



Article

# Knockdown of Quinolinate Phosphoribosyltransferase Results in Decreased Salicylic Acid-Mediated Pathogen Resistance in *Arabidopsis thaliana*

Shengchun Li \*, Haiyan Ding, Yi Deng and Jiang Zhang

State Key Laboratory of Biocatalysis and Enzyme Engineering, School of Life Sciences, Hubei University, Wuhan 430062, China; inghaiyan24@163.com (H.D.); dengyi10998@163.com (Y.D.); zhangjiang@hubu.edu.cn (J.Z.)

\* Correspondence: shengchun.li@hubu.edu.cn

**Abstract:** Nicotinamide adenine dinucleotide (NAD) is a pivotal coenzyme that has emerged as a central hub linking redox equilibrium and signal transduction in living cells. The homeostasis of NAD is required for plant growth, development, and adaptation to environmental stresses. Quinolinate phosphoribosyltransferase (QPRT) is a key enzyme in NAD de novo synthesis pathway. T-DNA-based disruption of *QPRT* gene is embryo lethal in *Arabidopsis thaliana*. Therefore, to investigate the function of *QPRT* in *Arabidopsis*, we generated transgenic plants with decreased *QPRT* using the RNA interference approach. While interference of *QPRT* gene led to an impairment of NAD biosynthesis, the *QPRT* RNAi plants did not display distinguishable phenotypes under the optimal condition in comparison with wild-type plants. Intriguingly, they exhibited enhanced sensitivity to an avirulent strain of *Pseudomonas syringae* pv. *tomato* (*Pst-avrRpt2*), which was accompanied by a reduction in salicylic acid (SA) accumulation and down-regulation of pathogenesis-related genes expression as compared with the wild type. Moreover, oxidative stress marker genes including *GSTU24*, *OX11*, *AOX1* and *FER1* were markedly repressed in the *QPRT* RNAi plants. Taken together, these data emphasized the importance of QPRT in NAD biosynthesis and immunity defense, suggesting that decreased antibacterial immunity through the alteration of NAD status could be attributed to SA- and reactive oxygen species-dependent pathways.

**Keywords:** NAD; QPRT; pathogen; pathogenesis-related genes; SA; oxidative stress



**Citation:** Li, S.; Ding, H.; Deng, Y.; Zhang, J. Knockdown of Quinolinate Phosphoribosyltransferase Results in Decreased Salicylic Acid-Mediated Pathogen Resistance in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **2021**, *22*, 8484. <https://doi.org/10.3390/ijms22168484>

Academic Editor: Marcello Iriti

Received: 30 March 2021

Accepted: 3 August 2021

Published: 6 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

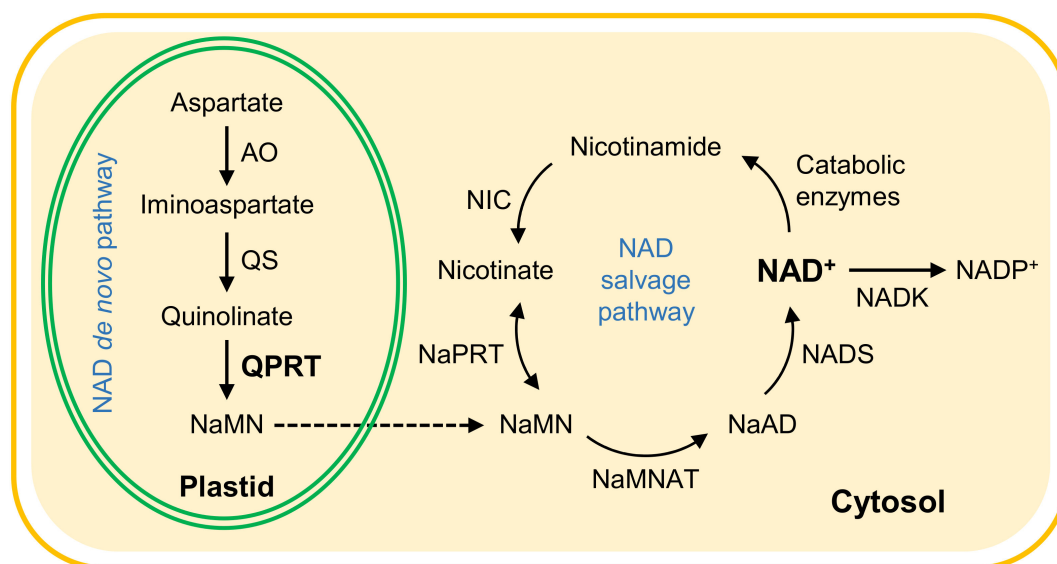
## 1. Introduction

Pyridine nucleotide co-enzymes are ubiquitous in living organisms [1]. Nicotinamide adenine dinucleotide (NAD) and its phosphate derivative NADP, exist in oxidized forms (NAD<sup>+</sup> or NADP<sup>+</sup>, respectively) or reduced forms (NADH or NADPH, respectively). They mediate a wide range of redox reactions, and thus impact virtually all metabolic reactions in the cell [2]. In addition to their functional roles in redox regulation, NADs also plays important roles in cell signaling, including intracellular Ca<sup>2+</sup> signalling via NAD-derived cyclic ADP-ribose [3], translational modification of target proteins by the transfer of ADP-ribose from NAD catalyzed by poly(ADP-ribose) polymerases and mono(ADP-ribose)transferase [4], and epigenetic regulations by the sirtuin histone deacetylases [5,6].

In plants, NAD has also been documented to act as a signal in response to environmental stresses, including pathogen infections [7–9]. For instance, quinolinate-induced stimulation of intracellular NAD in *Arabidopsis* expressing *nadC* gene from *Escherichia coli*, which encodes quinolinate phosphoribosyltransferase (QPRT), enhances defense gene expression and resistance to diverse bacterial and fungal pathogens [10,11]. Overexpression of *Arabidopsis* Nudix (nucleoside diphosphates linked to some moiety X) hydrolase gene, hydrolase homolog 6 (*AtNUDT6*), encoding an ADP-ribose/NADH pyrophosphohydrolase, and disruption of *AtNUDT6* [12], *AtNUDT7* [13,14], or *AtNUDT8* [15] all lead to

changes in intracellular NADH levels and salicylic acid (SA)-mediated immune signaling. Additionally, exogenous NAD(P) treatment in *Arabidopsis* can induce SA-dependent and -independent expression of pathogenesis-related (*PR*) genes and resistance to bacterial pathogens [16,17]. The recent identification of plasma membrane-localized NAD(P) receptors LecRK-I.8 and LecRK-VI.2 confirmed that NAD(P) act as extracellular signals [18,19].

