Temperature-Inducible Transgenic EDS1 and PAD4 in Arabidopsis Confer an Enhanced Disease Resistance at Elevated Temperature

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Abstract: Temperature is one of the most important environmental factors greatly affecting plant disease development. High temperature favors outbreaks of many plant diseases, which threaten food security and turn to be a big issue along with climate change and global warming. Here, we found that concurrent constitutive expression of the key immune regulators EDS1 and PAD4 in Arabidopsis significantly enhanced resistance to virulent bacterial pathogen Pseudomonas syringae pv. tomato at elevated temperature; however, autoimmunity-related growth retardation was also observed on these plants at a normal temperature. To balance this growth-defense trade-off, we generated transgenic plants dual expressing EDS1 and PAD4 genes under the control of a thermo-sensitive promoter from the HSP70 gene, whose expression is highly induced at an elevated temperature. Unlike constitutive overexpression lines, the proHSP70-EP transgenic lines exhibited enhanced resistance to bacterial pathogens at an elevated temperature without growth defects at normal condition. Thus, this study provides a potential strategy for genetic manipulation of plants to deal with the simultaneous abiotic and biotic stresses.

Keywords: temperature; disease resistance; immunity; EDS1; PAD4

1. Introduction

Plant diseases are one of the most important causes leading to crop losses; thus, the broad diversity and rapid evolution of pathogens bring a big challenge for modern plant breeding. To defend against pathogens, plants have evolved a sophisticated innate immune system, which recognizes infectious microbes and activates defense responses mainly through two types of receptors: (1) one is the cell surface localized pattern recognition receptors (PRRs) that perceive pathogen-associated molecular patterns (PAMPs) and activate immune responses, which is called PAMP-triggered immunity (PTI); (2) the other type is intracellular nucleotide-binding/leucine-rich-repeat (NLR) receptors that intercept pathogen effectors and induce a robust disease response called effector-triggered immunity (ETI) [1,2]. Both PTI and ETI induce similar immune responses, including transient calcium influx, a rapid production of reactive oxygen species, activation of mitogen-activated protein kinase (MAPK) cascades and defense-related transcriptional reprogramming [1]. The immune responses have to be tightly controlled, as prolonged or auto-activated immune responses result in plant growth retardation and cell death [3]. Due to intensive studies on the plant immune system, an increasing number of immune regulators have been identified, and their molecular mechanisms are being uncovered. Several immune regulators have been applied in breeding for resistant crops with expected benefits [4,5]. Ambient environment is one of the key determinants for plant disease epidemics [6]. Humidity and temperature are major climate factors that have profound impact on the
outcomes of plant–microbe interactions, as the continuous hot and humid weather is prone to outbreaks of many plant diseases [7]. How these specific environmental factors affect the host and the pathogen as well as their interactions is an important question to be addressed and will be very helpful for making new strategies in plant breeding. It has been shown that high temperatures significantly increase Arabidopsis susceptibility to bacterial pathogen Pseudomonas syringae pv. tomato DC3000 (Pst DC3000) [8,9]. Pst causes the bacterial speck disease on tomato leaves which has been used as a model for studying plant–bacterial interactions [10]. The high temperature-provoked disease susceptibility is associated with boosted PTI but reduced ETI in plants, consisting of the reports that several NLRs-mediated resistances are abolished at elevated temperatures [11–14]. Recently, Huot et al. discovered that an elevated temperature enhances disease susceptibility at least partially by suppressing biosynthesis of salicylic acid (SA), an essential phytohormone involved in plant resistance to biotrophic and semi-biotrophic pathogens [8]. Moreover, application of benzo-2-thiadiazole (BTH), an SA synthetic analogue, could provide enhanced disease resistance at elevated temperatures [8]. This provides a useful clue regarding the genetic manipulation of the SA-related plant immune system to enhance disease resistance at elevated temperatures.

