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Molecular hydrogen can take part in phytohormone signal pathways in wild rice

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Abstract

Molecular hydrogen (H_2) could be a novel signal in phytohormone signaling pathways in response to biotic and abiotic stresses. Here, we employed two wild rice species (*Oryza rufipogon* Griff. and *O. minuta* J. Presl) to test this hypothesis using hydrogen-rich water (HW). The expression differences of phytohormone and hydrogenase genes between conventional rice (*Oryza sativa* L,) and wild rice were determined by real-time quantitative polymerase chain reaction, and the effects of HW on gene expression of wild rice were detected during three growth stages. Expression of hydrogenase genes, synthesis genes, and receptor genes of salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signalling pathways was higher in six wild rice types than in conventional rice. Hydrogen-rich water up-regulated expression of two hydrogenase genes, SA, JA, and ET receptor genes and synthesis genes in the seedling stage of wild rice. But this positive regulation by HW was less significant in the vegetative and reproductive stages.

Additional key words: abiotic and biotic stresses, ethylene, hydrogen-rich water, jasmonic acid, *Oryza minuta*, *Oryza rufipogon*, *Oryza sativa*, salicylic acid.

Introduction

Hydrogen has been used in clinical treatments because it can selectively alleviate reactive oxygen species (ROS) and protect cells from oxidative damage (Huang *et al.* 2010), yet the physiological functions of H_2 in plants is almost unknown. Metabolism of H_2 was first reported in algae and then in bacteria (Gaffron 1939, Gaffron and Rubin 1942, Gest and Kamen 1949, Melis and Melnicki 2006). Hydrogen is produced through photosynthetic and non-photosynthetic processes with either hydrogenase or nitrogenase (Gaffron and Rubin 1942, Hatchikian *et al.* 1992, Shimada *et al.* 2008, Eroglu and Melis 2011). Genes encoding hydrogenase have been found in algae, such as *SoHydA1* in *Scenedesmus obliquus* and *CrHydA1* and *CrHydA2* in *Chlamydomonas reinhardtii* (Forestier *et al.* 2003, Weng *et al.* 2007). The existence of hydrogenase in some higher plants is also confirmed in isolated chloroplasts (Renwick *et al.* 1964). Metabolism of H_2 has been described in *Hordeum vulgare*, *Oryza sativa*, *Medicago sativa*, and *Arabidopsis thaliana* (Renwick *et al.* 1964, Xie *et al.* 2012, Jin *et al.* 2013, Zeng *et al.* 2013), and three putative hydrogenase gene homologs (*i.e.*, *OsHypA1*, *OsFhdB*, and *OsHypB*) have been found in rice (Zeng *et al.* 2013). Although the putative hydrogenase genes in rice may function in H_2 metabolism, its exact biological role remains unclear. Previous studies have reported that hydrogen-rich water (HW) plays a crucial role in plant stress tolerance (Xie *et al.* 2012, Jin *et al.* 2013, Zeng *et al.* 2013).

Plants possess several mechanisms to perceive external stress signals and induce optimal responses (Fujita *et al.* 2006). Four endogenous phytohormones, salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and abscisic acid (ABA), involve in plant defence (Pieterse and Van Loon 1999, Fujita *et al.* 2006). In these signal pathways, phytohormone receptor genes and synthesis genes regulate expression of defense genes

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Abbreviations: ABA - abscisic acid; AJ - Anjiashan; CL - Chaling; CK - cytokinin; DX - Dongxiang; ET - ethylene; GA - gibberellic acid; GORK - guard cell outwardly rectifying K^+ channel protein; HW - hydrogen-rich water; JA - jasmonic acid; LS - Lingshui; NC - Nanchang; qPCR - quatitative polymerase chain reaction; ROS - reactive oxygen species; SA - salicylic acid; ST - Shuitao.
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and induce systemic resistance (Ryals *et al.* 1996, Reymond *et al.* 2000, Ryan 2000, Kunkel and Brooks 2002). Phytohormone signaling pathways do not function independently, but rather with each other *via* synergistic or antagonistic ways (Kunkel and Brooks 2002, Fujita *et al.* 2006). Hydrogen may act as a novel signaling molecule in response to stresses and involve in phytohormone signaling pathways (Zeng *et al.* 2013). Xie *et al.* (2014) reported that HW can regulate closure of stomata to resist drought *via* the ABA signaling pathway. Hydrogen has a potential to alleviate NaCl toxicity through increasing expression of genes encoding antioxidants and maintaining ion homeostasis in plant cells (Xie *et al.* 2012). Heavy metals (*e.g.*, Hg^{2+} and Cd^{2+}) and pesticides (*e.g.*, paraquat) induce formation of ROS and lead to oxidative damage in plants (Cho and Park 2000, Sharma and Dietz 2009, Jin *et al.* 2013). Hydrogen rich water reduces uptake of Hg^{2+} and Cd^{2+} and alleviates seedling growth inhibition and oxidative damage through increasing expression of antioxidant enzyme genes (Cho and Park 2000, Jin *et al.* 2013, Cui *et al.* 2013, 2014).