NAD can be synthesized via both a de novo pathway and a salvage pathway in plants (Figure 1) [2,9,20]. The de novo pathway starts in plastids using aspartate as precursor. In plastids, quinolinate is produced from aspartate and dihydroxyacetone phosphate by aspartate oxidase (AO) plus quinolinate synthase (QS), and quinolinate is rapidly converted to nicotinate mononucleotide (NaMN) by QPRT. NaMN is thereafter converted to NAD in the cytosol through adenylation by NaMN adenylyltransferase (NaMNAT) and amidation by NAD synthase (NADS). *AO*, *QS*, *QPRT* and *NaMNAT* are single genes in the *Arabidopsis* genome. The T-DNA insertion of either of these genes exhibited developmental defects or was embryo lethal [20,21], indicating an essential role of the de novo pathway in plant growth and development. *NaMNAT* and *NADS* also participate in the NAD salvage pathway (Figure 1) [9]. Unlike the disrupted *AO* allele [20], *AO*-knockdown mutants are viable and fertile plants, but harbor impaired stomatal immunity against a coronatine-deficient strain of *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 [22]. *Arabidopsis* *QS* consists of a quinolinate synthase (*NadA*) and a SufE3 domain required for incorporation of the Fe-S cluster [23]. A non-lethal mutation in the SufE domain is responsible for the early senescence phenotype of the *onset of leaf death5* (*old5*) mutant [24]. Rather than decreased NAD, this effect was associated with increased level of NAD, which was attributed to enhanced activities of the salvage pathway in the *old5* mutant [24]. Recent studies have shown that a point mutation (Q288E substitution) in the region encoding the *NadA* domain of the *QS* gene resulted in markedly decreased levels of NAD, and caused salt and abscisic acid (ABA) hypersensitivity in *Arabidopsis* [25,26]. To date, no detailed studies of *QPRT* knockdown mutants of *Arabidopsis* have been reported.



**Figure 1.** Schematic representation of NAD biosynthesis pathway in plants. The de novo biosynthesis of NAD starts from aspartate in the plastid. Abbreviations: AO, aspartate oxidase; NaAD, nicotinate adenine dinucleotide; NADK, NAD kinase; NADS, NAD synthetase; NaMN(AT), nicotinate mononucleotide (adenylyltransferase); NaPRT, nicotinate phosphoribosyltransferase; NIC, nicotinamide; QPRT, quinolinate phosphoribosyltransferase; QS, quinolinate synthase.

The NAD salvage pathway starts from nicotinamide (Figure 1), which is sequentially catalyzed by nicotinamidase (NIC), nicotinate phosphoribosyltransferase (NaPRT), NaMNAT, and NADS. Three *NIC* genes (*NIC1*, *NIC2*, and *NIC3*) have been identified in *Arabidopsis* [27,28]. The major *NIC* appears to be *NIC1*, and null mutant *nic1-1* shows

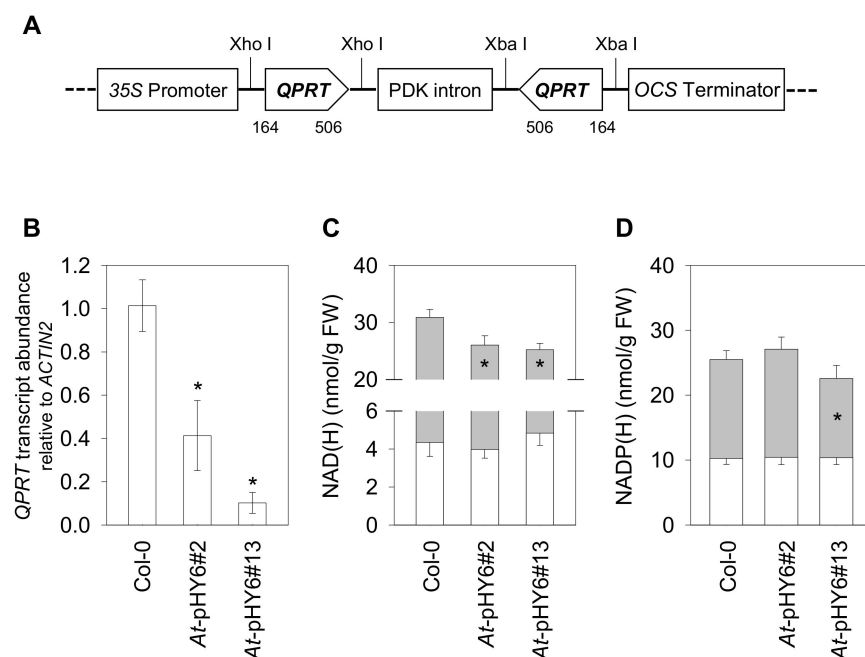
decreased pools of pyridine nucleotides in many tissues and is hypersensitive to salt and ABA treatments, pointing to a role for the pathway in recycling and maintaining NAD pools [27]. Moreover, a 60-fold increase in nicotinamide contents in *nic1-1* Arabidopsis mutants was shown to inhibit aphid reproductive potential [29]. The *NIC2* gene is most strongly expressed in mature seeds. Increased levels of NAD was observed in *nic2-1* mutant, and this was associated with increased seed dormancy [28].

Since disruption of *QPRT* causes lethality in Arabidopsis [20], *QPRT* knockdown RNAi lines were generated to assess the effects of a reduced level of *QPRT* on pathogen infection. Decreased NAD levels in the *QPRT* RNAi plants led to enhanced pathogen sensitivity accompanied by decreased expression of *PR* genes and oxidative marker genes, and also reduced SA accumulation.

