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Research article

Rice OsBT1 regulates seed dormancy through the glycometabolism pathway

Weihan Song[a], Qixian Hao[a], Mengying Cai[a], Yihua Wang[a], Xingjie Zhu[a], Xi Liu[a], Yunshuai Huang[a], Thanhliem Nguyen[a,c], Chunyan Yang[a], Jiangfeng Yu[a], Hongming Wu[a], Liangming Chen[a], Yunlu Tian[a], Ling Jiang[a,**], Jianmin Wan[a,b,*]

[a] State Key Laboratory of Crop Genetics and Germplasm Enhancement, Research Center of Jiangsu Plant Gene Engineering, Nanjing Agricultural University, Nanjing, 210095, China
[b] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100081, China
[c] Department of Biology and Agricultural Engineering, Quynhon University, Quynhon, Binhdinh, 590000, Viet Nam

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ABSTRACT

Seed dormancy and germination in rice (*Oryza sativa* L.) are complex and important agronomic traits that involve a number of physiological processes and energy. A mutant named *h470* selected from a ^{60}Co-radiated *indica* cultivar N22 population had weakened dormancy that was insensitive to Gibberellin (GA) and Abscisic acid (ABA). The levels of GA_4 and ABA were higher in *h470* than in wild-type (WT) plants. The gene controlling seed dormancy in *h470* was cloned by mut-map and transgenesis and confirmed to encode an ADP-glucose transporter protein. A 1 bp deletion in *Os02g0202400* (*OsBT1*) caused the weaker seed dormancy in *h470*. Metabolomics analyses showed that most sugar components were higher in *h470* seeds than the wild type. The mutation in *h470* affected glycometabolism.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food resources for humankind (Hong et al., 2019), and the level of production affects food security. Increased yield and superior processing quality are major foci of research (Wang et al., 2015). Seed dormancy in rice is an important agricultural trait on account of its association with pre-harvest sprouting (PHS), seedling vigor, quality and yield as in many different cereals (Nonogaki et al., 2018).

Seed dormancy is affected by many factors and is controlled by multiple proteins (Allen et al., 2018; Nonogaki et al., 2018). Numerous studies have shown a close correlation between seed dormancy and phytohormones. Among them, GA and ABA are particularly important in regulating seed dormancy (Urbanova and Leubner-Metzger, 2018). Mutants *ga1* and *ga2* in Arabidopsis cause defective GA biosynthesis that results in strong dormancy and sensitivity to exogenous GA (Holdsworth et al., 2008). Overexpression of *GID1* (*GA INSENSITIVE DWARF1*, GA receptor) significantly decreased seed dormancy (Hauvermale et al., 2015). A *SnRK2.2SnRK2.3SnRK2.6* (*Sucrose Non-fermenting1–Related Protein Kinase*, activated by ABA) triple mutant was insensitive to ABA and showed weak seed dormancy in humid conditions (Fujii and Zhu, 2009). A number of quantitative trait loci (QTLs) controlling seed dormancy in rice have also been reported, but only three, named *Sdr4*, *qSD7-1/qPC7* and *qSD1-2* have been isolated (Sugimoto et al., 2010; Gu et al., 2011; Ye et al., 2015). *Sdr4* was the first map-based cloned gene in rice, but the function of the protein was largely undetermined although possibly regulated by *OsVP1* (positive regulators in the ABA signaling pathway). *Sdr4* positively regulates expression of *OsDOG1-like* (*DELAY OF GERMINATION 1*, orthologous to *Arabidopsis thaliana DOG1*) genes during seed maturation, suggesting that it acts as an intermediate regulator of seed dormancy (Sugimoto et al., 2010). *qSD7-1/qPC7* defined as the *Os07g11020* or *Rc* gene encoding a basic helix-loop-helix (bHLH) transcription factor, functions in the regulation of seed dormancy through ABA biosynthesis and pigmentation pathways (Gu et al., 2011). *qSD1-2* encodes *GA20ox2*, which is involved in GA metabolism and regulates seed dormancy (Ye et al.,

Abbreviations: WT, wild-type; PHS, pre-harvest sprouting; ABA, abscisic acid; QTLs, quantitative trait loci; DPH, days post heading; QRT-PCR, quantitative real-time PCR; MCF, mitochondrial carrier family; HAI, hours after imbibition; SNPs, single nucleotide polymorphisms; InDels, insertions/deletions; Nip, nipponbare; PP2C, protein phosphatase 2C; TCA, tricarboxylic acid

* Corresponding author. State Key Laboratory of Crop Genetics and Germplasm Enhancement, Research Center of Jiangsu Plant Gene Engineering, Nanjing Agricultural University, Nanjing, 210095, China.
** Corresponding author.
E-mail addresses: jiangling@njau.edu.cn (L. Jiang), wanjm@njau.edu.cn (J. Wan).

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2015). It is important to explore more genes that regulate seed dormancy in rice.

The *BT1* gene was first identified in a *brittle-leaf* maize (*Zea mays*) mutant (Sullivan et al., 1991). BT1 proteins in cereal endosperms were proposed to be ADP-glucose transporters, major amyloplast membrane proteins containing three mitochondrial carrier family (MCF) domains (Haferkamp, 2007). *BT1* was also characterized in many other species including *Arabidopsis thaliana* (Kirchberger et al., 2008) and *Triticum aestivum* (Bowsher et al., 2007). Other research confirmed that *BT1* is important in starch biosynthesis where it affects the formation of composite starch granules in rice seeds (Cakir et al., 2016; Li et al., 2017). However, there was no report suggesting that *BT1* affected seed dormancy.

Here, we characterized a weakly dormant mutant, *h470*, which was insensitive to applied GA and ABA. GA and ABA contents were both increased in *h470* relative to the WT. Mut-map and transgenesis studies showed that a mutation in *OsBT1* caused the *h470* phenotype. Metabolomics studies showed that the mutation affected seed dormancy by its effects on glycometabolism.

