CRISPR/Cas9-Mediated Gene Editing of Vacuolar ATPase Subunit D Mediates Phytohormone Biosynthesis and Virus Resistance in Rice

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Research Article

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Abstract

Background: Vacuolar ATPases (v-ATPases) are proton pumps for proton translocation across membranes that utilize energy derived from ATP hydrolysis; Previous research revealed Osv-ATPases mediates phytohormes levels and resistance in rice. Osv-ATPase subunit d (Osv-ATPase d) is part of an integral, membrane-embedded V0 complex of V-ATPases complex, whether Osv-ATPase d involves in phytohormes biosynthesis and resistance in rice remains unknown.

Finding: The knockout mutant line (line 5) of *Osv-ATPase d* was generated using the CRISPR/Cas9 system, mutation of *Osv-ATPase d* did not show any detrimental effect on plant growth or yield productivity. Transcriptomic results showed *Osv-ATPase d* probably involved in mediating the biosynthesis of plant hormones and resistance in rice. Mutation of *Osv-ATPase d* significantly increased JA and ABA biosynthesis than wild type. Compared to wild type, mutation of *Osv-ATPase d* increased the resistance against *Southern rice black-streaked dwarf virus* (SRBSDV), however, decreased the resistance against *Rice stripe virus* (RSV) in rice.

Conclusion: Taken together, our data reveal the *Osv-ATPase d* mediates phytohormone biosynthesis and virus resistance in rice, which can be selected as a potential target for resistance breeding in rice.

Findings

Rice (*Oryza sativa*) is one of the most important staple foods for more than half of the world's population (Chen et al. 2011). However, many viral diseases severely challenge rice production in many global areas, such as *Southern rice black-streaked dwarf virus* (SRBSDV), which is severely epidemic and has caused 30-50% rice yield losses in southern China and Southeast Asia in the last decade (Alonso et al. 2019). Although SRBSDV has been successfully controlled by international cooperation, it still exists in the majority of rice producing areas of eastern China, with periodic outbreaks in a few rice production areas and the potential for additional widespread outbreaks (Zhou et al. 2013). One of the most effective strategies to prevent viral diseases is growing resistant or tolerant varieties; nevertheless, almost all cultivated rice varieties are susceptible to SRBSDV (Wang et al. 2017; Yu et al. 2017; Zhou et al. 2021). CRISPR/Cas-based genome editing is an alternative method for accelerating rice improvement (Ma et al. 2015). The availability of rice reference genome sequences and the CRISPR/Cas9-editing system has made it possible to develop disease-resistant or disease-tolerant rice by precisely editing endogenous genes (Zhao et al. 2020).

Vacuolar ATPases (v-ATPases) are proton pumps for proton translocation across membranes that utilize energy derived from ATP hydrolysis (Forgac et al. 2007; Mazhab-Jafari et al. 2017). Eukaryotic v-ATPases are multiprotein complexes, and v-ATPase subunit d (v-ATPase d) is part of an integral, membrane-embedded V0 complex (Hohlweg et al. 2018). The pathogens *Sarocladium oryzae* and *Pseudomonas fuscovaginae* cause rice sheath rot and produce cyclic lipopeptides to inhibit membrane-bound H^+-ATPase pumps in the rice plant, resulting in reduced abscisic acid (ABA), jasmonate acid (JA)

and auxin levels and grain yield in rice (Peeters et al. 2020). Plant hormones are pivotal for biotic and abiotic resistance, and rice hormones have diverse functions in rice resistance against different viruses (Xie et al. 2018; Yan et al. 2015; Zhang et al. 2020). Therefore, *Osv-ATPase d* could be an alternative target for gene editing by CRISP/Cas9 to enhance viral resistance in rice.

In this study, CRISPR/Cas9-based genome-editing technology was employed to edit *Osv-ATPase d* in Nipponbare (*Oryza sativa* L. cv. *Japonica*, NIP), which is highly susceptible to SRBSDV and Rice stripe virus (RSV) (Zhang et al. 2019). Two guide RNAs were designed to target the first exon of *Osv-ATPase d* by CRISPR Design (http://cripsr.mit.edu) (Fig. 1a). Specific single guide RNAs (sgRNAs) targeted to *Osv-ATPase d* were selected and constructed by universal primers (Table S1) , which was used to transform the rice cultivar NIP by the Agrobacterium mediated transformation as previously described (Ma et al., 2015). Five independent T0 lines were found to carry heterozygous mutations of *Osv-ATPase d*. From the T1 segregation population, a CAS9-free homozygous mutant with knockout of *Osv-ATPase d* (hereafter named line 5) was identified. Conventional Sanger sequencing verified that a “C” insertion resulted in a frameshift mutant with a “G” deletion in *Osv-ATPase d* (Figure 1a). This plant and its offspring were selected and used for further trait analysis.

The growth trial of editing line 5 grown in pots under greenhouse conditions showed normal growth with no morphological differences when compared to wild-type plants at 50 days of age (Figure 1b). No adverse effect was observed regarding spike morphology (Figure 1c) or the yield characteristics of spike length, number of spikelets, grain number per spike and 1000-grain weight between editing line 5 and wild-type plants (Table 1). These results suggested that there was no detrimental impact of knocking out *Osv-ATPase d* in rice.