## 2. Results

### 2.1. Generation of Transgenic Plants with Decreased *QPRT* Expression

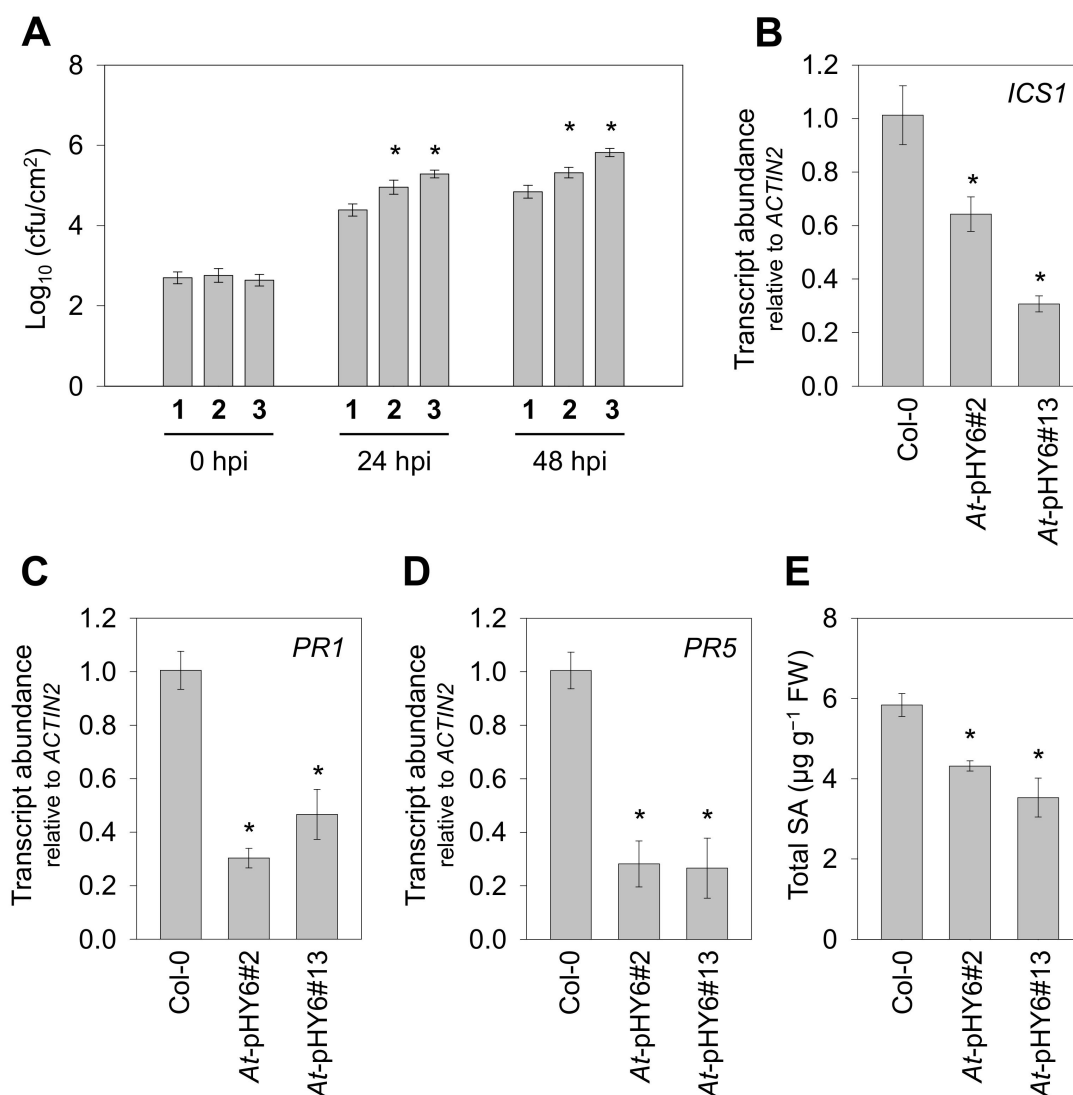
To assess the effects of decreased expression of *QPRT*, we generated RNAi knockdown lines by transforming wild-type Arabidopsis plants with a 343 bp fragment from cDNA in sense and antisense directions under the 35S promoter (Figure 2A). We selected two independent RNAi lines (*At-pHY6#2* and *At-pHY6#13*) with greatly reduced *QPRT* mRNA levels (Figure 2B) and used the homologous T3 progeny of these lines in subsequent analyses (Figure S1). As expected, the *QPRT* RNAi lines showed reduced levels of NAD (Figure 2C). Moreover, a significant decrease in NADP content was observed in *At-pHY6#13* plants (Figure 2D). Neither the NADH nor the NADPH pool was significantly affected in the RNAi lines (Figure 2C,D). However, reduced *QPRT* expression did not cause aberrant phenotypes when plants were grown under the conditions of these experiments (Figure S2).



**Figure 2.** Generation of *QPRT* RNAi lines and determination of pyridine nucleotides contents in Arabidopsis. (A) The RNAi construct pHY6 contained a 35S promoter, a sense fragment of *QPRT* cDNA from 164 to 506, the PDK intron, the *QPRT* fragment in antisense orientation, and an OCS terminator. (B) *QPRT* mRNA levels analyzed by qRT-PCR. Contents of NAD(H) (C) and NADP(H) (D) were quantified. White bars, reduced forms; black bars, oxidized forms. Data are means  $\pm$  SE of three biological replicates. Asterisk indicates difference relative to Col-0 for each form at  $* p < 0.05$ .

## 2.2. Decreased *QPRT* Enhances Sensitivity to *Pst-avrRpt2*

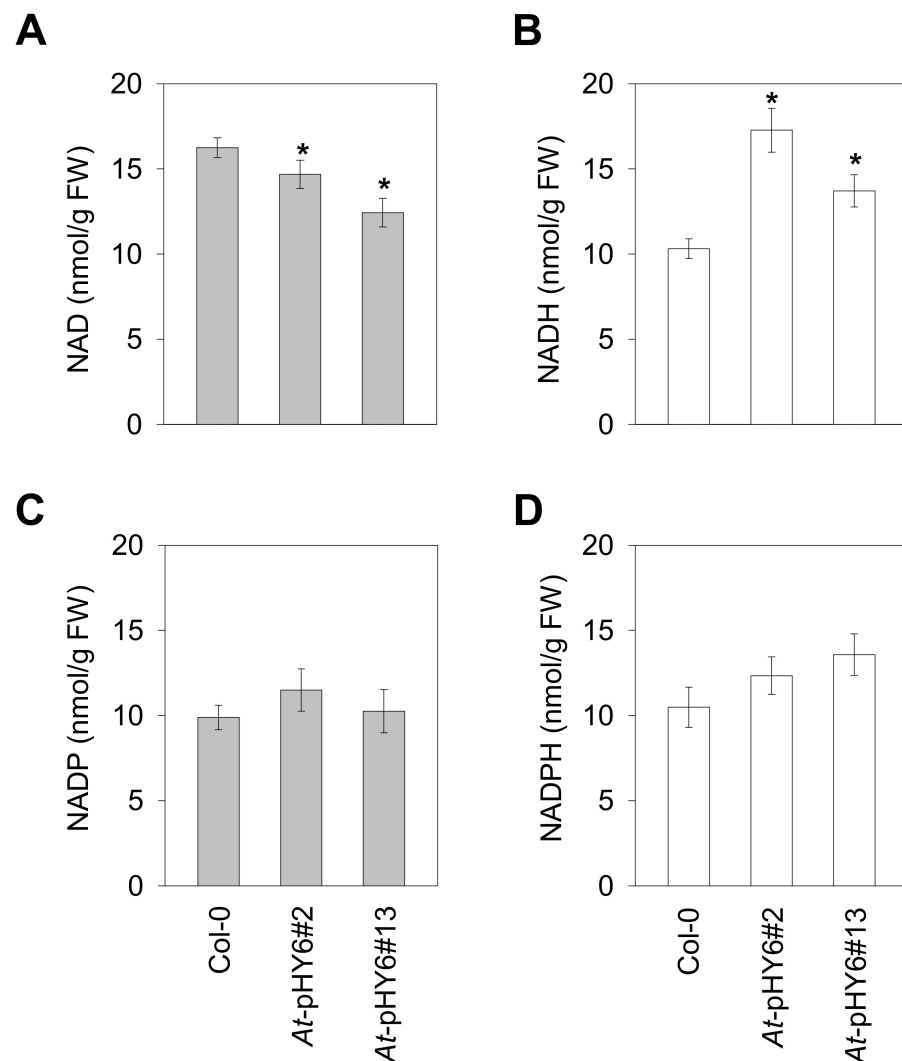
To determine whether decreased expression of *QPRT* affects plant resistance to biotic stress, we investigated the response of the RNAi lines to an avirulent pathogen of *Pst* DC3000 harboring the elicitor *avrRpt2* (*Pst-avrRpt2*). As shown Figure 3A, the growth of *Pst-avrRpt2* in the *QPRT* RNAi plants was significantly higher than that in the wild-type plants, both at 24 h post inoculation (24 hpi) and 48 hpi. To understand the mechanism by which the decreased *QPRT* expression led to enhanced disease sensitivity, we examined defense responses including transcripts of a key gene involved in SA synthesis (*isochorismate synthase 1*, *ICS1*) and *PR* genes, as well as SA accumulation. Quantitative RT-PCR analysis showed that transcripts of *ICS1*, *PR1* and *PR5* were reduced in response to *Pst-avrRpt2* in the *QPRT* RNAi plants in comparison with Col-0 at 48 hpi (Figure 3B–D). Moreover, down-regulation of these transcriptional levels coincided with reduced accumulation of total SA in the RNAi lines (Figure 3E).