Wild rice possesses valuable and elite genes which involve in resistance to cold, drought, diseases, pests and other stresses (Li *et al.* 2014), because wild rice is often exposed to various abiotic and biotic stresses in the nature (Chen *et al.* 2008). For example, *Oryza rufipogon* growing in Dongxiang, Jiangxi province, is the most northern distributed wild rice in the world (Chen *et al.* 2008). Wild rice exposed to various abiotic and biotic stresses in the natural environment is able to tolerate cold, drought, diseases, pests, *etc*. (Chen *et al.* 2008). Zeng *et al.* (2013) found that HW can enhance transcription of phytohormone receptor genes in conventional rice through interfering with SA, JA, and ET signaling pathways. Thus, this study aims to *1*) explore whether H_2 involves in plant stress tolerance and interacts with phytohormone signaling in wild rice, and *2*) detect whether regulating expression of phytohormone and hydrogenase genes by HW varies with rice growth stages.

Materials and methods

Hydrogen-rich water was produced according to Nakao *et al.* (2010). A plastic shelled product consisting of metallic magnesium (99.9 % pure) and natural stones in polypropylene containers combined with ceramics (*Doctor SUISOSUI*®, Friendear, Tokyo, Japan) was used to produce HW.

Conventional rice (*Oryza sativa* L.) and two wild rice species (*O. rufipogon* Griff. and *O. minuta* J. Presl) were employed. Wild rice *O. minuta* was taken from Lingshui (LS), Hainan province, and one type of *O. rufipogon* was taken from Chaling (CL), Hunan province. Other four types of *O. rufipogon* were taken from Jiangxi province, *i.e.*, NC from one research base at Nanchang, DX from Dongxiang, ST from Shuitao, and AJ from Anjiashan. These four types were Donxiang wild rice.

The seeds were soaked in distilled water for germination in Petri dishes (30 seeds per dish). The rice seedlings were cultivated in a growth chamber (a 16-h photoperiod, an irradiance of 340 μmol m^{-2} s^{-1}, day/night temperatures 30/25 °C, and a relative humidity of 70 %). Three leaves of 14-d-old rice seedlings were collected and immediately frozen in liquid nitrogen to next real-time polymerase chain reaction analyses.

To detect whether H_2 involves in expression of phytohormone and hydrogenase genes, the wild rice seeds were germinated with HW or distilled water in Petri dishes (30 seeds per dish) in an above mentioned growth chamber. To keep the H_2 concentration in the water, the HW was refreshed every day. The 14-d-old seedlings of six wild rice types (LS, CL, NC, DX, ST, and AJ) were transplanted to plastic barrels (27 cm in diameter and 29 cm in height) and grown in a greenhouse (under the same conditions). Each wild rice type was planted in two barrels (three plants per barrel), one with HW and the other with distilled water. Three leaves of each plant were sampled to RNA isolation at the seedling stage (14-d-old), vegetative growth stage (1-month-old) and reproductive stage (3-month-old). Leaves were collected at the same position of plants for all sampled plants and immediately frozen in liquid nitrogen.

All sampled leaves from the above two assays were used to isolate RNA using RNA plant kits (*Tiangen Biotech*, Beijing, China). Copy DNAs were synthesized from 2 μg of total RNA using *PrimeScript*™ 1st strand cDNA synthesis kits (*Takara*, Shuzo, Japan) in a 20 mm^3 reaction mixture. The cDNA was diluted to 1:50 by adding distilled water. Each sample that was used to perform real-time quantitative PCR (qPCR) included 10 mm^3 of a 2× *SYBR* solution, 0.6 mm^3 of a forward/reverse primer mix, 7.4 mm^3 of distilled water, and 2 mm^3 of cDNA. Real time qPCR was performed with a *Takara SYBR Premix Ex Taq* kit on a *CFX 96* machine (*Bio-Rad*, Hercules, USA). The PCR was triplicated for each cDNA sample. Gene-specific primers are listed in Table 1. *OsActin1* mRNA was used to normalize the expression of each gene (Zeng *et al.* 2013). Amplification conditions were following: 95 °C for 2 min followed by 40 PCR cycles of 95 °C for 15 s, 58 °C for 30 s, and 72 °C for 20 s. Changes in expression were calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008).

The relative expressions of phytohormone and hydrogenase genes are shown as means ± standard deviations (SDs) from three independent experiments. Statistical analysis was performed using the *Origin* software (*v. 8*). One-way analysis of variance was used to detect the effect of HW on gene expression.

Table1. A list of primers used in real-time qPCR.

