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ORIGINAL ARTICLE

# Benzothiadiazole-elicited defense priming and systemic acquired resistance against bacterial and viral pathogens of pepper under field conditions

**Hwe-Su Yi · Jung Wook Yang · Hye Kyung Choi · Sa-Youl Ghim · Choong-Min Ryu**

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**Abstract** Like the innate immunity in mammals, plants have developed an induced resistance, referred to as systemic acquired resistance (SAR). Recently, defense priming that is not related to the direct activation of defenses, but instead elicits more rapid induction of resistance mechanisms following trigger application, has been proposed to explain the long-lasting effect of SAR. However, the majority of previous studies have focused on understanding the molecular mechanism underlying priming under in vitro and laboratory conditions. This study examined whether defense priming occurred and was detectable with SAR marker genes by a chemical elicitor, benzothiadiazole (BTH), under field conditions. Pepper seedling application of 0.5 mM BTH was sufficient to prime the *CaPR4* gene for 20 days as well as to induce SAR against bacterial spot caused by *Xanthomonas axonopodis*. Transcriptome analysis revealed to prime defense hormonal signaling and antimicrobial compound production genes. At the end of the season, when bacterial spot and *Cucumber mosaic virus* disease outbreaks naturally occurred, BTH-treated plants demonstrated less disease symptoms. Our results indicate that the priming of SAR genes plays a critical role in plant protection against pathogens under natural conditions.

**Keywords** PGPR · ISR · SAR · BTH · Defense priming

H.-S. Yi and J.W. Yang contributed equally to this work.

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H.-S. Yi · J. W. Yang · H. K. Choi · C.-M. Ryu (✉)
Systems and Synthetic Biology Research Center, KRIBB, Daejeon 305-806, South Korea
e-mail: cmryu@kribb.re.kr

H.-S. Yi · S.-Y. Ghim
School of Life Science, Kyungpook National University, Daegu 702-701, South Korea

C.-M. Ryu
Field of Biosystems and Bioengineering, School of Science, University of Science and Technology (UST), Daejeon, South Korea

## Introduction

Plants have developed an array of defense mechanisms, including pre-existing physical barriers and chemical defenses (the new production of toxic chemicals against plant pathogens), by sophisticated pathway manipulation. In addition, Ross (1961) proposed a new plant defense referred to as systemic acquired resistance (SAR), which was induced in *Nicotiana tabacum* systemic leaves by the inoculation of the lower leaves with *Tobacco mosaic virus* (TMV). In his pioneering work, Ross reported the general features of SAR as appearance at 2–3 days after primary TMV inoculation and maintenance for at least 20 days. Moreover, SAR was not limited to TMV but appeared in response to four different plant viruses. The long-lasting effect of SAR and its induction of broad-spectrum resistance are attractive characteristics for plant disease management. Although chemical SAR inducers have been intensively studied for potential agrochemical development, the chemical inducers benzothiadiazole (BTH) (known as Actigard® in USA and BION® in Europe) and DL-3-aminobutyric acid (BABA) have been reported to exhibit a critical disadvantage in their negative effect on plant growth (Heil et al. 2000; Van Hulten et al. 2006). The phenomenon is known as "allocation fitness cost" or "trade-off".

An additional feature of SAR is "priming". Early studies of SAR revealed that low BTH and salicylic acid (SA) concentrations did not directly activate the defense response but rather triggered the expression of pathogenesis-related (PR) genes (Kohler et al. 2002; Mur et al. 1996). Priming of sensitized plant defense responses provides an effective way for plants to obtain resistance against plant pathogens. In addition to chemical induction by BTH, SA, or BABA, the primed state can also be induced biologically by root-associated bacteria, mycorrhizae, and even insect infestation (Conrath et al. 2006). To understand the biochemical and molecular mechanisms of SAR and priming, most studies have used *Arabidopsis thaliana* as a model system due to its short life cycle, low space requirement, and availability of specific gene knockout mutants (Van Loon 2007). However, the results obtained from studying *Arabidopsis* are not always comparable or relevant to crop plants (Van Loon 2007; De Vleesschauwer et al. 2008). To fully understand the mechanism of SAR and priming in crop species, sophisticated approaches are needed, with targeted chemical inducers acting on specific plant species to elicit SAR. Unfortunately, mechanistic studies of SAR in crop plants, with the exception of rice, are not available due to the limited genetic and molecular information available in crop species such as pepper.

To apply SAR chemicals in the field, maximum SAR capacity must be balanced with minimum growth influence. To determine minimum growth effect during elicitation of SAR, BTH-elicited SAR and its duration after pathogen challenge in peppers (*Capsicum annuum*) were assessed under field conditions. Defense priming was indicated by the expression of the *C. annuum* (*Ca*) *pathogenesis-related protein 4* (*PR4*) gene. Our results indicate that priming during BTH-elicited SAR was sustained up to 20 days after treatment.