Plant NLR receptors can be classified into two major groups depending on their distinct N-terminal domains: coiled-coil-NB-LRRs (CNLs) and Toll/interleukin-1 receptor (TIR)-NB-LRRs (TNLs). Enhanced disease susceptibility 1 (EDS1) is a key signaling component in TNL-mediated immunity and also serves as an important positive regulator in SA synthesis [15]. EDS1 is required for all tested TNLs-mediated ETI and for basal resistance to virulent pathogens [3,15]. Pst DC3000-induced SA accumulation is dramatically compromised in Arabidopsis eds1 mutant plants [16]. EDS1 interacts with its sequence-related protein phytoalexin deficient 4 (PAD4) and senescence-associated gene 101 (SAG101) forming a heteromeric regulatory unit for signaling [16–18]. Thus, overexpression of EDS1 or PAD4 alone does not render Arabidopsis-enhanced resistance to Pst DC3000, probably due to less amounts of their interacting partners [19]. Indeed, co-overexpression of EDS1 and PAD4 lead to autoimmunity and enhanced disease resistance [19]. Transcriptional profile analysis revealed that EDS1-PAD4 not only promotes expression of the isochorismate synthase 1 (ICS1) gene, encoding the key enzyme for pathogen-induced SA biosynthesis, but also a great portion of the SA-responsive genes in parallel with SA accumulation [19]. In addition, EDS1-PAD4 could boost SA pathways through antagonizing jasmonic acid (JA) signaling pathway by suppressing the master transcription factor MYC2 [20].

Since EDS1-PAD4-mediated immunity could bypass SA accumulation to induce expression of SA-responsive genes [19], we wondered whether the stimulation of EDS1-PAD4 signaling could compensate for the loss of SA synthesis at elevated temperatures. In this study, we examined the EDS1-PAD4-mediated immune functions and tested their potential application in enhancing plant resistance at elevated temperatures.

2. Results
2.1. The Autoimmunity Activated by Dual Overexpression of EDS1 and PAD4 Is Not Suppressed at Elevated Temperatures

Previously, we have shown that dual overexpression of EDS1 and PAD4 in Arabidopsis (OE.EP) results in autoimmunity and enhances resistance to a virulent bacterial pathogen Pst DC3000 at a normal temperature (22 °C) [19]. In Arabidopsis, many TNL-mediated immunities are suppressed at an elevated temperature (28 °C) [9]. Because EDS1 and PAD4 function downstream of TNLs [3,15], we wondered whether the autoimmunity in the OE.EP line is temperature dependent. To test this, we grew OE.EP plants at 28 °C. We found that the OE.EP plants exhibited a dwarf phenotype at both 22 and 28 °C compared with wild-type (WT) ecotype Wassilewskija-2 (Ws) (Figure 1A). Consistently, the expressions of the SA synthesis gene ICS1 and the defense marker gene PR1 in OE.EP plants were much higher than in WT plants in both conditions (Figure 1B), indicating that the enhanced SA pathway in OE.EP plants was maintained at an elevated temperature. Moreover, the growth of Pst DC3000 in OE.EP plants was more than ten times less compared with that
in WT plants at 28 °C (Figure 1C), indicating that OE.EP plants are more resistant to Pst DC3000 than WT plants at an elevated temperature. These data demonstrate that the autoimmunity mediated by dual overexpression of EDS1 and PAD4 is not suppressed at an elevated temperature.

Figure 1. Dual overexpression of EDS1 and PAD4 in Arabidopsis enhances resistance to bacterial pathogens at normal and elevated temperatures. (A) Developmental phenotype of wild-type Arabidopsis ecotype WS and transgenic line constitutively expressing EDS1 and PAD4 driven by 35S promoter (OE.EP) grown at 22 or 28 °C. When photographed, plants were 4 weeks old, grown at 22 °C or 3 weeks old grown at 28 °C. Bars = 1 cm. (B) Expression of salicylic acid pathway genes PR1 and ICS1 in leaves of plants in (a) measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Gene expression was normalized to AT4G26410. Error bars represent mean ± SD of three biological replicates. * indicates significant difference to WS in a Student’s t-test (p < 0.05). (C) Growth of bacterial strain Pst DC3000 in leaves of WS or OE.EP plants. Leaves of 4-week-old (22 °C) or 3-week-old (28 °C) plants were hand-infiltrated with bacterial suspensions (OD600 = 0.0002) and bacterial titers were determined at 3 dpi. Error bars represent mean ± SD of six biological replicates. * indicates significant difference to WS in a Student’s t-test (p < 0.05). The above experiments were repeated three times with similar results.