Genes	Forward (5'-3')	Reverse (5'-3')
OsActin	CGGTGTCATGGTCGGAAT	GCTCGTTGTAGAAGGTGT
OsHypB	GCTGCCTAGCAACAAAGGTC	TACACCTCGCTCACACCATC
OsFhdB	ACGAGTTGCGGGTCAATTAG	CATCCATGTCTTGCTTCCTG
OsAOS2	CTCGTCGGAAGGCTGTTGCT	ACGATTGACGGCGGAGGTT
OsLOX	GCATCCCCAACAGCACATC	AATAAAGATTTGGGAGTGACATA
OsCOI1	TTGCCGTGAATTGGAGTACATAG	GTCAAGTAGCACAAGCCGAAAG
OsEDS1	CATTCCAAGAACGAGGACACTG	CAAGACTCAAGGCTAGAACCGA
OsPAD4	CCAACATGTACCGCATCAAG	GGTTGTTTCGGTGGTAGTGG
OsNPR4	CAACGTCGAGCAAATGTACG	TCAAGCACTGGAGTCAGCTC
OsERS1	TCATGGTTCTGATGCTTCCA	TGCTCCATTAGCAGATCACG
OsERS2	CTCCCTCCAGACAGTGCAAG	CATTACGGGCACAAATAGCC
OsETR2	GTTCGTCATCCAGTCGGAGA	GAACTGAAGGGCAAGCATGA
OsACS1	GAGATGGTGAGCCAAGTGGT	CACTCCTCAAGCAGGTCGAA

Results

Comparing with conventional rice, most genes involved in the SA, JA, and ET signaling pathways were up-regulated in wild rice (Fig. 1). In the JA pathway, the receptor gene *OsCOI1* was up-regulated in all six wild rice types (Fig. 1*A*). The synthesis genes (*OsAOS2* and *OsLOX*) showed higher expressions in CL, NC, and DX than in conventional rice, whereas the two genes were not detected in wild rice LS (Fig. 1*B,C*). In the SA pathway, the receptor gene *OsNPR4* was up-regulated in all six wild rice types, but the expressions of the two synthesis genes were not different between wild rice and conventional rice except for higher *OsEDS1* in DX and lower *OsPAD4* in CL (Fig. 1*D-F*). In the ET pathway, wild rice LS, CL, NC, DX, and AJ showed up-regulated expressions of the synthesis gene *OsACS1* (Fig. 1*J*). For the three ET receptor genes compared to conventional rice, higher expressions were found in wild rice LS, NC, and DX for the *OsERS1* gene, in LS, DX, ST, and AJ for the *OsERS2* gene, and in LS for the *OsETR2* gene (Fig. 1*G-I*). Wild rice LS showed higher relative expressions of two hydrogenase genes *OsHypB* and *OsFhdB* than conventional rice (Fig. 1*K,L*). Wild rice NC, DX, and AJ showed a higher relative expression of *OsHypB* but not of *OsFhdB* (Fig. 1*K,L*).

Considering high expressions of phytohormone and hydrogenase genes in wild rice, we intended to determine whether H_2 could affect expression of these genes in different growth stages. In the seedling stage, HW increased expressions of *OsHypB* and *OsFhdB* in LS and DX, but did not significantly alter their expression in the other wild rice types (Fig. 2*K,L*). The JA receptor gene *OsCOI1* was up-regulated in LS, DX, and ST (Fig. 2*A*). The SA receptor gene *OsNPR4* showed a higher expression in LS and DX (Fig. 2*D*). For three ET receptor genes, expressions of *OsERS1*, *OsERS2*, and *OsETR2* increased in LS and DX, and *OsERS1* was up-regulated in CL and NC (Fig. 2*G-I*). For two JA synthesis genes, HW increased expressions of *OsAOS2* in DX and *OsLOX* in CL, DX, and ST (Fig. 2*B,C*). Expression of *OsAOS2* was down-regulated in NC and of *OsLOX* in AJ (Fig. 2*B,C*). Expressions of two JA synthesis genes (*OsAOS2* and *OsLOX*) were not found in LS (Fig. 2*B,C*). The ET synthesis gene *OsACS1* showed higher expressions in NC, ST, and AJ (Fig. 2*J*). For two SA synthesis genes, *OsEDS1* was up-regulated in LS, DX, ST, and AJ, and *OsPAD4* in ST and AJ, but *OsPAD4* was down-regulated in NC (Fig. 2*E,F*). These results show that HW could enhance expressions of the hydrogenase genes and phytohormone receptor genes in the wild rice seedlings, but the phytohormone synthesis genes were affected differently in the six wild rice types.

In the vegetative growth stage, *OsHypB* was up-regulated by HW in LS, NC, DX, and AJ, and *OsFhdB* in LS and NC, whereas both two hydrogenase genes were down-regulated in ST (Fig. 3*F,G*). For the JA receptor gene, expression of *OsCOI1* increased by HW in LS (Fig. 3*A*). The SA receptor gene *OsNPR4* was up-regulated in LS and down-regulated in DX by HW (Fig. 3*B*). For three ET receptor genes, higher expressions were found in LS for *OsERS1*, in LS, ST, and AJ for *OsERS2*, and in CL, NC, DX, and ST for *OsETR2* (Fig. 3*C-E*). A lower expression was found in CL for *OsERS2* (Fig. 3*D*).