## Materials and methods

### Plant preparation and field trial

The field trial was conducted at Cheongwon-gun, Chungcheongbuk-do, Korea (36°35′32.27″N, 127°30′34.75″E) in spring 2009. Pepper seeds (*Capsicum annuum* L. cv. Bukwang, which is a susceptible cultivar to *Xanthomonas axonopodis* pv. *vesicatoria* and *Cucumber mosaic virus*) were surface-sterilized using 5 % sodium hypochlorite (NaOCl) for 10 min and rinsed five times with sterile distilled water. The seeds were then placed on Murashige and Skoog (MS) medium (0.22 % MS salt including vitamins, 1.5 % sucrose, and 0.8 % plant agar, pH 5.8) in a transparent sterile container. The pepper seedlings were prepared as previously described (Yang et al. 2009). For BTH treatment, 6-week-old pepper seedlings that were grown in the plug were soaked in the 0.5 mM BTH solution for 1 h before transplanting. The pepper plants in each treatment were grown in beds 20 cm high and 30 cm × 880 cm in area. Single-row treatment plots covered with black and white polyethylene plastic consisted of 23 plants and were replicated four times in a completely randomized design.

### Systemic acquired resistance against *X. axonopodis* pv. *vesicatoria*

A virulent isolate of *X. axonopodis* pv. *vesicatoria* was gifted from Dr. Doil Choi in Seoul National University, South Korea. At 10, 20, and 40 days post-treatment (dpt), to verify defense priming by pathogen attack, leaf samples collected 0 and 6 h after inoculation with bacterial suspensions of $10^6$ cfu/ml *X. axonopodis* pv. *vesicatoria* on the abaxial side of pepper leaves using the needleless syringe method as described by Yang et al. (2009). At 30 dpt, we did not collect samples due to continuous rainy days during the rainy season. The disease severity was assessed as described previously (Yang et al. 2009). Briefly, the severity of symptoms was scored from 0 to 5 as follows: 0, no symptoms; 1, yellowish color; 2, chlorosis only; 3, necrosis and chlorosis; 4, partial necrosis of the inoculated area; and 5, complete necrosis of the inoculated area. The experiment was repeated with 4 blocks (20 plants per block).

### RT-PCR and qRT-PCR

Total RNA was isolated from inoculated leaf tissue according to the protocol of Kim et al. (2006). Polymerase chain reaction (PCR) was carried out according to the manufacturer's instructions. The candidate priming gene *CaPR4* was analyzed using the primers 5′-AACTGGGATTTGAGAA CTGCCAGC-3′ (*CaPR4*-F) and 5′-ATCCAAGGTACAT ATAGAGCTTCC-3′ (*CaPR4*-R), which were designed based on GenBank database sequence (GenBank ID: AF244122.1). The following additional genes and corresponding primer sets used to detect them were investigated: *Cagamma-thionin*, 5′-CAATGATTGTAGAGGTGCAAG G-3′ and 5′-TTTGTTGACATAGCGGGTCA-3′; *Cacytochrome P450*, 5′-TCCTTCTCGCATCAAGTCCT-3′ and 5′-TTGTTCCCGAAACACATCAA-3′; *Caserine carboxypeptidase*, 5′-AGCCACTCGTTTCTCCTCAA-3′ and 5′-GC CATTTCCCACCATGTAAC-3′; and *CaAP2 domain*, 5′-C AGCTCGAGTATGGCTAGGG-3′ and 5′-AGGGAATT CAGCATCATTGC-3′. As a control to ensure that equal amounts of RNA were analyzed in each experiment, *Caactin* was also analyzed using the primers 5′-CACTGAAG