**Figure 3.** Reduced transcript levels of *QPRT* are associated with increased susceptibility. (A) The *QPRT* RNAi plants showed enhanced sensitivity to *Pst-avrRpt2* compared with Col-0. Leaves from two 5-week-old independent lines of *QPRT* RNAi or Col-0 were inoculated with *Pst-avrRpt2* at a concentration of  $5 \times 10^5$  cfu·mL<sup>-1</sup>. Bacterial growth was measured at 0, 24, and 48 hpi. 1, Col-0; 2, At-pHY6#2; 3, At-pHY6#13. Expression of *ICS1* (B), *PR1* (C) and *PR5* (D) was measured by qRT-PCR in Arabidopsis challenged with *Pst-avrRpt2* at 48 hpi. (E) Quantification of SA in pathogen-challenged plants at 48 hpi. Means  $\pm$  SE of three repetitions are shown. Asterisk indicates means are significantly different from those of Col-0 at  $p < 0.05$ .

### 2.3. Decreased *QPRT* Affects Redox Homeostasis

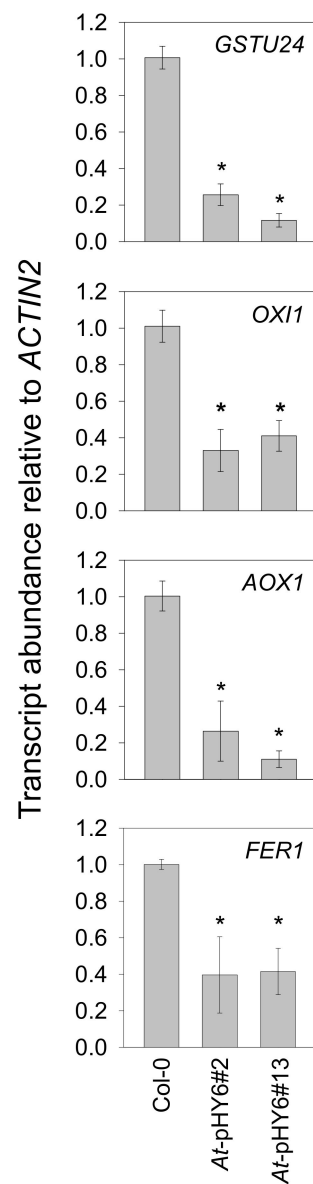
To test whether the observed sensitivity was accompanied by changes in NAD pools, we determined the expression level of *QPRT* gene and contents of pyridine nucleotides in the *QPRT* RNAi plants infected by *Pst-avrRpt2*. There was a relatively low level of *QPRT* expression in the RNAi lines in comparison with the wild-type plants at 48 hpi (Figure S3). Consistent with *QPRT* expression, the NAD levels were lower in the *QPRT* RNAi plants than that in Col-0 at 48 hpi, whereas much higher levels of NADH were seen in the RNAi lines (Figure 4A,B). In addition, the levels of NADP and NADPH did not differ significantly between the RNAi lines and Col-0 at 48 hpi (Figure 4C,D).



**Figure 4.** Contents of pyridine nucleotides in Col-0 and RNAi plants upon *Pst-avrRpt2* infection. Levels of NAD (A), NADH (B), NADP (C) and NADPH (D) in bacterium-challenged plants at 48 hpi were determined. Data are means  $\pm$  SE of four independent extracts. Asterisk indicates significant differences from Col-0. \*  $p < 0.05$ .

Reactive oxygen species (ROS) burst has been well established as an integral aspect of plant immunity [30,31], and NAD has been known to stimulate ROS production [11]. To further investigate overall cellular redox states, we measured changes in hydrogen peroxide ( $H_2O_2$ ) indirectly by measuring changes in the transcript levels of four  $H_2O_2$  marker genes, cytosolic glutathione *S*-transferase TAU 24 (*GSTU24*, AT1G17170) [32] and oxidative signal-inducible 1 (*OX11*, AT3G25250) [33], mitochondrial alternative oxidase 1 (*AOX1*, AT3G22370) [34], and chloroplast *ferritin 1* (*FER1*, AT5G01600) [35]. The expression

of these genes was reduced in the RNAi plants in comparison with wild-type plants at 48 hpi (Figure 5).



**Figure 5.** Transcript abundance of oxidative stress marker genes were suppressed in the RNAi plants compared with Col-0 at 48 hpi. Transcriptional expression is shown relative to the reference gene *ACTIN2*. Values shown indicate means of four biological replicates. Asterisks indicate that values are significantly different from those of Col-0. \*  $p < 0.05$ .