2. Materials and methods

2.1. Plant materials and growth conditions

The weakly dormant mutant *h470* was selected from the strongly dormant *indica* cultivar N22 following ^{60}Co irradiation. The *h470* mutant line with stable inheritance was established after several generations of self-crossing. A cross was made between *h470* and N22 to produce an F_2 population for subsequent mut-mapping. Rice seedlings were grown in a paddy field at Nanjing Agricultural University in 2018.

2.2. Germination test and trait measurement

Seeds of N22 and *h470* and individual F_2 plants were harvested at 7, 14, 21, 24, 28, and 35 days post heading (DPH) for the germination experiment. Fifty seeds of each parent and F_2 plant were put on filter paper in a 9 cm Petri dish to which 10 mL of water was added. The dishes were held in darkness at 30 °C and each germination test was replicated three times. Germination percentages were determined on the seventh day. Grain lengths and widths were measured by a Wansen automatic rice test analyser (Hangzhou). Plant height, numbers of tillers and grain number per panicle were recorded at 35 DPH. There were three biological repeats for each trait.

2.3. Evaluation of sensitivity to exogenously applied GA and ABA and quantification of endogenous GA_4 and ABA

N22 and *h470* seeds at 24 DPH were used for endogenous treatment experiments. Germination trials were set up as described above but different concentrations of GA_3 (0, 1, 10 μM) and ABA (0, 1, 4 μM) were added to the dishes. Germination percentages were recorded each day for 7 day at 30 °C. GA_4 and ABA levels were measured in WT and *h470* seeds collected at 24 DPH. Assays were carried out by ZhongDing Biological Company (Nanjing). GA_4 and ABA were extracted from sampled seeds using an isopropyl alcohol/water/hydrochloric acid extraction method, and analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) (Izumi et al., 2009). Three biological repeats were made for each sample.

2.4. Identification the mutant gene in h470

Thirty F_2 plants from the *h470* × N22 cross were selected and placed into weak and strong dormancy groups according to phenotype. Young leaves from each type were cut and pooled with equal numbers for whole-genome resequencing by Genepioneer Biotechnologies Company (Nanjing) using Illunima HiSeqXTen (Illumina, http://www.illumina.com/), the mut-map was performed according to a previous method based on whole-genome resequencing of pooled DNA from a segregating population of plants that showed significantly different phenotypes. The SNP/InDel ratio was determined as described previously (Abe et al., 2012). The 58.76 Gb of data showed 300 single nucleotide polymorphisms (SNPs) and 68 insertions/deletions (InDels).

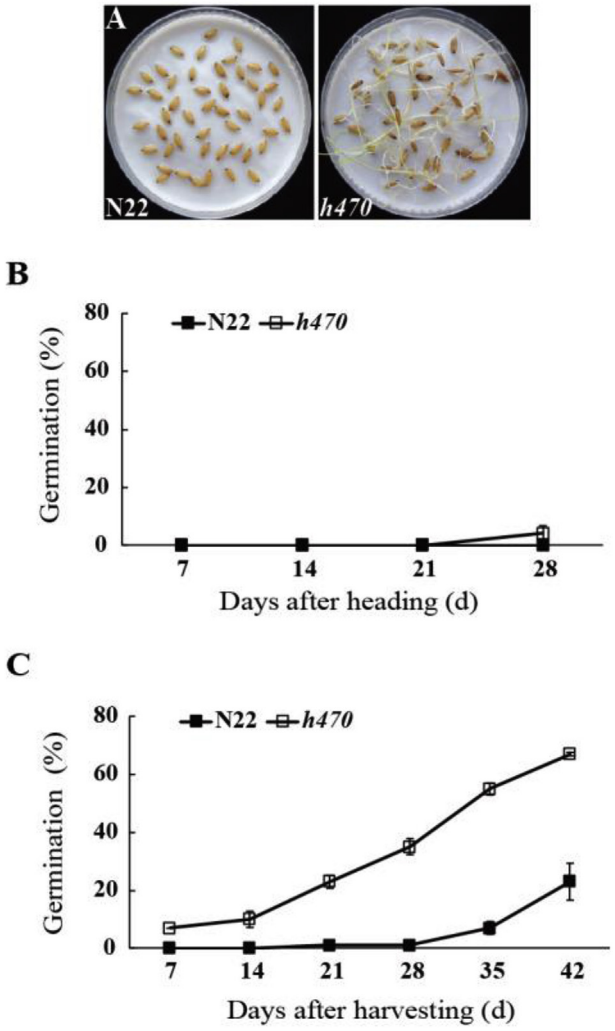

Fig. 1. Phenotypic comparison of N22 and *h470*. A Representative image of germinating N22 and *h470* seeds harvested at 35 DPH and stored at room temperature for 30 days before germination at 30 °C for 7 days. B Changes in the degree of seed dormancy during seed development in N22 and *h470*. C Germination percentages of N22 and *h470* following different storage periods. Three biological replicates of 50 seeds per Petri dish were measured. Values are means ± SD of three replicates.