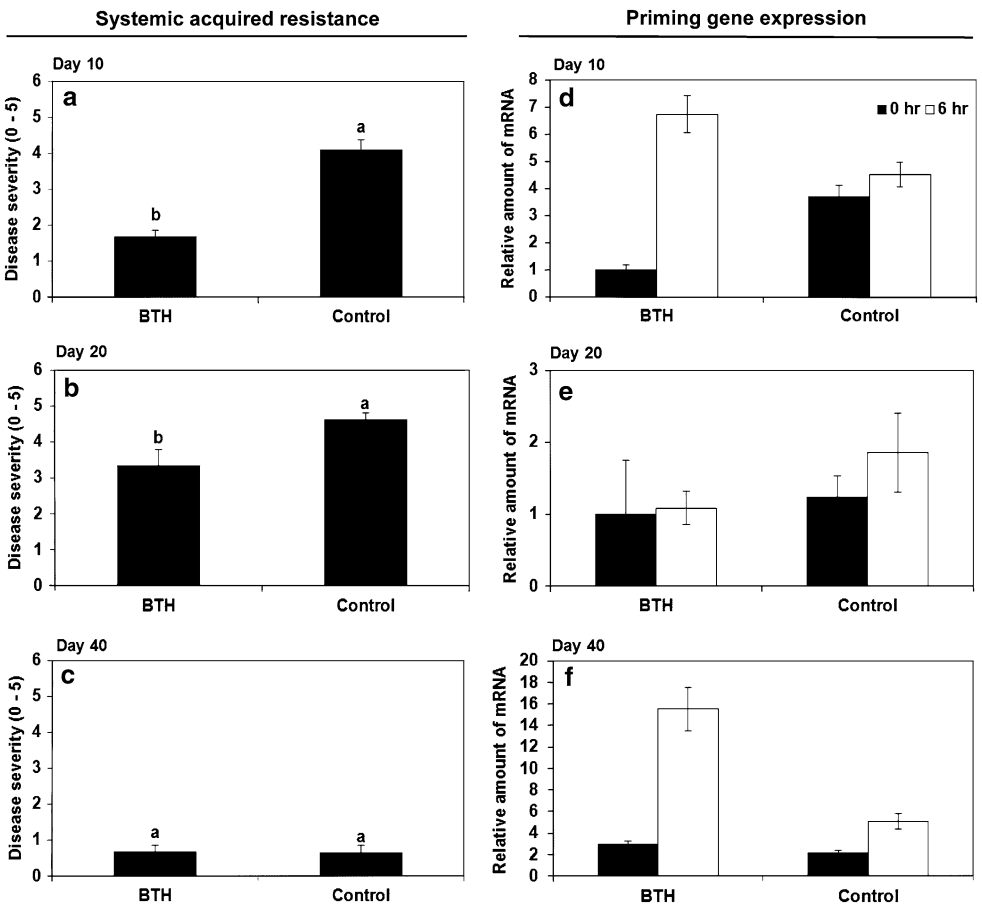


**Fig. 1** Systemic acquired resistance by BTH under field conditions. **a–c** SAR capacity induced by application of 0.5 mM BTH against *Xanthomonas. axonopodis* pv. *vesicatoria* at 10, 20, and 40 days post-transplanting (dpt). *Bars* represent mean ± SEM of 16 replications per treatment group. *Different letters* indicate significant differences between treatments ($P < 0.05$ according to LSD). **d–f** *CaPR4* gene expression level quantified by qRT-PCR at day 10, 20, 40 dpt respectively. The leaves were collected 0 and 6 h after infiltration of *X. axonopodis* pv. *vesicatoria* ($10^6$ CFU/ml) at 10, 20, and 40 dpt

CACCCTTGAACCC-3′ and 5′-GAGACAACACCGCCT GAATAGC-3′, which were designed based on the GenBank database sequence (GenBank ID: AY572427.1). Quantitative real-time PCR (qRT-PCR) was carried out using a Chromo4 real-time PCR system (Bio-Rad) according to a previously described protocol (Yang et al. 2009).

## Diagnosis of viral disease

For diagnosis, test samples were selected from areas of the plant that exhibited symptoms of disease. Samples were ground, and 50 mM $NaHPO_4$ (pH 7.0) buffer was added. The ImmunoStrip (Agdia, USA) was dipped into the ground sample and buffer mixture and determined whether it showed positive (two stripes) or negative (one stripe) after 30 min. The ImmunoStrip was then removed from the sample and the results were interpreted. One and two purple lines indicated a negative and positive result, respectively.

## Microarray analysis

For microarray analysis, pepper leaves were collected in liquid nitrogen at 0 and 6 h post-inoculation (hpi) with *X. axonopodis* pv. *vesicatoria*. The microarray was designed from 29,580 consensus sequences and manufactured at NimbleGen Inc. (http://www.nimblegen.com/). Random GC probes (40,000) to monitor hybridization efficiency and four corner fiducial controls (225) were included to assist with overlaying the grid on the image. Further information on this microarray including statistical analysis can be found at http://www.ggbio.com (GreenGene Biotech, Korea). The Database for Annotation, Visualization, and Integrated Discovery (DAVID) functional annotation was used for these microarray database analyses (Huang and Lempicki 2009).

## Statistical analysis

Analysis of variance for experimental datasets was performed using JMP software v.5.0 (SAS Institute, Cary, NC, USA). Significant effects of treatment were determined by the magnitude of the $F$ value ($P = 0.05$). When a significant $F$ test was obtained, separation of means was accomplished by Fisher's protected least significant difference (LSD) at $P = 0.05$ (Ryu et al. 2011).