The effect of HW on expressions of hydrogenase and phytohormone genes was less obvious in the reproductive stage than in the vegetative and seedling stages (Fig. 4). HW did not significantly alter expressions of two hydrogenase genes in all the wild rice types except for down-regulation of expression of *OsFhdB* in ST (Fig. 4*F,G*). Expression of the JA receptor gene *OsCOI1* was not influenced by HW in the six wild rice types (Fig. 4*A*). The SA receptor gene *OsNPR4* was

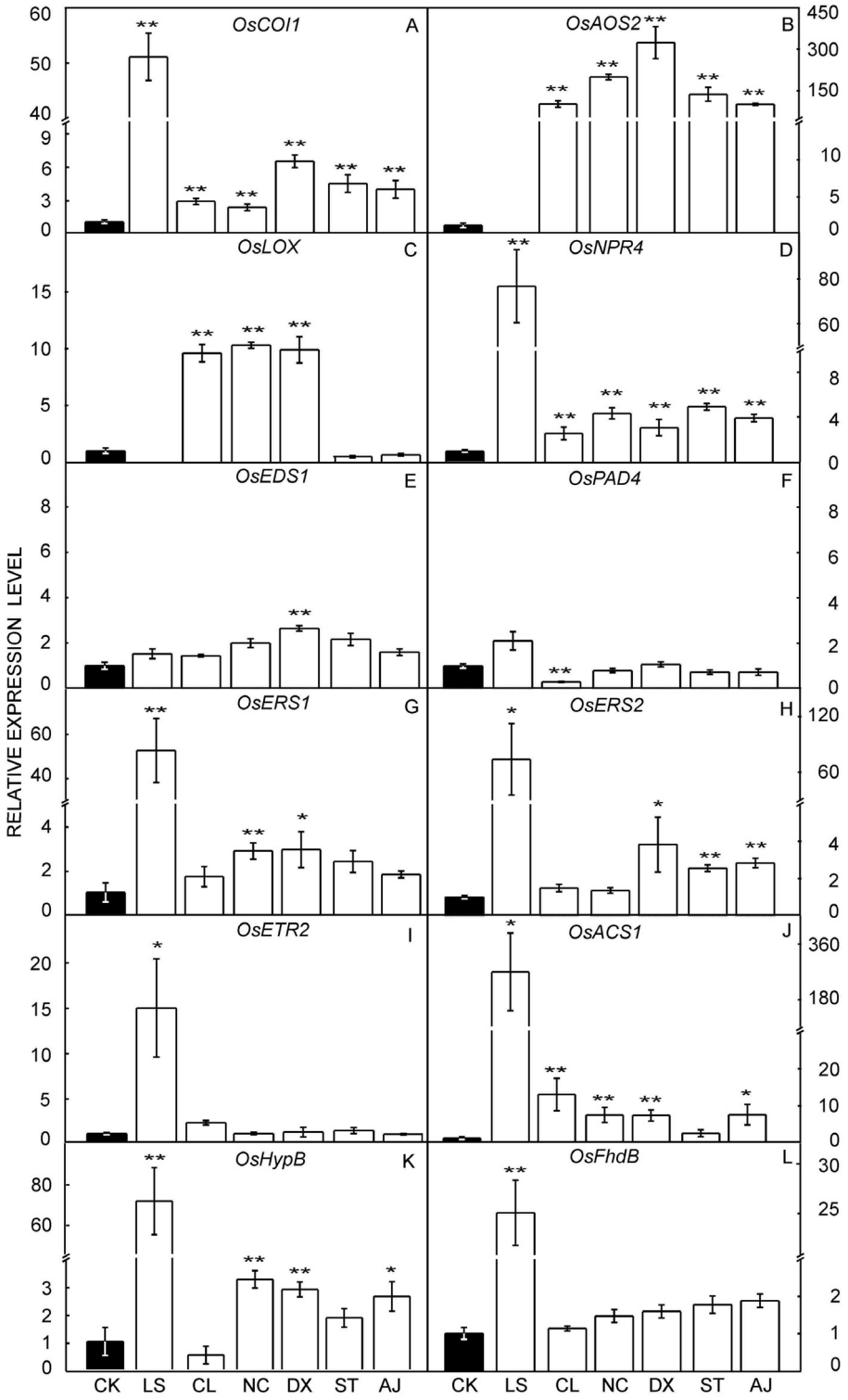

Fig. 1. Relative expressions of genes involved in phytohormone signaling pathways and H_2 production in wild rice compared to conventional rice. Leaves were harvested from 14-d-old rice seedlings grown in distilled water, and real-time qPCR was performed to analyze expression of jasmonic acid (JA) receptor gene *OsCOI1* (*A*), two JA synthesis genes *OsAOS2* (*B*) and *OsLOX* (*C*), salicylic acid (SA) receptor gene *OsNPR4* (*D*), two SA synthesis genes *OsEDS1* (*E*) and *OsPAD4* (*F*), three ethylene (ET) receptor genes *OsERS1* (*G*), *OsERS2* (*H*), and *OsETR2* (*I*), ET synthesis gene *OsACS1* (*J*), and two hydrogenase genes *OsHypB* (*K*) and *OsFhdB* (*L*). Relative expressions in wild rice types (LS, CL, NC, DX, ST, and AJ) are compared with conventional rice (CK = 1). Means ± SDs, $n = 3$, * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively.