2.5. Plasmid construction and isolation of transgenic lines

Among 368 potential mutant locations, there was only one InDel that showed a SNP/InDel ratio of 1. This InDel has a 1-bp deletion in the first exon of *Os02g0202400*. To test whether *Os02g0202400* was responsible for *h470* mutation, ~2 kb of promoter and full-length DNA of *OsBT1* were cloned into the pCUbi1390 binary vector. Fusion vector was transformed into *h470* callus by Agrobacterium-mediated transformation. The cDNA of *OsBT1* from Nipponbare (Nip) was cloned and fused into the binary vector LH-FAD2-1390RNAi under the control of the maize UBIQUTIN1 promoter to acquire an RNAi vector. The recombination plasmid then introduced into *Agrobacterium tumefaciens* strain *EHA105* and transformed into Nipponbare calli. The transgenic

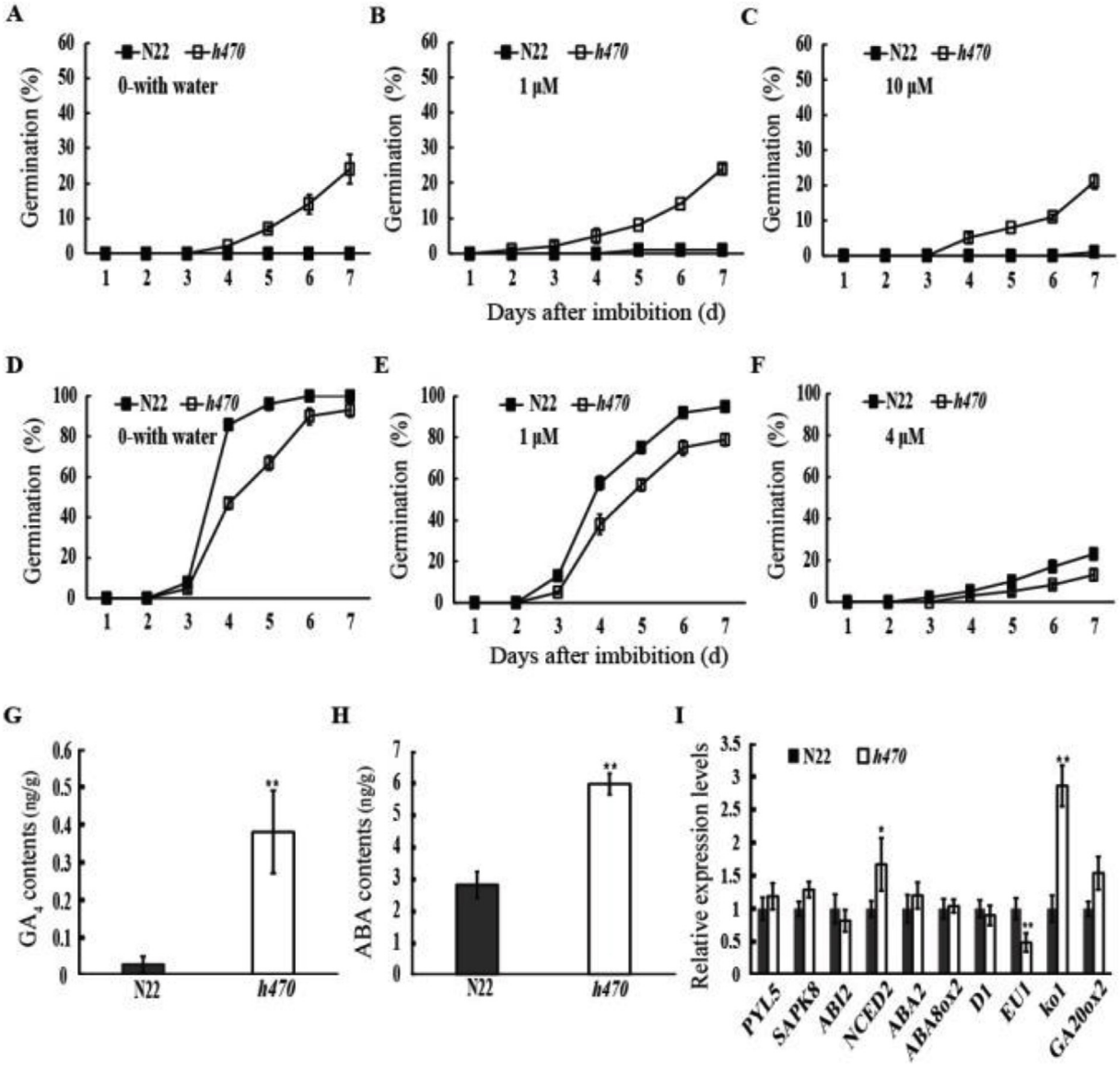

Fig. 2. *h470* was not involved in the GA or ABA pathways. A,B,C *h470* was insensitive to GA. WT and *h470* seeds were harvested at 35 DPH. GA_3 concentrations of 0 (with water as the control), 1 and 10 μM. D Loss of dormancy in seeds of N22 and *h470* after seven days of dry heat treatment at 50 °C. E,F Exogenous ABA treatment of N22 and *h470*. ABA concentrations were 1 and 4 μM. GA and ABA dissolved in absolute alcohol were diluted to relevant concentrations. G,H GA_4 and ABA contents in WT and *h470* seeds harvested at 24 DPH. I Expression of genes related to ABA and GA biosynthetic and signaling pathways. All germination results are means ± SD of three independent experiments. Three biological replicates of 50 seeds per Petri dish were subjected to germination tests. Values are means ± SD of three replicates. Asterisks indicate significant differences compared with WT (*$P < 0.05$; **$P < 0.01$) on the basis of Student's *t*-tests.

plants were grown in a paddy field. All primer sequences are listed in Supplementary Table 2.

2.6. RNA extraction, reverse transcription and qRT-PCR analysis

N22 and *h470* seeds were harvested at 24 DPH and total RNA was extracted using an RNApure Plant Kit (DNase I) (CWBIO, Beijing). A SuperScript II Kit was used for reverse transcribion (TaKaRa, Beijing). Real-time PCR was conducted using a SYBR Premix Ex TaqTM Kit (TaKaRa) on an ABI 7500 Real-Time PCR Analyser. The $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels of genes (Livak and Schmittgen, 2001). The rice *ubiquitin* gene (*Os03g0234200*) was used as a reference. The PCR procedure was: 95 °C for 30s, 40 cycles of 95 °C for 5s and 60 °C for 34s, then a melting curve was generated and analyzed.

2.7. Measurement of alpha-amylase activity

Alpha-amylase activity in seeds of N22 and *h470* harvested at 24 DPH was measured with a Megazyme Cat. No. K-CERA 09/11 kit (McCleary and Sheehan, 1987). Three biological repeats were measured for each sample.