**Table 1** List of genes encoding defense priming genes in pepper leaves under field condition

| KEGG ID | Functional category and description | Fold change hour(s) after pathogen challenge | | | |
|---|---|---|---|---|---|
| | | BTH | | Control | |
| | | 0 | 6 | 0 | 6 |
| Valine, leucine and isoleucine degradation | | | | | |
| 2.6.1.42 | [GO:0009081] branched chain family amino acid metabolic process | 2.10 | 7.43 | 1.81 | 5.04 |
| 2.1.4.4 | [GO:0008152] metabolic process | 1.06 | 2.25 | 1.44 | 1.57 |
| 1.8.1.4 | [GO:0045454] cell redox homeostasis | 0.60 | 1.38 | −0.26 | 0.30 |
| 2.3.3.10 | [GO:0019287] isopentenyl diphosphate biosynthetic process, mevalonate pathway | 0.20 | 1.00 | −0.02 | 0.69 |
| 2.3.1.16 | [GO:0006635] fatty acid beta-oxidation | 0.15 | 1.24 | −0.28 | −0.11 |
| | [GO:0009611] response to wounding | | | | |
| | [GO:0009695] jasmonic acid biosynthetic process | | | | |
| Cysteine and methionine metabolism | | | | | |
| 4.4.1.11 | [GO:0006520] cellular amino acid metabolic process | 1.17 | 3.74 | 0.44 | 0.45 |
| | [GO:0019458] methionine catabolic process via 2-oxobutanoate | | | | |
| 2.6.1.5 | [GO:0009058] biosynthetic process | 0.46 | 0.78 | 1.51 | 1.74 |
| | [GO:0010189] vitamin E biosynthetic process | | | | |
| 2.1.1.37 | [GO:0006306] DNA methylation | 0.59 | 0.72 | −0.50 | −0.16 |
| | [GO:0006342] chromatin silencing | | | | |
| | [GO:0010069] zygote asymmetric cytokinesis in embryo sac | | | | |
| 4.4.1.14 | [GO:0009693] ethylene biosynthetic process | 2.48 | 1.56 | 1.77 | 1.52 |
| | [GO:0009693] ethylene biosynthetic process | | | | |
| 1.14.17.4 | [GO:0009620] response to fungus | −0.28 | 1.28 | 0.27 | 1.84 |
| | [GO:0009693] ethylene biosynthetic process | | | | |
| 2.5.1.16 | [GO:0008295] spermidine biosynthetic process | 1.43 | 3.86 | 1.38 | 3.22 |
| Phenylpropanoid biosynthesis | | | | | |
| 1.2.1.44 | [GO:0009409] response to cold | 0.17 | 0.88 | 0.37 | 0.05 |
| | [GO:0009809] lignin biosynthetic process | | | | |
| 1.2.1.44 | [GO:0006694] steroid biosynthetic process | 1.20 | 1.72 | 1.95 | 2.25 |
| | [GO:0008152] metabolic process | | | | |
| | [GO:0009809] lignin biosynthetic process | | | | |
| 3.2.1.21 | [GO:0005975] carbohydrate metabolic process | 1.56 | 1.49 | 1.87 | 1.69 |
| 3.2.1.21 | [GO:0009809] lignin biosynthetic process | 0.57 | 0.65 | 0.62 | 1.02 |
| 1.11.1.7 | [GO:0050832] defense response to fungus | 0.42 | 1.40 | 0.00 | 1.22 |
| 1.2.1.68 | [GO:0009699] phenylpropanoid biosynthetic process | 0.67 | 0.90 | 0.71 | 1.04 |

Injected leaves were collected 0 and 6 h after *X. axonopodis* pv. *vesicatoria* challenge at 10 dpt. All the transcript levels used in this table are $\log_2$-transformed ratio. The genes were selected steps from KEGG pathways. The detaiedl protocol is provided in "Materials and methods"

## Results and discussion

### Evaluation of systemic acquired resistance and defense gene priming

To evaluate the duration of SAR and possible involvement of priming, the disease severity of bacterial spot caused by *X. axonopodis* pv. *vesicatoria* in pepper plants subjected to root application of 0.5 mM BTH was measured 10, 20, and 40 days post-transplantation (dpt) in 2009. SAR occurred at 10 and 20 dpt but not at 40 dpt. The disease severity of BTH-treated plants was 1.7, 3.1, and 0.7 at 10, 20, and 40 dpt, respectively, while that of water-treated control plants was 4.0, 4.4, and 0.6 (Fig. 1a–c). The absence of SAR at 40 dpt was the result of reduced disease development caused by environmental cues such as high temperature and vegetative growth stage. During the growing season, from 20 to 40 dpt, pepper plants established vegetative growth before flowering that lead to less symptom development, even in the control group, when leaves were