2.2. Selection of Promoters That Are Induced at Elevated Temperature

Constitutive dual overexpression of EDS1 and PAD4 enhances plant resistance, but also results in autoimmunity-related growth retardation, which limits its application in plant breeding for disease resistance. However, the EDS1-PAD4-mediated resistance is insensitive to an elevated temperature (Figure 1), which provides potential gene resources for enhancing plant resistance at an elevated temperature in plant breeding. Thus, a method is needed to control immune activity of EDS1-PAD4 regulon at a normal temperature but reinforce their activity at an elevated temperature. For this, we sought to use a temperature-responsive promoter to drive the expression of EDS1 and PAD4. Such promoter activity should be relatively low at a normal temperature but drive high expression of EDS1 and PAD4 at an elevated temperature.

To select promoters which fulfill the above criteria, we analyzed published microarray dataset (GSE50019) and RNA-seq dataset (GSE80448) of Arabidopsis plants shifted from 28 to 19 °C [21,22]. The genes with high expression level at 28 °C and low expression level when shifted to 19 °C were selected (Tables 1 and 2). Among those genes, AT5G12110 and AT3G12580 (HSP70) were listed on top with a high expression level at 28 °C in both datasets. We then verified the expression of these two genes in wild-type Col-0 plants shifted from 22 to 28 °C. Consistent with the microarray and RNA-seq data, the transcripts of AT5G12110 and HSP70 genes were highly induced when plants were shifted to 28 °C for
24 h (Figure 2). Thus, promoters of AT5G12110 and HSP70 were cloned to drive expression of both EDS1 and PAD4 genes in new transgenic lines.

### Table 1. Top 20 downregulated genes in *Arabidopsis* plants shifted from 28 to 19 °C identified from microarray dataset (GSE50019).

| Gene ID   | 0 h-Average | 24 h-Average | 2 h-Average | 8 h-Average | 0 h vs. 24 h-FD | 0 h vs. 2 h-FD | 0 h vs. 8 h-FD |
|-----------|-------------|--------------|-------------|-------------|----------------|----------------|----------------|
| AT3G12580 | 3982.4      | 54.5         | 140.4       | 42.1        | 73.0           | 28.4           | 94.5           |
| AT1G62510 | 3823.5      | 188.9        | 2727.7      | 52.1        | 20.2           | 1.4            | 73.4           |
| AT5G12110 | 3711.1      | 186.1        | 551.7       | 87.9        | 19.9           | 6.7            | 42.2           |
| AT3G28270 | 2741.2      | 130.0        | 595.2       | 57.4        | 21.1           | 4.6            | 47.8           |
| AT5G48570 | 1333.9      | 17.8         | 69.0        | 15.2        | 74.9           | 19.3           | 87.8           |
| AT5G12020 | 1093.1      | 14.9         | 128.0       | 12.5        | 73.4           | 8.5            | 87.6           |
| AT5G52640 | 952.1       | 25.8         | 32.7        | 15.2        | 37.0           | 29.1           | 62.8           |
| AT1G55260 | 833.1       | 114.6        | 619.2       | 404.2       | 7.3            | 1.3            | 2.1            |
| AT1G60590 | 667.1       | 90.7         | 52.7        | 12.0        | 7.4            | 12.7           | 55.4           |
| AT3G46230 | 512.1       | 17.1         | 52.4        | 12.5        | 29.9           | 9.8            | 40.8           |
| AT1G17870 | 386.1       | 53.2         | 134.4       | 34.6        | 7.3            | 2.9            | 11.2           |
| AT5G51440 | 372.0       | 10.3         | 14.1        | 9.7         | 36.1           | 26.3           | 38.2           |
| AT4G26790 | 331.2       | 16.1         | 90.9        | 17.2        | 20.5           | 3.6            | 19.2           |
| AT1G02205 | 304.0       | 15.4         | 478.5       | 39.6        | 19.7           | 0.6            | 7.7            |
| AT2G29500 | 273.2       | 15.4         | 22.9        | 17.9        | 17.7           | 11.9           | 15.3           |
| AT4G12400 | 270.0       | 11.4         | 15.6        | 10.4        | 23.8           | 17.3           | 26.0           |
| AT1G72970 | 208.8       | 30.6         | 251.8       | 64.2        | 6.8            | 0.8            | 3.2            |
| AT5G12030 | 200.2       | 9.7          | 9.8         | 9.2         | 20.6           | 20.3           | 21.7           |
| AT1G07400 | 158.2       | 11.5         | 10.1        | 10.6        | 13.8           | 15.6           | 15.0           |
| AT1G53540 | 120.0       | 13.5         | 22.2        | 14.1        | 8.9            | 5.4            | 8.5            |