2.8. Metabolomics and determination of starch, sugar and glucose contents

Metabolic assays on N22 and *h470* seeds harvested at 24 DPH were performed by UPLC-MS/MS at Metware Technology Company, Wuhan (Chen et al., 2013). Total starch contents were measured by a Megazyme assay kit (Wicklow, Ireland). Soluble sugar contents were assayed using a kit from the Nanjing Jiancheng Bioengineering Institute. Glucose contents were measured specifically by a kit from Pribolab (Beijing). Germination trials were set up as described above but 100 μM glucose was added with or without 5 μM of ABA to the dishes. Three

A

Chromosome	Positon	N22	*h470*	Gene	Note	SNP/Indel index
Chr. 2	5736606 - 5738853	GT	G	Os02g0202400	Deletion	1

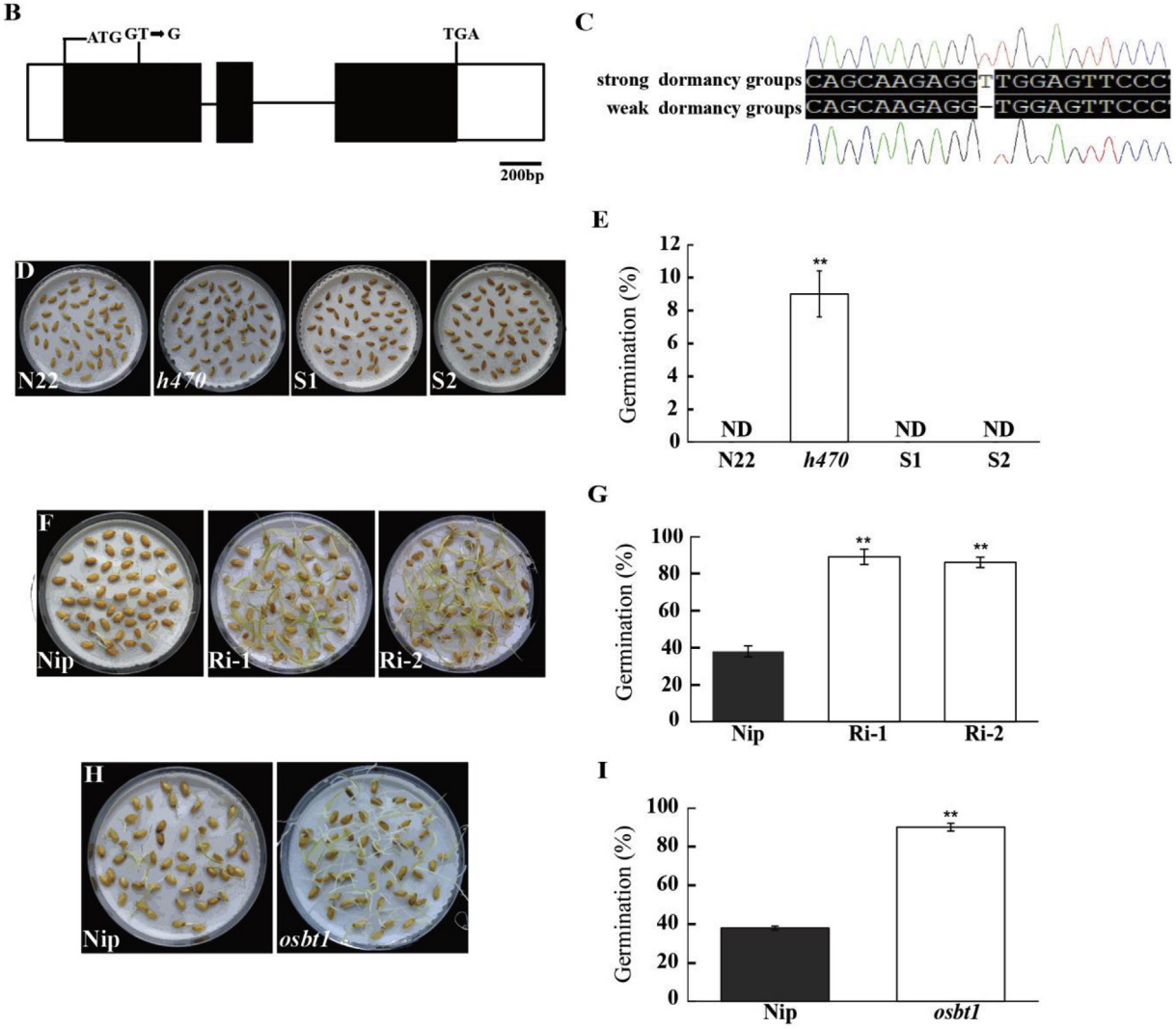

Fig. 3. Mut-map of *h470* and RNAi of gene *OsBT1*. A Identification of the *h470* mutation using the mut-map approach. Whole-genome sequencing detected one base deletion in gene Os02g0202400, and the SNP/InDel-index was 1. B The structure of the Os02g0202400 gene. Black box represents the exon; a line represents the intron. C Co-segregation analysis of the mutant site in weak versus strong dormancy groups. D Complementation of *h470* in transgenic lines (S1–S2) restored to WT appearance. E Germination percentages of N22, *h470* and two complementation lines. ND, not detected. F Representative images of germination in Nip and two Ri lines. G Germination percentages of Nip and two Ri lines. H Representative image of germinating Nip and *osbt1* seeds. I Germination percentages of Nip and *osbt1*. There were three biological replicates of 50 seeds per Petri dish. All germination percentages are means ± SD of three independent experiments.

biological repeats were made for each sample.

2.9. Data analysis

Three replicates for each experiment refers to the number of technical replicates that were used in statistical analysis. All values in figures were means ± SD (standard deviation). Results were subjected to Student's *t*-test to compare the statistically significant differences of the mean values between WT and *h470* (Microsoft Excel software). The mass spectrometry data of metabolites were analyzed by the software of Analyst 1.6.3. Statistically significance differences at $P < 0.05$ and $P < 0.01$ were indicated by asterisks * and **, respectively.