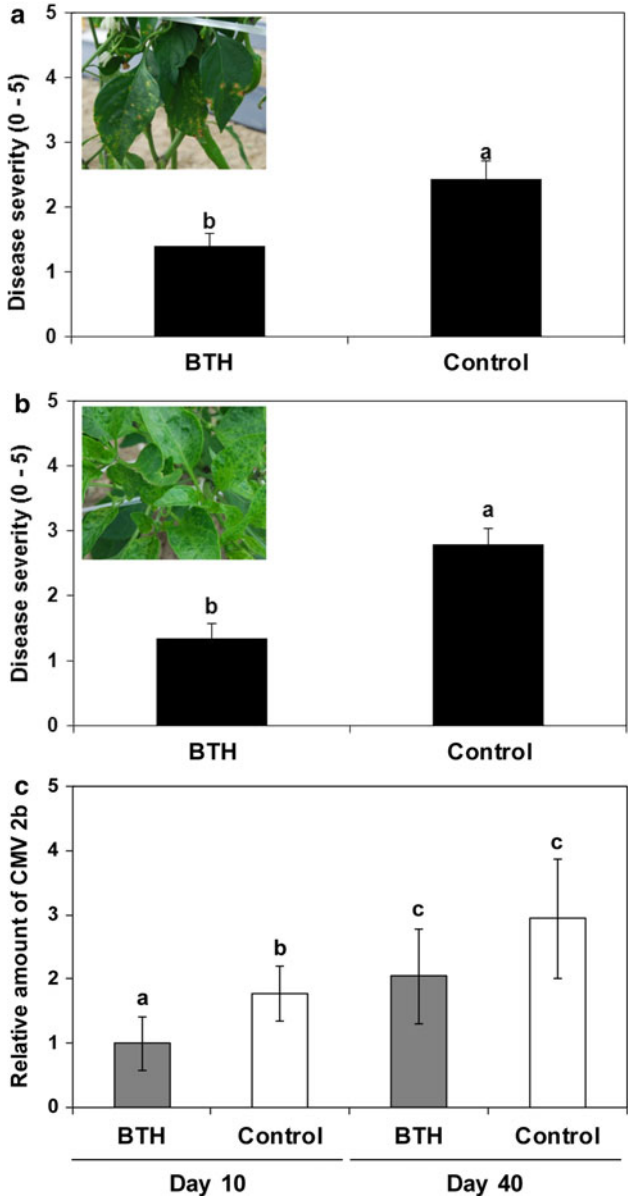


**Fig. 2** Systemic acquired resistance by BTH against naturally occurring bacterial spot and CMV diseases and its effect on plant growth. After 90 days, certain bacterial (**a**) and viral diseases (**b**) occurred in the field. *Insets* show bacterial and viral disease symptoms, respectively. *Different letters* indicate significant differences between treatments ($P < 0.05$ according to LSD)

infiltrated with *X. axonopodis* pv. *vesicatoria*. No symptom development was observed even at 14 days post-inoculation of pathogen. After that time point, measurement of symptom development after artificial infiltration of *X. axonopodis* pv. *vesicatoria* could not be used as an indicator of SAR due to the reduced disease occurrence among control plants, which suggested that the duration of experiments using artificial infiltration of bacterial pathogen to induce SAR may be limited beyond a certain time point. However, the possibility cannot be excluded that an unknown mechanism to elicit strong SAR may be involved between 20 and 40 dpt.

qRT-PCR was used to verify priming of the *CaPR4* gene, which was found to demonstrate priming during elicitation of SAR by rhizobacteria and BTH in our previous study (Yang et al. 2009). At 6 h after pathogen infiltration 10 and 40 dpt, the expression level of *CaPR4* in BTH-treated plants was higher than in controls (Fig. 1d, f; Table 1). Induced resistance and priming gene expression results were similar at 10 dpt but differed at 20 and 40 dpt. These results may be attributable to complex environment factors, such as the variety of pathogens and fickle weather. In a study of the cost of priming benefits in *Arabidopsis*, the fitness cost of priming was found to be lower than those of activated defenses (Van Hulten et al. 2006).

## SAR against naturally-occurring pathogens

After 90 dpt, several disease symptoms including spots, wilt, and chlorosis appeared across the whole field. Throughout the rainy season and high temperature, from late June to mid-August in Korea, the symptoms became more severe (Supporting Information Fig. S1a, b). Interestingly, the spot symptoms were similar to bacterial spot caused by *X. axonopodis* pv. *vesicatoria*. Isolation of leaf spots on LB medium revealed large yellow colonies typical of *Xanthomonas* spp. (data not shown). Unlike our introduced *X. axonopodis* pv. *vesicatoria* strain, normal pathovars of *Xanthomonas axonopodis* do not grow very well on LB medium, indicating that the isolated *Xanthomonas* spp. may have resulted from multiplication of the strain introduced for SAR experiments during the rainy season. To obtain more solid evidence on the bacterial classification, 16S rRNA analysis was used to confirm that the causal pathogen was *X. axonopodis* pv. *vesicatoria* (data not shown). According to the measurement of visual disease rate (0–5), the disease severity of plants treated with BTH (1.3) was significantly less than that of control plants (2.5; Fig. 2a, inset).

In addition to bacterial symptom development, severe leave chlorosis, abnormal development of leaves including twisted, mottling, and shoestring phenotypes, and stunting (dwarfism) typical of virus symptoms appeared throughout the field after the rainy season. The collective symptoms were very similar to those caused by *Cucumber mosaic virus* (CMV) and TMV in pepper. Antibody-based stick tests confirmed the presence of CMV on symptomatic leaves (data not shown). CMV RNA was confirmed by the qRT-PCR technique (Fig. 2c). The above-described viral symptoms, including the shoestring phenotype, matched those of CMV. A large population of aphids was also