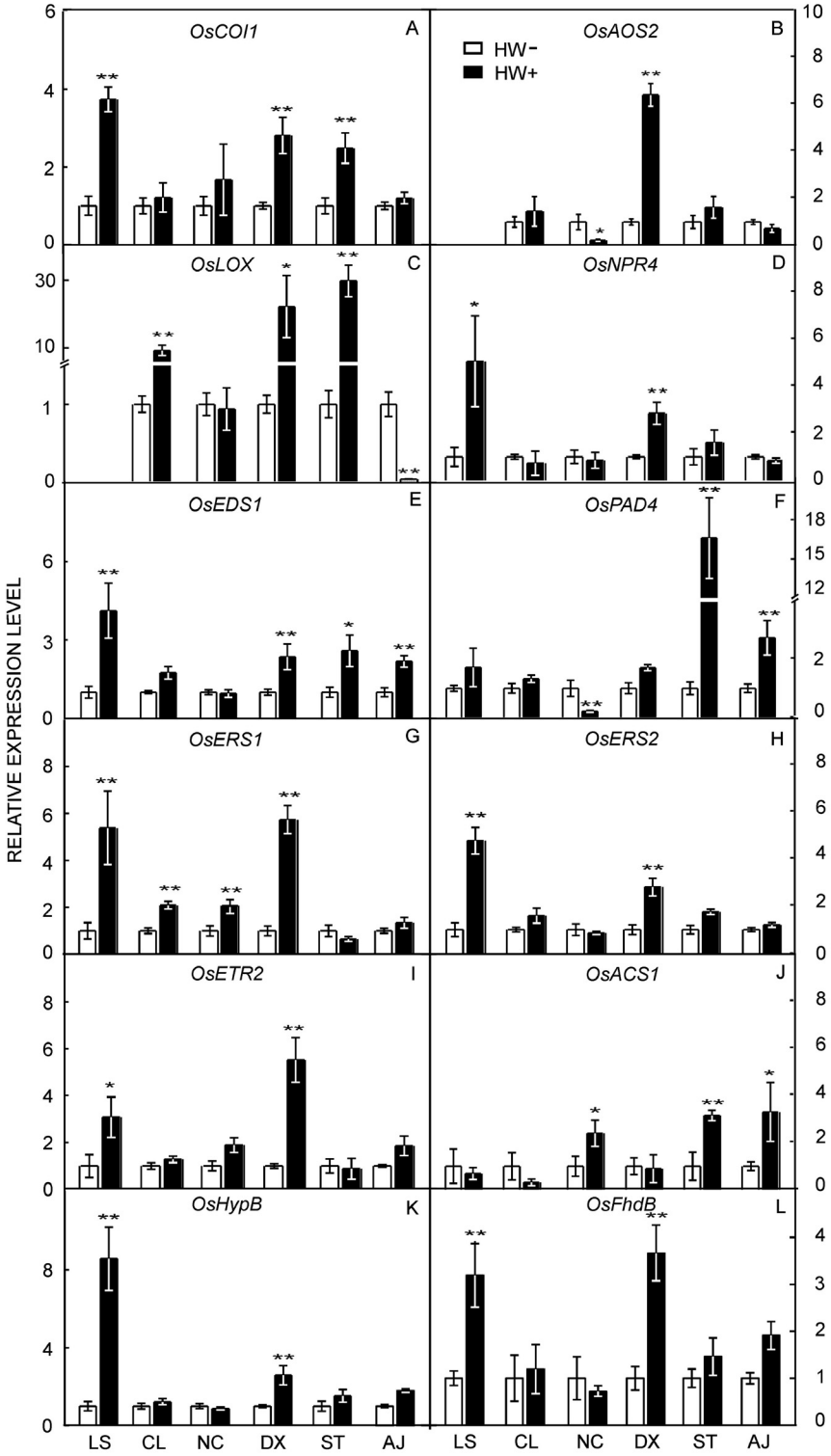

Fig. 2. Relative expressions of hydrogenase genes and phytohormone signaling pathway genes in wild rice seedlings (LS, CL, NC, DX, ST, and AJ) germinated and grown in hydrogen-rich water (HW+) or distilled water (HW-) for two weeks. Real-time qPCR was performed to analyze expressions of jasmonic acid (JA) receptor gene *OsCOI1* (*A*), two JA synthesis genes *OsAOS2* (*B*) and *OsLOX* (*C*), salicylic acid (SA) receptor gene *OsNPR4* (*D*), two SA synthesis genes *OsEDS1* (*E*) and *OsPAD4* (*F*), three ethylene (ET) receptor genes *OsERS1* (*G*), *OsERS2* (*H*), and *OsETR2* (*I*), ET synthesis gene *OsACS1* (*J*), and two hydrogenase genes *OsHypB* (*K*) and *OsFhdB* (*L*). Relative expressions in HW+ plants are compared with HW-. Means ± SDs, $n = 3$, * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively.