### Table 2. Top 10 downregulated genes in *Arabidopsis* plants shifted from 28 to 19 °C identified from RNA-seq dataset (GSE80448).

| Gene ID   | 0 h-Average | 4 h-Average | 8 h-Average | 0 h vs. 4 h-FD | 0 h vs. 8 h-FD |
|-----------|-------------|-------------|-------------|----------------|----------------|
| AT5G48570 | 12139.3     | 1279.0      | 242.0       | 9.5            | 50.2           |
| AT5G12110 | 11136.3     | 974.3       | 309.7       | 11.4           | 36.0           |
| AT1G02205 | 3978.5      | 1111.8      | 608.7       | 3.6            | 65             |
| AT3G12580 | 3647.3      | 368.7       | 14.0        | 9.9            | 260.5          |
| AT5G52640 | 3336.3      | 218.3       | 22.7        | 15.3           | 147.2          |
| AT4G12400 | 2415.5      | 114.3       | 22.7        | 21.1           | 106.6          |
| AT1G60590 | 1182.2      | 260.0       | 27.5        | 4.5            | 43.0           |
| AT1G17870 | 876.7       | 128.3       | 67.3        | 6.8            | 13.0           |
| AT5G51440 | 620.3       | 82.7        | 6.0         | 7.5            | 103.4          |
| AT4G26790 | 385.3       | 155.3       | 99.7        | 2.5            | 3.9            |

Average, average reads from 3 replicates. FD, foldchange.
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Figure 2. Quantitative expression of AT5G12110 and HSP70 genes in leaves of Arabidopsis wild-type Col plants shifted from 22 to 28 °C for 24 h. Gene expression was measured by quantitative reverse transcription polymerase chain reaction (qRT-PCR) and normalized to housekeeping gene AT4G26410. Error bars represent mean ± SD of three biological replicates. * indicates significant difference to 22 °C in a Student’s t-test (p < 0.05).

2.3. Construction of Transgenic Lines Harboring Temperature-Inducible EDS1 and PAD4

Using Goldengate cloning system, we made two plasmids containing the BASTA gene for Basta resistance, N-terminal tagged FLAG-EDS1, and GFP-PAD4. Both FLAG-EDS1 and GFP-PAD4 were driven by the 2 kb promoter of AT5G12110 on one plasmid, and by the 2 kb promoter of HSP70 on the other plasmid (Figure 3A). The two plasmids were transformed into Agrobacteria GV3101 and then the transgenic Arabidopsis lines were generated using Agrobacteria-mediated transformation. The transgenic lines were named as proHSP70-EP (promoter of HSP70 driving EDS1 and PAD4) and proAT5G12110-EP (promoter of AT5G12110 driving EDS1 and PAD4), respectively. The protein accumulation of FLAG-EDS1 and GFP-PAD4 in individual T1 lines was examined by immunoblotting. The FLAG-EDS1 and GFP-PAD4 proteins could be detected in several proHSP70-EP lines (Figure 3B), but FLAG-EDS1 was undetectable in all proAT5G12110-EP lines for unknown reason (Figure S1). Thus, we focused on the proHSP70-EP lines in the further studies. Two proHSP70-EP T3 homozygous lines were obtained, which did not show any growth defect either at a normal temperature or at an elevated temperature (Figure 3C). The expression level of EDS1 and PAD4 genes in proHSP70-EP lines was increased when plants were transferred from 22 to 28 °C for 24 h (Figure 3D). Consistent with the increased transcripts, the FLAG-EDS1 and GFP-PAD4 proteins accumulated in proHSP70-EP lines at 28 °C compared with those at 22 °C (Figure 3E). These data show that we have successfully obtained the transgenic lines expressing inducible EDS1 and PAD4 at an elevated temperature.
To examine whether temperature-controlled expression of *EDS1* and *PAD4* in *proHSP70-EP* lines confers enhanced pathogen resistance at elevated temperature, we tested *Pst* DC3000 resistance on *proHSP70-EP* plants shifted from 22 to 28 °C 24 h before pathogen infection. The results showed that *proHSP70-EP* lines exhibited WT-like resistance to *Pst* DC3000 at 22 °C, while they showed significantly enhanced resistance to *Pst* DC3000 at 28 °C (Figure 4). This result agrees that transgenes of *EDS1* and *PAD4* under the control of the temperature-responsive promoter could enhance resistance to bacterial pathogens at elevated temperatures in *Arabidopsis*.