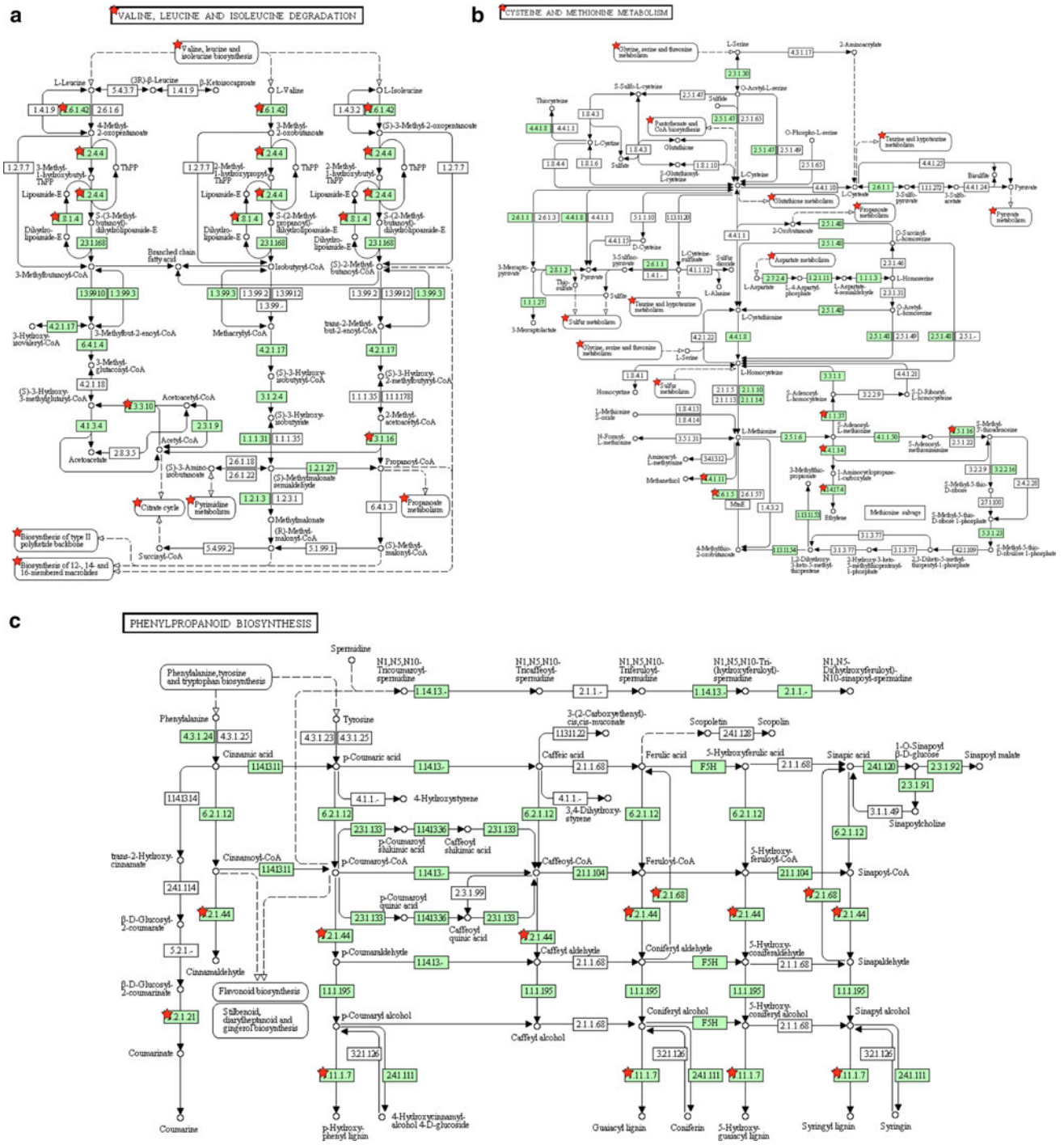


**Fig. 3** Analysis of pathways related to defense. Injected leaves were collected 0 and 6 h after *X. axonopodis* pv. *vesicatoria* challenge at 10 dpt. *Map* displays selected steps from KEGG pathways. *Red stars* indicate significant differences in expression, with higher relative levels in BTH-treated plants. **a–c** Valine/leucine and isoleucine degradation pathway, cysteine/methionine metabolism pathway, and phenylpropanoid biosynthesis pathway, respectively

observed to act as a CMV vector in the pepper plants because no pesticide was applied over the experimental period. The viral disease severity was 1.5 and 2.3 in BTH- and control-treated plants, respectively (Fig. 2b). In addition, qRT-PCR analysis revealed that CMV accumulation detected by CMV 2b protein was lower in BTH treatments at days 10 and 40 than control (Fig. 2c). However, the difference was not significant at day 40.