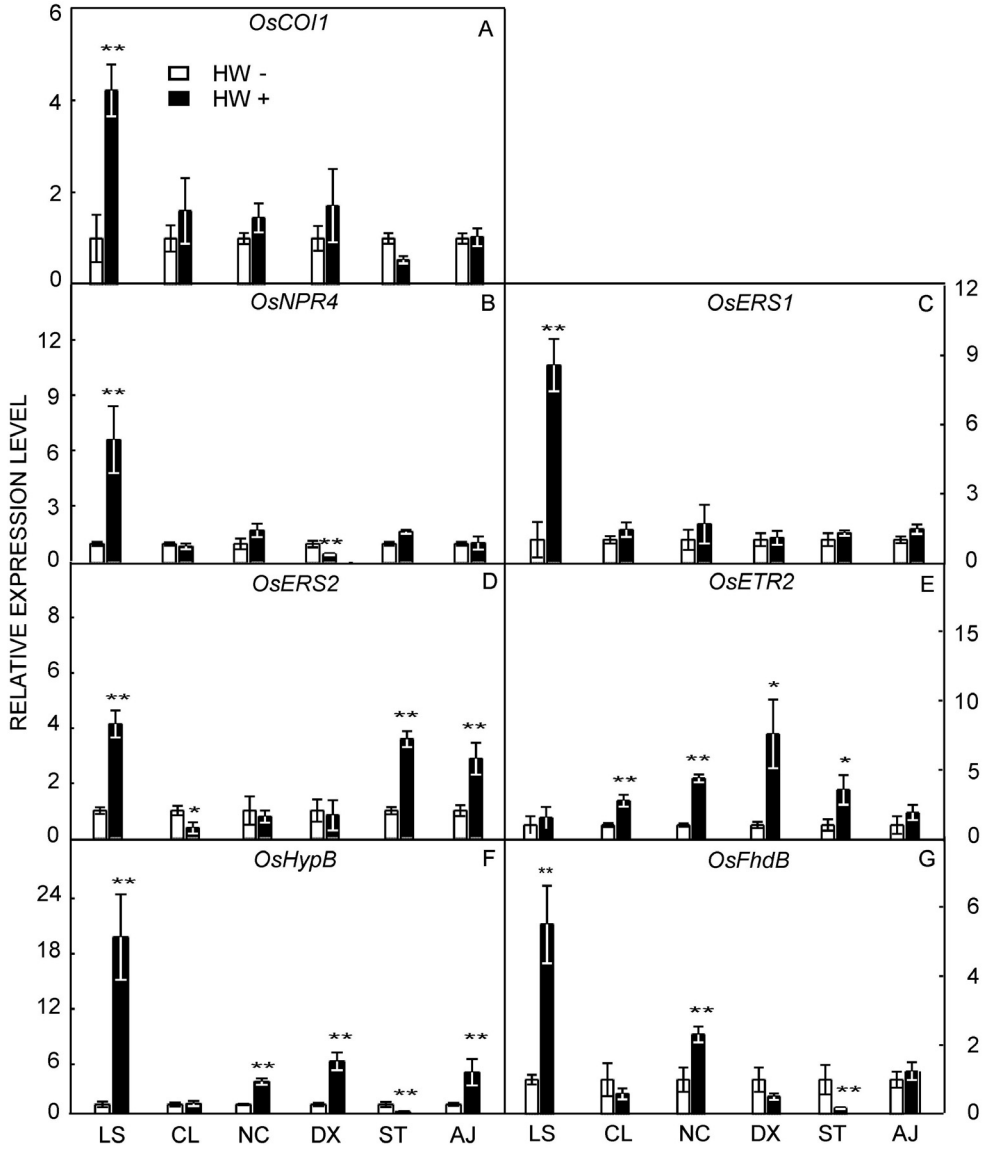

Fig. 3. Relative expressions of hydrogenase genes and phytohormone signaling pathway genes in 1-month-old wild rice plants (LS, CL, NC, DX, ST, and AJ) grown in hydrogen-rich water (HW+) or distilled water (HW-). Real-time qPCR was performed to analyze expressions of jasmonic acid (JA) receptor gene *OsCOI1* (*A*), salicylic acid receptor gene *OsNPR4* (*B*), three ethylene receptor genes *OsERS1* (*C*), *OsERS2* (*D*), and *OsETR2* (*E*), and two hydrogenase genes, *OsHypB* (*F*) and *OsFhdB* (*G*) in the vegetative growth stage. Relative expressions in HW+ plants are compared with HW- plants. Means ± SDs, $n = 3$, * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively.

down-regulated in ST (Fig. 4*B*). Expressions of three ET receptor genes were not changed by HW in most wild rice types except for a decreased expression of *OsERS1* in LS, increased expressions of *OsERS2* in NC and ST, and *OsETR2* in ST (Fig. 4*C-E*).