3. Results

3.1. h470 exhibits weak seed dormancy

To identify the differences in dormancy levels of N22 and h470, we detected the germination percentages after imbibition for seven days, the germination percentages of *h470* seeds harvested at 35 DPH and stored at room temperature (about 25 °C) for 30 days were about 40% higher than N22 (Fig. 1a). We also determined germination percentages after seven days of imbibitions using seeds harvested at 7, 14, 21 and 28 DPH. The seed dormancy of *h470* was broken at 28 DPH whereas N22 maintained strong dormancy (Fig. 1b). Then germination percentages of different storage periods after heading 35 days were detected. At 35 DPH N22 completed germination to about 8%, compared to 60% for

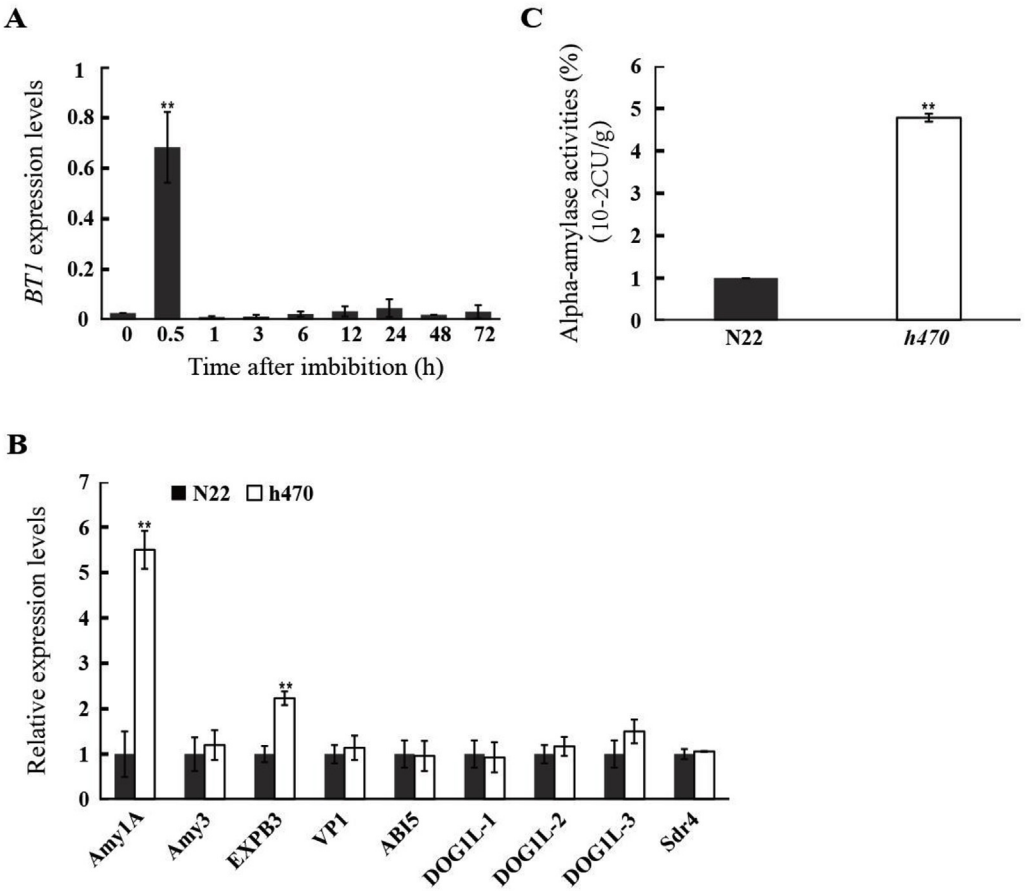

Fig. 4. Relative expression of *OsBT1* and genes related to seed dormancy in WT and *h470*. A QRT-PCR analysis of *OsBT1* expression after different times of imbibition. B Expression of genes related to seed dormancy. C Alpha-Amylase activities in N22 and *h470* seeds at 24 DPH. Data are means ± SD (n = 3). Asterisks indicated significant differences compared with WT (*$P < 0.05$; **$P < 0.01$).

h470 (Fig. 1c).

3.2. The weak dormancy phenotype of h470 was probably not regulated by the GA or ABA pathways

As *h470* had a high germination typical of many GA overexpressors or ABA-related mutants, we speculated that *h470* might involve the GA or ABA pathways. We therefore monitored germination over seven days of imbibition using different concentrations of GA_3 and ABA. Neither N22 or *h470* responded to GA treatment with germination percentages maintained at 0 and 20%, respectively (Fig. 2a–c). When dormancy was broken by a 7-day, 50 °C treatment the germination percentages of both N22 and *h470* exceeded 90% (Fig. 2d). Germination percentages of both lines was also inhibited at < 20% with 4 μM ABA (Fig. 2e–f).

Assays of endogenous GA or ABA contents at 24 DPH showed that the ABA level in seeds of *h470* was twofold higher than N22 (Fig. 2h), and the GA_4 content had increased nearly 13-fold relative to N22 (Fig. 2g). In regard to GA- and ABA-related genes the expression levels of *PYL5* (*PYROBACTIN RESISTANT LIKE5*, an ABA receptor), *SAPK8* (*SUCROSE NONFERMENTING1-RALATED PROTEIN KINASE8*), *ABI2* (a negative regulator in the ABA signaling pathway), and *D1* (a G protein *α* subunit, and positive regulator of the GA signaling pathway) were unchanged in *h470* relative to N22. These results were consistent with phenotypes following exogenous hormone treatments. *KO1* (*ENT-KAURENE OXIDASE*) and *NCED2* (*9-CIS-EPOXYCAROTENOID DIOXYGENASE*), which regulate synthesis of both GA and ABA, were more highly expressed in *h470* (Fig. 2i). The overall results suggested that the weaker dormancy of *h470* was not regulated by the GA or ABA pathways.