The naturally occurring bacterial and viral diseases appeared with different patterns compared with SAR at

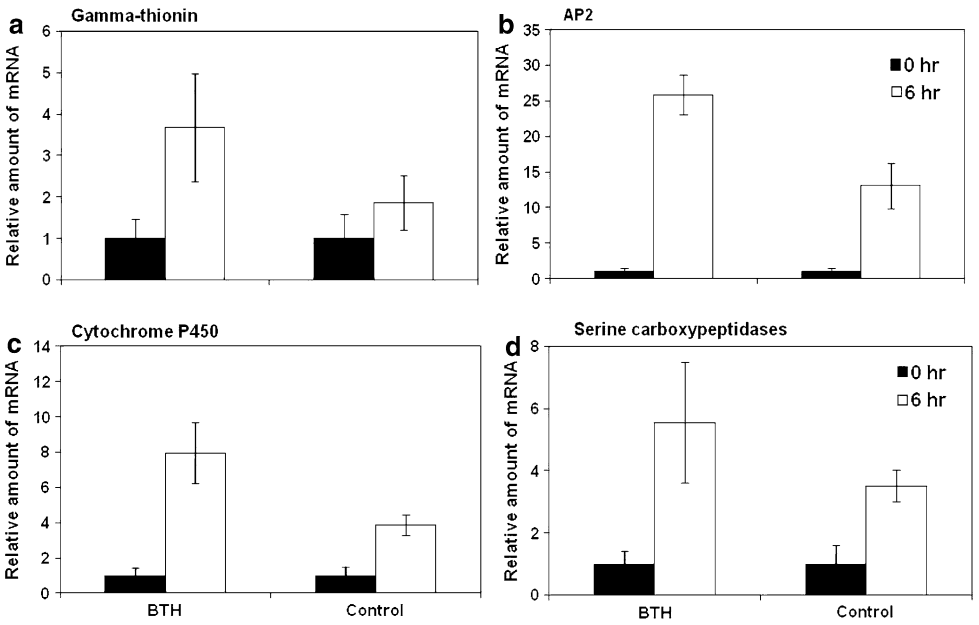


**Fig. 4** Priming of pepper resistance genes *Cagamma-thionin*, *CaAP2, Cacytochrome* P450, and *Caserine carboxypeptidase* by BTH in the field. The expression levels of *Cagamma-thionin* (**a**), *CaAP2* (**b**), *Cacytochrome* P450 (**c**), and *Caserine carboxypeptidase* (**d**) genes expected to demonstrate priming based on microarray findings were quantified by qRT-PCR. The leaves were collected 0 and 6 h after infiltration of *X. axonopodis* pv. *vesicatoria* at 10 dpt. The housekeeping gene *Caactin* was used as a control. *Bars* mean ± SEM of 4 replications per treatment

40 dpt (Figs. 1c, 2a, b). The results in Fig. 1a–c were obtained by artificial infiltration with a needleless syringe. We speculated that the infiltration of *X. axonopodis* pv. *vesicatoria* on the leaves in the rainy season allowed an outbreak of the disease due to the ideal conditions for pathogens to spread via rain splash, with temperatures as higher as 30 °C for more than 1 month. This possibility raises the question of how pepper plants treated with BTH at the time of transplantation are protected more effectively against bacterial and viral diseases. The precise answer is not known. As shown in Fig. 1f, priming as indicated by the expression of *CaPR4* still persisted at 40 dpt, although disease severity measurements had no different between treatment (Fig. 1c). We hypothesized that the priming of *CaPR4* did not play an important role on disease suppression. Thus, we attempted to employ microarray to search new molecular marker genes for defense priming in pepper plant under field conditions.

## Identification of new priming genes and pathways by transcriptome analysis

In an earlier study, we used the *CaPR4* gene as an indicator of SAR priming after pathogen infiltration in pepper plants pretreated with BTH before transplantation. In the greenhouse, consistent results were obtained using *CaPR4* as a priming marker gene in pepper (Yang et al. 2009). However, in the current study, the SAR phenotype did not correlate with the transcriptional expression of *CaPR4* (Fig. 1b, c, e, f). The reliability of *CaPR4* as a marker at 10 dpt may be the result of less exposure to environmental and biological factors. In contrast, at 20 dpt, BTH-treated plants demonstrated increased SAR against bacterial spot disease, but *CaPR4* expression did not differ between treatments. More interestingly, no difference in SAR capacity was observed at 40 dpt, although the priming of *CaPR4* expression was demonstrated at this time point. Collectively, these results indicate the necessity of identifying more sensitive and consistent marker genes of priming after BTH induction of SAR.

To isolate promising genes regulated by BTH, a pepper microarray system was employed (data not shown). Based on our microarray data, functional annotation and analyses were conducted using DAVID, and three Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified: valine/leucine and isoleucine degradation (VLILD pathway, Fig. 3a), cysteine/methionine metabolism (CMM pathway, Fig. 3b), and phenylpropanoid biosynthesis (PPB pathway, Fig. 3c) (http://www.genome.jp/kegg/) (Huang and Lempicki 2008; Dennis et al. 2003). Polyketide backbone biosynthesis via the VLILD pathway is related to polyketide-derived phytoalexins, low molecular weight secondary metabolites involved in the defense response (Hammerschmidt 1999). Ethylene (ET) synthesis via the CMM pathway is strongly stimulated by both biotic and abiotic stresses, such as pathogens, insects, mechanical wounding, water deficiency, and extreme temperature (Iriti and Faoro 2003). Various lignins are synthesized by the PPB pathway (Fig. 3c). Lignin is a complex hydrophobic phenylpropanoid network important for plant mechanical support, water transport, and defense (Campbell and Sederoff 1996).

