mutant *noe1* exhibited higher shattering value while *rhs1* had lower SNO content and lower shattering value. These results indicate a possible involvement of endogenous SNO contents in seed shattering. Moreover, the expression of *OsCPL1*, the negative regulator of seed shattering by repressing abscission layer differentiation in the process of shattering, was lower in the loss of function *rhs1* while significantly higher in the NO over accumulator *noe1* as compared to both of the wild type rice plants. Together all, these results reveal that *RHS1* negatively regulates seed shattering and that higher endogenous SNO levels may add to the lower seed shattering in rice.

Conclusion

This study reports the involvement of a novel gene *RHS1* in seed shattering. As mentioned earlier, most of the shattering related genes or QTLs reported so far in the literature positively regulate seed shattering as their knockout or loss-of-function mutants exhibit non-shattering phenotypes. Whereas, *RHS1* negatively regulates shattering as the loss of function *rhs1* plants exhibit high seed shattering with perturbed SNO contents. The molecular and mechanistic control of *RHS1* mediated regulation of seed shattering and its interaction with nitric oxide needs to be investigated further.

Methods

Plant material and phenotype evaluation

A population of 130 Ac/Ds transposon mutant rice lines in Dongjin background was obtained from Rural Development Administration (RDA), Korea. Initial screening revealed that the loss of function transposon mutant line of rice *LOC_Os04g41250* which encodes a putative armadillo/beta-catenin repeat family protein, exhibit high seed shattering and therefore, named as the *Rice High Shattering 1 (RHS1)*. Plants were grown in paddy at research field of Kyungpook National University, Gunwi, Korea. Two locally cultivated rice cultivars “Woonkwang” and “Drimi” were also cultivated together in the field as control. Normal agricultural practices were adapted for growing these cultivars. Data on phenotypic characteristics such as average number of tillers per plant, culm and panicle length, and number of spikelets per panicle were recorded for each line. For analyzing the response to nitrosative stress, rice seeds were sterilized with 25% prochloraz and germinated on sterile wet tissue papers for 12 days at 24°C. The plants were then transferred to 13 cm × 9 cm 6-well tissue culture plates (SPL, Korea) containing CySNO for 6, 12, and 24 h at 24 °C.

273   **Nitrosative stress**

274   CySNO solution was prepared by mixing equal volumes of 1 M L-cysteine dissolved in 1 N
275   HCL and 1 M sodium nitrate (NaNO_2). The resulting solution was diluted with 40 mM EPPS
276   (pH 7.8) to make 10 mM CySNO. This way, 2 weeks old MS grown plants were treated with
277   10 mM and 50 mM CySNO in 6 well plates for 6, 12 and 24 h. The plates were kept in dark to
278   prevent decomposition of CySNO.

279   **Measurement of seed shattering**

280   Rice seed shattering degree was measured as described by Qin et al [5]. Briefly, primary tillers
281   of rice plants were harvested 45 days after the start of heading and kept at room temperature
282   for a week or until the moisture of spikelets reached approximately 20% to 25%. Twenty grains
283   at three different points on each panicle were analyzed for their breaking tensile strength (BTS)
284   and the pulling strength (PS) required to shear them off their pedicels. For this purpose the
285   pedicle of each grain was fixed upside down to digital force gauge (Model No. DS2-5N,
286   IMADA, Japan) and the grain was pulled down with forceps. The value displayed by the digital
287   force gauge was recorded at the moment of pedicle breakage in gravitational force unit (gf).

288   **SNO measurement**

289   To measure SNO contents, rice plants were grown for four weeks with Hoagland solution in
290   Magenta box and then five plants from each box were harvested. Samples were ground with
291   liquid nitrogen until fine powder was obtained. Then 1ml of extraction buffer (1×PBS pH 7.4)
292   was added to the powdered samples and mixed well. The samples in PBS were centrifuged at
293   13,500 g for 10 min at 4°C. The supernatant was transferred and centrifuged again at 15,900 g
294   for 10 min at 4°C. Proteins were quantified by Bradford assay method using Coomassie
295   (Bradford) protein assay kit (Thermo Fisher Scientific) according to manufacturer's manual.
296   Briefly, 1.5 mL of Coomassie dye reagent was added to 30 µl of extracted protein and mixed
297   thoroughly. Optical density (OD) of all the samples were measured with spectrophotometer

(OPTIZEN α , Mecasys, Korea) at 595 nm. For SNO measurement, 100 μ L of the extracted proteins were injected into the reaction vessel of the Nitric Oxide Analyzer (NOA 280i, GE Water & Process Technologies, Ratingen, Germany) containing CuCl/cysteine reducing agent. The S-nitrosothiol concentration was derived from a standard curve generated by CySNO. As final step, SNO level was calculated as nM/ μ g protein.