3.3. Identification of the h470 gene

To identify the gene responsible for the phenotype of *h470*, the reciprocal cross between N22 and *h470* was made to produce a F_2 segregation population adapted for mut-map re-sequencing. Germination percentages of F_2 segregation ratio implied that a single gene determined the phenotype of *h470* (weak dormancy plants n = 255, strong dormancy plants n = 150, $\chi^2_{3:1} = 1.11 < \chi^2_{0.05} = 3.84$). Defined the SNP/InDel index as the ratio between the number of reads of a mutant SNP and the total number of reads corresponding to the SNP, this index would approach 1 near the mutant gene and 0.5 for unlinked location (Abe et al., 2012). Because the two pools of extreme individuals were selected from the F_2 population, the numerical value of SNP/InDel index of mutant site should be 1 (Fig. 3a). A total of eight putative genes were identified, but only *Os02g0*202400 had one InDel in exon 1. This was a 1 bp deletion (Fig. 3a–b). Moreover, these mutations between weak and strong dormancy groups existed in co-segregation assays (Fig. 3c). On the basis of these results we hypothesized that *Os02g0202400* was the mutated gene in *h470*.

Os02g0202400 encodes an ADP-glucose transporter protein containing 425 amino acids, and annotated as *OsBT1*. Further bioinformatic analysis indicated that the single base deletion caused premature termination of protein translation (Fig. 3b). For further confirmation that *OsBT1* was responsible for the mutant phenotype, we constructed a 4076 bp fragment containing the entire *OsBT1* DNA sequence and promoter region, cloned it into the pCUbi1390 complementation binary vector and transformed it into *h470*. Germination percentages of 10 independent positive transgenic lines were zero, similar to that of the WT (Fig. 3d–e). An RNAi knock-down plasmid (Ri) containing the cDNA of *OsBT1* was transformed into *japonica* cv. Nipponbare (Nip) and all 4 independent Ri transgenic lines exhibited increased germination

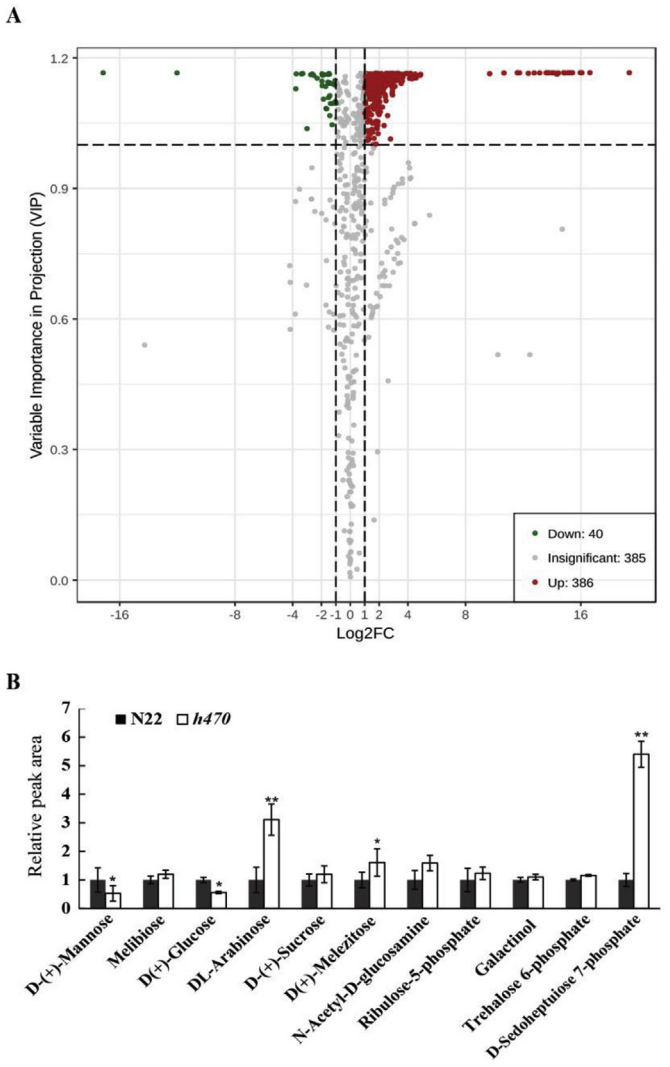

Fig. 5. Metabolomics analyses of N22 and *h470*. A Volcano plot of differential metabolite levels between N22 and *h470*. The abscissa represents the contents of differential metabolites between two samples, numbers mean fold change. The larger the ordinate, the more credible the difference. Green dots indicate reduced levels; red dots indicated increased levels; gray dots indicate no difference. B Contents of metabolotes involved in sugar biosynthesis. The contents of almost all components were increased in *h470*, except for D-(+)-mannose and D(+)-glucose. Data are means ± SD (n = 3). Asterisks indicated significant differences compared with WT (*$P < 0.05$; **$P < 0.01$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

percentages that were similar to *h470* (Fig. 3f–g). The germination percentage of an independently derived *osbt1* mutant obtained from the China National Rice Research Institute was also higher than that of WT, Nip (Fig. 3h–i). These experiments supported the above hypothesis that the mutation causing reduced dormancy in *h470* involved *Os02g0202400*.