*Gamma-thionin* (Fig. 4a), *AP2* (Fig. 4b), *cytochrome P450* (Fig. 4c), and *serine carboxypepdidase* (Fig. 4d) genes were selected on the basis of their relationship to the

four above pathways and defense signaling. Gamma-thionin isolated from wheat, barley, tobacco, potato, tomato, cowpea, soybean, and *Arabidopsis* is also known as a plant defensin that functions in defense mechanisms against a broad spectrum of plant pathogens (Pelegrini and Franco 2005). *Cytochrome P450* expression was increased in pepper following treatment with SA and abscisic acid (Kim et al. 2006). The APETALA2/ethylene response factor (AP2/ERF) domain transcription factor ORA59 has been suggested to be a positive regulator of the *PDF1.2* gene in ET signaling (Pre et al. 2008). The rice *serine carboxypeptidase-like* gene *OsBISCPL1* was significantly upregulated after treatment with BTH, SA, jasmonic acid, and 1-amino cyclopropane-1-carboxylic acid, a precursor of ET (Liu et al. 2008). These four genes were upregulated 6 h after pathogen challenge of pepper plants at 10 dpt and demonstrated significantly higher expression in BTH-treated plants than in controls (Fig. 4). Taken together, the defense gene expression pattern indicates the priming of defense genes during elicitation of SAR under field conditions, suggesting that plants modulate defense gene expression to minimize the trade-off effect.

## Conclusion

Our results provide the first evidence for occurrences of the defense priming during elicitation of systemic acquired resistance under field conditions. Seedling treatment of a commercial chemical inducer, BTH before transplanting was enough to maintain priming *CaPR4* gene until day 40. Transcriptome analysis revealed that diverse hormone-dependent defense genes and phenylpropanoid pathway-dependent phytoalexin genes were induced more rapidly following pathogen challenge. Such defense priming in pepper can be proposed to explain the long-lasting effect of SAR. However, the features of the internal translational signals that are involved remain to be determined.

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## References

Campbell MM, Sederoff RR (1996) Variation in lignin content and composition (mechanisms of control and implications for the genetic improvement of plants). Plant Physiol 110:3

Conrath U, Beckers GJM, Flors V, García-Agustín P, Jakab G et al (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071

Da Wei Huang BTS, Lempicki RA (2008) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57

De Vleesschauwer D, Djavaheri M, Bakker PAHM, Höfte M (2008) *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. Plant Physiol 148:1996–2012

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W et al (2003) DAVID: database for annotation, visualization, and integrated discovery. Genome Biol 4:P3

Hammerschmidt R (1999) PHYTOALEXINS: what have we learned after 60 years? Annu Rev Phytopathol 37:285–306

Heil M, Hilpert A, Kaiser W, Linsenmair KE (2000) Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? J Ecol 88:645–654

Huang DWSB, Lempicki RA (2009a) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37:1–13

Huang DWSB, Lempicki RA (2009b) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57

Iriti M, Faoro F (2003) Does benzothiadiazole-induced resistance increase fitness cost in bean. J Plant Pathol 85:265–270

Kim YC, Kim SY, Paek KH, Choi D, Park JM (2006) Suppression of CaCYP1, a novel cytochrome P450 gene, compromises the basal pathogen defense response of pepper plants. Biochem Biophys Res Commun 345:638–645

Kohler A, Schwindling S, Conrath U (2002) Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/NIM1 gene in *Arabidopsis*. Plant Physiol 128:1046–1056

Liu H, Wang X, Zhang H, Yang Y, Ge X et al (2008) A rice serine carboxypeptidase-like gene OsBISCPL1 is involved in regulation of defense responses against biotic and oxidative stress. Gene 420:57–65

Mur LAJ, Naylor G, Warner SAJ, Sugars JM, White RF et al (1996) Salicylic acid potentiates defence gene expression in tissue exhibiting acquired resistance to pathogen attack. Plant J 9:559–571

Pelegrini PB, Franco OL (2005) Plant [gamma]-thionins: novel insights on the mechanism of action of a multi-functional class of defense proteins. Int J Biochem Cell B 37:2239–2253

Pre M, Atallah M, Champion A, De Vos M, Pieterse CMJ et al (2008) The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiol 147:1347

Ross AF (1961) Systemic acquired resistance induced by localized virus infections in plants* 1. Virology 14:340–358

Ryu CM, Shin JN, Qi W, Ruhong M, Kim EJ, Pan JG (2011) Potential for augmentation of fruit quality by foliar application of *Bacilli* spores on apple tree. Plant Pathol J 27:164–169

Van Hulten M, Pelser M, Van Loon LC, Pieterse CMJ, Ton J (2006) Costs and benefits of priming for defense in *Arabidopsis*. Proc Natl Acad Sci USA 103:5602–5607

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Yang JW, Yu SH, Ryu CM (2009) Priming of defense-related genes confers root-colonizing bacilli-elicited induced systemic resistance in pepper. Plant Pathol J 25:303–440