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**Figure 3.** Construction of transgenic *Arabidopsis* Col lines expressing inducible *EDS1* and *PAD4* at elevated temperatures. (A) The diagrams show arrangement of BASTA, *EDS1*, and *PAD4* genes within left border (LB) and right border (RB) on the two T-DNA binary constructs. pHSP70: the promoter of *HSP70*, pAT5G12110: the promoter of *AT5G12110*. (B) Immunoblots probed with anti-FLAG and anti-GFP antibodies show FLAG-EDS1 and YFP-PAD4 proteins accumulation in T1 transgenic *Arabidopsis* lines expressing FLAG-EDS1 and YFP-PAD4 under the control of promoter of *HSP70* (*proHSP70-EP*) with WT Col as control. (C) Developmental phenotype of 4-week-old (22 °C) and 3-week-old (28 °C) plants of T3 homozygous lines of *proHSP70-EP* (line #1 and #7). Bars = 1 cm. (D) Expression of *EDS1* and *PAD4* in leaves of Col and *proHSP70-EP* lines shifted from 22 to 28 °C for 24 h measured by qRT-PCR. Gene expression was normalized to *AT4G26410*. Error bars represent mean ± SD of three biological replicates. * indicates significant difference to 22 °C in a Student’s t-test (*p* < 0.05). (E) Immunoblots probed with anti-FLAG and anti-GFP antibodies show FLAG-EDS1 and YFP-PAD4 protein accumulation in T3 homozygous lines of *proHSP70-EP* (line #1 and #7) shifted from 22 to 28 °C for 24 h. Ponceau staining of the blots indicates the equal loading.
It has been reported that EDS1 and PAD4 are prerequisites for several autoimmunity mutants, such as *snc1*, *bon1*, and *cpr1* [11,12,25]. The constitutive defense phenotypes of the *Arabidopsis constitutive induced resistance 1* (*cir1*) mutant require both EDS1 and PAD4 [26]. The enhanced resistance to *Pst* DC3000 in *cir1* was abolished at 25 °C [26]. The CIR1 gene has not been cloned yet. It is possible that *cir1* is a gain function mutation of an TNL gene, such as *snc1*, whose autoimmunity requires EDS1 [27], and is suppressed by an elevated temperature (28 °C) [13], or that the *cir1* mutant triggers activation of an TNL receptor.

Plant defense activation generally comes at the expense of plant growth [28]. The growth–defense trade-off is the one of the major issues that should be considered in plant breeding for biotic and abiotic resistance. Overexpression of positive regulators in plants enhances disease resistance, but is usually associated with a significant fitness cost [29,30], which limits the application of this approach in the practice of crop breeding. For example, overexpression of the SA receptor *NRPI* in rice enhances plant resistance to blast.
disease, but also results in dwarf plants and poor yield [31]. Dual overexpression of EDS1 and PAD4 also results in autoimmunity-related growth retardation [19]. To overcome the growth–defense trade-off mediated by overexpression of EDS1 and PAD4, we sought a more stringent way by expressing EDS1 and PAD4 genes under the control of temperature in transgenic plants. From published transcriptome datasets, we identified promoters of AT5G12110 and HSP70 that were highly induced at an elevated temperature. We successfully attained transgenic plants expressing EDS1 and PAD4 genes driven by promoter of HSP70 (proHSP70-EP), and both proteins were induced at an elevated temperature as expected. The proHSP70-EP plants growing at a normal temperature developed normally as seen in WT plants (Figure 3C). In pathogen infection assays, the proHSP70-EP plants exhibited enhanced resistance to Pst DC3000 (Figure 4), demonstrating this approach works in the model plant species. EDS1 has been documented to be a conserved key component in TNL ETI in dicots [15], including many important Solanaceous food plants [32]. Further study could be worth for application on the food plants, such as pepper, potato and tomato, to enhance disease resistance at an elevated temperature. Thus, our study provides a potential strategy for genetic engineering of environment-relevant components in plants to enhance disease resistance facing global warming.