Discussion

The genus *Oryza* is composed of approximately 24 species, 2 conventional rice species and 22 wild rice species. Advantageous traits are found in wild rice, especially its resistance to biotic stresses (Liu and Chen 2014). Here, two species, *O. rufipogon* and *O.minuta* were employed. *O. rufipogon* and *O.minuta* are resistant to blight, blast, and planthoppers (Liu and Chen 2014). To elucidate molecular mechanisms of biotic stress resistance, resistance genes have been map-based cloned and isolated in wild rice, and *Pib* and *Pita* confer

resistance to rice blast (Liu *et al.* 2002).

Besides the resistance genes, genes involved in phytohormone signal pathways may contribute to biotic stress defenses as well (Pieterse and Van Loon 1999, Kunkel and Brooks 2002, Fujita *et al.* 2006). The phytohormone genes that play a crucial role in biotic stress tolerance, and silencing genes that involved in JA, SA, and ET perception or production can enhance susceptibility to pathogens and chewing insects (Thomma *et al.* 1999, Norman-Setterblad *et al.* 2000, Kunkel and Brooks 2002, Ye *et al.* 2012). We compared the expression difference of the phytohormone genes between conventional rice and wild rice and found their higher expression in wild rice. This enhanced expression of these phytohormone genes might explain the wild rice advantages in biotic stress defense (Liu and Chen 2014). Meanwhile, expressions of two hydrogenase genes were higher in wild rice than in conventional rice. The hydrogenases response to biological H_2 production in microorganisms and plants (Gaffron and Rubin 1942, Gest and Kamen 1949, Renwick *et al.* 1964, Hatchikian *et al.* 1992, Forestier *et al.* 2003, Melis and Melnicki 2006, Weng *et al.* 2007, Shimada *et al.* 2008, Eroglu and Melis 2011, Xie *et al.* 2012, Jin *et al.* 2013, Zeng *et al.* 2013). Therefore, the hydrogenases or produced H_2 in wild rice may involve in biotic stress tolerance.

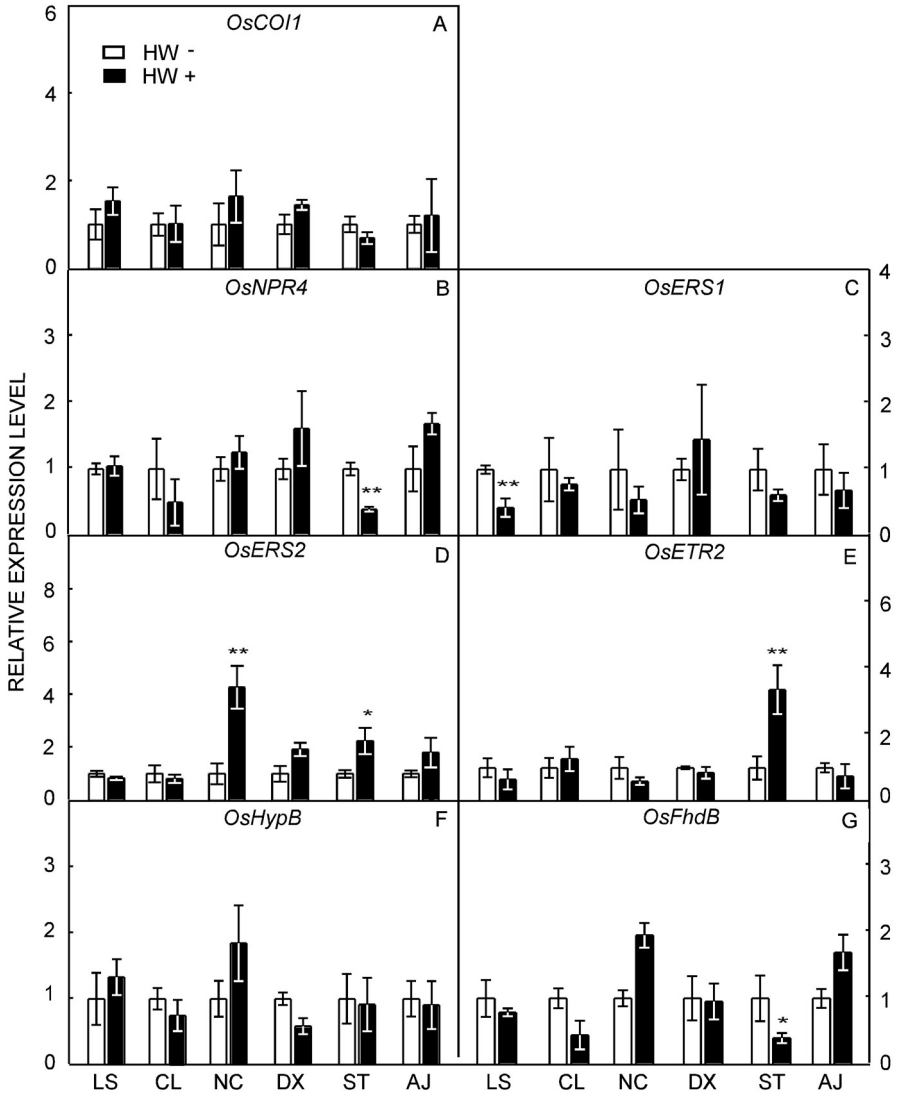

Fig. 4. Relative expressions of hydrogenase genes and phytohormone signaling pathway genes in the rice reproductive growth stage. Wild rice plants (LS, CL, NC, DX, ST, and AJ) were grown in hydrogen-rich water (HW+) or distilled water (HW-) for three months. Real-time qPCR was performed. Jasmonic acid receptor gene *OsCOI1* (*A*), salicylic acid receptor gene *OsNPR4* (*B*), three ethylene receptor genes *OsERS1* (*C*), *OsERS2* (*D*), and *OsETR2* (*E*), and two hydrogenase genes, *OsHypB* (*F*) and *OsFhdB* (*G*). Relative expressions in HW+ plants are compared with HW- plants. Means ± SDs, $n = 3$, * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively.