RNA extraction and RT-PCR

RNA was extracted from control and treated plants using Trizol® (Invitrogen, USA) according to manufacturer's instructions. cDNA was synthesized using cDNA synthesis kit (PhileKorea, Korea) according to manufacturer's instructions. Quantitative real time polymerase chain reaction (qRT-PCR) was performed in Eco^{TM} real-time PCR machine (Illumina, USA) using 2x Quantispeed SYBR MIX (PhileKorea, Korea) along with 100 ng of template DNA and 10 nM of each primer in a final volume of 20 μ l in a two-step PCR reaction for 40 cycles under the following conditions. Polymerase activation at 95°C for 2 min, subsequent denaturation steps at 95°C for 5 seconds and annealing and extension at 65°C for 30 seconds. Rice ubiquitin (*UBI*) and a "no template control (NTC)" were used as a control. Primer sequences are given in Table S1.

Statistical analysis

Each experiment was performed at least three time with multiple replicates. Means were analyzed for significant difference using the student's T-test.

Abbreviations

QTL: Quantitative Trait Locus; BEL1-type: Bentazon Lethal 1-type; CPL1: C-terminal domain Phosphatase-Like 1; AZ: Abscission Zone; PDH1: Prephenate Dehydrogenase 1; NO: Nitric Oxide; SNO: S-Nitrosothiol; CySNO: S-Nitrosocystein; GSNOR: S-Nitrosoglutathione Reductase; ROS: Reactive Oxygen Species; Ac/Ds: Activator/Dissociation; PCR: Polymerase Chain Reaction;

Declarations

Ethics approval and consent to participate

Not applicable

327 **Consent of publication**

328 Not applicable

329 **Availability of data and materials**

330 All data generated or analyzed during this study are included in this published article [and its
331 supplementary information files. Any other relevant information can be obtained from the
332 corresponding author on reasonable request.

333 **Competing interests**

334 The co-corresponding author (Dr. Adil Hussain) of the current manuscript is the Associate
335 Editor of BMC Plant Biology Journal.

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341

342 **Authors' contributions**

343 BGM and BWY designed the study. BGM, SUL and HHK performed experiments. BGM and
344 MS analyzed the data, BGM and AH wrote the manuscript. BGM, MS and AH revised
345 manuscript. GSL and BWY supervised the overall experiment and revised the manuscript.
346 All authors read and approved the final manuscript.

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Figure legends

Figure 1. *RHS1* is expressed in seeds and regulates plant development.

Expression of *RHS1* (LOC_Os04g41250) was highest in mature seeds followed by milk-stage seeds and roots (A). Loss of *RHS1* function negatively affects the overall yield and plant development as *rhs1* plants produced less number of tillers per plant (B) and number of spikelets per spike (C) and showed a stunted phenotype (D). Each data point represents the mean of three replications. Means were analyzed for significant difference using the student's T-test. Error bars represent standard deviation. Significant differences are represented by asterisk (*).

Figure 2. *RHS1* regulates seed shattering in rice.

Seeds of *rhs1* plants showed significantly lower shattering value in terms of the vertical and horizontal force required to mechanically detach them from the base of the spikelet (A). Significant variation was observed in the expression of shattering-related genes in *rhs1* plants under normal and nitrosative stress conditions (B). Each data point represents average of 3 data points. Means were analyzed for significant difference using the student's T-test. Error bars represent standard deviation. Significant differences are represented by asterisk (*).

Furthermore, we evaluated the sensitivity of *rhs1* mutants to nitrosative stress.

Figure 3. Response of *rhs1* seedlings to nitrosative stress.

Seedlings of *rhs1* tested for their response to nitrosative stress induced by 10 and 50 mM CysNO (A). Expression of rice *NIA1* (B), *NOA1* (C), *GSNOR* (D) and *NIA2* (E) before and after nitrosative stress treatment. Each data point represents the mean of three replicates. Means were analyzed for significant difference using the student's T-test. Error bars represent standard deviation. Significant differences are represented by asterisk (*).

Figure 4. *RHS1* regulates seed shattering and SNO contents in rice.

Seeds of *rhs1* plants showed significantly lower shattering value in terms of the vertical and horizontal force required to pluck them from the base of the spikelet (A). Endogenous level of SNO was significantly lower in *rhs1* plants compared to WT and *noe1*, a high SNO containing mutant (B). Expression of rice *CPL1*, the negative regulator of seed shattering (C). Each data point represents average of 3 data points. Means were analyzed for significant difference using the student's T-test. Error bars represent standard deviation. Significant differences are represented by asterisk (*).

Additional files

Additional file 1: Figure S1. Phenotypic comparison of the indicated genotypes.

Figure S2. Genetic structure (A) and verification of *rhs1* (B).

Additional file 2: Table S1. Primer sequences used for qRT-PCR in the study.

Table S2. Expression of *RHS1* in *A. thaliana* and rice after CysNO infiltration and its orthologs in different plant species.

Figures

Figure 1

RHS1 is expressed in seeds and regulates plant development. Expression of *RHS1* (LOC_Os04g41250) was highest in mature seeds followed by milk-stage seeds and roots (A). Loss of *RHS1* function negatively affects the overall yield and plant development as *rhs1* plants produced less number of tillers

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