4. Materials and Methods

4.1. Plant Materials, Growth Conditions and Pathogen Strains

Arabidopsis thaliana wild-type accessions used in this research are Wassilewskija-2 (Ws) and Columbia-0 (Col). Transgenic OE.EP line was published in Cui et al., 2017 [19]. Pseudomonas syringe pv. tomato (Pst) strain DC3000 was maintained as previously described [19]. The seeds were nursed in MS (Murashige and Skoog) medium and then were transplanted into soil after 1 week. All plants were grown in a growth chamber setting as 22 (normal) or 28 °C (elevated) and 65% relative humidity, with a 9 h/15 h light/dark period and the light intensity was 150 μE/m²/s.

4.2. Golden Gate Cloning and Generation of Arabidopsis Transgenic Lines

Promoters (2 kb upstream of start codon) of AT5G12110 and HSP70 were amplified by PCR and cloned into the level 0 vector pAGM1251, respectively. EDS1 and PAD4 coding sequence were cloned into the level 0 vector pICH41308. At level 1 vectors, EDS1 were cloned into pICH47811 (proAT5G12110: or proHSP70:3xFLAG-EDS1-35S_term), and PAD4 were cloned into pICH47822 (proAT5G12110: or proHSP70:YFP-PAD4-35S_term). For level 2 constructs in pAGM4673, the pNos:BASTA®-Nos_term—cassette was placed at position 1, EDS1—at position 2, and PAD4—at position 3. Backbones, tags, and the terminator module, as well as the BASTA® expression cassette, are from the Golden Gate cloning toolkit [13]. Primers used for cloning are provided in Supplemental Table S1.

The level 2 constructs were transformed into agrobacterium strain GV3101 and used to transform Arabidopsis plants. The transformants growing in soil were selected after spraying with 0.01% BASTA (Sangon, #A614229).

4.3. Pathogen Infection Assays

For bacterial growth assays, Pst DC3000 (OD600 = 0.0002) in sterilized water were hand-infiltrated into leaves of 4-week-old plants, and bacterial titers were measured as previously described [17]. Statistical analysis of bacterial growth data was done by student’s t-test.

4.4. RNA Analysis

Total RNA from leaves of 4-week-old plants was extracted using TRIzol Regent (Invitrogen, #15596018), and then cDNA was obtained using RNA as a template by GoScriptTM Reverse Transcription kit (Promega, #M170B). qRT-PCR analysis was performed on a CFX Connect machine (Biorad). Expression of the test genes were normalized to AT4G26410, as described previously [19].
4.5. Protein Extraction and Immunoblotting

Leaves from 4-week-old plants were frozen in liquid nitrogen and ground by TissueLyser (Qiagen). Then the total proteins were extracted in extraction buffer (50 mM of Tris pH7.5, 150 mM of NaCl, 10% (v/v) glycerol, 2 mM of EDTA, 5 mM of DTT, 0.1% Triton X-100, protease inhibitor cocktail (Roche, 1 tablet per 50 mL)). Lysates were centrifuged for 15 min at 14,000 rpm at 4 °C. After adding the same volume of 2X SDS loading buffer and boiled at 100 °C for 5 min, the supernatant was subjected to SDS-PAGE gel electrophoresis and blotting with anti-FLAG (Abcam, #ab49763) and anti-GFP (TransGen, #HT801) antibodies.

5. Conclusions

In this study, we found that constitutive expression of EDS1 and PAD4 simultaneously in Arabidopsis can enhance disease resistance at an elevated temperature, as well as at a normal temperature. To explore the potential application of this finding and evade the growth inhibition caused by constitutive overexpression of EDS1 and PAD4, we generated transgenic Arabidopsis lines expressing both EDS1 and PAD4 under control of the HSP70 promoter, whose expression is low at a normal temperature but could be highly induced at an elevated temperature. These transgenic lines exhibited enhanced resistance to *Pst* DC3000 at an elevated temperature without developmental defects. Thus, this study provided a new strategy for enhancing plant resistance to pathogens at an elevated temperature. The strategy could be potentially applied to breed disease-resistant crops.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10061258/s1, Figure S1: EDS1 and PAD4 protein accumulation in T1 transgenic lines. Table S1: Primers used in this study.

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