Table 1 Agronomic traits of the line 5 and the wild type

Sample	Spike length (cm)	Number of spikelets	grain number per spike	1000-grain weight (g)
line 5	20.26±0.56a	9.67±0.67a	114.33±1.45a	19.76±1.18a
NIP	19.37±0.47a	9.67±0.33a	104.00±4.04a	20.91±0.91a

Notes: Letters indicate significantly different values using Student’s *t* test ($\rho < 0.05$).

To gain insight into the functional profiles of *Osv-ATPase d* in rice, the transcriptomic response (dataset was permanently deposited in GenBank at accession number: PRJNA753714) of editing line 5 plants was comparatively analyzed with that of wild-type plants. Compared with wild-type plants, a total of 664 differentially expressed genes (DEGs) were induced in the editing line 5 seedlings (15 days old) (Fig 2a). KEGG pathways with enrichment of 443 significantly upregulated and 221 significantly downregulated genes revealed that several metabolic pathways were altered between editing line 5 and the wild type, such as phenylpropanoid biosynthesis, plant hormone signal transduction and the MAPK signalling pathway (Fig 2b). These findings showed that *Osv-ATPase d* is probably involved in mediating the

biosynthesis of plant hormones and resistance to pathogens of rice and may be involved in the molecular mechanisms of both pathways.

Transcriptomic analysis showed that *Osv-ATPase d* is involved in plant hormone mediation; thus, the plant hormones in editing line 5 and the wild type were then quantified by ultra-high-performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-MS/MS) (Hu et al. 2020; Peeters et al. 2020). As expected, *Osv-ATPase d* was indeed involved in mediating plant hormone biosynthesis. Compared with wild-type plants, editing line 5 showed significantly increased JA and ABA biosynthesis (Fig 2c and 2d), but there was no effect on the biosynthesis of five other plant hormones, including 1-aminocyclopropanecarboxylic acid (ACC), indoleacetic acid (IAA), salicylic acid (SA) and *trans*-zeatin (tZ) (Table S2).

Transcriptomic and plant hormone biosynthesis analysis showed that *Osv-ATPase d* may mediate resistance in rice. Three replicates of editing line 5 were evaluated for resistance against SRBSDV and RSV. SRBSDV disease symptom observations showed that at 30 dpi, the NIP plants showed more severe stunting upon SRBSDV infection (Fig 3a), and the SRBSDV disease incidence (Fig 3b) and accumulation of SRBSDV virions (Fig 3c) in the wild-type plants were significantly higher than those in the line 5 plants. In contrast, the editing line 5 plants displayed higher susceptibility to RSV than the wild-type plants (Fig 3d-3f). Further analyses showed that editing line 5 in rice had no significant effect on virus-transmitting vector infestation. These results indicated that *Osv-ATPase d* can differentially regulate rice resistance to SRBSDV and RSV infection.

Altogether, the *Osv-ATPase d* knockout mutant of rice showed different levels of resistance to important viruses, SRBSDV and RSV, and did not show any detrimental effect of gene knockout on plant growth or yield productivity. *Osv-ATPase d* can be selected as a potential target for resistance breeding in rice.

Abbreviations

ABA: abscisic acid; ACC: 1-aminocyclopropanecarboxylic acid; IAA: indoleacetic acid; JA: jasmonate acid; SA: salicylic acid; SRBSDV: Southern rice black-streaked dwarf virus; RSV: rice stripe virus; tZ: trans-zeatin; v-ATPase: vacuolar ATPase.

Declarations

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Author contributions

QL, XL, XY, TZ, YZ, YL and Ying Lan performed the experiments; Li Li, LZ and DZ performed data analyses; SZ and Yong Liu designed the research and wrote the manuscript.

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Availability of Data and Materials

All data supporting the conclusions of this article are provided within the article (and its Additional files).

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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Figures

Figure 1

CRISPR/Cas9-mediated editing of *Osv-ATPase d* in rice. **a** Illustration of *Osv-ATPase d* gene structure and the editing target. **b** Morphology of plants of the *Osv-ATPase d* knockout mutant (line 5) and the wild type at 50 days of age. **c** Morphology of spikes of the line 5 and the wild type.

Figure 2

Transcriptomic response and Quantitative determination of endogenous plant hormones by a UPLC-MS/MS system. **a** The volcano map presents the differentially expressed genes (FDR < 0.05 and >= 2-fold change) between the line 5 and the wild type based on leaf transcriptome analysis.**b** KEGG pathways with enrichment of significantly upregulated and downregulated genes. Plant hormone biosynthesis genes and plant defence genes are underlined in red. **c** ABA; **d** JA. *, $\rho < 0.05$ by the Student's *t* test

Figure 3

Osv-ATPase d modulates rice resistances to SRBSDV and RSV. **a** Representative images of mock-inoculated or *southern rice black-streaked dwarf virus* (SRBSDV)-infected NIP and line 5 plants. Bar = 2 cm (top). Bar = 1 cm (bottom). **b** The percentages of SRBSDV-infected NIP and line 5 plants. **c** Detection of SRBSDV levels by quantitative RT-PCR of *CP* gene RNA expression levels and by western blotting using antibodies against the SRBSDV P8 protein. CBB: Coomassie brilliant blue staining. **d** Representative images of mock-inoculated or *rice stripe virus* (RSV)-infected NIP and line 5 plants. Bar = 5 cm (top). Bar = 1 cm (bottom). **e** The percentages of RSV-infected NIP and line 5 plants. **f** Detection of RSV CP RNA expression levels by quantitative RT-PCR. *, $\rho < 0.05$ by the Student's *t* test; **, $\rho < 0.01$ by the Student's *t* test.

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