### 3. Discussion

Because the homozygous null alleles of *qprrt* mutants of *Arabidopsis* are embryolethal [20], *QPRT* RNAi plants were generated to assess the effects of constitutively reduced level of *QPRT*. The amount of NAD was lower in the *QPRT* RNAi plants, while the NADH levels were similar to those observed in control plants under optimal conditions (Figure 2C). Pyridine nucleotide measurements in this study represent total nucleotide pools, i.e., they include free pools and those bound to proteins. It was revealed that the free NADH level can be maintained at a more constant value than oxidized NAD and total NAD [36], partially providing an explanation for the NADH levels not changing significantly in the RNAi lines. No obvious phenotype was observed in the RNAi lines under the conditions of these experiments (Figure S2).

A growing body of evidence suggests that NAD plays a crucial role in plant immunity [10,11,16,22,37]. Notably, overexpression of the bacterial NAD biosynthesis gene *nadC*, which increased intracellular NAD levels with the addition of quinolinate, resulted in heightened resistance to avirulent pathogen *Pst-AvrRpm1*, but not to virulent strain *Pst* DC3000 [10]. In the current study, we tested whether a deficient NAD synthesis pathway in *QPRT* RNAi lines would have increased susceptibility to an avirulent bacterial strain. As expected, after infection with the avirulent *avrRpt2*-containing *Pst* DC3000 strain (*Pst-avrRpt2*), the *QPRT* RNAi plants exhibited increased bacterial proliferation compared with Col-0. The enhanced sensitivity to *Pst-avrRpt2* was associated with suppressed expression of the defense-related genes (*ICS1*, *PR1* and *PR5*), and this was consistent with decreased SA accumulation (Figure 3). In addition, the NAD levels were decreased following the infection with *Pst-avrRpt2*, whereas the NADH levels were increased (Figure 2C; Figure 4A,B). A much higher level of NADH was observed in the RNAi lines in comparison with Col-0 after *Pst-avrRpt2* infection. The observed alterations of NAD(H) pools led to a lower NAD/NADH ratio in the RNAi lines than that in wild-type plants. Indeed, the physiologically relevant ratio of NAD to NADH is generally high, favoring hydride transfer from a substrate to NAD to form NADH in plant cells [38]. The results of the current study support that *QPRT* enzyme is critical for the steady state of NAD and the homeostasis of NAD/NADH in *Arabidopsis*.

The cytosolic *GSTU24* gene is known to be H<sub>2</sub>O<sub>2</sub> inducible [32,39] and up-regulated by *Pst-avrRpt2* [40,41]. OX1, a serine/threonine kinase, activates the MAPK3/6 cascade in an H<sub>2</sub>O<sub>2</sub>-dependent manner, which has been implicated in some pathogen responses and root hair development [33,42]. AOX1 encodes a mitochondrial alternative oxidase that has been shown to be induced by *Pst* attack [43] and links mitochondrial ROS to cell death [44,45]. FER1, encoding chloroplast-localized ferritins, has been shown to play an important role in keeping Fe<sup>2+</sup> levels at a minimum upon increased intracellular H<sub>2</sub>O<sub>2</sub> [35,46]. In the current study, reduced expression of four H<sub>2</sub>O<sub>2</sub> marker genes suggests redox perturbations occurred in different subcellular compartments in *QPRT* RNAi plants. Given that the cytosol is the site of the final step of NAD biosynthesis (Figure 1), specific transport proteins are required to shuttle NAD across intracellular membranes for organellar import of NAD into chloroplasts, mitochondria and peroxisomes [47–51]. NAD transport could influence cellular redox balance, thus may lead to redox perturbations in different subcellular compartments of *QPRT* RNAi plants.

Collectively, these results indicate that *QPRT* is important in NAD biosynthesis, and contributes to plant resistance through SA- and ROS-dependent pathways. Additionally, the availability of the *QPRT* RNAi lines allow future detailed studies on the role of NAD metabolism in many aspects of plant biology including, but not limited to, immunity, development, signaling, and biosynthesis [8,9,38].

## 4. Materials and Methods

### 4.1. Plant Materials and Growth Condition

The RNAi lines used in this study were derived from the wild-type *Arabidopsis thaliana* Columbia (Col-0) ecotype. Plants were cultivated in controlled growth chambers in a photoperiod of 8 h light/16 h dark, an irradiance of 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  at leaf level, at temperatures of 22 °C day/20 °C night and 75–80% humidity.

### 4.2. Generation of RNAi Plants

To obtain RNAi plants, partial coding region for *QPRT* (AT2G01350) was amplified with RiQPRT-Fwd1/RiQPRT-Rev1 and RiQPRT-Fwd2/RiQPRT-Rev2 primers (Table S1). The primers harbor a short homologous sequence (underlined) to pHELLSGATE8 vector. Two *QPRT* PCR products were then cloned into the pHELLSGATE8 vector via homologous recombination between the XhoI-XhoI sites in the sense orientation and the XbaI-XbaI sites in the antisense orientation to generate RNAi construct pHY6 (Figure 2A) [52]. The construct was transformed into the *Agrobacterium tumefaciens* GV3101 strain, which was

then used to transform *Arabidopsis* Col-0 using the floral dip method [53]. Transgenic plants were selected on Murashige and Skoog (MS) media containing  $50\text{mg}\cdot\text{L}^{-1}$  kanamycin. Single insertion lines were selected by 3:1 segregation on selection media.

#### 4.3. Pathogen Test

The avirulence strain of *Pseudomonas syringae* pv. *tomato* DC3000 carrying the avirulent gene *avrRpt2* (*Pst-avrRpt2*; a gift from Prof. Jianmin Zhou, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences) was used for resistance tests with a medium titer of  $5 \times 10^5$  colony-forming units (cfu)  $\text{mL}^{-1}$ . Whole leaves of 5-week-old plants were infiltrated with a 1-mL needleless syringe in the middle of the photoperiod. Six to eight leaves were made by pooling two leaf discs ( $\varphi = 0.45$  cm each) harvested from different inoculated leaves of different plants. Bacterial growth was examined by grinding leaf discs in 400 mL of water, plating appropriate dilutions on solid King's B medium with  $100\text{mg}\cdot\text{L}^{-1}$  rifampicin and  $25\text{mg}\cdot\text{L}^{-1}$  kanamycin, and quantifying colony numbers. Bacterial cfu were counted at 0, 24 and 48 hpi. Experiments were repeated three times with similar results.