3.4. Expression analysis of OsBT1 and genes related to dormancy

We analyzed the temporal expression patterns of *OsBT1* at 0, 0.5, 1, 3, 12, 24, 48 and 72 h after imbibition (HAI). Expression of *OsBT1* at 0.5 h was significantly elevated but subsequently reverted to a level slightly higher than that at 0 h (Fig. 4a). Although expression levels of many genes regulating seed dormancy, including *Amy3* (*Alpha-Amylase3*), *VP1*, *ABI5* (positive regulators in the ABA signaling pathway), *DOG1L-1*, *DOG1L-2*, *DOG1L-3* and *Sdr4*, were not significantly different between N22 and *h470*, expression of others such as *Amy1A* (*Alpha-Amylase1A*) and *EXPB3* were increased in *h470* compared to N22 (Fig. 4b). The α-amylase enzyme activity in *h470* seeds was approximately four-fold higher than that in the WT (Fig. 4c). We concluded that *OsBT1* might regulate seed dormancy by affecting the expression of amylase-related genes.

3.5. OsBT1 influences glycometabolism in rice

The above results indicated that the weaker dormancy of *h470* was associated with changes in starch metabolism. Metabolites in seeds of N22 and *h470* at 24 DPH were qualitatively and quantitatively analyzed by UPLC-MS/MS. Among 811 metabolites, 386 were increased and 40 were decreased in *h470* relative to N22 (Fig. 5a; Supplementary Table 3). Using KEGG functional annotation and enrichment analysis, the differentially generated metabolites in *h470* were mostly involved in carbohydrate metabolism. These included melibiose, DL-arabinose, D-(+)-sucrose, D(+)-melezitose, N-acetyl-D-glucosamine, ribulose-5-phosphate, galactinol, trehalose 6-phosphate and D-sedoheptuiose 7-phosphate, indicative of increased sugar contents (Fig. 5b) and decreased total starch (Fig. 6a–c). These results suggested that OsBT1 was involved in glycometabolism to regulate seed dormancy in rice. Previous studies showed that sugar acted as a signaling molecule with ABA to inhibit seed germination in rice (Zhu et al., 2009). We examined the effects of 100 μM of exogenous glucose with or without 5 μM of ABA on germination of N22 and *h470*. Addition of glucose alone had no effect (Fig. 6d), but in combination with ABA there was a 20% increased in germination percentage of N22 whereas *h470* remained unchanged (Fig. 6e).

4. Discussion

Seed dormancy is a complex quantitative trait regulated by many proteins. Despite identification of many genes, there are more to be characterized. Our lab has been focusing research on rice seed dormancy for decades, most of the detected sites were from different populations using strongly dormant *indica* cultivar N22. We previously reported reduced dormancy mutants *Q4359* and *Q4646* in N22 following ^{60}Co irradiation. QTL analysis showed that *Q4359* lacked *qSdn-1* and *Q4646* lacked *qSdn-5* (Lu et al., 2011; Wu et al., 2016). At present, we report mutant *h470* with reduced dormancy but other agronomic traits were not different from N22 wild type (Supplemental Table 1). The germination percentages of *h470* seeds after seven days of imbibitions were higher than N22 after 28 DPH and storage periods (Fig. 1a–c).

It is well-known that GA and ABA play an antagonistic role in regulating seed dormancy. Many genes related to GA and ABA have been cloned and reported to regulate seed dormancy. GA2ox (GA2-oxidases) are involved in GA metabolism, and loss of function decreases seed dormancy (Yamauchi et al., 2007). RGL2 (RGA-like 2) and SPY (SPINDLY) are DELLA proteins that negatively regulate the GA signaling pathway, both expression up-regulated during seed imbibition and complement the non-dormant phenotype of a *ga1* mutant (Lee et al., 2002). *CYP707A1* and *CYP707A2* encode ABA 8′-hydroxylase, which inactivates ABA. Their mutants showed increased levels of ABA in dry and imbibed seeds, leading to the enhanced dormancy (Okamoto et al., 2006). *ABI1* and *ABI2* encode Ser/Thr PROTEIN PHOSPHATASE 2C (PP2C), as negative regulators in the ABA signaling pathway. Mutants *abi1-1* and *abi2-1* with weak dormancy were unable to bind to ABA receptors (Ma et al., 2009). As *h470* showed a similar phenotype to some GA and ABA mutants, we speculated that *OsBT1* was involved in the well-established hormonal pathways that regulate seed dormancy. However, *h470* was insensitive to exogenously applied GA_3 and ABA (Fig. 2a–f). We also identified the GA sensitivity of wild-type and mutant after-ripened seeds, and the results were the same as the newly