The putative hydrogenase genes have a function in H_2 metabolism in conventional rice, and their transcriptions were dramatically enhanced by HW (Zeng *et al.* 2013). Moreover, transcription of hydrogenase genes and H_2 production in rice increased by JA, ET, and ABA treatments (Zeng *et al.* 2013). In wild rice LS and DX, the two hydrogenase genes were up-regulated by HW in this study, with the increased transcription of all five receptor genes of the three pytohormone signal pathways in the seedling stage. In *Arabidopsis*, a rapid and sustained production of H_2 is detected in leaves under ABA treatment (Xie *et al.* 2014). Therefore, there is a positive regulation mechanism between hydrogenase genes and H_2 production, which interacts with phytohormone signal pathways.

Jasmonic acid, SA, ET, and ABA play important roles in response to stresses (Pieterse and Van Loon 1999, Kunkel and Brooks 2002, Fujita *et al.* 2006). Zeng *et al.* (2013) suggested that H_2 acts as a signaling molecule in response to phytohormones because they found that HW up-regulates transcription of phytohormone receptor genes of ET, indole-3-acetic acid, ABA, gibberellins, cytokinins, and SA in rice. Hydrogen regulates stomatal closure and enhanced drought tolerance through the ABA signaling cascade in *Arabidopsis* (Xie *et al.* 2014). Reactive oxygen species, nitric oxide, and a guard cell outwardly rectifying K^+ channel protein (GORK) are the key factors in ABA signaling (Bright *et al.* 2006, Yan *et al.* 2007, Hao *et al.* 2012). Hydrogen can induce ROS-dependent NO production and promote stomata closure through GORK (Xie *et al.* 2014). In this study, we found that HW could up-regulate expression of receptor genes of JA, SA, and ET in wild rice. The receptor genes could perceive and transfer signals in JA, SA, and ET signaling pathways (Pieterse and Van Loon 1999, Kunkel and Brooks 2002, Turner *et al.* 2002, Lorenzo and Solano 2005). Thus, an enhanced expression of receptor genes by H_2 could rapidly activate the phytohormone signaling pathways in response to biotic stresses.

Here, the transcription of *OsACS1* was enhanced by HW in wild rice *O. rufipogon*. Zeng *et al.* (2013) found that ET synthesis gene *OsACS1* in *O. sativa* is down-regulated by HW. Wild rice LS (*O. minuta*) and DX (*O. rufipogon*) are presented at different phylogenetic branches and have different genome types (Liu and Chen 2014), but they had the same responses to HW in the seedling stage. Wild rice CL, NC, DX, ST, and AJ belong to the same species from different habitats, but they had different responses to HW in expression of these genes. For example, compared to CL, DX and ST, and NC and AJ had opposite responses to HW in expression of synthesis genes *OsAOS2*, *OsLOX*, and *OsPAD4*. The effects of HW on transcription of phytohormone genes varied with rice species and types. This is likely attributable to the difference of rice living habitats.

In general, the effects of HW are detected using plant seedlings, such as 14-d-old rice, 4-week-old *Arabidopsis*, and 5-d-old alfalfa (Zeng *et al.* 2013, Cui *et al.* 2014, Xie *et al.* 2014). Here, we employed three growth stages to study the effects of HW on expression of the phyto-hormone genes and found that it varied with the rice growth stages. In the seedling stage, HW up-regulated the expression of the hydrogenase genes and phytohormone receptor and synthesis genes. In the vegetative and reproductive stages, HW increased the expression of the hydrogenase genes, but most of the receptor genes were not up-regulated. We propose the reason is that the soil environment might affect HW absorption by roots in the vegetative and reproductive stages. In addition, phytohormones mediate other physiological process, *e.g.*, JA regulates pollen maturation, ET plays a role in flower and fruit development, and SA is involved in cell cycle progression and shoot growth (Turner *et al.* 2002, Meguro and Sato 2014, Ju and Chang 2015). This likely decreased the effect of phytohormones on stress responses. Therefore, studies should be done employing different plant stages for deeply understanding HW effects.

In conclusion, the expressions of the SA, JA, and ET signaling pathway genes and hydrogenase genes were higher in wild rice than in conventional rice. These genes were up-regulated by HW in the wild rice seedlings. It is likely that H_2 participated in biotic stress resistance through regulating gene expression that involved in H_2 production and phytohormone signal pathways.

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