#### 4.4. RNA Isolation and Quantitative Reverse Transcription-PCR

Total RNA was isolated from leaf samples using RNAiso Plus Reagent (Takara, Dalian, China) according to the manufacturer's instructions. Hifair<sup>®</sup> II 1st Strand cDNA Synthesis Kit with gDNA digester (Yeasen, China) was used to synthesize cDNA. Quantitative reverse transcription-PCR (qRT-PCR) was performed on Bio-Rad CFX Connect Real-Time System (Bio-Rad, USA) using the SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II (Tli RNaseH Plus) kit (Takara, Dalian, China). The *ACTIN2* gene was used as an internal control to normalize for specific gene expression in the samples. Each treatment was replicated with at least three independent biological sample preparations. Quantitative analysis of gene expression was performed using the  $2^{-\Delta\Delta\text{CT}}$  method [54]. Gene primers are listed in Table S1.

#### 4.5. Metabolite Measurements

Reduced and oxidized forms of NAD and NADP were determined by spectrophotometric assays, as previously described [55,56]. Total SA concentration was measured by high-performance liquid chromatography-fluorescence according to Langlois-Meurinne et al. (2005) [57].

#### 4.6. Statistical Analysis

Statistical significance was determined using the Student's *t*-test. A *p* value of  $<0.05$  was considered significant and indicated by an asterisk in the Figures.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/ijms22168484/s1>. Figure S1. Seed assays confirming the homozygous status of the *QPRT* RNAi plants. Figure S2. Knockdown of *QPRT* expression by RNAi did not affect plant growth under optimal growth condition. Figure S3 *QPRT* mRNA levels at 48 hpi. Table S1. List of primers used in the study.

**Author Contributions:** Conceptualization, S.L.; methodology, H.D., Y.D., writing—original draft preparation, H.D. and Y.D.; writing—review and editing, J.Z. and S.L.; funding acquisition, S.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** S.L. was supported by the National Natural Science Foundation of China (32071477 and 31700227) and Innovation Base for Introducing Talents of Discipline of Hubei Province (2019BJH021).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** This study did not report any data.

**Conflicts of Interest:** The authors declare no conflict of interest.



## References

1. Noctor, G.; Hager, J.; Li, S. Biosynthesis of NAD and its manipulation in plants. *Adv. Bot* **2011**, *58*, 153–201. [[CrossRef](#)]
2. Noctor, G.; Queval, G.; Gakière, B. NAD(P) synthesis and pyridine nucleotide cycling in plants and their potential importance in stress conditions. *J. Exp. Bot.* **2006**, *57*, 1603–1620. [[CrossRef](#)] [[PubMed](#)]
3. Sánchez, J.; Duque, P.; Chua, N. ABA activates ADPR cyclase and cADPR induces a subset of ABA-responsive genes in *Arabidopsis*. *Plant J.* **2004**, *38*, 381–395. [[CrossRef](#)] [[PubMed](#)]
4. Briggs, A.G.; Bent, A.F. Poly(ADP-ribosyl)ation in plants. *Trends Plant Sci.* **2011**, *16*, 372–380. [[CrossRef](#)]
5. Kupis, W.; Palyga, J.; Tomal, E.; Niewiadomska, E. The role of sirtuins in cellular homeostasis. *J. Physiol Biochem* **2016**, *72*, 371–380. [[CrossRef](#)]
6. Imai, S.; Armstrong, C.; Kaeberlein, M.; Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **2000**, *403*, 795–800. [[CrossRef](#)]
7. Berger, F.; Ramirez-Hernandez, M.H.; Ziegler, M. The new life of a centenarian: Signalling functions of NAD(P). *Trends Biochem. Sci.* **2004**, *29*, 111–118. [[CrossRef](#)]
8. Gakière, B.; Fernie, A.R.; Petriacq, P. More to NAD(+) than meets the eye: A regulator of metabolic pools and gene expression in *Arabidopsis*. *Free Radic. Bio. Med.* **2018**, *122*, 86–95. [[CrossRef](#)]
9. Gakière, B.; Hao, J.; de Bont, L.; Pétriacq, P. NAD biosynthesis and signaling in plants. *Crit. Rev. Plant Sci.* **2018**, *37*, 1–49. [[CrossRef](#)]
10. Pétriacq, P.; de Bont, L.; Hager, J.; Didierlaurent, L.; Mauve, C.; Guérard, F.; Noctor, G.; Pelletier, S.; Renou, J.; Tcherkez, G.; et al. Inducible NAD overproduction in *Arabidopsis* alters metabolic pools and gene expression correlated with increased salicylate content and resistance to *Pst-AvrRpm1*. *Plant J.* **2012**, *70*, 650–665. [[CrossRef](#)] [[PubMed](#)]
11. Pétriacq, P.; Ton, J.; Patriot, O.; Tcherkez, G.; Gakiere, B. NAD acts as an integral regulator of multiple defense layers. *Plant Physiol.* **2016**, *172*, 1465–1479. [[CrossRef](#)]
12. Ishikawa, K.; Yoshimura, K.; Harada, K.; Fukusaki, E.; Ogawa, T.; Tamoi, M.; Shigeoka, S. AtNUDX6, an ADP-ribose/NADH pyrophosphohydrolase in *Arabidopsis*, positively regulates NPR1-dependent salicylic acid signaling. *Plant Physiol.* **2010**, *152*, 2000–2012. [[CrossRef](#)] [[PubMed](#)]
13. Ge, X.; Li, G.J.; Wang, S.B.; Zhu, H.; Zhu, T.; Wang, X.; Xia, Y. AtNUDT7, a negative regulator of basal immunity in *Arabidopsis*, modulates two distinct defense response pathways and is involved in maintaining redox homeostasis. *Plant Physiol.* **2007**, *145*, 204–215. [[CrossRef](#)]
14. Bartsch, M.; Gobbato, E.; Bednarek, P.; Debey, S.; Schultze, J.L.; Bautor, J.; Parker, J.E. Salicylic acid-independent enhanced disease susceptibility1 signaling in *Arabidopsis* immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. *Plant Cell* **2006**, *18*, 1038–1051. [[CrossRef](#)]
15. Fonseca, J.P.; Dong, X. Functional characterization of a Nudix hydrolase AtNUDX8 upon pathogen attack indicates a positive role in plant immune responses. *PLoS ONE* **2014**, *9*, e114119. [[CrossRef](#)] [[PubMed](#)]
16. Zhang, X.; Mou, Z. Extracellular pyridine nucleotides induce PR gene expression and disease resistance in *Arabidopsis*. *Plant J.* **2009**, *57*, 302–312. [[CrossRef](#)] [[PubMed](#)]
17. Wang, C.; Zhang, X.; Mou, Z. Comparison of nicotinamide adenine dinucleotide phosphate-induced immune responses against biotrophic and necrotrophic pathogens in *Arabidopsis thaliana*. *Plant Signal. Behav.* **2016**, *11*, e1169358. [[CrossRef](#)] [[PubMed](#)]
18. Wang, C.; Zhou, M.; Zhang, X.; Yao, J.; Zhang, Y.; Mou, Z. A lectin receptor kinase as a potential sensor for extracellular nicotinamide adenine dinucleotide in *Arabidopsis thaliana*. *Elife* **2017**, *6*, e25474. [[CrossRef](#)]
19. Wang, C.; Huang, X.; Li, Q.; Zhang, Y.; Li, J.L.; Mou, Z. Extracellular pyridine nucleotides trigger plant systemic immunity through a lectin receptor kinase/BAK1 complex. *Nat. Commun.* **2019**, *10*, 4810. [[CrossRef](#)] [[PubMed](#)]
20. Katoh, A.; Uenohara, K.; Akita, M.; Hashimoto, T. Early steps in the biosynthesis of NAD in *Arabidopsis* start with aspartate and occur in the plastid. *Plant Physiol.* **2006**, *141*, 851–857. [[CrossRef](#)]
21. Hashida, S.N.; Takahashi, H.; Kawai-Yamada, M.; Uchimiya, H. *Arabidopsis thaliana* nicotinate/nicotinamide mononucleotide adenyltransferase (AtNMNAT) is required for pollen tube growth. *Plant J.* **2007**, *49*, 694–703. [[CrossRef](#)] [[PubMed](#)]
22. Macho, A.; Boutrot, F.; Rathjen, J.; Zipfel, C. Aspartate oxidase plays an important role in *Arabidopsis* stomatal immunity. *Plant Physiol.* **2012**, *159*, 1845–1856. [[CrossRef](#)]
23. Murthy, U.M.N.; Ollagnier-de-Choudens, S.; Sanakis, Y.; Abdel-Ghany, S.E.; Rousset, C.; Ye, H.; Fontecave, M.; Pilon-Smits, E.A.H.; Pilon, M. Characterization of *Arabidopsis thaliana* SufE2 and SufE3: Functions in chloroplast iron-sulfur cluster assembly and Nad synthesis. *J. Biol. Chem.* **2007**, *282*, 18254–18264. [[CrossRef](#)]
24. Schippers, J.H.; Nunes-Nesi, A.; Apetrei, R.; Hille, J.; Fernie, A.R.; Dijkwel, P.P. The *Arabidopsis* onset of leaf death5 mutation of quinolinate synthase affects nicotinamide adenine dinucleotide biosynthesis and causes early ageing. *Plant Cell* **2008**, *20*, 2909–2925. [[CrossRef](#)] [[PubMed](#)]
25. Wei, M.; Zhuang, Y.; Li, H.; Li, P.; Huo, H.; Shu, D.; Huang, W.; Wang, S. The cloning and characterization of hypersensitive to salt stress mutant, affected in quinolinate synthase, highlights the involvement of NAD in stress-induced accumulation of ABA and proline. *Plant J.* **2020**, *102*, 85–98. [[CrossRef](#)]
26. Hong, Y.; Wang, Z.; Shi, H.; Yao, J.; Liu, X.; Wang, F.; Zeng, L.; Xie, Z.; Zhu, J.K. Reciprocal regulation between nicotinamide adenine dinucleotide metabolism and abscisic acid and stress response pathways in *Arabidopsis*. *PLoS Genet.* **2020**, *16*, e1008892. [[CrossRef](#)]