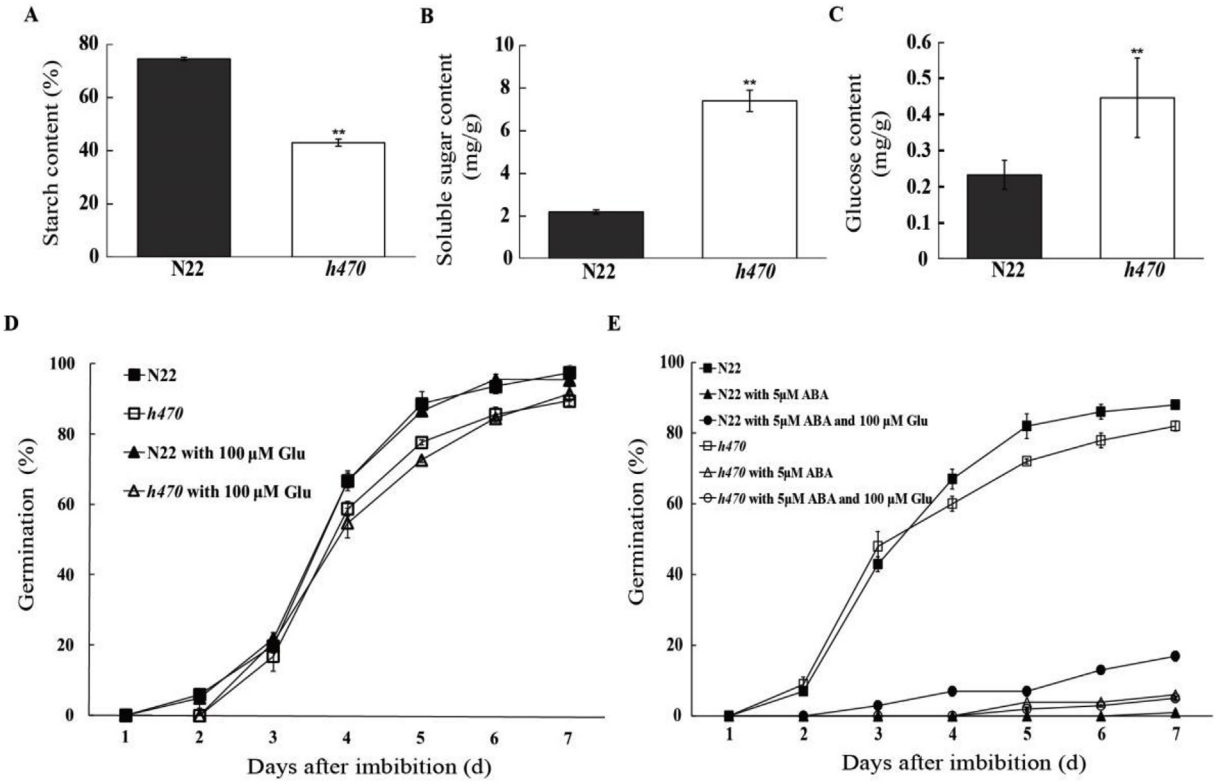

Fig. 6. Comparison of starch mobilization and glucose with ABA effects on N22 and *h470*. A,B,C Total starch, soluble sugar and glucose contents in N22 and *h470* seeds at 24 DPH. d,e Effects of glucose and ABA on N22 and *h470*. Three biological replicates of 50 seeds per Petri dish were assessed for germination. All germination percentages are means ± SD of three independent experiments. Asterisks indicate significant differences compared with WT (**$P < 0.01$).

harvested seeds of these both genotypes. Endogenous ABA content was increased to a small extent, along with a significantly increased level of GA_4 (Fig. 2g–h). Expression levels of *PYL5*, *SAPK8*, *ABI2* and *D1* were almost invariably, but *KO1* and *NCED2* expression levels were markedly higher in *h470* (Fig. 2i). These results were consistent with the previous consequences and phenotype (Miura et al., 2009; Kim et al., 2011; Li et al., 2015; Zhu et al., 2009). We assayed previously reported dormancy-related genes, including *Alpha-Amylase1A*, *Alpha-Amylase3*, *EXPB3* (an expansin gene), *VP1*, *ABI5*, *DOG1L-1*, *DOG1L-2*, *DOG1L-3* and *Sdr4*, there were no differences, except for *Alpha-Amylase1A* and *EXPB3* (Huang et al., 2000; Lee and Kende, 2001) (Fig. 4b). These latter changes in *h470* were consistent with the weaker dormancy phenotype and we concluded that the weak dormancy phenotype of *h470* was not regulated by the GA or ABA pathways.

Specific regulatory genes, such as *AlaAT* (*ALANINE AMINOTRANSFERASE*) in barley (Sato et al., 2016) and *Sdr4* (*Seed Dormancy 4*) in rice (Sugimoto et al., 2010), and metabolism-associated factors affecting dormancy were identified. In barley, Thioredoxin *h* (Thioredoxin of the h-type) converted from an oxidized to a partially reduced state during seed germination. Overexpressing Thioredoxin *h* increased the alpha-amylase activity, soluble protein and GA_1 content, ultimately regulated seed dormancy (Wong et al., 2002). *PHS8*, which encodes an isoamylase (ISA1), influenced the accumulation of low molecular weight sugars in the seed endosperm and reduced seed dormancy. Mutation of *PHS8* caused inhibited expression of *OsVP1* and *OsABI5*, two important transcription factors in the ABA signaling pathway causing PHS. Furthermore, overexpression of *OsABI3* or *OsABI5* could partially alleviate the PHS of *phs8* (Du et al., 2018). OsIPMS1 (ISOPROPYLMALATE SYNTHASE) affects the biosynthesis of free amino acids during seed germination and promotes GA synthesis, enhancing the glycolysis and tricarboxylic acid (TCA) reactions and provides more energy for during the seed germination and seedling growth (He et al., 2019). By mut-map, complementation and RNAi knock-down, we confirmed that mutation of *OsBT1* was the cause of the *h470* mutant phenotype (Fig. 3a–g). There are reports on the role of BT1 protein in various species such as maize, Arabidopsis, barley and wheat. Previous studies have shown that *ZmBT1* (maize), *HvNST1* (barley) and *TaBT1* (wheat) are able to transport ADPG into amyloplasts and used for starch biosynthesis. *OsBT1* encodes an ADP-glucose transporter that functions in starch synthesis (Cakir et al., 2016; Li et al., 2017). Analysis of metabolic differences between N22 and *h470* showed that *OsBT1* involved in the glycometabolism physiology processes, impacted the total starch, soluble sugar and glucose contents (Fig. 6). How *OsBT1* regulates seed dormancy through the glycometabolism pathway remains unclear.

5. Conclusions

We identified a mutant allele of *OsBT1* that caused reduced seed dormancy independent of the GA and ABA pathways and implied a glycometabolism disorder. This study provides important insights into the function of OsBT1 on seed dormancy in rice and the underlying metabolic mechanisms might be beneficial to cultivate new varieties resistance to PHS and increase quality in crops.

Contributions

Weihan Song, Ling Jiang and Jianmin Wan were responsible for establishing and designing the experiments. Weihan Song, Qixian Hao, Mengying Cai, Yihua Wang, Xingjie Zhu, Xi Liu, Yunshuai Huang, Nguyen Thanhliem, Chunyan Yang, Jiangfeng Yu and Hongming Wu operated the experiments. Liangming Chen and Yunlu Tian planted and managed the materials in the fields. Weihan Song and Ling Jiang wrote the manuscript and analyzed the data. All authors have read and approved the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2020.03.055.

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