27. Wang, G.; Pichersky, E. Nicotinamidase participates in the salvage pathway of NAD biosynthesis in *Arabidopsis*. *Plant J.* **2007**, *49*, 1020–1029. [[CrossRef](#)]
28. Hunt, L.; Holdsworth, M.J.; Gray, J.E. Nicotinamidase activity is important for germination. *Plant J.* **2007**, *51*, 341–351. [[CrossRef](#)]
29. Sattar, S.; Martinez, M.T.; Ruiz, A.F.; Hanna-Rose, W.; Thompson, G.A. Nicotinamide inhibits aphid fecundity and impacts survival. *Sci. Rep.* **2019**, *9*, 19709. [[CrossRef](#)] [[PubMed](#)]
30. Castro, B.; Citterico, M.; Kimura, S.; Stevens, D.; Wrzaczek, M.; Gitta Coakerg, G. Stress-induced reactive oxygen species compartmentalization, perception and signalling. *Nat. Plants* **2021**, *7*, 403–412. [[CrossRef](#)]
31. Torres, M.A. ROS in biotic interactions. *Physiol. Plantarum* **2010**, *138*, 414–429. [[CrossRef](#)]
32. Chaouch, S.; Queval, G.; Noctor, G. AtRbohF is a crucial modulator of defence-associated metabolism and a key actor in the interplay between intracellular oxidative stress and pathogenesis responses in *Arabidopsis*. *Plant J.* **2012**, *69*, 613–627. [[CrossRef](#)] [[PubMed](#)]
33. Rentel, M.; Lecourieux, D.; Ouaked, F.; Usher, S.; Petersen, L.; Okamoto, H.; Knight, H.; Peck, S.; Grierson, C.; Hirt, H.; et al. OX11 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature* **2004**, *427*, 858–861. [[CrossRef](#)] [[PubMed](#)]
34. Vanlerberghe, G.C. Alternative oxidase: A mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. *Int. J. Mol. Sci.* **2013**, *14*, 6805–6847. [[CrossRef](#)] [[PubMed](#)]
35. Petit, J.; Briat, J.; Lobréaux, S. Structure and differential expression of the four members of the *Arabidopsis thaliana* ferritin gene family. *Biochem. J.* **2001**, *359*, 575–582. [[CrossRef](#)]
36. Kasimova, M.R.; Grigiene, J.; Krab, K.; Hagedorn, P.H.; Flyvbjerg, H.; Andersen, P.E.; Moller, I.M. The free NADH concentration is kept constant in plant mitochondria under different metabolic conditions. *Plant Cell* **2006**, *18*, 688–698. [[CrossRef](#)]
37. Zhang, X.; Mou, Z. Expression of the human NAD(P)-metabolizing ectoenzyme CD38 compromises systemic acquired resistance in *Arabidopsis*. *Mol. Plant Microbe Interact.* **2012**, *25*, 1209–1218. [[CrossRef](#)] [[PubMed](#)]
38. Smith, E.N.; Schwarzländer, M.; Ratcliffe, R.G.; Kruger, N.J. Shining a light on NAD- and NADP-based metabolism in plants. *Trends Plant Sci.* **2021**. [[CrossRef](#)]
39. Mhamdi, A.; Mauve, C.; Gouia, H.; Saindrenan, P.; Hodges, M.; Noctor, G. Cytosolic NADP-dependent isocitrate dehydrogenase contributes to redox homeostasis and the regulation of pathogen responses in *Arabidopsis* leaves. *Plant Cell Environ.* **2010**, *33*, 1112–1123. [[CrossRef](#)]
40. De Vos, M.; Van Oosten, V.; Van Poecke, R.; Van Pelt, J.; Pozo, M.; Mueller, M.; Buchala, A.; Métraux, J.; Van Loon, L.; Dicke, M.; et al. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Mol. Plant Microbe Interact.* **2005**, *18*, 923–937. [[CrossRef](#)]
41. Gullner, G.; Komives, T.; Kiraly, L.; Schroder, P. Glutathione S-transferase enzymes in plant-pathogen interactions. *Front. Plant Sci.* **2018**, *9*, 1836. [[CrossRef](#)]
42. Petersen, L.N.; Ingle, R.A.; Knight, M.R.; Denby, K.J. OX11 protein kinase is required for plant immunity against *Pseudomonas syringae* in *Arabidopsis*. *J. Exp. Bot.* **2009**, *60*, 3727–3735. [[CrossRef](#)]
43. Simons, B.; Millenaar, F.; Mulder, L.; Van Loon, L.; Lambers, H. Enhanced expression and activation of the alternative oxidase during infection of *Arabidopsis* with *Pseudomonas syringae* pv tomato. *Plant Physiol.* **1999**, *120*, 529–538. [[CrossRef](#)] [[PubMed](#)]
44. Garmier, M.; Priault, P.; Vidal, G.; Driscoll, S.; Djebbar, R.; Boccara, M.; Mathieu, C.; Foyer, C.H.; De Paepe, R. Light and oxygen are not required for harpin-induced cell death. *J. Biol. Chem.* **2007**, *282*, 37556–37566. [[CrossRef](#)] [[PubMed](#)]
45. Vidal, G.; Ribas-Carbo, M.; Garmier, M.; Dubertret, G.; Rasmusson, A.G.; Mathieu, C.; Foyer, C.H.; De Paepe, R. Lack of respiratory chain complex I impairs alternative oxidase engagement and modulates redox signaling during elicitor-induced cell death in tobacco. *Plant Cell* **2007**, *19*, 640–655. [[CrossRef](#)] [[PubMed](#)]
46. Arnaud, N.; Murgia, I.; Boucherez, J.; Briat, J.F.; Cellier, F.; Gaymard, F. An iron-induced nitric oxide burst precedes ubiquitin-dependent protein degradation for *Arabidopsis* AtFer1 ferritin gene expression. *J. Biol. Chem.* **2006**, *281*, 23579–23588. [[CrossRef](#)] [[PubMed](#)]
47. Palmieri, F.; Rieder, B.; Ventrella, A.; Blanco, E.; Do, P.T.; Nunes-Nesi, A.; Trauth, A.U.; Fiermonte, G.; Tjaden, J.; Agrimi, G.; et al. Molecular identification and functional characterization of *Arabidopsis thaliana* mitochondrial and chloroplastic NAD<sup>+</sup> carrier proteins. *J. Biol. Chem.* **2009**, *284*, 31249–31259. [[CrossRef](#)]
48. De Souza Chaves, I.; Feitosa-Araujo, E.; Florian, A.; Medeiros, D.B.; da Fonseca-Pereira, P.; Charton, L.; Heyneke, E.; Apfata, J.A.C.; Pires, M.V.; Mettler-Altmann, T.; et al. The mitochondrial NAD(+) transporter (NDT1) plays important roles in cellular NAD(+) homeostasis in *Arabidopsis thaliana*. *Plant J.* **2019**, *100*, 487–504. [[CrossRef](#)] [[PubMed](#)]
49. Luo, L.; He, Y.; Zhao, Y.; Xu, Q.; Wu, J.; Ma, H.; Guo, H.; Bai, L.; Zuo, J.; Zhou, J.M.; et al. Regulation of mitochondrial NAD pool via NAD(+) transporter 2 is essential for matrix NADH homeostasis and ROS production in *Arabidopsis*. *Sci. China Life Sci.* **2019**, *62*, 991–1002. [[CrossRef](#)]
50. Van Roermund, C.W.; Schroers, M.G.; Wiese, J.; Facchinelli, F.; Kurz, S.; Wilkinson, S.; Charton, L.; Wanders, R.J.; Waterham, H.R.; Weber, A.P.; et al. The peroxisomal NAD carrier from *Arabidopsis* imports NAD in exchange with AMP. *Plant Physiol.* **2016**, *171*, 2127–2139. [[CrossRef](#)] [[PubMed](#)]
51. Bernhardt, K.; Wilkinson, S.; Weber, A.P.; Linka, N. A peroxisomal carrier delivers NAD(+) and contributes to optimal fatty acid degradation during storage oil mobilization. *Plant J.* **2012**, *69*, 1–13. [[CrossRef](#)] [[PubMed](#)]
52. Wu, Y.Y.; You, L.L.; Li, S.C.; Ma, M.Q.; Wu, M.T.; Ma, L.X.; Bock, R.; Chang, L.; Zhang, J. In vivo assembly in *Escherichia coli* of transformation vectors for plastid genome engineering. *Front. Plant Sci.* **2017**, *8*, 1454. [[CrossRef](#)] [[PubMed](#)]

53. Zhang, X.; Henriques, R.; Lin, S.S.; Niu, Q.W.; Chua, N.H. Agrobacterium-mediated transformation of *Arabidopsis thaliana* using the floral dip method. *Nat. Protoc.* **2006**, *1*, 641–646. [[CrossRef](#)]
54. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta C(T)}$  method. *Methods* **2001**, *25*, 402–408. [[CrossRef](#)] [[PubMed](#)]
55. Noctor, G.; Mhamdi, A.; Foyer, C.H. Oxidative stress and antioxidative systems: Recipes for successful data collection and interpretation. *Plant Cell Environ.* **2016**, *39*, 1140–1160. [[CrossRef](#)] [[PubMed](#)]
56. Queval, G.; Noctor, G. A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: Application to redox proWling during *Arabidopsis* rosette development. *Anal. Biochem* **2007**, *363*, 58–69. [[CrossRef](#)] [[PubMed](#)]
57. Langlois-Meurinne, M.; Gachon, C.M.; Saindrenan, P. Pathogen-responsive expression of glycosyltransferase genes *UGT73B3* and *UGT73B5* is necessary for resistance to *Pseudomonas syringae* pv *tomato* in *Arabidopsis*. *Plant Physiol.* **2005**, *139*, 1890–1901. [[CrossRef](#)] [[PubMed](#)]